Histol Histopathol (1997) 12: 799-806

Histology and Histopathology

Invited Review

Developmental anatomy of the primary olfactory pathway in the opossum *Monodelphis domestica*

M.I. Chuah, R. Tennent and R. Teague
Department of Anatomy and Physiology, University of Tasmania, Hobart, Tasmania, Australia

Summary. It has been shown in previous studies that the marsupial central nervous system is born at a relatively immature state. Although olfaction is thought to play a role in guiding the locomotion of the newborn, the cellular substrates on which this notion is based have not been systematically investigated. This review article summarises the anatomical development of the primary olfactory pathway in the postnatal *Monodelphis*. The olfactory epithelium and bulb appear morphologically immature at birth although some of the olfactory neurons are shown to express olfactory marker protein. The olfactory tissues subsequently undergo a rapid sequence of developmental events during the first two postnatal weeks. The evidence shows that the marsupial and eutherian olfactory system share a similar temporal sequence of developmental processes although the former proceeds at a lag time of about 10-14 days compared to that of mice (using the date of birth as a common reference point). Much physiological and behavioral studies remain to be done before we can be certain about the time at which full functional maturity is attained in this system.

Key words: Olfactory epithelium, Olfactory bulb, Differentiation, Synaptogenesis, Ultrastructure

Introduction

The olfactory system plays an important role in the social biology of marsupials; it conveys information regarding individual identity, sex, reproductive state and dominance status (Biggins, 1984). These physiological states are the ones that will dictate in the long term whether a certain group of animals can be successful in their adaptation to the environment.

Studies of neocortical development suggest that marsupials are born at an immature stage showing a gradient of differentiation along its long axis, with the more developed regions located in the cervical, thoracic regions of the spinal cord and a few specific brain stem nuclei (Reynolds et al., 1985; Saunders et al., 1989). The CNS located rostral and caudal to these regions is less mature. Hence one would expect that the olfactory system, the most rostral part of the CNS, should be poorly developed at birth. However, it is widely observed that newborn marsupials are able to climb from the birth canal to attach themselves to the teats on the mother's abdomen (Renfree et al., 1989). It is unknown whether this locomotor behavior is mediated by both the tactile or olfactory sense or contribution from both.

Although much has been written about the development and anatomy of the primary olfactory pathway in eutherian mammals (e.g. Graziadei, 1971; Cuschieri and Bannister, 1975; Farbman, 1991; Chuah and Farbman, 1995), detailed description of the marsupial system is comparatively scarce. Furthermore, data from published reports have given rise to some uncertainties regarding the development of the marsupial primary olfactory system. Based on the identification of sensory neurons in the olfactory epithelium, Hughes and Hall (1984) and Gemmell and Nelson (1988) concluded that the olfactory system in newborn marsupials may be sufficiently differentiated so as to play a role in guiding the newborn to the pouch and nipples. However, these studies did not include the olfactory bulb which bears the terminals of the olfactory axons. On the other hand, several investigators have demonstrated that the marsupial olfactory bulb remains immature at birth (Kratzing, 1986; Brunjes et al., 1992; Malun and Brunjes, 1996) because the mitral cells of the olfactory bulb which are postsynaptic to the olfactory neurons are not morphologically differentiated until about 2 weeks after birth. Hence, conflicting conclusions have been drawn from current data.

We have undertaken a morphological examination of the developing olfactory system in the *Monodelphis* as a groundwork for future studies into the functional maturation of this sensory system. In this review, we highlight some of the features of the *Monodelphis* olfactory epithelium and discuss them in the light of results from previous investigations on the olfactory

Offprint requests to: M.I. Chuah, Department of Anatomy and Physiology, University of Tasmania, Box 252-24, Hobart, Tasmania, Australia 7001

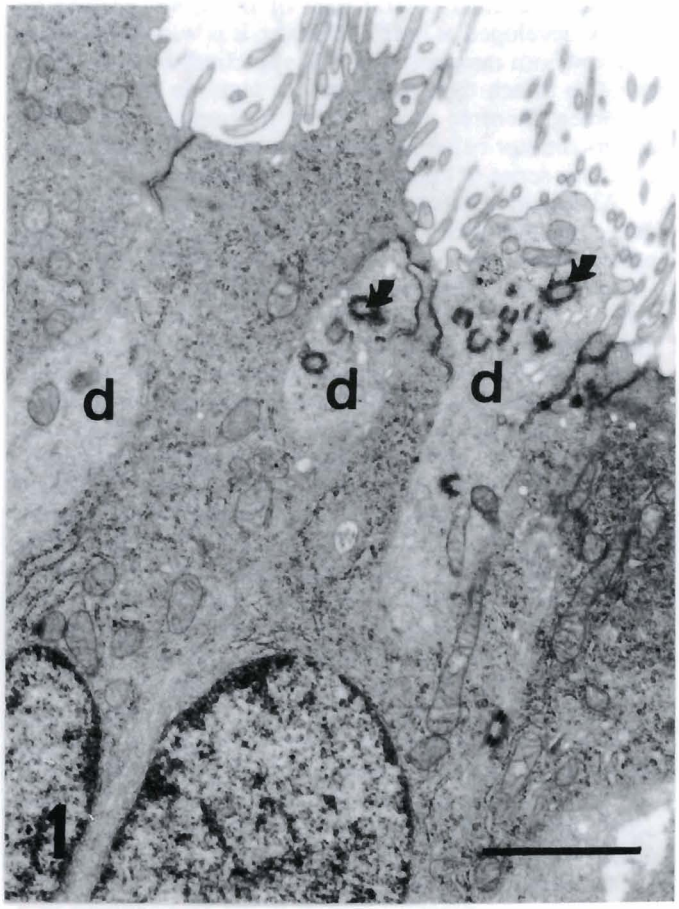
system in eutherian mammals such as mice and rats.

