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**Sawitzke, Sharon L., Ph.D.**

**City University of New York, 1994**

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THE MAMMALIAN OLFACTORY BULB: A COMPARATIVE ANALYSIS

by

Sharon L. Sawitzke

A dissertation submitted to the Graduate Faculty in Anthropology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1994

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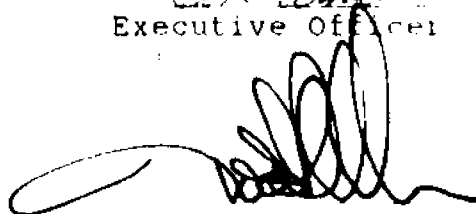
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THE CITY UNIVERSITY OF NEW YORK

## Abstract

## THE MAMMALIAN OLFACTORY BULB: A COMPARATIVE ANALYSIS

by

Sharon L. Sawitzke

Advisors: Professor Warren J. Kinzey

Professor Ilya I. Glezer

Traditionally, the size of the olfactory bulb has been directly related to the functional ability of the olfactory system. The catarrhine primates, because of their small olfactory bulbs (microsmatic), have been assumed to have a regressive olfactory system with reduced olfactory function. Behavioral studies within the last two decades have refuted the belief that olfactory function in these primates is minimal. The goal of this study was to evaluate the olfactory bulb of several mammalian species to determine if additional features corroborate the belief that olfactory system function is correlated with bulb size.

Two species of *Macaca* (*M. mulatta* and *M. fascicularis*) and four macrosmatic mammals (cat, rabbit, squirrel, and rat) were analyzed for bulb morphology, density of cells, and volume of those layers comprising the bulb.

Data showed that the only feature that could be construed as indicating decreased functional ability of catarrhine primates was reduced bulb size relative to brain size. In all other features the olfactory bulb in *Macaca* was comparable to the bulbs of the four macrosmatic mammals. One result, suggestive of the change that has occurred within the evolution of the neocortex, may indicate an improvement in the processing of information: the ratio of interneurons to identified second order neurons was increased in *Macaca*. One factor invalidates olfactory bulb size as an indicator of functional ability. Small olfactory bulbs (rat and *Macaca*) lack an olfactory ventricle and have a small subventricular layer, a layer of nonsynaptic, immature neurons. In larger bulbs, part of the volume which contributes to large size is the enlargement of the subventricular layer and the presence of an olfactory ventricle. This indicates that a larger portion of the bulb of *Macaca* and the rat is concerned with processing information than is found in larger olfactory bulbs.

In conclusion, the morphology of the olfactory bulb of the supposedly microsmatic catarrhine primate (*Macaca*) does not provide any evidence for reduced function in the olfactory system. At least one feature is strongly suggestive of a more highly developed mechanism for processing information within the bulb.

This dissertation is dedicated to the memory of my father, Mr. Joseph Sawitzke Jr., who lives on in my thoughts, and who speaks to me in happy moments. My father was one of the smartest people I have ever known, and one of the kindest.

Thanks Dad, for everything!

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## CHAPTER 1

### INTRODUCTION AND STATEMENT OF HYPOTHESIS

In terms of their sense of smell, mammals have traditionally been classified as either macrosmatic or microsmatic. Macrosmatic animals use their sense of smell to survive, that is: to find food, detect predators, identify territory, recognize kin and conspecifics, identify receptivity of a mating partner. Macrosmia has been correlated with a relatively large area of the brain involved in olfaction. Vertebrates designated as microsmatic have been thought to depend more extensively on vision and/or audition rather than olfaction in order to survive. Correlated with microsmia is a reduction in the size of regions of the brain having to do with olfaction, particularly in the size of the olfactory bulb.

Keverne (1980) has suggested that two findings invalidate the concept of macrosmatic vs microsmatic: 1) olfactory receptor neurons are not narrowly tuned to particular odors but can respond to a number of different odors; 2) neural projections have access to the neocortex via the thalamus, implying that animals with a more highly

developed neocortex have the potential for a more sophisticated sense of smell. "The fact that olfactory information is coded as a pattern which requires a higher order of recognition means that animals with a greater neural back-up have the potential for a more sophisticated sense of smell. Since the olfactory bulb seems to be acting mainly as a filter, and decoding of the olfactory message is a more central event, then animals with a greater neocortical support system are ones with the greatest ability to make use of this system." (Keverne, 1980, p. 314). Keverne adds (1983, p. 80), "Primates do not therefore have a poorly developed sense of olfactory perception, and the ways in which this is employed may well be the most evolved of all species."

For many years it was accepted that primates were microsmatic when compared with most other mammals. Within the primates, the strepsirrhines were accepted as having the keenest sense of smell, based on observed behavior and a larger olfactory bulb. All haplorhines were considered to be microsmatic, based on a lack of behavioral data indicating the importance of olfaction in the lives of these animals, and more obviously, a reduction in size of the olfactory bulb. In the last twenty years, several studies have indicated that many haplorhine primates have a keen sense of smell. Epplé (1974) found that *Saguinus fuscicollis* can distinguish between the following: 1) male

and female conspecifics based on the scent marking odor; 2) individual conspecifics, for example between the scent marking odor of a conspecific it has had a recent aggressive encounter with and the scent marking odor of a more neutral conspecific. In her review of chemical communication in primates, Eppler (1985, 1986, 1993) showed that in haplorhines, scent marking and examination of scent marks (smelling and licking) are common. Male spider monkeys (*Ateles*) frequently sample the urine of females during all stages of the reproductive cycle including pregnancy. Female howler monkeys (*Alouatta*) urine-wash their hands, feet and tails; male howler monkeys have been observed tracking estrous females by sniffing branches the females have traveled along. *Macaca mulatta* females who have had their ovaries removed stimulate copulatory behavior in males when vaginal secretions from receptive females are smeared on them. While it is known that in rodents, pheromones carried in the urine bind to receptor cells located within the vomeronasal organ, it cannot be assumed that all pheromones in all mammals are received by the vomeronasal organ and none are received by olfactory receptor neurons. Nor can it be said that all pheromones are chemical constituents of urine. Although the presence of accessory nasochemosensory systems complicates our understanding of how odorant information is transmitted to the brain, not all mammals have a vomeronasal organ or accessory olfactory

bulb. It is likely that some, and perhaps many pheromone odorant molecules bind to receptor cells located within the olfactory epithelium. This is especially likely to be true in those primates that lack a functioning vomeronasal organ in the adult.

In addition to the recent primate behavioral data indicating that olfactory bulb size may not be an accurate indication of function, the process of parcellation as described by Ebbeson (1980, 1984) has provided a theoretical framework upon which this study has been built. Briefly, neuronal systems that have to handle an increase in complexity of information may improve the processing of that information by decreasing extraneous information or "noise". They may do this through a process of parcellation, that is, through a decrease in number of cells within a nucleus, a decrease in size of nuclei, or a decrease in connectivity. Since parcellation within a system is often associated with an increase in lamination (layering) of cell populations, and since lamination itself is often associated with a shift in relative proportions of classes of cells, we have a model with which to investigate differences in the mammalian olfactory bulb that is not based on the assumption that a decrease in olfactory bulb size indicates a decrease in function.

The underlying hypothesis for this study is that the functional capability of the olfactory system is not

directly correlated with size of the olfactory bulb. A series of mammalian olfactory bulbs including two species of primates has been examined for: 1) differences in degree of lamination; 2) relative proportions of classes of cells within the olfactory bulb; 3) relative differences in volume. The reduced olfactory bulb size of some mammals may be indicative of a sensory system that handles an increased complexity of information by a change in processing rather than by increasing bulb volume.

A rather extensive background of the nasochemosensory system in mammals is included as part of this thesis. Chapter 2 includes a description of the ontogeny, morphology, and function of the cells that form the peripheral olfactory system. It also includes the ontogeny, morphology and function of the remaining four components of the nasochemosensory system. Chapter 3 discusses in great detail the ontogeny of the olfactory bulb, the morphology of the several component layers of the adult olfactory bulb and function of the cells within these layers. A summary of synaptic connections between the layers of the bulb and the implications this has for function is also included within this chapter. Chapter 4 is a discussion of the phylogenetic and ontogenetic changes that have occurred within neural systems in general: these changes form the theoretical framework for this investigation. Chapter 5 includes the

material and methods used in this study. Chapter 6 includes both the descriptive and statistical data generated by this study, and interprets the findings. In Chapter 7, the findings are discussed in a functional and evolutionary context, and the significance of the study is discussed.



## CHAPTER 2

### THE NASOCHEMOSENSORY SYSTEM: BACKGROUND

#### I. INTRODUCTION

The nasochemosensory system in mammals is a complex of five component nasal cavity senses by which animals relate to their external environment. This complex includes olfaction in the strict sense (the primary component, or the "main olfactory system") and four accessory components (the accessory nasochemosensory or olfactory system): vomeronasal organ, septal organ, free trigeminal nerve endings within the nasal cavity, and the terminal nerve. In mammals, the components of the nasochemosensory system contribute to: finding food, defining and maintaining territory, finding mates, distinguishing kin from strangers, reciprocal recognition between mother and infant, recognizing danger, warning against intruders, and many physiological functions such as sexual maturation, ovulation, and the maintenance or termination of pregnancy. Much work has been done to separate out, both functionally and anatomically, the main olfactory system from the four components of the accessory olfactory system. This study focuses on the olfactory bulb,

just one part of the main olfactory system. However, to appreciate the sense of smell more fully, the background section of this thesis will include a description of the entire main mammalian olfactory system and each of the four components of the accessory system. Each section will include the embryology of the system in mammals, the general function of the system, and a morphological description of its peripheral parts, that is, its nasal components and the first, more central region of synapse (the olfactory bulb in more than one case).

The main olfactory system includes the olfactory epithelium within the nasal cavity and the olfactory bulb, into which fibers from the accessory system also project. Since the main thrust of this thesis concerns the olfactory bulb, background on this structure will be discussed after: 1) the olfactory epithelium of the main olfactory system and the primary receptor neurons located therein; 2) the accessory nasochemosensory system.

## **II. ONTOGENY OF THE GENERALIZED MAMMALIAN MAIN OLFACTORY SYSTEM**

Areas of thickening within cephalic surface ectoderm in the early embryo form specialized regions of sensory cells with specialized functions. These bilateral ectodermal

regions form the sensory placodes which include the olfactory, lens, and otic placodes.

The first step in the formation of the sensory placodes is the formation of a "primitive placodal thickening". This arises very early in the formation of the nervous system, during the development of the neural plate. The neural plate forms as a midline region of ectoderm whose component cells differentiate into elongated columnar cells. It is the invagination into underlying mesoderm and subsequent folding of this plate that forms the neural tube, the primordium of the central nervous system. Lateral to each side of the developing neural plate (but not cranial to it), a column of neural crest cells forms. Cranial to the neural plate and flanking the neural crest cells laterally, the primitive placodal thickening develops. (Brunjes and Frazier, 1986).

The primitive placodal thickening is made up of pseudo-stratified epithelium with two layers: an outer layer of non-neural ectoderm and an inner layer of neural ectoderm. At the time that the neural plate begins to invaginate and form the neural groove, there is a dissociation of the developing neural tube and neural crest cells from the primitive placode. The primitive placode then undergoes separation into the three sensory placodes and a thickening of stomodeal ectoderm from which the adenohypophysis eventually develops. (Brunjes and Frazier, 1986).

The sensory placodes thicken in a manner similar to that of the neural plate and invaginate into the layer of mesoderm internal to them. In the olfactory region, the placode furnishes the neurosensory cells of the olfactory epithelium, cells that are unusual in that they function as both receptor cell and neuron. The olfactory placode is the last of the three sensory placodes to appear.

#### **A. ONTOGENY OF THE PERIPHERAL OLFACTORY SYSTEM**

In mammals, the olfactory placodes appear anteroinferiorly on either side of the frontal prominence. In the human, this occurs on day thirty of the embryo, after the optic and otic placodes have appeared, and after full closure of the neural tube (Tuchmann-Duplessis, et al., 1982). Formation of the nasal cavity appears to be induced by both underlying mesoderm and by the deep layer of ectoderm. Evidence for this inductive process includes the following: 1) transplants of placode tissue which include the underlying mesoderm differentiate into nasal pits much more rapidly and completely than transplants consisting of placode tissue alone (Zwilling, 1940). 2) When the placode is dissociated into deep and superficial layers of ectoderm, deep layers develop into nasal pits while the superficial layers don't (Zwilling, 1940). [It should be pointed out, however, that in the amphibian embryo studied by Klein and

Graziadei (1983), the boundary between these two tissue layers becomes difficult to distinguish because of migration and mixing of cells from the inner layer (neural) to the outer layer (non-neural ectoderm). The neural layer gives rise to the receptor cells of the olfactory mucosa while the superficial non-neural ectoderm gives rise to mucosal secretory cells and supporting cells (Klein and Graziadei, 1983).]

The developing nasal placode invaginates inwardly due to proliferation of mesodermal cells at its lateral and medial borders. The invagination proceeds towards the brain, the base of the invagination developing into olfactory epithelium while the surrounding walls become the surface ectoderm lining the nasal cavity. The cells of the placode differentiate into neurosensory cells which, at about six weeks in the human, give rise to axons which traverse the inner epithelium and mesenchyme and make contact with the olfactory region of the telencephalon.

In the twelve day old mouse embryo, the base of the nasal epithelium, the basal lamina, is perforated at many sites by exiting axons of olfactory receptor neurons and by basal cells. Basal cells are a type of stem cell that gives rise to olfactory neurons and also affords structural strength to the basal layer of olfactory epithelium. It appears that some basal cells follow the migration of the axons, using the same exit perforations in the basal lamina

and directly trailing a small group of axons (Marin-Padilla and Amieva B., 1989). As soon as they leave the epithelium, these basal cells ensheath small groups of axons usually consisting of 10-20 axon fibers. These migrating basal cells are the progenitors of olfactory sheath cells, frequently referred to as Schwann cells (Rafols and Getchell, 1983; Farbman and Squinto, 1984) in the literature. Each axon group ensheathed by its specific sheath cells is termed an olfactory nerve fascicle. Nerve fascicles from different epithelial regions converge to form progressively larger ones as they approach the telencephalon. The relationship of specific sheath cells to a specific axon group is retained throughout the merging of fascicles. Mitotic sheath cells are seen throughout the length of the advancing axons in order to ensheath the axon groups from olfactory epithelium to the telencephalon. Marin-Padilla and Amieva B. state that the immediate and close association of sheath cells with a specific axon group must be important because it is retained up to the point that axon contact is made with the anteromedial region of the telencephalon. This region of the telencephalon represents the embryonic anlage from which the olfactory bulb develops.

Brunjes and Frazier (1986) state that another type of cell to migrate out of the early olfactory epithelium is the primordium of the ganglion cells of the terminal nerve

(nervus terminalis). The cells remaining within the olfactory epithelium consist of original stem cells of the placode, differentiating neurons, and a decreased number of original cells that will give rise to supporting cells and Bowman's glands. (Brunjes et al., 1986). At tissue maturity, Bowman's glands will be located within the lamina propria and will provide the solvent for odorant substances.

Toward the end of the third month of development in the human, the mesenchyme between the olfactory epithelium (placode-derived) and the olfactory bulb (telencephalon-derived) gives rise to the cribriform plate of the ethmoid bone. The cribriform plate organizes itself around axon bundles of the receptor neurons and separates them from each other (Tuchmann-Duplessis et al., 1982).

The formation of axonal processes of the receptor cells always precedes development of the dendritic processes. With formation of the dendrites, the bipolar shape of the neurons becomes evident and terminal expansions of dendrites develop distally. During the final stages of ontogeny of the receptor cells, the terminal endings develop cilia, organelles mature within the cytoplasm of the cells, and supporting cells proliferate and mature between receptor cells. It has been observed by Cuschieri and Bannister (1975) that receptor cells develop in clumps which communicate with each other through gap junctions and which may ultimately encode for a particular stimulus.

Olfactory receptor neurons continue to regenerate throughout life: the life span of this cell in the olfactory epithelium of the mouse and hamster is between 30 to 35 days (Graziadei and Monti Graziadi, 1978; Samanen and Forbes, 1984). Cells in different stages of differentiation can be seen throughout the life of the individual. There is, however, a general but substantial decline in the total number of receptor cells in the olfactory epithelium as aging progresses. This decline in receptor neurons precedes a decline in mitral cell size and number of axons within the glomerular layer of the olfactory bulb (Hinds and McNelly, 1981).

#### **B. NORMAL OLFACTORY BULB DEVELOPMENT IS DEPENDENT UPON RECEPTOR CELL AXONS**

The ontogeny of the olfactory bulb is mentioned here due to the dependance of bulb development on the presence of receptor neuron axons. A more in-depth discussion of olfactory bulb development is included in Chapter 3.

The olfactory bulb, comprised of main and accessory divisions, receives axons from three peripheral sources: olfactory receptor neurons within the olfactory mucosa; receptor neurons within the vomeronasal organ; receptor neurons within the septal organ. Axons of receptor neurons within the olfactory epithelium project to the main



olfactory bulb. Since the focus of this study is the main olfactory bulb, projections from the accessory olfactory system to the accessory olfactory bulb will not be dealt with in great detail.

Telencephalic vesicles evaginate about the same time that the nasal placodes invaginate into the underlying mesoderm to form the nasal pits. Axons of olfactory receptor cells extend toward the forebrain of the developing telencephalon. Olfactory nerve fibers encircle and contact the rostral pole of the developing telencephalon long before cells characteristics of the olfactory bulb have developed. Surgical removal of the olfactory placode (in amphibians) results in a reduction in size or complete failure of the olfactory bulb to form, indicating that the development of this target tissue is dependant upon the presence of olfactory receptor neurons (Stout and Graziadei, 1980; Klein and Graziadei, 1983). The influence of the axons of developing olfactory receptor neurons often extends to the development of a particular region of the telencephalon. Removal of the olfactory placode in amphibians prevents the telencephalon from developing to its full size (Stout and Graziadei, 1980). The power of the developing receptor cell is such that when the olfactory placode is transplanted with a fragment of forebrain tissue, it can still induce the formation of a glomerulus-like structure within that fragment (Stout and Graziadei, 1980).

Axons of the olfactory receptor neurons synapse with dendrites of mitral and tufted cells within the glomeruli of the olfactory bulb. Mitral and tufted cells are the second order neurons of the main olfactory system: they transmit the signal to more central regions of the brain and are, to date, the only known output neurons of the olfactory bulb.

### **III. MORPHOLOGY OF THE ADULT PERIPHERAL OLFACTORY SYSTEM**

#### **A. THE NASAL CAVITY**

The nasal cavity is the beginning of the respiratory passageway and is contained within the face. It begins with the nostrils or nares and ends with the choanae (posterior nasal apertures), where it becomes continuous with the nasopharynx. The nasal cavity is divided into two more-or-less equal chambers by a midline nasal septum, formed primarily by the septal cartilage of the nose as well as by bony elements from the vomer and the perpendicular plate of the ethmoid. The lateral wall of each chamber of the nasal cavity is formed by the maxillary bone anteroinferiorly, by the perpendicular plate of the palatine bone posteriorly, and by the conchae (also referred to as turbinates). The conchae are a variable number of curved processes which

protrude from the lateral wall of each nasal chamber (Figure 2, page 37). There are three pair of conchae in humans while macrosmatic primates (*Nycticebus coucang*, for example) may have as many as eight pair (Loo and Kanagasuntheram, 1971). A complex system of concha (turbينات) projects into the nasal cavity of strepsirhines<sup>1</sup> from the lateral nasal cavity wall. Lemurs possess a nasoturbinate, maxilloturbinate and up to four main ethmoturbinate processes (Hill, 1953). In addition, two small scroll-like processes (ectoturbينات) are present. In the lorises (Hill, 1955), the nasal fossae are less spacious and contain four ethmoturbينات, the first one being large and which hides the maxilloturbinate. There is one nasoturbinate, and few ectoturbينات. The haplorhines have a simplified maxilloturbinate that lacks the furrows found in the strepsirhines (Hill, 1955). There are two or three endoturbينات but only the foremost one is well developed. The nasoturbinate is reduced or absent, and there are no ectoturbينات. The nasal cavity of *Tarsius* is characteristic of that found in other haplorhines (Hill, 1955). Within the platyrrhines, there is generally wide variation in the relative size and arrangement of the turbينات.

1. Szalay and Delson (1979) define the suborder Strepsirhini as including the infraorders Adapiformes (all species extinct) and Lemuriformes. The suborder Haplorhini includes the infraorders Tarsiiformes, Platyrrhini, and Catarrhini.

The floor of the nasal cavity is formed by the maxillary and palatine bones. The roof of the cavity is formed by the nasal bones, the sphenoid bone posteriorly, and the cribriform plate of the ethmoid bone which separates the nasal cavity from the anterior fossa of the cranial cavity. In the Strepsirhini, the nasal fossae are spacious because of the complex system of turbinate processes they contain (Hill, 1953). In the Haplorhini, the nasal fossa is reduced secondary to reduction of the muzzle. The fossa is relatively short and high, with a reduced choanal region (Hill, 1955). The nasal fossae of monkeys differs from that in humans in that there is an absence of frontal, ethmoidal and sphenoidal air sinuses and a retention of a perforate nasopalatine canal (Hill, 1955). Nasal cartilages form the mobile part of the external nose in humans and some non-human primates. In all primates except man, they tend to fuse with each other early in life (Hill, 1957). [For greater detail on the morphology of the nose and nasal cavity, refer to Williams et al., 1989; Maier, 1980; Evans and Christensen, 1979); Hill, Volumes I-VII, 1953-1957.]

Each nasal chamber consists of three regions delineated by their epithelial linings: the vestibule, the respiratory region, and the olfactory region. The naris or nostril is the opening into each nasal vestibule. In the human, the vestibule is primarily an antechamber or dilatation lined with a continuation of the stratified squamous epithelium of

the facial skin. Coarse hairs and associated sebaceous glands within the vestibule act as an early line of respiratory defense by trapping particles brought in with inspired air (Sorokin, 1983). In the dog (Evans et al., 1979), a large bulbous extension of the ventral nasal concha obliterates much of the vestibule and diverts incoming air medially and ventrally across the nasal conchae. Internal to the vestibule lies the respiratory region.

The respiratory region comprises a large portion of the surface area of the nasal conchae (turbinates) as well as the remainder of the nasal cavity. The conchae are scrolls of cartilaginous or slightly ossified tissue which greatly increase the surface area of each nasal chamber and create increased turbulence of air flow. This increased turbulence facilitates humidification and warming of the inhaled air and probably slows the passage of air across the olfactory epithelium (Williams et al., 1989), enhancing its contact with odoriferous molecules. In macrosmatic mammals, (those whose olfactory sense is important for their survival), the nasal conchae may be elaborately scrolled, making a large contribution to the surface area of the nasal chamber.

The respiratory region is lined with nasal mucosa specifically referred to as respiratory epithelium. Respiratory epithelium consists of columnar or pseudo-stratified ciliated epithelium containing goblet cells, non-ciliated columnar cells with microvilli, and basal cells.

Serous and mucous glands are located below the basal lamina and open by branched ducts onto the surface of the epithelium. Mucous is secreted in abundance by glands and by mucous cells, thereby creating a sticky epithelial surface which traps dust and other particles carried into the chamber by inspired air. The mucous is continually moved toward the nasopharynx by the motion of the epithelial cilia. The secretions of the nasal mucosa also contain antibacterial lysozyme and immunoglobins to prohibit growth of inhaled microbes (Williams *et al.*, 1989).

The olfactory region of the nasal chamber is lined with olfactory epithelium and is located within or near the roof of each nasal chamber. In the human, the olfactory region is limited to the superior nasal concha, the wall of the nasal septum facing this concha, and the surface area of the roof between the two (Morrison and Costanzo, 1990). In the haplorhine primates, the olfactory epithelium is restricted to the upper region of the nasal fossa on both the medial and lateral walls, and is relatively more extensive than in humans (Hill, 1955). In macrosmatic mammals like the dog, the surface area of nasal mucous membrane committed to olfactory epithelium is proportionately much more extensive than that found in the human, but it continues to be located on the same structures within the caudal nasal chambers: 1) on the caudal part of the nasal septum; 2) across the roof of each nasal cavity, and 3) covering the ethmoturbinates,

the conchae derived from the ethmoid bone (Evans and Christensen, 1979). In some mammals, such as the bat *Artibeus jamaicensis* (Bhatnagar et al., 1974), and the mouse *Peromyscus* (Adams, 1972), the olfactory epithelium may extend onto the nasoturbinates as well. Like the respiratory epithelium, the olfactory epithelium is covered with a layer of fluid which may have enzymatic or bacteriocidal properties, and is likely to aid in the diffusion of airborne molecules to receptor sites on the apical cilia of neuronal dendrites (Williams et al., 1989). This fluid is secreted by Bowman's glands which are located deep to the basal lamina and whose ducts extend to the surface of the olfactory epithelium.

## **B. HISTOLOGY OF THE OLFACTORY EPITHELIUM**

The olfactory mucosa is readily distinguished in living mammals by its yellow pigmentation (Dodd and Squirrell, 1980). It is considerably thicker than the adjacent respiratory mucosa and is composed of pseudostratified columnar epithelium. In most species, it is characterized by three cell types: olfactory receptor neurons, sustentacular cells and basal cells (Figure 1, page 22). Yamamoto (1976) identified what he called interstitial or intercalated cells present in large numbers within the bat olfactory epithelium but interpreted these cells as

Figure 1. Olfactory epithelium in the squirrel (X 100).

R = cell bodies of olfactory receptor neurons

S = cell bodies of sustentacular (supporting)  
cells

arrow = basal cell layer





Figure 1

representing undifferentiated basal cells. More recently, Carr et al., (1991) identified a small population of cells within the adult rat olfactory epithelium that are not neurons (based on immunohistochemical and morphological characteristics including absence of axons) and are most likely a specialized type of sustentacular cell, although immunohistochemically atypical. As many as two additional receptor cell types have been identified in fish and in the human: Type II cells and microvillar cells in the goldfish (*Carassius auratus*) and channel catfish (*Ictalurus punctatus*) (Muller and Marc, 1984; Ichikawa and Ueda, 1977) and microvillar cells in salmonid fishes (Thommesen, 1983). In mammals, the only species in which microvillar cells have been carefully identified and described is the human (Moran et al., 1982; Morrison and Costanzo, 1990).

In the following sections, the cell types located within the olfactory epithelium will be described from the deepest to the most superficial layer.

### **1. Basal Cells**

Basal cells are confined to the innermost layer of epithelium, along the basal lamina. Basal cells appear to be of two types, one type contributing strength to the epithelial base while the second type differentiates into receptor neurons. These basal cells maintain the ability

throughout their life to differentiate into receptor neurons, and it is this ability that accounts for a most unusual characteristic concerning receptor neurons in most mammals: their continuous regeneration after a routine life cycle of approximately thirty to sixty days (Sorokin, 1983). Graziadei and Monti Graziadei (1978) found that within 30 days of severing the olfactory receptor neuron axons in rodents, the morphology of the olfactory epithelium had returned to normal: the neuronal population had regenerated so that a typical distribution of these cells once again existed within the underlying basal cell and sustentacular cell population. Although it was previously thought that basal cells retained the ability to differentiate into both neurons and sustentacular cells throughout the life of the individual (Sorokin, 1983, pg. 791), Graziadei and Monti Graziadei's (1978) documentation of mitosis occurring within the upper one-third of the olfactory epithelium indicates that sustentacular cells divide *in situ* within that layer and do not migrate up as differentiated basal cells. This is consistent with Klein and Graziadei's (1983) observation that in the placode stage, the neural layer gives rise to receptor cells while nonneural ectoderm gives rise to secretory and sustentacular cells. Graziadei and Monti Graziadei (1978) also observed that the rate of turnover of sustentacular cells is slower than that of neurons.

## *2. Olfactory Receptor Neurons*

Olfactory receptor neurons are bipolar cells with a peripherally extended apical pole dendrite and a fine, unmyelinated centrally extended basal pole axon. Each cell body consists of a very large nucleus surrounded by scant cytoplasm. Cell bodies of receptor neurons are located within the basal two thirds of the olfactory epithelium. The dendrite of each cell ends in an enlarged dendritic knob which extends above the surface of the epithelium and bears a radial tuft of cilia. The cilia are not freely motile as they are covered by a fluid layer and enmeshed with cilia of adjacent receptor neurons and microvilli of neighboring sustentacular cells. Most investigations (Dodd and Squirrell, 1980; Getchell and Getchell, 1987; Lancet, et al., 1987) have led to the conclusion that the receptor sites for odoriferous substances are located on the plasma membrane of the dendritic knob cilia. Herein lies the second unique feature of olfactory receptor neurons: their somata are situated very near the sensory surface and their apical endings are separated from the external environment only by a layer of fluid secreted by underlying Bowman's glands. It is likely that this exposure to the external environment and the noxious substances contained therein causes constant attrition of olfactory neurons and requires that these cells be continuously replaced (Graziadei and

Graziadei, 1979; Dodd et al., 1980). Numerous cilia extending from the dendritic knob increase the area of exposure of receptor sites to odoriferous substances, thereby enhancing each receptor neuron's sensitivity to low concentrations of inhaled volatile substances. Olfactory cell cilia in the human may vary within the wide range of 1  $\mu\text{m}$  to over 30  $\mu\text{m}$  in length (Morrison and Costanzo, 1990).

The olfactory neuron has been determined to be the primary receptor for olfaction based on the following factors: 1) when olfactory nerves are sectioned, olfactory receptor cells degenerate but sustentacular and basal cells do not. 2) If an odoriferous substance is added to the surface fluid, a change occurs in the electrical potential recorded along olfactory nerve fibers (axons of the receptor neuron). 3) Electrical recordings made from single olfactory cells indicate that these cells respond to particular odors. 4) When sustentacular cells are isolated in degenerating epithelium, they do not respond electrically to odorants. (Sorokin, 1982; Dodd and Squirrell, 1980).

Ichikawa and Ueda (1977) documented two morphologically distinct types of olfactory receptor neurons in the goldfish. The types were similar except that the apical process of Type I contained five to seven radially oriented cilia extending from its superficial surface, and the apical process of Type II neurons had many microvilli extending from its superficial end. The authors suggested that the

two types might represent different progressive stages in the differentiation of the receptor cell. Thommesen (1983) corroborated the presence of two morphologically distinct types of receptor neurons within the olfactory epithelium of several species of salmon. However, electrophysiological studies on these species demonstrated that the cells with ciliated apical surfaces were sensitive to bile salt-like substances while cells with microvillous receptor endings were sensitive to amino acids. In addition, there was a correspondence between the preponderance of neuron type and the ecology of the species. Migratory species had twice as many ciliated receptor cells as microvillous ones, while in stationary species, the proportion was reversed.

Muller and Marc (1984) documented three morphologically distinct classes of receptor neurons in the goldfish and channel catfish. Type I and microvillar cells are similar morphologically to the cells described above while Type II ciliar cells resembled respiratory epithelial cells with motile, synchronously beating cilia that moved fluid over the olfactory epithelium. These cells were categorized as receptor neurons based on the identification of axons using retrograde HRP transport studies.

In mammalian species, microvillar cells have been identified without question only in the olfactory epithelium of the human. These cells will be discussed below under **Microvillar Cells.**

Axons of the olfactory receptor neurons extend through the basal lamina and immediately join to form numerous axon bundles. The axon bundles become invested in sheath cells as soon as they leave the basal lamina and are then referred to as fascicles. These fascicles traverse the underlying connective tissue and foramina within the cribriform plate to reach the olfactory bulb. Although the fascicles are invested with sheath cells, the individual axons themselves remain unmyelinated. Many of these axons are in direct contact with each other, leading some investigators to suggest that electrotonic interactions exist between these fibers (Jones, 1983). When the fascicles penetrate the cribriform plate they are often referred to as fila olfactoria. They must grow through the outermost olfactory bulb layer, the olfactory nerve layer, before penetrating the layer that contains dendrites of the second order neurons.

The olfactory nerve layer of the bulb consists of the nerve fascicles which traverse it to enter the periglomerular layer. It is within the periglomerular layer that synapse with the dendrites of second order neurons occurs.

### 3. Microvillar Cells

Although there are suggestions that microvillar cells may be present in the olfactory epithelium of other mammalian species (see Moran et al., 1982), to date this cell type has been unquestionably identified only within the olfactory epithelium of humans (Moran et al., 1982; Morrison and Costanzo, 1990). These flask or pear shaped cells are the most superficially located cells within the olfactory epithelium and resemble neurons in their fine structure, in the clear quality of the cytoplasm, and in the presence of a cluster of microvilli extending from their apical surface into the nasal cavity. Moran et al. (1982) and Morrison and Costanzo (1990) were unable to trace the continuation of the axon-like process extending from the basal region of these cells and were only able to determine that it extended toward the basal lamina. They could not determine whether the process joined olfactory receptor neuron axons or other microvillar cell axons within the epithelium or lamina propria. The function of these cells is unknown although Moran et al. (1982) suggested that within the human epithelium, they may be secondary chemoreceptive cells, similar to the chemosensitive microvillar cells found within the vomeronasal organ. Ichikawa and Ueda (1977) found that in the goldfish, microvillar cells degenerated after olfactory nerve bundles were cut, indicating that these



cells are receptor neurons. It has been assumed that microvillar cells in the human are receptor neurons based on their morphological similarity to these cells in fish.

#### **4. Sustentacular Cells**

Sustentacular cells are positioned among receptor neurons. Their nuclei are located superficial to the cell bodies of the receptor neurons, but their more extensive cytoplasm extends along the full length of the adjacent neurons and reaches the basal lamina. In histological sections for routine light microscopy, the sustentacular cells can be recognized by their more superficial and ovoid nuclei, while receptor neurons are distinguished by round nuclei located between the basal cell layer and the more superficially located sustentacular cell nuclei. The apical surface of each sustentacular cell is covered with microvilli that extend into the fluid layer covering the surface of the olfactory epithelium. The presence of these numerous microvilli further enmesh the cilia of the receptor neurons. Sustentacular cells have many properties of secretory cells such as the ability to reduce NADP and to synthesize complex molecules (Sorokin, 1983). Getchell and Getchell (1987) state that mucopolysaccharides are released from sustentacular cells into the overlying fluid layer and that other functions of these cells may include the

following: maintenance of an ionic reservoir for the electrical activity of the receptor neurons, uptake and transport of odorants, isolation of receptor units, guidance of migrating receptor neurons, and maintenance of the structural integrity of the epithelium. Interestingly, Yamamoto's electron microscopic study (1976) of bat and rabbit olfactory mucosa failed to find cytological evidence for secretory activity of sustentacular cells. He states that evidence for production of secretory granules was completely absent in these mammalian species but does cite several publications indicating a secretory role for sustentacular cells in amphibians, the turtle and the turkey.

Sustentacular cells separate olfactory receptor cells, and share adjacent surfaces with them as well as with other sustentacular cells. Metabolic exchanges apparently occur between the two cell types (Sorokin, 1983), and for this reason, sustentacular cells are also aptly referred to as "supporting cells". Reese and Brightman (1970) identified an extensive system of tight junctions between the epithelial surfaces of bipolar and supporting cells and demonstrated that these tight junctions completely seal off the underlying extracellular spaces from the overlying mucous layer. These tight junctions form a protective interface preventing the diffusion of harmful substances across the epithelium.

Rafols and Getchell (1983) documented two morphologically distinct types of sustentacular cells in the olfactory epithelium of the salamander. Type I sustentacular cell has a broad cylindrical cell body, the apex of which partially forms the epithelial surface while the base constricts into an unbranched stalk. Type II sustentacular cell is thinner and has a more irregular contour. The central stalk lacks a discrete boundary with the cell body and follows a tortuous course through the width of the epithelium. This central stalk gives rise to rib-like processes from which cytoplasmic veils or curtain-like structures extend. These veils unfurl around adjacent receptor neurons and interdigitate with nearby sustentacular cells. The interdigitation of these veils forms a lattice throughout the depth of the epithelium and the compartments formed by adjacent veils probably house the receptor neuron cell bodies. Costanzo and Morrison (1989) suggest that the population of sustentacular cells within the hamster olfactory epithelium is also heterogeneous.

Rafols and Getchell state that Type I sustentacular cells share many morphological features with tanycytes of the ependyma lining the ventricles of the brain as well as tanycytes within the median eminence. Tanycytes of the median eminence have been found to transport substances between the cerebrospinal fluid and the hypophyseal portal system, suggesting to Rafols and Getchell that Type I

sustentacular cells may transport materials across the avascular olfactory epithelium. Type II sustentacular cells have been compared functionally to glial cells. The authors also suggest that the central stalk of both Type I and II sustentacular cells act as a mechanical pathway guiding post-mitotic receptor neurons through the depth of the olfactory epithelium, a function similar to that of the glial cells in the developing cortex.

### ***5. Lamina Propria***

The lamina propria separates the basal lamina of the olfactory epithelium from the underlying bone. Located within this connective tissue layer are nerve fibers, components of a rich vascular system, and Bowman's glands. Bowman's glands secrete the serous fluid which bathes the epithelial surface of the olfactory mucosa. This fluid leaves the glands by way of excretory ducts which extend through the layers of the olfactory epithelium and open onto the epithelial surface. The fluid is secreted continuously and provides a solvent for dissolving inhaled odorant substances (Sorokin, 1983; Dodd et al., 1980), and may have enzymatic and bacteriocidal properties as well (Williams, et al., 1989). While the olfactory epithelium itself is avascular, blood vessels are located adjacent to it within the lamina propria. Nerve fibers within the lamina propria

include axon fascicles of olfactory receptor neurons as well as fibers of the trigeminal nerve. The trigeminal fibers extend into the nasal epithelium where they terminate. Trigeminal fibers play a role in chemosensation and are discussed below (page 70).

#### **IV. THE ACCESSORY NASOCHEMOSENSORY SYSTEM**

Of the five nasochemosensory structures located within the mammalian nasal chambers, only one represents olfaction in the strictest sense. Although interest in the other four structures, collectively referred to as the accessory system, has grown in the last twenty years, it is still unclear just how each of these four structures are related functionally to each other and to the main olfactory system. Although this study focuses on one component of the main olfactory system, the olfactory bulb, the accessory system must also be discussed in order to give a more accurate representation of the complexity of mammalian nasal chemoreception.

The first area of chemosensitive neural tissue within the nasal chambers, the olfactory epithelium, has been discussed in some detail. This is the location of the primary receptor cells responsible for olfaction, and it is these neurons that project to the olfactory bulb, the focus

of this study. The remaining four nasochemosensory structures include the vomeronasal organ (Jacobson's organ), the septal organ (organ of Rodolfo-Masera), the terminal endings of the trigeminal nerve, and the terminal nerve (nervus terminalis).

The review of each of the accessory nasochemosensory structures will include, in sequence: 1) morphology, 2) function, 3) embryological development.

#### **A. THE VOMERONASAL ORGAN**

Most and probably all Orders of terrestrial mammals have vomeronasal organs although certain species within each order may lack them. Many aquatic mammals have reduced or absent vomeronasal structures. The presence of the vomeronasal organ in mammals will be discussed in greater detail after a review of the morphology of the system.

##### ***1. Morphology***

In most vertebrates studied, the bilateral vomeronasal organ is located in the rostral portion of the nasal region, ventral to the nasal chambers. It is usually a blind sac or tubular pouch located on either side of the nasal septum (Figure 2). It is of varying length with an os or duct that

Figure 2. The nasal cavity of the squirrel (X 10).

C = nasal conchae

S = nasal septum

V = vomeronasal organ

vc = cartilage adjacent to vomeronasal organ

arrow = neurosensory epithelium

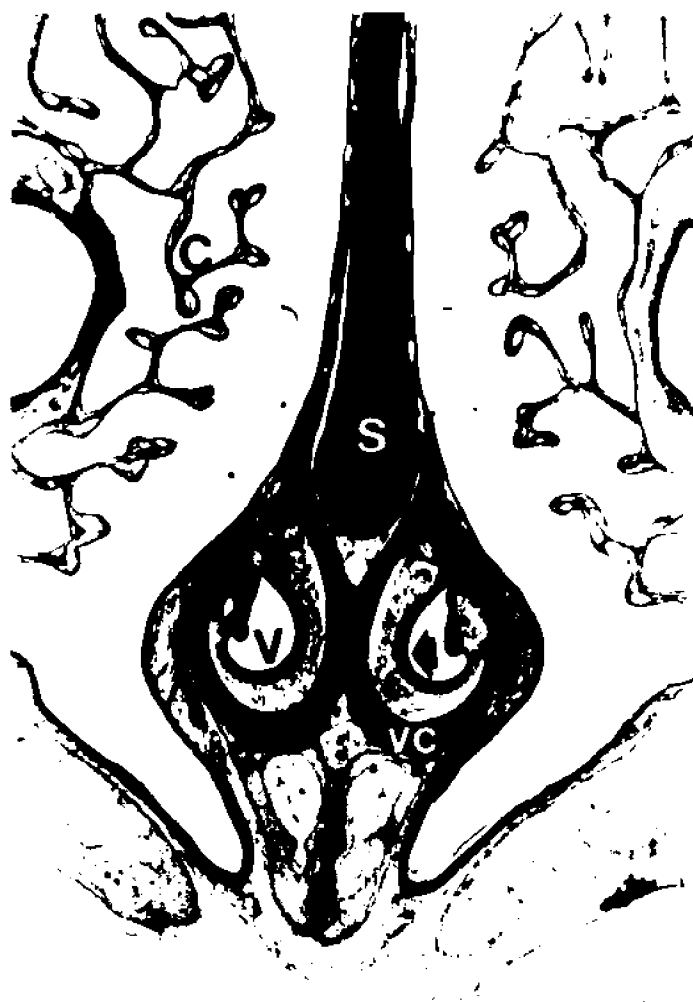


Figure 2



opens into either the nasal chamber, the nasopalatine duct, or the oral cavity. When the vomeronasal organ is present, it is most commonly associated with the ipsilateral nasopalatine duct. As the nasopalatine duct connects the nasal and oral cavities, nonvolatile substances entering either of these cavities may have access to the vomeronasal organ. This morphology is found in most mammals (Estes, 1972) including the dog (Evans and Christensen, 1979), in the tree shrew (Loo and Kanagasuntheram, 1972), and in some primates (Maier, 1980). In rodents, the vomeronasal organ opens into the nasal cavity rostral to the nasal end of the nasopalatine duct. External stimuli are thought to reach the vomeronasal organ through the external nares (Vaccarezza et al., 1981). In bats, the nasopalatine ducts open anteromedially into the roof of the oral cavity; the ostium of the vomeronasal organ is located near where the nasopalatine duct joins the nasal cavity (Cooper and Bhatnagar, 1976). In snakes, the vomeronasal organ opens directly into the mouth without any connection to the nasal cavity (Wysocki and Meredith, 1987).

There is also variation as to the extent the vomeronasal organ is surrounded by cartilage. In most mammals, including the dog and mouse (Adams and Wiekamp, 1984; Barber and Raisman, 1978), the vomeronasal organ is crescent shaped transversely and is enclosed within a cartilaginous shell. In bats (Cooper and Bhatnagar, 1976),

the cartilage surrounding the vomeronasal organ is more variable in shape being either crescent or hooked shaped, and in the most rostral regions, the cartilage may completely surround the vomeronasal organ. Even in those species of bats that lack a vomeronasal organ, a remnant of the vomeronasal cartilage, the paraseptal cartilage, is present. Both vomeronasal cartilage or paraseptal cartilage have anterior processes which accompany and may support the nasopalatine ducts. In the dog, the nasopalatine ducts are not encased within a bony canal (Evans and Christensen, 1979). In humans and in all species of bats, the nasopalatine ducts are supported by cartilage which functions to maintain its patency (Cooper and Bhatnagar, 1976).

The lining of the vomeronasal organ is at its opening, either a continuation of the epithelial lining of the ventral nasal cavity or the epithelium lining the nasopalatine duct. However, the lining soon changes to that typical of the vomeronasal organ in mammals: pseudostratified columnar epithelium lining the medial walls, and either ciliated columnar epithelium (Negas, 1958; Cooper and Bhatnagar, 1976) or pseudostratified columnar epithelium (Adams and Wiekamp, 1984; Loo and Kanagasuntheram, 1972) lining the lateral walls. Neurosensory receptor cells are usually restricted to the epithelium of the medial wall (Figure 2, page 37) (Loo and

Kanagasuntheram, 1972; Cooper and Bhatnagar, 1976; Barber and Raisman, 1978; Adams and Wiekamp, 1984) although in one species of bat (*Artibeus jamaicensis*) investigated by Cooper and Bhatnagar, the sensory epithelium lined all surfaces of the vomeronasal organ. The medial wall epithelium strongly resembles the olfactory epithelium of the nasal cavity.

The non-neurosensory epithelium of the vomeronasal organ is usually a combination of the following cell types: non-ciliated, ciliated, or cells with a microvillous luminal surface (Adams and Wiekamp, 1984). In the dog, goblet cells are infrequently seen within the epithelium of both medial and lateral walls. Cooper and Bhatnagar (1976) have also documented the presence of goblet cells and "mucus-producing secretory cells" in some species of bats. In most mammalian species studied, basal cells are sparse and scattered, and there is no distinct basal cell layer adjacent to the lamina propria as there is in the olfactory epithelium.

The receptor cells of the vomeronasal organ are histologically similar to olfactory receptor neurons: they are bipolar cells with dendrites extending to the surface and cell bodies located deep to the supporting cells of the epithelial layer. The dendrites are unlike olfactory receptor cell dendrites in that they lack cilia but possess microvilli on their apical surface (Allison, 1953; Cooper and Bhatnagar, 1976; Moulton, 1979). However, Adams and Wiekamp (1984) have described vomeronasal receptor cells in

the dog as frequently having from one to four non-motile apical cilia.

Sectioning of the vomeronasal nerve causes retrograde degeneration and death of the existing receptor neurons (Barber, 1981). These cells are replaced by new receptor neurons which arise from a population of stem cells. Unlike the olfactory epithelium, there is little evidence to indicate that new neurons arise from the sparse vomeronasal organ basal cell population. In the adult mouse, Barber and Raisman (1978a, b) identified a population of mitotic cells located on the periphery of the neurosensory epithelium and directly adjacent to the non-neurosensory epithelium. These mitotic cells gave rise to slowly migrating cells which eventually replaced the degenerated neurons. The greatest concentration of these stem cells were located at the caudal end of the vomeronasal organ. Cell migration followed a course from the periphery of the neurosensory epithelium toward the more central regions. Barber and Raisman's impression was that of a much slower turnover for neurosensory cells in the vomeronasal organ than is seen in the olfactory epithelium. This is consistent with their observation that the rate of cell migration from peripheral to central regions was slow.

