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The Development of Nasal Mucosa
in the Non-human primate, *Macaca nemestrina*:
Qualitative and Quantitative Analyses

By
Cheryl Sinkevitch

A thesis
Submitted to the Faculty of Graduate Studies and Research
Through the Department of Biological Sciences
in Partial Fulfillment
of the Requirements for the degree of
Master of Science
at the University of Windsor

Windsor, Ontario, Canada
1992

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ABSTRACT

The development of the respiratory and olfactory mucosae from the old world monkey, *Macaca nemestrina*, from fetal (F) 121 days to postnatal (P) 9.5 years, has been examined by light and electron microscopy. By F121 days, respiratory and olfactory mucosae appeared to be morphologically capable to carry out their required functions. Olfactory receptor cells were well developed with the presence of a ciliated olfactory knob and abundant dendritic microtubules. At the light and electron microscopic levels, prenatal receptor cells were differentiated into pale, intermediate and dark cells. The lightly stained receptor cells appeared to be young. They were sparsely ciliated and had dendritic centrioles and mitochondria. The receptor cells with intermediate staining appeared to be mature. The olfactory knobs contained mitochondria and basal bodies associated with the cilia. The nuclei of the darkly stained electron dense receptor cells were located distally in the olfactory epithelium. Although these receptor cells retained their tight junctions and nuclear structure, they showed intracellular changes indicative of apoptotic degeneration, including disrupted plasma membranes, cytoplasmic multivesicular bodies and an apparent contraction of cytoplasmic volume.

The number of olfactory receptor cells counted along a 1 mm length of olfactory epithelium showed the greatest density of cells just prior to and just after birth. This apparent high density of olfactory receptor cells during the perinatal period correlates with evidence from other studies that the olfactory system is highly discriminatory at these ages. The number of receptor cells were quantified in a 1 mm² surface area and percentage of dark receptor cells was found to be 8% in the F121 day stage and 12% in the F156 day stage. This 4% increase in the number of degenerating olfactory receptor cells may be the result of the increased density of receptor cells competing for neurotrophic factors at the later prenatal stage. The locations of receptor cells were mapped. These receptor cells were located in small clusters scattered through the olfactory epithelium. Degenerating receptor cells appeared to be scattered over the surface, more frequently occurring near the clusters.
To my dear husband, David Punga.
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INTRODUCTION

Objectives

The purpose of this study was to qualitatively and quantitatively describe the morphological development of nasal mucosa in the non-human primate, *Macaca nemestrina*. This type of study has not been attempted with primates previously; most earlier studies involved amphibian and rodent models (Cuschieri and Bannister, 1975; Farbman *et al.*, 1988; Graziadei and Dehan, 1973; Menco and Farbman, 1985). Data on human nasal mucosa mainly comes from studies using adult autopsy or biopsy tissue (Jafek, 1983; Moran *et al.*, 1982; Morrison and Costanzo, 1990). Tissue from *M. nemestrina* closely resembles human tissue, therefore, conclusions drawn from this study may be applied to humans. One important reason for investigating development, including prenatal stages, is so that possible parallels can be made between structure and function of the olfactory epithelium during early life.

Morphology of nasal mucosa

Nasal mucosa is a collective term for the cellular layers that line the nasal cavity. It consists of two types of tissue, respiratory mucosa and olfactory mucosa. Respiratory mucosa functions to warm, filter and humidify incoming air as it passes through the nose toward the lungs during the inspiratory cycle. Olfactory mucosa is able to detect odourants in the incoming air and to encode and transmit information about their identity, concentration and persistence to the central nervous system.

The respiratory epithelium is a pseudostratified columnar epithelium that contains three cell types; ciliated cells, goblet cells and basal cells. The lamina propria lies beneath the epithelium and contains blood vessels, blood sinuses and respiratory glands. Respiratory mucosa occupies approximately 120 cm² in the adult human nose (Jafek, 1983). Ciliated cells provide support to the epithelium while their motile cilia provide protection against foreign particles. The number of cilia per cell, in humans, is approximately 50 and they beat at a rate of approximately 1,000 times a minute (Proctor, 1982).
The major secretory cells, in the respiratory mucosa, are epithelial goblet cells and subepithelial glandular acinar cells (Tos, 1982). These cells contribute to the layer of mucus that covers the epithelial surface. There are three types of glands identified in vertebrate respiratory mucosa. One type lies under columnar goblet cells and consists of superficial serous acini, as well as, deeper large clusters of acini composed of mucous acini (Tos, 1982). Another type lies under cuboidal goblet cells and consists of isolated acini lined with cuboidal epithelium. The third type is an invagination of the surface epithelium, usually the columnar type (Tos, 1982).

The olfactory epithelium is also a pseudostratified columnar epithelium that rests upon a highly cellular lamina propria containing blood vessels, nerve bundles and mucus secreting Bowman's glands, composed of serous cells containing secretory granules and occasional vesicles. Olfactory mucosa covers approximately 1 cm² on each side in the human nose (Jafek, 1983). There are three major cell types in the epithelium; ciliated olfactory receptor cells, sustentacular cells and basal cells.

The olfactory receptor cells are bipolar neurons which send a dendrite to the epithelial surface lining the nasal cavity and an axon to the olfactory bulb in the brain. The tip of the dendrite is called the olfactory knob and bears many, long cilia. The ciliary membranes have been shown to contain the "cellular machinery" that enables transduction to take place such as receptor proteins, G-proteins, adenylate cyclases and specific cation channels (Boekhoff and Breer, 1992; Buck and Axel, 1991; Dhallan et al., 1990; Lancet et al., 1985; Lancet, 1987). Excitation of the olfactory receptor cells by odourants is thought to be initiated at these ciliary membranes. Olfactory receptors are abundant occurring at 3-5 µm intervals, in adult humans, along the surface and have a population density that approximates 30,000 receptors per mm² of tissue (Kanda et al., 1973).

The epithelial surface is covered by a layer of mucus which bathes the cilia that extend from the tips of the olfactory receptor cells, or olfactory knobs, and the microvilli from the sustentacular cells. This region is known as the mucociliary matrix. Bowman's glands, located in the lamina propria, are specialized for secretion and contribute greatly to the overlaying mucus.
layer. These glands produce a secretion rich in glycoconjugates, antioxidants and proteins including an odourant binding protein, immunoglobulins A and M, secretory component, J chain, lactoferrin and lysozyme (Mellert et al., 1991). In the olfactory mucosa of non-mammalian vertebrates, epithelial sustentacular cells secrete mucus (Getchell et al., 1984). In mammalian olfactory mucosa, sustentacular cells do not contain the glycoconjugates characteristic of mucus and are not specialized for mucus synthesis, ultrastructurally (Foster et al., 1991). Sustentacular cells are also known as supporting cells and their function is to maintain the integrity of, or to provide support to mammalian olfactory epithelium.