Differentiation of the olfactory mucosa

The offsprings of the *Monodelphis* are born after a 14-day gestation period and the stage of neural development at birth is thought to correspond to that of the 13 to 15-day gestation rat (Iqbal et al., 1995; Tarozzo et al., 1995). Coronal sections through the nasal cavity show that the olfactory epithelium is preferentially located in the dorsal region, while most parts are lined with respiratory epithelium. The structure of the cavity appears relatively simple initially but elaborate scrolling of turbinates develops soon after. Because olfactory epithelium is pseudostratified as opposed to the simple columnar feature of the respiratory epithelium, the former can be distinguished easily by its greater thickness. The characteristic pattern of organization and distribution of the different cell types is not readily apparent in neonatal pups, unlike that in the mature opossum (Tennent and Chuah, 1996). Only in electron microscopy do we detect cell differentiation as indicated by the emergence of variation in electron density of different cell types, similar to what has been observed in the rat on the 14th embryonic day (Farbman, 1992) and the mouse on the 10th day of gestation (Cuschieri and Bannister, 1975).

Supporting cells are characterized by the presence of elongated nuclei aligned closest to the surface and bearing microvilli on the apical membrane. Although a mucus layer has been laid down on the surface of the epithelium, the supranuclear region of the supporting cell perikaryon still lacks secretory vesicles. During the second postnatal week, secretory vesicles are observed clearly in the supranuclear region of some supporting cells.

In the early neonatal period, the regular arrangement of supporting cells is punctuated by the more electron light olfactory dendrites coursing towards the surface of the olfactory epithelium (Fig. 1). Most of the olfactory



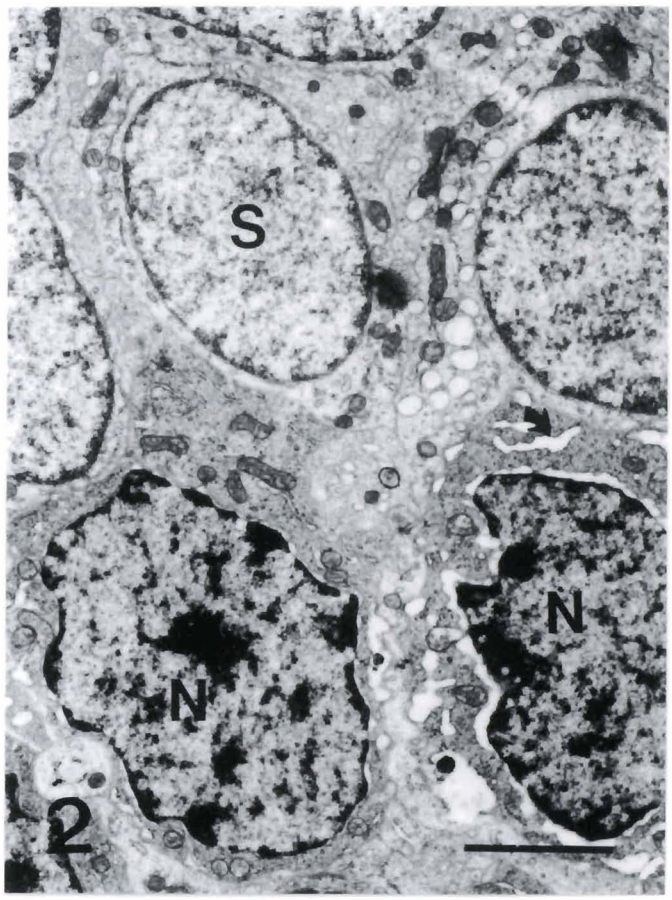


Fig. 1. Olfactory epithelium of three day-old *Monodelphis*. Differentiating olfactory dendrites (d) grow towards the surface; the one on the right is most mature having just reached the surface. Basal bodies (arrows) will subsequently give rise to cilia. Bar: 3.5 μm.

Fig. 2. Tangential section through the olfactory epithelium of a nine day-old *Monodelphis*. At this stage, it is easy to identify the supporting cells (S) and olfactory neurons (N). Supporting cells are characterised by a more oval nucleus and less electron dense cytoplasm. The rough endoplasmic reticulum of the olfactory neurons sometimes appear as dilated citernae (arrow). Bar: 2.5 μm.

neurons appear morphologically immature because few dendritic knobs are present on the epithelial surface, and most of them are devoid of cilia. The scarcity of olfactory cilia in the newborn *Monodelphis* bears remarkable resemblance to that of rat olfactory epithelium on the 14th day of gestation (Menco and Farbman, 1985). It should be noted that G-proteins and adenylate cyclase, which are involved in transduction of the olfactory stimulus, are expressed in rat olfatory cilia shortly after they are formed (Mania-Farnell and Farbman, 1990; Dau et al., 1991). As such, it is debatable whether the *Monodelphis* olfactory function is fully operational at birth.

With differentiation, olfactory neurons increase in electron density while the perikaryal difference compared to supporting cells become more marked. The rough endoplasmic reticulum in olfactory neurons is more extensive, some of which are composed of dilated cisternae (Fig. 2). Clumping of dense heterochromatin around the nuclear periphery is also more prominent in the olfactory neurons.