Axons of the receptor neurons are unmyelinated. They join together to become fascicles deep to the epithelium of the medial wall of the vomeronasal organ. On either side of

the nasal septum, these fascicles converge to become larger axon bundles that traverse the septum in a dorsocaudal direction until they approach the cribriform plate bilaterally. In bats, the axon bundles on either side form the vomeronasal nerve bilaterally, each nerve passing into the cranial cavity through a dorsomedial foramen of the cribriform plate (Cooper and Bhatnagar, 1974). In the rat, (Bojsen-Moller, 1975), at least four or five vomeronasal nerves pass through the cribriform plate. The axons of the vomeronasal receptor neurons synapse with second order neurons within the accessory olfactory bulb.

Numerous nerve cell bodies resembling ganglion cells have been located along vomeronasal nerve fibers in bats and other mammals (Bhatnagar and Kallen, 1974) and are most likely cell bodies associated with the terminal nerve (refer to the Terminal Nerve, page 75).

The second category of cells associated with the epithelium of the vomeronasal organ are the sustentacular (supporting) cells. These cells are easily identified within the neurosensory epithelium by their oval nuclei and by the superficial position of their nuclei relative to the receptor cells (Cooper and Bhatnagar, 1976; Barber and Raisman, 1978; Adams and Wiekamp, 1984). Sustentacular cells extend from the luminal surface to the basal surface of the epithelium. Microvilli are present on the luminal surface of both sustentacular and receptor cells in the dog

(Adams and Wiekamp, 1984). According to Barber and Raisman's investigation (1978a) of cell division in the vomeronasal neuroepithelium of the mouse, mitosis in a population of cells located within the fringes of the neuroepithelium gives rise to both sustentacular and receptor cells. As mentioned above, this would indicate that the stem cell population within the vomeronasal organ is not associated with a population of basal cells as in the olfactory epithelium, but rather with this population of cells located peripherally between the boundaries of the neural and non-neural epithelium.

The lamina propria of the vomeronasal organ is unlike that of the olfactory mucosa in that it lacks Bowman's glands (Allison, 1953; Barber and Raisman, 1978 a; Moulton, 1979). However, there are acinar glands located dorsal and lateral to the vomeronasal organ with ducts that open into the lumen of the organ. Epithelium that lines the lumen of the vomeronasal organ continues upward to line the walls of the ducts (Cooper and Bhatnagar, 1976). In the dog and mouse (Adams and Wiekamp, 1974; Barber and Raisman, 1978a), the ducts usually open into the commissures of the crescent shaped organ, the region where neuroepithelium meets respiratory epithelium. Indeed, in the illustration of the glandular duct in the bat (Cooper and Bhatnagar, 1976), the duct is present at just this juncture. In the dog, the ducts occasionally open through the lateral epithelium, and

least frequently through the medial epithelium. Cooper and Bhatnagar found that there is a correlation between the size and presence of the vomeronasal organ and the size and presence of these glands in the bat. Presumably this signifies a strong functional association of these structures.

Adams and Weikamp (1984) describe the lamina propria of both convex and concave surfaces of the vomeronasal organ in the dog as highly vascularized. Large veins are commonly located within the lamina propria of the lateral surface; smaller veins intervene between the lamina propria and the basal region of the epithelium. Microvessels are abundantly found within the lamina propria of the medial surface. In the mouse, Cuschieri and Bannister (1975) and Barber and Raisman (1978a) describe the neurosensory epithelium as containing capillary loops, in contradistinction to the olfactory epithelium which has a rich subepithelial vascularity but lacks blood vessels within its layers. Allison (1953) describes the submucosa of the vomeronasal organ as extremely vascular, being formed to a large degree of thin-walled blood sinuses. Cooper and Bhatnagar (1976) found that vascular sinuses accompany the vomeronasal organ in each species of bat they studied.

The function of the vascularity surrounding the vomeronasal organ is alternately to draw in and expel its contents according to the degree of engorgement of these

blood vessels. Constriction of these vessels results in dilation of the vomeronasal lumen, which in turn draws in fluid from the nasopalatine duct (Adams and Weikamp, 1984). In species with well developed vomeronasal organs, the sinuses are located dorsolaterally where they can compress the vomeronasal organ against cartilage when distended with blood. Compression of the vomeronasal organ would facilitate the emptying of its contents. Eccles' (1982) study of the cat found that autonomic innervation to the vomeronasal organ consisted of both sympathetic and parasympathetic fibers. Secretions within the vomeronasal organ were produced by stimulation of parasympathetic fibers to the organ. Stimulation of sympathetic fibers caused fluid to be drawn into the organ. The secretions may act as a sampling medium for airborne odorants and may continuously flush out the lumen. The pumping mechanism would function routinely to draw fluid into the vomeronasal organ for sampling.

## ***2. Presence of the Vomeronasal Organ in Mammals***

The vomeronasal organ has been identified in many, but not all, mammalian species, and is best developed in monotremes and marsupials (Johnson et al., 1983). When it is absent in the adult form, it frequently develops during the embryological period and then regresses.



A vomeronasal organ is present in the insectivore *Tupaia glis* (Loo and Kanagasuntheram, 1972; Maier, 1980), and in all strepsirrhine primates that have been examined: *Microcebus murinus*, *Otolemur crassicaudatus*, *Loris tardigradus*, *Galagoides demidoff* (Maier, 1980), *Nycticebus coucang* (Loo and Kanagasuntheram, 1972), and "in all lemurs" (Tattersall, 1982). Starck (1975) states that *Tarsius bancanus* has a vomeronasal organ in fetal and adult stages, and that a well-formed sensory epithelium is present. According to Maier (1980), all platyrrhine primates have a vomeronasal organ although in varying degrees of differentiation or reduction. He states that marking behavior is well known in this group and therefore presence of the vomeronasal organ is not surprising. Eppler (1986) states that documentation of the presence or absence of a vomeronasal organ in the Catarrhini differs according to author. Some authors report presence of a rudimentary vomeronasal organ in *Cercopithecus*, *Macaca fascicularis*, *Hylobates*, and *Pan*. Other authors report it to be absent in *Cercopithecus*, *Macaca fascicularis* and *Hylobates*. Maier (1980) found its presence in *Pan troglodytes* questionable. Likewise, the accessory olfactory bulb was reported as present or absent in *Macaca*, and absent in *Macaca mulatta*, *Cercopithecus ascanius* and *C. mitis*, *Colobus badius*, *Papio*, *Pan troglodytes* and *Gorilla gorilla*. A vomeronasal organ has been observed in the embryonic development of the human

(O'Rahilly, 1976; Johnson et al., 1983). In adult human cadavers, part or all of the vomeronasal organ was identified in 70% of all nasal septa sectioned, and in living subjects, 39% had at least one vomeronasal pit present (Johnson et al., 1983).

In bats, Cooper and Bhatnagar (1976) found the vomeronasal organ complex to be partly or fully present in five out of the six chiropteran families they studied. In the Family Rhinopomatidae, *Rhinopoma microphyllum* has reduced vomeronasal structures. *Megaderma lyra* (Family Megadermatidae) has a complete vomeronasal organ but lacks an accessory olfactory bulb. *Rhinolophus lepidus* and *Hipposideros lankadiva* (Family Rhinolophidae) retain some vomeronasal structures but not others (nerve fibers and accessory olfactory bulbs were not seen in either species, while *Rhinolophus* also lacked vomeronasal nerve fibers). In the Family Phyllostomatidae, *Glossophaga soricina*, *Artibeus jamaicensis*, and *Desmodus rotundus*, the vomeronasal organ and related structures including the accessory olfactory bulb are present. Within the Family Vespertilionidae, the vomeronasal organ and accessory olfactory bulbs are present in *Miniopterus schreibersi* while *Myotis lucifugus*, *Eptesicus fuscus*, and *Pipistrellus subflavus* lack accessory olfactory bulbs but retain the vomeronasal organ. Within the Family Pteropodidae, the species studied by Cooper and Bhatnagar confirmed a previous conclusion that the vomeronasal organ

is absent in this family.

Some aquatic mammals are notable for their complete lack of an olfactory and vomeronasal organ system. Mackay-Sim et al. (1985) state that completely aquatic marine mammals of the Order Odontoceti (toothed whales, porpoises and dolphins), have a reduced or absent olfactory system and lack a vomeronasal organ and accessory olfactory bulb. To the list of aquatic mammals lacking a vomeronasal organ, the authors have added the West Indian Manatee, *Trichechus manatus*, a member of the Order Sirenia which includes dugongs, sea cows, and manatees. They hypothesize that aquatic mammals that spend most of their lives submerged will lose the structures associated with nasochemoreception, due to the lack of functional significance of airborne chemicals to these mammals.

### 3. Function

In many mammals, the vomeronasal organ plays an important role in the maturation of reproductive organs and in reproductive behavior. Since the reproductive behavior of bats is not well known, Cooper and Bhatnagar focused on what they perceived to be a functional association between vomeronasal organ size and feeding behavior in Chiroptera. They found that bats with the best developed vomeronasal organs were those whose food-finding ability was probably

dependent upon nasal chemoreception: frugivorous, nectivorous, and sanguivorous bats. Insectivorous bats, known to rely more on their sense of echolocation, have either rudimentary or absent vomeronasal organs.

The correlation between flehman<sup>1</sup>, the presence of a vomeronasal organ and accessory olfactory bulb, and the presence of pheromones in urine, strongly suggests that the vomeronasal organ functions in reproductive behavior (Estes, 1972). Wysocki and Meredith (1987) state that the mammalian vomeronasal organ has been implicated in the following mammalian functions: advancement or delay of puberty by chemical signals from conspecifics; regulation of the estrous cycle by chemical cues of conspecifics; testosterone surge in males resulting from exposure to a novel conspecific female; termination of pregnancy in the house mouse by exposure to a strange male; male mating behavior. Aggression between conspecific males can be decreased or eliminated by vomeronasal lesions. Neuronal activity within the accessory olfactory bulb of the fetus may indicate that the vomeronasal organ functions to test the quality of the

1. Flehman: A response of facial expression to an odor or airborne molecules, particularly urine or glandular secretions. It is seen in many mammals and is characterized by lifting the nose, retraction of the upper lip and holding the mouth open, sometimes followed by rapid licking movements to the nose. It is thought to facilitate the exposure of the vomeronasal organ to airborne molecules. (Eisenberg and Kleiman, 1972; Estes, 1972; Walther, 1984).

intrauterine milieu (Pedersen et al., 1983). Investigations of function based on the sectioning of vomeronasal nerves have to be interpreted with caution since terminal nerve and vomeronasal nerve fibers run so closely together (Wysocki and Meredith, 1987).

The vomeronasal organ is important in the mediation of aggressive behavior in lactating female mice (Bean and Wysocki, 1987). Lactating females who had their vomeronasal organs removed exhibited uncharacteristically nonaggressive behavior towards intruder males. In several rodent studies, the vomeronasal organ has been functionally implicated in the role of reproductive behavior (Wysocki and Meredith, 1987). Female prairie voles, *Microtus ochrogaster*, are dependent upon urinary chemosignals from conspecific males for the growth and maturation of their ovaries and uterus (Lepri and Wysocki, 1987). In normal and sham-operated female prairie voles, ovarian and uterine weights doubled and tripled after a 12 and 60 hours (respectively) when exposed to stud males during this period of time. Removal of the vomeronasal organs from adult females resulted in low uterine and ovarian weights even when pairing with stud males was maintained over a prolonged period of time. Bean (1981) found that normal precopulatory ultrasonic vocalizations by male mice depended upon reception of female chemosignals by a functional male vomeronasal organ.

Wysocki et al. (1980) exposed guinea pigs to the tagged

urine of conspecifics. After test animals examined the tagged urine, they were sacrificed. Subsequent histological examination of the nasal structures of these animals demonstrated that tagged substances of low volatility such as those found in urine, were present within the epithelium of the vomeronasal and septal organs, but not in the olfactory epithelium. Patent external nares and nasopalatine ducts were also determined to be important in conducting these molecules to the vomeronasal organ.

In mammals, several mechanisms may facilitate sampling of fluid by the vomeronasal organ. Forceful inhalation introduces fluid into the nasal chamber, while licking introduces molecules into the oral cavity. Grimacing, characteristic of flehmen, would also facilitate the passage of molecules into the region of the vomeronasal organ (Epple, 1988). Head-bobbing, sniffing and licking are movements exhibited by guinea pigs when examining odorants (Wysocki et al., 1980). All of these movements would contribute to introducing odorants into the region of the vomeronasal organ (Epple, 1988).

#### ***4. Embryology***

The vomeronasal organ begins to form slightly after the invagination of the olfactory pit. In the mouse, it is

first recognizable on embryonic day 11 as a thickened region within the medial wall of the pit. By embryonic day 12, this region invaginates to form a groove. Fusion of the lips of the groove forms a tubular structure which maintains an open channel with the developing olfactory pit. Within the epithelium lining the medial and inferior walls of the developing vomeronasal organ, cells in a designated region develop processes which terminate in rounded endings at the epithelial surface. These dendritic processes mark the developing neurosensory tissue. The remainder of the epithelium is respiratory epithelium. (Cuschieri and Bannister, 1975; Brunjes and Frazier, 1986).

The normal development of the vomeronasal organ is dependent upon the presence of at least one hormone. As was stated previously, the vomeronasal organ functions in sexual and maternal behaviors. In addition, normal development of the accessory olfactory bulb has been shown to be dependent upon the presence of gonadal steroids. Segovia et al. (1982) tested the effects of removal of the thyroid gland on the development of the vomeronasal organ and found a dramatic decrease in all of the following parameters: decrease in vomeronasal volume; decrease in volume of the neurosensory epithelium; decrease in the number and nuclear size of the neurons.

### 5. The Accessory Olfactory Bulb<sup>1</sup>

Although the accessory olfactory bulb is located adjacent to and is mechanically inseparable from the main olfactory bulb, it is not functionally related to olfaction but rather to the vomeronasal organ, and possibly to the terminal nerve as some fibers of this nerve have also been traced to the accessory olfactory bulb (Wirsig, 1987). Because it is not functionally related to neurons of the main olfactory bulb, the accessory olfactory bulb will be discussed in its entirety here.

Axons of the vomeronasal organ receptor neurons project to the ipsilateral accessory olfactory bulb but not to the main olfactory bulb (Barber, 1981a). The accessory olfactory bulb is located dorsocaudally and directly adjacent to the main olfactory bulb (Figure 3, page 55). Allison (1953) states that the presence and size of the accessory olfactory bulb varies according to the presence and size of the vomeronasal organ: it is relatively large in rodents, of moderate size in carnivores and ungulates, and small in many bats and in primates. Bhatnagar and Kallen (1974) found that in *Artibeus jamaicensis* and *Myotis lucifugus*, this association holds true: *Artibeus* has both a

1. Many terms used in this discussion presume a knowledge of the morphology of the olfactory bulb. For a review of the olfactory bulb, refer to page 85.



Figure 3. The accessory olfactory bulb in the rat (X 10).

aob = accessory olfactory bulb

mob = main olfactory bulb



Figure 3

vomeronasal organ and an accessory olfactory bulb while *Myotis* has neither. Cooper and Bhatnagar (1976) state that in bats having only rudimentary vomeronasal organ structures but lacking identifiable accessory olfactory bulbs, the possibility of sensory function still exists in that vomeronasal nerve fibers, paravomeronasal ganglia, and receptor-like cells are still present. The degree to which function is retained in these structures has not yet been determined.

Allison (1953) stated that the accessory olfactory bulb differed in details of structure from the main olfactory bulb, although the general pattern is recognizable. He described relatively poorly defined and small glomeruli, mitral cells scattered throughout the external plexiform layer, a reduction in number of periglomerular cells, and a complete absence of tufted cells. The degree of organization of the cellular constituents of the accessory olfactory bulb are apparently related to the relative size of that structure. Macrides and Davis (1983), and Roos et al. (1988) described a laminar organization similar to the layers present within the main olfactory bulb, with the exception of a reduced or absent external plexiform layer. In the bat *Artibeus*, Bhatnagar and Kallen (1974) described the accessory olfactory bulb as containing all cell types of the main olfactory bulb but without the presence of a laminar organization. They described the bulb in this

species as principally being made up of very small glomeruli.

Central projections of the accessory olfactory bulb (Macrides and Davis, 1983) are limited compared to the projections of the main olfactory bulb. They consist of projections to the amygdaloid regions of the temporal lobe. Terminal fields within this region have been specifically identified as the bed nucleus of the accessory olfactory tract in the medial nucleus of the amygdala, and the posteromedial division of the cortical nucleus of the amygdala. Also, efferent projection of the accessory olfactory bulb enter the stria terminalis and proceed to a region near the anterior commissure. The accessory olfactory bulb receives reciprocal inputs from its target regions.

Roos *et al.* (1988) studied the fetal and postnatal development of the accessory olfactory bulb in the rat. At its earliest documented stage of development (fetal day 16), the accessory bulb consisted of one layer, a differentiating mitral cell layer, capped by a zone of primitive nerve fibers originating from the vomeronasal organ. This acellular region became the differentiating glomerular layer and what little existed of the external plexiform layer. On fetal day 19, an acellular layer appeared between the mitral cell layer and the subependymal zone. This became the

internal plexiform layer. At birth, and in subsequent days, a layer developed in contact with the inner plexiform layer. The cell population of this layer arose from differentiating cells within the mantle layer. Processes of the granule cells subsequently extended through the internal plexiform layer where they came into contact with the axons of the mitral cells.

Bayer (1983) avoided calling the output cells of the accessory olfactory bulb mitral cells, since Golgi impregnations failed to reveal true mitral cell structure. Bayer refers to these cells simply as "output cells", and found that neurogenesis within this cell population of the rat is at its peak during the 13th embryonic day. Interneurons develop later. The peak for neurogenesis of granule cells is in the embryonic period between day 17 and 19. The periglomerular interneuron population is the last to develop, the peak period for their neurogenesis being within the postnatal period, up to the sixteenth day after birth. The neurogenesis of the specific cell types within the accessory olfactory bulb precedes the neurogenesis of these cell types within the main olfactory bulb.

It is interesting to note that during fetal and postnatal development, the size of the accessory olfactory bulb in the male rat is consistently larger than that of the female (Roos et al., 1988). The experimental aspects of the study by these researchers confirms previous research

indicating that castration in the neonatal male and androgenization in the young female alter the size of the developing accessory bulb. The work by Roos et al. (1988) demonstrates that castration in the neonatal male reduces the accessory bulb size to that of the developing female or smaller. It can be concluded from these studies that in the rat, sex steroids, particularly the secretion of testosterone by the testes, is necessary for full development of the accessory olfactory bulb in the male. It would be interesting to determine if this sexual dimorphism applies to the size of the vomeronasal organ and if so, is it also influenced by testosterone levels in the developing male? This is the second example of the influence of hormones on the normal early development of the vomeronasal system in mammals.

Barber (1981b) found that after axotomy of vomeronasal nerves in the mouse, some axons of regenerated neurons do grow back along the nasal septum to reach the olfactory bulb. However, these regenerated axons do not make synaptic contact with second order neurons within the accessory olfactory bulb as expected, but instead form glomeruli within the main olfactory bulb, making synaptic contact with main bulb neurons. Glomeruli formed by the axons of these replacement cells fill in sites of the main olfactory bulb which were damaged or deafferented by the axotomy. Many

axons of the vomeronasal replacement neurons end up within loose connective tissue external to the olfactory bulb without being drawn to the neural structures of the bulb (main or accessory). Examination of the accessory olfactory bulb post-axotomy revealed that the glomerular layer effectively disappeared and that what once was a very limited external plexiform layer subsequently become covered with glial lamellae on the dorsal bulb surface. This raises the question of whether the vomeronasal organ continues to function after damage to the vomeronasal nerves or after regeneration of damaged vomeronasal epithelium. If it does continue to function after regeneration of the receptor cells, the question becomes in what capacity, as central projections from the main and accessory olfactory bulbs differ.

## **B. THE SEPTAL ORGAN**

The septal organ (of Maserà) has only been described in a limited number of mammals Miragall et al. (1984), and in the absence of contradictory information, it is possible that this organ is present to some degree in most mammalian orders. There is essentially no information available concerning its embryological development (Brunjes and Frazier, 1986). In addition to the species mentioned below, a septal organ is present in the rodents *Sciurus*

*carolinensis* (gray squirrel), *Zagodontomys brevicauda*, and the laboratory mouse (Harlan Sprague Dawley) (Sawitzke, present study).

## 2. Morphology

The paired olfactory septal organ (of Masera) is located on either surface of the septal wall ventral to the olfactory epithelium and at the entrance to the nasopharynx (Figure 4, page 63). It is composed of pseudostratified columnar epithelium and is surrounded by respiratory epithelium (Figure 5, page 65). In structure it resembles olfactory epithelium, in that it is composed of receptor, sustentacular and basal cells, with Bowman's glands located within the underlying lamina propria (Miragall et al. 1984; Pederson and Benson, 1986). Nuclear stratification characterizes the epithelium of the septal organ: there is a superficial row of supporting cell nuclei, a wider zone of receptor cell nuclei, and a single deep row of basal cell nuclei. Cell nuclei within the septal organ appear to be more tightly packed than nuclei in other regions of the nasal septum. Although the sensory epithelium lacks goblet cells, they are common to the respiratory epithelium surrounding the septal organ (Kratzing, 1978).

The surface of the septal organ in the deer mouse, *Peromyscus* (Adams and McFarland, 1971), is covered by a



Figure 4. Spetal organ in the rodent *Zagodontomys brevicauda*  
(X 5).

C = cartilage of nasal septum

arrow = spetal organ



Figure 4

Figure 5. Epithelium of the septal organ in the rodent *Zagodontomys brevicauda*. The septal organ epithelium is located between two regions of respiratory epithelium (X 25).

arrows = septal organ epithelium



Figure 5

mass of intertwining, thread-like structures believed to be cilia. These cilia are smaller than the large cilia covering the surface of the surrounding respiratory epithelium. In the mouse, olfactory knobs bear from none to more than ten cilia; microvilli are also observed on some knobs (Miragall et al., 1984). Also common to the surface of the septal organ are a large number of globules believed to be secretory droplets of the glands beneath. These globules are closely associated with numerous holes in the surface, believed to be the ducts of the underlying glands (presumably Bowman's). The glands, located beneath the septal organ epithelium, are typical in structure and have ducts that open onto the epithelial surface.

Although the septal organ and the main olfactory epithelium are both located on the nasal septum, in the adult the septal organ is well isolated from the main olfactory epithelium by respiratory mucosa. [In the fetal and newborn rat, Astic and Saucier (1988) found that no clear border existed between the respiratory epithelium and either of the sensory epithelia. By the end of the first postnatal week, a sharp demarcation between the respiratory epithelium and the two sensory epithelia it separates had been formed.]

Pederson and Benson (1986) investigated the central projections of septal organ receptor axons. Using a

modified technique of retrograde horseradish peroxidase (HRP), they found that HRP delivered into the posterior dorsomedial region of the glomerular layer of the main olfactory bulb resulted in labeled neurons within the ipsilateral septal organ epithelium. When HRP was delivered into the confines of the accessory olfactory bulb however, no neurons within the septal organ epithelium were labeled. Septal organ axons that were labeled by this process indicated that these axons did not travel in direct association with the vomeronasal nerves. This confirmed the earlier study by Bojsen-Moller (1975) that described septal organ nerve fibers as traversing the nasal septum on a course superficial to the vomeronasal nerves. It appears from these data that the septal organ and the vomeronasal organ are anatomically, and probably functionally, separate chemosensitive organs that project to different central structures by separate nerve routes. In the Pederson and Benson study, only the posterior dorsomedial region of the main olfactory bulb was injected, and only some of the receptor neurons within the septal organ were labeled. However Astic and Saucier (1988), using retrograde HRP tracing techniques, showed that only injections of HRP into the posterior ventromedial aspect of the olfactory bulb labeled the septal organ. Injections of WGA-HRP into the septal organ of rats confirmed that axons from this organ projected exclusively to the posterior half of the main

olfactory bulb and are mainly restricted to the ventromedial region (Giannetti et al., 1992).

Although the main olfactory epithelium also projects to the olfactory bulb, its axons are distributed all along the anteroposterior axis of the bulb (Gianetti et al., 1992).

## 2. Function

Not too much is known about septal organ function. In the bandicoot, a marsupial with a distinct septal organ, Kratzing (1978) suggested that this ventrally situated olfactory area could monitor air flow in quiet respiration, when inspired air would be unlikely to reach the dorsal regions of the nasal cavity. Since the nasal septum in the bandicoot does not separate the two nasal chambers except in the most rostral portion, Kratzing suggested that in these animals in particular, the septal organ may help to determine the direction from which air-flow, and any odors carried with it, originate. Pederson and Benson (1986) agree with Kratzing that the septal organ is in a position to monitor incoming air that may never reach the olfactory epithelium. Since nonvolatile substances have access to both the septal and vomeronasal organs, but not the olfactory epithelium, nonvolatile substances may stimulate the main olfactory bulb by way of the septal organ. Marshall and Maruniak (1986) found that the most sensitive

septal organ receptor sites have a high sensitivity to low concentrations of odorant molecules: its threshold is lower than that of the olfactory epithelium. Also, it is responsive to a variety of odorants. Marshall and Maruniak agree that the septal organ is likely to be the first to signal the arrival of weak odors, and thus has an alerting function. Given its location, it may also provide maximum exposure to odors during sniffing, and may dictate the manipulation of air flow allowing for the distribution of odorant molecules to the less accessible olfactory epithelium (Marshall and Maruniak, 1986).

### **C. TRIGEMINAL NERVE FIBERS WITHIN THE NASAL CAVITY**

One of the functions of chemoreception is to detect potentially harmful environmental chemicals. What has been termed the "common chemical sense" is one of the chemosensory components mediated by free nerve endings within the epithelium. Fish, for example, will turn away from noxious chemical stimuli even when their senses of olfaction and taste have been destroyed (Silver, 1987). The main function of trigeminal chemoreception within the nasal cavity appears to be avoidance behavior or initiation of inhibitory reflexes in response to noxious chemical stimuli (Moulton, 1979).



### 1. *Morphology*

The trigeminal nerve is responsible for sensory innervation of the epithelial surfaces of the head. In addition to the perception of pain, touch, temperature, and position, some trigeminal fibers are chemosensitive and innervate the epithelial surfaces of the nasal and oral cavities. The ophthalmic and maxillary divisions of the trigeminal nerve provide chemosensory fibers to the nasal cavity by way of the ethmoid, nasopalatine, and infraorbital nerves. Most of these fibers are either finely myelinated or unmyelinated and arborize into free nerve endings either adjacent to the basal lamina or within the epithelium itself. (Silver, 1987).

The nasal septum is innervated by the following trigeminal nerve branches: the medial nasal nerve from the anterior ethmoidal nerve; the posterior nasal branches and the nasopalatine nerve from the pterygopalatine ganglion; the internal nasal branches from the infraorbital nerve (Bojsen-Møller, 1975). Branches from the anterior ethmoidal nerve pass to the vestibule to innervate the dorsal and apical regions. The nasopalatine nerve appears to supply the vomeronasal organ before ending in the nasopalatine canal. The posterior nasal branches form a large meshed network which supplies the entire respiratory region. Fibers from the infraorbital nerve supply part of

the vestibule. Bojsen-Moller states that trigeminal nerve branches are mainly myelinated nerves.

The respiratory region of the nasal cavity appears to be supplied by trigeminal and perivascular nerves whose functions include: somatic reception, chemoreception, regulation of air conductivity, and regulation of heat and water exchange (Bojsen-Moller, 1975). Bojsen-Moller states that all nasal glands are innervated by parasympathetic fibers. In the respiratory part of the nasal septum, these fibers are associated with nasopalatine and posterior nasal branches arising from the pterygopalatine ganglion within the pterygopalatine fossa. Although conclusive evidence is lacking, it is likely that Bowman's glands are innervated by autonomic fibers following meningeal branches of the anterior cerebral artery through the cribriform plate.

## *2. Function*

When presented with irritants, the following protective reflexes have been attributed to an initial trigeminal chemoreceptive response within the nasal cavity: decrease in respiratory rate; decrease in heart rate; constriction or dilation of the bronchi; increase in nasal secretion; sneezing; closure of the nares; closure of the glottis, and peripheral vasoconstriction (Silver, 1987).

The role of trigeminal fibers in chemoreception within

the nasal cavity has been demonstrated by Silver et al. (1985). Capsaicin, a substance found in red peppers, has the ability to render animals insensitive to noxious stimuli by depletion of the putative neurotransmitters, substance P and somatostatin. Substance P is the neurotransmitter released at the synapse between the pain transmitting fibers of the lateral spinothalamic tract and the second order neurons within the dorsal horns of the spinal cord (Snell, 1987). Substance P has recently been found in trigeminal nerve fibers (but not in olfactory nerve fibers) at the base of the olfactory epithelium. Depletion of substance P by capsaicin may be responsible for its antinociceptive (decreased perception of pain) effect. In laboratory studies, capsaicin injections abolished or severely limited nasal trigeminal responses to the noxious stimuli amyl acetate, cyclohexanone, and propionic acid. Behavioral avoidance of two strong trigeminal stimuli, capsaicin and formaldehyde, were also eliminated by capsaicin injections. Within the olfactory epithelium, olfactory receptor neurons are not sensitive to painful stimuli and did not exhibit any measurable deficits after treatment with chronic capsaicin injections. This study illustrated that chemosensitive fibers of the trigeminal nerve and pain fibers in other parts of the nervous system respond to a lack of substance P in a like manner: they failed to elicit a painful response to painful (noxious) stimuli.

Electrophysiological experiments in tiger salamanders indicate that trigeminal chemoreceptors respond to the intensity of odorants but are unable to distinguish between types of odorant (Silver et al., 1987). Qualitatively then, all odorants appear to be perceived as the same by the trigeminal system.

### 3. *Embryology*

The embryological development of the trigeminal nerve has been documented in the baboon by Gasser and Hendrickx (1969). The primordium of the trigeminal nerve is first seen as a small collection of cells located at the ventral surface juncture of the neural fold, between it and the ectoderm lateral to it. This collection of cells develops into a prominent trigeminal crest which projects ventrally and is located between the optic placode and the first pharyngeal pouch.

When the neural tube is formed, the trigeminal primordium is located at the level of the rhombencephalon's juncture with the mesencephalon, and extends ventrally into the first branchial arch. At the stage of development of the 22nd somite, the three divisions of the trigeminal nerve can be distinguished. Between the stage of 26 and 36 somites, the primordium of the ganglion is definable by the concentration of nuclei within it. At this stage, the

ophthalmic division can be followed dorsal to the optic primordium while the maxillary division is cellular proximally and loosely arranged distally. The mandibular division is less cellular than the other two and courses into the mandibular division of the first branchial arch. From this point onward, the distal-most portions of the divisions form fibrous sprays. These fibrous sprays will eventually give rise to many of the nerves which are derived from the three divisions of the trigeminal nerve.

#### **D. THE TERMINAL NERVE (NERVUS TERMINALIS)**

The terminal nerve is partly associated with the lamina terminalis and derives its name from that association. It has been designated as "cranial nerve 0" due to its position dorsal to cranial nerve I and the fact that it was discovered after all the other cranial nerves had been identified and named.

##### ***1. Morphology***

The terminal nerve has been found in virtually every vertebrate studied (Wirsig, 1987). In most species, this small nerve is distributed as loosely grouped and individual fibers that traverse the nasal septum, pass through the cribriform plate, and follow the ventromedial surface of the

olfactory bulb and forebrain to points of entry into the cerebrum just caudal to the olfactory bulb.

Cell bodies of the terminal nerve are located individually or within ganglia along the entire course of the nerve to its region of entry into the brain (Denski and Schwanzel-Fukuda, 1987). In mammals, the largest ganglion associated with the terminal nerve is the ganglion terminale, located just caudal to each olfactory bulb and on the ventromedial surface of the forebrain. Within the vomeronasal epithelium, nerve cell bodies resembling ganglion cells in the bat, *Artibeus*, (Bhatnagar and Kallen, 1974; Cooper and Bhatnagar, 1976) and free nerve endings both here and within nasal cavity epithelium of the white rat, have been identified as terminal nerve structures (Bojsen-Moller, 1975). Adams and Wiekamp (1984) consider the function of these nerve fibers to be to control the secretions of non-ciliated epithelial cells or to alter surface membrane permeability.

The terminal nerve appears to innervate two regions in the rat: the region of the vomeronasal organ and the vestibule of the nasal cavity (Bojsen Moller, 1975). Deep within the mucous membrane of the vestibule of the nose, the fibers of the terminal nerve form a plexus which interconnects with 20 to 30 small ganglia. From this region, fibers traverse the nasal septum caudally and accompany the medial septal branch of the anterior ethmoid

nerve. Unmyelinated terminal nerve branches can be distinguished from branches of the trigeminal nerve which are myelinated, and from vomeronasal nerve fibers which are of larger diameter and are located more ventrally within the septum. Fibers of the terminal nerve gather at the cribriform plate to form a discrete branch which accompanies the vomeronasal nerve into the cranial cavity. At the rostral end of the olfactory bulb, the fibers of the terminal nerve are so intertwined with the vomeronasal fibers that they are difficult to identify. Caudal to the entrance of the vomeronasal nerve fibers into the accessory olfactory bulb, the terminal nerve forms a plexus of fibers and ganglia, and then enters the telencephalon at two or three locations. (Bojsen-Moller, 1975).

In the hamster (Wirsig, 1987), terminal nerve fibers follow the same general course but run individually and are dispersed among the fibers of the olfactory and vomeronasal nerves.

Terminal nerve ganglia generally contain more than one type of neuron (Denski and Schwanzel-Fukuda, 1987; Ridgeway et al., 1987; Wirsig, 1987). In the hamster (Wirsig, 1987), neuron types within the ganglia are distinguished in part by their intracellular products: acetylcholinesterase has been identified in one neuron type while the second type contains leuteinizing hormone-releasing hormone (LHRH). The presence of LHRH has functional significance which will be discussed

under terminal nerve function. In the cetaceans *Tursiops* and *Delphinus* (Ridgeway et al., 1987), ganglion cells are round or fusiform, and LHRH immunoreactivity was detected only in the fusiform cells.

Recent advances in immunocytochemical techniques have led to the identification and mapping of terminal nerve fibers both centrally and peripherally. Terminal nerve fibers project to structures within the forebrain by way of several nerve bundles. In most mammals, terminal nerve axons project to the medial and lateral septal nuclei, olfactory tubercle, and to preoptic areas (Demski and Schwanzel-Fukuda, 1987). In the hamster, Wirsig (1987) identified projections of the terminal nerve that accompany the vomeronasal nerves into the accessory olfactory bulb. The root of the terminal nerve enters the cerebrum just caudal to the olfactory bulbs near the hippocampal rudiment. In the medial region of the olfactory peduncle, terminal nerve fibers terminate in or continue through the ganglion terminale to more central connections within the medial septal area, and to a secondary ganglion associated with the anterior cerebral artery (Wirsig, 1987).

In fetal *Macaca mulatta* (Witkin, 1987), LHRH immunoreactive fibers identified as belonging to the terminal nerve reached the medial septum, accompanied the anterior cerebral artery as it penetrated the forebrain, and followed an extracerebral course to the olfactory tubercle.



Additionally in this primate, LHRH immunoreactivity was seen within the optic nerve and optic chiasm, a situation which may be analogous to the LHRH innervation of the optic system via the olfactoretinalis nerve in bony fishes: the olfactoretinalis nerve in these fishes is a branch of the terminal nerve.

## 2. Function

The presence of LHRH immunoreactivity within the terminal nerve system is functionally significant. LHRH is a neurohormone known to mediate reproductive functions through its effect on the gonadotrophs of the anterior pituitary gland. The role of LHRH in reproduction and in mating behavior, the presence of LHRH within the terminal nerve, and the direct terminal nerve connection between the nasal cavity peripherally with regions of the brain implicated in reproductive behavior centrally all imply a functional role in mating and reproductive behavior for the terminal nerve (Schwanzel-Fukuda, 1987). Wirsig (1987) found that after terminal nerve axotomy, male hamsters displayed obvious deficits in mating behavior and were less interested in vaginal smears of female hamsters than were control animals. In fish, excitation of areas receiving terminal nerve fibers caused spawning behavior.

Wysocki and Meredith (1987) caution that assumptions of

vomeronasal function based on sectioning vomeronasal nerves could result from sectioning the terminal nerve, since fibers of the two systems run so closely together in most mammals.

### 3. *Embryology*

Brown (1980, 1987) was able to follow the development of the terminal nerve in bat and human embryos due to lack of development of vomeronasal nerve fibers in these mammals. Where both systems are present, the close anatomical association between the two obscures the exact identification of each.

At the nasal placode stage, large numbers of ganglion cells separate from the thickened placode of the nasal septum in the approximate region of the vomeronasal organ (in species that have them) and migrate to the deeper mesenchyme of the developing septum. The basal lamina in the region of these migrating cells is deficient. Fibers can be traced from these migrating ganglion cells back into the epithelium. Farbman and Squinto (1985) state that two cell types previously identified as migrating from the olfactory epithelium are likely to represent the precursors of sheath cells in one case and ganglion cells in the other case. This is in agreement with Brown (1980, 1987).

Brown (1980) traced the migration of the terminal nerve ganglion cells and their fibers through the mesenchyme of the nasal septum in a dorsocaudal direction, joining olfactory nerve fibers at the posterior aspect of the septum. The fibers reach the posterior border of the olfactory evagination where they separate from the olfactory fibers. In this region, the terminal nerve divides into two bundles, fibers of each penetrating the ventromedial forebrain. Fully differentiated ganglion cells follow the nerve fibers into the structure of the developing forebrain. For a time, increasing numbers of ganglion cells migrate from the periphery towards more central structures. In the bat and human embryo, however, the number of ganglion cells increasingly diminishes, with a subsequent disappearance of these cells and their fibers. In insectivorous bats and humans, no evidence of the terminal nerve exists beyond the embryonic period. In species with a terminal nerve, it is reasonable to assume that the development of this system is the same as that in the bat but without the subsequent degeneration of ganglion cells and nerve fibers.

#### **E. SUMMARY OF THE ACCESSORY NASOCHEMOSENSORY SYSTEM**

The vomeronasal organ is present in embryonic forms of all mammals studied, but is reduced or absent in the adult forms in some species of many mammalian orders. It is

notably reduced or absent in completely aquatic mammals presumably due to the lack of airborne chemical stimuli. Receptor neurons are present within the epithelium of the vomeronasal organ; axons of these cells project to the accessory olfactory bulb. In those mammals where a vomeronasal organ and accessory olfactory bulb are present, the system appears to be functionally associated with those behaviors that are mediated by hormones: for example reproductive behavior and aggressive behavior. This is most clearly documented in rodents. It may also play a role in food finding and recognition of conspecifics.

There is less information available for the septal organ. Receptor cells are present within the epithelium of the ventral nasal septum. Axons of these neurons have been traced to the posterior dorsomedial main olfactory bulb but may have additional target areas as well. The function of this organ may be to monitor airflow and any odors carried with it.

Trigeminal nerve fibers within the nasal cavity are end branches of the ophthalmic and maxillary divisions of the trigeminal nerve. As such, the somata of these neurons are located within the semilunar ganglion of the trigeminal nerve and the central processes project to nuclei within the brain stem. The nasal trigeminal nerve fibers are sensitive to noxious stimuli.

The terminal nerve is present in all vertebrate species

studied thus far. Its receptor ends are located in the region of the vomeronasal organ and in the vestibule of the nasal cavity. Its cell bodies are located along the course of the nerve fibers, from the nasal septum peripherally to the ventromedial surface of the brain centrally. The terminal nerve axons project to nuclei within the forebrain, and some fibers may terminate within the accessory olfactory bulb. Through its association with the neurotransmitter leuteinizing hormone-releasing hormone, the terminal nerve may be involved in the mediation of reproductive behavior.

There does not appear to be a neat division of labor when considering function in the five systems that together comprise nasochemosensory system. In those mammals lacking a fully developed vomeronasal organ, the question arises as to what part of the nasochemosensory system carries signals normally transmitted by vomeronasal organ fibers. It is unlikely that the answer to this is a simple one, considering that within the accessory nasochemosensory systems, two pathways appear to be associated with reproductive behavior: the vomeronasal organ and the trigeminal nerve. The function of the olfactory epithelium has yet to be discussed (Chapter 3, page 170) but it is likely that the majority of odorant information is received and transmitted by receptor cells of the olfactory epithelium. This assumption is based on the following

observations:

1) damage to the olfactory nerve fibers or the olfactory bulb profoundly affects the sense of smell; 2) the receptor surface area of the olfactory epithelium is much greater than the surface area associated with any of the accessory nasochemosensory systems. Within mammalian orders, much variation exists in size and the presence or absence of the vomeronasal organ and accessory olfactory bulb. Because of the limited information regarding the other components of the nasochemosensory systems, and the limited physiological evidence for the transmission of specific odorant molecules by a specific nasochemosensory system neurons, a definitive statement regarding the function of each of these systems cannot be offered at this time. However, the question is addressed in greater detail when the function of the main olfactory system is discussed (Chapter 3, page 170).

## CHAPTER 3

### THE MAIN OLFACTORY BULB

#### I. REVIEW OF MAIN OLFACTORY BULB MORPHOLOGY

The axons of olfactory epithelial primary receptor neurons synapse within the olfactory bulb. Second order neurons within the bulb transmit the olfactory information to structures within the brain. A brief description of basic neuronal types encountered within the main olfactory bulb, and a brief description of the cellular and fiber layers that comprise the bulb is offered as a reference before describing the details of olfactory bulb development.

Three types of neurons are involved in the transmission of olfactory information from the nasal mucosa to cerebral structures central to the main olfactory bulb: 1) olfactory receptor neurons which have been discussed previously; 2) mitral and tufted cells, second order neurons whose axons leave the olfactory bulb to synapse with third order neurons in more centrally located nuclei; 3) interneurons. Interneurons are usually small-bodied cells with short axons that are involved in local circuitry. In cortical regions of the brain, varying numbers of interneurons intervene

between the afferent and efferent fibers that will ultimately transmit information to the next synapse (Barr and Kiernan, 1988). Interneurons function either to inhibit or to allow excitatory responses to pass through, thereby regulating and modulating the signal that proceeds to the final output pathway.

Moving from superficial to deep, the main olfactory bulb consists of the following layers: 1) olfactory nerve layer; 2) glomerular layer; 3) external plexiform layer; 4) mitral cell layer; 5) internal plexiform layer; 6) granule cell layer (Figure 6, page 87).

The olfactory bulb is surrounded by olfactory receptor neuron axons that form the **olfactory nerve layer**. The axons are surrounded by sheath cells that accompany them from epithelium to main olfactory bulb, and whose cell bodies are located anywhere along the length of the axons as well as within the olfactory nerve layer. Also present within the olfactory nerve layer are the somata of glial cells. The receptor neuron axons penetrate the next layer, the glomerular layer.

The **glomerular layer** consists of the somata of the following cell types: one subdivision of tufted cells, the external tufted cells; interneurons including periglomerular and short axon cells; glial cells. The periglomerular cells are interneurons that are morphologically similar to the granule cells located in the deepest neuronal layer of the



Figure 6. The olfactory bulb of the rat (X 10).

o = olfactory nerve layer

gl = glomerular layer

e = external plexiform layer

m = mitral cell layer

arrow = i = internal plexiform layer

G = granule cell layer

s = subventricular layer



Figure 6

bulb. In addition, there are predominantly cell free areas called glomeruli where axons of receptor neurons synapse with dendrites of second order neurons, the mitral and tufted cells, and in some species with periglomerular cell dendrites.

The **external plexiform layer** is located deep to the glomerular layer. There are somata of all subdivisions of tufted cells located within this layer along with the somata of a variety of interneurons and glial cells. This is a layer of fibers, both axons and dendrites, and of synapses involving second order neurons with interneurons.

The fourth layer is the **mitral cell layer**, a relatively narrow layer located between the more superficial external plexiform layer and the deeper internal plexiform layer (when it is present). The mitral cell layer contains the somata of the large mitral cells, and interneurons which appear to be morphologically identical to granule cells of the granule cell layer.

In many mammalian species, a relatively cell-free **internal plexiform layer** constitutes the fifth layer within the main olfactory bulb. This layer often has a few to moderate number of granule cells located within it but is predominantly a layer of myelinated fibers. If the internal plexiform layer is absent, it is due to a merging of the mitral cell layer with the granule cell layer deep to it.

The final and deepest layer containing mature neurons

(forming synapses) is the **granule cell layer** which is nearly filled with rows or clumps of granule cell interneurons, and contains a small population of other interneurons.

Myelinated mitral and tufted cell axons traverse the granule cell layer and exit the main olfactory bulb, forming the olfactory tract.

Deep to the granule cell layer is the **subependymal** or **subventricular layer**, and in some species, an extension of the anterior lateral ventricle which produces a cavity (the olfactory ventricle) within each olfactory bulb. The subependymal layer is the remnant of the second zone of cell proliferation in the embryo which has substantially regressed in the adult. The subependymal layer will not be discussed beyond its embryological significance. The olfactory ventricle is present in the embryonic form of most mammals but frequently regresses and is occluded by the subependymal layer in the adult.

In summary, receptor cells are located in the olfactory epithelium; second order cells are mitral and tufted cells, located within the glomerular, external plexiform and mitral cell layers; and interneurons (predominantly granule-type cells), located in all layers of the olfactory bulb except the olfactory nerve layer. The six layers that comprise the main olfactory bulb in the adult will be discussed in greater detail in the section, **Morphology of the Adult Main Olfactory Bulb**.

## II. DEVELOPMENT OF THE OLFACTORY BULB

### A. EARLY DEVELOPMENT

As was discussed briefly in Chapter 2, gross development of the early olfactory bulb begins with the early interaction between the olfactory placodes and the telencephalon. As the bilateral olfactory placodes deepen within the invaginating nasal pits, olfactory receptor axons and a specific type of basal cell exit the basal lamina of the olfactory epithelium and migrate toward the putative olfactory bulb (Marin-Padilla and Amieva B., 1989). Close proximity of the migrating axons to the developing telencephalon are necessary for the evagination of the early olfactory bulbs from the developing telencephalon. In the embryonic mouse, the olfactory placode is present as a pronounced thickening on embryonic day 10 (E10) and the olfactory placode invaginates on E11, the same day that evagination of the cerebral vesicle begins (Hinds, 1972a). Evagination of the olfactory bulbs from the rostral cerebral vesicles does not occur until E13 (Hinds, 1972a). The telencephalon-directed receptor cell axons are accompanied by migrating basal cells that appear to be the precursors of sheath cells (Marin-Padilla and Amieva B., 1989). Several axons, along with their accompanying sheath cells, form bundles called olfactory nerve fascicles. Upon reaching the

primordium of the olfactory bulb, the olfactory nerve fascicles and their ensheathing cells form the olfactory nerve layer (approximately E16).