Development of olfactory receptor cells

The first sign of olfactory organ development in mammalian embryos is the appearance of bilateral placodes or epithelial thickenings, on the anterior surface of the embryo heads (Farbman, 1986). The placodes form depressions that deepen into two nasal pits. Even at this early stage, receptor cells elongate and some display a bulbous surface process. First, axons sprout and grow toward the olfactory bulb where they will eventually form synapses (Carr and Farbman, 1992). As the axon is elongating, at the other end of the differentiating receptor cell a dendrite grows toward the epithelial surface (Carr and Farbman, 1992). When the dendrite reaches the surface it takes on a bulbous shape and a single cilium sprouts, called the primary cilium (Menco, 1985). Olfactory receptor cells are said to be mature when the dendrite bears cilia (Chuah et al., 1985; Menco and Farbman, 1985), or when olfactory marker protein is present (Farbman and Margolis, 1980; Verhaagen et al., 1989). In humans, ciliogenesis occurs early in gestation, at ten weeks. Olfactory marker protein is not expressed until the twenty-eighth week or two-thirds of the way through gestation which suggests that olfactory receptor cells do not mature until late in gestation (Chuah and Zheng, 1987).
Function of the perinatal olfactory system

The olfactory system is morphologically and functionally developed well before birth in the well studied animal, the rat. The rat fetus exhibits olfactory function in utero and it has been suggested that central processing of sensory information exists, including evidence of habituation or growing accustomed to an odour, a fetal orienting reflex to novel stimuli, and the existence of prenatal behavioural states associated with different patterns of response (Rockwood and Riley, 1990; Schaal and Orgeur, 1992; Smotherman and Robinson, 1988, 1991; Teicher and Blass, 1977).

Human newborns have been shown to have a highly discriminatory olfactory system (Makin and Porter, 1989; Samat, 1978; Sullivan et al., 1991). Makin and Porter (1989) observed that breast odours from lactating females were attractive to 2-week-old infants who were unfamiliar with the lactating woman used as the stimulus and who had no prior breast-feeding experience. Whereas most infants appear capable of discriminating among lactating and nonlactating individuals, breast-feeding infants can further recognize the characteristic olfactory signature of their own mother in particular (Cemoch and Porter, 1985; MacFarlane, 1975; Russell, 1976; Schaal et al., 1980). Based on studies such as these, mammalian fetuses appear to use the olfactory system before birth to identify odourants and to begin to build up an odour memory. Odourant molecules are able to be detected in utero as they are detected postnatally due to the fact that both provide a fluid environment; prenatally being amniotic fluid and postnatally being the layer of mucus that bathes the olfactory epithelium.

Regenerative capacity of olfactory receptor cells

It is known that in the nervous system of adult mammals, neurons cannot be replaced and intrinsic axons to the central nervous system fail to regrow over long distances (Graziadei et al., 1980). Replacement of neurons has never been documented and only sprouting of axons has been observed following partial deafferentation of neurons in the brain (Goodman et al., 1973) and in the spinal cord (Bernstein et al., 1978). Vertebrate olfactory neurons are unique among neurons in that they are continuously replaced throughout the life of the animal. When mature
receptor neurons reach a critical stage or become injured, they degenerate and are subsequently replace by newly formed receptor cells (Graziadei et al., 1980; Schwartz Levey et al., 1991). Under conditions of experimentally induced degeneration of olfactory receptor cells or under normal conditions of cellular turnover, basal cells are believed to proliferate and provide a source of new receptor neurons (Schwartz Levey, 1991). Recent evidence indicates the life span of olfactory receptor cells is highly variable, some living for 3 months to 1 year or more (Hinds et al., 1984; Mackay-Sim and Kittel, 1991) while others die before reaching maturity (Breipohl et al., 1988; Farbman, 1990).

Experiments involving degeneration and regeneration of olfactory receptor cells have focused on the regenerating cells rather than the appearance of dying cells or the mechanisms leading to cell death. It is probable that many of these changes, although associated with injury, may be reversible and therefore are not sufficient to define death at all. Also, appearances which are distinguishable from each other in static electron micrographs may represent different phases in a single process. Taking these facts into account, two major types of cells death have been presented in the literature. They are termed necrosis and apoptosis.

Morphology of dying cells

With very few exceptions necrosis occurs exclusively in circumstances departing from physiological conditions. It has been observed in cells injured by hypoxia (Ganote et al., 1975; Jennings et al., 1975; Saladino and Trump, 1968), inhibition of oxidative phosphorylation, glycolysis or Krebs cycle enzymes (Hawkins et al., 1972; Prieto et al., 1967) or exposure to toxins (Laiho and Trump, 1975; McDowell, 1972a, 1972b). Necrosis is characterized by cellular edema and ends in the rupture of plasma and internal membranes and leakage of cellular contents (Wyllie, 1981). The characteristics of necrotic cell death are consistent with a failure of osmotic regulation that may be triggered by a loss of cellular energy supplies.

The second type of cell death is termed apoptosis. It occurs in normal tissue turnover, embryogenesis and metamorphosis. It involves a progressive contraction of cell volume and widespread chromatic condensation, but with the initial preservation of the integrity of
cytoplasmic organelles (Kerr et al., 1972; Wyllie et al., 1980; Wyllie, 1981). The mechanism that leads to this type of cell death is not known. It is known that, unlike necrotic cells, apoptotic cells retain normal ATP levels, despite major morphological changes in chromatin (Anilkumar et al., 1992; Dive et al., 1992; Fidzianska et al., 1991). Cell death is not a single entity but is very diverse in structure, mechanisms, circumstances of initiation and biological function.

In many populations of developing neurons, natural death of some cells occurs which is not a pathological process, but is a normal feature of neurogenesis (Oppenheim, 1985; 1991). Some examples of putative genetic differences that could confer an advantage on certain neurons in a population are: earlier birthdate; faster growth; more extensive target or afferent contacts; increased numbers of receptors for the binding and uptake of extra-cellular trophic molecules (Oppenheim, 1985). It has been suggested that size of the synaptic target affects neuronal survival (Oppenheim, 1981; Jacobson, 1991), but what causes naturally occurring neuronal death remains unresolved.