Tarozzo and co-workers (1995) showed in a recent study that the olfactory marker protein (OMP) was expressed in olfactory neurons situated in the dorso-caudal region of the nasal cavity of the newborn *Monodelphis*. Olfactory marker protein is a 19kDa acidic protein of undetermined function which is present in olfactory neurons (Margolis, 1972). It is highly conserved phylogenetically and is accepted as a marker for mature olfactory neurons in several vertebrates (Farbman and Margolis, 1980; Chuah and Zheng, 1987; Rama Krishna et al., 1992). Studies from our laboratory indicate that although OMP is present in the newborn *Monodelphis*, its distribution is patchy and mirrors the initial expression of this protein in the E14 rat (Allen and Akeson, 1985). It has been shown that the majority of the OMP-positive cells in the *Monodelphis* also express carnosine (Tarozzo et al., 1995) a major constituent of

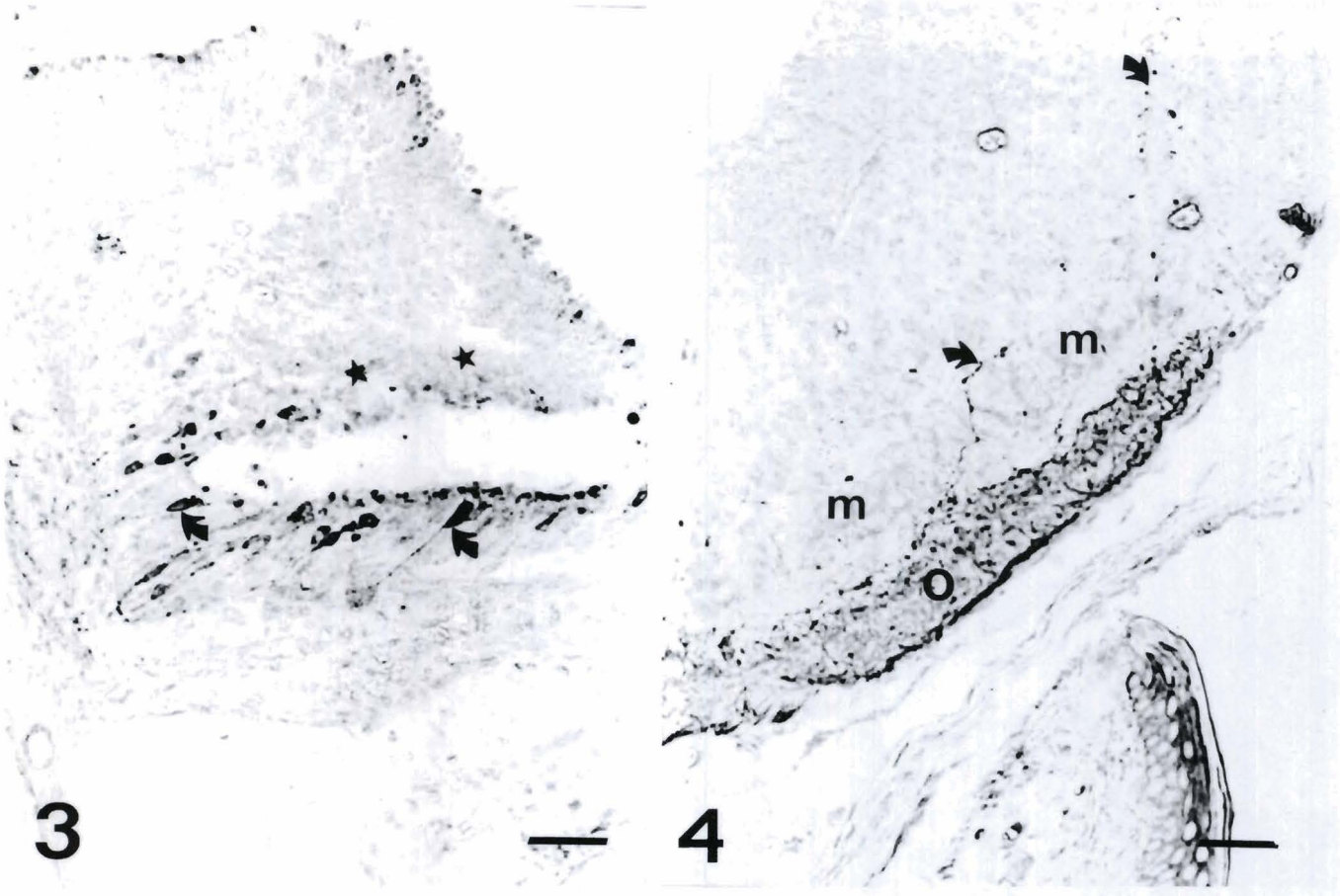


Fig. 3. Immunostaining for OMP in olfactory mucosa of 1 week-old *Monodelphis*. Cell bodies of OMP-positive neurons (arrows) are mainly restricted to the apical region of the epithelium. Olfactory marker protein is present in a patchy distribution in the epithelium with some regions (stars) showing virtually no immunoreactivity. Bar: 30 μm.