On E12, three of the primitive cell producing layers within the olfactory bulb (discussed below) are present and many receptor neuron axons have penetrated deep within the developing bulb. Some neurons are distinguishable at this time. The mitral cell layer is apparent by E15, followed in one day by the olfactory nerve, external plexiform and glomerular layers. Mitral cells are the earliest cells which can be recognized within the developing olfactory bulb and are quickly followed by the development of radially migrating tufted cells. There is a slight internal-to-external time gradient of output neuron (second order neuron) development within the bulb with the sequence of appearance being: mitral cell, internal tufted, middle tufted, and external tufted cells. By E18, primitive glomeruli are present as is the granule cell layer (Hinds, 1968b). Interneurons, neurons which function as modifiers of either second order neurons or other interneurons, develop late, closer to the end of the tufted cell production. There is a slight external-to-internal time gradient in the appearance of the different types of interneurons with the more superficially located periglomerular population appearing first and the most internally located granule cell population appearing

slightly later (Brunjes and Frazier, 1985).

Olfactory receptor axons are important in the formation of glomeruli and in the orientation of mitral cells: this will be discussed later in this section.

## **B. DEVELOPMENT OF OLFACTORY BULB LAYERS AND DIFFERENTIATION OF NEURONS**

In the development of the central nervous system in mammals, the neural plate invaginates, folds, and forms the neural tube, enclosing within it the neural groove. It is the neural groove that becomes the ventricular system of the brain and the central canal of the spinal cord. The region of neural tube that surrounds the neural groove is called the ventricular zone or ependymal layer (Moore, 1988). It is this layer that gives rise to all neurons and glial cells of the central nervous system.<sup>1</sup>

1. In 1970, the "Boulder Committee" devised a standard terminology to describe the four layers of cells and their processes encountered in the developing central nervous system. This was deemed necessary due to the proliferation of confusing terms used by different researchers. The Boulder Committee terminology is offered below because it is the terminology used in most recent publication. However, Hinds, in describing the development of the olfactory bulb, used terms that do not exactly correspond to the Boulder Committee schema. In the text of this chapter, I have chosen to follow Hinds as his publications on the development of the olfactory bulb are the most comprehensive: following his terminology avoids the possibility of incorrectly stating his findings.

In the embryonic mammalian brain, the rostral end of the lateral ventricles extends into the developing olfactory bulb creating the olfactory ventricle. The rostral part of the developing cerebral vesicles and the olfactory bulbs that evaginate from them are comprised of two layers: 1) the inner (deep) layer referred to as the matrix or primitive ependymal layer (ventricular zone as defined by the Boulder Committee). This layer is derived from the ventricular zone of the neural tube. 2) The outer

Boulder Committee terminology for the zones of development within the vertebrate fetal nervous system are as follows:

1. The *ventricular zone*, the first zone to appear, contains only ventricular cells. These cells are the ultimate progenitors of all neurons and macroglial cells within the central nervous system. The ventricular zone has alternately been referred to by a variety of terms including the *matrix layer*, *primitive ependyma*, and *neuroepithelial layer*.
2. The *marginal zone*, appears slightly later. This layer contains no primary cells of its own but is composed of cytoplasmic processes of cells located within the ventricular layer. This layer expands as ventricular cell processes elongate, and although its inner region become replaced by the next two developmental layers, the marginal zone always remains the most peripheral embryological zone.
3. The *intermediate zone* is the third zone to form and is composed of peripherally migrating, dividing, and differentiating ventricular cells. This zone is located between the ventricular and marginal zones. The intermediate zone has been referred to as the *mantle layer* and *intermediate zones of His*.
4. The *subventricular zone*, particularly prominent in the cerebral hemispheres, is located between the ventricular and intermediate zones. The cells of this layer are very proliferative and give rise to special classes of neurons and to most classes of macroglia within the central nervous system. The subventricular zone has been referred to as the *subependymal layer*.



(superficial) layer, referred to as the mantle layer (Hinds, 1968b) (possibly the intermediate zone of the Boulder Committee), becomes the substrate for several of the olfactory bulb layers as neuroblasts and glioblasts migrate into it and differentiate into various types of neurons and glial cells. Although these two layers contain cells, they are undifferentiated in the early embryo.

The matrix layer lies adjacent to the ventricle and consists of epithelium in which cell mitosis occurs and from which cells migrate outward to more peripheral regions. Hinds (1968a,b) injected mouse embryos with tritiated thymidine during various embryonic stages and sacrificed animals within each group at varying intervals after injection. The following sequence of cell and layer development within the main olfactory bulb is based on these studies and on analysis of Golgi stained and electron microscopy sections (Hinds 1972a, b).

In animals injected on E12, labeled cells are evident in the matrix layer very close to the ventricle. Within 16 hours, tagged cells have migrated outward to distribute more evenly within the matrix layer and have moved into the mantle layer (Figure 7A, 96). The outward migration continues over subsequent days, and most of these cells will differentiate into mitral cells. Some of these cells continue migrating into more superficial regions and are precursors of external tufted cells and periglomerular

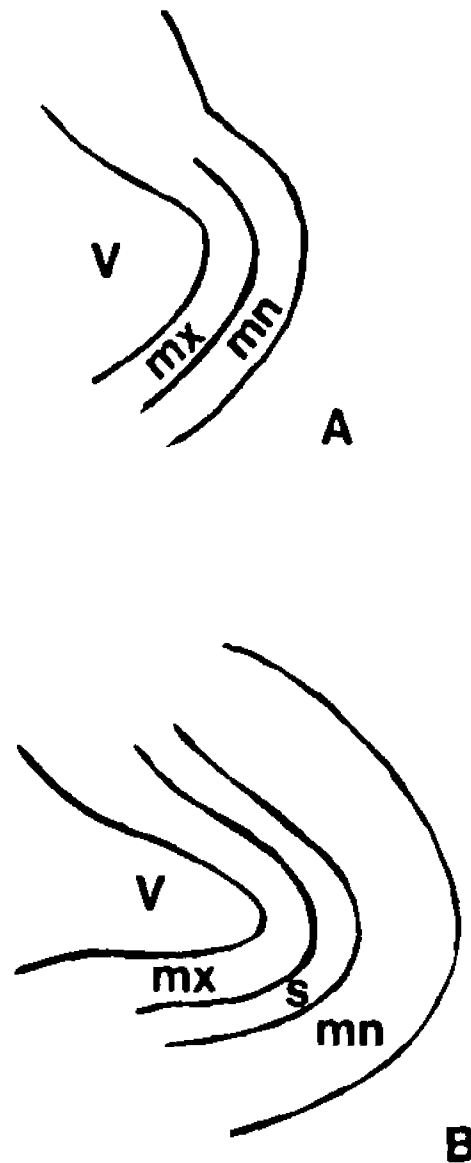


Figure 7. Development of olfactory bulb layers. A. The developing olfactory bulb has evaginated from the telencephalon and two cellular layers are present. **Mx** = matrix layer; **Mn** = mantle layer; **V** = ventricle.

B. **SE** = subependymal layer, an important layer of cell proliferation that will replace the matrix layer.

(Modified from Hinds, 1968b).

cells.

A second zone of cell proliferation is identified 22 hours after injection on E12. These cells are located within the olfactory nerve layer and are glial cells which differentiate and remain within this layer.

The olfactory bulbs of animals injected on E15 demonstrate four populations of proliferating cells: 1) within the matrix layer (ventricular zone); 2) the subependymal layer (subventricular zone), an important layer of cell proliferation that forms between the matrix and mantle layers (Figure 7b, page 96); 3) populations of glial cells proliferating throughout the substance of the olfactory bulb; 4) the olfactory nerve layer (glial cells). At this stage of embryonic development, Doucette (1989) states that within the olfactory nerve layer, the only cell bodies present are the somata of sheath cells. According to Doucette, astrocytes, the second type of glial cell located within this layer, arise later from progenator cells that must be located within the olfactory bulb, not peripherally. This is in agreement with Hinds' (1972a, b) observation that glial cell proliferation occurs within the olfactory nerve layer on E14 of the mouse. There is a discrepancy in the time between Doucette's presumed differentiation of astrocytes and Hinds' observations in the embryonic mouse. However, it does appear that the two glial cell populations within this layer are derived from entirely different

sources: sheath cells from stem cells within the olfactory epithelium, and astrocytes from progenator cells within the olfactory bulb (Doucette, 1989).

Tagged precursors of neurons differentiating approximately three days after the E15 injection are primitive tufted and granule cells. Animals injected on E17 have only three proliferative layers: the matrix layer has completely receded and the subependymal layer extends deeply to the olfactory ventricle. It is the subependymal layer that exists in the adult as the layer deep to the granule cell layer. Cells located in the subependymal layer on E17 migrate into the granule cell layer and will continue to migrate into more superficial layers. These cells are precursors of periglomerular and tufted cells.

On the first postnatal day (P1), the layers of the mature olfactory bulb are clearly present. Glomeruli surrounded by periglomerular cells are present. Just deep to this layer is a population of tufted cells (external tufted) which loosely defines the most superficial region of the external plexiform layer. Additional tufted cells are located in no particular order within the remainder of the external plexiform layer. Two or three rows of mitral cells clearly demarcate the mitral cell layer (Hinds, 1968b) although in most adult species, the mitral cells are more randomly placed within the boundaries of what is termed the mitral cell layer. The granule cell layer is characterized

by granule cells which have aggregated into rows or clumps. Although it is clearly visible between mitral cell and granule cell layers, the internal plexiform layer is not labeled as a distinct layer in Hinds' (1968b) illustrations of sectioned olfactory bulbs at days E16, P1, and P7.

In summary, the matrix layer is present in the earliest embryos studied by Hinds (E11), and produces more matrix cells, mitral cells, cells of the subependymal layer, and a few tufted and granule cells (including periglomerular and granule cells located within the mitral cell layer). The matrix layer disappears around E17.

The subependymal layer originates on E14 and is present throughout the life of the animal. This layer gives rise to more subependymal cells, glioblasts (future astrocytes and oligodendrocytes), precursors of some tufted cells, and perhaps precursors of some granule cells. Although granule cells differentiate within the olfactory bulb, Kishi (1987) has demonstrated that the precursors of the majority, and perhaps all granule cells migrate in from the subependymal region caudal to the olfactory bulb. This will be discussed in greater detail below.

To summarize Hinds' observations of neuron development in the olfactory bulb of the embryonic mouse (1968a), the time of origin of mitral cells precedes that of tufted cells, which precedes that of most interneurons. There is

only slight overlap in time in the production of the interneurons. Granule cells within the mitral cell layer are produced earliest, followed shortly thereafter by production of periglomerular cells. While some interneurons within the granule cell layer are produced in the late embryonic period, the majority differentiate after birth.

Hinds and McNelly (1977) found that granule cells in the mouse increase in number for up to 24 months after birth. Glial cell production peaks between the last embryonic day (E18) and postnatal day 5 in the mouse. Therefore, the majority of glial cells arise later than the majority of neurons within the bulb, with the exception of granule cells within the granule cell layer whose proliferation time is greatest after birth.

Since most neuroblasts are derived from the matrix and subependymal layers, migration of developing neurons through previously formed cell layers is extensive. This is not unusual in cortical structures and is seen in the development of the cortex of the cerebral hemispheres and the cerebellum (Hinds, 1968b).

### **C. GRANULE CELL PRECURSORS MIGRATE INTO THE OLFACTORY BULB**

It was previously stated that a layer of mitotically active cells surrounding the ventricular system of the brain is the source of all brain cells, both neurons and glial.

Both the rostral portion of the lateral ventricle and the layer of cells surrounding it extend into the developing olfactory bulb. It has been discussed in detail how precursors of neurons and glial cells are derived from the layers immediately surrounding the olfactory ventricle. The matrix layer, the initial source of olfactory bulb cells, is eventually replaced by the cell-producing subependymal layer. Since granule cells arise in the late embryonic period and throughout an extended postnatal period, a period that coincides with a low number of mitotic cells within the subependymal layer of the olfactory bulb, the question of where precursors of granule cells arise has been asked. Could these precursor cells migrate into the olfactory bulb where they differentiate into granule cells?

Kishi (1987) studied the mitotic indices and golgi stained sections of the subependymal layer of the anterior lateral ventricle and its extension into the olfactory bulb of embryonic and postnatal rats. In newborn rats, the subependymal layer around the anterior horn of the lateral ventricle extends as follows: it descends vertically just caudal to the olfactory peduncle, then turns horizontally following the olfactory ventricle into the olfactory bulb. Within the bulb, it surrounds the olfactory ventricle and is itself surrounded superficially by the granule cell layer. Around the time of birth, the number of mitotic cells around the anterior horn of the lateral ventricle and in the

descending part of the subependymal layer is high while the number of mitotic cells within the horizontal portion, particularly within the olfactory bulb, is low. Over the next three weeks, there is a shift in mitotic cells away from the anterior lateral ventricle towards a concentration within the descending portion of the subependymal layer: the subependymal layer of the olfactory bulb continues to contain few mitotic cells. Since 90% of granule cells arise during the first three postnatal weeks (Kishi, 1987), the identification of mitotic activity is important.

Analysis of golgi impregnated subependymal cells (Kishi, 1987) during the postnatal period indicates a stream of cells spanning from the rostral anterior horn of the lateral ventricle, down the vertical stretch in the region of the olfactory peduncle, and into the horizontal layer associated with the olfactory bulb. Nowhere along this subependymal route is the orientation towards any structure other than the olfactory bulb. The majority (72%) of the subependymal cells within this stream are bipolar with a leading growth cone and a trailing process. 89% of the leading growth cones are oriented toward the olfactory bulb, and the migration of what are at this stage bipolar cells is always towards the olfactory bulb.

Until day 15, there is a steady and often massive rostrally directed stream of bipolar subependymal cells which reach the olfactory bulb and migrate superficially



into the granule cell layer. At the border of the subependymal layer with the granule cell layer, two cell morphologies are found: bipolar cells which are migrating peripherally, and immature granule cells. Immature granule cells can be found throughout the granule cell layer. Between days 37 and 60, most of the granule cells have matured, although there are still a small number of bipolar cells migrating into the olfactory bulb. After migration into the granule layer of the olfactory bulb, the leading processes of bipolar cells differentiate into peripheral processes by extending, branching, and developing gemmules. The trailing processes develop into basal dendrites. Thus there is morphological continuity between the migrating bipolar cells and mature granule cells.

Autoradiographic studies using  $H^3$ -thymidine confirm the migration of cells from the dorsocaudal subependymal region around the anterior lateral ventricle into the subependymal layer, followed by migration into the granule layer of the olfactory bulb (Kishi, 1987).

#### **D. RECEPTOR CELL AXONS INFLUENCE MORPHOLOGY WITHIN THE BULB**

In the mouse, olfactory receptors project axons towards the forebrain on day E11. Olfactory nerve fibers contact and encircle the rostral pole of the telencephalon before evagination of the bulb (Brunjes and Frazier, 1986; Marin-

Padilla and Amieva B., 1989), which occurs on E13 in the mouse (Hinds, 1972a), and before any specific class of cells forms within the bulb. Hinds' (1972b) electron microscopic study of the developing mouse olfactory bulb revealed that receptor cell axons penetrate the deep into the mantle layer and occasionally reach into the deepest layer, the matrix layer. The differentiation of the developing olfactory bulb neurons occurs after penetration by these incoming receptor cell axons, but communication between these axons and developing neurons has not yet been proven to exist (Brunjes and Frazier, 1986). Monti Graziadei et al. (1980) found that olfactory marker protein, a protein specific to the olfactory sensory neurons, is first present in the olfactory epithelium of the 14-day embryonic mouse. By embryonic day 17, olfactory neurons and axons covering most of the surface of the olfactory bulb stained positive for olfactory marker protein. These researchers demonstrated that the presence of olfactory marker protein in the receptor neurons and their axons coincides with orientation of the mitral cells towards these sensory axons and, as importantly, with the development of synapses between olfactory receptor cell axons and the mitral cell dendrites.

There is much evidence to support the idea that olfactory bulb development is dependant upon the presence of olfactory receptor axons (Stout and Graziadei, 1980; Klein and Graziadei, 1983). The production of the olfactory

glomeruli, the morphological structure that assures the close and somewhat topographical approximation of incoming receptor cell axons with the dendrites of second order neurons, is determined by the presence of the incoming receptor neuron axons and not by the presence of second order neurons (Graziadei and Monti Graziadei, 1986). In partially bulbectomized neonatal mice, Graziadei and Samanen (1980) found that incoming olfactory axons formed the characteristic pattern of normal glomeruli in whatever layer of the olfactory bulb they were exposed to. A recent study by Monti-Graziadei and Graziadei (1992) found that newly formed "replacement" glomeruli were positioned randomly, that ectopic locations were not uncommon even when only very small lesions were made within the bulb, and that incoming axons penetrated the bulb in a disorganized manner, forming glomeruli within all layers of the olfactory bulb. When olfactory tissues were extensively disturbed, the dendrites of the remaining mitral cells reoriented themselves towards the newly formed glomeruli and formed branches within them. Graziadei et al. (1979) demonstrated that in bulbectomized mice, synaptic contact is established between post-trauma axons and the dendrites of remaining olfactory bulb neurons.

Newly formed olfactory receptor axons can innervate other telencephalic tissue when the olfactory bulb is removed. When the adjacent forebrain invades the vacated region left by removal of the olfactory bulb, incoming axons

form glomeruli within the brain tissue. Graziadei and Kaplan (1980) demonstrated that incoming olfactory axons also reorganize non-olfactory related telencephalic tissue to form glomeruli and that synapses are formed with the host cells (Graziadei, 1979). In the embryonic mouse, when presumptive occipital lobe cortical tissue was transplanted into the bulbectomized olfactory bulb region, incoming receptor axons invaded the transplanted tissue and formed glomeruli-like structures. Dendrites of the neurons within the transplanted brain tissue oriented towards these glomeruli, and in some cases extended dendritic arborizations directly into these newly formed glomerular structures. This suggests that olfactory axons are programmed to reorganize and form glomeruli-like structures in rostrally placed telencephalic nervous tissue, irrespective of the origin or presumptive function of the tissue located there.

#### **E. THE DEVELOPING OLFACTORY BULB IN THE HUMAN**

Although the preceding discussion has been based on the work of Hinds with the embryonic mouse, Humphrey's study (1940) of the main and accessory olfactory bulb in human embryos shows a similar sequence of developmental events.

The study begins in embryonic week 6.5 (EW 6.5) when the vomeronasal groove is present and both main and

accessory olfactory bulbs lack any evidence of lamination. By EW 8 - 8.5, the vomeronasal organ has formed a deep pocket, vomeronasal nerve fibers are seen entering the olfactory bulb, and towards the end of this period, lamination of cell layers in the main and accessory olfactory bulbs has begun. The vomeronasal organ begins to degenerate as early as EW 9.5 although in most fetuses, this process begins between 10 and 20 embryonic weeks. There is a correspondence between a fully developed vomeronasal organ and complete differentiation of the accessory olfactory bulb. As was seen in the mouse, accessory olfactory bulb differentiation occurs somewhat earlier than that in the main olfactory bulb, but by EW 10, lamination in both main and accessory olfactory bulbs is clear, with the mitral cell layer exhibiting the clearest differentiation. By EW 12, the internal and external plexiform and immature granule cell layers are present, but the glomerular layer is not yet distinguishable as such. Also, the accessory olfactory bulb has begun its degeneration. In EW 15.5, the glomeruli are beginning to differentiate and the accessory olfactory bulb has degenerated to no more than a vestigial structure in most (but not all) specimens. By EW 18, the lamination within the main olfactory bulb is typical of that found in the adult, and it is at this embryonic stage that Humphrey's study was concluded.

Development in the olfactory bulb of the mouse and

human correspond in the following ways: the mitral cell layer differentiates first, the glomerular layer last; accessory bulb differentiation occurs slightly ahead of main olfactory bulb differentiation. In those vertebrate that lack a functioning vomeronasal organ in the adult, the accessory olfactory bulb degenerates. As mentioned above, degeneration of the accessory olfactory bulb in the human embryo begins in embryonic week 12 (Humphrey, 1940). Hinds (1968a) and Brunjes and Frazier (1986) state that in the formation of the accessory olfactory bulb, the granule cells of the granule cell layer may arise before the periglomerular cells, a reversal of the order seen in the main olfactory bulb.

#### **F. FORMATION OF SYNAPSES**

The formation of synapses is crucial to the functioning of any system of neurons. Hinds (1976a) states that of all systems currently investigated, synapses are functional at about the time they are recognizable. In the olfactory system of the mouse, all basic synapses within the olfactory bulb are present at the time of birth. The sense of smell is known to be critical for survival of newborn rodents. Five day old neonatal pups subjected to bilateral olfactory bulbectomy were unable to distinguish the ventral nursing surface of their mother from either her dorsal surface or

the covering of a heating pad (Teicher et al., 1984).

In the embryonic mouse, synapse formation within the olfactory bulb begins on embryonic day 14 in the region just underneath the olfactory nerve layer (Hinds, 1976a). By E 15, there is a much greater concentration of synapses, particularly in regions where axons of olfactory neurons are penetrating the glomerular layer. This increase in synapse formation correlates with a reorientation of the mitral cell somata which directs its developing dendrites towards the receptor neuron axons within the glomerular layer.

By E18, synaptic density in the glomeruli layer is about ten times that of the external plexiform layer. Not surprising is the fact that the granule cell layer has the least synaptic density, about ten times less than the external plexiform layer. As discussed above, the majority of cells within the granule cell layer develop after birth. Synaptic formation, particularly in the external plexiform and granule cell layers continues to increase for up to 44 days after birth (Hinds, 1976a).

There is evidence that the axon of a neuron must reach its target cell before the developing dendrites of that neuron will extend and mature (Hinds, 1976a). This implies that synapse formation associated with the dendrites of a neuron is also dependent upon that neuron's axon reaching its target cell. Although the axons of olfactory receptor cells are present within the future glomerular layer days

before mitral cell processes mature, function within the system must await full development of the mitral cell axon and dendrites.

### III. MORPHOLOGY OF THE ADULT OLFACTORY BULB

The olfactory bulb is made up of several types of neurons and glial cells, yet only two types of cells (receptor cells and second order neurons) are responsible for receiving and transferring olfactory information to more central regions of the brain. While input into the main olfactory bulb is by way of olfactory receptor neuron axons, output from the bulb is through the axons of second order neurons, the mitral and tufted cells. The somata of these output neurons are located within the mitral cell layer and external plexiform layer/glomerular layer, respectively. As output cells, mitral and tufted cells are excitatory neurons that receive transmitted odorant information from the olfactory receptor neurons by way of axodendritic synapses within the glomeruli: they transmit this information to more centrally located nuclei. Axons of the mitral and tufted cells traverse the olfactory bulb and exit by way of the lateral olfactory tract<sup>1</sup>. They synapse with more centrally

1. In most mammalian studies, the efferent olfactory fiber systems form the olfactory tracts. Three separate bundles



located third order neurons within the brain, or in some cases, in a nucleus located within the lateral olfactory tract, the anterior olfactory nucleus. Axons that synapse within the anterior olfactory nucleus do so with third order neurons whose axons project to the contralateral olfactory bulb.

A series of interneurons located within the olfactory bulb modify and synthesize the output of the mitral and tufted cells. These neurons will be discussed within the context of the bulb layer in which their cell bodies and synaptic processes are located. Since the transfer of information from primary to second order neurons occurs within the glomeruli, primary dendrites of mitral and tufted cells and the synapses occurring on these dendrites are included within the discussion of the glomerular layer. Their somata and other cell processes will be discussed with the olfactory bulb layer in which they are located.

form the olfactory fiber systems: the medial, intermediate and lateral olfactory tracts (LOT). The majority of efferent fibers reach more central telencephalic structures by way of the LOT, by far the largest of the three (Zeman and Innes, 1963). In humans, efferent fibers are referred to as forming the olfactory tract. Centrally, this long tract splits to form a smaller medial olfactory stria and a larger lateral olfactory stria. In keeping with nomenclature used in most mammalian studies, I will use the term lateral olfactory tract when quoting studies where this term has been used.

### **A. THE OLFACTORY NERVE LAYER**

In the adult, the olfactory nerve layer consists of the axons of olfactory receptor neurons and the somata of two types of glial cells: 1) sheath cells that surround and accompany unmyelinated receptor neuron axons as they grow toward the olfactory bulb, and 2) astrocytes. The function of sheath cells is comparable to that of Schwann cells within the peripheral nervous system, that is to support axonal growth either by providing an adhesive substrate or by secretion of neurotropic molecules (Doucette, 1989).

Axons of the olfactory receptor neurons have been discussed earlier in this chapter and will be again in the discussion of the glomerular layer. The two types of cells whose somata are located within this layer are both glial cells. Since no synapses occur within the olfactory nerve layer, interactions between these axons and the neurons they interact with will be discussed with the glomeruli, the sites within which the synapses occur.

### **B. THE GLOMERULAR LAYER**

Much of the information included below on the glomerular and periglomerular regions comes from the work of Pinching and Powell (1971a, b).

The glomerulus functions as a region of transfer of

information through the extensive interconnections of a large number of cells representing four (or more) types of neurons. This is the region where the input from peripheral receptor neurons in contact with the external environment is transferred to the dendrites of second order neurons and ultimately becomes the output stimulus of the olfactory bulb. As regions of transfer of information, the glomeruli contain numerous axons and dendrites but no neuronal cell bodies.

The periglomerular region which surrounds each glomeruli is loaded with the somata of neurons and glial cells. The neurons include small second order neurons (external tufted cells) and at least two types of interneurons whose synaptic influence is within the local region.

### **1. The Glomeruli**

The glomeruli are round or oval structures that contain a mass of nerve fibers consisting of the terminal ends of olfactory receptor axons and dendrites and dendritic appendages of mitral, tufted and periglomerular cells (Pinching and Powell, 1971b). The glomeruli are generally free of cell bodies; occasionally they contain a few glial cells but never the somata of neurons. Glomeruli are located between the superficial olfactory nerve layer and

the deeper bulb layers: in cross-section, they form a series of circles surrounding the deeper layers (Figure 6, page 87).

Although not always present, a glial wrapping composed of several thin lamellae can often be found partially enclosing each glomerulus and separating it from the periglomerular region. This glial wrapping is discontinuous and is never found in conjunction with the entry of olfactory receptor axons or primary dendrites (Pinching and Powell, 1971b). However, it is not unusual to find one or more thin lamellae covering the surface of a single dendrite or dendrites within the periglomerular region (Pinching and Powell, 1971a). The origin of these lamellae are obviously glial as in some cases they have been determined to be continuous with the somata of glial cells.

The axons of most olfactory receptor neurons enter a glomerulus across its superficial border. Some (but not all) olfactory nerve fascicles penetrate the periglomerular region, the cellular area surrounding each glomerulus, before entering the glomerulus. Others enter the superficial rim of the glomerulus which often is not demarcated by periglomerular cells. As soon axons leave the periglomerular region to enter the glomerulus, they do so as single axons, separating from the nerve bundle within which they traversed the lamina cribrosa. Once into the neuropil of the glomerulus, the nerve axons run for various distances

giving off short branches and becoming irregular in shape (Pinching and Powell, 1971b; White, 1973). One axon may synapse with the dendrites of several cells associated with that glomerulus (Pinching and Powell, 1971b). In the rat, axonal terminal endings synapse mostly onto fine dendritic branches of mitral, tufted and periglomerular cells, but may also synapse on more proximal regions of the dendrites (Pinching and Powell, 1971b). In certain species of mice, receptor cell axons synapse with dendrites of mitral and tufted cells, but do not synapse with periglomerular cell processes (White, 1973). The terminal axonal endings of olfactory receptor neurons do not extend beyond the glomerular border.

The dendrites that enter the glomerulus are primary dendrites of mitral and tufted cells and dendritic arborizations of periglomerular cells. In most species, the primary dendrite of a second order neuron is a single structure that enters the glomerulus either at a right or oblique angle, depending on the position of the second order neuron cell body to the glomerulus. The dendrite forms an arborization or tuft either before or within the glomerulus. In the hedgehog, the primary dendrites of tufted cells usually enter one glomerulus but occasionally divide into two tufts that enter different glomeruli (Lopez-Mascaraque et al., 1990).

In the rat, Pinching and Powell (1971a) found that the

primary dendrites of the second order neurons pass through the periglomerular region and branch before entering a single glomerulus. Within this region, large segments of dendrites are covered by several layers of glial lamellae, the function of which is unknown but which may be to insulate the dendrite from surrounding cell activity or to facilitate conduction, similar to a myelin sheath. In the hedgehog, Lopez-Mascaraque et al. (1990) state that portions of the primary dendrites of mitral cells fail to absorb stain due to myelination.

Synaptic activity within the glomerulus occurs between the axons of incoming olfactory receptor cells and the dendrites of mitral and tufted cells. Some inhibitory dendrodendritic synapses between periglomerular and second order cell dendrites probably occur within the glomerulus. (Pinching and Powell, 1971a).

## ***2. The Periglomerular Region***

The glomeruli are surrounded by numerous cells, many of which are neurons but with a variety of glial cells comprising at least 33% of the cell population (Willey, 1973). This periphery, which usually surrounds each glomerulus with a cellular abundance, is termed the periglomerular region due to the most numerous type of neuron located here, the periglomerular cell. Also located

within this region are short axon cells (Pinching and Powell, 1971a) which are also interneurons, and external tufted cells (Pinching and Powell, 1971a; Macrides and Schneider, 1982), the most superficially located second order neuron found within the olfactory bulb.

#### *a. Periglomerular cells*

Periglomerular cells are referred to by some (Hinds, 1968a, b,; Price and Powell, 1970a) as external or superficial granule cells because they are small interneurons whose somata closely resemble the granule cells of deeper layers (mitral, internal plexiform and granule cell layers). The most numerous neuron found within the periglomerular region is the periglomerular cell. By conventional staining methods (Nissl), these small bodied neurons are difficult to distinguish from glial cells. The two categories of cells can be distinguished only by the presence of synapses (electron microscopy) in the case of the neurons (Pinching and Powell, 1971a), or by immunocytochemical techniques. Occasionally, the somata of a periglomerular cell is wrapped within a glial lamellae which does not extend beyond the body of the periglomerular cell.

Golgi impregnated periglomerular cells have demonstrated dendritic arborizations contributing to one or

more glomeruli. The arborizations may show many secondary and tertiary branches with many spine-like appendages. They also have short dendrites which extend into the periglomerular region. The somata and large dendrites of periglomerular cells have many spines which commonly receive synapses from axon terminals of neurons intrinsic to this layer. Periglomerular spines also occasionally receive synapses from mitral cell dendrites or the somata or dendrites of tufted cells (Pinching and Powell, 1971a). White (1973) found that periglomerular dendrites also synapse with each other, and that this always occurs within close proximity to a pre- or post-mitral cell synapse.

Although they are often difficult to stain, periglomerular cells have an axon which may branch, the terminals of which encompass one or more glomeruli in the immediate region. Periglomerular axons do not enter into a glomerulus but are presumed to synapse with dendrites of tufted cells in the region immediately adjacent to the glomerulus (Pinching and Powell, 1971a). Reciprocal synapses by tufted cells onto periglomerular cells occur within this same region.

Periglomerular cells are superficially located granule cells (Pinching and Powell, 1971a): they are interneurons, and as such, are thought to be inhibitory to the second order neurons with which they synapse. They receive input directly or secondarily from olfactory receptor cell axons



and although their dendrites are associated with one or two glomeruli indicating a rather localized effect, their axons reach and influence more distant regions of the glomerular layer.

*b. External tufted cells*

Tufted cells are second order neurons that Cajal (1911) originally subdivided into three major types according to their location: external tufted cells in the glomerular/superficial external plexiform region, middle tufted cells within the middle region of the external plexiform layer, and internal tufted cells deep within the external plexiform layer and immediately adjacent to the mitral cell layer. There appears to be some correlation in the size of the tufted cell soma and its location: the further a tufted cell is from the mitral cell layer, the smaller the size of its soma.

External tufted cells are located within the periglomerular region and within the superficial region of the external plexiform layer immediately adjacent to the periglomerular region. Based on specific dendritic morphology, Macrides and Schneider (1982) have subdivided external tufted cells into three types, one type with somata located predominantly within the glomerular layer: they are characterized by their lack of secondary dendrites.

Most tufted cells are fusiform or oval in shape, are considerably larger than periglomerular cells (Macrides and Schneider, 1982) but are usually smaller than the tufted cells located in deeper regions of the olfactory bulb. External tufted cells rarely or never have secondary dendrites but as demonstrated in the rat and hedgehog (Pinching and Powell, 1971a; Lopez-Mascaraque et al., 1990), give rise to one or more primary dendrites that exit from one or both sides of the cell body and may arborize within the same or neighboring glomeruli (Macrides and Schneider, 1982). Primary dendrites divide into several branches that form tufts and enter a glomerulus from any angle, depending on the position of the cell body. Branches of the primary dendrites arise at an acute angle and tend to occupy a triangular portion of the glomerulus (Pinching and Powell, 1971a); the main branches of the primary dendrites often enter different but neighboring glomeruli (Lopez-Mascaraque et al., 1990). The dendrites are usually smooth and typically do not show spines. Because of their location within the periglomerular region, tufted cell somata and proximal dendritic segments rarely receive synapses from any cell processes other than from periglomerular cells. (Pinching and Powell, 1971a).

Thin glial lamellae may cover tufted cell dendrites in the periglomerular or superficial external plexiform layer, except in regions of synapse (Pinching and Powell, 1971a).

The glial covering does not extend into the glomeruli. Because external tufted cells located within the periglomerular region often lack secondary dendrites, their synaptic interaction is restricted to periglomerular cell dendrites within the glomerular layer.

Axons of periglomerular cells synapse with tufted cell dendrites within the periglomerular region and at the border directly adjacent to the glomerulus. The subdivision of external tufted cells most commonly found within the periglomerular region (those cells lacking secondary dendrites) have an axon emerging most commonly from the soma but occasionally from the proximal portion of a primary dendrite. The axons follow an oblique course within the periglomerular region and can be traced to the external plexiform layer but not beyond.

Synapses between the axons of primary receptor neurons and primary dendrites of all subdivisions of tufted cells within the glomeruli have already been discussed.

Tufted cells will be discussed in greater detail within the section, **External Plexiform Layer**.

#### *c. Short Axon Cells*

Short axon cells are described as fusiform or oval in the hamster (Schneider and Macrides, 1977) and spherical or slightly ovoid in the rat (Pinching and Powell, 1971a). The

cell body is larger and has a more irregular outline than that of the periglomerular cells and is approximately the same size as external tufted cells. The nuclear membrane of these cells is frequently indented, unlike the smooth nuclear membrane in tufted and mitral cells (Willey, 1973). The somata and dendrites may or may not have spines. The superficial short axon cells are located in the glomerular layer in the rat (Pinching and Powell, 1971a), and also within the superficial half of the external plexiform layer in the hamster (Schneider and Macrides, 1977). Short axon cells give rise to several dendrites which vary in number (between two to seven in the hamster) and in complexity of branching patterns. Dendritic branchings are generally located within the periglomerular region in the rat but are also found within the superficial region of the external plexiform layer in the hamster. Arborizations within the glomerular layer are located throughout the region of periglomerular cells and sometimes appear to "grasp" the adjacent glomerulus (Pinching and Powell, 1971a; Schneider and Macrides, 1977).

The axons of the short axon cells and their minimal number of collateral branches are found throughout the superficial external plexiform layer and the glomerular layer. Axons seldom extend beyond the limits of two glomeruli and restrict synaptic contact to the somata, dendrites and axon initial segments of periglomerular cells.

They do not establish direct contact with mitral or tufted cells within the superficial layers (Pinching and Powell, 1971c). Occasionally an axon extends past the mitral cell layer with ramifications within the granule cell layer (Schneider and Macrides, 1977), but its target is not identified.

Unlike external tufted cells, short axon cells lack reciprocal synapses on their cell bodies and dendrites, a character used by Pinching and Powell in their identification. Short axon cells receive synapses from all types of axon terminals with the exception of olfactory receptor neuron axons. However, synapses with tufted cell collaterals predominate (Pinching and Powell, 1971c).

### **C. THE EXTERNAL PLEXIFORM LAYER**

At this point, it should be stated that there are several types of interneurons within the olfactory bulb. Other than granule cells, the additional types are predominantly located within the glomerular, external plexiform, and granule cell layers. Schneider and Macrides (1978) state that there are eight clearly identifiable classes of interneurons within the main olfactory bulb: superficial short axon cells, two types of deep short axon cells (Golgi and Blanes cells), horizontal cells, vertical cells of Cajal, Van Gehuchten cells, granule cells, and what

some consider to be a superficially located granule cell, the periglomerular cells. In their study of the superficial layers of the hedgehog olfactory bulb, Lopez-Mascaraque et al. (1990) omit some of the above cell types and describe what they refer to as satellite cells. Price and Powell (1970d) state that cell types are identified by size, position and dendritic arrangement. Schneider and Macrides (1978) state that cell type is characterized by location of the cell body, and by dendritic and axonal properties. Different researches use different techniques to analyze cells: Nissl stain; golgi stain; electron-microscopy studies of cell somas, their processes and synaptic endings; immunocytochemical techniques to study neurotransmitters and the extent of cell processes; HRP tracing of cell processes and synaptic endings. The final word on how many cell types (interneurons) actually exist within the olfactory bulb may require a comprehensive study using most of the above techniques: some cells currently accepted as discrete types may turn out to be variations of a single type. Finer subdivisions of certain cell types may be deemed necessary. I have chosen to briefly discuss most of the several categories of interneurons currently acknowledged in the literature. This is to highlight the fact that several cell types are normally found within the olfactory bulb of mammals, and that to differentiate these types is far beyond the limits of Nissl staining techniques. For more

information on each of the cell types mentioned, please refer to the publications cited.

There are two olfactory bulb layers which exhibit a paucity of cells relative to the remainder of the olfactory bulb. These are the external and internal plexiform layers (Figure 6, Page 87). The external plexiform layer is a constant within the olfactory bulb and contains within it a majority of the rather heterogenous group of second order neurons known as tufted cells. The internal plexiform layer is less constant, but when present it is located between the mitral cell and granule cell layers

The external plexiform layer is made up of dense neuropil consisting of the dendrites and axons of neurons whose somata are located both within and outside of this layer. Neurons intrinsic to the external plexiform layer and their cell processes will be discussed first.

#### *a. Tufted Cells: A Heterogenous Group*

Mitral and tufted cells are the second order neurons of the olfactory bulb. The primary dendrites of these cells synapse with axons of the olfactory receptor neurons within the glomeruli. The impulse is transmitted by way of mitral and tufted cell axons to more centrally located regions of the brain. Although subpopulations of tufted cells were defined by Cajal as early as 1911, many recent studies have

redefined and expanded upon this subdivision based on morphological and/or functional differences (Macrides and Schneider, 1982; Orona et al., 1984; Mouradian and Scott, 1988). This study will continue to use the traditional subdivisions of tufted cells into external, middle and internal tufted cells, but will refer to additional subdivisions when this contributes to an understanding of the function of the system.

#### *b. External Tufted Cells*

Many external tufted cells are located within the periglomerular region and have been previously been described in this chapter under the heading of "Periglomerular Region".

Using Macrides and Schneider's (1982) subcategory for external tufted cells in the adult hamster, one type of external tufted cell whose soma is located within the superficial region of the external plexiform layer has one primary dendrite associated with one glomerulus while the second category of external tufted cell has a primary dendrite that terminates within more than one glomerulus at least 30% of the time.

External tufted cells whose somata lie along the glomerular/external plexiform layer border or within deeper layers of the external plexiform layer all have secondary



dendrites. Although Macrides and Schneider further subdivide these border tufted cells according to the degree of secondary dendritic branching, these secondary dendrites always branch, extend, and remain within the most superficial 25% or 33% of the external plexiform layer (Orona et al., 1984; Macrides and Schneider, 1982, respectively). The distribution of cell processes within particular layers or sublaminae of the olfactory bulb appears to have functional significance that will be discussed at the end of this chapter.

The axons of the border external tufted cells characterized by densely branching secondary dendrites (Macrides and Schneider, 1982) arise from the proximal portion of a secondary dendrite and give off an elaborate system of collateral fibers. The axonal branching occurs within the region of dendritic branching in the superficial external plexiform layer. Axon collaterals also occupy the periglomerular region.

In their study of the rat, Orona et al., (1984) lump all external tufted cells into one category and describe their axons as having extensive collaterals within the internal plexiform and granule cell layers.

The work of Schoenfeld et al. (1985) on the hamster has demonstrated that the majority of external and middle tufted cells located within the more superficial external plexiform layer and along the border with the glomerular layer have

intrabulbar projections to the opposite side of the olfactory bulb. Axons of these neurons extend through the external plexiform and mitral cell layers, project dorsally and ventrally within a confined region of the internal plexiform layer, and terminate within this layer on the opposite side of the olfactory bulb. Retrograde labeling of neurons (predominantly tufted cells) on the side of the bulb opposite the injection site indicates that a reciprocity of axonal projections exists within this intrabulbar associational system. This association exists between specific populations of external and middle tufted cells within medial and lateral sides of the olfactory bulb. Schoenfeld et al., (1985) functionally categorize all external tufted cells into three functional groups: 1) intrabulbar local circuit neurons located superficially and distributing their axons locally within the periglomerular region; 2) intrabulbar association neurons projecting to the opposite side of the olfactory bulb; 3) true output neurons located at the border between the glomerular and external plexiform layer and projecting to central brain structures. A subset of these true output neurons, (primarily external tufted cells) project axons to third order neurons within the anterior olfactory nucleus. The third order neurons project in turn to the contralateral olfactory bulb, comprising a fourth functional group, that of an interbulbar associational system.

*c. Middle Tufted Cells*

Middle tufted cells are located within the superficial and middle two thirds of the external plexiform layer in the hedgehog, hamster and rabbit, but appeared most abundant in the region close to the glomerular layer in the hedgehog (Lopez-Mascaraque et al., 1990; Macrides and Schneider, 1982; Mori et al., 1983).

Lopez-Mascaraque et al. (1990) described variation within middle tufted cells of the hedgehog based on characteristics of the primary dendrites. The more superficially placed cells often entered a single glomerulus by way of a short, stubby dendrite while primary dendrites of other cells branched into two terminal tufts which entered different glomeruli. Still other cells had a long obliquely or horizontally projecting primary dendrite that approached and entered a distant glomerulus. In the hamster (Macrides and Schneider, 1982) and the rabbit (Mori et al., 1983) the middle tufted cells emitted a single primary dendrite which projected more or less radially and arborized within a single glomerulus. Macrides and Schneider (1982) and Mori et al. (1983) observed that the angle of entry into the glomerulus was dependant upon the position of that cell within the external plexiform layer: the closer the somata to the granular layer, the more oblique the course of the trajectory into the glomerulus. The obliquity of dendritic

course implies that the length of the primary dendrite cannot be directly correlated with the position of the somata within the external plexiform layer (Macrides and Schneider, 1982).

In the rabbit, Mori et al. (1983) found that on the average, two to four secondary dendrites arose from the proximal portion of the middle tufted cell's primary dendrite. Most of these dendrites projected tangentially within the superficial one-third or one-half of the external plexiform layer. Secondary dendrites of middle tufted cells in the hedgehog tended to be restricted to the superficial one-third of the external plexiform layer although a curved trajectory toward the deep part of the external plexiform layer was not uncommon (Lopez-Mascaraque et al., 1990). In their study of the rat, Orona, et al. (1984) describe the secondary dendrites as being confined to the intermediate regions of the external plexiform layer but overlapping to some degree the dendritic fields of both the external tufted cells and mitral cells (Type II, discussed below).

The axons of middle and internal tufted cells in the hedgehog (Lopez-Mascaraque et al., 1990), did not fully stain and could only be traced to the granule cell layer but not beyond. In young animals, the axons were described as thick fibers that gave off several axon collaterals within the external plexiform layer and then projected to the granule cell layer. In the rat, Orona et al. (1984) found

middle tufted cell axons to project similarly to those of external tufted cell axons, that is they branched extensively within the internal plexiform and granule cell layers. Kishi et al. (1984) described axons of middle tufted cells in the rabbit as most frequently arising from the proximal portion of a secondary dendrite and passing through the external plexiform layer without giving off collateral branches. However, collateral branches were seen within the granule cell layer, and they spread out along a projectory parallel to the mitral cell layer. Occasionally these collaterals curved superficially and entered concentrations of granule cells within the mitral cell layer. After collaterals were given off in the more superficial regions of the granule cell layer, the main projection of the axon proceeded through to the deeper regions and turned caudally, eventually joining the lateral olfactory tract (Kishi et al., 1984).

#### *d. Internal Tufted Cells*

Mori et al. (1983) refer to internal tufted cells as displaced mitral cells because the somata are located immediately superficial to the mitral cell layer. The cell body size (and shape) of internal tufted cells is either the same or close to that of the mitral cells. Because of cell body size, their location, and the morphology of cell

processes (Golgi stain), it is often unclear whether some cells should be classified as internal tufted or mitral cells (Lopez-Mascaraque, et al., 1990). However, Mori et al. state that in the rabbit, retrograde axonal transport of horseradish peroxidase (HRP) can clearly distinguish internal tufted cells from mitral cells by their dendritic patterns.

Because of the similarity between internal tufted cells and mitral cells, Macrides and Schneider (1982) described these cells together, noting that internal tufted cell somata are only slightly smaller than those of mitral cells. In both the rabbit and the hamster, each internal tufted cell gave rise to one primary dendrite which arborized within one glomerulus (Mori et al., 1983; Macrides and Schneider, 1982).

In the hamster, each internal tufted cell gave rise to an average of three to four secondary dendrites which were very long, sparsely branched, and extended parallel to the mitral cell layer within the deep region of the external plexiform layer (Macrides and Schneider, 1982). In the rabbit, an average of three secondary dendrites were associated with each internal tufted cell and usually one of them emerged from the primary dendrite (Mori et al., 1983). Secondary dendrites in this species were more inclined to follow a course within the middle or slightly superficial region of the external plexiform layer. The course of the

secondary dendrites in the hedgehog followed a similar course to those in the rabbit in that they followed a radially inclined course and were not directed tangential to the mitral cell layer until they reached the superficial one-half of the external plexiform layer (Lopez-Mascaraque *et al.*, 1990).