In summary, this study focuses upon structural changes concerning olfactory development with an emphasis on olfactory receptor cells. The methods of light and transmission electron microscopy used allowed for a detailed morphological study of nasal mucosa in the non-human primate, M. nemestrina. This study indicates that the density of olfactory receptor cells, in the perinatal animals studied, is quite high which appears to be related to the sensitivity of the perinatal sense of smell observed in other studies.
MATERIALS AND METHODS

Experimental Animals

The olfactory mucosae from Macaca nemestrina monkeys were studied from prenatal (F121day and F158day) and postnatal (P7day, P12week, P13month and P9.5year) specimens. Respiratory mucosa was also studied from the prenatal and postnatal stages mentioned above, including P6weeks. Birth occurred in the University of Washington Regional Primate Research Center M. nemestrina colony at F165-F170days. All prenatal animals were the result of timed matings with gestational age known ± 1 day. Postnatal animals were full term as judged by birth weight and gestational age.

Tissue Preparation

Fetal animals were delivered by aseptic caesarian sections under halothane anesthesia and were immediately perfused. Postnatal animals were perfused intravascularly, first with a body temperature rinse of surgical Ringer solution containing 5% dextrose and 0.5% heparin, followed by 4% paraformaldehyde, 0.05% glutaraldehyde, 0.2% picric acid in 0.1 M phosphate buffer, pH 7.3.

The olfactory and respiratory mucosae were removed and postfixed in 1% osmium tetroxide for 1 hour, dehydrated in ethanol and embedded in Medcast resin to be used for analyses at the light and electron microscopic levels.

Light microscopic analysis

For qualitative analysis, 1 μm sections were cut with an RMC ultramicrotome MT6000-XL and stained with the modified Richardson's stain (1% methylene blue, 1% azure II, 1% sodium tetraborate in a 1:1 dimethyl sulfoxide mixture). For quantitative analysis, the number of olfactory receptor cells was determined per surface area of the olfactory mucosa by the disector method of Gunderson et al., 1988. Ten 1 μm serial sections were cut, stained with the modified Richardson's stain and photographed. The montages, consisting of approximately 100 photographs each, were used to determine the number of olfactory receptor cells per 1 mm².
surface area of olfactory epithelium. This was done in triplicate for the F121day and F156day specimens and in duplicate for the P9.5year adult specimen. These data were used to map the locations of moderately stained olfactory receptor cells (ORC) compared to the darkly stained ORC and to determine the number of ORC per mm length of olfactory epithelium for the F121day, F156day, P7day and P9.5year specimens. All numbers of ORC obtained per mm are biased high, therefore the graph in Figure 35 represents a trend rather than absolute values.

Electron microscopic analysis

Ultrathin sections, 95 nm, were cut and stained with a 2% uranyl acetate solution, then with lead citrate. Prenatal tissue (F121day and F156day) was used for qualitative analysis. For quantitative analysis, 3 thin sections were cut, stained and photographed. The montages of the photographs were used to determine the average number of olfactory receptor cells per mm length of olfactory epithelium for the P7day, P12week and P13month specimens. Due to the tight packing and uniform staining of olfactory receptor cells in the epithelium at P12weeks and P13months, it was not possible to count these cells at the light microscopic level and they were counted at the electron microscopic level, using a Philips 201 electron microscope.
RESULTS

Respiratory mucosa

Prenatal Stages

Prenatally, the surfaces of the ciliated cells bore cilia reaching 6 μm in length. Ciliated cells stained moderately dark in colour compared to the lightly stained goblet cells (Figure 1). In comparison with the ciliated cells, the goblet cells appeared to be fewer in number. They also contained a small number of secretory vesicles (data not shown). The lamina propria of the prenatal stages contained very few glands. Small blood vessels were evident beneath the basal lamina, while large blood sinuses were found deeper in the lamina propria (Figure 1).

Postnatal Stages

At the earliest postnatal stage, P7day, the epithelium stained differentially dark and light (Figure 2). The goblet cells appeared lighter in colour compared to the surrounding ciliated cells. The number of goblet cells appeared to have increased from the prenatal stages. In the lamina propria, the glands appeared to be more abundant at this stage.

At the P6week stage, as in the P7day stage, the goblet cells were filled with lightly stained secretory vesicles. They appeared swollen and the non-ciliated surfaces protruded from the surface of the epithelium (Figure 3). The outer membranes of the goblet cells were very dark in colour.

At the P12week stage, the goblet cells appeared to have increased in number when compared to the previous stage, P7days. In the lamina propria, the glands were more abundant than previous postnatal stages. The glands of the respiratory mucosa were small and contained secretory granules (Figure 4).

The epithelium, at the P13month stage, stained differentially dark and light. There was an abundance of goblet cells containing many, lightly stained vesicles. In the lamina propria, there were more glands apparent at this stage compared to the previous stages. The glands were large, moderately stained and contained many secretory granules.
At the adult stage, P9.5 years, the mucosa appeared to be similar to the previous postnatal stage. However, at this stage the goblet cells seemed to have increased in number; approximately equaling the number of ciliated cells present.

Olfactory mucosa

Prenatal Stages

The olfactory epithelium at the earliest prenatal stage, F121 days, appeared to be uniformly stained and the layering of the nuclei was very evident (Figure 5). Most olfactory receptor cells stained moderately and appeared darker than the surrounding sustentacular cells. A population of receptor cells stained darker than the more abundant moderately stained receptor cells and had nuclei located superficially. Receptor cells appeared mature by the presence of a well defined knob bearing many cilia reaching 4.7 μm in length (Figure 6). Microvilli on the sustentacular cells appeared to be as long as, or longer than receptor cell cilia reaching 6.4 μm in length (Figure 7). A population of sustentacular cells stained very darkly, in contrast to the more abundant lightly stained sustentacular cells.

At the F121 day stage, large blood vessels were present deep within the lamina propria with smaller, more abundant blood vessels just under the basement membrane. Bowman’s glands were abundant with ducts that opened to the mucociliary matrix (Figure 5). These glands were large and contained numerous secretory granules (Figure 11).

The olfactory epithelium at the latest prenatal stage, F156 days, stained differentially dark and light (Figure 8). The majority of the olfactory receptor cells stained a moderate intensity, as with the F121-day stage. A population of olfactory receptor cells, with vesicles, that stained very darkly was prominent (Figure 9). These receptor cells had nuclei located superficially; higher in the epithelium than sustentacular cell nuclei. There was also a population of lightly stained cells (Figure 10). The location of their nuclei appeared to be normal in that they were located in the receptor cell nuclear layer.

The lamina propria at the F156 day stage appeared very similar to the earlier F121 day stage with the presence of many blood vessels and Bowman’s glands. The blood vessels appeared
larger at the later prenatal stage. Bowman's glands were prominent, containing secretory granules and secretion in the lumens (Figure 12).