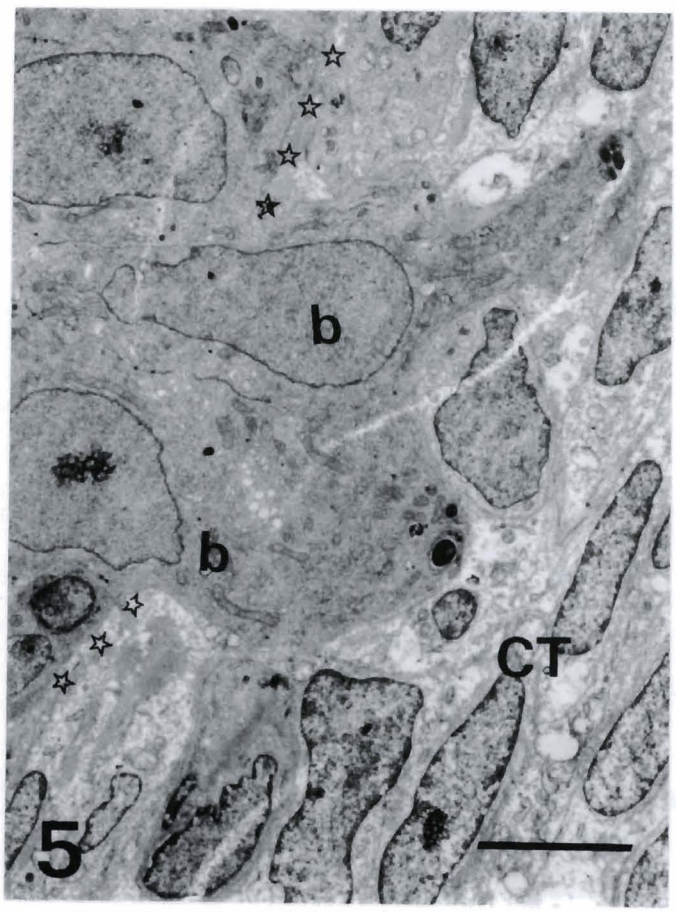
Fig. 4. One week-old *Monodephis*. Positive immunostaining for OMP is present in the olfactory nerve layer (o). Some of the fibers (arrows) are observed to course deep into the olfactory bulb, beyond the mitral cell layer (m). The fiber shown on the right is extremely fine; the punctate staining probably represents the varicosities of the axon. Bar: 30 μm.

mature olfactory neurons which is demonstrated immunohistochemically in rat at E17 (Biffo et al., 1992).

The uneven distribution of OMP-positive cells remains apparent as late as one week after birth, suggesting that their rate of differentiation is relatively slow (Fig. 3). The pattern of axonal OMP immunoreactivity in the olfactory bulb confirms the ongoing state of differentiation of the olfactory neurons. Some of these OMP-positive fibers are observed to extend deep into the olfactory bulb, beyond the mitral cell layer (Fig. 4). These exuberant projections of olfactory axons have also been observed in the perinatal rat and mouse; with subsequent postnatal development they usually undergo a gradual regression (Monti Graziadei et al., 1980; Santacana et al., 1992). The elimination of axons is a general phenomenon present in several developing systems and is thought to contribute to the final pattern of connectivity in adult animals (Crepel, 1982; Finlay and Slattery, 1983).

The sparse presence of OMP in the neonatal *Monodelphis* raises questions regarding the functional state of the olfactory system. Although the evidence indicates clearly that there are mature olfactory neurons, it also reveals that much of the sensory cells are still developmentally immature. One can speculate whether this cluster of mature olfactory neurons are sufficient to impart olfactory function that is crucial for survival in the early days after birth. It is worth noting that Harding and co-workers (1978) showed in an earlier study that mice can maintain sufficient olfactory function, at least for locating food, with significantly less than 10% of its population of olfactory cells functioning.

In addition to the presence of OMP in the *Monodelphis* olfactory mucosa, Cummings and Brunjes (1995) have shown that S-100 protein, which is often used as a marker for glial cells, is present in olfactory nerve fascicles at birth. Although observations from our laboratory indicate that GFAP, another glial cell marker, is absent in the olfactory nerves of the newborn *Monodelphis*, this protein can be demonstrated on the 5th postnatal day (Cummings and Brunjes, 1995). The presence of both these antigens indicate that the



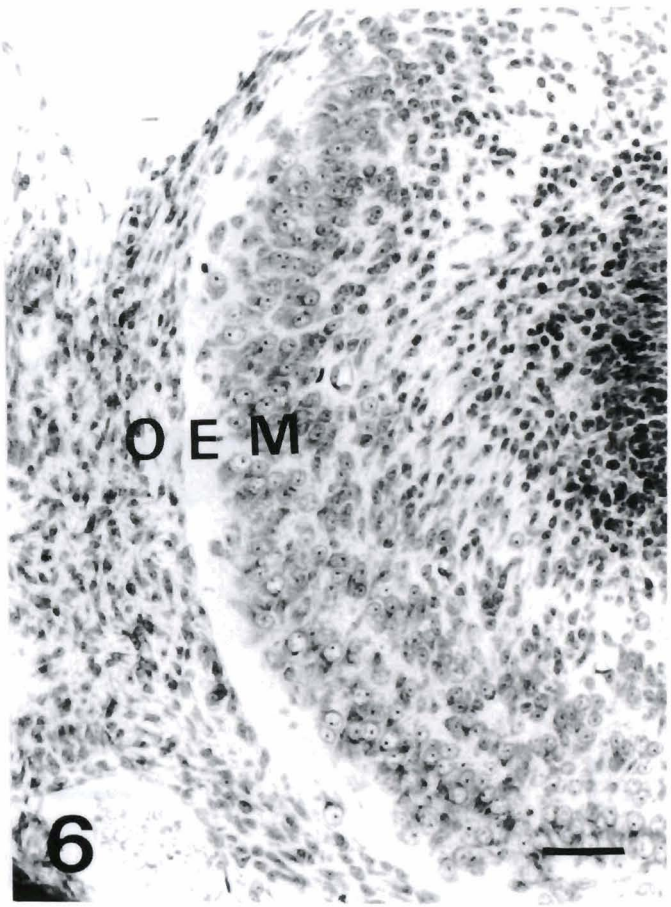


Fig. 5. Olfactory mucosa of nine day-old *Monodelphis*. Cells (b) which will eventually form Bowman's glands protrude into the underlying connective tissue (CT). The stars indicate the boundary between the olfactory epithelium and the connective tissue. Bar: 5.0 μm.

