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Stem cells and their niche in the adult olfactory mucosa

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ABSTRACT

It is well known that new neurons are produced in the adult brain, in the hippocampus and in the subventricular zone. The neural progenitors formed in the subventricular zone migrate forward and join in neural circuits as interneurons in the olfactory bulb, the target for axons from the olfactory sensory neurons in the nose. These neurons are also continually replaced during adulthood from a stem cell in a neurogenic niche in the olfactory epithelium. The stem cell responsible can regenerate all the cells of the olfactory epithelium if damaged by trauma or toxins. This stem cell, the horizontal basal cell, is in a niche defined by the extra cellular matrix of the basement membrane as well as the many growth factors expressed by surrounding cells and hormones from nearby vasculature. A multipotent cell has been isolated from the olfactory mucosa that can give rise to cells of endodermal and mesodermal origin as well as the expected neural lineage. Whether this is an additional stem cell or the horizontal basal cell is still an open question.

Key words

Neurogenesis • Adult stem cell • Neural stem cell • Subventricular zone • Olfactory epithelium • Human

Introduction

In humans, as in other mammals, there is a continuing neurogenesis in the subventricular zone generating neurons that migrate and become interneurons in the olfactory bulb (Curtis et al., 2009). The olfactory bulb is that part of the brain which receives axonal input from the sensory neurons in the olfactory epithelium in the nose. The olfactory epithelium is the most superficial layer of the olfactory mucosa which also comprises a lamina propria containing the specialized olfactory glands ("Bowman's glands") and the olfactory sensory axons surrounded by their specialized glia, the olfactory ensheathing cells (Fig. 1). In mammals, the olfactory mucosa is typically located dorsally and posteriorly in the nasal cavity covering the nasal septum medially, and laterally, covering the turbinate bones, to provide a very large surface area of sensory cilia projecting from the dendrite of the sensory neurons (Fig. 2). The human olfactory mucosa is similar in structure to other vertebrates with the same cell types in a pseudostratified olfactory epithelium (Moran et al., 1982; Nakashima et al., 1984; Morrison and Costanzo, 1990; Nakashima et al., 1991). In human adult, compared to fetus, there is a loss of olfactory epithelium from the superior posterior regions of the nasal cavity and its replacement with respiratory and squamous epithelium (Nakashima et al., 1984). The "olfactory region" in the adult has a non-contiguous and patchy distribution of olfactory epithelium (Moran et al., 1982; Nakashima et al., 1984; Morrison and Costanzo, 1990; Paik et al., 1992). On the other hand, the distribution of olfactory epithelium in adult human extends more anteriorly and inferiorly than in the fetus (Feron et al., 1998; Leopold et al., 2000).

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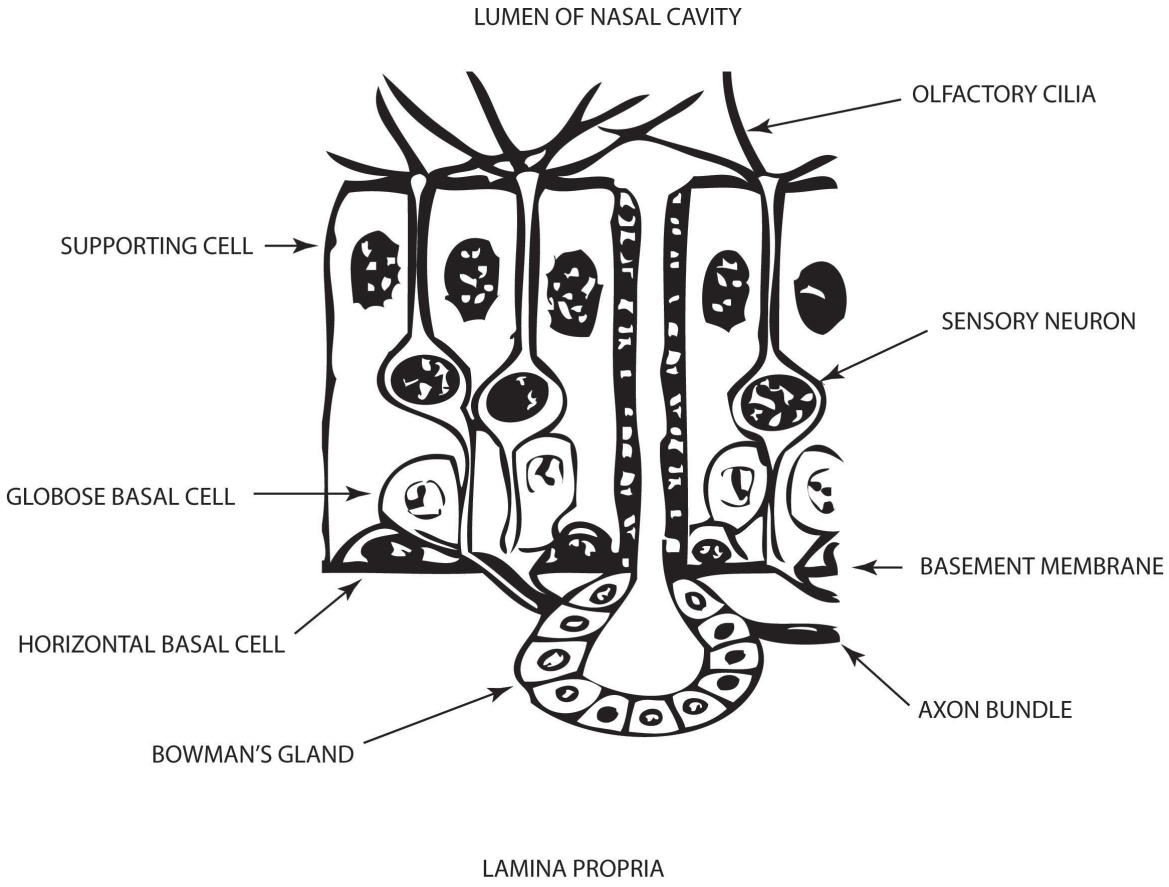

Fig. 1. - Olfactory epithelium.
The olfactory mucosa is divided into epithelium and lamina propria, separated by the basement membrane. The sensory neurons in the epithelium send a dendrite to the apical surface where their cilia extend into the mucus lining the lumen of the nasal cavity. The sensory axons leave the epithelium and enter the lamina propria where they gather in bundles surrounded by olfactory ensheathing cells. The Bowman's glands are specialised glands of the olfactory mucosa located in the lamina propria with their ducts open into the lumen. Most of the luminal surface is made up by supporting cells, covered in microvilli, whose end-feet extend to the basement membrane. The horizontal basal cell, the neural stem cell, sits adjacent to the basement membrane. The globose basal cell, a multipotent precursor, migrates away from the basement membrane and gives rise to the sensory neuron.