**Postnatal Stages**

The olfactory epithelium at the earliest postnatal stage, P7 days, stained very darkly and uniformly. Cells of the epithelium appeared to be densely packed and the nuclei were tightly packed together (Figure 13). The olfactory receptor cells stained uniformly dark and the presence of sub-populations were not evident, contrary to the prenatal stages. In the lamina propria, Bowman's glands (Figure 19) and blood vessels were abundant, as in the prenatal stages.

At the P12 week stage, the olfactory epithelium stained very darkly and uniformly, as in the earlier postnatal stage. The epithelium appeared thicker and the cells were tightly packed (Figure 15). The Bowman's glands, in the lamina propria, appeared to have increased in size and cell number. Non-myelinated nerve bundles were prominent.

At the P13 month stage, the epithelium stained lighter than the previous postnatal stages, therefore the regular arrangement of cells and nuclei were evident (Figure 17). From the top of the epithelium were the nuclei of the sustentacular cells followed by the nuclei of the olfactory receptor cells, lying in a broad band that covered one-half of the epithelial thickness (Figure 17). The nuclei of the basal cells were found at the base of the epithelium. The epithelium appeared to be tightly packed. There were few lightly stained olfactory receptor cells. The darkly stained olfactory receptor cells, evident during the prenatal stages, were not apparent at this stage. The receptor cell knobs were located buried in the microvilli of the sustentacular cells, rather than protruding high above the microvilli and appeared to be smaller in diameter (Figure 18).

The lamina propria, at the P13 month stage, contained many Bowman's glands and non-myelinated nerve bundles (Figure 17); more than the stages examined previously. Some of the glands stained differentially dark and light (Figures 20 and 21). The darkly stained cells of the Bowman's glands contained an abundance of secretory granules (Figure 22) and the lighter cells
were filled with vesicles and contained no secretory granules (Figure 20). The blood vessels found deep within the lamina propria were very large.

The cells of the olfactory epithelium appeared to be tightly packed and uniformly stained at the latest postnatal stage, P9.5years. Changes were evident in the lamina propria. The blood vessels under the basement membrane were larger and less abundant than the previous stages. Bowman's glands were abundant and stained variably dark and light (Figure 16). The lightly stained cells contained much mucus and no secretory granules (Figure 21). However, the darkly stained cells did contain secretory granules (Figure 23). Non-myelinated nerve bundles were very prominent.

**Electron microscopic analysis of prenatal olfactory receptor cells**

Olfactory receptor cells were identified by the presence of the following structures: olfactory knob, tight junctions at the base of the knob, cilia extending from the knob, basal bodies in the knob and longitudinally-oriented microtubules in the knob and the dendrite. At both prenatal stages there were three types of olfactory receptor cells based on their electron densities and ultrastructural characteristics.

The first type of olfactory receptor cell was lightly stained. These receptor cells had one or few cilia on the knobs. Mitochondria were located at least 1 μm below the knob and were not numerous (Figures 24, 25 and 26). The centrioles in the knob were near the base and appeared to be still migrating (Figures 25 and 26). Centrioles were in the dendrite, apparently migrating toward the knob (Figure 24). These receptor cells had intact tight junctions.

Most receptor cells were of intermediate density. Receptor cells appeared to be mature with well defined knobs with many cilia and basal bodies immediately subjacent to the knob plasma membrane (Figures 27 and 28). These cells showed the presence of tight junctions and mitochondria that filled the dendrite and appeared in the knob (Figures 29, 30 and 31). Large nuclei contained one or more prominent nucleoli. The olfactory knobs of these cells were well developed, bearing many cilia (Figure 31). Below the knob, there were many longitudinally-
oriented microtubules (Figure 31) and numerous mitochondria in the dendrite (Figure 29). These receptor cells contained few multivesicular bodies (Figures 30 and 31).

The third type of olfactory receptor cell showed electron-dense cytoplasm and nucleus and had a superficially located nucleus (Figure 32). These cells contained many multivesicular bodies and had disrupted plasma membranes. They had conserved tight junctions and retained nucleolar structure and nuclear pores (Figure 32). The olfactory knobs appeared "normal" bearing many cilia (Figure 32).

Quantitative analysis of the olfactory and respiratory epithelia

At every stage of development studied, the olfactory epithelium was significantly thicker than the respiratory epithelium due to the presence of an olfactory receptor cell nuclear layer (Figure 34). The olfactory epithelium, from the earliest prenatal to the latest postnatal stage, increased in thickness from 69 μm to 84 μm. Similarly, the respiratory epithelium, from the earliest prenatal to the latest postnatal stage, increased in thickness from 45 μm to 50 μm.

The number of olfactory receptor cells per 1 mm length of olfactory epithelium was determined by counting the number of knobs present. Olfactory knobs were identified by the bulbous shape, staining intensity compared to sustentacular cells and the presence of cilia. At the earliest prenatal stage, F121days, receptor cell density was relatively high at 85.0±2.9 receptor cells/mm (Figure 35). By P7days, the density increased by 46% from F121days, to a value of 158.7±6.0 receptor cells/mm. The density decreased during the postnatal stages, P12weeks and P13months, and by P9.5-years it had decreased by 32% from P7days, to a value of 106.7±6.0 receptor cells/mm (Figure 35).

The number of olfactory receptor cells per 1 mm² surface area of olfactory epithelium was determined by counting the number of knobs present in serial sections of tissue. Olfactory knobs were identified as above. At the earliest prenatal stage, F121days, receptor cell density per mm² was 25,667±1644 (Table 1). The density increased, by F156days, to 48,778±3119 receptor cells/mm². By the postnatal stage, P9.5-years, the density decreased compared to F156days, to
39,167±235 receptor cells/mm². The number of darkly stained olfactory receptor cells in the 1mm² surface area increased from F121 days to F156 days from 8% to 12% (Table 1).

The locations and densities of olfactory receptor cells were mapped in a surface area measuring 10 μm wide and 670 μm long (Figures 36). It is apparent that the density of olfactory receptor cells increased from F121 days to F156 days and decreased from F156 days to P9.5 years. Receptor cells appeared to be in small clusters, scattered in the epithelium, at all three stages. The dark olfactory receptor cells in both of the prenatal stages also appeared to be scattered in the epithelium (Figures 36). The ducts of Bowman's glands appear to have no effect on the distribution of olfactory receptor cells in the epithelium.