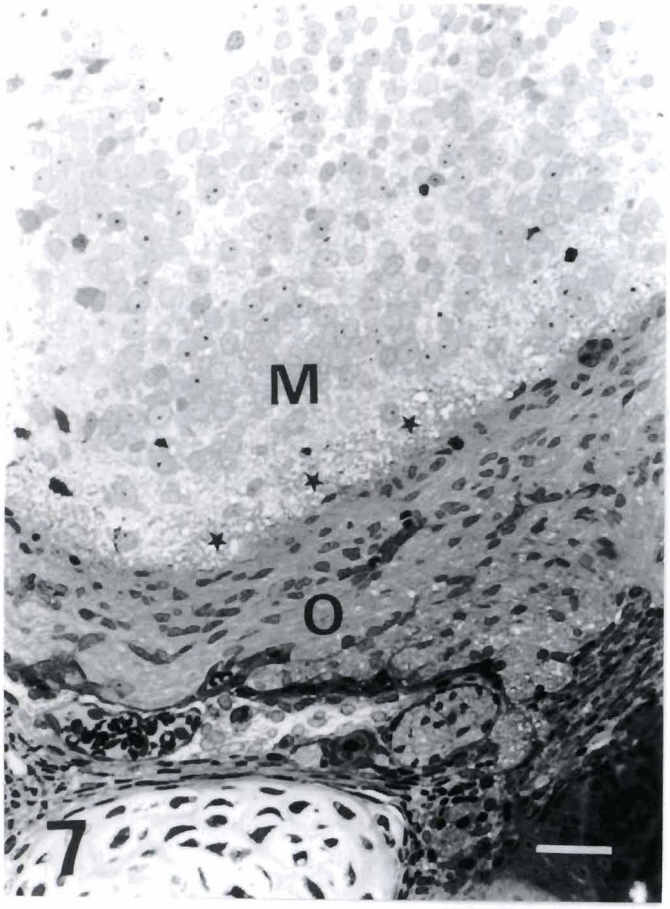
Fig. 6. Olfactory bulb of 1 week-old *Monodelphis*. Toluidine blue staining of paraffin section shows that the olfactory nerve (O), external plexiform (E) and mitral cell layers (M) have formed in the bulb. Bar: 40 μm.

ensheathing cells are quite well differentiated in the early postnatal period and that they may be instrumental in guiding the nerves to their appropriate target.

Given that no distinct basal cell population can be discerned in the newborn *Monodelphis*, it is not surprising that mitotic figures are restricted to the apical region of the olfactory epithelium. A basal cell population emerges at about the 9th postnatal day as evidenced by clusters of flat, electron dense cells near the basal lamina. This is accompanied by the appearance of mitotic figures in the basal part of the olfactory epithelium. The apical location of dividing cells is typically observed in fetal mice up to about the 11th day of gestation, after which the mitotic figures undergo a similar progressive shift to the basal region where postnatally they become most numerous (Smart, 1971).

It should be noted that no Bowman's glands are present in the lamina propria up to the 9th postnatal day although glands associated with the respiratory epithelium may be present earlier in development. On the 9th postnatal day, formation of Bowman's glands is initiated by the outgrowth of presumptive cells from the epithelium (Fig. 5). It appears then that onset of differentiation of supporting and basal cells and Bowman's glands all happens within a relatively brief period of time at around the 9th day after birth. Interestingly, ontogeny of the non-neural elements of the mouse olfactory mucosa also occur abruptly, albeit at an earlier time, on the 17th day of gestation (Cuschieri and Bannister, 1975).

Secretions from Bowman's glands normally contribute to most of the mucus covering the olfactory epithelial surface. The mucus is known to contain a soluble odorant binding protein which modifies stimulus molecules, particularly hydrophobic molecules, so that they become accessible to the receptors on the cilia of the olfactory neurons (Pelosi et al., 1982; Pevsner et al., 1986). The absence of Bowman's glands in the



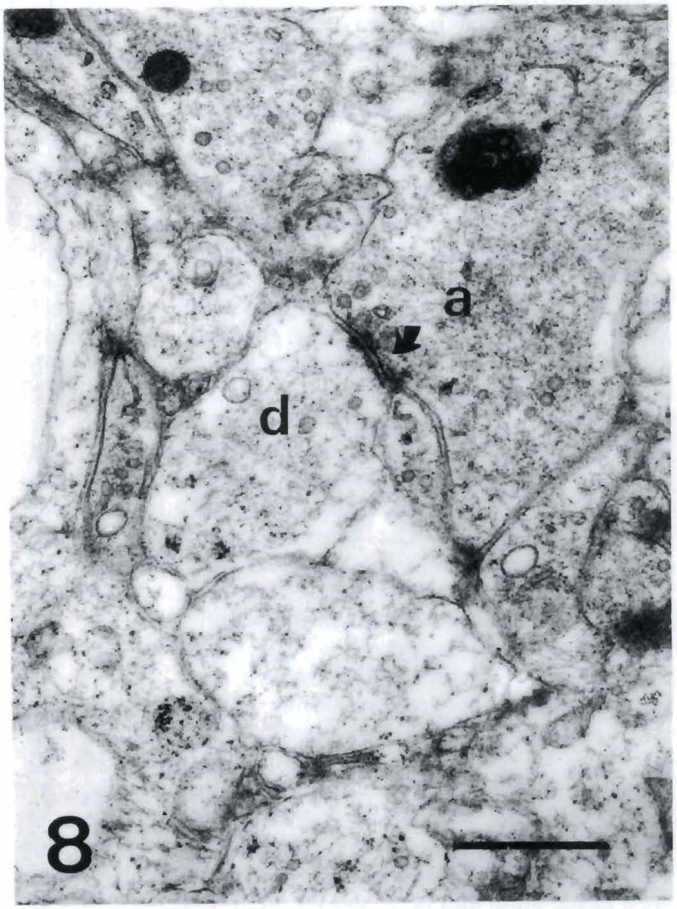


Fig. 7. Olfactory bulb of 9 day-old *Monodelphis*. Toluidine blue of plastic section shows the presence of a fringed border (stars) deep to the olfactory nerve layer (O). This region marks the incursion of olfactory axons into the bulb neuropil and it also contains dendrites from the mitral cells (M). Bar: 40 μm.

Fig. 8. Olfactory bulb of 9 day-old *Monodelphis*. Ultrastructural observations of the fringed border (labelled with stars in Fig. 7) show the presence of synapses, one of which is shown here (arrow). A small number of synaptic vesicles are present in the presynaptic axon (a). The dendrite (d) presumably from a mitral cell appears electron light. Bar: 0.8 μm.

Monodelphis during the first postnatal week throws doubt on the functional capability of the olfactory epithelium.

Differentiation of the olfactory bulb

Although the first strands of olfactory nerves have established contact with the olfactory bulb in the newborn *Monodelphis*, the latter remains morphologically immature as evidenced by the lack of lamination. Generally there is a higher density of cells towards the centre of the bulb with mitotic figures around the ventricular region. Abundant free ribosomes taking up much of the cytoplasm confirms the immaturity of these cells in this region. Larger cells with pale stained nuclei, presumably representing very immature mitral cells, are found towards the outer regions of the bulb. Ultrastructural examination shows that with the exception of the site at which olfactory nerves have penetrated the bulb, much of the peripheral region is interspersed with numerous spaces. No synapses have been observed in the bulb at the 3rd postnatal day although apposing membrane densities are present.

By the application of fluorescent tracers onto the lateral olfactory tract, Malun and Brunjes (1996) showed that labelled cells representing differentiating mitral cells are present in the bulb by the third postnatal day. These cells generally display a tangential orientation with numerous widespread dendrites. By the end of the first postnatal week, some degree of lamination is apparent as indicated by the presence of the olfactory nerve, external plexiform and mitral cell layer (Fig. 6). The next 2 days mark the incursion of the olfactory axons into the bulb neuropil, giving rise to a fringed border between the olfactory nerve and external plexiform layer (Fig. 7). Ultrastructural observations reveal synaptogenesis taking place between olfactory axons and mitral cell dendrites (Fig. 8). Generally, no more than 10 vesicles are found at each synaptic site. The sparsity of vesicles in the terminals suggests that the synapses are newly formed and that increasing numbers can be expected in view of the numerous processes containing vesicles. The overall pattern of synaptogenesis in the olfactory bulb is generally from the outside in, i.e development of synapses in the presumptive glomerular layer precedes that in the granular layer (Farbman, 1992). Hence it is not surprising that in the second postnatal week, synapses are rarely found in the more central regions of the *Monodelphis* bulb. In comparison, synaptogenesis in the mouse olfactory bulb takes place much earlier, at the 14th gestational day and essentially all synaptic types seen in the adult can be found on the day of birth (Hinds and Hinds, 1976a,b). However, for both the eutherian mammal and marsupial, synaptogenesis between olfactory axons and mitral dendrites takes place prior to the formation of a definitive glomerular layer (Hinds and Hinds, 1976a,b).

At the same time that synapses are forming between incoming olfactory axons and mitral cells, the latter are undergoing transformation into their typical mature morphology. During the second week after birth, the number of dendrites projecting from the mitral cell body decreases until a primary dendrite which ends in a terminal tuft remains (Malun and Brunjes, 1996); well developed glomeruli are only apparent in the third postnatal week (Shnayder and Halpern, 1992; Malun and Brunjes, 1996).

In summary, the sequence of events in the differentiating olfactory bulb of the *Monodelphis* is generally in agreement with that reported in the mouse and rat (Hinds, 1972; Mair et al., 1982). The major differences are that the initiation of the developmental processes are delayed and that they progress more slowly.

Migration of OMP-containing cells from the olfactory epithelium

Recent studies show the existence of OMP-containing cells migrating from the olfactory epithelium of the mouse and rat, beginning from E14 and E16 respectively (Valverde et al., 1993; Tarozzo et al., 1994). These cells were present along the course of the olfactory fibers and upon entering the cranium, they remained as a cluster above the cribriform plate. Interestingly, in the rat they seemed to disappear soon after birth. A similar migrating group of OMP-containing cells were first found in the *Monodelphis* at birth (Tarozzo et al., 1994). Throughout the postnatal eleven days, these cells were present in the rostral aspect of the primordial olfactory bulb and in the lamina propria along the length of the nasal septum. Although some OMP-positive cells could still be found in restricted areas of the forebrain in the adult *Monodelphis*, they appeared glial-like in morphology and did not correlate with those found in the neonatal *Monodelphis*.

The functional significance of the migrating OMP-positive cells has yet to be elucidated. What is known is that they are a separate population from the cells expressing luteinizing hormone-releasing hormone (LHRH, also known as GnRH) which are found in association with the nervus terminalis (Schwanzel-Fukuda et al., 1988).

Concluding remarks

In describing the morphological development of the primary olfactory pathway in the *Monodelphis*, and comparing it with that of the rodent, it appears that eutherian mammals and marsupials share similarities in the temporal sequence of developmental processes. The major difference lies in the exact time in ontogeny that each developmental process commences. Although several lines of evidence from behavioral (e.g. Rudy and Cheatle, 1977; Pedersen and Blass, 1981), electrophysiological (Gesteland et al., 1982) and morphological

(Cuschieri and Bannister, 1975; Hinds and Hinds, 1976a,b) studies support the notion that the eutherian olfactory system is functional at birth, the existing data does not provide conclusive evidence to confirm that this is also the case in marsupials.

The bulk of the data on the *Monodelphis* olfactory system is derived from morphological and immunohistochemical studies. These findings point to the immaturity of the olfactory system at birth and the slower progression of development. An issue worth pursuing is to determine the exact time of expression of molecules that are thought to be directly related to the olfactory tranducing apparatus, i.e. G proteins and adenylate cyclase. A knowledge of when odorant binding proteins are present will also be helpful in gauging more accurately the functional status of the olfactory system. Nevertheless, it is a well known fact that sightless newborn *Monodelphis* are able to manouvre their way from the birth canal to the mother's nipples. Whether that capability is largely dependent upon the few mature olfactory neurons present at birth (as determined by OMP immunoreactivity) or whether tactile input comes into play remains to be elucidated. Furthermore, if olfactory input is crucial to the survival of the marsupial neonate, we need to determine to what extent its locomotor behavior is regulated by a general sensitivity to odours versus a selective response to specific odours. Information from well controlled behavioral and physiological studies are essential before any firm conclusions can be drawn.

Acknowledgements. The authors thank Dr. Frank Margolis for the generous gift of anti-OMP, Mr. Edward Garrard for photographic assistance and Dr. Graham Knott for providing some of the *Monodelphis* tissue.

References

Allen W.K. and Akeson R. (1985). Identification on an olfactory receptor neuron subclass: cellular and molecular analysis during development. Dev. Biol. 109, 393-401.

Biffo S., Marti E. and Fasolo A. (1992). Carnosine, nerve growth factor receptor and tyrosine hydroxylase expression during ontogeny of the rat olfactory system. J. Chem. Neuroanat. 5, 51-62.

Biggins J.G. (1984). Communications in possums: a review. In: Possums and Gliders. Smith A.P. and Hume I.D. (eds). Aust. Mammal. Soc. Sydney. pp 35-37.

Brunjes P.C., Jazaeri A. and Sutherland M.J. (1992). Olfactory bulb organization and development in *Monodelphis domestica* (grey short-tailed opossum). J. Comp. Neurol. 320, 544-554.

Chuah M.I. and Farbman A.I. (1995). Developmental anatomy of the olfactory system. In: Handbook of olfaction and gustation. Doty R.L. (ed). Marcel Dekker Inc. New York. pp 147-171.

Chuah M.I. and Zheng D.R. (1987). Olfactory marker protein is present in olfactory receptor cells of human fetuses. Neuroscience 23, 363-370.

Crepel F. (1982). Regression of functional synapses in the immature mammalian cerebellum. Trends Neurosci. 5, 266-269.

Cummings D.M. and Brunjes P.C. (1995) Migrating luteinizing hormone-releasing hormone (LHRH) neurons and processes are associated with a substrate that expresses S100. Dev. Brain Res. 88, 148-157.

Cuschieri A. and Bannister L.H. (1975). The development of the olfactory mucosa in the mouse: electron microscopy. J. Anat. 119, 471-498.

Dau B., Menco B.P.M., Bruch R.C., Danho W. and Farbman A. (1991). Appearance of the transduction proteins G_s , G_{olf} and adenylate cyclase in the olfactory epithelium of rats occurs on different prenatal days. Chem. Senses 16, 511-512.

Farbman A.I. (1991). Developmental neurobiology of the olfactory system. In: Smell and taste in health and disease. Getchell T.V., Doty R.L., Bartoshuk L.M. and Snow Jr., J.B. (eds). Raven Press, N.Y. pp 19-33.

Farbman A.I. (1992). Cell biology of olfaction. Cambridge Univ. Press, U.K. pp 167-206.

Farbman A.I. and Margolis F.L. (1980). Olfactory marker protein during ontogeny: immunohistochemical localization. Dev. Biol. 74, 205-215.

Finlay B.L. and Slattery M. (1983). Local differences in the amount of early cell death in neocortex predict adult local specializations. Science 219, 1349-1351.

Gemmell R.T. and Nelson J. (1988). Ultrastructure of the olfactory system of 3 newborn marsupial species. Anat. Rec. 221, 655-662.

Gesteland R.C., Yancey R.A. and Farbman A.I. (1982). Development of olfactory receptor neuron selectivity in the rat fetus. Neuroscience 7, 3127-3136.

Graziadei P.P.C. (1971). The olfactory mucosa of vertebrates. In: Handbook of sensory physiology. Vol. IV. Chemical senses 1, Olfaction. Beidler L.M. (ed). Springer-Verlag. Berlin. pp 27-58.

Harding J.W., Getchell T.V. and Margolis F.L. (1978). Denervation of the primary olfactory pathway in mice. V. Long-term effect of intranasal $ZnSO_4$ irrigation on behavior, biochemistry, and morphology. Brain Res. 140, 271-285.

Hinds J.W. (1972). Early neuron differentiation in the mouse olfactory bulb. I. Light microscopy. J. Comp. Neurol. 146, 233-252.

Hinds J.W. and Hinds P.L. (1976a). Synapse formation in the mouse olfactory bulb. I. Quantitative studies. J. Comp. Neurol. 169, 15-40.

Hinds J.W. and Hinds P.L. (1976b). Synapse formation in the mouse olfactory bulb. II. Morphogenesis. J. Comp. Neurol. 169, 41-62.