Until now, analysis of human olfactory mucosa has been restricted to sampling of biopsy and autopsy material and no systematic and complete distribution study of human olfactory mucosa has been done. The axons of the olfactory sensory neurons leave the olfactory epithelium and form bundles and fascicles in the lamina propria before entering the olfactory bulb where they synapse with mitral/tufted cells and interneurons, some of which are generated by neurogenesis in the subventricular zone (Breton-Provencher et al., 2009; Curtis et al., 2009).

Seventy years ago mitotic activity was first observed in the basal cells of the olfactory epithelium of adult mice (Nagahara, 1940) and soon after, regeneration of olfactory sensory neurons was observed in monkey after toxic damage (Schultz, 1941). Numerous reports confirmed these observations in frog (Smith, 1951), fish (Westerman and Baumgarten, 1964), cat and dog (Andres, 1966), lamprey (Thornhill, 1970), mouse (Moulton et al., 1970; Thornhill, 1970; Graziadei and Metcalf, 1971; Smart, 1971), monkey (Graziadei et al., 1980) and human (Wolozin et al., 1992; Murrell et al., 1996). The vulnerability of the olfactory sensory neurons to environmental factors makes neurogenesis in the olfactory epithelium necessary in order to maintain the sense of smell, essential for most aspects of daily life in most vertebrates including food seeking, pathfinding, social interactions and sexual behaviour. It is worth considering that a primary drive for neurogenesis in the

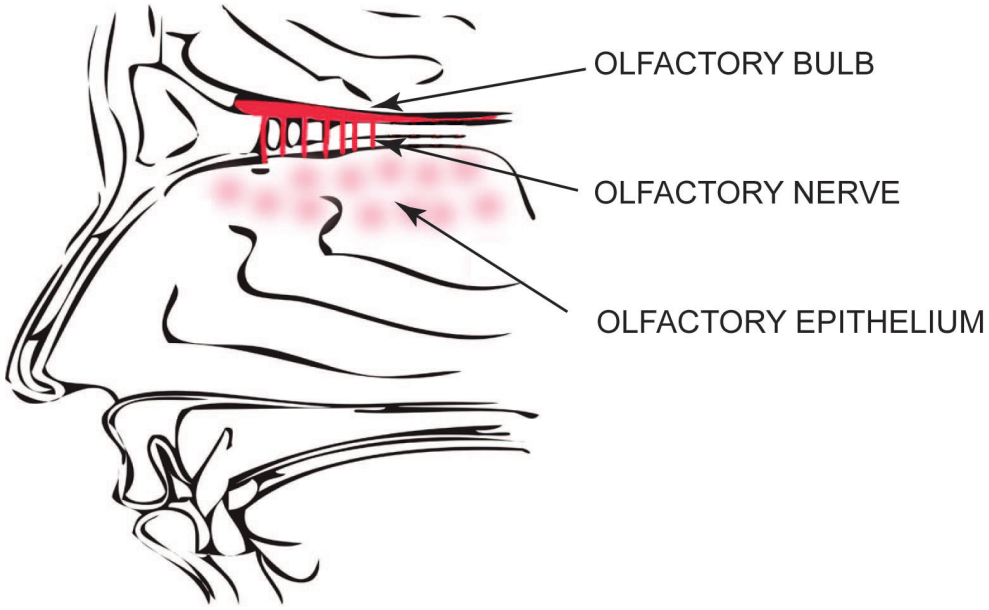

Fig. 2. - Anatomy of the human olfactory system.
In the adult nose, the olfactory mucosa is patchily distributed in the superior-posterior region of the nasal cavity interspersed with respiratory mucosa. The olfactory sensory axons gather in bundles and penetrate the skull at the cribriform plate to synapse in the olfactory bulb lying on the skull base under the forebrain.

adult forebrain, that replenishes interneurons in the olfactory bulb, is the requirement to provide new central circuits because of the continual neurogenesis occurring in the periphery. For example, there is evidence that olfactory bulb interneurons are lost after chemical destruction of the sensory neurons and up-regulated when sensory innervation returns (Nadi et al., 1981; Baker et al., 1993). The olfactory mucosa is accessible in living adult humans (Feron et al., 1999b) and so presents an accessible source of tissue for understanding the biology of adult neurogenesis in health and disease (Mackay-Sim and Silburn, 2008). It is also a source of cells for transplantation repair of the nervous system (Feron et al., 2005; Mackay-Sim et al., 2008). Understanding the biology of olfactory neural stem cells and their niche will be very important for optimizing clinical applications.

The requirement for stem cells in olfactory mucosa

A major difference between olfactory sensory neurons and other neurons is their proximity to the external environment which increases their risk of death (Fig. 1). The dendrites of the olfactory sensory neurons penetrate the epithelium and are decorated with multiple cilia containing the sensory transduction proteins. These cilia extend into the overlaying mucus and may be only a few microns below the surface (Menco, 1980). Inhaled toxic gases can destroy the olfactory epithelium and the neurons within it. Inhalation of N-methyl-formiminomethylester and methyl bromide lead to reversible loss of the sensory neurons with temporary loss of smell (Rehn et al., 1981; Schmidt et al., 1984; Hurtt et al., 1988). Even nasal lavage with wheat germ agglutinin-horse radish peroxidase, induces loss of sensory neurons from the epithelium and stimulates basal cell proliferation (Moon and Baker, 1998), indicating the potential for large molecules to affect sensory neuron survival. Olfactory neurons provide a route for viruses and bacteria to enter the brain (Owen et al., 2009) and for this reason they may be vulnerable to apoptosis as a protective mechanism, part of an innate response to infection (Harris et al., 2009). Environmental susceptibility is also suggested by evidence that olfactory neurogenesis is vulnerable to usage. For example, if over-usage is induced by closure of one naris in adult mice, the

open side epithelium is reduced in thickness and sensory neurons are lost, with a lack of ability to maintain regeneration (Maruniak et al., 1989). The mechanism for this loss is unknown but it may be due to overuse, to toxins or to infections, as indicated by the large number of polymorphonuclear leukocytes on the open side after 7 and 8 months of closure (Maruniak et al., 1990). With increasing age, the proliferative and neurogenic capacity of the olfactory epithelium declines, especially in the antero-dorsal region in the rat (Loo et al., 1996; Weiler and Farbman, 1997). This may reflect usage, rather than age, per se, because neurogenesis in the posterior nasal cavity is better preserved (Loo et al., 1996; Weiler and Farbman, 1997). Histologically the olfactory epithelium in the antero-dorsal region in the ageing rat has reduced lamination, a loss of neurons and increased proliferation of supporting cells (Loo et al., 1996). Similar changes are seen in olfactory epithelium from aged human with reduced thickness, reduced numbers of sensory neurons and patchy distribution of olfactory epithelium within the respiratory epithelium (Naessen, 1971; Nakashima et al., 1984).