Values obtained for epithelial thickness (Figure 34) and number of olfactory receptor cells per 1 mm length of olfactory epithelium (Figure 35) and per 1 mm² surface area of olfactory epithelium (Table 1) represent an average from a number of values obtained from one animal at a given developmental stage. Standard errors represent variations within one individual animal (Table 2).
Prenatal and Postnatal Respiratory Mucosa

Figure 1. Low power light micrograph of respiratory mucosa at P156 days. The epithelium (RE) stains uniformly. In the lamina propria, there are many large blood sinuses (BS).

Figure 2. Low power light micrograph of postnatal respiratory mucosa at P7 days. The epithelium stains differentially dark and light. There is a respiratory gland (RG) in the lamina propria. The magnification is the same as in Figure 1.

Figure 3. High power light micrograph of respiratory epithelium at P6 weeks. The goblet cells (GC) are filled with lightly stained vacuoles and have round nuclei (arrow) located at the base of the cell. The surrounding cells stain moderately and bear many cilia (\(\ast\)). The magnification is the same as in Figure 4.

Figure 4. High power light micrograph of a respiratory gland (RG) in the respiratory lamina propria at 12 weeks. The gland stains uniformly dark and contains secretory granules (arrows).
Prenatal Olfactory Mucosa

Figures 5 and 8 are the same magnification and figures 6, 7, 9 and 10 are the same magnification.

Figure 5. Low power light micrograph at F121 days. The olfactory mucosa is composed of three regions: the mucociliary matrix (MC), the olfactory epithelium (OE) and the lamina propria (LP). The regular arrangement of nuclei is apparent in the epithelium. There are many, large blood vessels (BV) deep within the lamina propria. Below the epithelium, just under the basement membrane (arrows), there are smaller blood vessels. A Bowman’s gland (BG) is present with a duct (D) that opens to the mucociliary matrix.

Figures 6 and 7 are high power light micrographs of the olfactory epithelium at F121 days.

Figure 6. Olfactory receptor cells stain darker than the surrounding sustentacular cells (SC). They appear to be mature with a well defined knob (arrows) and many cilia (*).

Figure 7. Microvilli (open arrow) on sustentacular cells (SC) are long.

Figure 8. Low power light micrograph at F156 days. The nuclei in the epithelium have a regular arrangement. In the lamina propria (LP) are Bowman’s glands (BG), non-myelinated nerve bundles (NN) and many blood vessels (BV). The boxed in area contains a dark olfactory receptor cell and is shown in Figure 9 at a higher magnification.

Figures 9 and 10 are high power light micrographs of the olfactory epithelium at F156 days.

Figure 9. An olfactory receptor cell (arrow), containing a vesicle, stains darkly and has a nucleus located superficially.

Figure 10. Olfactory receptor cells stain variably from dark to light. The lightly stained cells (short, bold arrows) with well defined knobs (long arrows) have their nuclei located within the olfactory receptor cell nuclear layer.
High Power Light Micrographs of the Lamina Propria from Prenatal Olfactory Mucosae.

Figure 11. At F121 days, blood vessels (BV) and non-myelinated nerve bundles (NN) are prominent. The Bowman’s gland (BG) contains secretory granules (arrows).

Figure 12. At F158 days, blood vessels (BV) and non-myelinated nerve bundles (NN) are prominent. The Bowman’s gland (BG) contains secretory granules (arrows) and has secretions in the lumen (open arrow). The magnification is the same as in Figure 11.
Low Power Light Micrographs of Postnatal Olfactory Mucosa

Figures 13 - 16 are at the same magnification.

Figure 13. At P7 days, the epithelium (OE) stains uniformly dark and the cells are tightly packed. In the lamina propria (LP), there are many Bowman's glands (BG) and blood vessels (BV).

Figure 14. At P6 weeks, the transition between olfactory (OE) and respiratory (RE) epithelia is very evident. Cells in the olfactory epithelium appear densely packed and stain darker than the cells in the respiratory epithelium. A respiratory gland (RG) is apparent in the lamina propria below the respiratory epithelium.

Figure 15. At P12 weeks, the cells of the olfactory epithelium stain uniformly and appear closely packed. Bowman’s glands (BG) are large and prominent. A non-myelinated nerve bundle (NN) is present in the lamina propria.

Figure 16. At P9.5 years, the cells of the olfactory epithelium stain uniformly and appear closely packed. Blood vessels under the basement membrane (arrows) appear large. Bowman’s glands (BG) are abundant and the cells stain variably light and dark. A non-myelinated nerve bundle (NN) is prominent.
Olfactory Mucosa at P13 months

Figure 17. The low power light micrograph shows the very regular arrangement of nuclei in the epithelium (OE) is very regular and evident. Bowman's glands (BG) are very abundant. A Bowman's gland with a duct (D) that empties into the mucociliary matrix is evident and a non-myelinated nerve bundle (NN) is prominent.

Figure 18. The high power light micrograph shows olfactory receptor cells that stain darker than the surrounding sustentacular cells and have their knobs (arrows) buried within the thick layer of microvilli (open arrows).
High Power Light Micrographs of Postnatal Bowman’s Glands

Figures 19 - 23 are at the same magnification.

**Figure 19.** At P7 days, the glands are large, composed of many cells. Secretory granules are present (arrows).

At P13 months (Figure 20) and P9.5 years (Figure 21), these glands contain large, mucus-containing vesicles (LV) and the lumens are filled with secretory product (open arrows).

At P13 months (Figure 22) and P9.5 years (Figure 23), these glands contain dark cells with numerous secretory granules (arrows) and the lumens are filled with secretory product (open arrows).
Electron Micrographs of Prenatal Olfactory Receptor Cells

Figures 24 - 26 are of lightly stained olfactory receptor cells and are at the same magnification.

Figure 24. At F156 days, the knob of this receptor cell bears one cilium (*) and contains several centrioles. Microtubules are evident in the knob and dendrite (arrowhead). Mitochondria (m) are at least 1 μm below the knob. There is a tight junction between the receptor cell and the adjacent sustentacular cell (SC), followed by a hemidesmosome (h). The mitochondria of the sustentacular cell fill the cytoplasm as far as the apical plasma membrane.

Figure 25. At F121 days, there are two adjacent knobs; both are ciliated (*). The proximal portion of one olfactory knob contains four centrioles (arrows).

Figure 26. At F156 days, the knob bears a few cilia (*) and contains centrioles (arrows). The dendrite contains small mitochondria (m) and the tight junctions are apparent (open arrows).

Figures 27 and 28 are of F121 days and are at the same magnification.

Figure 27. There is an abundance of microtubules (arrowhead) in the knob and the mitochondria reach the level of the tight junctions (open arrows).

Figure 28. Basal bodies are immediately subjacent the olfactory knob plasma membrane (arrows) and each is associated with a cilium (*). Mitochondria are present in the knob (m). Sustentacular microvilli are very long (mv).