Hughes R.L. and Hall L.S. (1984). Embryonic development in the common brushtail possum (*Trichosurus vulpecula*). In: Possums and Gliders. Smith A.P. and Hume I.D. (eds). Aust. Mammal. Soc., Sydney. pp 197-212.

Iqbal J., Elmquist J.K., Ross L.R., Ackermann M.R. and Jacobson C.D. (1995). Postnatal neurogenesis of the hypothalamic paraventricular and supraoptic nuclei in the Brazilian opossum brain. Dev. Brain Res. 85, 151-160.

Kratzing J.E. (1986). Morphological maturation of the olfactory epithelium of Australian marsupials. In: Ontogeny of olfaction. Breipohl W. (ed). Springer-Verlag. Berlin. pp 57-70.

Mair R.G., Gellman R.L. and Gesteland R.C. (1982). Postnatal proliferation and maturation of olfactory bulb of the neonatal rat. Neuroscience 7, 3105-3116.

Malun D. and Brunjes P.C. (1996). Development of olfactory glomeruli: temporal and spatial interactions between olfactory receptor axons and mitral cells in opossums and rats. J. Comp. Neurol. 368, 1-16.

Mania-Farnell B. and Farbman A.I. (1990). Immunohistochemical localization of guanine nucleotide-binding proteins in rat olfactory epithelium during development. Dev. Brain Res. 51, 103-112.

Margolis F.L. (1972). A brain protein unique to the olfactory bulb. Proc. Natl. Acad. Sci. USA. 69, 1221-1224.

Menco B.Ph.M. and Farbman A.I. (1985). Genesis of cilia and microvilli of rat nasal epithelia during pre-natal development. I. Olfactory epithelium, qualitative studies. J. Cell Sci. 78, 283-310.

Monti Graziadei G.A., Stanley R.S. and Graziadei P.P.C. (1980). The olfactory marker protein in the olfactory system of the mouse during development. Neuroscience 5, 1239-1252.

Pedersen P.E. and Blass E.M. (1982). Prenatal and postnatal determinants of 1st suckling episode in albino rats. Dev. Psychobiol. 15, 349-355.

Pelosi P., Baldaccini N.E. and Pisanelli A.M. (1982). Identification of a specific olfactory receptor for 2-isobutyl-3-methoxypyrazine. Biochem. J. 201, 245-248.

Pevsner J., Sklar P.B. and Snyder S.H. (1986). Odorant-binding protein: localization to nasal glands and secretions. Proc. Natl. Acad. Sci. USA 83, 4942-4946.

Rama Krishna N.S., Getchell T.V., Margolis F.L. and Getchell M.L. (1992). Amphibian olfactory receptor neurons express olfactory marker protein. Brain Res. 593, 295-298.

Renfree M.B, Fletcher T.P., Blanden D.R., Lewis P.R., Shaw G., Gordon K., Short R.V., Parer-Cook E. and Parer D. (1989). Physiological and behavioural events around the time of birth in macropodid marsupials. In: Kangaroos, Wallabies and Rat-Kangaroos. Gridd G., Jarman P. and Hume J. (eds). Surrey Beatty & Sons Ltd. New South Wales. pp 323-337.

Reynolds M.L., Cavanagh M.E., Dziegielewska K.M., Hinds L.A., Saunders N.R. and Tyndale-Biscoe C.H. (1985). Postnatal development of the telencephalon of the tammar wallaby (*Macropus eugenii*). An accessible model of neocortical differentiation. Anat. Embryol. 173, 81-94.

Rudy J.W. and Cheatle G.D. (1977). Odor aversion learning in neonatal rats. Science 198, 845-846.

Santacana M., Heredia M. and Valverde F. (1992). Transient pattern of exuberant projections of olfactory axons during development in the rat. Dev. Brain Res. 70, 213-222.

Saunders N.R., Adam E., Reader M. and Mollgard K. (1989). *Monodelphis domestica* (grey short-tailed opossum): an accessible model for studies of early neocortical development. Anat. Embryol. 180, 227-236.

Schwanzel-Fukuda M., Fadem B.H., Garcia M.S. and Pfaff D.W. (1988). Immunocytochemical localization of luteinizing hormone-releasing hormone (LHRH) in the brain and nervus terminalis of the adult and early neonatal gray short-tailed opossum (*Monodelphis domestica*). J. Comp. Neurol. 276, 44-60.

Shnayder L. and Halpern M. (1992). Developmental expression of OMP and N-CAM in the nasal chemosensory systems of the postnatal Brazilian short-tailed opossum. *M. domestica*. Chem. Senses 17, 698.

Smart I.H.M. (1971). Location and orientation of mitotic figures in the developing mouse olfactory epithelium. J. Anat. 109, 243-251.

Tarozzo G., Peretto P., Perroteau I., Andreone C., Varga Z., Nicholls J. and Fasolo A. (1994). GnRH neurons and other cell populations migrating from the olfactory neuroepithelium. Ann. Endocrinol. Paris 55, 249-254.

Tarozzo G., Peretto P., Biffo S., Varga Z., Nicholls J.G. and Fasolo A. (1995). Development and migration of olfactory neurones in the nervous system of the neonatal opossum. Proc. R. Soc. Lond. B. 262, 95-101.

Tennent R. and Chuah M.I. (1996). Ultrastructural study of ensheathing cells in early development of olfactory axons. Dev. Brain Res. 95, 135-139.

Valverde F., Heredia M. and Santacana M. (1993). Characterization of neuronal cell varieties migrating from the olfactory epithelium during prenatal development in the rat. Immunocytochemical study using antibodies against olfactory marker protein (OMP) and luteinizing hormone-releasing hormone (LH-RH). Dev. Brain Res. 71, 209-220.