Regulation of neurogenesis in the olfactory epithelium

Neurogenesis in the olfactory epithelium is tightly regulated such that the number of mature sensory neurons is maintained with a constant surface density of sensory dendrites (Mackay-Sim et al., 1988), although there is a large overproduction of immature neurons with only a subset surviving (Hinds et al., 1984; Mackay-Sim and Kittel, 1991b; Mackay-Sim and Kittel, 1991a). Neurogenesis is stimulated by the death of the sensory neurons from olfactory nerve transection (Nagahara, 1940; Graziadei, 1973) and from toxins like zinc sulphate and methimazole (Schultz, 1941; Smith, 1951; Margolis et al., 1974; Genter et al., 1995) which cause apoptotic cell death (Michel et al., 1994; Holcomb et al., 1995; Deckner et al., 1997). The loss of neurons increases basal cell mitosis (Graziadei, 1973; Camara and Harding, 1984) with recovery of the sensory neuron population (Costanzo and Graziadei, 1983; Samanen and Forbes, 1984) and recovery of function (Harding et al., 1978; Costanzo, 1985). Despite a high rate of proliferation of neuronal precursors, the thickness of the olfactory epithelium remains static in the adult rat from 60-330 days of age (Hinds and McNelly, 1981; Weiler and Farbman, 1997). Without an increase in epithelial thickness it follows that cell proliferation is balanced by apoptotic cell death. Apoptosis occurs at all stages of olfactory neuron development from recently born basal cells through to immature and mature neurons (Carr and Farbman, 1993; Holcomb et al., 1995; Magrassi and Graziadei, 1995; Mahalik, 1996).

Some of the growth factor signals that regulate stem and progenitor proliferation have been identified. Horizontal basal cell proliferation is stimulated by EGF and TGFα (Mahanthappa and Schwarting, 1993; Farbman and Buchholz, 1996; Newman et al., 2000; Carter et al., 2004). Globose basal proliferation is stimulated by FGF2 (DeHamer et al., 1994; Newman et al., 2000). *In vivo*, appropriate FGF receptor subtypes (FGFR1 and FGFR2) are present and FGF2 immunoreactivity is also present in globose basal cells and supporting cells (Hsu et al., 2001). EGF and FGF2 are important indicators of neural stem cell proliferation and are required to generate multipotent neurospheres from olfactory mucosa (Murrell et al., 2005, 2008; Tome et al., 2009) just as they are required to generate neurospheres from adult brain (Reynolds and Weiss, 1992; Kilpatrick and Bartlett, 1993).

Tissue homoeostasis is maintained by positive feedback from dying neurons which release LIF to stimulate basal cell proliferation and survival (Bauer et al., 2003; Carter et al., 2004). Similarly, nitric oxide released during inflammation or cell death stimulates basal proliferation independently of other growth factors (Harris et al., 2009; Sulz et al., 2009). Negative feedback of basal cell proliferation is provided by the local density of developing neurons *in vivo* (Mackay-Sim et al., 1988) and *in vitro* (Mumm et al., 1996) probably via BMP2/4/7 (Shou et al., 1999, 2000). Sensory neuron differentiation is induced *in vitro* by TGFβ2, IGF1 and dopamine (Mahanthappa and Schwarting, 1993; Feron et al., 1999a; Newman et al., 2000; McCurdy et al., 2005). IGF1 and dopamine are available in the mucus bathing the olfactory epithelium (Lucero and Squires, 1998; Federico et al., 1999) and could act as target-derived factors that provide a selective advantage to developing neurons whose dendrite reaches the

epithelial surface. Dopamine may serve a similar role as a target-derived factor for sensory axons as it is available from periglomerular interneurons with which they synapse in the olfactory bulb (Ennis et al., 2001). Survival of immature olfactory neurons *in vitro* is enhanced by PDGF and, to a lesser extent, BDNF (Newman et al., 2000). Survival of immature, but not mature sensory neurons *in vivo* requires thyroxine (Mackay-Sim and Beard, 1987).

Olfactory stem cells

Numerous studies suggest that there is a hierarchical stem cell lineage in the adult olfactory mucosa which normally regenerates the olfactory epithelium, including its neural and non-neural components. This evidence is generated in rat and mouse but, given the similarity in the olfactory mucosa in all vertebrates, including humans, it is expected that the same principles apply, although some differences in human tissues are reported (Hahn et al., 2005). Following destruction of the olfactory nerve, the sensory neurons regenerate and the animals regain their sense of smell (Costanzo, 1984, 1985). The weight of evidence indicates that the horizontal basal cell is the stem cell responsible for regeneration. The horizontal basal cell proliferates slowly, and self-renews (Mackay-Sim and Kittel, 1991b) and it generates all olfactory epithelial cell types *in vivo* and *in vitro* (Carter et al., 2004; Leung et al., 2007). Chemical ablation of the olfactory epithelium induces proliferation of the horizontal basal cells and subsequent regeneration of the epithelium and recovery of smell function (Rehn et al., 1981; Youngentob and Schwob, 2006). Reconstitution of the olfactory epithelium after chemical ablation is also achieved by transplantation of globose basal cells which, like horizontal basal cells, give rise to sensory neurons, supporting cells and Bowmans glands and duct cells(Huard et al., 1998; Chen et al., 2004). This leads to discussion of the roles of globose versus horizontal basal cells in a lineage hierarchy: which is the "true" stem cell?