Axons of internal tufted cells in the hamster were unstained while in the hedgehog, they could not be traced beyond the granule cell layer (Macrides and Schneider, 1982; Lopez-Mascaraque *et al.*, 1990). In the rabbit (Kishi *et al.*, 1984), axons of both mitral and internal tufted cells (displaced mitral cells) projected into the deeper regions of the granule cell layer before giving off collateral branches. At that time, the axons gave off two or three collateral projections before proceeding caudally to contribute to the lateral olfactory tract. The axon collaterals projected into more superficial regions of the granule cell layer, then followed a long tangential course parallel to the mitral cell layer. Generally, the axon collaterals of the internal tufted cells traversed through more superficial regions of the granule cell layer than did the axon collaterals of the mitral cells (Kishi *et al.*, 1984).

*e. Van Gehuchten Cells*

These interneurons have been described as fusiform in the hamster (Schneider and Macrides, 1977) or as trigonal or polygonal in the hedgehog (Lopez-Mascaraque et al., 1990). The somata are spineless and are usually located within the internal regions of the external plexiform layer but can also be found within the mitral cell layer (Lopez-Mascaraque et al., 1990; Schneider and Macrides, 1977). The somata gave rise from one to five cell processes (generally more in the hedgehog), all of which appeared to be dendrites. In the hedgehog, the processes usually extended from the polar regions of the cell body as two distinct groups, one typically having one or more processes that were thicker than the remainder of the processes. The thinner processes were shorter but arborized extensively; the thicker processes were longer and also arborized extensively. Schneider and Macrides could not perceive any particular orientation to these processes.

In the hedgehog, Lopez-Mascaraque et al. (1990) describe the processes as smooth and dendritic-like, and giving rise to secondary fibers. The secondary fibers resemble axons in that they are thin, beaded fibers which themselves give rise to short collateral branches that arborize within the mitral cell layer and penetrate the granule cell layer. In the hamster, Schneider and Macrides



(1977) failed to find either an axon or clearly defined initial segment projecting from any Van Gehuchten cell although some of the thinner processes had smooth outlines with periodic varicosities, similar to axons.

#### *f. Satellite Cells*

Lopez-Mascaraque et al. (1990) named and described these cells in the hedgehog. They are round, small-bodied interneurons located within the middle and superficial portions of the external plexiform layer. Two or more rather elaborately branched, extremely varicose processes arise from the somata and give off terminal clusters close to that somata. In some cells, a thin but varicose axon was present while in other cells, no axon was identifiable. These cells were always found in close association to the secondary dendrites of mitral cells (thus the designation "satellite"). In many cases, golgi staining demonstrated that the terminal clusters of the satellite cells enveloped portions of the mitral cell dendrites, suggesting a functional relationship.

#### *g. Horizontal Cells*

These cells in the hedgehog may equate to the short axon cells described in the hamster (below), and in the rat

(Pinching and Powell, 1971a). The fusiform somata are located in the superficial region of the external plexiform layer and are slightly larger than the satellite cells. Relatively smooth dendrites with an occasional hair-like appendage exit the polar ends of the somata. When axons were definable, they branched between different glomeruli or ran horizontally within the external plexiform layer. The horizontal cells are interneurons.

#### *g. Superficial Short Axon Cells*

These cells are found within the periglomerular region in the rat (Pinching and Powell, 1971a) and in the periglomerular layer and superficial half of the external plexiform layer in the adult hamster (Schneider and Macrides, 1978). Superficial short axon cells have been described previously and in greater detail under the heading of "Periglomerular Region". In the hamster, the dendrites of these interneurons ramify in the glomerular and superficial region of the external plexiform layer, but never extend to the deeper regions of this layer. Axonal processes of superficial short axon cells were identified within the glomerular and superficial external plexiform layers. These processes branch infrequently. Occasionally, a lengthy axon passes deeply through the width of the olfactory bulb, into the granule cell layer. These axons

ramify within the internal plexiform and granule cell layers. Most short axon cells whose somata are located within the periglomerular region have axons that appear to ramify within the glomerular layer.

#### **D. THE MITRAL CELL LAYER**

The mitral cell layer is located deep to the external plexiform layer and is situated between the external and internal plexiform layers when the latter is present. It is a predominantly cellular layer which varies in its thickness according to species. The mitral cell layer contains the somata of the largest and farthest reaching second order neuron (output cell) of the olfactory bulb, the mitral cell (Figure 8, page 138). In addition, the mitral cell layer contains the somata of granule cells which migrate into the developing mitral cell layer during embryonic development. These granule cells are interneurons, and share a like morphology with the granule cells of the layers deep to them.

##### *a. Mitral Cells*

Mitral cells have the largest somata within the olfactory bulb and in many species have a triangular shape or are shaped like a bishop's miter. In the rat, Price and

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Figure 8. Olfactory bulb layers in the rat (X 25).

arrow = mitral cells within the mitral cell layer

ip = internal plexiform layer

gc = granule cell layer

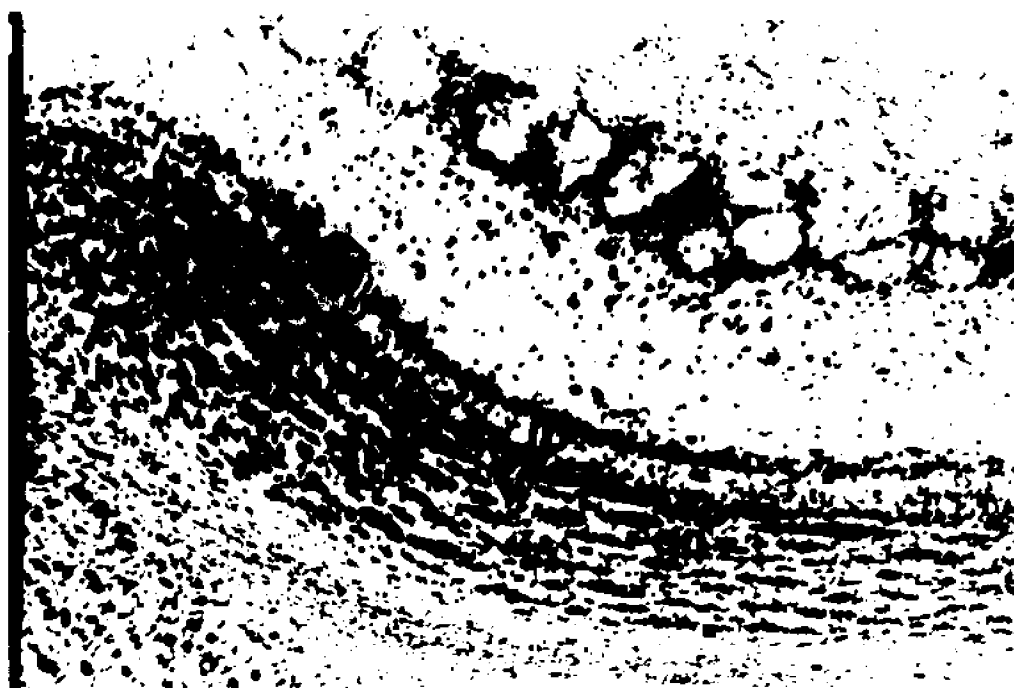


Figure 8

Powell (1970d) found mitral cells to have a diameter of from 20 to 30 microns, although the values varied. The somata are located within the mitral cell layer which is usually narrow but varies in width in different mammalian species. In both young and adult hedgehogs (Lopez-Mascaraque et al., 1990), mitral cells are located within a broad band with some mitral cells displaced superficially into the external plexiform layer, and some displaced deeply into the granule cell layer. Switzer and Johnson (1977) found that in monotremes (*Ornithorhynchus anatinus* and *Tachyglossus aculeatus*), the mitral cell layer does not exist as such but is more broadly dispersed throughout the external plexiform layer. What does exist in what would normally be termed the mitral cell layer is a vague line formed by some granule cells. Deep to this line of granule cells, the internal plexiform layer can be distinguished by its greater degree of myelination compared with the external plexiform layer superficial to it. Among different species of reptiles and birds, a range of variation exists for the width of the mitral cell layer, from a narrow band of cells typical of what is seen in all placental mammals, to the diffuse distribution of mitral cells characteristic of the monotremes (Switzer and Johnson, 1977). Mitral cells resemble other large neurons in that they have a large, pale nucleus surrounded by a relatively generous amount of cytoplasm. The nucleolus is a prominent structure within

the nucleus (Price and Powell, 1970,d). The primary dendrite of the mitral cell arises from the superficial surface of the somata, crosses the external plexiform layer either vertically or slightly obliquely, and enters a glomerulus where it arborizes. In the rat (Orona et al., 1984, Pinching and Powell, 1971b), the primary dendrite appears to be associated with a single glomerulus which it enters across its deep border. In the adult hamster (Macrides and Schneider, 1982), the single primary dendrite arises from either the somata or from the proximal portion of a secondary dendrite. It extends either radially or obliquely into a single glomerulus where its arborization is one of the most extensive known within the olfactory bulb. In the rabbit, 20% or 30% of the mitral cells were identified as having two or three primary dendrites, but the multiple dendrites were also associated with a single glomerulus (Mori et al., 1983). In the newborn and juvenile hedgehog, Lopez-Mascaraque et al. (1990) found examples of mitral cells with two to four primary dendrites which entered and arborized within adjacent glomeruli. Older hedgehogs, however, appeared to have only a single primary dendrite, associated with a single glomerulus. These primary dendrites in the adult hedgehog frequently gave rise to horizontally running secondary dendrites.

In the adult hamster (Macrides and Schneider, 1982), the somata of mitral cells give rise to an average of 4.5

secondary dendrites per neuron. The secondary dendrites are sparsely branched, extremely lengthy, and remain within and following the curvature of the deep portion of the external plexiform layer. Within this layer, these dendrites make serial and reciprocal synapses (Price and Powell, 1970b) with distal dendrites of granule cell processes. This is similar to that observed in the hedgehog (Lopez-Mascaraque et al., 1990) and the rabbit (Mori et al., 1983), where two to five secondary dendrites projected extensively within the deeper region of the external plexiform layer, also remaining parallel to the curvature of this and the mitral cell layer. Most secondary dendrites in the rabbit branch on the average of two to four times.

Orona et al. (1984) categorize mitral cells into Type I and Type II, based on whether secondary dendrites ramify within the deep or middle regions of the internal plexiform layer, respectively. The significance of this may be functional, in that each of these two types may synapse with the differing granule cell populations. However, Orona et al. also consider that the two mitral cell types may represent two ends of a continuum.

In the hedgehog, the axons of mitral cells were stained in only newborn or juvenile hedgehogs. These axons entered the granule cell layer, emitted a few collateral fibers, and could not be traced further (Lopez-Mascaraque, 1990).

Macrides and Schneider (1982) found that axons in the adult



hamster did not impregnate well with Golgi stain, but could be identified as extending from either the soma or the proximal part of a secondary dendrite. Although they projected towards the internal plexiform layer, they could not be followed beyond this.

Kishi *et al.* (1984) identified mitral cell axons in the rat as extending from the soma and projecting into the deeper regions of the granule cell layer where they changed course towards a caudal direction. At this point, from two to seven collateral branches diverged from the axon. These axon collaterals projected into the middle or superficial region of the granule cell layer, following a course parallel to the mitral cell layer. Nearly all of the axon collaterals remained within the granule cell layer for the entirety of its lengthy course. Occasionally an axon collateral followed a course from the deep granule cell layer to the deep edge of the mitral cell layer, but no axon collateral was located superficial to this border. As the main axon projected caudally towards the lateral olfactory tract, one or two additional collateral branches were occasionally emitted in the most caudal region of the olfactory bulb.

In keeping with their categorization of two types of mitral cells, Orona *et al.* (1984) identified different degrees of axonal branching corresponding to each type. Type II mitral cells had axons which branched extensively

within the internal plexiform and superficial granule cell layers. Axons of Type I mitral cells demonstrated the least amount of branching exhibited by any intrabulbar cell. There were no axon collaterals within the internal plexiform or superficial granule cell layers of this type. Occasionally axon collaterals occurred very caudally within the granule cell layer and were not very extensive.

#### *b. Granule Cells*

The granule cells of the mitral cell and internal plexiform layers will be discussed with the population of cells within the granule cell layer. The thickness of the mitral cell layer appears to be more dependant upon the density of granule cells than on that of mitral cells.

#### **E. THE INTERNAL PLEXIFORM LAYER**

The internal plexiform layer is variably present: in some mammals, the layer is very obvious throughout the bulb; in other species, it is obvious in some regions of the bulb while in other regions, the merging of the granule cell layer with the mitral cell layer largely obliterates the internal plexiform layer. Since the internal plexiform layer primarily contains the cell processes of local neurons, its presence or absence is related to the density

of cell bodies within the two layers contiguous with it (Figure 6, page 87).

The internal plexiform layer is located deep to the mitral cell layer and superficial to the granule cell layer. Some researchers consider it to be the most superficial region of the granule cell layer (Price and Powell, 1970a). The internal plexiform layer is a region of cell processes with a relative paucity of neuron somata (all interneurons). In species where an internal plexiform layer is clearly present, the somata of granule cells are scattered throughout the layer in small clumps or as solitary cells. Also present within the internal plexiform layer is one subtype of short axon cells (Price and Powell, 1970d). Granule cells and short axon cells located within the deeper half of the olfactory bulb will be discussed with the granule cell layer, the region in which they predominate.

In some species, and to varying degrees throughout various regions of many olfactory bulb sections, it is often difficult to determine where the internal plexiform layer ends and the granule cell layer begins. In many olfactory bulbs, the internal plexiform layer is obvious in certain regions of the bulb only to be crowded out by a proliferation of granule cells in other regions of the bulb.

The internal plexiform layer is predominantly a layer of fiber tracts. Axon collaterals of mitral and tufted cells project for long distances within the internal

plexiform layer and superficial region of the granule cell layers (Price and Powell, 1970d; Orona et al., 1984; Kishi et al., 1984). Intrabulbar axons of external tufted cells that project to neurons on the opposite side of the olfactory bulb reach their target area by following a course entirely within the internal plexiform layer (Schoenfeld et al., 1985).

#### **F. GRANULE CELL LAYER**

The granule cell layer is the deepest layer to contain neurons within the main olfactory bulb. It contains the somata of two types of interneurons, the granule cells and short axon cells. Axons of second order neurons and their axon collaterals are located within the granule cell layer. The main axon fibers of mitral and tufted cells turn caudally within the granule cell layer to contribute to the formation of the lateral olfactory tract. The deep border of this tract also contains some centrifugal axons that project to the main olfactory bulb from more centrally located neurons, although it is not the principal pathway for these fibers as previously thought (Macrides and Davis, 1983). Incoming axons from more centrally located neurons may traverse the granule cell layer. The axon collaterals of mitral cells run horizontally within the superficial granule cell and/or internal plexiform layers, following a

course parallel to the mitral cell layer (Mori et. al., 1983).

Granule cells are found in abundance within the granule cell layer where they aggregate into "islands": in cross-section they appear to be or loosely constructed rows of cells (Figure 8, page 138). The volume of granule cells drops considerably in the internal plexiform layer where fiber tracts rather than neurons predominate. Within the mitral cell layer, granule cells contribute to the thickness of the mitral cell layer and are interspersed with mitral cell bodies. Also located within these three main olfactory bulb layers are the somata of a second type of interneuron, the short axon cells. Granule cells and short axon cells, irrespective of the location of their somata within the granule cell, internal plexiform, or mitral cell layers, are discussed below.

#### *a. Granule Cells*

Granule cells are inhibitory interneurons. Electrophysiological studies of the rabbit olfactory bulb have shown that through dendrodendritic synapses, mitral cells activate granule cells and activated granule cells in turn elicit inhibitory potentials in mitral cells (Rall et al., 1966; Nicoll, 1969). Granule cells are the most common cell type within the granule cell layer. They are small,

round or fusiform-shaped interneurons that have a relatively large nucleus, small amount of cytoplasm, and lack a true axon (Price and Powell, 1970a). Processes that arise from the superficial region of the soma are difficult to classify as either axonal or dendritic and are termed peripheral processes by Price and Powell (1970a), Mori and Kishi (1982); Orona et al. (1983) refer to them as distal dendrites. Peripheral processes are directed towards the mitral cell layer and arise as thickened trunks from the soma that taper slightly as they project superficially. At the mitral cell layer, each peripheral process branches repeatedly, ramifying among mitral cell dendrites within the external plexiform layer (Price and Powell, 1970a; Orona et al., 1983).

From the deep side of the granule cell, one to four dendrites arise from the soma and extend into the deep region of the granule cell layer, usually branching at least once. These dendrites are referred to as deep dendrites by Price and Powell (1970a) and Mori and Kishi (1982); Orona et al. (1983) refer to them as proximal dendrites. Compared to the peripheral processes, the dendritic (deep) processes of granule cells arise sharply from the soma, are finer, and have a beaded appearance due to localized swellings along their shaft (Price and Powell, 1970a). The dendrites project into the deep regions of the granule cell layer and rarely can be followed for any distance. Occasionally a

deep dendrite turns superficially and projects parallel to the mitral cell layer.

In the rat (Price and Powell, 1970a), appendages referred to as spines are located on all parts of the granule cell. While located most frequently along the length of the dendrites, they are also found on the soma and on proximal portions of the peripheral processes. The frequency of spines decreases as the peripheral process projects superficially towards and into the mitral cell layer. In the external plexiform layer, most appendages on peripheral processes no longer appear spine-like but are described morphologically as long, large, bulbous appendages referred to as gemmules. Unlike the shafts of peripheral processes in the deeper regions, the regions of peripheral processes located within the external plexiform layer are densely covered with gemmules (spine-like appendages) (Price and Powell, 1970a; Mori and Kishi, 1982).

Away from the granule cell somata, the peripheral processes increasingly resemble dendrites. In the external plexiform layer, the peripheral processes are distinguished from the dendrites of mitral and tufted cells by the presence of their appendages (particularly gemmules), small size, and the irregularity of their outline (Price and Powell, 1970a).

Spines are evaginations of the neuronal surface; most frequently they form a slight elevation along one surface of

a process, although they may have a stem. All spines are post-synaptic structures in that they receive at least one synapse which is usually characterized as having an asymmetric type of membrane complex. (Price and Powell, 1970a)

Gemmules are located on the distal portions of peripheral processes, most commonly within the external plexiform layer. Gemmules are postsynaptic to axon terminals but form unusual "reciprocal" dendrodendritic synapses with mitral and tufted cells, as demonstrated by electron microscopy (Price and Powell, 1970a; Orona et al., 1983). Presence of the presynaptic membrane indicate that gemmules are the efferent processes of the granule cells, that is, they function in part as axons even though they are morphologically aligned more closely with dendrites (Price and Powell, 1970a; Mori and Kishi, 1982).

In their study of the rat, Orona et al. (1983) have found that different populations of granule cells innervate superficial or deep regions of the external plexiform layer, apparently interacting with different populations of tufted or mitral cells. Small extracellular injections of horseradish peroxidase into the superficial region of the external plexiform layer labeled granule cells with somata located predominately within the superficial granule cell layer. When HRP was injected into the superficial region of the granule cell layer, dendrites of the labeled granule



cells extended across the entire external plexiform layer, frequently reaching the edge of the glomerular layer. Golgi studies indicated that the greatest number of branches and greatest gemmule density occurred within the superficial external plexiform layer. These granule cells corresponded to Type III granule cells defined by Mori et al. (1983) in the rabbit. In the hedgehog, Lopez-Mascaraque et al. (1986) found that granule cell peripheral processes that branched within the superficial external plexiform layer occasionally penetrated a glomerulus.

Conversely, injections of HRP into the deep external plexiform layer labeled granule cell somata in both superficial and deep regions of the granule cell layer. When HRP was injected into the deep granule cell layer, the dendritic processes of labeled deep granule cells extended into the deep external plexiform layer, but very seldom to sublaminae superficial to this. As with the superficially placed granule cells, branching of the peripheral processes occurred away from the cell body, and greatest gemmule density occurred distal to the superficial granule cell/mitral cell layer, that is, distal to the primary branching (Orona et al., 1983). These cells correspond to Type II granule cells of Mori et al., (1983). In summary, in the rat and rabbit, the dendrites of superficial granule cells extend to the superficial external plexiform layer while dendrites of deep granule cells are confined to the

deep regions of the external plexiform layer (Orona et al., 1983). As the majority of synapses occur on the gemmules of peripheral processes, the majority of interactions between the peripheral processes of superficial granule cells and the cell processes they interact with occur within the superficial external plexiform layer. For the deep granule cells, the majority of interactions between their peripheral processes and the cell processes they interact with occur within the deep external plexiform layer. In the hedgehog, Lopez Mascarague et al. (1986) failed to find a clear correlation between position of granule cell soma and the distribution of peripheral processes. They favored the interpretation that a cell body at any level of the granule cell layer could have a distribution of peripheral processes into any sublayer of the external plexiform layer.

Mori et al. (1983) classified granule cells into three subgroups according to distribution of the peripheral processes. Types II and III have already been correlated with the sub-classification of Orona et al. (1983). An additional category, Type I granule cell, had peripheral processes that branched at the border between the mitral and external plexiform layers, with one branch reaching the most superficial region of the external plexiform layer. Gemmules in this cell were distributed in all sublayers but predominated within the deeper portion of the external plexiform layer.

Kaplan et al. (1985) investigated the granule cell layer increase in volume during adult life. They found that in the rat, the number of granule cells per volume of granule cell layer increases linearly with age; there is approximately a twofold increase in the number of granule cell during adult life. The increase in cell number occurred during several different periods of life. Granule cell death also occurred during adulthood; new granule cells were produced, often in great numbers, but many of these cells subsequently underwent cell death. However, not all granule cells formed in adulthood died and many survived for over eighteen months. The authors suggest that an increase in olfactory receptor neuron number may correlate with an increase in size of mitral cells and their dendrites, creating an increase in postsynaptic space on the mitral cells. This in turn could be correlated with an increase in granule cell number to utilize these potential synaptic connections. Kaplan et al. state that more research is required to investigate this hypothesis, but the interesting point is that the olfactory system sustains two populations of regenerating neurons, one within the olfactory epithelium and the second, interneurons within the telencephalon-derived olfactory bulb.

*b. (Deep) Short Axon Cells*

Short axon cells are interneurons whose somata lie scattered throughout the granule cell layer. These cells are thought to be inhibitory to granule cells: they receive excitatory inputs from mitral and tufted cell axon collaterals as well as centrifugal fibers, and act to cancel the inhibitory response granule cells have on mitral and tufted cells (Macrides and Davis, 1983). Deep short axon cells are not uncommon, but are much less abundant than are the granule cells. Somata of short axon cells are larger than granule cells. They have a large pale nucleus and are surrounded by a moderate amount of cytoplasm, long fingers of which indent the nucleus in a way considered to be characteristic of these cells. Cajal (1911) identified four types of short axon cells, although only one of these types predominated in the rat olfactory bulbs studied by Price and Powell (1970d). Lopez-Mascaraque et al. (1986) described two basic subcategories of short axon cells in the hedgehog, based on distribution of the axons. These will be described below. Schneider and Macrides (1978) subdivided the deep short axon cells into two types: Golgi cells and Blanes cells.

The short axon cells most frequently seen by Price and Powell had numerous dendrites which emerged from all sides of the soma. Although the soma and proximal dendrites were

devoid of spines, the remainder of the dendrites had numerous spines. The other types of short axon cells described by Cajal were relatively spine free although occasional spines were found on the proximal dendrites, the soma, and most frequently, on distal parts of dendrites.

As described by Cajal (1911), axons of the spiny short axon cells and one other type of short axon cell (Golgi cells) had long axons that remained within the granule cell layer. A third short axon cell type described by Cajal (Cajal cells) had an axon that extended through to the external plexiform layer. A fourth type of short axon cell was described by Cajal as having the cell body located within the internal plexiform layer, dendrites extending parallel to the mitral cell layer, and an axon that entered the external plexiform layer (Price and Powell, 1970d).

Lopez-Mascaraque et al. (1986) describe two categories of short axon cells in the deep layers of the hedgehog olfactory bulb: local and extended cells. Local cells have ovoid or piriform somata and are subcategorized as being spinous or smooth, depending upon the presence of spines on the secondary dendrites. In both subcategories of local short axon cells, the axons primarily remain within the granule cell layer and form axon plexuses here. However, some portions of the main trunk or axon collaterals may enter the internal plexiform layer. In the extended short axon cells, the subcategory of spinous versus smooth was

again determined by the presence or absence of spines on the dendrites. The axons of extended short axon cells, entered, formed collaterals, and often modestly arborized somewhere between the mitral cell layer and the border between the external plexiform layer with the glomerular layer. In some cells, the axon terminals "clasped" a glomerulus, while a few had fine axon collaterals that entered glomeruli.

Lopez-Mascaraque et al. (1986) felt that the variations and complexity of axonal arborizations found in the hedgehog were beyond that found in other mammalian species examined by them. Schneider and Macrides (1978) subdivided deep short axon cells into Blanes cells and Golgi cells. They characterized Blanes cells as having a larger cell body compared to Golgi cells; somata usually restricted to the deeper regions of the granule cell layer; a large number of dendritic spines concentrated distally. Golgi cell somata are slightly smaller, are located more peripherally, sometimes within the internal plexiform and mitral cell layers. Golgi cell dendrites extended further than those of Blanes cells, sometimes into the external plexiform layer. According to Schneider and Macrides, the most notable difference was the relatively lower incidence of dendritic spines.

Price and Powell (1970d) state that electron microscopy studies indicate that axon terminals of at least some of these cells form synapses with granule cells. Based on

electrophysiological studies, these short axon cells may be inhibitory to granule cells.

## **H. BULBOFUGAL AND CENTRIFUGAL FIBERS OF THE MAIN OLFACTORY BULB**

### ***1. Bulbofugal fibers***

In their review of olfactory bulb anatomical and neurochemical organization, Macrides and Davis (1983) list the following structures as central recipients of second order neuron axons of the main olfactory bulb: anterior olfactory nucleus, olfactory tubercle, ventral hippocampal rudiment, dorsal peduncular cortex, piriform cortex, cortical amygdaloid regions, the entorhinal cortex, and in marsupials, the dorsal bank of the entorhinal sulcus. In the accessory olfactory bulb, second order axons project to the amygdaloid regions of the piriform lobe, and the stria terminalis.

The research of Scalia and Winans (1975), Skeen and Hall (1977), Davis et al., (1978), and Macrides and Davis, (1983) all support the belief that the main and accessory olfactory bulbs are totally segregated in their peripheral inputs and central projections. More recently, Licht and Meredith (1987) have provided evidence that in one region within the amygdaloid nucleus, input from the main and

accessory olfactory bulbs do overlap. In their recording of field potentials in the posteromedial cortical nucleus of the amygdala, Licht and Meredith demonstrated a physiological convergence of input from the main and accessory olfactory systems onto a small but definite number of specific neurons. Other neurons in this nucleus responded either to inputs from the accessory olfactory bulb or from the main olfactory bulb, but not to both. In addition, in some neurons, inputs from one system suppressed inputs from the other system. Although the number of neurons driven by both main and accessory olfactory bulb projections was small, Licht and Meredith believed that electrophysiological convergence within the posteromedial cortical nucleus may be more prevalent than documented by this study, and that functional or behavioral convergence between the two systems is likely to exist.

## *2. Centrifugal fibers*

Centrifugal inputs are also summarized by Macrides and Davis (1983). Centrifugal fibers to the main olfactory bulb project from neurons within the following central structures: anterior olfactory nucleus, ventral hippocampal rudiment, dorsal peduncular cortex, piriform cortex, nucleus of the lateral olfactory tract, medial septum/diagonal band complex, regions of the hypothalamus, dorsal and median



raphe nuclei, and the locus ceruleus. Many of the central projection targets of the main olfactory bulb are reciprocally connected. The accessory olfactory bulb is also reciprocally connected to its central targets, that is the amygdaloid region of the piriform cortex and the stria terminalis. Macrides and Davis state that although the concept of totally separated main and accessory systems exists, it is likely that both receive subcortical central projections from the following regions: medial septum/diagonal band complex, hypothalamus, raphe nuclei, locus ceruleus, and from a group of LHRH immunoreactive neurons within the cerebral hemispheres. Although many fibers destined for the main olfactory bulb pass through the accessory olfactory bulb, it has yet to be ruled out or confirmed whether these fibers have terminal endings within the accessory olfactory bulb (Macrides and Davis, 1983).

#### **I. THE SIGNIFICANCE OF STRATIFICATION OF NEURONAL PROCESSES WITHIN THE MAIN OLFACTORY BULB**

Output neurons within the main olfactory bulb project to different central structures (Macrides and Davis, 1983; Lopez-Mascaraque et al., 1990). Retrograde transport of horseradish peroxidase indicates that the greatest proportion of tufted cells in the external plexiform layer, and at least a few of the tufted cells within the

periglomerular layer, project more rostrally into telencephalic regions than do internal tufted and mitral cells which project relatively more caudally. External and middle tufted cells project to the rostral part of the piriform cortex near the lateral olfactory tract (Haberly and Price, 1977), the anterior olfactory nucleus, and the olfactory tubercle (Mori et al., (1983). A few neurons located within the granule cell layer also project to this region and are tentatively identified as displaced mitral cells by Haberly and Price (1977). The terminal ends of mitral cells and internal tufted cell axons extend somewhat more caudally into central structures than do more superficially located tufted cells. Although decreasing numbers of mitral cell axons reach more caudal structures, (for example, the caudal piriform cortex and lateral entorhinal cortex), proportionately far fewer tufted cell axons terminate in these areas than do mitral cells (Haberly and Price, 1977). The somata of all middle or external tufted cells remained unlabeled by retrograde horseradish peroxidase injected into these caudal regions indicating that axons of these cells do not project this far.

The fact that the sublaminal location of second order neuron cell bodies within the olfactory bulb correlates to some degree with location of axonal termination within the brain implies that the stratification found within the olfactory bulb has functional significance. Knowledge of

laminar distribution of cell processes is important in order to determine from which cells a neuron can receive synaptic input and to which cells it directs synaptic output. Output neurons (mitral and tufted cells) can be acted upon wherever synaptic input occurs with these cells and their processes. Mitral and tufted cells can also affect the output of neighboring neurons through reciprocal synapses associated with these interneurons. Different populations of interneurons affect differing regions of mitral and tufted cell dendrites as well as different subclassifications of these cells. Within the glomerular layer, mitral and tufted cell dendrites are presynaptic to periglomerular cell dendrites, gemmules and spines. In close proximity to this, primary dendritic shafts of tufted and mitral cells receive synapses from periglomerular dendrite gemmules and periglomerular axon terminals (Pinching and Powell, 1971b). The close approximation of these synapses are termed reciprocal synapses, and although they are unusual within the central nervous system, they are a common finding within the olfactory bulb (Rall et al., 1966). Axon terminals of superficial short axon cells may also come into close synaptic relationship with the periglomerular part of the reciprocal synapse (Pinching and Powell, 1971b). Since periglomerular cells are believed to be inhibitory, inhibition applied at this point by periglomerular cell axons could be highly effective in reducing or preventing

the spread of excitation by the mitral and tufted cell beyond the glomerular region (Pinching and Powell, 1971a).

Along the glomerular/external plexiform layer border, gemmules of periglomerular cell dendrites participate in reciprocal synapses with tufted cells and their processes. In the hedgehog, Lopez-Mascaraque et al. (1990) identified the following fibers within this region: terminal ramifications of axons of superficial short axon cells located within the granule cell layer, terminal endings of granule cell peripheral processes, axons of horizontal cells intrinsic to the external plexiform layer, the majority of secondary dendrites of external tufted and granule cells, and terminal endings of axons of centrifugal afferent fibers. It is possible that many of these cells interact with surrounding interneurons. According to Orona et al., (1983), only the peripheral process of granule cells located within the superficial granule cell layer (Type I granule cells of Mori et al., 1983) would reach the superficial half of the external plexiform layer and would synapse upon the secondary dendrites of external tufted cells. Granule cells, like periglomerular cells, inhibit mitral and tufted cells through granule cell gemmules and spines (Orona et al., 1984).

Secondary dendrites of middle tufted cells distribute for long distances within the superficial sublaminae of the external plexiform layer and in the region between it and

the and the deep sublaminae (Mori et al., 1983; Orona et al., 1984; Macrides and Schneider, 1982). Secondary dendrites of Type II mitral cells (Orona et al., 1984), and internal tufted cells (referred to as "displaced mitral cells" by Mori et al., 1983) also ramify and project tangentially within this region. According to Mori et al. (1983), Type I granule cells project through the entire external plexiform layer and could have synaptic interactions with second order neurons throughout the entire layer. Type III granule cells project specifically to the superficial half of the external plexiform layer and would synapse only with second order neurons having dendritic processes within that region.

The deep external plexiform layer contains secondary dendrites of mitral cells (specifically Type I mitral cell of Orona et al., 1984), and internal tufted cells (Macrides and Schneider, 1982; Lopez-Mascaraque et al., 1990). Secondary dendrites of deep granule cells described by Orona et al., (1983) and Type II granule cells (Mori et al., 1983) project tangentially within the deep sublaminae of the external plexiform layer. This would be the granule cell population having an inhibitory effect upon the mitral and internal tufted cells through dendrodendritic synapses. Mori et al., (1983) state that among the subclassifications of all second order neurons, mitral cells have the most extensive ramifications and projections of secondary

dendrites and therefore may have the most dendrodendritic interactions with granule cells. Orona et al. (1984) confirm this with the observation that the deeper the tufted cell is located, the longer the secondary dendrites, providing more surface for granule cells to synapse with.

Kishi et al., (1984) state that mitral and tufted cells have two types of output pathways for synaptic interaction with local interneurons, that is through dendrites and through axon collaterals. Axon collaterals of middle and external tufted cells project horizontally within the border region of external plexiform/granule cell layers in the hedgehog (Lopez-Mascaraque et al., 1990). In the rabbit (Kishi et al., 1984), the layer in which axon collaterals of tufted cells project is correlated to some degree with location of cell body: axon collaterals of more superficially placed tufted cells distribute more superficially than those of deeper tufted cells. Middle tufted cell axon collaterals project within the internal plexiform/superficial granule cell layer and occasionally within the mitral cell layer. Internal tufted cells (displaced mitral cells) have axon collaterals that project within all regions of the granule cell layer, but are concentrated most heavily within the superficial granule cell layer. Most mitral cell axon collaterals are emitted within the deeper regions of the granule cell layer but ascend to the mid or superficial sublaminae of the granule

cell layer, sometimes reaching the deep border of the mitral cell layer but never entering the external plexiform layer. The length, and proximity of the axon collaterals with granule and short axon cells may indicate that synaptic influence from a single mitral cell is spread more widely than that through a secondary dendrite course. Also, the depth of the axon collaterals near to or within the granule cell layer affects which granule cell subtype (superficial or deep) and short axon cell subtype these axon collaterals synapse with. In some instances, axonal terminations make dense arborizations in particular regions (Kishi, 1984), perhaps selecting for particular subpopulations of cells.

Kishi et al., (1984) discuss the significance of two types of monosynaptic pathways responsible for the activation of granule cells. Within the external plexiform layer, the dendrodendritic synaptic pathway from secondary dendrites of mitral cells to the peripheral processes of granule cells is the principal route of synaptic input to these cells. A second synaptic pathway exists between intrabulbar axon collaterals and granule cells within the granule cell layer. The two synaptic pathways provide for self and lateral inhibition of mitral cells through granule-to-mitral cell inhibitory synapses.

Output neurons within the olfactory bulb act and are acted upon by interneurons in three regions of the main olfactory bulb. In the glomerular layer, mitral and tufted

cells receive the odorant signal from olfactory receptor neurons and then interact (primarily) with periglomerular cells. Throughout the external plexiform layer, secondary dendrites interact in a reciprocal fashion with peripheral processes of granule cells. Within the deeper layers, that is the internal plexiform layer, granule layer, and possibly mitral cell layer, axon collaterals of mitral and tufted cells interact with the somata of granule cells and the proximal shafts of their peripheral processes.

Schneider and Macrides (1978) identified five interneurons whose processes could interact with granule cells. Blanes and Golgi cells could interact with the proximal processes of granule cells while horizontal, Cajal and Van Gehuchten cells could interact with the peripheral processes. Also, all of these cells overlap to some degree and could interact with each other. It is unlikely that any of these cells interact with periglomerular cells. Short axon cells within the periglomerular and superficial external plexiform layer receive synaptic contact from other short axon cells, tufted cells, and centrifugal fibers; they form reciprocal synapses with periglomerular cells.

Schneider and Macrides (1978) state that in addition to the importance of periglomerular and granule cells in the processing of sensory information within the bulb, the contribution to this process by all other categories of interneurons has yet to be defined.



Centrifugal axons also influence the final output of the second order neurons by interaction on differing populations of interneurons. Axon terminals predominantly end on periglomerular, granule and short axon cells within the main olfactory bulb. Centrifugal projections from the following structures terminate within deep olfactory laminae: pars medialis of the anterior olfactory nucleus, ventral hippocampal rudiment, the piriform cortex (Macrides and Schneider, 1982), and nuclei within the diagonal band (Price and Powell, 1970c,e; De Olmos et al., 1978). De Olmos et al., (1978) state in the rat and the rhesus monkey, a multitude of widespread and large centrifugal pathways indicate the significance of olfactory stimuli in mammals and the importance of the brain in integrating it. These centrifugal terminations can affect mitral and tufted cell output via the granule and deep short axon cells. Centrifugal fibers from the anterior olfactory nucleus terminate in both the glomerular and deeper bulb layers and could affect the output of mitral and tufted cells through cell interactions within all bulb layers.

Price and Powell (1970c) state that evidence suggests that different groups of central fibers to the olfactory bulb terminate on different parts of the granule cell: anterior commissure fibers end on deep dendritic spines and somata; the ipsilateral anterior olfactory nucleus fibers end on spines and gemmules of the peripheral processes.

Pinching and Powell (1972) state that centrifugal terminals ending within the glomerular layer end predominantly on periglomerular cells, but may also terminate on short axon or tufted cells. These terminals never enter a glomerulus but always remain within the periglomerular region.

The observation that mitral and tufted cells differ in central projections, and the laminar distribution found within the main olfactory bulb has led to the conclusion that there are two different but overlapping circuits for the processing of olfactory information (Lopez-Mascaraque et al., 1990). In one circuit, tufted cells project to the rostral piriform cortex where neurons send projections back to the bulb that terminate on the superficial granule cells: these cells preferentially innervate tufted cells. In the other circuit, mitral cell axons project throughout the rostrocaudal extent of the piriform cortex: neurons in the caudalmost regions send projections back to deep granule cells. Deep granule cells, in turn, preferentially innervate mitral cell dendrites within the external plexiform layer (Orona et al., 1983). Selectivity of termination sites of centrifugal fibers on particular populations of granule cells is further demonstrated by the fact that fibers from the ventral hippocampal rudiment and pars medialis of the anterior olfactory nucleus terminate in the deep half of the granule cell layer while fibers from

the pars posterior, ventralis, dorsalis and lateralis of the anterior olfactory nucleus terminate in the superficial granule cell layer. As projections from the piriform lobe project from increasingly caudal positions within the lobe, their termination sites upon granule cells occurs at progressively deeper levels within the granule cell layer.

Functional comparisons of the significance of dendritic distribution within the olfactory bulb and the retina in the fish have been made by Mori et al., (1983). Retinal ganglion cells within the inner plexiform layer have been functionally correlated with distribution of their dendrites within the distal sublamina of the inner plexiform layer. In sublamina b, ganglion cell dendrites receive synaptic input from ON-type bipolar cells while in sublamina a, ganglion cells whose dendrites terminate in this sublayer receive synapses from OFF-type bipolar cells. The analogy can be carried to the primary visual cortex where principal cells located in different layers have dendritic arborizations (both basal and apical) within different laminae. These cells also have different functional properties and different sites of axonal projections. This is very similar to the arborization of secondary dendrites and projections of axon collaterals found within the olfactory bulb (Mori et al., 1983).

Another analogous feature between the retina and the olfactory bulb is the presence of reciprocal synapses: in

the retina they are between the bipolar cells and amacrine cells while in the olfactory system, they are between the gemmules of periglomerular cells and the secondary dendrites of mitral and tufted cells Price and Powell, 1970d).

While the olfactory bulb has characteristics which are unique to it (receptor cells receiving the odorant signal from the environment are also the primary sensory neurons; olfactory epithelial neurons and granule cells degenerate and are replaced throughout the life of the organism), it shares morphological and functional similarities with other sensory systems, particularly that of vision.

#### **IV. MAIN OLFACTORY SYSTEM FUNCTION VS. FUNCTION IN THE ACCESSORY NASOCHEMOSENSORY SYSTEMS**

Although it is difficult to determine which of the five nasochemosensory systems is responsible for the reception of odorant molecules and transmission of that odorant information, it is fair to assume that many (and probably most) of the odors that reach mammalian noses are received by receptors within the olfactory mucosa. This assumption is based on: 1) the observation that the area covered by receptors within the olfactory epithelium in any species is much greater than the receptor area for all the accessory nasochemosensory systems combined (in that species); 2)

functions attributed to any of the accessory nasochemosensory systems are fairly circumscribed, for example, the molecules of pheromones present in urine bind to receptors within the vomeronasal organ epithelium of rodents. It has not been determined that other pheromones bind exclusively or at all to vomeronasal organ receptors.

The following are but a few examples of how the sense of smell functions in the daily lives of humans and other mammals: food finding and selection, territorial recognition, recognition of dangerous substances (smoke, commercial natural gas, food spoilage), recognition of disease (diabetic acidosis, internal bleeding or tissue necrosis to those who are familiar with it), the smell of seasonal change, the recognition of a perfume or after-shave lotion and its association with past or present acquaintances; the attractiveness or avoidance of another individual based on odor. The reception of most or all of these odors can be assumed to be received and transmitted by receptor cells within the olfactory epithelium based on the fact that in humans, the vomeronasal organ is lacking (although remnants of it are found in some adults), and that there is lack of evidence for reception of these odorant molecules by the other nasochemosensory systems. Meredith (1983) states that the olfactory system seems capable of responding to almost all molecules that can reach its receptors. This suggests that its role is one of a general

molecular analyzer rather than as a specific detector for particular chemical signals.

Function of the accessory nasochemosensory systems has been discussed in Chapter 2. That there may be functional overlap between these systems is suggested by the observation that both the vomeronasal organ and terminal nerve cells are believed to be involved in reproductive behavior in rodents and other mammalian species. The pursuit of functional clarification is further complicated by the fact that fibers of the vomeronasal organ and terminal nerve run very close together so that sectioning of nerve fibers to determine function (or more correctly, loss of function) is likely to damage fibers of both systems, rendering functional interpretations unreliable (Wysocki and Meredith, 1987).

The fact that components of the accessory nasochemosensory system cannot be the exclusive receptor sites of pheromone molecules is demonstrated by the fact that many species do not appear to have a developed vomeronasal organ or accessory olfactory bulb at any time other than during embryological development. Some investigators have reported this to be true for many primate species, particularly the Catarrhini, but conflicting observations have been reported. Species reported to lack these structures include the following: *Homo sapiens*, *Macaca fascicularis*, *Macaca mulatta*, *Cercopithecus ascanius*,

*Cercopithecus mitis*, *Colobus badius*, *Papio doguera*, *Pan troglodytes* and *Gorilla gorilla* (Epple, 1986). In the human, the vomeronasal organ is formed and regresses during embryonic development. What may persist are unilateral or bilateral pits within the nasal septum, or a patch of epithelial cells (Stoddart, 1990). Whether these pits and their associated epithelia are functional, and for what, has yet to be determined.

In spite of what appears to be an absence of the vomeronasal organ system in many catarrhine primates, vaginal secretions in *Macaca mulatta* apparently play a role in reproductive behavior. Field and laboratory observations indicate that the vaginae of females coming into heat are examined by males by sniffing, licking, or touching, with subsequent sniffing or licking of fingers that touched the area. Sexual interactions sharply peak around the time of ovulation in both free and laboratory macaques (Stoddart, 1990). Examination of the vaginal secretions of *Macaca mulatta* indicated that the only component that elicited male sexual behavior were free fatty acids. When a synthetic mixture of these acids was applied to test females, a huge increase in male sexual behavior was seen. Actual amounts of acids within vaginal secretions doubled immediately prior to ovulation (Stoddart, 1990).

In the human female there is evidence for menstrual synchrony (reviewed by Stoddart, 1990). In one study, the

daily axillary secretions of a woman who had observed that her roommates always developed menstruations synchronous to hers were mixed with alcohol and frozen. A dab of this mixture was placed on the upper lip of sixteen volunteers three times a week for four months. Half of the women were controls: in this group the routine was identical except that the dab lacked the axillary secretion. The experimental group receiving the axillary secretion began to menstruate in unison with the donor's cycle: the change was statistically significant at  $p < 0.01$ . The results suggest that some pheromonal communication exists between humans living in close proximity. Given the lack of evidence for a functioning vomeronasal system, it seems likely that pheromones are received by other components of the nasochemosensory system, and probably by the main olfactory system.

Meredith (1983, page 201) defines a pheromone as "a chemical emitted by one member of a species which when detected by another member results in behavioral or physiological changes that are likely to benefit both individuals. Chemicals meeting this definition have many potential sources and conceivably could range from ions to macromolecules, making all known chemoreceptor systems possible pheromone detectors." This implies that functions attributed to the vomeronasal organ based on the secretion and reception of pheromones could in fact be main olfactory



system functions with receptor sites on the olfactory receptor cells. Based on the observation that there may be functional overlap of nasochemosensory system in mammals, the question arises as to why two systems are present in the first place. Meredith speculates that: 1) by being very sensitive to some chemicals, vomeronasal receptors may require protection from other potentially damaging chemicals; 2) different glandular secretions may be required for the reception of certain molecules; 3) some afferent information transmitted by the vomeronasal organ may be so behaviorally or physiologically potent that to expose inexperienced or immature individuals to the stimuli might be disadvantageous in an evolutionary sense.

Meredith states that it is necessary to demonstrate which nasochemosensory system is involved in a particular pheromonal response and that it should not be assumed that these molecules bind with vomeronasal organ receptor cells rather than receptor cells located within the olfactory epithelium.

Defining function in any of the nasochemosensory system depends on providing evidence that an odorant molecule is received by particular receptor cells and that information is transmitted centrally along fibers of that system. This may differ between species as does the presence, reduction, or absence of the vomeronasal system differ between species. In most cases, this evidence is yet to be obtained.

Therefore a concise picture of olfaction and the specific function of the various systems that contribute to this important sense must await further studies.

## **CHAPTER 4**

# **NEOCORTICAL CHANGES IN BRAIN DEVELOPMENT: A MODEL FOR INTERPRETATION OF THE OLFACTORY BULB**

### **I. INTRODUCTION**

Much of the literature concerned with the evolution of the brain has examined microscopic and macroscopic changes occurring within the cerebral cortex. Since the olfactory bulb is a derivative of the telencephalon, it is believed that an understanding of principles of ontogenetic and phylogenetic change occurring within the cerebral cortex can serve as a model for understanding species differences within the olfactory bulb. Therefore, in order to interpret better the data presented here, the theoretical background reviews literature concerned with: 1) neuronal number and how changes in neuronal number occur; 2) the degree of lamination or layering present within cortical structures and its significance; 3) the density of cells within the cerebral cortex and how it relates to brain/body size; 4) how ontogenetic changes can be related to phylogenetic

differences.

## II. NEOCORTICAL DEVELOPMENT: INCREASE IN INTERNEURON NUMBER AND DEGREE OF LAMINATION

A discussion of the evolutionary changes that have occurred within cortical regions of the brain requires a brief explanation of the two major cell types found within the neocortex. These two principal types of neurons are the *pyramidal cells* and the *stellate, or granule cells*.

**Stellate cells** are small, multipolar neurons with short axons that remain within the cortex, often confined to the local cortical region. They are the principal *interneurons* of the cortex and therefore act on other regional neurons. They are thought to be inhibitory in function.

**Pyramidal cells** are more triangular in shape, with a long apical dendrite projecting from the superficial cell surface and extending towards the pial surface. From one to several basal dendrites project from the deep cell surface and spread out horizontally. The somata of pyramidal cells (10 - 100 $\mu$ ) are larger in diameter than those of stellate cells (less than 10  $\mu$ ). The axons of most pyramidal cells are long and leave the cortex to synapse with neurons in other cortical or subcortical regions. Therefore, pyramidal cells are the *principal output neurons* of the cortex. (The description of these cells is based on the description of

human cortex by Nolte, 1988).

Regions of the cortex characterized by a preponderance of pyramidal cells and a paucity of stellate cells are termed "agranular": this is exemplified by the motor cortex of haplorhine primates (Sanides, 1969). Cortical regions with a high number of small-bodied cells (stellate cells) are referred to as granular, exemplified in its greatest degree by the hypergranular or koniocortex ("powder cortex") of the visual and auditory neocortical regions in primates. It is an important point that lamination within the koniocortex is accentuated due to the fact that the increased density of granule cells stands out in contrast to the adjacent layers (Sanides, 1969).

In 1955, Mitra published a study of the relative percentages of major cell types within the cerebral cortex of cats, rabbits and one specimen of *Macaca mulatta*. Mitra describes the major cell types as pyramidal, stellate (or granular), with a small percentage of fusiform cells. The morphology of the pyramidal cells is similar to that described above for the human: the soma has a long apical dendrite extending toward the pial surface, often reaching the molecular layer (the layer located immediately beneath the pia mater). The basal (deep) dendrites extend horizontally or obliquely from the basal surface of the cell. Stellate cells are described by Mitra as having

spherical, ellipsoidal or polygonal somata. Dendrites emanate from anywhere on the cell surface and ramify uniformly around it. Fusiform cells are bipolar cells with one dendrite extending from each pole: their long axes are usually situated parallel to the cell surface. Mitra was able to classify eighty percent of the cells into the three cell types. The relative percentages of pyramidal and stellate cells in different species are a major interest to this study.

In the macaque visual cortex, the pyramidal cells were distributed within the upper and lower one-thirds of the cortex, with stellate cells sandwiched in between: the middle zone of stellate cells measured approximately  $700\mu$  in width. In the upper and lower zones where the pyramidal cells predominate, there were very few stellate cells to be found. Within the middle, "stellate zone", the stellate cells outnumbered the pyramidal cells. Of the total number of neurons classified in the visual cortex of the macaque, 45% were stellate cells, 52% were pyramidal cells, and 3% were fusiform. The number of stellate cells came close to reaching the number of pyramidal cells.

In the visual cortex of the cat, the pattern of stellate and pyramidal cells mirror that of the monkey, but there is a greater overlap between the cells of the upper zone and the "stellate zone", and between the "stellate zone" and the lower zone. This merging of zones can be

stated as a decrease in lamination (layering) of the cellular zones within the cortex, relative to that of the monkey. Of the total number of neurons classified, stellate cells comprised 35% while pyramidal cells comprised 60%. There are considerably fewer stellate cells than there are pyramidal cells.

In the visual cortex of the rabbit, there is greater overlap between the zones with the "stellate zone" measuring only 300 to 400  $\mu$  thick and a concomitant reduction of lamination of the cellular layers. In the rabbit, the stellate cells comprise only 31% of the total number of neurons classified while the pyramidal cells comprised 66%: there are over twice as many pyramidal cells as stellate cells.

Ontogenetically, changes occur in the proportions of stellate cells to pyramidal cells. In younger animals (a six week old kitten and ten and seventeen day old rabbits), there are proportionately fewer stellate cells to pyramidal cells than are found in adults of these species. In the kitten, stellate cells comprised 31% of the total neuron number compared to the 35% in the adult cat. In the 10 and 17 day old rabbit, the percentage of stellate cells was 13% and 23% respectively, compared to the 31% in the adult. In the species examined, the percentage of stellate cells increases with age.

In all species and within all cortical regions of the

brain examined, the fusiform cell population contributed between 2% and 6% of the total neuron population. The percentages of fusiform cells was not considered to be revealing of any evolutionary significance.

In the motor cortex of the monkey and cat, the change in the proportions of major neuronal types found within that region was similar to the comparative change in proportions seen within the visual cortex of these species. In the monkey, pyramidal cells made up 74% of the total neuron population while stellate cells made up 22%. In the cat, the pyramidal cell population was up to 85% while the stellate cell population comprised only 9% of total neuron number.

Three conclusions can be drawn from these data: 1) ontogenetic changes within the cerebral cortex includes an increase in the percent of stellate cells that comprises the total neuronal population; 2) within the mammalian species studied, the species (primate) known to have the most highly developed neocortex also has the highest percentage of stellate cells proportionate to other neuronal types. It is significant that primates, who have a more highly developed ability to discriminate between visual patterns than do the cat or rabbit, and whose visual sense is known to be of the highest importance for their survival and social behavior, have a ratio of stellate cells to pyramidal cells nearing 50% within the visual cortex. In the cat and rabbit,



species whose dependance on sensory information is more obviously shared among the senses of vision, olfaction and hearing, the stellate cell to pyramidal cell ratio within the visual cortex is approximately 33% to 66%. This implies that the more discriminatory a sensory sense is (vision, in the case of the monkey), the per cent of stellate cells associated with that cortical region increases. 3) There appears to be a correlation between the proportionate increase in stellate cells with an increase in degree of lamination or layering of the cellular zones of the cortex. Diamond and Hall (1969) state that in modern reptiles, the neurons within the cerebral hemispheres are concentrated on the surface and represents a single layer of cells. In all mammals, the cortex is characterized by from few to several neuronal layers, the degree of lamination varying from one particular cortical region to the next. In insectivores, mammals who have long been considered to have the most primitive type of mammalian brain (see below), the degree of lamination (less) remains typical of those regions of the cortex not considered to have attained the level of morphological development characteristic of the neocortex. In humans, lamination reaches its greatest extent of six or more layers in certain neocortical regions. This appears to corroborate the belief that the degree of neocortical lamination increases with the expansion and complexity of the vertebrate neocortex.

#### **A. THE NEOCORTEX OF THE HEDGEHOG EXHIBITS PRIMITIVE FEATURES**

Sanides and Sanides (1974) studied the cerebral cortex of the hedgehog (*Hemiechinus*) and found it to exhibit primitive cortical organization as exemplified by poor lamination (also Valverde and Lopez-Mascaraque, 1981) and a paucity of granularization to the degree that cortical layer IV is difficult to discern. No trace of a hypergranular koniocortex was found in the hedgehog neocortex. The relative lack of cortical differentiation is also seen in the elephant shrew and in bats (Sanides and Sanides, 1974).

Another primitive cortical feature identified in Layer II of the neocortex of insectivores and bats is the preponderance of cells whose somata are larger and are more densely packed than comparable cells found in neocortical Layer II of the majority of most mammals. Golgi analysis demonstrated that these cells are pyramidal cells based on the presence of dendrites rich in spines. (Sanides and Sanides [1972] describe pyramidal cells as having dendrites rich in spines while stellate cells have dendrites with few or no spines.) In addition, pyramidal cells in the hedgehog neocortex have a wide, externally directed dendritic spread which enters Layer I, and a much more underdeveloped internally directed dendritic expression, often represented by a single, short dendrite extending into deeper regions. This is in contrast to the small pyramidal cells found in

Layer II of the majority of mammals, whose basal and apical dendritic spread is approximately equal in diameter and whose overall cell morphology roughly forms a cylindrical shape. The configuration of these neurons, found within Layer II of insectivores and bats and termed "extraverted neurons" by Sanides and Sanides, is also characteristically found within the amphibian endbrain and in the more primitive cortical regions of placental mammals (the archiocortex and paleocortex). This categorizes the neocortex of insectivores and bats as primitive relative to the neocortex of many placental mammals, and is particularly demonstrated within Layer II (Sanides and Sanides, 1974). Sanides and Sanides (1972) refer to "the expanded apical bouquet as a conservative feature and the basal skirt as a progressive feature in pallial neuron evolution". Sanides and Sanides (1974) also found that the size of stellate somata per thickness of cortex in the insectivore (hedgehog) and bat group was large compared to stellate somata size in other placental mammals (rat, guinea pig and rabbit). The conclusions of the Sanides and Sanides study were thus: 1) As the neocortex enlarges, there is an increasing trend towards finer (smaller) stellate soma size culminating in the koniocortex of the haplorhine primates: the greater the expansion of the neocortex, the more granular the stellate cell population. This is substantiated by the observation that the average size of the stellate cells in the monkey

was less than one half that of the guinea pig and rabbit.

2) With regard to pyramidal cell size, two trends occur concurrently: some pyramidal cells enlarge culminating in the large Betz cells found in the human neocortex while the remainder of pyramidal cells decrease in size as the neocortex expands.

### III. DENSITY OF CELLS WITHIN THE NEOCORTEX

Within the cerebral cortex, the density of neurons decreases as brain size increases. Cell density must not be confused with absolute cell number: as brain size increases, the total number of neurons also increases, although these cells are packed less densely within the brain (Williams and Herrup, 1988). The relationship between decrease in cell density to increase in brain size appears to exist within the agranular (motor) cortex, the koniocortex (visual cortex), and most likely within the eulamine<sup>1</sup> cortex (isocortex) as well (Tower, 1954). Shariff (1953) demonstrated that a correlation exists between relative brain size and the density of cells within

1. Eulamine cortex or isocortex refers to the regions of neocortex which have six designated cellular layers. These are typically the associative regions of the cerebral cortex.

the neocortex of primates. Table 1 (page 188) illustrates Shariff's findings.

Shariff also demonstrated an inverse relationship between cell density and cell size: there is a decrease in density of cells within the cerebral cortex as cell size (absolute) increases (Table 2, page 188).

It is much harder to demonstrate a direct correlation between function (degree of intelligence, for example) and density of cells. For instance, difference in density of cells within the cerebral cortex of five primate species (Table 1) is more likely to be directly correlated with differences in body size and therefore brain size rather than with the degree of intelligence often associated with degree of neocorticalization. For example, in the elephant and the whale, the packing density of cells is less than in the human, but this does not indicate that elephants are more intelligent than humans. Also, the 6000 gram brain of an elephant contains two to three times as many neurons as does a 1350 gram human brain (Williams and Herrup, 1988), indicating a lack of correlation between absolute neuron number and function. In humans, when seeking a size-related explanation for what is considered to be our unusual intelligence, it is the disproportionate increase in the expansion of the neocortex ("brain size") to body size that is offered as the connection between size and function.

Table 1

The density of cells per 0.001 cubic millimeter within the eulamine (E), agranular (A), and koniocortex (K) of various primates (n = 1 for each species):

|                      | E     | A     | K     |
|----------------------|-------|-------|-------|
| Human                | 29.0  | 18.2  | 97.2  |
| Chimpanzee           | 60.1  | 49.3  | 174.0 |
| <i>Cercopithecus</i> | 114.0 | 62.0  | 265.0 |
| <i>Callithrix</i>    | 87.6  | 87.8  | 398.0 |
| <i>Tarsius</i>       | 304.0 | 156.0 | 702   |

Table 2

Average cell size is measured in cubic microns (n = 1 for each species):

|                      | E    | A    | K   |
|----------------------|------|------|-----|
| Human                | 1621 | 2468 | 895 |
| Chimpanzee           | 865  | 1035 | 616 |
| <i>Cercopithecus</i> | 718  | 855  | 468 |
| <i>Callithrix</i>    | 502  | 638  | 284 |
| <i>Tarsius</i>       | 536  | 580  | 339 |

Glezer (1968) states that the packing density of cells is inversely related to dendrite development: in areas 4 and 6 of the human neocortex, it is the extensive span of highly developed dendrites that is responsible for the low density of cells. Holloway (1968) cites several studies demonstrating that a decrease in cortical neuronal density is correlated with: 1) ontogenetic development of the brain; 2) an increase in dendritic branching of cortical neurons. Williams and Herrup (1988) state that although the ratio of neurons within a selected nucleus, or the ratio of neurons across interconnected parts of a system are an important determinant of functional capability, the differences in these ratios may vary substantially. The substantial variation in cell ratio may be compensated for by size and degree of branching of axons and dendrites and by the distribution of synapses. The example quoted by Williams and Herrup is that the ratio of sympathetic preganglionic neurons to postganglionic neurons drops in mammals as body size increases: this change is compensated for by an increase in size and complexity of axons and dendrites.

Although absolute neuron number shifts during the ontogeny of the mammalian brain, there is a progressive reduction in density of cells during ontogeny of the human cerebral cortex (Glezer, 1968). During fetal development and postnatally, the main cause of density reduction is 1)

the growth of the afferent connections of cells, and 2) the development of glial cells and vascular structures.

Although decrease in cell density continues into adulthood of the human, it does so at a reduced rate (Glezer, 1968). This again brings up the importance of neuronal processes in understanding the morphology and functioning of the brain. It is obvious that knowledge of absolute cell number or cell density contributes less to a functional understanding of a system than does knowledge of cell number or density combined with a knowledge of dendritic and axonal connection's and synaptic frequency.

#### **IV. PARCELLATION: CELL DEATH OR NEURONAL PROCESS DEGENERATION AND EVOLUTION OF THE BRAIN**

The parcellation process, as conceived by Sven Ebbesson (1980, 1984), states that diffuse, undifferentiated neuronal systems existed within the nervous system of early vertebrates. As more complex behaviors evolve and are modified, there is a parcellation and differentiation of these diffuse connections which involves competition of inputs, redistribution of inputs and the loss of connections. The variation within these systems found amongst different species of extant vertebrates is due to the response to selective pressures for a modification of



behavior. According to Ebesson, the degree of parcellation is dependent on: 1) the state of the organization when new selective pressures appear and 2) the strength of that pressure.

Theoretically, there are three mechanisms for the evolution of new structures within the various neuronal systems of the central nervous system: 1) a genetic mutation creating a new structure or system *de novo*; 2) the invasion of one system by another; 3) parcellation (loss of connections). In his analysis of neural systems in advanced forms, Ebesson (1980) states that he failed to find a single example of "new connections" between unrelated neural systems and that no example of invasion of one system into another was found. However, in species typified by less brain development or less differentiation within the central nervous system, overlap of certain modalities exists. For example, in the opossum, the somatosensory and motor cortex are completely overlapping while in the rat, overlapping is restricted to the area of the cortex concerned with the hind limbs (Ebesson, 1984). In primates, the somatosensory and motor cortices are completely separated. Ebesson concludes that parcellation is the process responsible for the isolation of the two areas, and that segregation through parcellation is a common event in the evolution of the nervous system.

Ebesson (1980) found that when a structure becomes

highly differentiated, the structures that it is functionally (and synaptically) related to also become more highly differentiated. One example they cite is increased differentiation (lamination) within the optic tectum: this is correlated with increased parcellation of synaptically related cell aggregates within the diencephalon and pretectum. As far as laminated structures are concerned, there is also a striking correlation between the degree of lamination and degree of parcellation of inputs.

According to Ebesson, two types of parcellation, horizontal and vertical, occur simultaneously. Horizontal parcellation accounts for the development of new nuclei as opposed to extensive aggregations of cells. Increased horizontal parcellation is accompanied by a loss of inputs to the regions associated with the dominant modality. Ebesson states this is seen in the visual cortex where the primary cortex loses some commissural and thalamic inputs, and in the optic tectum where direct spinal input is lost to large areas. The analytic capabilities of a primary input increase as afferents to particular areas are reduced or lost. This isolation of function is associated with an increase in specialized neurons and a vertical segregation of inputs, as presumably specialized circuits are formed.

Vertical parcellation leads to increased lamination or stratification within a system. Data suggest that the significance of vertical parcellation is that the processing

capability of the nervous system can increase by a change in circuitry secondary to losing certain inputs. In vertical parcellation, there is greater segregation of inputs from different sources, specialization of neuronal types, and loss of particular connections as specialization of neurons increases (Ebbesson, 1980). The decrease or loss of particular connecting fibers within a commissure found in those animals with better vision or finer tactile discrimination is one example given by Ebbesson. The loss of specific inputs and the increase in specialization of certain cells associated with lamination may reflect the evolution of more complex and better tuned functional circuits. Therefore, the functional outputs in a highly differentiated structure differ from functional outputs in an ancestral, less differentiated structure. As Ebbesson states (1980, p. 197), "In a sensory structure, this could mean responding to a more defined class of stimuli or resolving more accurately within that class. It may also have to do with increasing the number of behavioral alternatives and enabling more proper responses to a given stimulus". An increase in segregation secondary to horizontal and vertical parcellation, would decrease "noise" between circuits and lead to an increase in sensitivity for selected signals as well as an increased selectivity in the processing of information in general.

Parcellation appears to be a normal process in

ontogeny. Ebesson's literature review (1980) cites the following as evidence for ontogenetic parcellation: 1) Layer IV C of the neocortex in the fetal macaque does not contain cortical columns; by six weeks post partum, there is complete segregation of cortical columns. 2) Optic afferents to the superior colliculi and the geniculate nuclei in the macaque synapse throughout each structure before segregating into the layers characteristic of the adult form of these structures. 3) Within the visual cortex, commissural connections are much more extensive in young cats than in adults.

Ebesson adds that there is a correlation between the degeneration of normal neuronal processes with function. This is supported by the findings that various neural systems degenerate at the approximate time of imprinting.

Parcellation has been proposed by Ebesson as an evolutionary hypothesis that uses cell death or loss of neuronal processes and their connections as a means for evolutionary change within the central nervous system. In terms of ontogeny, Cowan et al. (1984) state that an essential feature of ontogenetic development is the loss of previously acquired properties by: 1) restriction of cellular potential; 2) the death of cells; 3) the progressive elimination of features of cell morphology or function. During vertebrate neurogenesis, elimination of

neuron processes and/or neuron cell death play a major role in the formation of the fully developed adult nervous system. During ontogeny, a significant number of neurons (up to half) degenerate, die and are rapidly removed by surrounding glial cells: this neuronal death has been documented in all major classes of vertebrates and in a wide range of species. During a phase apparently specified for each neuronal population, overproduced neurons cannot effectively synapse with cells of the target field and therefore die. Thus, naturally occurring cell death matches the size of the neuronal population to the size and functional needs of its target. Neurons with axons reaching the wrong areas within the target field, or missing the correct target altogether, die. Catsicas et al. (1987) state that in the isthmo-optic nucleus (ION) of the chick, 60% of the initial neuronal population dies between days E12 and E17. Neurons within the isthmo-optic nucleus project to the contralateral retina: this is true during early and late embryonic periods. However, projections in younger embryos were much less precise. Projections were most densely packed in a region that corresponded topographically between the isthmo-optic nucleus and the retina (the medial part of the ION projects temporally: the ventral part of the ION projects dorsally). Some cells known as "outsiders" projected outside of the densely labeled region while other cells known as "wrong-halfers" projected from the opposite

half of the isthmo-optic nucleus. On day E13, 49% of the cells were outsiders and 16% of the cells were wrong-halfers. At longer survival times, the number of outsiders and wrong-halfers decreased; by E18, the number of outsiders and wrong-halfers was 7.5% and 1.5% respectively. Although neurons within the ION projected to the correct target (the contralateral retina), neurons projecting to regions outside of a specific circumscribed area were selected against and subsequently died.

It is thought that axons compete for some limited entity within the target field and that this may be the trophic factor, nerve growth factor (NGF). One possible mechanism that leads to preferential death of certain neurons may be that the limited amount of trophic factor available to any neuron may be increased by the near synchronous activity of adjacent presynaptic cells. This would clearly select for the survival of neighboring cells and not more distant cells that would not be activated simultaneously. Cowan et al. (1984) state that this kind of activity-dependent mechanism has been thought to be responsible for the fine-tuning of the visual system connections and explains the formation of eye dominance lamination found in animals where each optic tectum receives bilateral retinal projections.

Cell death can also be related to the absence of a particular circulating hormone or hormones. In songbirds,

for example, several forebrain structures are necessary for the development of song in both males and females. At a specific stage, females become songless as cells within relevant nuclei degenerate and die. This degeneration of cells in the female can be arrested by the administration of certain hormones (Cowan, et al., 1984).

In addition to cell death, connections can be eliminated by the degeneration of neuronal processes while the neuron itself survives (Purves and Lichtman, 1980; Cowan et al., 1984; Katz and Calloway, 1992). The withdrawal or elimination of axon terminals is widespread during the development of the nervous system. One example of this is discussed below: in the cat visual cortex, there is selective degeneration of neuronal processes without cell death during ontogeny (Katz and Calloway, 1992). In their review of the literature, Cowan et al. (1984) cite several examples of this, including reduction of callosal connections being due to elimination of collateral projections without cell death. Degeneration of neuron processes without cell death has also been shown to occur secondary to the removal of NGF. In muscle cells, the selective elimination of redundant synapses increases the specificity of muscle innervation (Changeux and Danchin, 1976). In the diaphragm of the fetal rat, the appearance of acetylcholinesterase in the middle of the muscle fiber is correlated with the development of multiple axonal branches

of the motor neuron which forms a complex and increasingly large network up until birth. At the motor endplate several synaptic profiles are in contact with the same synaptic folds, and several motor axons are functional. After birth, eventually all of the synapses are innervated by a single nerve terminal, and most of the functional contacts have disappeared. The number of motor axons and the number of muscle fibers does not change, however. This indicates that the number of muscle fibers innervated by a single motor neuron decreases: the size of the "motor unit" decreases. As there is no overlap between the fields of innervation, and a single motor axon causes the contraction of a smaller number of muscle fibers, graded contraction of a muscle is possible and the specificity of muscle innervation is increased (Changeux and Danchin, 1976). Thus, regression leads to an improvement in the motor command of a muscle.

#### **V. INTERCONNECTIVITY WITHIN CORTICAL TISSUE IS BOTH VERTICAL AND HORIZONTAL**

The significance of and development of connections within layers is addressed by Katz and Callaway (1992). They reviewed what is presently known about the emergence of intrinsic processing within the neocortex (particularly visual) of the mammalian brain. Most synapses within the



mammalian cerebral cortex are components of intrinsic or local circuits, and the elaboration of these local connections are likely to be a key element in generating the neuronal codes that convey sensory data and elicit behavior. Katz and Callaway state that in the mammalian primary visual cortex, the precise arrangement of *local vertical and horizontal connections* (not to be confused with Ebbesson's vertical and horizontal parcellation discussed above) is crucial if a function as fundamental as the detection of orientation and direction of moving visual stimuli is to be assimilated by cortical neurons.

Vertical connections (linking layers) were initially thought to be the predominant, if not exclusive dimension of connectivity in the cortex. However, extensive horizontal connections, also referred to as intralaminar or tangential connections, have been revealed by tracing techniques (Katz and Callaway, 1992). Therefore, synaptic connectivity takes place along vertically and horizontally aligned circuits.

Vertical connections develop from the elaboration of local collaterals within the appropriate cortical layers and are highly specific. Laminar specificity occurs early in both cortical cells that project beyond the cortex and in cortical neurons whose synaptic interactions occur more locally: this includes spiny stellate cells and nonprojecting inhibitory interneurons (Katz and Callaway, 1992). Specificity of vertical connections is achieved in

one or all of several ways: 1) selective degeneration of poorly specified collaterals; 2) sprouting of collaterals in all layers followed by differential growth of specific optimally located collaterals; 3) initial collateral outgrowths are specific to appropriate layers and sites of synapse. There is evidence that all three mechanisms participate in the development of the mature nervous system, and specifically within the developing visual system (Katz and Callaway, 1992).

Within the cat visual cortex, horizontal connections (*tangential or intralaminar connections*) depend on aggregates of synaptic terminals located at the distal ends of the tangentially spread neuronal processes. These processes radiate from the cell body like the spokes of a wheel: each aggregate consists of two or three spokes that have moved close to each other and radiate out together. The terminal arborizations of these "sister spokes" form a synaptic aggregation. Radiating spokes spread out from the cell body at fairly consistent intervals and have reciprocal connections to distinct clusters of cortical neurons. Because the span of horizontal connections is large, the central neuron's response may be modified according to the net input of the surrounding stimuli. The development of appropriate horizontal links within a cortical layer may be required for the integration of information leading to

perceptual capabilities (Katz and Callaway, 1992).

Katz and Calloway found that there is developmental stasis regarding cluster number and tangential extent but that early clusters are crude, with many retrogradely labeled cells present within the spaces between clusters. With age, distinct clusters of cells are retrogradely labeled but the between-cluster cells are not, demonstrating that they no longer make connections to previous injection sites. This indicates that a process of selective loss of non-essential connections has occurred, but that cell death has not: in other words, cluster refinement requires elimination of selective processes, but not cell death (Katz and Calloway, 1992).

In ontogeny, an unclustered distribution of unbranched tangentially-spread collaterals changes to a progressive clustering of some branches while other branches regress. The final stage in the refinement in the development of clustered horizontal connections is formation of arborizations at the terminal ends of the radially directed collaterals. (Katz and Calloway, 1992)

The initial emergence of crude clusters does not require patterned visual experience. However, Katz and Calloway (1992) found that within the visual system of the cat, normal cluster development is altered by prolonged binocular deprivation, or by rearing infant laboratory animals in the dark. Therefore, patterned visual activity

is necessary if cluster organization of horizontal connections is to develop normally. Without normal patterned visual activity, the progression from crude to refined clusters does not occur: the precision of grouping of horizontal collaterals, and the elaboration of distal branches is much reduced. Postnatally, the refinement of clusters may be related to the increased precision of orientation tuning.

In terms of evolution, intrinsic cortical circuits may have consisted of entirely vertical connections. An increasing complexity in processing of information may have necessitated the evolution of a horizontal system of connections (Katz and Calloway, 1992). Katz and Calloway state that although stereotypic vertical connections are observed in the cortex of all vertebrate species, highly specific horizontal connections are especially prominent in the primate cortex. The modification of horizontal connections, driven by changing neuronal activity, may provide the opportunity for the evolution of new perceptual or cognitive capabilities.

Finlay et al.(1987) discuss how heterochrony in the process of cell death or neuronal process retraction (regressive events) would effect parcellation or the evolution of nervous system structures. In the ontogenetic development of the nervous system, the overproduction of

cells or cell processes means that afferents to a particular nucleus compete to become the presynaptic part of permanently functional synapses. Timing appears to be an important part of the selective process amongst competing afferent axons. Changes in the arrival time of converging inputs at a common target could alter the outcome of an activity-dependent sorting mechanism. Variation in the amount of time available for segregation of overlapping fibers may determine the degree of parcellation that occurs. "In closely related species, alterations in the genetic control of the timing of developmental events (produced by ecological pressures favoring shortened or extended gestational periods) could respectively minimize or extend the period of axonal segregation. A restricted 'critical period' for fiber sorting could result in a greater overlap of inputs and less specificity in the adult nervous system. A prolongation of development might exaggerate the segregation of populations, resulting in greater adult parcellation." (Finlay et al., 1987).

## **VI. INCREASE IN DENDRITIC TUFT SIZE AND SYNAPTIC NUMBER AS A PROCESS IN BRAIN DEVELOPMENT**

Although regressive events in the development of the brain have been emphasized in the previous discussion,

Pomeroy et al. (1990) state that the ongoing growth of the brain postnatally may indicate that there is a progressive increase in neuronal number and complexity of neural circuits. In their study of the olfactory bulb in mice, Pomeroy et al. found that: 1) the number of glomeruli increased from 410 in the fetus to 1710 at birth; 2) the diameter of the circumference of the apical dendritic tuft of mitral cells increases in diameter by two to three times during the first 10-12 weeks postnatally; the dendritic tuft synapses with the axons of primary receptor neurons within the glomeruli; 3) The number of synapses within the glomeruli increases disproportionately to the increase in size of the glomerulus or the bulb; 4) the overall volume of the external plexiform layer increases substantially as do the number of synapses per unit area within it. The continuous addition of glomeruli, together with increasing dendritic tuft complexity in mitral cells and increasing numbers of synapses in at least two bulb layers indicate that large, complex neural circuits can be made and elaborated throughout the postnatal period and beyond the onset of sexual maturity (at 6-7 weeks). Pomeroy et al. believe that the dominant quantitative theme within the olfactory bulb is progressive addition of synapses, neuronal branches and functional circuits, and not regressive changes. They feel that the "selectionist view", as put forth by Changeux and Danchin (1976) and others, is not

supported by the ontogenetic changes they have described as occurring within the olfactory bulb.

## **VII. ENVIRONMENTAL EFFECTS ON THE DEVELOPMENT OF THE NERVOUS SYSTEM**

In their search for evidence that experience can modify the anatomy or chemistry of the brain, Bennett et al. (1964) separated rat litter mates into three groups. Individuals in the Social Condition (SC) group shared a cage with two littermates, were exposed to normal activity within the lab but were not provided with any additional stimulation. Individuals in the Isolated Condition (IC) group were housed singly in isolated cages and kept in a dimly lit, quiet room with no environmental stimulation. Individuals in the Environmental Complexity and Training (ECT) group were housed in a large cage with at least nine other littermates, had several toys available to them, were taken out of the cage for daily play periods, and ran mazes for sugar rewards. When the animals were sacrificed, brain weight and thickness of the cortex for the ECT group was higher than that of the IC group (the SC group was not included in this comparison). In addition, the level of acetylcholinesterase, the enzyme that deactivates the neurotransmitter acetylcholine after it acts on the post-

synaptic membrane, was highest in the cortex of the ECT group, lowest in the IC group, and slightly lower than the ECT group in the SC group. Diamond (1964) measured the size of the neuronal cell bodies and their nuclei in the ECT and IC groups. The mean for neuron cell body size and nucleus size was found to be larger in the ECT group than the IC group, but cell body and nuclear size was greatest in the upper level of the cortex and less so for the middle and lower levels.

Holloway (1966) found that neurons in the visual cortex of rats raised in an ECT environment had increased dendritic branching compared to the neurons of rats raised in isolation.

Uylings et al. (1978), studied adult rats that were exposed to normal and enriched stimuli, enriched in this study being defined as a cage provided with six or more toys. A baseline group (B) was killed at the onset of the study, the standard caged group (SC) was taken as the standard for changes occurring with age, and the enriched caged group (EC) was studied for the effect of environmental enrichment on particular regions of the neocortex. This study confirmed the findings of Bennett et al. (1964): the frontal and occipital regions of the cortex in the EC rats were significantly thicker than that in the either the B or SC animals. Also, an increase in branching and length of the terminal segments of dendrites of layers II to III



pyramidal neurons was seen in both standard and enriched groups as compared to baseline groups. In addition, the radial extension of basal dendrites in the enriched group indicated an increase in dendritic surface area in these rats, and it may be assumed, an increase in synaptic contact.

Diamond et al. (1964) found that rats raised in an environment that was enriched with toys and with maze running experiments had an increase in brain weight, an increase in depth of neocortex (particularly the visual cortex) and a decrease in cell density. They questioned whether the increase in weight and volume could be related to an increase in dendritic branching. In a study by Bhide and Bedi (1985), two groups of rats, one undernourished and one well-fed were exposed to environmental diversity for thirty days beginning either at 35 days of age or at 85 days of age. The only group that exhibited alterations in either neuronal or synaptic measurements was the well-fed group exposed to environmental diversity at the later stage (adulthood) beginning at 85 days. These rats had a significantly smaller neuronal density and a greater synaptic disc diameter; the significant increase in synapse to neuron ratio seen in any group exposed to environmental diversity probably reflected reduced neuronal densities rather than an increase in the number of these synapses. It

appears that even well after birth, timing plays a role in morphological change: what is unusual about these results is that environmental diversity produced changes in the neurons only after adulthood had been reached. Bhide and Bedi question whether the normal developmental curves so pronounced in early postnatal life are so strong that they mask the small changes in features produced by environmental diversity. However, the authors cite several studies that appear to substantiate their findings that environmental enrichment can effect neuronal and synaptic features. Although this was not addressed by Bhide and Bedi, one wonders if the decrease in numerical density represents cell death associated with fine tuning driven by the stimulus of an enriched environment.

Based on research showing that 1) monocular deprivation induces changes in spine density in the rat occipital and specifically visual cortex, and that 2) olfactory deprivation effects the size of mitral cells and the number of synapses they have with granule cells, Rehn et al. (1988) studied how inhalation of a single odor (cyclohexanone) or inhalation of deodorized air over time affects spine density of granule cells. Granule cells were selected because the majority of these cells develop postnatally: therefore, they are forming functional synapses during and after this period and should be particularly

sensitive to environmental (olfactory) manipulation. In response to inhalation of deodorized air, they found a decrease in granule cell spine density within both the lateral and medial halves of the bulb; mitral cell size in rats reared in deodorized air was also decreased. This was correlated with a decrease in olfactory acuity. Inhalation of cyclohexanone in deodorized air produced a decrease in spine density in the lateral, but not the medial half of the olfactory bulb: control rats and rats exposed to deodorized air normally had a higher density of spines within the lateral half of the bulb. The formation of spines generally reflects the formation of synapses: hence, the decrease in spine formation implies both a decrease in synaptic formation and a decrease in function.

From the preceding studies, it is obvious that environment can effect the development of central nervous system structures and that this in turn modifies functional ability. Primary olfactory receptor neurons are directly exposed to the external molecular environment through air currents that enter the nasal cavity. Receptor neurons axons synapse with dendrites of second order neurons (mitral and tufted cells) within the olfactory bulb; the axons of these cells project primarily to the piriform cortex and the amygdaloid nucleus. Both of these structures are components of the limbic system, a system with many connections to

diencephalic structures, particularly the hypothalamus (Nolte, 1988; Martin, 1989). Environmental changes that might be transmitted to the hypothalamus by way of olfactory system/limbic system pathways could effect the timing of hormonal secretion by endocrine system cells, and thereby influence many systems of the body. In terms of the development of the nasochemosensory system, it was mentioned previously that removal of the thyroid gland effected the development of the vomeronasal organ (Segovia et al., 1982), and that removal of the testes affected the development of the accessory olfactory bulb (Roos et al., 1988). In their discussion of the effects of heterochrony on the development of nervous system structures, Finlay et al. (1987), point out that manipulation of thyroxin levels in rats results in alteration of rate of neurogenesis, duration of axon outgrowth, synaptic density and cell survival within the cerebellum. Although it is way beyond the scope of this thesis to do more than ask the question, one wonders if the components of the nasochemosensory system, as a direct conduit from the external molecular environment to the limbic system, might be an instrument for producing changes in developmental timing based on responses to environmental signals.

## CHAPTER 5

### MATERIALS AND METHODS

#### I. MATERIALS

Mammalian olfactory bulbs were received from a variety of laboratory sources for two reasons: 1) I preferred to avoid sacrificing animals for the sole purpose of obtaining olfactory bulbs; 2) the animal facilities at City College limit the species from which specimens could be obtained locally. Because specimens were obtained from a variety of sources, it was not possible to control for variables such as exact age or sex. Most animals in the study were of reproductive age; in the cases where age was not obtainable, the animals were reported to be of adult size, although sexual maturity in these cases cannot be guaranteed. Table 3, (page 212) gives the average age for reaching reproductive maturity in the laboratory rat (Donaldson, 1924), and in *Macaca mulatta* and *M. fascicularis* (Napier and Napier, 1967). Table 4 (page 213) lists the species, number of specimens (n), weight (when known), age (when known), and method of fixation for the specimens included in this study.

Table 3

Depending on the study quoted, the average age for reaching sexual maturity (first estrus) in the Albino Rat *Mus norvegicus albinus* is (Donaldson, 1924):

*Mus norvegicus albinus*

54.1 days (range 40-67 days)

40.9 days (range 36-51 days)

The difference in average age that estrus is reached is attributed to an improved diet in the group reaching estrus earlier.

Average age for reaching sexual maturity as reported by Napier and Napier (1967):

*Macaca mulatta*

female 3.5 years

male 4.5 years

*Macaca fascicularis*

female 4.3 years

male 4.2 years

Table 4  
Specimens included in this study:

| <u>Species</u>                                  | <u>N</u> | <u>Sex</u> | <u>Age</u> | <u>Weight</u> | <u>Meth. Fix.</u> |
|-------------------------------------------------|----------|------------|------------|---------------|-------------------|
| Taconic white rat<br>(Fisher 344)<br>(#711-713) | 3        | M          | 10wk*      | 190-205 Gms** | Immers. F         |
| Taconic white rat<br>(Fisher 344)<br>(#714-715) | 2        | M          | 10wk*      | 190-205 Gms** | Perf. G/P         |
| <i>Sciurus</i><br><i>carolinensus</i><br>(#810) | 1        | M          | U          | 435 Gms       | Immers. F         |
| <i>Macaca mulatta</i><br>(#118)                 | 1        | F          | 14yr       | 8.71 Kg       | Immers. F         |
| <i>Macaca mulatta</i><br>(#119)                 | 1        | F          | 19yr       | U             | Immers. F         |
| <i>Macaca</i><br><i>fascicularis</i><br>(#128)  | 1        | F          | 5+yr       | 9.5 Kg        | Immers. F         |
| New Zealand white<br>rabbit<br>(#902)           | 1        | M          | U          | U             | Immers. F         |
| <i>Felis catus</i><br>(#604)                    | 1        | U          | U          | U             | Perf. F           |

\* Taconic white rats: age 10 weeks reported by supplier  
(Taconic) to be sexually mature.

\*\* Taconic white rats: all animals were weighed but weights  
recorded as a range of between 190-205 grams.

U = unknown. U for age: supplying laboratories were  
requested to send adult specimens so that specimens of  
unknown age are presumed to be adult or near-adult in  
age.

Perf. = perfusion fixed

Immer. = immersion fixed

P/G = paraformaldehyde/glutaraldehyde

F = buffered formalin 10%

Weights = Last weight recorded by supplying laboratory.

Six litter-mates (all male) of an inbred, homozygous strain of Taconic white rats (Fischer 344), stated to be raised under the same environmental and dietary conditions, were received in our laboratory and sacrificed one day after arrival. Three individuals (#711-713) were sacrificed by ether inhalation. When dead, a coronal section was made through the skull in line with the external auditory meatus using a Stryker autopsy saw. The rostral portion of the skull containing the rostral brain and olfactory bulbs was immersed in 10% buffered formalin and refrigerated. The remaining three individuals (#714-716) were deeply anesthetized with ether and injected with 0.8ml of Pentobarbital. Ether inhalation continued throughout the procedure. Intracardiac perfusion was begun with Hanks solution followed by 4% Paraformaldehyde, 2.5% Glutaraldehyde in phosphate buffer solution. The head was removed, a coronal cut using a Stryker autopsy saw was made, and the rostrum of the skull containing brain and olfactory bulbs was immersed in the same fixative and refrigerated. Only two of the perfused specimens (#714 and #715) could be used in the study: after full preparation including embedding, sectioning and staining, the histological sections of #716 were found to be torn and fragmented to a degree that rendered them unusable for this study.

Specimen #810, a wild-caught *Sciurus carolinensis*, was received from Dr. Todd Olson as a rostral skull with the



majority of the brain intact. The rostral skull and brain were fixed by immersion in 10% buffered formalin. The entire rostrum was decalcified, embedded and sectioned so that histological sections included the nasal cavity structures, olfactory epithelium and olfactory bulbs. .

Specimen #118, *Macaca mulatta*, was received from Dr. Mark Harrison, University of California at Davis. It was received as a formalin fixed (10% buffered) rostral skull with rostral brain and olfactory bulbs intact. Upon arrival in our laboratory, both bulbs were carefully removed from the surrounding bone without obvious damage, immersed in 4% buffered formalin and refrigerated until further processing.

Specimen #119, *Macaca mulatta*, was received from Delta Regional Primate Center. This rostral skull and brain with olfactory bulbs intact was fixed and shipped in 10% buffered formalin. Upon receipt of the specimen in our laboratory, both bulbs were carefully removed from the surrounding bone without obvious damage, immersed in 4% buffered formalin and refrigerated until further processing.

Specimen #128, *Macaca fascicularis*, was received from Buckshire Corporation as a rostral skull and brain with intact olfactory bulbs. It was fixed and shipped in 10% buffered formalin. Upon arrival by our laboratory, the specimen was stored in 10% buffered formalin and refrigerated for one month. At this time the olfactory bulbs were removed and stored in 4% buffered formalin.

Specimen #604, the olfactory bulbs of a domestic cat (*Felis catus*), were received from Dr. Jack Martin at Columbia Presbyterian Medical Center, New York. The cat had been perfused with 10% formalin, the olfactory bulbs were removed and stored in the same fixative and refrigerated until received by our laboratory. No information other than fixation technique was available for this specimen.

Specimen #910, a New Zealand white rabbit, was received from Dr. Kalsner's laboratory at City College of N.Y. The head was received in our laboratory immediately after death. A coronal section of the skull was made, exposing the rostral brain and olfactory bulbs; this portion of the skull was immediately immersed in 10% buffered formalin until the brain tissue was fixed (six days). At that time the olfactory bulbs were removed and stored in buffered formalin. No information was available for this specimen.

Numerous people and institutions contributed specimens throughout the years of this study. Although many of the specimens were not included in this study for various reasons, all of the contributors are sincerely thanked and are listed under Acknowledgements.

## II. METHODS

### A. REMOVAL OF OLFACTORY BULBS

Fixation by immersion requires that the brain and olfactory bulbs be exposed to the fixative solution immediately after death. The calvaria is opened using a Stryker autopsy saw. A coronal (frontal) section is made that passes immediately rostral to both external acoustic meatus. In most mammals, the olfactory bulbs are located rostral and ventral to the cerebral hemispheres and can be easily sectioned from the brain when it is removed from the skull. In the haplorhine primates included in this study, the olfactory bulbs are encased within the bone of the frontal-ethmoidal region and are not easily removed from this bone. Therefore fixation of the olfactory bulbs has to occur while *in situ*. Once the calvaria is sectioned, the rostral portion of the skull including the brain and olfactory bulbs it contains are immediately immersed in fixative solution. The surrounding dura mater is loosened from the brain tissue so that fixative can more readily reach the olfactory bulbs.

Brain tissue needs to be fully fixed before the olfactory bulbs can be removed from the skull. Fixation is determined by color of the tissues (pale tissues are more fixed), absence of blood, and firmness of the tissue. Brain

and olfactory bulbs need a minimum of three days in fixative before the bulbs can be removed from the surrounding bone. When fixation is complete, the bone surrounding the olfactory bulbs can be carefully removed using microronguers. Frequent examination of the dissection site using a dissecting microscope is necessary to prevent damage to the small olfactory bulbs during bone removal. In many cases, an extension of the dura mater afforded some protection to the olfactory bulbs during removal of surrounding bone. In some cases, the bone surrounding the olfactory bulbs was decalcified and the olfactory bulbs were not removed: this is discussed under "Decalcification of Bone".

#### **B. DECALCIFICATION OF BONE**

In the early stages of this study, the entire rostrum of the skull was decalcified and sectioned in order to study the density of cells within the olfactory epithelium (Specimen #810). Although this part of the study was discontinued due to technical difficulties, decalcification was continued to be used in some cases to soften the bone surrounding the olfactory bulbs. In these cases, once fixation had occurred, the frontal-ethmoidal region of the skull which encased the olfactory bulbs was removed from the remainder of the skull and decalcified in a solution of EDTA

(Sequestrene) disodium salt. Decalcification of these small specimens took less than one week when large amounts of EDTA solution were used and the solution was changed daily. Decalcification was considered to be complete when bony tissues could be easily compressed between two fingers. At this time, excess decalcified bone was removed and the olfactory bulbs with the remainder of the surrounding decalcified bone were embedded. This technique of decalcifying the bone surrounding the olfactory bulbs was found to be preferable to removal of the bulbs from the bone because of the damage that was sometimes sustained by the superficial layers of the bulb during removal.

Decalcification did not appear to effect the morphology of the cells, but the difference in densities between bone and neural tissue led to an increase in tearing of tissues during sectioning. See Appendix (page 328) for the full decalcification procedure.

### **C. EMBEDDING**

The specimens were embedded in Paraplast Plus, a paraffin based embedding material. When the entire rostrum including the nasal cavity was embedded (as in Specimen #810), it was necessary to carry out the final Paraplast Plus immersion in a vacuum oven set at 58 degrees Celsius. This insured that Paraplast reached the spaces surrounding

the nasal conchae. When olfactory bulbs with or without a limited amount of decalcified bone were embedded, simple immersion into Paraplast Plus was adequate. See Appendix (page 328) for the full embedding procedure.

#### **D. SECTIONING**

A standard microtome for paraffin embedded tissues was used to section all specimens at a thickness of 10 microns. All olfactory bulbs were sectioned coronally: the first sections represented the rostral end of the bulb while each subsequent section represented a slightly more caudal section of the bulb. Paraffin ribbons of ten sections each were placed in a warm water bath: every tenth section was floated onto the slide and kept for staining. Most of the problems in sectioning were encountered in the decalcified specimens where separation or tearing of tissues occurred. The slides bearing the paraffin sections were placed in a warm oven (40 degrees Celsius) overnight and then stored in a dust-free container until staining.

#### **E. STAINING**

A solution of Cresyl Violet was used with excellent results. See Appendix (page 328) for the full staining procedure.

## F. ANALYSIS OF DENSITY OF CELLS AND LAYER VOLUME

### *1. Density of Cells*

Microscopic analysis of density of cells and area of . layers was carried out using a combined system of MicroComp Integrative Image Analysis System (Southern Micro Instruments, Inc.) and an IBM PC-XT microcomputer. In this system, the image is transferred from the light microscope to the computer monitor by way of a Videcon tube. The parameters were measured and statistically analyzed using the SMI planar morphometry software program. A 45X objective was used to visualize cells within each layer. All measurements were made in microns. To obtain the absolute data in these units, the system was calibrated in both perpendicular axes, x and y, using the stage micrometer (with smallest subdivision of 10 microns). The calibration program assigned specific number of pixels on the monitor to specific number of microns the stage micrometer. The area within which cells were to be counted was outlined using a digitizing pad (Houston H-Pad) and a "mouse": both area and cell count were automatically computed by the Image Analysis program, giving the value for density of cells. In this study, the area chosen for measurement was determined by the borders of the particular olfactory bulb layer within which the density of cells was being determined. The border

between layers was indicated by a change in density of cells. Between the glomerular layer and external plexiform layer, for example, the density of cells surrounding the glomeruli was much higher than the density of cells within the external plexiform layer. It was much easier to determine borders between layers in specimens where an abrupt change in density of cells created an obvious line of demarcation. In specimens where there was pronounced overlap of cells from one layer into the next, areas of higher density of cells were considered to belong to that layer characterized by a higher density of cells whereas areas along the border containing a lower density of cells were considered to belong to that layer characterized by a low density of cells (Figure 9, page 223). In two instances, the border between layers was determined by a change in cell morphology. This occurred between the olfactory nerve layer within which the non-neuronal cells were able to be distinguished from the neurons within the glomerular cell layer. The neurons of the granule cell layer were also distinguishable from the cells within the subventricular layer. For some measurements of density of cells, the layer containing the cells to be counted was larger than the area of the screen. In this case the four borders of the screen were outlined as the boundary within which cells were counted.

Within the series of histological slides for each



**Figure 9. Defining boundaries of layers**

Olfactory bulb of *Macaca mulatta* (#118) (25X)

line = line of demarcation between two layers:

a = between glomerular and external plexiform

b = between mitral cell and external plexiform

boxes = blocks, from superficial to deep, within which  
density of cells was measured.



Figure 9

specimen, every other slide was selected for measurement, (one out of every twenty bulb sections initially obtained by sectioning). The first slide to be measured was the most rostral section of the bulb in which clear glomeruli could be distinguished. The first slides of the bulb contained only glomeruli; in the next few slides, the external plexiform layer formed the only layer deep to the glomerular layer. A separation into mitral cell, granule cell and internal plexiform layers became evident within the next few slides. Within each histological slide selected for measurement, two blocks from different regions of that slide were selected for estimation of density of cells within each layer containing mature neurons. Keeping within the block selected for measurement, I started measuring density of cells within the glomerular layer, followed by the external plexiform layer, and deeper into the bulb until ending with measurements of the granule cell layer (Figure 9, page 223). When the first block (within a slide) that was selected for measurement contained a high density of cells, the second block (within that same slide) was selected for measurement due to its decrease in density of cells, and vice versa. This was done because the density of cells within the bulb consistently varied throughout each histological slide and within each layer of that section. Also, areas in different regions of the bulb were alternately selected so that dorsal, ventral, medial, and

lateral regions of the bulb were equally represented in the measurements. The density of cells was measured in every layer containing mature neurons (that is, neurons making synaptic contact with other neurons). This includes the following layers: glomerular, external plexiform, mitral cell, internal plexiform, and granule cell layers. Each block selected for determination of density of cells represented one sample. Therefore, within each olfactory bulb layer measured, the density of cells represents the mean for the total number of blocks measured.

Most cells within the designated area of study were counted. All cells that demonstrated a distinct nucleolus were counted. A vast majority of cells were darkly stained and it was difficult to distinguish nucleoli: these cells were included in the count. The only cells that were omitted from the cell count were those with very irregular shapes. Some of these cells appeared to be slivers, some were very small, irregularly shaped, and very deeply stained. In many cases, there was a question as to whether these were cell fragments. The greatest problem in determining which structures should be counted as cells and which should be omitted occurred within the external plexiform layer and the glomerular layer. It was estimated that of the total cells comprising the glomerular cell layer, approximately 14% were irregular enough to be omitted from the cell count. Within the external plexiform layer,

the percentage sometimes rose to as high as 25%, possibly due to the fact that irregularly shaped cells showed up clearly against the relatively cell-poor background. In the remainder of the layers, cell size and shape appeared to be more standardized. In the mitral cell layer, only those mitral cells whose nucleoli were clearly visible were counted. Since the diameter of the mitral cell of any specimen never exceeded 55 microns (this was in the cat; the range of mitral cell diameter in smaller specimens was between 19 and 31 microns), Abercrombie's rule was not used: only one paraffin-embedded section out of the twenty initially obtained from sectioning (10 microns out of 200 microns) was used for analysis in this study. The largest cell (55 micron diameter) was too small to span the distance between the consecutive sections (190 microns) included in the analysis: therefore no cell was counted twice.

At the onset of the data collection, the area for density measurement was demarcated and the cell count was taken, the value noted, the data erased and the cell count taken again. This was repeated several times with virtually identical results, thus assuring that observer error was minimal. Observer error was also minimized by the fact that I was the only person who collected the density and volume data throughout the entire study.

## *2. Area of Layers*

The Planar Morphometry program also calculated the sum of all blocks of each olfactory bulb layer and computed the statistical analysis of the data. For determination of total layer area, it was necessary to change the objective to 10X: thus, the measuring system was recalibrated for this magnification. The area of each olfactory bulb layer was measured using those histological sections that had been used in the study of density of cells. Since the size of most bulbs exceeded the limits of the computer screen at this magnification, layers had to be measured as continuous fragments of the whole. The same procedure for determining layer boundaries defined above for density of cells was used to demarcate the layers in this part of the study.

The value for the volume of each area was determined by multiplying the Planar Morphometry sum by: 1) the thickness of each slide (10 microns); 2) the total number of sections comprising the entire length of the olfactory bulb. The final value obtained was the total volume for each olfactory bulb layer in that specimen. In addition to the layers containing synaptically active neurons, the subventricular layer (the layer between the ventricular space and the granule cell layer) and the ventricular space, when present, were also measured. At the caudal pole of the bulb, where the glomerular layer disappeared, all of the other layers

were also either greatly diminished in size or gone, so that area measurements were discontinued at this point.

#### **G. STATISTICAL ANALYSIS**

The Planar Morphometry program contains a statistical package that provides automatic statistical analysis of the measured parameters. For density (the number of cells per area per layer) and area data, the program provided the following: N = total number of blocks measured, data sum, mean, range, standard error and standard deviation. Tables 5, 6, 11, 12 and 13 (Pages 239, 244, 272, 274, 281) provide data sum, range, standard deviation and standard error for density and area of each layer measured in each specimen. In every case, the data were received as square microns and were converted to cubic millimeters. To convert density per square microns to density per cubic millimeters, the following formula was used:

$$N = \frac{n \times 10^9}{10}$$

N = mean density per cubic millimeter

n = mean density in square microns

10 = is the thickness of each section in microns

To convert area in square microns to volume in cubic millimeters:

$$\frac{S_L \times 10 \times M}{10^9} = Y, \quad \frac{Y}{N} = V_L$$

$V_L$  = Volume of layer

$S_L$  = Area of layer in square microns

$M$  = Total number of sections that comprise the length of the olfactory bulb. This includes the nine out of ten sections of the paraffin ribbons that were discarded as well as the one out of ten sections that were saved and stained.

$N$  = Number of histological slides that were measured

The statistical package of the Planar Morphometry program automatically provides the value of  $t$  for the student  $t$ -test and degrees of freedom between any pair of values.  $P \leq 0.01$  was accepted the level of significance for all  $t$ -tests.

Variance and correlation coefficient were analyzed using the statistical program "Statistica". Because this program reported numerical values for  $F$  and level of significance for  $p \leq 0.2$ , but no higher, the statistical program "Sigmastat" was randomly used to confirm that  $p$  values were indeed this large: "Sigmastat" confirmed that differences within the analysis were due to random variation and that  $p \geq 0.9$ .



## H. LAMINATION: DETERMINATION OF A RELATIVE SCALE

Part of this study was an attempt to determine if the overlap of cells between adjacent layers of some species represented a difference that was significant enough to be considered as a change in the degree of lamination. If a difference in lamination existed among species, was it correlated with olfactory bulb size? An increase in lamination can be described as an increase in difference in the density cells of adjacent layers. Macroscopically, this would be indicated by a reduction in the number of cells that spread from one cellular layer into adjacent layers. Therefore, the boundaries between layers in a less laminated bulb would be unclear due to the number of cells spreading from one layer into adjacent layers while a more laminated bulb would be characterized by a sharp boundaries between the layers. For each specimen, I obtained the t-values for difference in the density of cells between each layer and its adjacent layer (or layers). In the majority of cases,  $p < .001$ , indicating a significant difference in the density of cells of most adjacent layers. In an attempt to determine if the difference in density of adjacent olfactory bulb cell layers was greater or lesser than that of other species in the study, the t-values for each specimen were summed, the mean for that specimen was obtained, and the value received was used as a relative gauge for degree of

lamination. This value is not meant to imply any degree of significance for the differences seen between species, but merely provides a way of quantifying what microscopic examination suggested: that some species have an increase in overlap of cells between adjacent layers relative to other species.

# I. COMPARISON OF LOG BULB VOLUME TO LOG BRAIN VOLUME

Using the allometric formula  $K = \frac{\log \text{bulb volume}}{\log (\text{brain volume}^x)}$

K was accepted as 1 since small sample size limited our ability to determine a more accurate constant'.

1. In numerous quantitative studies (Count, 1947; Shariff, 1953; Glezer, 1968), it has been shown that in most biological systems, the relationship between the size of the part and the size of the whole system is defined by allometric equation

$$K = \frac{a}{b^x}, \text{ where } K = \text{a constant,}$$

a = size of the part, b = size of the whole system, x = species specific constant. To define the constant x when the constant K is also unknown, it can be hypothesized that  $K = 1$  (Huxley, 1932; Glezer, 1968).

$$\text{Therefore, } 1 = \frac{a}{b^x} \rightarrow x = \frac{\log a}{\log b}$$

Theoretically the parameters of olfactory bulb size and brain size are related allometrically to each other, in spite of the immense difference in their absolute size. Since their relationships are not linear, the logarithms were plotted against each other rather than the absolute values.

$$\log \text{ brain volume}^x \times 1 = \log \text{ bulb volume}$$

$$x = \frac{\log \text{ bulb volume}}{\log \text{ brain volume}}$$

x = species value for use in an allometric plot of brain to bulb volume. .

The log of brain volume was plotted against the log of bulb volume. For each species, this was done for: 1) total bulb volume; 2) volume of layers containing mature neurons.

Brain weights in grams were obtained from Table 128 of Blinkov and Glezer (1968). Brain weights were converted to volume by using the formula:

$$\frac{\text{brain weight (gms)}}{1.2} \times 1000 = \text{brain volume in cubic millimeters}$$

The constant 1.2 was derived by Glezer (personal communication) using a series of known brain weights and brain volumes for the human brain.

## CHAPTER 6

### RAT STUDY

#### I. INTRODUCTION TO THE COMPARATIVE ANALYSIS

The main olfactory bulb of mammals is subdivided into several layers, but only five of the layers contain mature neurons, their axonal and dendritic process, and synapses between the cell processes and somata. For the purposes of the rat study and the comparative mammalian study, these layers are referred to as "layers containing mature neurons" and include the glomerular, external plexiform, mitral cell, internal plexiform and granule cell layers. Excluded from this category are the olfactory nerve layer, the subventricular layer, and the ventricular space when it is present: no synapses between primary, second order neurons, or interneurons are known to occur within these layers. The olfactory nerve layer was discussed in Chapter 3 and does not contain olfactory bulb neurons. The subventricular layer is made up of exiting or entering neuron fibers and supporting cells. Neurons (immature cells), when they are present in this layer, are precursors of granule cells and

have the capacity to differentiate into mature granule cells. As discussed in Chapter 3, granule cells are unusual in that they are constantly renewed throughout the life of the individual. As they approach maturity, they migrate from the subventricular region into the granule cell layer where they function as interneurons. Due to the lack of evidence for the presence of functioning synapses within the subventricular layer, this layer has not been included with those layers considered to contain mature neurons. The ventricular space, when it is present, is an extension of the ventricular system, and as such, is a fluid filled cavity, not a cellular layer.

Data collection for the rat study, and for the comparative study of five mammalian species includes: 1) a description of the component layers of the olfactory bulb of each species; 2) density of cells within each of the layers containing mature neurons; 3) volume of each of the layers comprising the olfactory bulb of each specimen. This includes the volume for the subventricular layer and ventricular space, when present. It does not include the olfactory nerve layer as this layer seldom remains undamaged by removal of the olfactory bulb from surrounding bone. Additional information generated by the numerical data will be presented within the statistical analysis and discussion sections.

All data were computed in square microns by the Planar

Morphometry program, but were converted to cubic millimeters, the standard unit of measurement used in most publications.

## **II. RAT STUDY: DOES METHOD OF FIXATION AFFECT RESULTS?**

Since method of fixation could not be controlled with each the olfactory bulbs I received from various laboratories, a comparison of three formalin fixed rat olfactory bulbs with two perfused rat olfactory bulbs (the third perfused bulb was unusable due to fragmentation) was done to determine: 1) if mean density of cells for the entire bulb or any of its layers was significantly altered by method of fixation; 2) if total volume of the olfactory bulb or volume of any its layers was significantly altered by the method of fixation. The rat specimens included six male littermates of inbred, homozygous, Taconic white rats stated to have been raised under identical conditions, and who were sacrificed in our laboratory on the same day (same age). One olfactory bulb of each of the three formalin fixed and two perfused Taconic white rats were included in this analysis.

### III. RESULTS

#### A. MORPHOLOGY OF OLFACTORY BULB LAYERS

The **glomerular layer** of the olfactory bulb of the rat is characterized by a single row of glomeruli. Most glomerular layer cells are located along the deep border of the glomeruli, between this layer and the external plexiform layer. Some cells extend between the glomeruli and completely surround it, separating the glomerulus from the olfactory nerve layer. In many cases, there are few or no cells separating the glomeruli from the olfactory nerve layer. The **external plexiform layer** is neither unusually narrow nor wide. It contains a number of cells, many of which are irregular or fragmented. In some regions, large cells comparable in size and shape to mitral cells are located throughout the external plexiform layer: these cells are considered to be large tufted cells of the external plexiform layer. In the caudal regions of the bulb, these large tufted cells are most frequently located close to and bordering the glomerular layer. There are many large mitral cells located within the **mitral cell layer** although in some regions, these cells are more sparsely located. The width of the mitral cell layer spans the diameter of approximately four granule cells. The **internal plexiform layer** is narrow when present: in many areas it is obliterated by granule

cells. The **granule cell layer** is comprised of narrow "tracks" of granule cells running parallel to the mitral cell layer. Caudally, the granule cell layer is wide and loaded with cells. A relatively cell free area separates the granule cell layer from the subventricular layer. There is no extension of the ventricle into the olfactory bulb of any of the rat specimens. In the caudal olfactory bulb, the accessory olfactory bulb is present.

## **B. NUMERICAL DATA**

### *1. Density of cells*

Density of cells was computed by the Planar Morphometry program in square microns. Mean, standard deviation, standard error for density of cells within each layer in each rat specimen are given in Table 5 (page 239). Mean, standard deviation and standard error were converted into cubic millimeter: these values are also listed in Table 5.

Figure 10 (page 242) is a line graph demonstrating the density of cells within each layer of the olfactory bulb for the five rat specimens.



Table 5

Density of cells within the olfactory bulb of five rat specimens:

## Rat #711: Non-Perfused

| Density in square microns    |            |             |            |
|------------------------------|------------|-------------|------------|
|                              | Mean       | Stand. Dev. | Stand. Er. |
| Glo.l                        | 0.003104   | 0.001099    | 0.000162   |
| Epl.T                        | 0.000068   | 0.000092    | 1.388142   |
| Epl.O                        | 0.000587   | 0.000180    | 2.711534   |
| Mcl.M                        | 0.000426   | 0.000200    | 3.158075   |
| Mcl.O.                       | 0.005133   | 0.000935    | 0.000144   |
| Ipl                          | 0.002944   | 0.000799    | 0.000139   |
| Gcl                          | 0.006602   | 0.000754    | 0.000118   |
| Density in cubic millimeters |            |             |            |
|                              | Mean       | Stand. Dev. | Stand. Er. |
| Glo.l                        | 3104000.00 | 1099000.00  | 162000.00  |
| Epl.T                        | 68320.76   | 92078.95    | 13881.42   |
| Epl.O                        | 587000.00  | 180000.00   | 27115.34   |
| Mcl.M                        | 426000.00  | 200000.00   | 31580.75   |
| Mcl.O                        | 5133000.00 | 935000.00   | 144000.00  |
| Ipl                          | 2944000.00 | 799000.00   | 139000.00  |
| Gcl                          | 6602000.00 | 754000.00   | 118000.00  |

Glo.l = Glomerular layer  
 Epl.T = External plexiform layer, large tufted cells  
 Epl.O = External plexiform layer, all other cells  
 Mcl.M = Mitral cell layer, mitral cells  
 Mcl.O = Mitral cell layer, all other cells  
 Ipl = Internal plexiform layer  
 Gcl = Granule cell layer

Table 5 continued

## Rat #712: Non-Perfused

| <u>Density in square microns</u>    |             |                    |                   |
|-------------------------------------|-------------|--------------------|-------------------|
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 0.002864    | 0.000752           | 0.000111          |
| Epl.T                               | 0.000039    | 0.000036           | 0.000005          |
| Epl.O                               | 0.000579    | 0.000148           | 0.000022          |
| Mcl.M                               | 0.000425    | 0.000793           | 0.000120          |
| Mcl.O                               | 0.004745    | 0.000973           | 0.000148          |
| Ipl                                 | 0.002719    | 0.000688           | 0.000112          |
| Gcl                                 | 0.006000    | 0.000615           | 0.000093          |
| <u>Density in cubic millimeters</u> |             |                    |                   |
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 2864000.00  | 752000.00          | 111000.00         |
| Epl.T                               | 39886.51    | 3637.25            | 5612.39           |
| Epl.O                               | 579000.00   | 148000.00          | 22319.13          |
| Mcl.M                               | 425000.00   | 793000.00          | 120000.00         |
| Mcl.O                               | 4745000.00  | 973000.00          | 148000.00         |
| Ipl                                 | 2719000.00  | 688000.00          | 112000.00         |
| Gcl                                 | 6000000.00  | 615000.00          | 93774.54          |

## Rat #713: Non-perfused

| <u>Density in square microns</u>    |             |                    |                   |
|-------------------------------------|-------------|--------------------|-------------------|
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 0.003016    | 0.000888           | 0.000140          |
| Epl.T                               | 0.000059    | 0.000048           | 0.000007          |
| Epl.O                               | 0.000556    | 0.000139           | 0.000021          |
| Mcl.M                               | 0.000460    | 0.000683           | 0.000109          |
| Mcl.O                               | 0.006836    | 0.013492           | 0.002189          |
| Ipl                                 | 0.003181    | 0.000968           | 0.000166          |
| Gcl                                 | 0.006174    | 0.000794           | 0.000127          |
| <u>Density in cubic millimeters</u> |             |                    |                   |
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 3016000.00  | 888000.00          | 140000.00         |
| Epl.T                               | 59372.30    | 48741.62           | 7520.99           |
| Epl.O                               | 556000.00   | 139000.00          | 21656.47          |
| Mcl.M                               | 460000.00   | 683000.00          | 109000.00         |
| Mcl.O                               | 6830600.00  | 13492000.00        | 2189000.00        |
| Ipl                                 | 3181000.00  | 968000.00          | 166000.00         |
| Gcl                                 | 6174000.00  | 794000.00          | 127000.00         |

Table 5 continued

Rat #714: Perfused

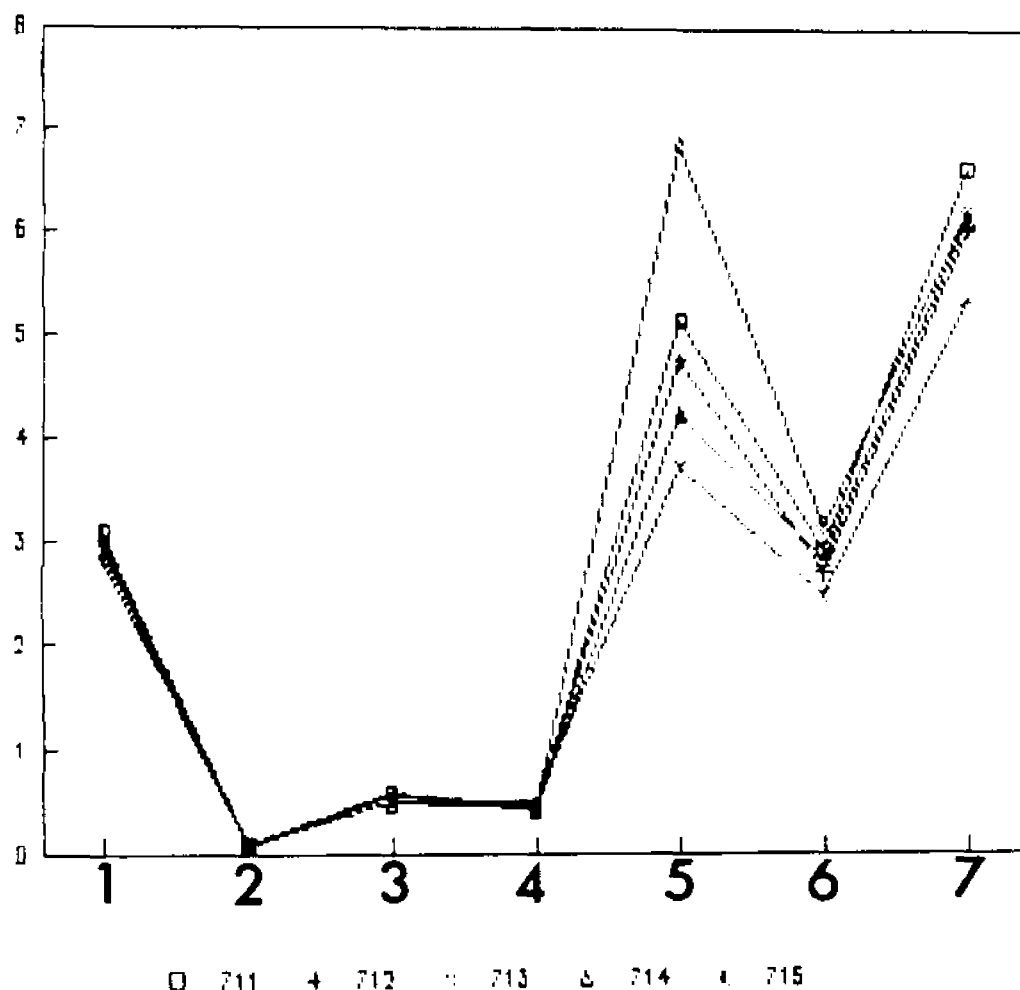
| <u>Density in square microns</u>    |             |                    |                   |
|-------------------------------------|-------------|--------------------|-------------------|
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 0.003083    | 0.000926           | 0.000136          |
| Epl.T                               | 0.000066    | 0.000075           | 0.000011          |
| Epl.O                               | 0.000473    | 0.000194           | 0.000029          |
| Mcl.M                               | 0.000408    | 0.000173           | 0.000027          |
| Mcl.O                               | 0.004239    | 0.000928           | 0.000145          |
| Ipl                                 | 0.002848    | 0.000765           | 0.000123          |
| Gcl                                 | 0.006119    | 0.000675           | 0.000105          |
| <u>Density in cubic millimeters</u> |             |                    |                   |
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 3083000.00  | 926000.00          | 136000.00         |
| Epl.T                               | 66883.84    | 75433.28           | 11639.61          |
| Epl.O                               | 473000.00   | 194000.00          | 29248.14          |
| Mcl.M                               | 408000.00   | 173000.00          | 27330.22          |
| Mcl.O                               | 4239000.00  | 928000.00          | 145000.00         |
| Ipl                                 | 2848000.00  | 765000.00          | 123000.00         |
| Gcl                                 | 6119000.00  | 675000.00          | 105000.00         |

Rat #715: Perfused

| <u>Density in square microns</u>    |             |                    |                   |
|-------------------------------------|-------------|--------------------|-------------------|
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 0.002943    | 0.000915           | 0.000140          |
| Epl.T                               | 0.000074    | 0.000056           | 0.000008          |
| Epl.O                               | 0.000476    | 0.000155           | 0.000023          |
| Mcl.M                               | 0.000481    | 0.000156           | 0.000023          |
| Mcl.O                               | 0.003732    | 0.000953           | 0.000147          |
| Ipl                                 | 0.002499    | 0.000766           | 0.000120          |
| Gcl                                 | 0.005349    | 0.001165           | 0.000174          |
| <u>Density in cubic millimeters</u> |             |                    |                   |
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 2943000.00  | 915000.00          | 140000.00         |
| Epl.T                               | 74874.00    | 56600.00           | 8839.58           |
| Epl.O                               | 476000.00   | 155000.00          | 23345.68          |
| Mcl.M                               | 481000.00   | 156000.00          | 23857.66          |
| Mcl.O                               | 3732000.00  | 953000.00          | 147000.00         |
| Ipl                                 | 2499000.00  | 766000.00          | 120000.00         |
| Gcl                                 | 5349000.00  | 1165000.00         | 174000.00         |

Figure 10.

Line graph comparing density of cells per layer for five rat specimens.



711, 712, 713 = Non-perfused rats; 714, 715 = Perfused rats

Values represent mean density of cells within a layer.

- 1 = Glomerular layer.
- 2 = External plexiform layer, large tufted cells
- 3 = External plexiform layer, all other cells
- 4 = Mitral cell layer, mitral cells
- 5 = Mitral cell layer, all other cells
- 6 = Internal plexiform layer
- 7 = Granule cell layer

## ***2. Volume of Layers Containing Mature Neurons***

The values generated by the Planar Morphometry program represent the total area of each layer for those histological slides measured. These data are computed in square microns by the Planar Morphometry program and are listed in Table 6 (page 244). The total volume of each layer within the olfactory bulb of each rat specimen has been converted to cubic millimeters, the standard unit used in most publications, and is also listed in Table 6. The number of histological sections retained and stained for each specimen, and the number of sections actually measured are given in Table 6: this information is necessary to convert square microns to cubic millimeters (see Methods). Figure 11 (page 247) is a line graph illustrating the volume of each layer in each of the five rat specimens.

Table 6

Volume of layers containing mature neurons within the olfactory bulb:

- 1) Original data in square microns: this sum represents the total ~~area~~ of all histological sections measured within one specimen.
- 2) Data in cubic millimeters: this sum represents the total ~~volume~~ of that layer within the olfactory bulb of the specimen.

Rat #711: Non-Perfused

| <u>Area in square microns: Original data</u> |            |                                |
|----------------------------------------------|------------|--------------------------------|
|                                              | <u>Sum</u> |                                |
| Glo.l                                        | 18797450   | # histological sections        |
| Epl                                          | 21734860   | <u>stained: 43</u>             |
| Mcl                                          | 4659230    |                                |
| Ipl                                          | 2596717    | <u># sections measured: 22</u> |
| Gcl                                          | 26026090   |                                |
| <u>Volume of layer in cubic millimeters</u>  |            |                                |
|                                              | <u>Sum</u> |                                |
| Glo.l                                        | 3.674047   |                                |
| Epl                                          | 4.248177   |                                |
| Mcl                                          | 0.910667   |                                |
| Ipl                                          | 0.507540   |                                |
| Gcl                                          | 5.086917   |                                |

Glo.l = Glomerular layer  
 Epl = External plexiform layer  
 Mcl = Mitral cell layer  
 Ipl = Internal plexiform layer  
 Gcl = Granule cell layer

Table 6 continued

## Rat #712: Non-Perfused

| <u>Area in square microns: Original data</u> |            |                                 |
|----------------------------------------------|------------|---------------------------------|
|                                              | <u>Sum</u> |                                 |
| Glo.1                                        | 18019130   | # of histological sections      |
| Epl                                          | 19802150   | <u>stained: 46</u>              |
| Mcl                                          | 4781573    |                                 |
| Ipl                                          | 2446280    | <u># sectioned measured: 22</u> |
| Gcl                                          | 23836660   |                                 |
| <u>Volume of layer in cubic millimeters</u>  |            |                                 |
|                                              | <u>Sum</u> |                                 |
| Glo.1                                        | 3.767636   |                                 |
| Epl                                          | 4.140449   |                                 |
| Mcl                                          | 0.999783   |                                 |
| Ipl                                          | 0.511494   |                                 |
| Gcl                                          | 4.984028   |                                 |

## Rat 713: Non-Perfused

| <u>Area in square microns: Original data</u> |            |                                   |
|----------------------------------------------|------------|-----------------------------------|
|                                              | <u>Sum</u> |                                   |
| Glo.1                                        | 14284090   | # of histological sections        |
| Epl                                          | 20439650   | <u>stained: 42</u>                |
| Mcl                                          | 4291169    |                                   |
| Ipl                                          | 2329600    | <u># of sections measured: 20</u> |
| Gcl                                          | 22243390   |                                   |
| <u>Volume of layer in cubic millimeters</u>  |            |                                   |
|                                              | <u>Sum</u> |                                   |
| Glo.1                                        | 2.999658   |                                   |
| Epl                                          | 4.292326   |                                   |
| Mcl                                          | 0.901145   |                                   |
| Ipl                                          | 0.489216   |                                   |
| Gcl                                          | 4.671111   |                                   |

Table 6 continued

## Rat #714: Perfused

| <u>Area in square microns: Original data</u> |            |                                                  |
|----------------------------------------------|------------|--------------------------------------------------|
|                                              | <u>Sum</u> |                                                  |
| Glo.1                                        | 13934960   | # of histological sections<br><u>stained: 45</u> |
| Epl                                          | 22202800   |                                                  |
| Mcl                                          | 4210528    | # sections measured: <u>22</u>                   |
| Ipl                                          | 1807708    |                                                  |
| Gcl                                          | 23954420   |                                                  |
|                                              |            |                                                  |
| <u>Volume of layer in cubic millimeters</u>  |            |                                                  |
|                                              | <u>Sum</u> |                                                  |
| Glo.1                                        | 2.850332   |                                                  |
| Epl                                          | 4.541481   |                                                  |
| Mcl                                          | 0.861244   |                                                  |
| Ipl                                          | 0.369758   |                                                  |
| Gcl                                          | 4.899766   |                                                  |

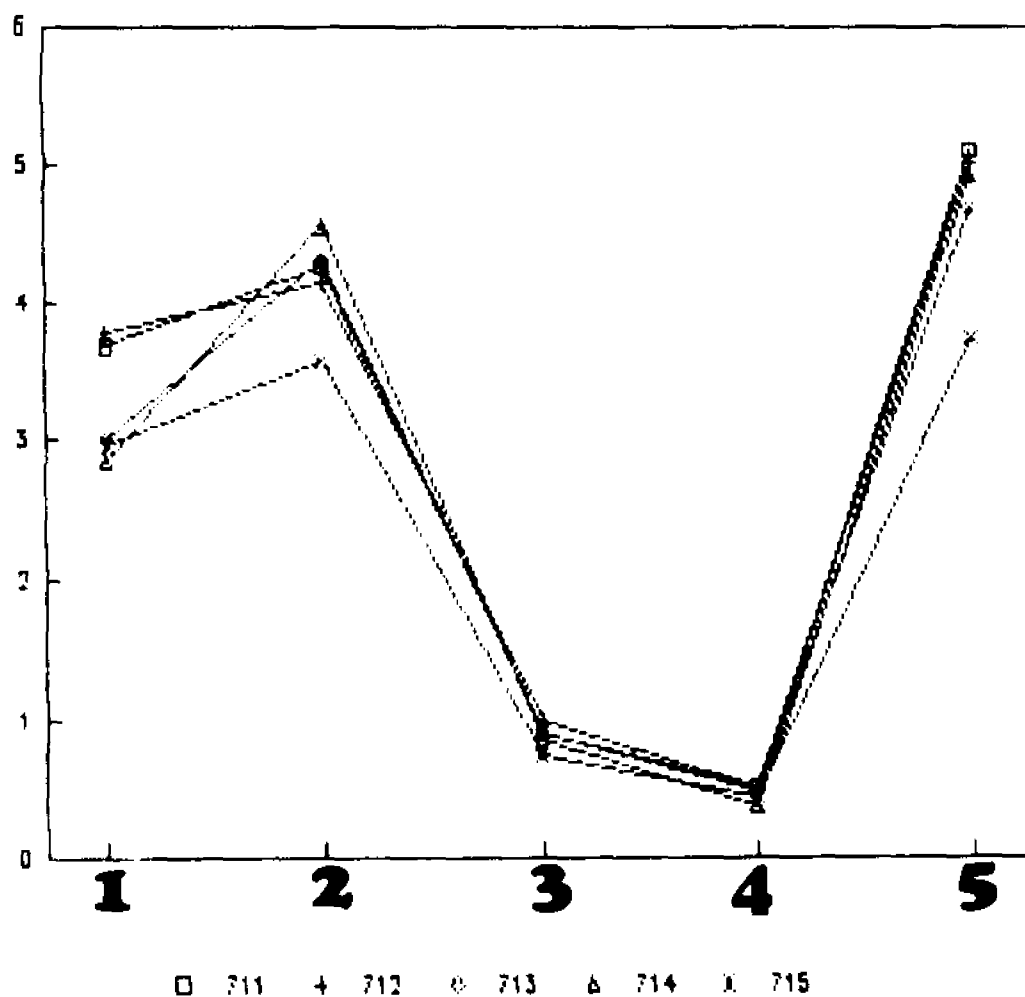
## Rat #715: Perfused

| <u>Area in square microns: Original data</u> |            |                                               |
|----------------------------------------------|------------|-----------------------------------------------|
|                                              | <u>Sum</u> |                                               |
| Glo.1                                        | 17410830   | # histological sections<br><u>stained: 39</u> |
| Epl                                          | 21012680   |                                               |
| Mcl                                          | 4405906    | # sections measured: <u>23</u>                |
| Ipl                                          | 2599818    |                                               |
| Gcl                                          | 22014830   |                                               |
|                                              |            |                                               |
| <u>Volume of layer in cubic millimeters</u>  |            |                                               |
|                                              | <u>Sum</u> |                                               |
| Glo.1                                        | 2.952271   |                                               |
| Epl                                          | 3.563019   |                                               |
| Mcl                                          | 0.747088   |                                               |
| Ipl                                          | 0.440838   |                                               |
| Gcl                                          | 3.732949   |                                               |



Figure 11

Line graph comparing volume of olfactory bulb layers for five rat specimens.



711, 712, 713 = Non-perfused rats; 714, 715 = Perfused rats

- 1 = Glomerular layer
- 2 = External plexiform layer
- 3 = Mitral cell layer
- 4 = Internal plexiform layer
- 5 = Granule cell layer

#### IV. STATISTICAL ANALYSIS

The analysis of variance (ANOVA) consisted of comparing mean density of cells of all layers containing mature neurons in the five rats. This was computed using the software program Sigmastat. Sigmastat was also used to compute an analysis of variance comparing volume of those layers containing mature neurons for the five rat specimens.

Based on degrees of freedom computed by the Planar Morphometry program for t-test analysis (range: 53-149; mean 101),  $p$  of  $\leq 0.01$  was accepted as representing statistically significant differences for both analysis of variance and T-test. However, all values representing  $p \leq 0.05$  are listed in the following tables.

##### A. RESULTS: ANALYSIS OF VARIANCE (ANOVA)

###### *1. Density of Cells*

The mean, standard deviation, and standard error in cubic millimeters for density of cells in the olfactory bulb of each rat specimen is given in Table 5 (page 239). Non-perfused (#711, 712, 713) and perfused (#714, 715) specimens were included in the analysis. The analysis of variance for mean density of cells is given in Table 7 (page 250).

## *2. Volume of Layers*

The volume (in cubic millimeters) of each layer containing mature neurons in the five rats was used in this analysis: these data are listed in Table 6 (page 244). Non-perfused (#711, 712, 713) and perfused (#714, 715) specimens were included in the analysis. The analysis of variance for volume of layers containing mature neurons in five rat specimens is given in given in Table 8 (page 250).

Table 7

Analysis of variance (ANOVA): Mean density of cells for five rat specimens.

Five rats included in analysis:

711, 712, 713, 714, 715

Layers included in analysis:

(Layers containing mature neurons)

Glo.1, Epl.T, Epl.O, Mcl.M, Mcl.O, Ipl, Gcl

| F     | P     |
|-------|-------|
| 0.081 | 0.988 |

Table 8

Analysis of variance: Volume of individual layers in cubic millimeters for five rat specimens.

Five rats included in analysis:

711, 712, 713, 714, 715

Layers included in analysis:

(Layers containing mature neurons)

Glo.1, Epl, Mcl, Ipl, Gcl

| F     | P     |
|-------|-------|
| 0.078 | 0.988 |

### ***3. Interpretation of Results***

The F value for one way analysis of variance for mean density of cells within all layers containing mature neurons of the five rat specimens is 0.081,  $p = 0.988$ . The differences that exist are not statistically significant. Therefore, it is impossible to rule out that all existing differences between the five specimens are due entirely to random variation.

One way analysis of variance for volume of all layers within the olfactory bulbs of five rat specimens gives an F value of 0.078,  $p = 0.988$ . Again, the differences are not statistically significant and cannot be ruled out as being due entirely to random variation.

### **B. ADDITIONAL STATISTICAL ANALYSIS: T-TEST**

#### ***1. Introduction and Results***

Figures 10 and 11 (pages 241, 247) graph the comparative density of cells and total volume of each olfactory bulb layer for the five rat specimens. Although analysis of variance for mean density of cells and total volume of all layers included in the study demonstrates that any differences that exist are probably due to random variation, t-test analyses were performed comparing: 1) the

density of cells in each layer of one rat against the identical layer in each other rat; 2) the mean volume for each layer containing mature neurons of three non-perfused rats compared with the mean for two perfused rats.

For mean density of cells, individual cell populations in each rat were compared against the same population in every other rat. This analysis of pairs was done to determine whether a particular cell population within the olfactory bulbs of the five rats was more variable in mean density of cells than were other layers within the bulb. For comparisons of the density of cells between an individual layers of two specimens, t-values were computed using the statistical package incorporated within the Planar Morphometry program: these values are derived using all of the data originally computed by the Planar Morphometry program. The t-values for level of significance of  $p \leq 0.05$  are listed in Table 9 (page 253).

For volume, a t-test analysis of the mean of three non-perfused rats compared with the mean of the two perfused rats was computed: results are given in Table 10 (page 254).

Table 9

T-Test results: comparison of density of cells within individual layers of olfactory bulbs of five rats.

Comparison of Five Rats: T Values for  $p \leq 0.05$

| Layer | Var. 1 | Var. 2 | DF | T val. | P        |
|-------|--------|--------|----|--------|----------|
| Glo.L |        |        |    |        |          |
| Epl.T | 712    | 713    | 82 | 2.072  | <0.05    |
|       | 712    | 714    | 82 | 2.089  | <0.05    |
|       | 712    | 715    | 81 | 3.356  | <0.01 *  |
| Epl.O | 711    | 714    | 86 | 2.858  | <0.01 *  |
|       | 711    | 715    | 86 | 3.102  | <0.01 *  |
|       | 712    | 714    | 86 | 2.881  | <0.01 *  |
|       | 712    | 715    | 86 | 3.189  | <0.01 *  |
|       | 713    | 714    | 83 | 2.254  | <0.05    |
|       | 713    | 715    | 83 | 2.502  | <0.02    |
| Mcl.M | 714    | 715    | 81 | 2.020  | <0.05    |
| Mcl.O | 711    | 714    | 81 | 4.371  | <0.001 * |
|       | 711    | 715    | 82 | 6.799  | <0.001 * |
|       | 712    | 714    | 82 | 2.436  | <0.02    |
|       | 712    | 715    | 83 | 4.847  | <0.001 * |
|       | 714    | 715    | 81 | 2.436  | <0.02    |
| Ipl   | 711    | 715    | 72 | 2.437  | <0.02    |
|       | 712    | 713    | 70 | 2.352  | <0.05    |
|       | 713    | 715    | 73 | 3.407  | <0.01 *  |
|       | 714    | 715    | 78 | 2.038  | <0.05    |
| Gcl   | 711    | 712    | 82 | 4.019  | <0.001 * |
|       | 711    | 713    | 78 | 2.473  | <0.02    |
|       | 711    | 714    | 80 | 3.057  | <0.01 *  |
|       | 711    | 715    | 84 | 5.859  | <0.001 * |
|       | 712    | 715    | 86 | 3.257  | <0.01 *  |
|       | 713    | 715    | 82 | 3.733  | <0.001 * |
|       | 714    | 715    | 84 | 3.704  | <0.001 * |

Var. = Variable

Glo.l = Glomerular layer

Epl.T = Large tufted cells, external plexiform layer

Epl.O = All remaining cells, external plexiform layer

Mcl.M = Mitral cell, mitral cell layer

Mcl.O = All remaining cells, mitral cell layer

Ipl = Internal plexiform layer

Gcl = Granule cell layer

Table 10

T-Test for volume: comparison of total volume of individual olfactory bulb layers between the mean for three non-perfused rats with the mean for two perfused rats.

| Var. 1                    | Var.2                | T Value | P     |
|---------------------------|----------------------|---------|-------|
| mean for 711,<br>712, 713 | mean for<br>714, 715 | 0.026   | 0.079 |



## 2. Interpretation of Results

The analysis of t-values for density of cells within each layer indicates that the greatest variation occurs between the comparison of a non-perfused rat with a perfused rat. Seventy t-tests were conducted between individual rats on density of cells within specific layers. Of the fifteen differences in density of cells that reached a significance of  $P \leq 0.01$ , thirteen out of the fifteen occur in comparisons between a non-perfused and a perfused rat. This indicates that a greater degree in variation between these groups exists than is found within either group. Only one out of fifteen of the differences indicated by the t-tests are within a populations of cells known to be second order neurons, indicating that the density of these cells is less subject to variation than populations of other cells located within the olfactory bulb. There may be an association between this finding, and the finding that within the glomerular layer, there is no variation in density of cells between the five rat specimens. This will be discussed under Conclusions (page 256).

The t-analysis comparing the mean values for volume of three non-perfused rats with two perfused rats confirmed the results of the analysis of variance. With a t- value of 0.026,  $P = 0.079$ , it must be concluded that variation in volume within the rat sample is due strictly to random

effects.

Based on the statistical analysis of variance, there is no evidence that either the mean density of cells or volume of layers within the olfactory bulb of the five rat specimens analyzed were significantly altered due to a change in fixation technique.

## V. CONCLUSIONS

Based on the analysis of variance, all of the variation found within the series of five rats must be attributed to random variation; none of the variation can be attributed to differences in fixation technique: none of the variation is statistically significant. Therefore, comparing the olfactory bulbs of six additional mammals fixed either by perfusion or immersion technique is valid: any differences that occur between these various mammalian species cannot be attributed to differences in fixation technique based on the analysis of variance of the five rat specimens. Chapter 7 discusses the comparative analysis of additional mammalian species.

As previously stated, t-test analyses comparing density of cells of a particular layer in any two rat specimens were done in the interest of identifying whether random variation occurred with a greater frequency in any particular cell population. Results of these analyses indicated that the

least amount of variation for density of cells is found within the known second order neurons population and within that layer in which these cells receive their input stimulus, the glomerular layer. There may be two explanations for this. 1) The density of these cells is tightly controlled whereas the density of all other cells, particularly the interneuron population may fluctuate more without compromising function within the system. Given that the second order neurons both receive sensory information from the olfactory epithelium and transmit this information to more central regions within the brain, the stability of their numbers may be necessary to insure a certain level of information processing within the species whereas the various interneuron populations may be more flexible. The variety of interneuron populations within the several layers of the olfactory bulb may compensate for one another; an increase in dendritic endings or number of spines may compensate for a decrease in cell density of a particular interneuron type. 2) An alternate hypothesis is that the findings are an artifact of the methodology used, particularly its limitations. The mitral cells and large tufted cells are morphologically unmistakable due to their large size, and identifying and counting them is relatively easy. In all other layers and cell populations, the density of cells represents not only various types of neurons but also an unknown number of glial cells. This variety of cell

type comprising the population of cells being counted may be responsible for greater variation between individual specimens as is indicated by the t-test analyses.

## CHAPTER 7

### COMPARATIVE MAMMALIAN STUDY

#### I. INTRODUCTION

A morphological and statistical analysis of five mammalian species was conducted. Of primary interest was whether the olfactory bulb of the catarrhine primate, historically considered to be microsmatic, significantly differed in general morphological appearance or statistically in terms of density of cells or relative volume per layer when compared with four macrosmatic mammals. Included in the study are two specimens of *Macaca mulatta*, one specimen of *Macaca fascicularis*, one squirrel (*Sciurus carolinensis*), one cat, (*Felis cattus*), one laboratory rabbit (New Zealand White), and the Taconic white rat, represented by the mean of the data for five rat specimens which were discussed in Chapter 6.

Data collection includes: 1) a description of the component layers of the olfactory bulb of six species (the olfactory bulb of the rat is described in Chapter 6, page 237); and 2) density of cells and volume of each of the layers containing mature neurons within the olfactory bulb.

The original data are given in square microns and are converted into cubic millimeters, the standard unit of measurement used in most publications. In addition, the volume of each layer is listed as percent of the total volume for all layers containing mature neurons. Three additional were made: 1) the relationship of volume of those layers containing mature neurons to those layers that do not contain functioning synapses (subventricular layer and ventricular space); 2) a comparative analysis of log bulb to log brain size in the various mammalian species; and 3) the proportion of second order neurons to all other cells within each species.