Figures 29 - 31 are of moderately stained olfactory receptor cells, at F156 days and are at the same magnification.

Figure 29. The dendrite contains many mitochondria (m).

Figure 30. The knob bears many cilia (*) and the dendrite contains a multivesicular body (v).

Figure 31. The knob bears many cilia (*) and contains a mitochondrion (m). Microtubules are apparent in the knob and dendrite (arrowhead). A multivesicular body appears in the dendrite (v).
Electron Micrographs of Olfactory Receptor Cells at F156days

Figure 32 is a three photograph montage. The receptor cell is stained darkly and the cell body is located very high up in the epithelium. The knob bears many cilia (*). Tight junctions are intact (open arrows). There are numerous mitochondria (m) and they are present in the cell body and high up in the dendrite. Multivesicular bodies are abundant throughout the dendrite and cell body (v). The plasma membrane appears to be disrupted (double arrow). Nucleoli (n) and nuclear pores (arrows) are evident.

Figure 33. The knob of the moderately stained receptor cell bears many cilia (*) with basal bodies located subjacent to the knob plasma membrane (arrows). Mitochondria (m) are located high in the dendrite.
Figure 34. Respiratory (open circles) and olfactory (filled circles) epithelial thicknesses measured at prenatal (F121days and F156days) and postnatal (P7days, P6weeks, P12weeks, P13months and P9.5years) stages of development. Conception occurred at 0days and birth occurred at 165days post-conception (arrow).
Figure 35. Total number of olfactory receptor cells determined per 1 mm length of olfactory epithelium at prenatal (F121days and F156days) and postnatal (P7days, P12weeks, P13months and P9.5years) stages of development. Receptor cells from the F121day, F156day and P9.5year specimens were counted at the light microscopic level. Receptor cells from the P7day, P12week and P13month specimens were counted at the electron microscopic level. Conception occurred at 0days and birth occurred at 165days post-conception, as indicated (arrow).
Table 1. Number of ORC/mm² and number of darkly stained ORC/mm² surface area of olfactory epithelium in the monkey, *M. nemestrina*.

<table>
<thead>
<tr>
<th>Age</th>
<th>ORC/mm²</th>
<th>Very darkly stained ORC/mm²</th>
<th>Percent very darkly stained ORC*</th>
<th>Areas counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>F121days</td>
<td>25,667±1644</td>
<td>2,166±167</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>F156days</td>
<td>48,778±3119</td>
<td>5,667±882</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>P9.5years</td>
<td>39,167±235</td>
<td>none</td>
<td>NA</td>
<td>2</td>
</tr>
</tbody>
</table>

ORC, olfactory receptor cells

NA, not available

*percent of very darkly stained ORC to total number of ORC/mm².
The Mapped Locations of Olfactory Receptor Cells

Figure 36 represents topographical distribution maps indicating the locations and relative densities of olfactory receptor cells in the olfactory epithelium for three developmental ages; F121 days, F156 days and P9.5 days, as indicated. The areas analyzed were 10 \( \mu \text{m} \) wide and 670 \( \mu \text{m} \) long and show the relative positions and densities of: Dark receptor cells (dark blue), non-dark receptor cells (light blue) and ducts of Bowman's glands (yellow). The sustentacular cells (gray) were assumed to fill the areas not mentioned above.
Table 2. Summary of olfactory epithelial thickness, number of ORC/mm and number of ORC/mm²

<table>
<thead>
<tr>
<th>Age</th>
<th>ORC/mm</th>
<th>ORC/mm²</th>
<th>Thickness of olfactory epithelium (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F121days</td>
<td>85.0±2.9</td>
<td>25,667±167</td>
<td>69.0±1.1</td>
</tr>
<tr>
<td>F156days</td>
<td>125.0±5.0</td>
<td>48,778±3119</td>
<td>74.0±0.4</td>
</tr>
<tr>
<td>P7days</td>
<td>156.7±6.0</td>
<td>NA</td>
<td>64.0±0.9</td>
</tr>
<tr>
<td>P12weeks</td>
<td>135.0±11</td>
<td>NA</td>
<td>81.0±0.5</td>
</tr>
<tr>
<td>P13months</td>
<td>99.0±6.1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>P9.5years</td>
<td>106.7±6.0</td>
<td>39,167±235</td>
<td>84.0±1.2</td>
</tr>
</tbody>
</table>

ORC, olfactory receptor cells

NA, not available

*Standard errors represent variations within one individual animal. n=10.
DISCUSSION

Comparative analysis of the development of respiratory and olfactory mucosae

At the light microscopic level, the nasal mucosa of the monkey, *Macaca nemestrina*, appeared to be morphologically mature and fully capable of carrying out its required functions by the prenatal stage F121days. By this age the respiratory mucosa had a complete epithelium containing ciliated cells, goblet cells and basal cells resting upon a lamina propria containing blood vessels and respiratory glands. The olfactory epithelium contained olfactory receptor cells with well defined knobs bearing many :silia as well as supporting cells and basal cells, which rested upon a lamina propria containing blood vessels, non-myelinated nerve bundles and Bowman's glands. Although the prenatal nasal mucosa appeared to be morphologically mature, many changes were observed as different stages of development were examined.

The mucus secreting systems of both respiratory and olfactory mucosae seemed to take on a greater role with age. Prenatally, the respiratory epithelium stained uniformly with very few goblet cells observed. By the earliest postnatal age examined, P7days, goblet cells were very evident, staining much lighter than the ciliated cells due to the accumulation of mucus-containing vesicles in their cytoplasm. As the age of the animal increased, the density of the goblet cells in the respiratory epithelium appeared to have increased. The density of goblet cells in human fetal respiratory epithelial is patchy and much lower in number than in adults (Cauna, 1982).

It is generally believed that the secretion of nasal glands is responsible for the humidification of inhaled air (Cauna, 1982). In the respiratory lamina propria, the number and size of the glands increased significantly after birth and continued to increase in number and size with age. Prenatally, in the olfactory lamina propria, Bowman's glands appeared to be quite large and showed the presence of secretory granules. Postnatally, these glands appeared to have increased in number and size and appeared to continue to increase in number and size with age. Both respiratory and olfactory glands contained large mucus-filled droplets intracellularly in the P13month and P9.5year specimens which is consistent with large amounts of mucus production.
Small serous secretory granules and occasional vesicles are signs of electrolyte and water secretion in both Bowman's and respiratory glands (Getchell and Mellert, 1991). Therefore, prenatal production of mucus is limited probably due to the fact that the nasal mucosa is protected significantly by being bathed in amniotic fluid.