An analysis of cell dynamics identified two dividing populations among the basal cells of the olfactory epithelium, a rapidly dividing population (1 division/day) and a slowly dividing population (1 division/~60 days) (Mackay-Sim and Kittel, 1991b). The rapidly dividing population migrated from the basement membrane into the neuronal layer whereas the slowly dividing population remained on the basement membrane (Mackay-Sim and Kittel, 1991b). These behaviours are consistent with a slowly dividing stem cell giving rise to a rapidly dividing, transit amplifying neural progenitor as seen *in vitro* (Calof and Chikaraishi, 1989) and its location is consistent with the horizontal basal cell as the stem cell (Rehn et al., 1981; Mackay-Sim and Kittel, 1991b; Carter et al., 2004; Leung et al., 2007) and the globose basal cell as the rapidly dividing precursor (Huard et al., 1998; Newman et al., 2000). Under the electron microscope there appear to be transitional cell types from globose basal cell through to sensory neuron with evidence that some globose basal cells are immature neurons (Graziadei, 1973; Graziadei and Monti Graziadei, 1979). *In vitro* it is the globose basal cells that are the immediate neuronal precursors (Newman et al., 2000) and single horizontal basal cells can proliferate and differentiate into globose basal cells, olfactory neurons and olfactory ensheathing cells (Carter et al., 2004). These experiments suggest that the horizontal basal cell can generate all the cells of the olfactory epithelium. Because the globose basal cell arises from the horizontal basal cell *in vivo* (Leung et al., 2007), it is likely that the horizontal basal cell is "true" tissue stem cell that maintains long-term regenerative capacity of the olfactory epithelium, whereas the globose basal cell is a multipotent, transit amplifying cell and immediate neuronal precursor (Calof and Chikaraishi, 1989; Newman et al., 2000). The source of the olfactory ensheathing cells below the basement membrane has not been definitively established but electron microscopic investigations suggest they emerge from the basal layer of the epithelium, that is, from the horizontal basal cells (Chuah and Au, 1991).

Although not yet identified positively as a particular cell type, an additional stem cell has been grown *in vitro* from biopsies of adult mouse, rat and human olfactory mucosa. This cell is multipotent and can generate cells not only from the epithelial and neural lineages but also from embryonic mesodermal and endodermal lineages (Murrell et al., 2005, 2008, 2009; McDonald et al., 2010). A multipotent stem

cell was also cultured from embryonic rat olfactory mucosa which expresses three mesenchymal markers that are expressed by some cells in the lamina propria (Tome et al., 2009). Cells cultured from adult human olfactory lamina propria expressed 19/32 CD markers, also expressed by human bone marrow mesenchymal stem cells, prompting the olfactory cells to be named "ecto-mesenchymal" stem cell while still retaining a neural lineage phenotype (Feron et al., 2009). These studies raise the possibility that there may be a stem cell in the olfactory lamina propria different from the horizontal basal cell in the epithelium above it. Although such a cell can be derived in cell culture, the identity of such a tissue-resident cell type is unknown. This will require prospective isolation as demonstrated for the mouse neural stem cell (Rietze et al., 2001).

The neurogenic niche in the olfactory epithelium

A cellular "niche" is defined by the local extracellular matrix, the local paracrine and autocrine signalling milieu, contact with neighbouring cells, as well as factors acting at a distance such as hormones diffusing from nearby blood vessels. A cellular niche is also defined by the cell surface receptors present on a cell that will define its capability to respond to extracellular signals. Horizontal basal cells express ICAM-1 and the integrins β1, β4, α1, α3 and α6 which act as receptors for collagens, fibronectin and laminin in the basement membrane (Carter et al., 2004). When selected for ICAM-1 expression and grown as clones, these horizontal basal cells demonstrated the stem cell properties of self-renewal and capable of differentiating into globose basal cells, olfactory sensory neurons and glia (Carter et al., 2004). An important aspect of a stem cell niche is to provide a mechanism to establish polarity for the regulation of symmetrical versus asymmetrical cell division. In the development of the nervous system, the neural stem cells are bipolar cells extending from basement membrane to apical surface. It is proposed that proteins in the apical membrane determine whether the cell undergoes symmetric versus asymmetric division (Gotz and Huttner, 2005). In the case of epithelia in which the stem cell lies on a basement membrane, the surface membrane exposed to the matrix proteins of the basement membrane may define polarity (Fuchs, 2009). The specific role of the basement membrane in determining symmetric versus asymmetric divisions in horizontal basal cells is not yet determined.

The complexity of the neurogenic niche of the olfactory epithelium is illustrated by the very large number of growth factors and their receptors in the olfactory epithelium (Mackay-Sim and Chuah, 2000). For example the neurotrophins and their receptors are each expressed by specific sets of cells (Feron et al., 2008). Horizontal basal cells express TrkA and NT4 whereas sensory neurons express TrkB abd TrkC and all the neurotrophins (Feron et al., 2008). Supporting cells, developing neurons and olfactory ensheathing cells express other combinations (Feron et al., 2008). Basal cells and neurons express CNTF (Buckland and Cunningham, 1999) and the dopamine D2 receptor (Feron et al., 1999a) Supporting cells, neurons, basal cells all express FGF2 (Matsuyama et al., 1992; Gall et al., 1994; Goldstein et al., 1997; Chuah and Teague, 1999; Hsu et al., 2001) whereas only neurons express GDNF (Buckland and Cunningham, 1999). The horizontal basal cell expresses the epidermal growth factor receptor, EGFR and proliferates in response to its ligands EGF and TGFα (Farbman et al., 1994; Farbman and Buchholz, 1996; Getchell et al., 2000; Newman et al., 2000; Carter et al., 2004). A full repertoire of receptors of horizontal basal cells is not known but it can be expected that there will be factors that inhibit horizontal basal cell proliferation as well as those that stimulate it, like EGFR activation. This prediction arises from the observation that horizontal basal cells appear to divide only every 30-60 days (Mackay-Sim and Kittel, 1991b) and they contribute to the olfactory cell lineages only when severe insult kills the sensory neurons and their globose basal cell progenitors (Rehn et al., 1981; Leung et al., 2007; Iwai et al., 2008). Otherwise the sensory neurons are maintained by the multipotent globose basal cells (Huard et al., 1998; Chen et al., 2004). There may be multiple factors to suppress horizontal basal cell proliferation in order to maintain its proliferative capacity for the lifetime of the organism. Some factors may derive from blood in the capillaries that are present in the lamina propria close to the basement membrane as demonstrated by the regulation of neuronal progenitor survival by thyroxine (Mackay-Sim and Beard, 1987).