An additional comparison generated by the statistical analysis consists of an analysis of the degree of lamination that exists within the olfactory bulb of the various species. The evidence is considered as to whether parcellation has occurred within the evolutionary development of the catarrhine olfactory bulb.

## **II. RESULTS**

### **A. MORPHOLOGY OF OLFACTORY BULB LAYERS**

#### **1. *Macaca mulatta* (#118, 119)**

**Glomeruli** generally form a single row encircling the

deeper layers of the bulb, but in one specimen (#119) there are regions where the glomeruli are more randomly placed forming clumps or rows of glomeruli. The majority of cells within the **glomerular layer** are usually found along the deep border adjacent to the external plexiform layer: within the caudal bulb, the density of these cells increases. The visual impression is one of a series of hammocks slung from glomerulus to glomerulus, forming a border with the external plexiform layer. These cells extend into the regions between the glomeruli, particularly in regions where more than a single layer of glomeruli exist. The extension of these cells superficially is never pronounced, but in the rostral portion of the bulb, there are areas where the cells extend around the glomeruli and form a superficial border between them and the olfactory nerve layer: this is not the predominant picture for the entire bulb. There are many cells within the **external plexiform layer** and the borders of this layer are hard to determine due to the spread of cells from the glomerular and mitral cell layers into it (Figure 12 (page 264)). In some regions, there are many large tufted cells located throughout the breadth of the external plexiform layer, but a greater number of these cells are located closer to the mitral cell layer. The cells of the **mitral cell layer** spread into the external plexiform and internal plexiform layers, forming a layer that is quite broad and creating borders that are not well defined. The

broadness of this layer is due mainly to granule cells since there is a paucity of mitral cells compared with granule cells in this layer. Also, it is the spread of granule cells that is responsible for the lack of formation of clear borders in this layer. In some regions of the bulb, the mitral cells are spaced widely apart while in other regions they form aggregations. The **internal plexiform layer** is often difficult to distinguish due to the density of granule cells located within it. In some regions, the granule cells of the granule cell layer and mitral cell layers merge, and there is no distinguishable internal plexiform layer. In other regions, an internal plexiform layer is revealed because the granule cells within the granule cell layer line up in rows leaving a relative cell free region between themselves and the mitral cell layer: this occurs most frequently in the caudal half of the bulb. There are many cells within the **granule cell layer**: clumping and formation of cell-rows does occurs, but rows, when present, are short and do not give a "railroad track" appearance. The subventricular layer consists only of a cell-free region central to the glomerular cell layer. Spaces between the aggregations of granule cells are loaded with individual granule cells.



*Macaca fascicularis* (#128)

The **glomeruli** form a well-defined row with clear borders. The majority of glomerular layer cells are located at the border with the external plexiform layer, although the region between adjacent glomeruli are always clearly indicated by cells which have extended up into this region. The **external plexiform layer** is not wide. The borders between the external plexiform layer and adjacent glomerular and mitral cell layers are well defined (Figure 12, page 264). There are few large tufted cells present in this layer. The width of the **mitral cell layer** is about five granular cells wide. Mitral cells are less well stained in the rostral end of the bulb: their density is greater in the mid and caudal regions of the bulb where they are better stained. The cells of the mitral cell layer form a well defined row of cells clearly demarcated from adjacent layers. The **internal plexiform layer** is a distinct layer containing few granule cells rostrally, with an increase in number of granule cells caudally. The **granule cell layer** is loaded with cells forming rows resembling discontinuous railroad tracks; although clumps of cells are present, rows are more common. The granule cells of the lateral half of the bulb cross the midline and form a near continuous layer with the granule cells of the medial half raising the question as to why there is no definable subventricular

Figure 12. Definition of borders between layers is affected by overlap of cell populations.

- A. *Macaca mulatta* (X 25). Notice the irregularity of borders due to overlap of cells into adjacent layers (arrows).
- B. *Macaca fascicularis* (X 25). Borders are sharp: cells do not overlap into adjacent layers.

E = External plexiform layer

M = mitral cell layer

I = internal plexiform layer

arrows = overlap of cells into adjacent layers

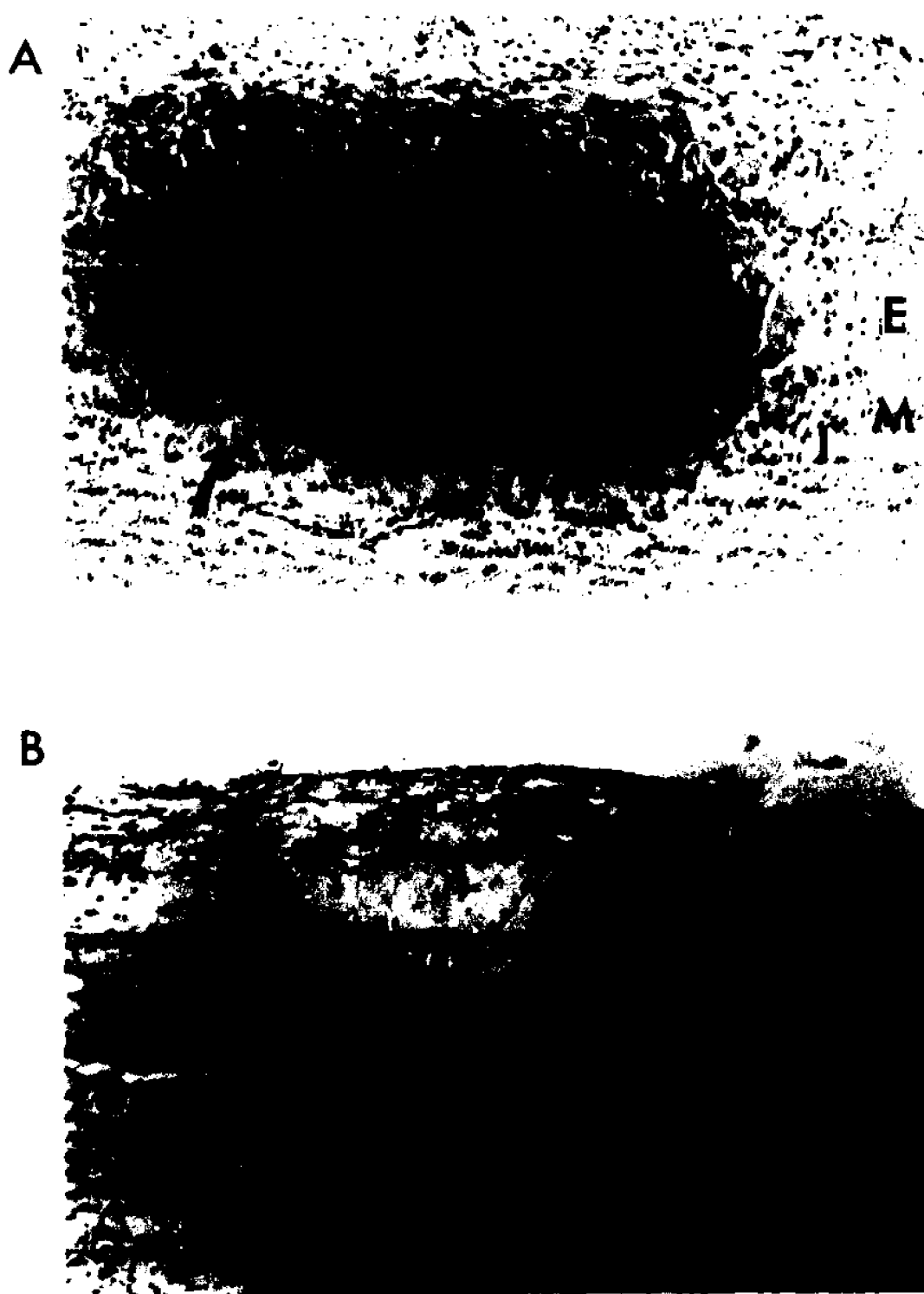


Figure 12

layer in this specimen. There is a very small slit down the center of this bulb which could be mistaken for a small ventricular space but is not: fragmentation of the borders can be seen when this region is closely examined under the microscope. This very thin tear does not account for the lack of a subventricular region in this specimen.

### 3. Cat (#604)

In the olfactory bulb of the cat, the **glomeruli** form single rows in some areas and more than one row in other areas. This layer is thick with cells particularly along the border with the external plexiform layer and with cells extending between adjacent glomeruli. Some of these superficially located cells form a narrow border between the glomeruli and the olfactory nerve layer. The **external plexiform layer** is large rostrally and loaded with cells, but the borders between this layer and adjacent glomerular and mitral cell layers are clearly demarcated. The external plexiform layer narrows caudally but remains clearly demarcated from the adjacent layers. What is striking in the rostral part of the bulb is that the majority of large tufted cells are located within the superficial half of this layer, close to the glomerular layer. Few of these cells are located adjacent to the mitral cell layer. The **mitral cell layer** is relatively narrow, approximating the diameter

of five to six granule cells. Mitral cells are moderate in number, their density increasing caudally. The borders of the mitral cell layer are clearly demarcated. The **internal plexiform layer** is present in most regions of the bulb and is clearly demarcated rostrally but somewhat less so caudally. There are a moderate number of granule cells present within this layer: rostrally these cells do not obscure the boundaries of the internal plexiform layer with the adjacent layers, but caudally, the increase in their density makes border demarcation less clear. The **granule cell layer** is characterized by clumps of cells rather than rows, although rows are formed to a minor degree in some regions (particularly caudally). Throughout the length of the bulb, the distribution of the granule cells provides a border between this layer and the internal plexiform layer. There is a large subventricular region which is populated by small cells. There is a region of overlap between the borders of this region with the granule cell layer, particularly rostrally. Caudally, there are collections of cells within this region which resemble cortical neurons. There is an extension of the ventricle into the olfactory bulb. Surprisingly, the accessory olfactory bulb was not seen in this specimen.

#### 4. Rabbit (# 902)

The **glomeruli** in the rabbit form a single layer which contains a low density of cells. Some of the cells extend between the glomeruli: infrequently they form a border between glomeruli and the adjacent olfactory nerve layer. The density of cells within the **external plexiform layer** is unremarkable. Large tufted cells are present throughout the external plexiform layer and are not concentrated along the border with either the external plexiform or mitral cell layers. In the rostral bulb, the cells of the glomerular layer extend into the external plexiform layer and form a region of overlap. More caudally in the bulb, both the glomerular, external plexiform and mitral cell layers have formed sharper borders. Although there is some migration of cells from the **mitral cell layer** into the external plexiform layer, the border between the two layers is always clearly demarcated. The mitral cell layer is approximately four to five granule cells wide. The density of mitral cells varies: rostrally, many regions have very few mitral cells while the density of these cells increases the mid and caudal regions of the bulb. The **internal plexiform layer** is present throughout the bulb, but in some regions, is obscured by rows of granule cells which extend into it. The **granule cell layer** is characterized by clumping of cells but there are some rows present. There is a large

subventricular region, and a ventricular space is present in the caudal region of the bulb. An accessory olfactory bulb is present caudally.

#### 5. *Squirrel (#810)*

In the squirrel, glomeruli form a single row with some regions characterized by small double-tiered groups. The glomeruli are surrounded by cells, many located in great numbers along the border with the olfactory nerve layer as well as along the border with the external plexiform layer. The external plexiform layer is wide. Rostrally, it is clearly demarcated from the glomerular layer; caudally the boundary between the two layers is less clearly demarcated due to an overlap of glomerular cells into the external plexiform layer. The border between the external plexiform layer and mitral cell layer is clearly demarcated although there is some spread of the mitral layer cells into the external plexiform layer. Those cells resembling mitral cells but located within the external plexiform layer are considered to be large tufted cells: rostrally there are not many large tufted cells present, but those that are located close to the border with the mitral cell layer. Caudally, there is an increase in the number of large tufted cells within the external plexiform layer and these are positioned near the glomerular layer. The width of the mitral cell

**layer** equals the diameter of many granule cells. This layer forms a clearly demarcated row. The **internal plexiform layer** is not clearly demarcated in many sections, and when it is, it contains numerous cells. The **granule cell layer** is wide, and is characterized by many clumps of granule cells. Although the granule cell layer merges with the **subventricular layer**, there is a decrease in the density of cells which indicates the approximate border between the two layers. An extension of the ventricle is present in the olfactory bulb of the squirrel. The accessory olfactory bulb is present caudally.

## **6. Rat**

For the description of the olfactory bulb of the rat, refer to Chapter 6 (page 237).

## **B. NUMERICAL DATA**

### **1. Density of Cells**

Density of cells was computed by the Planar Morphometry program in square microns. Mean, standard deviation, standard error for density of cells within each layer of the olfactory bulb in the two specimens of *Macaca mulatta* and



one specimen of *Macaca fascicularis* are given in Table 11 (page 272). In addition, the data for density of cells for the three *Macaca* specimens have been combined and the mean for the three specimens is given in Table 12 (page 274). This mean represents the genus *Macaca* in the comparative analysis: the justification for this is based on the analysis of variance discussed below (page 300). Based on the results of the comparative analysis of the five rat specimens, the data for cell density within each layer of the five rats has been combined and is given as the mean for the five specimens (Table 12). Table 12 also includes the density of cells for each layer of the olfactory bulb in the cat, rabbit and squirrel. All data have also been converted to cubic millimeters, the standard unit of measurement used in most publications.

Figure 13 (page 277) is a line graph demonstrating the density of cells within each layer of the olfactory bulb of the five species being compared.

Table 11

Density of cells within the olfactory bulb of two specimens of *Macaca mulatta* and one specimen of *Macaca fascicularis*.

*Macaca mulatta* # 118

| Density in square microns: Original Data |          |             |            |
|------------------------------------------|----------|-------------|------------|
|                                          | Mean     | Stand. Dev. | Stand. Er. |
| Glo.l                                    | 0.001944 | 0.000631    | 0.000081   |
| Epl.T                                    | 0.000029 | 0.000033    | 0.000004   |
| Epl.O                                    | 0.000615 | 0.000226    | 0.000030   |
| Mcl.M                                    | 0.000152 | 0.000106    | 0.000014   |
| Mcl.O                                    | 0.002908 | 0.000961    | 0.000130   |
| Ipl                                      | 0.001659 | 0.000432    | 0.000094   |
| Gcl                                      | 0.003301 | 0.000728    | 0.000098   |

| Density in cubic millimeters |            |             |            |
|------------------------------|------------|-------------|------------|
|                              | Mean       | Stand. Dev. | Stand. Er. |
| Glo.l                        | 1944000.00 | 631000.00   | 81483.10   |
| Epl.T                        | 29627.34   | 33009.66    | 4372.23    |
| Epl.O                        | 615000.00  | 226000.00   | 30423.13   |
| Mcl.M                        | 152000.00  | 106000.00   | 14455.60   |
| Mcl.O                        | 2908000.00 | 961000.00   | 130000.00  |
| Ipl                          | 1659000.00 | 432000.00   | 94237.25   |
| Gcl                          | 3301000.00 | 728000.00   | 98193.01   |

Glo.l = Glomerular layer

Epl.T = External plexiform layer, large tufted cells

Epl.O = External plexiform layer, all other cells

Mcl.M = Mitral cell layer, mitral cells

Mcl.O = Mitral cell layer, all other cells

Ipl = Internal plexiform layer

Gcl = Granule cell layer

Table 11 continued

*Macaca mulatta* #119

| <u>Density in square microns: Original Data</u> |             |                    |                   |
|-------------------------------------------------|-------------|--------------------|-------------------|
|                                                 | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                                           | 0.001784    | 0.000506           | 0.000070          |
| Epl.T                                           | 0.000046    | 0.000063           | 0.000009          |
| Epl.O                                           | 0.000780    | 0.000433           | 0.000061          |
| Mcl.M                                           | 0.000122    | 0.000099           | 0.000014          |
| Mcl.O                                           | 0.003648    | 0.000934           | 0.000136          |
| Ipl                                             | 0.001398    | 0.000600           | 0.000092          |
| Gcl                                             | 0.004373    | 0.001186           | 0.000175          |

| <u>Density in cubic millimeters</u> |             |                    |                   |
|-------------------------------------|-------------|--------------------|-------------------|
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 1784000.00  | 506000.00          | 70165.61          |
| Epl.T                               | 46157.16    | 63077.27           | 90110.39          |
| Epl.O                               | 780000.00   | 433000.00          | 61899.56          |
| Mcl.M                               | 122000.00   | 99617.39           | 14530.69          |
| Mcl.O                               | 3648000.00  | 934000.00          | 136000.00         |
| Ipl                                 | 1398000.00  | 600000.00          | 92560.93          |
| Gcl                                 | 4373000.00  | 1186000.00         | 175000.00         |

*Macaca fascicularis* #128

| <u>Density in square microns: Original data</u> |             |                    |                   |
|-------------------------------------------------|-------------|--------------------|-------------------|
|                                                 | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                                           | 0.002871    | 0.000759           | 0.000120          |
| Epl.T                                           | 0.000021    | 0.000025           | 0.000003          |
| Epl.O                                           | 0.000668    | 0.000208           | 0.000032          |
| Mcl.M                                           | 0.000148    | 0.000118           | 0.000018          |
| Mcl.O.                                          | 0.004400    | 0.000985           | 0.000160          |
| Ipl                                             | 0.001581    | 0.000444           | 0.000074          |
| Gcl                                             | 0.003510    | 0.000638           | 0.000097          |

| <u>Density in cubic millimeters</u> |             |                    |                   |
|-------------------------------------|-------------|--------------------|-------------------|
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 2871000.00  | 759000.00          | 120000.00         |
| Epl.T                               | 21318.13    | 25529.08           | 3986.97           |
| Epl.O                               | 668000.00   | 208000.00          | 32863.11          |
| Mcl.M                               | 148000.00   | 118000.00          | 18411.84          |
| Mcl.O                               | 4400000.00  | 98500.00           | 16000.00          |
| Ipl                                 | 1581000.00  | 444000.00          | 74993.63          |
| Gcl                                 | 3510000.00  | 638000.00          | 97318.12          |

Table 12

Density of cells within the olfactory bulb of the following genera: the mean for three specimens of *Macaca*; one specimen of cat; one specimen of rabbit; one specimen of squirrel; the mean for five Taconic white rat specimens.

*Macaca*: mean for three specimens

| Density in square microns    |            |             |            |
|------------------------------|------------|-------------|------------|
|                              | Mean       | Stand. Dev. | Stand. Er. |
| Glo.l                        | 0.002134   | 0.000768    | 0.000062   |
| Epl.T                        | 0.000033   | 0.000044    | 0.000003   |
| Epl.O                        | 0.000686   | 0.000315    | 0.000026   |
| Mcl.M                        | 0.000141   | 0.000108    | 0.000009   |
| Mcl.O                        | 0.003562   | 0.001126    | 0.000095   |
| Ipl                          | 0.001519   | 0.000520    | 0.000052   |
| Gcl                          | 0.003706   | 0.000990    | 0.000082   |
| Density in cubic millimeters |            |             |            |
|                              | Mean       | Stand. Dev. | Stand. Er. |
| Glo.l                        | 2134000.00 | 768000.00   | 62200.00   |
| Epl.T                        | 33000.00   | 44000.00    | 3600.00    |
| Epl.O                        | 686000.00  | 315000.00   | 26200.00   |
| Mcl.M                        | 141000.00  | 108000.00   | 9000.00    |
| Mcl.O                        | 3562000.00 | 1126000.00  | 95100.00   |
| Ipl                          | 1519000.00 | 520000.00   | 52500.00   |
| Gcl                          | 3706000.00 | 990000.00   | 82000.00   |

Glo.l = Glomerular layer

Epl.T = External plexiform layer, large tufted cells

Epl.O = External plexiform layer, all other cells

Mcl.M = Mitral cell layer, mitral cells

Mcl.O = Mitral cell layer, all other cells

Ipl = Internal plexiform layer

Gcl = Granule cell layer

Table 12 continued

Cat #604

| <u>Density in square microns: original data</u> |             |                    |                   |
|-------------------------------------------------|-------------|--------------------|-------------------|
|                                                 | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                                           | 0.001756    | 0.000586           | 0.000063          |
| Epl.T                                           | 0.000053    | 0.000056           | 0.000006          |
| Epl.O                                           | 0.000319    | 0.000428           | 0.000046          |
| Mcl.M                                           | 0.000250    | 0.000152           | 0.000017          |
| Mcl.O                                           | 0.004090    | 0.008141           | 0.000905          |
| Ipl                                             | 0.001325    | 0.000381           | 0.000043          |
| Gcl                                             | 0.003706    | 0.000496           | 0.000055          |

| <u>Density in cubic millimeters</u> |             |                    |                   |
|-------------------------------------|-------------|--------------------|-------------------|
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 1756000.00  | 586000.00          | 63210.97          |
| Epl.T                               | 53852.71    | 56337.39           | 6110.64           |
| Epl.O                               | 319000.00   | 428000.00          | 46652.63          |
| Mcl.M                               | 250000.00   | 152000.00          | 17000.48          |
| Mcl.O                               | 4090000.00  | 8141000.00         | 905000.00         |
| Ipl                                 | 1325000.00  | 381000.00          | 43125.07          |
| Gcl                                 | 3706000.00  | 496000.00          | 55470.73          |

Rabbit #902

| <u>Density in square microns: Original data</u> |             |                    |                   |
|-------------------------------------------------|-------------|--------------------|-------------------|
|                                                 | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                                           | 0.002044    | 0.000638           | 0.000084          |
| Epl.T                                           | 0.000017    | 0.000023           | 0.000003          |
| Epl.O                                           | 0.000370    | 0.000118           | 0.000015          |
| Mcl.M                                           | 0.000225    | 0.000133           | 0.000018          |
| Mcl.O                                           | 0.003949    | 0.000781           | 0.000107          |
| Ipl                                             | 0.001299    | 0.000482           | 0.000067          |
| Gcl                                             | 0.002844    | 0.000558           | 0.000076          |

| <u>Density in cubic millimeters</u> |             |                    |                   |
|-------------------------------------|-------------|--------------------|-------------------|
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 2044000.00  | 638000.00          | 84552.66          |
| Epl.T                               | 17799.28    | 23299.71           | 3086.12           |
| Epl.O                               | 370000.00   | 118000.00          | 15919.44          |
| Mcl.M                               | 225000.00   | 133000.00          | 18613.09          |
| Mcl.O                               | 3949000.00  | 781000.00          | 107000.00         |
| Ipl                                 | 1299000.00  | 482000.00          | 67505.74          |
| Gcl                                 | 2844000.00  | 558000.00          | 76700.33          |

Table 12 continued

## Squirrel #810

| <u>Density in square microns: Original data</u> |             |                    |                   |
|-------------------------------------------------|-------------|--------------------|-------------------|
|                                                 | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                                           | 0.003082    | 0.000917           | 0.000114          |
| Epl.T                                           | 0.000035    | 0.000069           | 0.000009          |
| Epl.O                                           | 0.000538    | 0.000802           | 0.000103          |
| Mcl.M                                           | 0.000376    | 0.000646           | 0.000086          |
| Mcl.O                                           | 0.004691    | 0.000978           | 0.000132          |
| Ipl                                             | 0.002472    | 0.000746           | 0.000099          |
| Gcl                                             | 0.004964    | 0.001161           | 0.000152          |

|       | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
|-------|-------------|--------------------|-------------------|
| Glo.l | 3082000.00  | 917000.00          | 114000.00         |
| Epl.T | 35165.89    | 69414.27           | 9036.96           |
| Epl.O | 538000.00   | 802000.00          | 103000.00         |
| Mcl.M | 376000.00   | 646000.00          | 86313.22          |
| Mcl.O | 4691000.00  | 978000.00          | 132000.00         |
| Ipl   | 2472000.00  | 746000.00          | 99735.52          |
| Gcl   | 4964000.00  | 1161000.00         | 152000.00         |

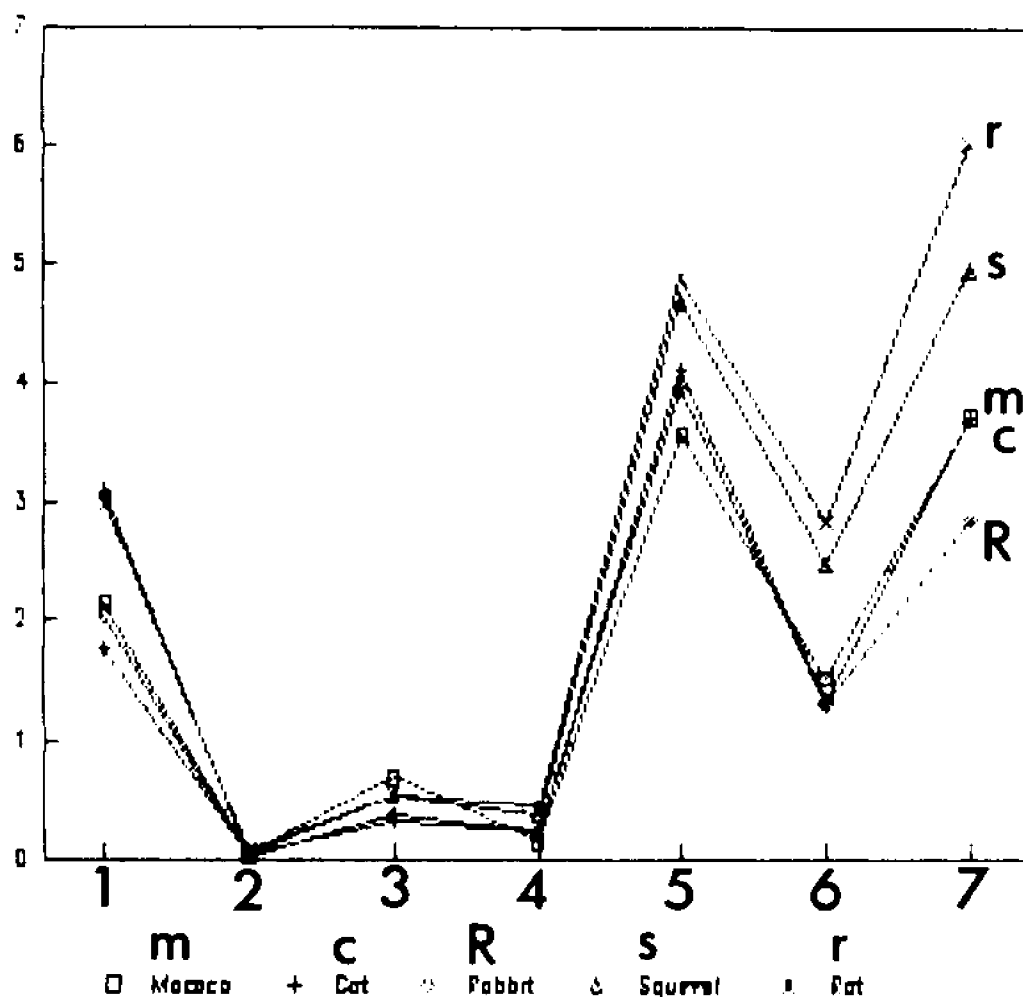
## Rat: Mean for five specimens

| <u>Density in square microns</u> |             |                    |                   |
|----------------------------------|-------------|--------------------|-------------------|
|                                  | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                            | 0.003003    | 0.000919           | 0.000061          |
| Epl.T                            | 0.000061    | 0.000065           | 0.000004          |
| Epl.O                            | 0.000534    | 0.000171           | 0.000011          |
| Mcl.M                            | 0.000440    | 0.000487           | 0.000033          |
| Mcl.O                            | 0.004903    | 0.005886           | 0.000410          |
| Ipl                              | 0.002823    | 0.000821           | 0.000060          |
| Gcl                              | 0.006034    | 0.000919           | 0.000063          |

| <u>Density in cubic millimeters</u> |             |                    |                   |
|-------------------------------------|-------------|--------------------|-------------------|
|                                     | <u>Mean</u> | <u>Stand. Dev.</u> | <u>Stand. Er.</u> |
| Glo.l                               | 3003000.00  | 919000.00          | 61800.00          |
| Epl.T                               | 61800.00    | 65700.00           | 4520.00           |
| Epl.O                               | 534000.00   | 171000.00          | 11600.00          |
| Mcl.M                               | 440000.00   | 487000.00          | 33900.00          |
| Mcl.O                               | 4903000.00  | 5886000.00         | 410000.00         |
| Ipl                                 | 2823000.00  | 821000.00          | 60300.00          |
| Gcl                                 | 6034000.00  | 919000.00          | 63500.00          |

Figure 13  
Line graph comparing density of cells per layer for five mammals.



Values represent mean density of cells within a layer.

- 1 = Glomerular layer
- 2 = External plexiform layer, Large tufted cells
- 3 = External plexiform layer, All other cells
- 4 = Mitral cell layer, mitral cells
- 5 = Mitral cell layer, all other cells
- 6 = Internal plexiform layer
- 7 = Granule cell layer

## 2. Volume of Layers Containing Mature Neurons

The Planar Morphometry program computes the area of each layer containing mature neurons for those histological slides measured in square microns. These data have to be converted to: 1) total volume in cubic microns for each layer within the olfactory bulb; 2) total volume in cubic microns is converted to cubic millimeters, the standard unit of measurement used in most publications.

Due to damage to the caudal olfactory bulb in one specimen of *Macaca mulatta*, the area for each layer in this species is based on a single specimen (#118). The original data for area of layers containing mature neurons in those slides measured for *Macaca mulatta* and *Macaca fascicularis* (computed by Planar Morphometry in square microns), and its conversion into total volume in cubic millimeters is given in Table 13 (page 281). Based on: 1) the difference in size of the olfactory bulbs of *Macaca mulatta* and *Macaca fascicularis*, and; 2) the fact that the subventricular layer (discussed below) in *Macaca fascicularis* could not be identified, the data for volume in specimen #118 (*Macaca mulatta*) is the only specimen representing the genus *Macaca* in Table 14. Based on the results of the comparative analysis of the five rat specimens, the data for area and for volume of each layer of the five rat specimens has been combined and is given as the mean for the five specimens



used in the comparative mammalian analysis (Table 14, page 282). Table 14 also includes the original data for the rabbit and squirrel as computed by the Planar Morphometry program as well as the total volume of each layer in cubic millimeters in these species. The number of histological sections comprising the length of the olfactory bulb, and the number of sections actually measured are necessary to determine the total volume of each layer. This information is included in Table 13 and Table 14.

In the cat olfactory bulb, a region of the superficial layers were torn away: this affected the glomerular and external plexiform layer in particular. As this missing portion approximated the shape of a rectangle, it was reconstructed without great difficulty. Therefore, area of layers containing mature neurons in the cat consists of direct measurement of individual layers plus measurements of reconstructed missing portions (also computed using Image Analysis and the Planar Morphometry program). The sum of these data is included in Table 14 and is given as area in square microns and its conversion into volume in cubic millimeters.

The volume of the olfactory bulb representing the sum of those layers containing mature neurons is given in Table 15 (page 284).

The size of the olfactory bulb in the mammalian species included in this analysis varies greatly. Because of this,

the comparative analysis for volume of those layers containing mature neurons had to be based on volume as percent of the total sum of all layers containing mature neurons. The values for each layer as percent are given in Table 16 (page 285). For the rat, the volume as percent is based on the mean values representing the five Taconic white rat specimens.

Figure 14 (page 286) is a line graph comparing the volume of each layer as percent of the total sum in each of the five mammalian genera included in this analysis.

Table 13

Volume of layers containing mature neurons within the olfactory bulb for *Macaca mulatta* and *Macaca fascicularis*:

1) Original data in square microns: this sum represents the total area of all histological sections measured within one specimen.

2) Data in cubic millimeters: this sum represents the total volume for that layer within the olfactory bulb.

*Macaca mulatta* #118

| <u>Area in square microns: Original data</u> |            |                                 |
|----------------------------------------------|------------|---------------------------------|
|                                              | <u>Sum</u> |                                 |
| Glo.1                                        | 25100160   | # of histological sections      |
| Epl                                          | 19452910   | <u>stained and retained: 58</u> |
| Mcl                                          | 5452899    |                                 |
| Ipl                                          | 1804175    | <u># sections measured: 28</u>  |
| Gcl                                          | 29965390   |                                 |
| <u>Volume of layer in cubic millimeters</u>  |            |                                 |
|                                              | <u>Sum</u> |                                 |
| Glo.1                                        | 5.1993     |                                 |
| Epl                                          | 4.0295     |                                 |
| Mcl                                          | 1.1295     |                                 |
| Ipl                                          | 0.3737     |                                 |
| Gcl                                          | 6.2074     |                                 |

*Macaca fascicularis* #128

| <u>Area in square microns: Original data</u> |            |                                 |
|----------------------------------------------|------------|---------------------------------|
|                                              | <u>Sum</u> |                                 |
| Glo.1                                        | 11840170   | # of histological sections      |
| Epl                                          | 10811410   | <u>stained and retained: 42</u> |
| Mcl                                          | 3961554    |                                 |
| Ipl                                          | 1679811    | <u># sections measured: 20</u>  |
| Gcl                                          | 17377010   |                                 |
| <u>Volume of layer in cubic millimeters</u>  |            |                                 |
|                                              | <u>Sum</u> |                                 |
| Glo.1                                        | 2.4864     |                                 |
| Epl                                          | 2.2704     |                                 |
| Mcl                                          | 0.8319     |                                 |
| Ipl                                          | 0.3528     |                                 |
| Gcl                                          | 3.6492     |                                 |

Table 14

Volume of layers containing mature neurons within the olfactory bulb. Included are the data for the following mammals: one cat; one rabbit; one squirrel; the mean for five Taconic white rat specimens.

Cat #604

| <u>Area in square microns: Original data</u>     |                                                  |                                      |            |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------|------------|
| <u>Undamaged portion</u>                         |                                                  | <u>Reconstructed segment</u>         |            |
|                                                  | <u>Sum</u>                                       |                                      | <u>Sum</u> |
| Glo.1                                            | 46530380                                         | Glo.1                                | 4500324    |
| Epl                                              | 54103840                                         | Epl                                  | 5338918    |
| Mcl                                              | 10082170                                         | Mcl                                  | 370499     |
| Ipl                                              | 11145430                                         | Ipl                                  | 250841     |
| Gcl                                              | 88848740                                         | -                                    | -          |
| # of histological sections stained and retained: |                                                  | # sections stained: damaged portion  |            |
| 84                                               |                                                  | 45                                   |            |
| # sections measured: undamaged portion           |                                                  | # sections measured: damaged portion |            |
| 23                                               |                                                  | 11                                   |            |
| <u>Volume of layer in cubic millimeters</u>      |                                                  |                                      |            |
|                                                  | <u>Sum: undamaged plus reconstructed portion</u> |                                      |            |
| Glo.1                                            | 18.8347                                          |                                      |            |
| Epl                                              | 21.9437                                          |                                      |            |
| Mcl                                              | 3.8338                                           |                                      |            |
| Ipl                                              | 4.1735                                           |                                      |            |
| Gcl                                              | 32.4491                                          |                                      |            |

Glo.1 = Glomerular layer  
 Epl = External plexiform layer  
 Mcl = Mitral cell layer  
 Ipl = Internal plexiform layer  
 Gcl = Granule cell layer

Table 14 continued

Rabbit #902

| <u>Area in square microns: Original data</u> |            |                                 |
|----------------------------------------------|------------|---------------------------------|
|                                              | <u>Sum</u> |                                 |
| Glo.1                                        | 20646530   | # of histological sections      |
| Epl                                          | 26143590   | <u>stained and retained: 57</u> |
| Mcl                                          | 51870000   |                                 |
| Ipl                                          | 3959610    | <u># sections measured: 15</u>  |
| Gcl                                          | 33140560   |                                 |
| <u>Volume of layer in cubic millimeters</u>  |            |                                 |
|                                              | <u>Sum</u> |                                 |
| Glo.1                                        | 7.8457     |                                 |
| Epl                                          | 9.9357     |                                 |
| Mcl                                          | 1.9711     |                                 |
| Ipl                                          | 1.5046     |                                 |
| Gcl                                          | 12.5934    |                                 |

Squirrel #810

| <u>Area in square microns: Original data</u> |            |                                 |
|----------------------------------------------|------------|---------------------------------|
|                                              | <u>Sum</u> |                                 |
| Glo.1                                        | 41438660   | # of histological sections      |
| Epl                                          | 55198170   | <u>stained and retained: 58</u> |
| Mcl                                          | 8206223    |                                 |
| Ipl                                          | 10628100   | <u># sections measured: 21</u>  |
| Gcl                                          | 58695550   |                                 |
| <u>Volume of layer in cubic millimeters</u>  |            |                                 |
|                                              | <u>Sum</u> |                                 |
| Glo.1                                        | 11.4449    |                                 |
| Epl                                          | 15.2452    |                                 |
| Mcl                                          | 2.2665     |                                 |
| Ipl                                          | 2.9354     |                                 |
| Gcl                                          | 16.2111    |                                 |

Table 14 continued

Rat: Mean for five specimens combined

| <u>Area in square microns: Mean of five specimens</u> |            |
|-------------------------------------------------------|------------|
|                                                       | <u>Sum</u> |
| Glo.1                                                 | 16489292   |
| Epl                                                   | 21038428   |
| Mcl                                                   | 4469681    |
| Ipl                                                   | 2356024    |
| Gcl                                                   | 23615078   |
| <u>Volume in cubic millimeters: Layer volume</u>      |            |
|                                                       | <u>Sum</u> |
| Glo.1                                                 | 3.248788   |
| Epl                                                   | 4.157090   |
| Mcl                                                   | 0.883985   |
| Ipl                                                   | 0.463769   |
| Gcl                                                   | 4.674954   |

Table 15

Volume for sum of those layers containing mature neurons

Volume in cubic millimeters

| <u>Macaca</u>   | <u>Macaca</u>       | <u>Cat</u> | <u>Rabbit</u> |
|-----------------|---------------------|------------|---------------|
| <u>mulatta</u>  | <u>fascicularis</u> |            |               |
| 16.9391         | 9.5907              | 81.2348    | 33.8494       |
| <u>Squirrel</u> | <u>Rat</u>          |            |               |
| 48.1031         | 13.4286             |            |               |

Table 16

Volume of individual layers containing mature neurons as percent of total volume of these layers. This comparative analysis includes the volume as percent derived from the mean value representing the five Taconic white rats; percent volume for *Macaca mulatta*, *Macaca fascicularis*, the cat, rabbit and squirrel.

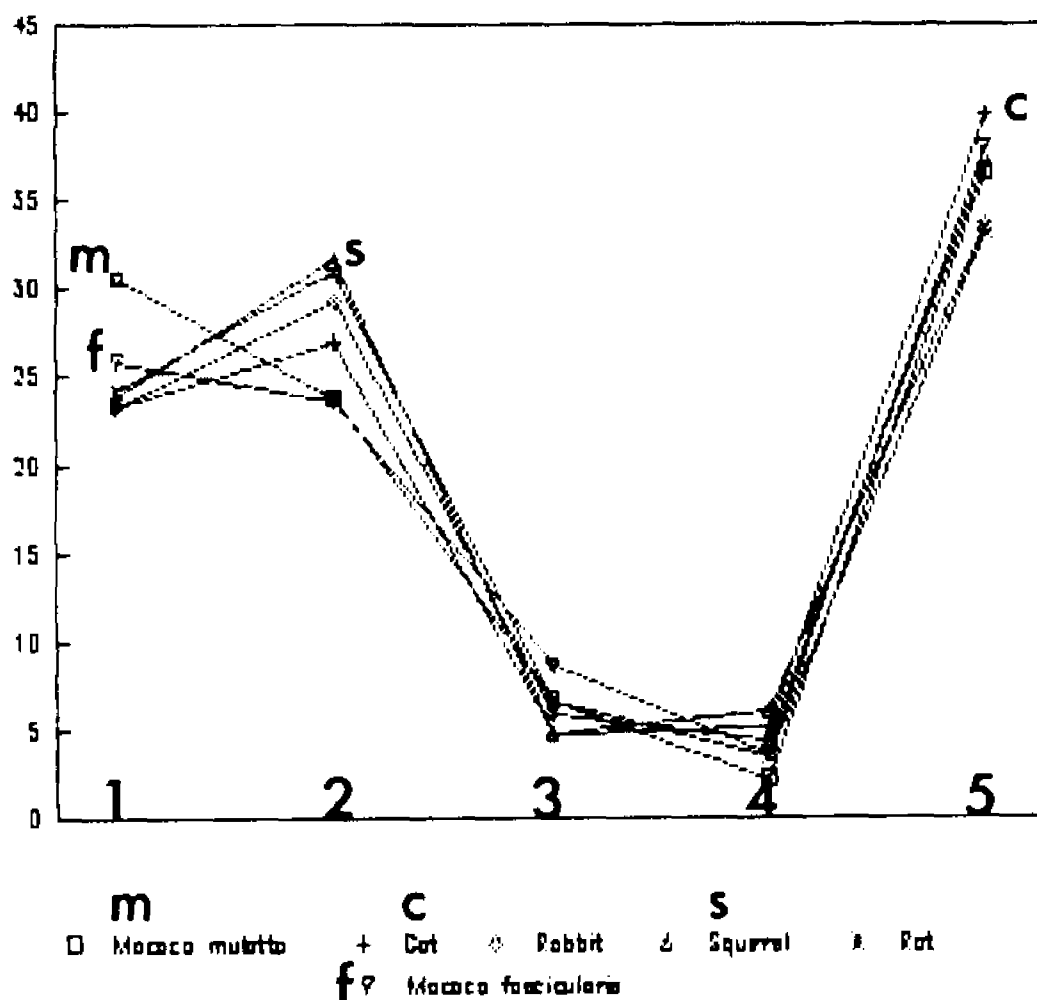
Volume as percent of sum of those layers containing mature neurons.

|       | Macaca<br>mulatta | Macaca<br>fascicularis | Cat<br>#604 | Rabbit<br>#902 |
|-------|-------------------|------------------------|-------------|----------------|
| Glo.1 | 30.69%            | 25.92%                 | 23.18%      | 23.18%         |
| Epl   | 23.79             | 23.67                  | 27.01       | 29.35          |
| Mcl   | 6.67              | 8.67                   | 4.72        | 5.82           |
| Ipl   | 2.21              | 3.68                   | 5.14        | 4.44           |
| Gcl   | 36.64             | 38.05                  | 39.94       | 37.20          |
|       | Squirrel<br>#810  | Rat<br>(mean)          |             |                |
| Glo.1 | 23.79%            | 24.19%                 |             |                |
| Epl   | 31.69             | 30.96                  |             |                |
| Mcl   | 4.71              | 6.58                   |             |                |
| Ipl   | 6.10              | 3.45                   |             |                |
| Gcl   | 33.70             | 33.32                  |             |                |

Glo.1 = Glomerular layer  
 Epl = External plexiform layer  
 Mcl = Mitral cell layer  
 Ipl = Internal plexiform layer  
 Gcl = Granule cell layer

Figure 14

Line graph comparing volume of individual layers containing mature neurons as percent of total volume of these layers.



- 1 = Glomerular layer
- 2 = External plexiform layer
- 3 = Mitral cell layer
- 4 = Internal plexiform layer
- 5 = Granule cell layer



### *3. Volume of the Subventricular Layer and Ventricular Space*

A ventricular space is present in the three largest olfactory bulbs included in this study. Under microscopic examination, it is evident that the subventricular layer is largest in those species with a ventricular space (Figure 15, page 288). The question arose as to whether the inclusion of subventricular layer and ventricular space substantially changes the total volume of the olfactory bulb. Since synapses occur only within those layers containing mature neurons, does an increase in olfactory bulb size indicate a proportionate increase in volume of regions able to process information or is some of the increase attributable simply to a presence of fluid-filled space (olfactory ventricle) and the non-synaptic layer containing immature neurons (the subventricular layer) that surrounds it? Although the olfactory bulb of the catarrhine primate has historically been considered to be unable to process olfactory information as effectively as those species with larger olfactory bulbs, the relative percent of layers containing mature neurons to total olfactory bulb size may be a more accurate assessment of ability to process information than is that of olfactory bulb size alone.

Area of the subventricular layer and ventricular space

Figure 15. Subventricular layer and ventricular space .

A. Olfactory bulb of the squirrel (X 5).

B. Olfactory bulb of the rat (X 5)

S = subventricular layer

arrow = subventricular layer

v = ventricular space



Figure 15

for those slides measured was computed using the Planar Morphology program: data were generated in square microns and are listed in Table 17 (page 291). The number of histological sections containing these regions is also given, as well as the number of sections actually measured. The total volume of the subventricular layer and ventricular space are given in cubic millimeters (Table 17).

The total volume for the olfactory bulb including subventricular layer and ventricular space is given in Table 18 (page 291). This is contrasted with the total volume of layers containing mature neurons. The volume of each layer as percent of total bulb volume is listed in Table 19 (292). The percent of the all layers containing mature neurons combined is compared with the percentage of the subventricular layer and ventricular space combined.

Table 17

Subventricular layer and ventricular space: area in square microns represents sum of the area for those slides measured. Volume in cubic millimeters represents **total sum** of the subventricular layer and ventricular space in each olfactory bulb included in the comparison. Values for the rat represent the mean for the five specimens.

Volume of subventricular layer and ventricular space

| <u>Area in square microns: Original data</u> |                   |          |          |          |               |
|----------------------------------------------|-------------------|----------|----------|----------|---------------|
|                                              | Macaca<br>mulatta | Cat      | Rabbit   | Squirrel | Rat<br>(mean) |
| SVL                                          | 945077            | 42841520 | 15974910 | 21531860 | 1948332       |
| VS                                           | none              | 10396740 | 751087   | 4155250  | none          |
| <u>Total volume in cubic millimeters</u>     |                   |          |          |          |               |
|                                              | Macaca<br>mulatta | Cat      | Rabbit   | Squirrel | Rat<br>(mean) |
| SVL                                          | 0.6048            | 15.4229  | 6.3899   | 5.2292   | 0.7614        |
| VS                                           | none              | 3.5189   | 0.4256   | 1.3158   | none          |

Table 18

Bulb volumes in cubic millimeters

| <u>Layers containing mature neurons: total volume</u> |          |         |          |               |
|-------------------------------------------------------|----------|---------|----------|---------------|
| Macaca<br>mulatta                                     | Cat      | Rabbit  | Squirrel | Rat<br>(mean) |
| 16.9391                                               | 81.2348  | 33.8494 | 48.1031  | 13.4286       |
| <u>Total bulb volume: includes SVL and VS</u>         |          |         |          |               |
| 17.5439                                               | 100.1767 | 40.6650 | 54.6481  | 14.1899       |

SVL = subventricular layer

VS = ventricular space

Table 19

Individual layers as percent of total bulb volume.

Volume of layers as % of total bulb

|       | Macaca<br>mulatta | Cat    | Rabbit | Squirrel | Rat<br>(mean) |
|-------|-------------------|--------|--------|----------|---------------|
| Glo.l | 29.63%            | 18.80% | 19.29% | 20.94%   | 22.89%        |
| Epl   | 22.97             | 21.90  | 24.43  | 27.90    | 29.30         |
| Mcl   | 6.44              | 3.83   | 4.58   | 4.15     | 6.23          |
| Ipl   | 2.13              | 4.17   | 3.70   | 5.37     | 3.27          |
| Gcl   | 35.38             | 32.39  | 30.97  | 29.66    | 32.94         |
| Svl   | 3.45              | 14.40  | 15.71  | 9.57     | 5.36          |
| Vs    | 0                 | 3.51   | 1.05   | 2.41     | 0             |

Percent of layers containing mature neurons compared with percent of layers not containing synapses (subventricular layer and ventricular space).

|     | Macaca<br>mulatta | Cat    | Rabbit | Squirrel | Rat<br>(mean) |
|-----|-------------------|--------|--------|----------|---------------|
| MNL | 96.55%            | 80.24% | 83.24% | 88.02%   | 94.63%        |
| NSL | 3.45              | 19.75  | 16.36  | 11.98    | 5.37          |

Glo.l = Glomerular layer  
 Epl = External plexiform layer  
 Mcl = Mitral cell layer  
 Ipl = Internal plexiform layer  
 Gcl = Granule cell layer  
 Svl = Subventricular layer  
 Vs = Ventricular space

MNL = Layers containing mature neurons  
 NSL = Non-synaptic layers (subventricular layer and ventricular space)

The data indicate that compared with the cat, 16% more of the total volume of the olfactory bulb of *Macaca* is composed of layers containing mature neurons. The cat has the largest olfactory bulb in this series followed in decreasing order of size by the rabbit and squirrel: *Macaca* has 13.31% and 8.53% more volume, respectively, of layers containing mature neurons than do these species.

#### *4. Log Brain to Log Bulb Volume*

Historically, it has been the ratio of olfactory bulb size to brain size underlying the morphological criterion for categorization of a vertebrate as macrosmatic vs microsmatic. This has been particularly true of the haplorhine primates whose olfactory bulb is small relative to the size of its cerebral cortex. Although it was not possible to do an allometric comparison of brain size to olfactory bulb size due to the small sample size of this study, two comparisons were made: 1) log brain volume to the log bulb volume where bulb volume consists only of those layers containing mature neurons; 2) log brain volume to log of total olfactory bulb volume (layers containing mature neurons plus the subventricular layer and ventricular space). Figure 16 (page 295) is a line graph comparing log brain volume to both log bulb volumes.

Table 20

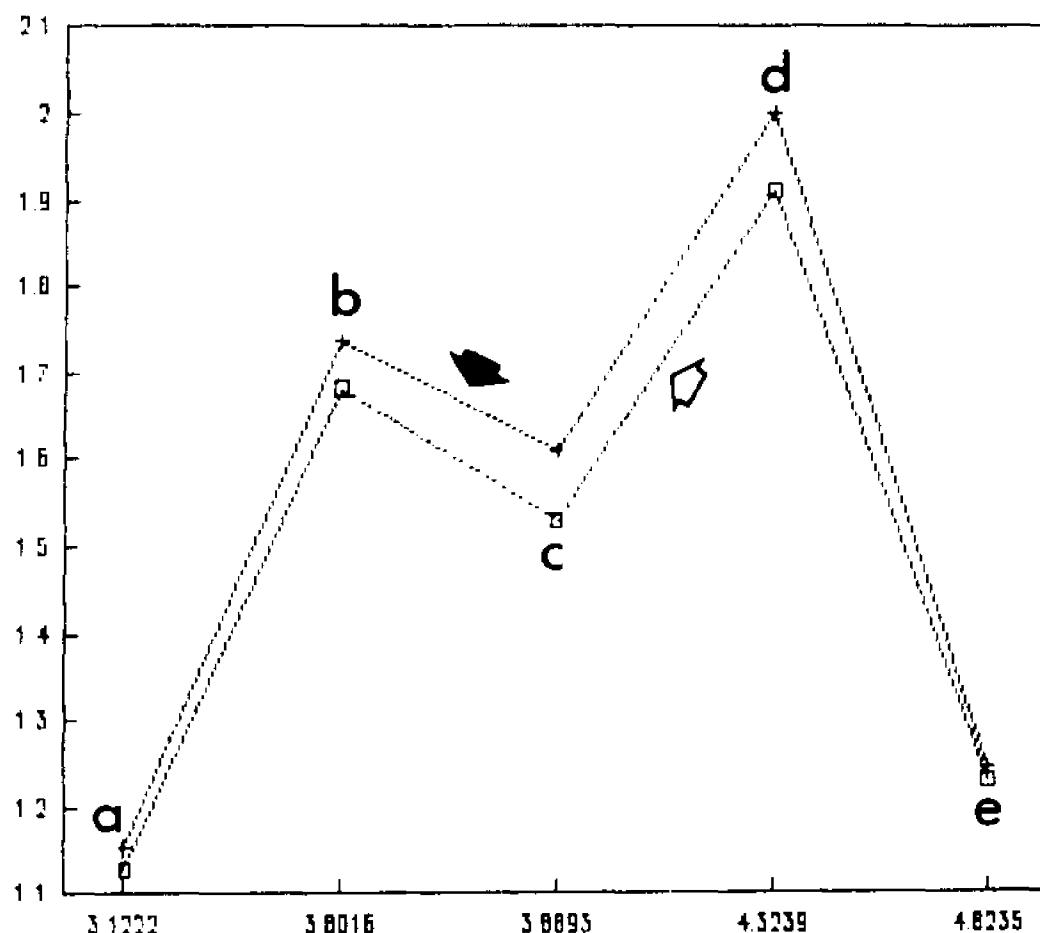
Brain volume in cubic millimeters  
 Log brain volume

| <u>Brain volume in cubic millimeters</u> |                 |               |            |               |
|------------------------------------------|-----------------|---------------|------------|---------------|
| <u>Rat</u>                               | <u>Squirrel</u> | <u>Rabbit</u> | <u>Cat</u> | <u>Macaca</u> |
| 1325                                     | 6333            | 7750          | 21,083     | 66,616        |
| <u>Log brain values</u>                  |                 |               |            |               |
| 3.1222                                   | 3.8016          | 3.8893        | 4.3239     | 4.8235        |



Figure 16

Comparison of log brain to log bulb volume where: 1) bulb volume consists of all layers including subventricular layer and ventricular space; 2) where bulb volume consists only of those layers containing mature neurons.



X axis = Log brain volume in order of increasing brain size  
Y axis = Log bulb volume

Closed arrow = Total bulb volume: all layers included  
Open arrow = Bulb volume includes only the volume of layers containing mature neurons

a = Rat  
b = Squirrel  
c = Rabbit  
d = cat  
e = *Macaca mulatta*

The sample size is small but for those species included in the analysis, there is a line whose slope runs in the proximity of the rat, squirrel, rabbit and cat. No matter at what slope the line is drawn, the rabbit is located above the line while *Macaca* is located below it. The rat, rabbit and cat fall either on or close to the line, depending on the angle of the slope. The shape of the graph does not change significantly by the addition or deletion of the volume for the subventricular layer and ventricular space combined. In either case, *Macaca mulatta* falls far below the line. This indicates that if a correlation exists between brain volume and olfactory bulb volume in mammals, *Macaca* has a very low bulb size compared with brain size (in this small sample).

#### **5. Relationship of Known Second Order Neurons To All Other Cells**

As discussed in Chapter 5, Mitra (1955) found that within the cerebral cortex, the proportion of interneurons to pyramidal cells increases as the neocortex expands. A functional significance may be attributed to this in view of the fact that primates, whose behavioral repertoire appears to be most dependent upon a highly developed visual system, also have the highest proportion of stellate cells (interneurons) to pyramidal cells within the visual cortex.

In this analysis, known (identifiable) second order neurons consist of mitral cells and large tufted cells which are both easily recognizable because of their large size and shape. Because some tufted cells are small and are therefore not identifiable by Nissl staining techniques, the second order neuron population does not represent the full number of tufted cells. Also, the remainder of the general cell population is comprised of several cell types: interneurons, small second order neurons, and a population of supporting cells. However, since interneurons must make up the majority of cells within the "other cell" population, it was felt that an analysis of the ratio of known second order neurons to "other cells" (considered to consist of a high percentage of interneurons) would be instructive. Using the data on density of cells located in Table 12 (page 274), the mean density of cells representing mitral and large tufted cells, and the mean density of cells representing all remaining cell populations was computed. These values, along with the ratio of known second order neurons to all other cells are given in Table 21 (page 298). Figure 17 (page 299) is a line graph demonstrating this ratio in the five species included in the comparative analysis.

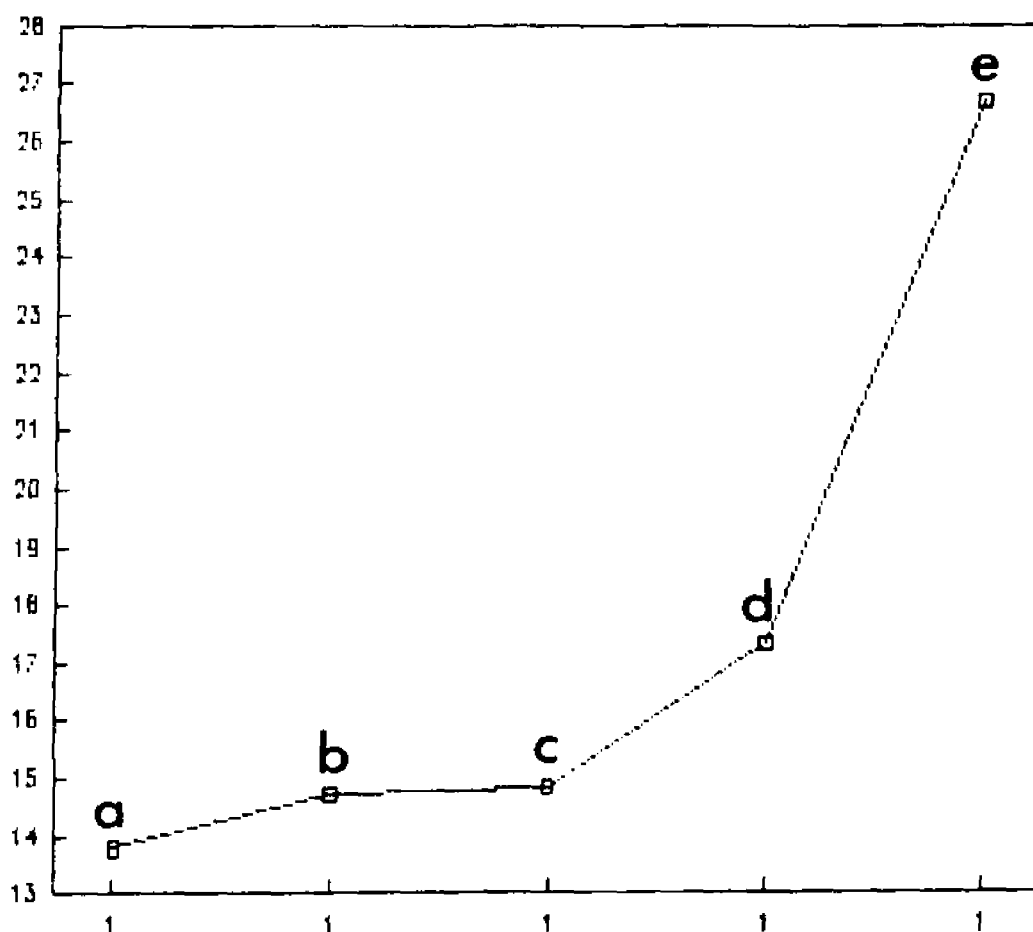
Table 21

Ratio of known second order neurons (mitral and large tufted cells) to "all other cells".

| <u>Mean density of cells: second order neurons</u>   |            |               |                 |            |
|------------------------------------------------------|------------|---------------|-----------------|------------|
| <u>Macaca</u>                                        | <u>Cat</u> | <u>Rabbit</u> | <u>Squirrel</u> | <u>Rat</u> |
| 87000                                                | 151926     | 121400        | 205583          | 250900     |
| <u>Mean density of cells: all other cells</u>        |            |               |                 |            |
| <u>Macaca</u>                                        | <u>Cat</u> | <u>Rabbit</u> | <u>Squirrel</u> | <u>Rat</u> |
| 2321400                                              | 2239200    | 2101200       | 3149400         | 3459400    |
| <u>Ratio of second order neurons/all other cells</u> |            |               |                 |            |
| <u>Macaca</u>                                        | <u>Cat</u> | <u>Rabbit</u> | <u>Squirrel</u> | <u>Rat</u> |
| 1:26.7                                               | 1:14.7     | 1:17.3        | 1:14.8          | 1:13.8     |

Figure 17

Ratio of known second order neurons to all other cells.



X axis = known second order neurons (as a unitary value)  
Y axis = all other cells (as a multiple of second order neurons)

a = rat  
b = cat  
c = squirrel  
d = rabbit  
e = Macaca

The analysis demonstrates that in *Macaca*, there is a significantly higher number of interneurons for every second order neurons than is found in any other mammalian species in this analysis.

### C. STATISTICAL ANALYSIS: ANALYSIS OF VARIANCE

#### 1. *Density of Cells*

An analysis of variance (ANOVA) for the two *Macaca mulatta* and one *Macaca fascicularis* specimens was conducted using the software program Sigmastat. This consisted of comparing the density of cells for all layers containing mature neurons for the three specimens. The result of this analysis is given in Table 22 (page 301) and indicates that the variation within the group is random. Therefore, the value for density of cells within each layer for the three specimens was combined, and the mean value used to represent *Macaca* in the analysis of variance for five mammalian species. The values for density of cells in each layer of the five rat specimens was combined and the mean represents the rat in this analysis. The analysis of variance for density of cells in five mammalian species is given in Table 23 (page 301).

Table 22

Analysis of variance: density of cells in three specimens of *Macaca*.

Two specimens of *Macaca mulatta* and one specimen of *Macaca fascicularis* included in the analysis: 118, 119, 128

Layers included in the analysis:  
(Layers containing mature neurons)  
Glo.1, Epl.T, Epl.O, Mcl.M, Mcl.O, Ipl, Gcl

| F     | P     |
|-------|-------|
| 0.635 | 0.542 |

Table 23

Analysis of variance: density of cells in five mammalian species.

Five mammalian species included in analysis:

*Macaca* (mean for three specimens), cat, rabbit, squirrel, rat (mean for five specimens)

Layers included in analysis (layers containing mature neurons):

Glo.1, Epl.T, Epl.O, Mcl.M, Mcl.O, Ipl, Gcl

| F     | P     |
|-------|-------|
| 0.745 | 0.569 |

The analysis of variance for density of cells in five mammalian species indicates that there is not a statistically significant difference among the treatment groups and therefore any variation that occurs is considered to be due random causes.

## *2. Volume of Layers*

The olfactory bulbs in the five mammalian species included in this study varied in volume as is demonstrated in Table 18 (page 291). It was not expected that an accurate assessment of the significance of variation that existed in specimens of obviously different volumes could be computed using actual values. However, an analysis of variance was performed for volume of the five mammalian species and the results are given in Table 24 (page 303).



Table 24

Analysis of variance of volume in cubic millimeters

Five mammals included in analysis:  
*Macaca mulatta*, cat, rabbit, squirrel, rat  
(mean for five specimens)

Layers included in analysis:  
(Layers containing mature neurons)  
Glo.1, Epl, Mcl, Ipl, Gcl

| F     | P     |
|-------|-------|
| 3.315 | 0.031 |

Pairwise multiple comparison shows  $P \leq 0.05$   
between the following specimens:  
cat vs rat  
cat vs *Macaca mulatta*

Analysis of variance for volume (actual values) indicate that if we accept  $p \leq 0.01$  as statistically significant, then the differences exhibited by this group of five mammals are not statistically significant. A better assessment of the differences, or lack of differences can be visualized in Figure 14 (page 286), a line graph of the volumes as percent of all layers containing mature neurons.

#### **D. ADDITIONAL ANALYSES**

##### ***1. T-Test Analysis***

Although the analysis of variance for mean density of cells illustrated that there was no statistically significant difference within the mammalian series, t-test analysis of individual layers between pairs of all mammals included in this study was conducted. It was of interest to see if the density of cells was more variable in particular layers or cell populations than in other layers. For comparisons between an individual layer of two specimens, t-values were derived using the statistical package incorporated within the Planar Morphometry program: these values were computed by Planar Morphometry using all original data stored within the data disc. The density of cells for individual layers in the three specimens of *Macaca* were combined and the mean was used in the comparative

mammalian analysis. The same procedure was used for the five rat specimens: the density of cells for each layer was combined for the five specimens and the mean was used in the comparative mammalian analysis. Because the mean values were computed by Planar Morphometry using the original data files, the degree of freedom in comparisons including *Macaca* (mean of three specimens), or the rat (mean of five specimens) are unusually high. The t-values for level of significance of  $p \leq 0.05$  are listed in Table 25 (page 306). As volume was generated from the data sum of the measurements on a given specimen, not on the mean as in density of cells, t-analysis of volume was not done. Also, it not was felt that knowledge of the variation between relative volume as percent of those layers containing mature neurons would be illuminating to questions based on how processing of information may differ.

Table 25

T-test results: Comparison of density of cells in layers containing mature neurons in five species of mammals.

| Layer | Var. 1 | Var. 2 | DF  | T val. | P      |
|-------|--------|--------|-----|--------|--------|
| Glo.1 | rat    | Macaca | 371 | 9.57   | 0.001  |
|       | rat    | cat    | 305 | 11.68  | 0.001  |
|       | rat    | rabbit | 276 | 7.42   | 0.001  |
|       | Macaca | cat    | 236 | 3.96   | 0.001  |
|       | Macaca | squir. | 215 | 7.84   | 0.001  |
|       | cat    | squir. | 149 | 10.81  | 0.001  |
|       | cat    | rabbit | 141 | 2.77   | 0.01   |
|       | squir. | rabbit | 120 | 7.15   | 0.01   |
| Epl.T | rat    | Macaca | 356 | 4.65   | 0.001  |
|       | rat    | rabbit | 266 | 4.97   | 0.001  |
|       | Macaca | cat    | 230 | 3.12   | 0.01   |
|       | Macaca | rabbit | 202 | 2.41   | 0.02 - |
|       | cat    | rabbit | 140 | 4.57   | 0.001  |
| Epl.O | rat    | Macaca | 359 | 5.91   | 0.001  |
|       | rat    | cat    | 299 | 6.24   | 0.001  |
|       | rat    | rabbit | 270 | 6.70   | 0.001  |
|       | Macaca | cat    | 226 | 7.42   | 0.001  |
|       | Macaca | squir. | 206 | 7.63   | 0.001  |
|       | Macaca | rabbit | 197 | 7.24   | 0.01   |
|       | Cat    | squir. | 143 | 2.12   | 0.05 - |
| Mcl.M | rat    | Macaca | 346 | 7.19   | 0.001  |
|       | rat    | cat    | 284 | 3.42   | 0.001  |
|       | rat    | rabbit | 255 | 3.12   | 0.02 - |
|       | Macaca | cat    | 220 | 6.22   | 0.001  |
|       | Macaca | squir. | 196 | 4.20   | 0.001  |
|       | Macaca | rabbit | 191 | 4.48   | 0.001  |
| Mcl.O | rat    | Macaca | 344 | 2.66   | 0.01   |
|       | Macaca | squir. | 193 | 6.53   | 0.001  |
|       | Macaca | rabbit | 191 | 2.30   | 0.05 - |
|       | squir. | rabbit | 106 | 0.001  |        |

Table 25 continued

| Layer | Var. 1 | Var. 2 | DF  | T val. | P      |
|-------|--------|--------|-----|--------|--------|
| Ipl   | rat    | Macaca | 281 | 14.27  | 0.001  |
|       | rat    | cat    | 261 | 15.42  | 0.001  |
|       | rat    | squir. | 239 | 2.86   | 0.01   |
|       | rat    | rabbit | 234 | 12.66  | 0.001  |
|       | Macaca | cat    | 174 | 2.75   | 0.01   |
|       | Macaca | squir. | 152 | 9.30   | 0.001  |
|       | Macaca | rabbit | 147 | 2.50   | 0.02 - |
|       | cat    | squir. | 132 | 11.63  | 0.001  |
|       | squir. | rabbit | 105 | 9.55   | 0.001  |
| Gcl   | rat    | Macaca | 351 | 66.65  | 0.001  |
|       | rat    | cat    | 287 | 21.46  | 0.001  |
|       | rat    | squir. | 265 | 7.38   | 0.001  |
|       | rat    | rabbit | 260 | 24.12  | 0.001  |
|       | Macaca | squir. | 200 | 7.77   | 0.01   |
|       | Macaca | rabbit | 195 | 5.99   | 0.01   |
|       | cat    | squir. | 137 | 8.67   | 0.001  |
|       | cat    | rabbit | 131 | 9.33   | 0.001  |
|       | squir. | rabbit | 109 | 12.07  | 0.001  |

T-test analysis for density of cells in those layers containing mature neurons fails to reveal any pattern to the variation found. The only observation of interest is that the least amount of variation found is between the rat and squirrel: two out of seven layers exhibited variation that was significant on t-test analysis. This is not surprising as both are members of the Order Rodentia. The rat and *Macaca* exhibited significant variation in all seven cell populations compared. The greatest degree of variation is found between *Macaca* and all other species (21), and between the rat and all other species (19). The cat (15), rabbit (15) and squirrel (12) showed less variation between species. However, as illustrated by the analysis of variance, all of the variation present must be attributed to random causes and is not considered to be statistically significant.

## ***2. An Analysis of Lamination Within the Bulb***

Mitra (1955) demonstrated that there is greater lamination within the visual cortex of *Macaca* than is found in the visual cortex of the cat or rabbit. Decreased lamination was characterized by an overlap in cells between the layers and by a greater proportion of pyramidal cells to stellate cells (interneurons): both features characterize the visual cortex of the cat and rabbit.

In the morphological description of the olfactory bulb of the six mammalian species included in this study, two observations were made: 1) that all bulbs are laminated and contain layers which are similar in general morphology; 2) that in some species, the overlap of cells from one layer into adjacent layers makes definition of layer borders difficult, even though all layers are present (the presence of an internal plexiform layer could be disputed in some cases). This variation in degree that cells overlap into adjacent layers could indicate that within the olfactory bulb of mammals, there is a slight difference in the degree of lamination that occurs between species. Because of the subjectivity of the categorization of greater vs. less lamination, the following scale was devised. A t-test analysis was conducted between the cell populations of adjacent layers within the olfactory bulb of each mammalian species. The analysis included the mean for density of cells of the three *Macaca* specimens, the mean for the five rat specimens, the cat, squirrel and rabbit. Most of the differences in cell density between adjacent layers of the same species were statistically significant ( $p \leq 0.001$ ) and therefore uninformative. By summing the t-values for each species and obtaining the mean value, a scale was developed that demonstrated which species had the least difference in density of cells between layers, and which had the most. Greatest lamination would be indicated by the highest value

on the scale (greatest difference in density of cells between adjacent layers). The t-test analysis of density of cells between cell populations of adjacent layers is given in Table 26 (page 311) as is the scale generated by this analysis.



Table 26

T-test analysis (density) between cell populations located within adjacent layers of a species.

|          | Var.1  | Var.2 | DF  | T-val. | P     |
|----------|--------|-------|-----|--------|-------|
| Macaca   | glo.l  | epl.t | 297 | 35.861 | 0.001 |
|          | glo.l  | epl.o | 294 | 21.011 | 0.001 |
|          | mcl.m  | epl.t | 287 | 11.221 | 0.001 |
|          | mcl.m  | epl.o | 284 | 19.530 | 0.001 |
|          | mcl.o  | epl.t | 285 | 37.998 | 0.001 |
|          | mcl.o  | epl.o | 282 | 29.506 | 0.001 |
|          | ipl    | mcl.m | 238 | 30.639 | 0.001 |
|          | ipl    | mcl.o | 236 | 16.754 | 0.001 |
|          | ipl    | gcl   | 240 | 20.064 | 0.001 |
| Cat      | glo.l  | epl.t | 169 | 26.649 | 0.001 |
|          | glo.l  | epl.o | 168 | 18.225 | 0.001 |
|          | mcl.m  | epl.t | 163 | 11.112 | 0.001 |
|          | mcl.m  | epl.o | 162 | 1.363  | ≥ 0.1 |
|          | mcl.o  | epl.t | 164 | 4.571  | 0.001 |
|          | mcl.o  | epl.o | 163 | 4.240  | 0.001 |
|          | ipl    | mcl.m | 156 | 23.406 | 0.001 |
|          | ipl    | mcl.o | 157 | 2.996  | 0.01  |
|          | ipl    | gcl   | 156 | 33.776 | 0.001 |
| Rabbit   | glo.l  | epl.t | 112 | 23.948 | 0.001 |
|          | glo.l  | epl.o | 110 | 19.132 | 0.001 |
|          | mcl.m  | epl.t | 106 | 11.577 | 0.001 |
|          | mcl.m  | epl.o | 104 | 5.947  | 0.001 |
|          | mcl.o  | epl.t | 108 | 37.994 | 0.001 |
|          | mcl.o. | epl.o | 106 | 33.592 | 0.001 |
|          | ipl    | mcl.m | 100 | 15.337 | 0.001 |
|          | ipl    | mcl.o | 102 | 20.724 | 0.001 |
|          | ipl    | gcl   | 102 | 15.078 | 0.001 |
| Squirrel | glo.l  | epl.t | 122 | 25.435 | 0.001 |
|          | glo.l  | epl.o | 124 | 16.528 | 0.001 |
|          | mcl.m  | epl.t | 113 | 4.030  | 0.001 |
|          | mcl.m  | epl.o | 115 | 1.197  | ≥ 0.1 |
|          | mcl.o  | epl.t | 112 | 36.495 | 0.001 |
|          | mcl.o  | epl.o | 114 | 25.111 | 0.001 |
|          | ipl    | mcl.m | 110 | 15.891 | 0.001 |
|          | ipl    | mcl.o | 109 | 13.456 | 0.001 |
|          | ipl    | gcl   | 112 | 13.576 | 0.001 |

Table 26 continued

|     | Var. 1 | Var. 2 | DF  | T-Val. | P     |
|-----|--------|--------|-----|--------|-------|
| Rat | glo.l  | epl.t  | 258 | 35.861 | 0.001 |
|     | glo.l  | epl.o  | 259 | 29.351 | 0.001 |
|     | mcl.m  | epl.t  | 249 | 6.934  | 0.001 |
|     | mcl.m  | epl.o  | 250 | 2.461  | 0.02  |
|     | mcl.o  | epl.t  | 249 | 8.221  | 0.001 |
|     | mcl.o  | epl.o  | 250 | 7.470  | 0.001 |
|     | ipl    | mcl.m  | 226 | 25.957 | 0.001 |
|     | ipl    | mcl.o  | 226 | 3.501  | 0.001 |
|     | ipl    | gcl    | 226 | 31.389 | 0.001 |

Relative scale in ascending order

|       |       |          |        |        |
|-------|-------|----------|--------|--------|
| Rat   | Cat   | Squirrel | Rabbit | Macaca |
| 13.23 | 14.04 | 16.86    | 20.37  | 24.43  |

Glo.l = Glomerular layer

Epl.T = External plexiform layer, large tufted cells

Epl.O = External plexiform layer, all other cells

Mcl.M = Mitral cell layer, mitral cells

Mcl.O = Mitral cell layer, all other cells

Ipl = Internal plexiform layer

Gcl = Granule cell layer

The scale generated by the t-test analysis indicates that there is a range in the density of cells between adjacent layers which I am interpreting as affecting the clarity of borders between cell layers. Adjacent layers with an increased overlap of cells between borders will have less of a difference in density of cells. This will be registered as a lower numerical value on the scale. If this interpretation is valid, the mean of the three *Macaca* specimens exhibit the greatest degree of lamination while the rat exhibits the least.

## CHAPTER 8

### DISCUSSION

The majority of the analyses conducted as a part of this study indicates that the olfactory bulbs of six mammalian species, including that of the catarrhine primate *Macaca*, are very similar. In spite of the characterization of the haplorhine primates as microsmatic, the olfactory bulb of *Macaca* in its general morphology, mean density of cells, and volume of those layers containing mature neurons is virtually identical to the olfactory bulb of several mammals characterized as macrosmatic. Only one finding, the ratio of known second order neurons to all other cells, is firmly suggestive of a proportional difference in the cellular make-up of *Macaca*. It is this difference that lends credence to the hypothesis that a smaller structure can facilitate more sophisticated processing of information by a shift in cell populations.

Olfactory bulb morphology in mammals is characterized by lamination to the degree that five layers containing mature neurons are present in all eutherian mammals reviewed in the course of this thesis. By virtue of the fact that

all synapses occur within these layers, these are the layers that process information and the overall morphology is conservative throughout the range of the species studied. This would imply a similarity of functional capability. The density of known second order neurons, that is the mitral cells and large tufted cells, is remarkably similar both within the rat series and across the mammalian series. This finding has been discussed in Chapter 6, pages 255-257, but in brief, may be attributed to tighter control of the number of second order neurons, the cells responsible for processing input from the receptor neurons and output to the more central regions within the cerebrum. Figure 13 (page 277) illustrates the similar density of second order neurons. It also indicates that the two members of the Order Rodentia have a higher density of cells in four of the layers containing interneurons (glomerular, internal plexiform, and granule cell layers, and in the granule cell population of the mitral cell layer) than do the other three mammalian species.

The most interesting finding associated with density of cells is the relationship of mean density of second order neurons to the mean density of "all other cells". Although the cell populations of all layers containing mature neurons include a percentage of glial cells (up to 30% in the glomerular layer according to Willey, 1973), and although a percentage of neurons within the glomerular and external

plexiform layers are small tufted cells, it still can be safely assumed that the majority of "other cells" consists of interneurons. Even with these qualifications, the difference in the ratio of known second order neurons to other cells between *Macaca* and the other mammalian species is meaningful. As Figure 17 (page 299) illustrates, between 13 and 17 other cells are associated with every mitral cell and large tufted cell in the cat, rabbit, rat and squirrel. In *Macaca*, each mitral and tufted cell interacts with 27 other cells. In the evolution of the neocortex, Mitra documented an increase in proportion of interneurons to pyramidal cells. In the visual cortex of *Macaca*, a species that relies heavily upon its keen visual sense for survival, the proportion of interneuron to pyramidal cell was particularly high. The correlation between a relative increase in density of interneurons to an increase in functional capability was made. The increase in ratio of interneurons is the only finding of this study which may indicate that a change in processing of information within the olfactory bulb of *Macaca* has occurred. If this change is real, then it is in the direction of an improvement of function within the confines of a small structure: it disputes the assumption that a reduction in structure size necessarily infers a reduction in functional ability.

Another morphological observation associated with density of cells is that concerning the overlap of cells

from one layer into adjacent layers. This is seen most clearly in the internal plexiform layer, which is only marginally present in the bulbs of some of the species. It becomes less visible when granule cells from the granule cell layer merge with granule cells located in the mitral cell layer. This does not appear to be an artifact of total olfactory bulb size as the internal plexiform layer in *Macaca mulatta* (with a larger bulb), is more difficult to distinguish than in *Macaca fascicularis* (with a smaller bulb). A reverse situation is seen in the cat, which has the largest bulb in the series and has a clearly demarcated internal plexiform layer while the rat and squirrel do not. Within the order Rodentia, the rat bulb is smaller in size and has a very poorly defined internal plexiform layer. In the squirrel, although the internal plexiform layer is not clearly demarcated, it is more so than in the rat.

The significance of cell overlap from one layer into the next is unclear. As one who has looked at the histological slides of the olfactory bulb in this study over numerous hours, I find the presence or absence of cell overlap intriguing: to those who observed the slides for the first time, the difference was non-existent. Whether the degree of lamination within the olfactory bulb varies at all may be questionable. The method that generated the scale in Table 26 (page 311) could also be questioned. However, assuming that it does indicate some difference in degree of

cell overlap, it is interesting that *Macaca* has the greatest degree of lamination, one of the features of the process of parcellation. I would have to concede however, that although some degree of variation in cell overlap exists within this series, the variation is small and in some cases is present within a single bulb. At this time, the only statement that can be made with assurance regarding lamination within the olfactory bulbs of eutherian mammals is that lamination exists at approximately the same level based on the observation that all of the bulbs studied consist of five layers containing mature neurons. In this feature too, the olfactory bulb of *Macaca* falls within the range consistent with that of all other mammals.

Table 16 (page 285) indicates that if the five layers containing mature neurons are taken as percent of the total of those layers, the volume of these layers within the series of mammals studied is remarkably similar. This is visualized in the line graph of Figure 14 (page 286). The presence of a large subventricular layer and ventricular space in some species but not in others was surprising. Historically, assumptions have been made about the functional capabilities of the olfactory bulb relative to volume of the brain. Most values of olfactory bulb size have been based on weight or cross-section measurements. It follows that if size has been correlated to function, then



assumptions about function have also been based on weight or cross-section measurements. Table 19 (page 292) illustrates that layers containing mature neurons, that is the layers where synapses occur and impulses are transmitted or inhibited, constitute only a percentage of the weight or cross-sectional area of the olfactory bulb. The subventricular layer and ventricular space also contribute to the weight and cross-sectional area of those bulbs in which they are present. Therefore neither weight nor cross-sectional area is an accurate measurement of that volume of the bulb where information is processed. Table 18 (page 291) illustrates that the cat olfactory bulb increases from 81.23 cubic millimeters when layers containing mature neurons are measured to 100.17 cubic millimeters when the subventricular layer and ventricular space are added. In the cat, the volume of the bulb concerned with processing information is 80.25%. In *Macaca*, there is no ventricular space and the subventricular layer is small compared with that in the cat. The size of the olfactory bulb increases from 16.94 cubic millimeters when layers containing mature neurons are measured to 17.54 cubic millimeters when the subventricular layer is added. In *Macaca*, the volume of the bulb concerned with processing information is 96.55%, a substantial increase over the 80.25% seen in the cat. A closer look at Table 18 (page 291) and Table 19 (page 292) illustrates that the presence of a ventricular space and the

size of the subventricular layer may be correlated with the size of the olfactory bulb. The two smallest olfactory bulbs have a small subventricular layer and lack a ventricular space. As the size of the olfactory bulb increases, the subventricular layer disproportionately increase in size and a ventricular space appears. The rat and *Macaca* have the smallest bulbs: the volume of the subventricular layer is 5.37 and 3.45 percent respectively. In order of increasing olfactory bulb size, the rabbit, squirrel and cat have a combined volume of subventricular layer/ventricular space of 16.36, 11.98 and 19.75 percent respectively. In small olfactory bulbs, most of the volume of the bulb is reserved for those layers which process information. This finding points out the importance of avoiding the strict correlation of olfactory bulb size with function. In the olfactory bulb, it is the volume of those layers containing mature neurons that is a more accurate assessment of volume associated with processing information. Within that volume, differences in the proportion of cells or slight changes in neuronal morphology may be responsible for improvement in processing of information.

The underlying hypothesis for this study has been that the functional capability of the olfactory system is not directly correlated with size of the olfactory bulb. It was thought that there would be evidence for the process of

parcellation as having occurred within the olfactory bulb of the catarrhine primate *Macaca*. Indications for this would include: 1) differences in degree of lamination; 2) a difference in the relative proportions of classes of cells within the olfactory bulb. The indication for any change in degree of lamination throughout the series is very weak. I believe the strongest indication that the olfactory bulb of *Macaca* may be capable of compensating for its small size by an improvement in the way it processes information is its increased ratio of interneurons to known second order neurons. However, the strongest indication that the olfactory bulb of the catarrhine primate *Macaca* has not lost its ability to process olfactory information as effectively as other mammals is its obvious similarity to all of the mammals included in this series. The only feature that is reduced in the olfactory bulb of *Macaca* is its size. I don't believe there is any additional morphological evidence to support the hypothesis that in the olfactory bulb of catarrhine primates, olfactory capability is reduced.

## CHAPTER 9

### CONCLUSION

Understanding the function of any of the special senses in vertebrates is not an easy task due to the complexity of neuronal connections within the brain. Understanding olfaction in any mammal is made even more difficult due to complexity that occurs peripherally, and the several routes by which information can reach the brain. In addition, odorant signals may reach the brain through more than one nasochemosensory route. Although a function may be known to be present, the component of the accessory nasochemosensory system traditionally thought to carry that information may be absent. The apparent absence of the vomeronasal system in *Macaca*, a species that detects cyclical changes in reproductive status through pheromonal cues, is one example of this. Because of this complexity in the delivery of information into the brain, a real understanding of the morphology and function of the nasochemosensory system will not be fully understood in any species until we have a better understanding of its component parts.

The purpose of this study has been to investigate whether the small olfactory bulb in the catarrhine primate *Macaca* differs in its morphology, density of cells, and relative volume of the layers comprising the bulb when compared with four macrosmatic mammalian species (cat, rabbit, squirrel, and rat). Due to the growing behavioral evidence that catarrhine primates have a keener sense of smell than was previously appreciated, it was expected that the olfactory bulb of *Macaca* would differ from macrosmatic mammals in features indicating an improvement in information processing. Features that characterize an evolutionary advancement in the neocortex include an increase in the degree of lamination, and an increase in ratio of interneurons to second order neurons. If this has occurred within the macaque olfactory bulb, it could explain how function is maintained or improved in a structure of reduced size. As discussed in Chapter 8, one finding is firmly suggestive of this as having occurred: the ratio of "all other cells" (including interneurons) to known second order neurons is higher in the *Macaca* olfactory bulb than in the other four species included in this study. To assess lamination, a numerical scale was created. *Macaca* does have a higher value on the "lamination scale", although this could not be demonstrated statistically.

Although none of the data produced by this study supports the hypothesis that the olfactory bulb of *Macaca* is

reduced in its ability to process odorant information, one has to resolve the small size of these primate olfactory bulbs relative to their brain size. I believe the answer to this lies partly in the observation that within the central nervous system, cellular regions receiving widespread input often reduce in size to form discrete nuclei when information processing becomes more sophisticated and/or specialized. This has been observed in the visual system but has not previously been suggested to occur within the olfactory system.

I believe that the olfactory system controls bulb size by channeling input from large receptor areas into the confines of a smaller structure. Allison and Warwick (1949) demonstrated that in the rabbit, 26,000 receptor neurons synapse with 94 second order neurons, a convergence that is similar to information convergence in the retina. Bhatnagar and Kallen (1975) compared two species of bats, one considered to be microsmatic and the other considered to be macrosmatic. In the macrosmatic bat, while the bulb size doubled, volume of the receptor rich olfactory epithelium increased 450 times. This is mentioned here to emphasize that olfactory bulb size is not a good criterion for function. The morphology of neurons, their dendrites and axon collaterals, and particularly the number and nature of their synaptic connections provide a much more accurate assessment of the ability to process information.

Output neurons, that is the mitral and tufted cells, can be acted upon wherever synaptic input occurs on these cells and their processes. Mitral and tufted cells can also affect the output of neighboring interneurons through reciprocal synapses with these interneurons. Different populations of interneurons affect differing regions of mitral and tufted cell dendrites, and are themselves acted upon by axon collaterals of the mitral and tufted cells. Within the glomerular layer, the dendrites of mitral and tufted cells are presynaptic to the interneurons within this layer (periglomerular cells) as well as being postsynaptic to dendritic spines and axon terminals of these same cells. These reciprocal synapses, common within the olfactory bulb (Rall et al., 1966), are the first point of information processing. It is significant that the percent volume of the glomerular layer in *Macaca mulatta* and *Macaca fascicularis* is larger than in the other species studied while density of cells has not (Table 16, page 285; Figure 14, page 286). This is suggestive of an increase in volume of dendritic or axonal fibers (not just neuron number) with a concomitant increase in synapse sites. This would fit with a picture of restructuring of neuron cell processes to enhance processing of information. One is tempted at first to say that the restructuring and reprocessing would be secondary to the reduction in olfactory bulb size. However, the reverse may also be true: the improvement in processing

of information may have facilitated a reduction in size of the olfactory bulb.

Further determination of the degree of sublamination of neuronal processes and their synaptic connections will be necessary in order to determine how processing of information differs between species. The extensiveness of dendrites and axon collaterals may be responsible for the presence or absence of an internal plexiform layer: hence, the extensiveness of connections may make an important contribution to lamination. For example, the axon collaterals of mitral cells and some tufted cells project within the internal plexiform/superficial granule cell layer to interact with the somata of granule cells and the proximal shafts of their peripheral processes. Many neuron processes project tangentially within the external plexiform layer: throughout this layer, secondary dendrites interact in a reciprocal fashion with peripheral processes of granule cells.

One final piece of evidence indicates that olfactory bulb size tells us little about bulb functional ability. The presence of an enlarged nonsynaptic layer and ventricular space in larger olfactory bulbs means that a disproportionately larger region of these bulbs does not process information.

The fact that lamination exists to the degree that it



does in all observed mammalian olfactory bulbs is itself worthy of comment. If lamination is an expression of advancement in the development of cortical structures and the way information is processed, then the olfactory bulb itself must be a very sophisticated structure. This is in contrast to the traditional view of the olfactory system as being an "old" system in terms of evolution, and synonymously, a "primitive" system. Even though a well laminated olfactory bulb is present in all mammals either reviewed in the literature or directly examined in the course of this thesis, it may be a derived eutherian feature compared with old telencephalic structures such as the paleocortex.

This study demonstrates that olfactory bulb size is not a good indicator of the ability to process information (i.e. functional ability). Functional ability must be based on knowledge of internal structure of the olfactory bulb at a cellular level together with physiological evidence of function and behavioral observation.

## **APPENDIX**

### **1. DECALCIFICATION PROCEDURE**

EDTA (Disodium Ethylenediamine Tetraacetate): 250 grams.

Distilled water: 1,750 ml

Adjust pH to 7.0 by adding Sodium Hydroxide pellets,  
approximately 26 grams.

Mix thoroughly until solution is clear (Need automatic mixer  
as this takes up to one hour).

Preparing specimen: clear away as much unnecessary bone from  
the fixed specimen as possible without risking damage to the  
tissues to be studied. Teeth take a long time to decalcify:  
if they are unnecessary to the study, remove them.

The specimen should be fully immersed in the solution until  
decalcification is complete. The more decalcifying solution  
used, the more quickly the specimen will decalcify.

Change the solution every 24 hours if possible.  
If one is available, an automatic rotator speeds  
decalcification.

Small specimens immersed in large amounts of solution will  
decalcify within a few days to one week. Large specimens  
(large portions of cranium) will take much longer.

The optimum way to tell if decalcification is complete is by  
taking an X-ray. This was not available at my institution.  
If I could easily compress the bone between my fingers, the  
decalcification was considered to be complete.

## 2. EMBEDDING IN PARAPLAST PLUS

The specimen must be well fixed.

All bone surrounding the specimen must be decalcified or removed if decalcification is not complete.

The embedding procedure takes three consecutive days to complete.

The specimen to be embedded is totally immersed in the following solutions:

- Ethanol 70% for 3 hours

- Ethanol 90% for 3 hours

- Ethanol 100% for 2 hours

- N-butanol\* for 15 hours

- N-butanol\* for 8 hours

- Mixture of N-butanol\* and melted (liquified) Paraplast plus in equal proportions (1:1) in an oven set between 56-58°C (not higher). It must remain in this solution for 15 hours.

- Paraplast plus for 3 hours

- Paraplast plus (new solution) for 3 hours

- Paraplast plus (new solution) for 3 hours

Final embedding is as follows:

Specimen is placed in embedding pan filled with melted Paraplast plus and aligned according to how it will be sectioned.

Return it to the oven for 10 minutes to 1 hour.

Remove it so that it hardens in the air.

Put the base of the embedding pan in a dish of ice.

Refrigerate embedded specimen overnight or until you plan to section it.

\* N-butanol is highly toxic and must be used under a hood. Wear gloves while handling it.

### 3. PREPARATION OF SLIDES; DEHYDRATION AND STAINING OF SECTIONS

Histological slides must be absolutely clean. Slides were washed in soapy water, rinsed well in distilled water, and rinsed again in 70% alcohol. They were dried in a warmer.

Slides were coated with chrome alum/gelatin solution (page 331).

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#### Preparation of sections:

Slides supporting the histological sections must be totally immersed in the following solutions:

- Xylene\* for 12-15 min.

- Xylene for 12-15 min.

- Alcohol 100% for 2-3 min.

- Alcohol 96% for 2-3 min.

- Alcohol 70% for 2-3 min.

- Alcohol 50% for 2-3 min.

- Distilled water for 2-3 min.

- Cresyl violet/buffer solution for 2.5 min. However, slides can be immersed as long as up to 6 minutes if staining is weak. Stain should only be used 2-3 times and then replaced. (Page 331 for preparing staining solution).

- Rinse slides quickly by dipping into 70% alcohol 1 or 2 times.

- Dip into 95% alcohol 1 or 2 times.

- Immerse in:

  - 100% alcohol for 3-4 min.

  - 100% alcohol for 3-4 min.

  - Xylene 3-4 min.

  - Xylene 3-4 min.

Mount and cover.

\* Xylene should be used under a hood with gloves.

Preparation of chrome alum/gelatin solution to coat slides.

Heat one liter of water to boiling.

Add the following to the boiling water:

5 grams of chrome alum

5 grams of gelatin

Stir until dissolved

Cool

Filter

Quickly dip (very clean) slides into room temperature solution.

Dry slides in warmer for one hour and store them in a dust-free environment until use.

The chrome alum/gelatin solution should be stored in the refrigerator. It must be filtered before each use.

Preparation of cresyl violet staining solution.

5% solution of cresyl violet:

Add 0.5 grams to each 100ml of distilled water

Buffer Solution (approximately 0.1 M, pH 3.5)

1. 0.1 M acetic acid (6 ml of glacial acetic acid\* in 1000ml of distilled water). Use 94.0ml

2. 0.1 M sodium acetate (13.6 grams sodium acetate in 1000ml of distilled water). Use 6.0ml

The working solution is:

1. Buffer solution 100 ml.

2. Cresyl violet solution between 6-12ml, depending on desired strength of stain (I used 10ml.)

Always filter the buffer solution before use.

\* Glacial acetic acid must be used under a hood and handled with gloves.

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