The lamina propria is structured differently in respiratory and olfactory mucosae according to their different functions. There were many very large blood vessels and sinuses observed in the respiratory lamina propria which function to warm inspired air. Even prenaturally, the blood vessels were very large and took up most of the space in the respiratory lamina propria as opposed to the smaller, less abundant blood vessels in the lamina propria of olfactory mucosa. The olfactory lamina r. opria contained non-myelinated nerve bundles which were bundles of fine axons originating from the olfactory receptor cell bodies. These nerve bundles were very prominent even at the earliest prenatal stages studied and appeared to have increased in size and number with age, as the number of olfactory receptor cells increased.

There are many differences between respiratory and olfactory epithelia. Respiratory epithelium functions as a physical barrier to foreign particles and the majority of its cells bear motile cilia which beat rhythmically in the overlying mucous, contributed to by goblet cells, and carry particles away. Olfactory epithelium is specialized for odour detection and contains olfactory receptor cells for this purpose. Respiratory epithelium of M. nemestrina, because of its cellular make-up, was much thinner than olfactory epithelium at every stage of development examined. Both respiratory and olfactory epithelia increased in thickness significantly from F121 days to P12 weeks presumably because during this period the density of the cells in the epithelium have increased at a rapid rate. The thickness of olfactory epithelium varies from species to species. It is generally thin in warm-blooded animals; for instance, 70 μm in the adult human (Moran et al., 1982). The thickness of adult human respiratory epithelium is 45 μm (Moran et al., 1982). The thicknesses of both olfactory and respiratory epithelia in the adult human were similar to the thicknesses found in the adult monkey at 84 μm and 50 μm, respectively.
Human olfactory epithelium appears to be morphologically mature by the twenty-eighth week of gestation (Chuah and Zheng, 1987). Comparably, at the light microscopic level, the nasal mucosa of the prenatal monkey, M. nemestrina, appeared to be morphologically mature. Although the cellular make-up of both respiratory and olfactory mucosa was such that they would be able to perform their required functions, prenatally, they continued to develop extensively late in the prenatal period to the newborn stage.

**Light and electron microscopic analyses of olfactory receptor cells**

One of the most striking observations in the olfactory epithelium was that of the variations in the staining of the olfactory receptor cells, at the light microscopic level. Most receptor cells stained moderately, but prenatally there were very lightly and very darkly stained cells. The light cells appeared lighter against a background of darker sustentacular cells and contained many dark mitochondria just below the knob. These cells appeared to be "normal" with a well defined knob bearing many cilia and had nuclei that were located in the middle of the epithelium. Dark receptor cells were very visible appearing very dark against a lighter background of sustentacular cells, at the light level. They were very irregular in that they had nuclei located near the epithelial surface, well above the normal receptor cell nuclear layer and contained vesicles. These distinctive features were observed in a study of chemically induced degeneration of cells in the olfactory bulb in which the cells were also stained with Richardson's stain. The nucleus and cytoplasm were darkened and there was a shrinkage of the cell with many vesicles indicating degeneration of these cells (Doving and Pinching, 1973).

Ultrastructurally, these dark cells appeared to share some of the features described in injured cells just before the development of apoptosis, including an apparent contraction of cytoplasmic volume due to a loss of intracellular fluid with initial preservation of organelle structure in apoptotic cells (Kerr et al., 1972; Wyllie et al., 1980; Wyllie, 1981). Many prominent vesicles were present and may have been dilated endoplasmic reticulum which is consistent with what is known about cells undergoing apoptosis (Wyllie, 1981). It is not certain that changes in "very dark" cells are irreversible, however, it is evident that they are degenerating or in an "altered
functional state" (Doving and Pinching, 1973). Electron radioautography suggests that these cells are unable to undertake synthetic functions seen in adjacent cells with normal morphology (Nussdorfer, 1970). Since these dark cells were surrounded by well-fixed cells, it is probable that they represent stages in cell death rather than preparative artifacts caused by inadequate fixation.

Different types of olfactory receptor cells, based on their staining intensities were found at the electron microscopic level as well as at the light microscopic level. The light receptor cells appeared to be young receptor cells having one or few cilia on the knob with many migrating centrioles located in the dendrite and at the base of the knob (Mulvaney and Heist, 1971). Some of these cells had a single cilium located at the top of the knobs at right angles to the epithelial surface which may have been the primary cilium. Primary cilia are the first to sprout from immature olfactory receptor cell knobs (Menco and Farbman, 1985). Light receptor cells appeared to have fewer mitochondria in the dendrite which is also consistent with what is known about young olfactory receptor cells (Cuschieri and Bannister, 1975).

Moderately stained receptor cells had cilia on the knobs indicating receptor cell maturity (Chuah and Zheng, 1987). These cells contained many mitochondria in the dendrite and some in the knob. The mitochondria, in the dark receptor cells, appeared to be more compact with less intervening cytosol than in the moderately stained cells and contained many multivesicular bodies which is consistent with cells undergoing apoptosis (Wyllie, 1981). Reasons for prenatal olfactory receptor cell degeneration are discussed below.

Quantitative analyses of olfactory receptor cells

One of the most important aspects of this study was to determine whether structure of the olfactory epithelium could be related to olfactory function. The olfactory system seems to develop very early in gestation and therefore may play a critical role in the life of the fetus. In humans, ciliogenesis occurs early in gestation, at ten weeks. The presence of olfactory marker protein is associated with receptor cell maturity and is expressed by the twenty-eighth week which is two-thirds of the way through gestation (Chuah and Zheng, 1987). This suggests that
olfactory receptor cells are mature and potentially functional before birth. In fact, several studies have shown that the olfactory system is functioning before birth (Makin and Porter, 1989; Rockwood and Riley, 1990; Schaal and Orgeur, 1992; Smotherman and Robinson, 1988, 1991; Teicher and Blass, 1977).

The number of olfactory receptor cells per mm² surface area of olfactory epithelium varies with species, as indicated in the following table.

Table 3. Number of ORC/mm² surface area of olfactory epithelium varies with species.