As well as diffusible growth factors and hormones, intercellular signalling by direct contact is normally an important part of regulation of development of the nervous system. In the case of the adult olfactory epithelium, whose cells are subject to direct environmental damage, it could be advantageous for a direct feedback from the differentiated cells to the stem cells generating them. The location of the horizontal basal cell places it in direct contact with all its progeny which could deliver continuous feedback signals about their function. As the horizontal basal cell divides and differentiates its globose basal cell progeny are apically in direct contact (Graziadei and Monti Graziadei, 1979). Supporting cells extend from the luminal surface of the epithelium with their endfeet on the basement membrane in close association with the horizontal basal cell (Graziadei and Monti Graziadei, 1979; Morrison and Costanzo, 1990). The supporting cells surround the sensory neuron dendrites (Graziadei and Monti Graziadei, 1979) and make tight junctions at the epithelial surface (Menco, 1980). They are in a location to monitor the external environment with their microvilli extending into the mucus above. With the sensory neurons, the supporting cells could provide a pathway for conducting signals from the environment to the horizontal basal cell. As neurons differentiate their axons extend basally to be wrapped in bundles by the horizontal basal cells as they leave the epithelium through the basement membrane (Holbrook et al., 1995). Entering the lamina propria the axon bundles are wrapped by olfactory ensheathing cells which are very closely associated with the basement membrane and appear to derive from the epithelium above (Doucette, 1984; Chuah and Au, 1991; Gong et al., 1994). Finally, the horizontal basal cell is also intimately associated with the Bowman's glands in the lamina propria and their ducts in the epithelium (Graziadei and Monti Graziadei, 1979; Morrison and Costanzo, 1990).

Summary

The adult olfactory epithelium provides an interesting model for understanding neural development and regeneration. Uniquely, the olfactory epithelium is accessible in humans giving access to stem cells, neural progenitors and neurons in every adult. This allows investigations of regulatory genes, growth factors and cytokines that control neurogenesis from stem cell to mature neuron in the human. The tissue-specific stem cell is identified as the horizontal basal cell, which can regenerate all the neural and non-neural cell types of the peripheral olfactory system. The "stem cell niche" of the horizontal basal cell is dominated by its location on the basement membrane and its direct contact with all other cell types of the epithelium. This is an ideal location to monitor the state of its progeny and to respond to the multiple growth factors present in the extracellular space. This niche keeps the normal rate of proliferation very low (~50 days) but triggers rapid proliferation when the olfactory epithelium is damaged sufficiently that the multipotent transit amplifying cell, the globose basal cell, is damaged or otherwise unable to regenerate the epithelium.

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References

Andres K. Der Feinbau der Regio olfactoria von Makrosmatikern. *Z. Zellforsch. Mikrosk. Anat.*, **69**: 140-154, 1966.

Baker H., Morel K., Stone D.M., Maruniak J.A. Adult naris closure profoundly reduces tyrosine hydroxylase expression in mouse olfactory bulb. *Brain Res.*, **614**: 109-116, 1993.

Bauer S., Rasika S., Han J., Mauduit C., Raccurt M., Morel G., Jourdan F., Benahmed M., Moyse E. and Patterson P.H. Leukemia inhibitory factor is a key signal for injury-induced neurogenesis in the adult mouse olfactory epithelium. *J. Neurosci.*, **23**: 1792-1803, 2003.

Breton-Provencher V., Lemasson M., Peralta M.R. 3rd, Saghatelyan A. Interneurons produced in adulthood are required for the normal functioning of the olfactory bulb network and for the execution of selected olfactory behaviors. *J. Neurosci.*, **29**: 15245-15257, 2009.

Buckland M.E. and Cunningham A.M. Alterations in expression of the neurotrophic factors, glial cell line-derived neurotrophic factor, ciliary neurotrophic factor and brain-derived neurotrophic factor, in the target-deprived olfactory neuroepithelium. *Neuroscience*, **90**: 333-347, 1999.

Calof A.L. and Chikaraishi D.M. Analysis of neurogenesis in a mammalian neuroepithelium: proliferation and differentiation of an olfactory neuron precursor in vitro. *Neuron*., **3**: 115-127, 1989.

Camara C.G. and Harding J.W. Thymidine incorporation in the olfactory epithelium of mice: early exponential response induced by olfactory neurectomy. *Brain Res.*, **308**: 63-68, 1984.

Carr V.M. and Farbman A.I. The dynamics of cell death in the olfactory epithelium. *Experimental Neurology*, **124**: 308-314, 1993.

Carter L.A., MacDonald J.L., Roskams A.J. Olfactory horizontal basal cells demonstrate a conserved multipotent progenitor phenotype. *J. Neurosci.*, **24**: 5670-5683, 2004.

Chen X., Fang H., Schwob J.E. Multipotency of purified, transplanted globose basal cells in olfactory epithelium. *J. Comp. Neurol.*, **469**: 457-474, 2004.

Chuah M.I. and Au C. Olfactory Schwann cells are derived from precursor cells in the olfactory epithelium. *J. Neurosci. Res.*, **29**: 172-180, 1991.

Chuah M. and Teague R. Basic fibroblast growth factor in the primary olfactory pathway: mitogenic effect on ensheathing cells. *Neuroscience*, **88**: 1043-1050, 1999.

Costanzo R.M. and Graziadei P.P.C. A quantitative analysis of changes in the olfactory epithelium following bulbectomy in hamster. *J. Comp. Neurol.*, **215**: 370-381, 1983.

Costanzo R.M. Comparison of neurogenesis and cell replacement in the hamster olfactory system with and without a target (olfactory bulb). *Brain Res*., **307**: 295-301, 1984.

Costanzo R.M. Neural regeneration and functional reconnection following olfactory nerve transection in hamster. *Brain Res.*, **361**: 258-266, 1985.

Curtis M.A., Monzo H.J., Faull R.L. The rostral migratory stream and olfactory system: smell, disease and slippery cells. *Prog. Brain. Res*., **175**: 33-42, 2009.

Deckner M.-L., Risling M., Frisen J. Apoptotic death of olfactory sensory neurons in the adult rat. *Experimental Neurology*, **143**: 132-140, 1997.

DeHamer M.K., Guevara J.L., Hannon K., Olwin B.B., Calof A.L. Genesis of olfactory receptor neurons in vitro: regulation of progenitor cell divisions by fibroblast growth factors. *Neuron*, **13**: 1083-1097, 1994.

Doucette J.R. The glial cells in the nerve fibre layer of the rat olfactory bulb. *Anat. Rec.*, **210**: 385-391, 1984.

Ennis M., Zhou F.M., Ciombor K.J., Aroniadou-Anderjaska V., Hayar A., Borrelli E., Zimmer L.A., Margolis F., Shipley M.T. Dopamine D2 receptor-mediated presynaptic inhibition of olfactory nerve terminals. *J. Neurophysiol.*, **86**: 2986-2997, 2001.

Farbman A., Buchholz J., Bruch R. Growth factor regulation of olfactory cell proliferation. pp. 45-48. In: Kurihara K., Suzuki N., Ogawa H. (Eds.) *Olfaction and Taste XI*. Tokyo, Springer-Verlag, 1994.

Farbman A. and Buchholz J. Transforming growth factor-α and other growth factors stimulate cell division in olfactory epithelium in vitro. *J. Neurobiol.*, **30**: 267-280, 1996.

Federico G., Maremmani C., Cinquanta L., Baroncelli G.I., Fattori B., Saggese G. Mucus of the human olfactory epithelium contains the insulin-like growth factor-I system which is altered in some neurodegenerative diseases. *Brain Res.*, **835**: 306-314, 1999.

Feron F., Perry C., McGrath J.J., Mackay-Sim A. New techniques for biopsy and culture of human olfactory epithelial neurons. *Arch Otolaryngol. Head Neck Surg*., **124**: 861-866, 1998.

Feron F., Vincent A., Mackay-Sim A. Dopamine promotes differentiation of olfactory neuron in vitro. *Brain Res.*, **845**: 252-259, 1999a.

Feron F., Perry C., Hirning M.H., McGrath J., Mackay-Sim A. Altered adhesion, proliferation and death in neural cultures from adults with schizophrenia. *Schizophr. Res.*, **40**: 211-218, 1999b.

Feron F., Perry C., Cochrane J., Licina P., Nowitzke A., Urquhart S., Geraghty T., Mackay-Sim A. Autologous olfactory ensheathing cell transplantation in human spinal cord injury. *Brain*, **128**: 2951-2960, 2005.

Feron F., Bianco J., Ferguson I., Mackay-Sim A. Neurotrophin expression in the adult olfactory epithelium. *Brain Res.*, **1196**: 13-21, 2008.

Feron F., Delorme B., Nivet E., Gaillard J., Haupl T., Ringe J., Deveze A., Magnan J., Sohier J., Khrestchatisky M., Roman F., Charbord P., Sensebe L., Layrolle P. The human nose harbours a niche of olfactory ecto-mesenchymal stem cells displaying neurogenic and osteogenic properties. *Stem Cells Dev*., 2009.

Fuchs E. Finding one's niche in the skin. *Cell Stem. Cell*, **4**: 499-502, 2009.

Gall C., Berschauer R., Isackson P. Seizures increase basic fibroblast growth factor mRNA in adult rat

forebrain neurons and glia. *Mol. Brain Res.*, **21**: 190-205, 1994.

Genter M.B., Deamer N.J., Blake B.L., Wesley D.S., Levi P.E. Olfactory toxicity of methimazole: dose-response and structure-activity studies and characterization of flavin-containing monooxygenase activity in the Long-Evans rat olfactory mucosa. *Toxicol. Pathol.*, **23**: 477-486, 1995.

Getchell T., Narla R., Little S., Hyde J., Getchell M. Horizontal basal cell proliferation in the olfactory epithelium of transforming growth factor-alpha transgenic mice. *Cell Tissue Res.*, **299**: 185-192, 2000.

Goldstein B.J., Wolozin B.L., Schwob J.E. FGF2 suppresses neurogenesis of a cell line derived from rat olfactory epithelium. *J. Neurobiol.*, **33**: 411-428, 1997.

Gong Q., Bailey M.S., Pixley S., Ennis M., Liu W., Shipley M.T. Localization and regulation of low affinity nerve growth factor receptor expression in the rat olfactory system during development and regeneration. *J. Comp. Neurol.*, **344**: 336-348, 1994.

Gotz M. and Huttner W.B. The cell biology of neurogenesis. *Nat. Rev. Mol. Cell. Biol.*, **6**: 777-788, 2005.

Graziadei P. and Metcalf J. Autoradiographic and ultrastructural observations on the frog's olfactory mucosa. *Z. Zellforsch.*, **116**: 305-318, 1971.

Graziadei P.P.C. Cell dynamics in the olfactory mucosa. *Tiss. Cell*, **5**: 113-131, 1973.

Graziadei P.P. and Monti Graziadei G.A. Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons. *Journal of Neurocytology*, **8**: 1-18, 1979.

Graziadei P.P.C., Karlan M.S., Monti Graziadei G.A., Bernstein J.J. Neurogenesis of sensory neurons in the primate olfactory system after section of the filia olfactoria. *Brain Res.*, **186**: 289-300, 1980.

Hahn C.G., Han L.Y., Rawson N.E., Mirza N., Borgmann-Winter K., Lenox R.H., Arnold S.E. In vivo and in vitro neurogenesis in human olfactory epithelium. *J. Comp. Neurol.*, **483**: 154-163, 2005.

Harding J.W., Getchell T.V., Margolis F.L. Denervation in the primary pathway of mice. V. long term effect of intranasal ZnSO4 irrigation on behavior, biochemistry and morphology. *Brain Res.*, **140**: 271-285, 1978.

Harris J.A., West A.K., Chuah M.I. Olfactory ensheathing cells: nitric oxide production and innate immunity. *Glia*, **57**: 1848-1857, 2009.

Hinds J.W. and McNelly N.A. Aging in the rat olfactory system: correlation of changes in the olfactory epithelium and olfactory bulb. *J. Comp. Neurol.*, **203**: 441-453, 1981.

Hinds J.W., Hinds P.L., McNelly N.A. An autoradiographic study of the mouse olfactory epithelium: evidence for long-lived receptors. *Anat. Rec.*, **210**: 375-383, 1984.

Holbrook E.H., Szumowski K.E., Schwob J.E. An immunochemical, ultrastructural, and developmental characterization of the horizontal basal cells of rat olfactory epithelium. *J. Comp. Neurol.*, **363**: 129-146, 1995.

Holcomb J., Mumm J., Calof A. Apoptosis in the neuronal lineage of the mouse olfactory epithelium: regulation in vivo and in vitro. *Developmental Biology*, **172**: 307-323, 1995.

Hsu P., Yu F., Feron F., Pickles J.O., Sneesby K., Mackay-Sim A. Basic fibroblast growth factor and fibroblast growth factor receptors in adult olfactory epithelium. *Brain Res.*, **896**: 188-197, 2001.

Huard J.M., Youngentob S.L., Goldstein B.J., Luskin M.B., Schwob J.E. Adult olfactory epithelium contains multipotent progenitors that give rise to neurons and non-neural cells. *J. Comp. Neurol.*, **400**: 469-486, 1998.

Hurtt M.E., Thomas D.A., Working P.K., Monticello T.M., Morgan K.T. Degeneration and regeneration of the olfactory epithelium following inhalation exposure to methyl bromide: pathology, cell kinetics, and olfactory function. *Toxicol. Appl. Pharmacol.*, **94**: 311-328, 1988.

Iwai N., Zhou Z., Roop D.R., Behringer R.R. Horizontal basal cells are multipotent progenitors in normal and injured adult olfactory epithelium. *Stem Cells*, **26**: 1298-1306, 2008.

Kilpatrick T. and Bartlett P. Cloning and growth of multipotential neural precursors: requirements for proliferation and differentiation. *Neuron*, **10**: 255-265, 1993.

Leopold D.A., Hummel T., Schwob J.E., Hong S.C., Knecht M., Kobal G. Anterior distribution of human olfactory epithelium. *Laryngoscope*, **110**: 417-421, 2000.

Leung C.T., Coulombe P.A., Reed R.R. Contribution of olfactory neural stem cells to tissue maintenance and regeneration. *Nat. Neurosci.*, **10**: 720-726, 2007.

Loo A.T., Youngentob S.L., Kent P.F., Schwob J.E. The aging olfactory epithelium: neurogenesis, response to damage, and odorant-induced activity. *Int. J. Dev. Neurosci.*, **14**: 881-900, 1996.

Lucero M.T. and Squires A. Catecholamine concentrations in rat nasal mucus are modulated by trigeminal stimulation of the nasal cavity. *Brain Res.*, **807**: 234-236, 1998.

Mackay-Sim A. and Beard M.D. Hypothyroidism disrupts neural development in the olfactory epithelium of adult mice. *Brain Res.*, **433**: 190-198, 1987.

Mackay-Sim A., Breipohl W., Kremer M. Cell dynamics in the olfactory epithelium of the tiger salamander: a morphometric analysis. *Exp. Brain Res.*, **71**: 189-198, 1988.

Mackay-Sim A., Kittel P.W. On the Life Span of Olfactory Receptor Neurons. *Eur. J. Neurosci.*, **3**: 209-215, 1991a.

Mackay-Sim A. and Kittel P. Cell dynamics in the adult mouse olfactory epithelium: a quantitative autoradiographic study. *J. Neurosci.*, **11**: 979-984, 1991b.

Mackay-Sim A. and Chuah M.I. Neurotrophic factors in the primary olfactory pathway. *Prog. Neurobiol.*, **62**: 527-559, 2000.

Mackay-Sim A. and Silburn P. Stem cells and genetic disease. *Cell. Prolif.*, **41** Suppl 1: 85-93, 2008.

Mackay-Sim A., Feron F., Cochrane J., Bassingthwaighte L., Bayliss C., Davies W., Fronek P., Gray C., Kerr G., Licina P., Nowitzke A., Perry C., Silburn P.A., Urquhart S., Geraghty T. Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial. *Brain*, **131**: 2376-2386, 2008.

Magrassi L. and Graziadei P. Cell death in the olfactory epithelium. *Anatomy and Embryology (Berlin)*, **192**: 77-87, 1995.

Mahalik T. Apparent apoptotic cell death in the olfactory epithelium of adult rodents: death occurs at different developmental stages. *J. Comp. Neurol.*, **372**: 457-464, 1996.

Mahanthappa N.K. and Schwarting G.A. Peptide growth factor control of olfactory neurogenesis and neuron survival in vitro: roles of EGF and TGF-beta s. *Neuron*, **10**: 293-305, 1993.

Margolis F.L., Roberts N., Ferriero D., Feldman J. Denervation in the primary olfactory pathway of mice: biochemical and morphological effects. *Brain Res.*, **81**: 469-483, 1974.

Maruniak J.A., Taylor J.A., Henegar J.R., Williams M.B. Unilateral naris closure in adult mice: atrophy of the deprived-side olfactory bulbs. *Develop. Brain Res.*, **47**: 27-33, 1989.

Maruniak J.A., Henegar J.R., Sweeney T.P. Effects of long-term unilateral naris closure on the olfactory epithelia of adult mice. *Brain Res.*, **526**: 65-72, 1990.

Matsuyama A., Iwata H., Okumura N., Yoshida S., Imaizumi K., Lee Y., Shiraishi S., Shiosaka S. Localization of basic fibroblast growth factor-like immunoreactivity in the rat brain. *Brain Res.*, **587**: 49-65, 1992.

McCurdy R.D., Feron F., McGrath J.J., Mackay-Sim A. Regulation of adult olfactory neurogenesis by insulin-like growth factor-I. *Eur. J. Neurosci.*, **22**: 1581-1588, 2005.

McDonald C., Mackay-Sim A., Crane C., Murrell M. Could cells from your nose fix your heart? Transplantation of olfactory stem cells in a rat model of cardiac infarction. *ScientificWorldJournal*, **10**: 422-433, 2010.

Menco B.P.M. Qualitative and quantitative freeze-fracture studies on olfactory and nasal respiratory epithelial surfaces of frog, ox, rat and dog. III. Tight-junctions. *Cell Tiss. Res.*, **211**: 361-373, 1980.

Michel D., Moyse E., Brun G., Jourdan F. Induction of apoptosis in rat olfactory neuroepithelium by synaptic target ablation. *Neuroreport*, **5**: 1329-1332, 1994.

Moon Y. and Baker H. Induction of cell division in olfactory basal epithelium following intranasal irrigation with wheat germ agglutinin-horseradish peroxidase. *J. Comp. Neurol.*, **393**: 472-481, 1998.

Moran D.T., Rowley J.C. 3rd, Jafek B.W., Lovell M.A. The fine structure of the olfactory mucosa in man. *J. Neurocytol.*, **11**: 721-746, 1982.

Morrison E.E. and Costanzo R.M. Morphology of the human olfactory epithelium. *J. Comp. Neurol.*, **297**: 1-13, 1990.

Moulton D.G., Çelebi G., Fink R.P. Olfaction in mammals - two aspects: proliferation of cells in the olfactory epithelium and sensitivity to odours. pp. 227-250. In: Wolstenholme G.E.W., Knight J. (Eds.) *Ciba Foundation on Taste and Smell in Vertebrates*. London, J. & A. Churchill, 1970.

Mumm J.S., Shou J., Calof A.L. Colony-forming progenitors from mouse olfactory epithelium: evidence for feedback regulation of neuron production. *Proc. Natl. Acad. Sci. USA*, **93**: 11167-11172, 1996.

Murrell W., Bushell G.R., Livesey J., McGrath J., MacDonald K.P., Bates P.R., Mackay-Sim A. Neurogenesis in adult human. *Neuroreport*, **7**: 1189-1194, 1996.

Murrell W., Feron F., Wetzig A., Cameron N., Splatt K., Bellette B., Bianco J., Perry C., Lee G.,

Mackay-Sim A. Multipotent stem cells from adult olfactory mucosa. *Dev. Dyn.*, **233**: 496-515, 2005.

Murrell W., Wetzig A., Donnellan M., Feron F., Burne T., Meedeniya A., Kesby J., Bianco J., Perry C., Silburn P., Mackay-Sim A. Olfactory mucosa is a potential source for autologous stem cell therapy for Parkinson's disease. *Stem Cells*, **26**: 2183-2192, 2008.

Murrell W., Sanford E., Anderberg L., Cavanagh B., Mackay-Sim A. Olfactory stem cells can be induced to express chondrogenic phenotype in a rat intervertebral disc injury model. *Spine J.*, **9**: 585-594, 2009.

Nadi N.S., Head R., Grillo M., Hempstead J., Grannot-Reisfeld N., Margolis F.L. Chemical deafferentation of the olfactory bulb: plasticity of the levels of tyrosine hydroxylase, dopamine, norepinephrine. *Brain Res.*, **213**: 365-377, 1981.

Naessen R. An enquiry on the morphological characteristics and possible changes with age in the olfactory region of man. *Acts Olotlaryngol.*, **71**: 49-62, 1971.

Nagahara Y. Experimentelle Studien uber die histologischen Veranderungen des Geruchsorgans nach der Olfactorius durchschneidung. Beutrage zur Kenntnis des feineren Baus des Geruchsorgans. *Jap. J. Med. Sci. V. Pathol.*, **5**: 46-63, 1940.

Nakashima T., Kimmelmann C., Snow J. Structure of human fetal and adult olfactory neuroepithelium. *Acts Olotlaryngol.*, **110**: 641-646, 1984.

Nakashima T., Tanaka M., Inamitsu M., Uemura T. Immunohistopathology of variations of human olfactory mucosa. *Eur. Arch. Otorhinolaryngol.*, **248**: 370-375, 1991.

Newman M.P., Feron F., Mackay-Sim A. Growth factor regulation of neurogenesis in adult olfactory epithelium. *Neuroscience*, **99**: 343-350, 2000.

Owen S.J., Batzloff M., Chehrehasa F., Meedeniya A., Casart Y., Logue C.A., Hirst R.G., Peak I.R., Mackay-Sim A., Beacham I.R. Nasal-associated lymphoid tissue and olfactory epithelium as portals of entry for Burkholderia pseudomallei in murine melioidosis. *J. Infect. Dis.*, **199**: 1761-1770, 2009.

Paik S.I., Lehman M.N., Seiden A.M., Duncan H.J., Smith D.V. Human olfactory biopsy. The influence of age and receptor distribution. *Arch. Otolaryngol. Head. Neck Surg.*, **118**: 731-738, 1992.

Rehn B., Breipohl W., Schmidt C., Schmidt U., Effenberger F. Chemical blockade of olfactory perception by N-methyl-formimino-methylester in albino mice. II. light microscopical investigations. *Chem. Senses*, **6**: 317-328, 1981.

Reynolds B.A. and Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*, **255**: 1707-1710, 1992.

Rietze R.L., Valcanis H., Brooker G.F., Thomas T., Voss A.K., Bartlett P.F. Purification of a pluripotent neural stem cell from the adult mouse brain. *Nature*, **412**: 736-739, 2001.

Samanen D.W. and Forbes W.B. Replication and differentiation of olfactory receptor neurons following axotomy in the adult hamster: a morphological analysis of postnatal neurogenesis. *J. Comp. Neurol.*, **225**: 201-211, 1984.

Schmidt U., Schmidt C., Breipohl W., Effenberger F. The effects of N-methyl-formimino-methylester on the neural olfactory threshold in albino mice. *Arch. Otorhinolaryngol.*, **239**: 25-29, 1984.

Schultz E. Regeneration of olfactory cells. *Proc. Soc. Exp. Biol. Med.*, **46**: 41-43, 1941.

Shou J., Rim P., Calof A. BMPs inhibit neurogenesis by a mechanism involving degradation of a transcription factor. *Nat. Neurosci.*, **2**: 339-345, 1999.

Shou J., Murray R., Rim P., Calof A. Opposing effects of bone morphogenetic proteins on neuron production and survival in the olfactory receptor neuron lineage. *Development*, **127**: 5403-5413, 2000.

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

Smith C.G. Regeneration of sensory epithelium and nerves in adult frogs. *Anat. Rec.*, **109**: 661-671, 1951.

Sulz L., Astorga G., Bellette B., Iturriaga R., Mackay-Sim A., Bacigalupo J. Nitric oxide regulates neurogenesis in adult olfactory epithelium in vitro. *Nitric Oxide*, **20**: 238-252, 2009.

Thornhill R. Cell division in the olfactory epithelium of the lamprey, *Lampetra fluviatilis*. *Zeitschrift fur Zellforschungen und Mikroskoic Anatomie*, **109**: 147-157, 1970.

Tome M., Lindsay S.L., Riddell J.S., Barnett S.C. Identification of nonepithelial multipotent cells in the embryonic olfactory mucosa. *Stem Cells*, **27**: 2196-2208, 2009.

Weiler E. and Farbman A.I. Proliferation in the rat olfactory epithelium: age-dependent changes. *J. Neurosci.*, **17**: 3610-3622, 1997.

Westerman R. and Baumgarten R.V. Regeneration of olfactory paths in carp (*Cyprinus carpio* L.). *Experientia*, **20**: 519-520, 1964.

Wolozin B., Sunderland T., Zheng B.B., Resau J., Dufy B., Barker J., Swerdlow R., Coon H. Continuous culture of neuronal cells from adult human olfactory epithelium. *J. Mol. Neurosci.*, **3**: 137-146, 1992.

Youngentob S.L. and Schwob J.E. Odorant identification and quality perception following methyl bromide-induced lesions of the olfactory epithelium. *Behav. Neurosci.*, **120**: 1346-1355, 2006.