<table>
<thead>
<tr>
<th>Animal</th>
<th>ORC/mm²</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(adult)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog and Cat</td>
<td>5,000-10,000</td>
<td>Andres (1969)</td>
</tr>
<tr>
<td>Human</td>
<td>30,000</td>
<td>Kanda et al. (1973)</td>
</tr>
<tr>
<td>Monkey</td>
<td>39,000</td>
<td>This study</td>
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<tr>
<td>(M. nemestrina)</td>
<td></td>
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<tr>
<td>Pig</td>
<td>60,000</td>
<td>Gasser (1956)</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>80,000-100,000</td>
<td>Kanda et al. (1973)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>120,000</td>
<td>Allison and Warwick (1949)</td>
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ORC, olfactory receptor cells

The density of olfactory receptor cells found in the adult monkey, M. nemestrina, was 39,000/mm² which is comparable to the 30,000/mm² found in the adult human (Kanda et al., 1973). Kanda et al. (1973) used the method of scanning electron microscopy to obtain their value for the number of receptor cells per mm² surface area in the adult human. This study employed the dissector method (Gunderson et al., 1988) to obtain the value for the number of receptor cells per mm² surface area in the adult monkey. The accuracy of this method of quantitation is reflected in the similarity in these values.
In this monkey, the number of olfactory receptor cells per mm of olfactory epithelium increased from the prenatal stage, F121 days, to the early newborn stage, P7 days and then decreased until the postnatal stage, P13 months where the number remained relatively constant until the P9.5 year stage. This study indicates that there is a high density of olfactory receptor cells during the perinatal period, in *M. nemestrina*, with the largest density occurring early postnatally. Apfelbach et al. (1991) observed a high density of olfactory receptor cells in neonatal rats. They also administered the odourant, ethyl acetate, to rats and observed an increase in odour sensitivity in neonatal and young rats. They suggested that the increased odour sensitivity was related to the increase in olfactory receptor cell density.

The increase in olfactory receptor cell density observed in this study corresponds to the time when olfaction appears to be very important; late in the prenatal period and early after birth. It has been shown that rat fetuses are able to detect odours in utero and that odours present in the uterus are detected by newborn rats (Rockwood and Riley, 1990; Schaal and Orgeur, 1992; Smotherman and Robinson, 1988, 1991; Teicher and Blass, 1977). It has also been shown that neonatal humans have a very discriminating olfactory system (Makin and Porter, 1989; Samat, 1978; Sullivan et al., 1991). Therefore, the apparent high density of olfactory receptor cells, observed in this study, during the late prenatal stages and early neonatal stages correlates with the evidence that the olfactory system is highly discriminatory at these ages. This system appears to be important to the survival of many neonatal mammals.

The numbers of olfactory receptor cells per mm² surface area of olfactory epithelium were found to be 25,667±1644 in the F121 day stage and 48,778±3119 in the F158 day stage. The numbers of dark receptor cells per mm² surface area of olfactory epithelium were found to be 2,166±167 in the F121 day stage and 5,667±882 in the F158 day stage. The percentage of dark receptor cells per mm² increased from 8% in the F121 day stage to 12% in the F158 day stage. This 4% increase in the density of degenerating receptor cells may indicate that olfactory receptor cell turnover had increased by the later prenatal stage. There are a number of reasons the olfactory receptor cells could have been degenerating at these prenatal stages. Since
receptor cells are being used extensively in utero, normal tissue turnover could be occurring; replacing the older cells with newly formed cells. However, the rate of degeneration in the later prenatal stage is 4% higher than the earlier stage. Late in gestation, oxygen demand increases as the fetus grows. Since olfactory receptor cells are sensitive to the environment (Hinds et al., 1984), a slight state of hypoxia may cause increased degeneration in these cells. Degrees of hypoxia inadequate to produce necrosis enhance apoptosis in some tissues (Wylie, 1981).

Small clusters of olfactory receptor cells seemed to be randomly spaced out along the surface of the epithelium. Dark receptor cells appeared to be scattered over the surface, more frequently occurring near the clusters. This suggests that increased receptor cell degeneration, indicated by dark cells, resulted from the increased density of receptor cells at this stage. It has been suggested that neuronal survival depends on the balance between the potentially lethal mechanisms and the neurons' access to trophic factors. Competition between neurons for neurotrophic factors has been observed which resulted in naturally occurring death of some neurons in the population (Jacobson, 1991). It appears that the increased degeneration, observed in this study, in the later prenatal stage, F156 days, may be due to the competition between the olfactory receptor cells for one or more neurotrophic factors. The surviving receptor cells observed had an advantage over the degenerating receptor cells; possibly increased numbers of receptors for the binding and uptake of neurotrophic factors.

Apoptotic neuronal death during late prenatal stages of development is consistent with what takes place during naturally occurring cell death in neurons (Jacobson, 1978; Oppenheim, 1981, 1991). It appeared that the location of olfactory receptor cell growth, dark or light, was not affected by proximity to Bowman's gland ducts.
Summary

This study has focused upon some of the qualitative and quantitative aspects of the development of nasal mucosa of the non-human primate, *Macaca nemestrina*. Qualitatively, the nasal mucosa appeared to be morphologically mature at the earliest prenatal stage studied, F121 days. At this stage, respiratory mucosa had a complete and intact epithelium consisting of ciliated cells, goblet cells and basal cells resting upon a lamina propria containing many blood vessels, blood sinuses and respiratory glands. The olfactory mucosa also had a complete epithelium containing olfactory receptor cells having well defined knobs bearing many cilia, as well as, sustentacular cells and basal cells. The olfactory lamina propria contained many blood vessels, non-myelinated nerve bundles and Bowman's glands. Although the nasal mucosa appeared to be intact at F121 days, it continued to develop extensively in the postnatal stages examined.

One of the most prominent features of the qualitative study of olfactory mucosa included the presence of a population of olfactory receptor cells that stained darkly, at the light microscopic level. These receptor cells had well defined knobs bearing many cilia, but contained cytoplasmic vesicles and had their nuclei located unusually high in the epithelium. At the electron microscopic level, these darkly stained receptor cells displayed characteristics of apoptotic degeneration having disrupted membranes, many cytoplasmic multivesicular bodies throughout the dendrites and cell bodies and an apparent shrinkage of cytoplasmic volumes.

Quantitatively, this study has indicated an apparent high density of olfactory receptor cells in the perinatal period of development. This corresponds to the time when the olfactory system appears to be very discriminatory and important to the survival of many neonatal mammals. The increased degeneration of receptor cells, in the later prenatal stage examined, occurred in the stage of development that had the greatest density of receptor cells per mm² surface area of olfactory epithelium, at F156 days. This indicates that there may be competition between olfactory receptor cells for neurotrophic factors.
This study was the first, in any species, to demonstrate topographical distribution maps of olfactory receptor cell density. From the topographical distribution maps of receptor cells, at three developmental stages, the following observations were made: receptor cells were located in small clusters scattered through the epithelium, degenerating receptor cells were scattered through the epithelium occurring more frequently near the clusters and the location of receptor cell growth was not affected by proximity to Bowman's gland ducts.
REFERENCES:


