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Peer reviewed

Neuronal Homeostasis in Mammalian Olfactory Epithelium: A Review

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ABSTRACT

The neuronal lineage of the olfactory epithelium (OE) is a cell lineage that includes the neuronal stem cell and its progeny (ultimately the mature olfactory receptor neuron [ORN]). Recent studies, including further characterization of the neuronal lineage of the OE, and of factors that influence proliferation, survival, and death of cells of this lineage, have contributed significantly to understanding of neuronal homeostasis, i.e., normal maintenance of neuronal number, in mammalian OE. Our recent studies indicate that in adult mice, all cell types of the neuronal lineage of the OE—neuronal precursors, immature ORNs and mature ORNs—undergo constitutive death, i.e., a normal, basal level of cell death, that is characteristic of programmed cell death or apoptosis. To some extent, constitutive cell death in this lineage may reflect random environmental insults; however, this may also be the result of an ongoing developmental program that acts to control both numbers and phenotypic organization of olfactory neurons. Although a variety of extrinsic and intrinsic factors are likely to contribute to cell death in the neuronal lineage of the OE, most have not been thoroughly studied. Detailed analysis of one of these factors, effects of target deprivation, suggests that survival of individual cell types of the neuronal lineage of the OE may be differentially regulated with mature

ORNs, but not immature ORNs or neuronal precursors, dependent upon the olfactory bulb for their survival. Factors normally provided to cells of the ORN lineage, as in other neuronal systems, are likely to promote survival by inhibiting an endogenous genetic program of cell death. Whether candidate polypeptide growth factors, e.g., the neurotrophins, or other pharmacological inhibitors of apoptosis will eventually play a role in the treatment of specific anosmias remains to be determined. (American Journal of Rhinology 10, 125–134, 1996)

The olfactory epithelium (OE) is a pseudostratified columnar epithelium specialized for transducing the sense of smell. Studies of the physiology and pathophysiology of the OE have been spurred by the frequency of clinical disorders of olfaction and associated disorders of gustation¹ for which no treatment is currently available, and by the ability of this tissue to replace its sensory neurons (olfactory receptor neurons), at a low level under normal conditions^{2,3} and on a much larger scale following injury.^{4–11}

The OE contains three main types of histologically identifiable cells: basal cells, olfactory receptor neurons (ORNs), and supporting (or sustentacular) cells.¹² Another cell type that has been described, the microvillar cell, may be a subtype of the receptor neuron.¹³ More recent studies using molecular markers indicate an even greater cellular complexity of the OE. *Basal cells* consist of at least three cell types, all of which lie in the basal one-fourth of the OE: (1) the “horizontal” or “dark” basal cells lie directly apposed to the basal lamina of the epithelium, and are distinguished by their expression of keratin intermediate filaments,^{14–16} (2) *Immediate Neuronal Precursors* (INPs), also referred to as “globose” or “light” basal cells, lie above the horizontal basal cells in the basal compartment of the OE.^{14,15,17} INPs, as their name suggests, divide to directly give rise to postmitotic ORNs,^{14,15,17} but INPs themselves

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appear to be the progeny of the third type of basal cell, (3) another "globose" basal cell which is distinguished by expression of the transcription factor MASH1.¹⁸ ORNs can be subdivided into two types, based on their expression of molecular markers and their degree of morphological maturity: Immature ORNs express the neural cell adhesion molecule NCAM and the growth-associated protein GAP-43 (B-50).^{8,9,14,19} Mature ORNs retain expression of NCAM, lose expression of GAP-43, and acquire expression of Olfactory Marker Protein (OMP) as they attain a more apical position within the OE, elaborate extensive cilia, and establish stable synaptic contacts with the olfactory bulb.^{8,9,14,19,20} Sustentacular or supporting cells of the OE have their cell bodies located in the apical one-fourth of the OE, and express at least two unique markers: Sus-1, an antigen present on sustentacular and Bowman's gland cells in the rat,²¹ and a mucin that can be detected in both rat and mouse using a monoclonal antibody, 3C2.²² These cell types are depicted in the drawing shown in Figure 1.

An understanding of neuronal homeostasis in the OE, i.e., normal maintenance of neuronal number, requires knowledge of factors that influence survival and death of different cells of the ORN lineage. While much descriptive work exists concerning proliferation, death, and survival of cells of this lineage, the factors that regulate these processes are only beginning to be elucidated. In this review, we explore factors and processes that affect maintenance of neuronal number in normal, adult OE. We draw upon studies of the OE that employ both *in vitro* (tissue culture) and *in vivo* (surgical manipulation, immunohistochemistry, etc.) experimental approaches, in both developing and adult animals.

While these experimental studies of the OE have largely used rodents, the results are likely to be directly applicable to man, since the anatomy and physiology of the OE appears to be quite similar among mammals.^{7,12}

NEURONAL HOMEOSTASIS IN THE OE

At least two major processes affect neuronal number in adult OE: These are neurogenesis (proliferation of neuronal precursors and differentiation into postmitotic ORNs) and neuronal death. These two processes appear to be interrelated in their regulation, as well as being subject to independent factors. Understanding of neuronal homeostasis in adult OE is facilitated both by knowledge of factors influencing neurogenesis and neuronal death in the OE and by recalling certain aspects of the developmental biology of this tissue, since many of the developmental processes that occur *en utero* and during early postnatal life persist in adult OE.

Neurogenesis and Neuronal Differentiation

Neurogenesis in adult OE is an ongoing process that serves to replace lost ORNs. In contrast with other neuronal systems in adult animals, the capacity of the OE for continual neurogenesis reflects the persistence into adulthood of some of the characteristics of developing neuronal systems, e.g., proliferative neuronal precursor cells^{2,5,7,9,18,19} and expression by ORNs of intermediate filament and microtubule-associated proteins typically found only during development in other areas of the nervous system.²³⁻²⁵ In any given region of normal OE, the number of proliferating neuronal precursors may be relatively high or low compared with other areas of the OE³ (also J.D.

Figure 1. Molecular markers in mammalian olfactory epithelium. The sustentacular cells are located in the apical one-fourth of the OE and can be detected with 3C2, an anti-mucin monoclonal antibody,²² or with antibodies against the antigen Sus-1.²¹ ORNs are located in the middle one-half of the OE: mature ORNs express both NCAM and OMP,^{8,9,14,19,20} whereas immature ORNs express NCAM and GAP43, but not OMP.^{8,9,14,19} Immediate neuronal precursors, located in the basal one-fourth of the OE, are best distinguished by the absence of molecular markers expressed in other OE cell types and their ability to be labeled with nuclear labeling agents.^{14,29} However, a subset of these cells express the transcription factor MASH1.¹⁸ Horizontal basal cells, directly apposed to the basement membrane of the OE, are identified with antibodies against keratin intermediate filaments.¹⁴⁻¹⁶

Figure 2. Proposed neuronal lineage of mammalian olfactory epithelium (OE), shown in schematic form. Adapted from Calof and Chikaraishi,¹⁴ Gordon et al.,¹⁸ and DeHamer et al.²⁹ The presence of a self-renewing stem cell (1) is thought to underlie the OE's capacity for continual replacement of ORNs. The recently described cell in the basal compartment of the OE (2) that expresses the transcription factor MASH1^{18,29,94} may be the cell in the ORN lineage that precedes the INP. The INP (3) gives rise to two immature olfactory receptor neurons (4), which express the neural cell adhesion molecule, NCAM. Both the MASH1-expressing cell and the INP appear to be capable of multiple divisions (amplification) before producing their final progeny. Immature ORNs migrate apically in the OE, extending an axon toward the olfactory bulb and a dendrite toward the lumen of the nasal cavity. Further steps in maturation of ORNs (5) include expression of Olfactory Marker Protein and elaboration of dendritic cilia.

Holcomb and A.L. Calof, unpublished data). Areas with relatively high levels of proliferating neuronal precursors have been termed "active zones," while areas with relatively low levels have been termed "quiescent zones."³ Although a cause and effect relationship appears to exist between induced neuronal death and upregulated neurogenesis in the OE (see below), it is not known whether such a relationship also holds for ongoing neurogenesis in active zones of normal OE.

Neurogenesis appears to be linked to neuronal death in the OE in a dependent manner in that neurogenesis is upregulated following experimental surgical manipulations that result in widespread death of ORNs, e.g., severing the fila olfactoria or removing the olfactory bulb.^{4,10,11,18,19,26-28} This relationship suggests several possibilities for the signal for induced neurogenesis in the OE. For example, actively dying ORNs might provide a positive signal for up-regulated neurogenesis; alternatively death of ORNs may remove a negative signal, normally provided by living ORNs, which inhibits neurogenesis. *In vitro* studies of neurogenesis in explants of embryonic OE indicate that members of the fibroblast growth factor (FGF) family are able to stimulate proliferation of INPs.²⁹ Other studies have reported effects of transforming growth factor-beta-2 (TGFβ2),³⁰ epidermal growth factor (EGF),³¹ as well as factors produced by astrocytes,³² on neurogenesis in cultured OE. Whether any of these factors regulate neurogenesis *in vivo* is not known.

In vivo, horizontal basal cells proliferate at a low level under normal conditions.⁵ However, they do not undergo increased proliferation following induced death of ORNs⁵ and their function and role, if any, in the neuronal lineage of the OE, remains in question.^{5,17,29,33} *In vitro* and *in vivo* studies suggest that the INP functions as a transit amplifying cell, undergoing several rounds of division before producing postmitotic ORNs.^{17,29} The INP, the direct precursor of ORNs, itself appears to be the progeny of an earlier neuronal precursor, the MASH1-expressing "globose" basal cell; the MASH1-expressing precursor also appears to function as a transit amplifying cell in the ORN differentiative pathway.¹⁸ A self-renewing stem cell, for which no molecular marker yet exists, may give rise to the MASH1-expressing cell, and is thought to underlie the OE's capacity for continual neurogenesis throughout life. Following their production by INPs, immature postmitotic ORNs mature, a process estimated to take approximately 7 days *in vivo* based on appearance of OMP expression in ORNs previously labeled with a nuclear labeling agent.³⁴

Neuronal Death and Survival-Promoting Factors

Studies of cell turnover in the OE initially suggested that newly-generated ORNs matured, lived an average of 30 days, and then died.³ Subsequent studies of the life span of ORNs, however, showed that many ORNs lived as long as 90 days,³⁵ and when mice were raised in a chemical- and

pathogen-free environment (caged in a laminar flow hood), some ORNs lived for one year, or even longer.³⁶ This led to the hypothesis that constitutive death of ORNs, i.e., a normal, basal level of cell death, was largely due to failure of immature ORNs to establish synaptic contact with the olfactory bulb, rather than from regular turnover of mature ORNs which were apparently quite long-lived.³⁶ The hypothesis that immature ORNs die in adult OE, a continually developing neuronal tissue, is consistent with the normal occurrence of extensive neuronal death in the developing nervous system, wherein many more neurons (≥ 2 -fold) are initially produced than are ultimately left after synaptic connections with targets have been made.³⁷ This normal developmental death of neurons has been ascribed to limiting amounts of target-derived survival factors (trophic substances or neurotrophic factors), such that many neurons die because they are unable to acquire sufficient amounts of these factors (see reviews by Oppenheim³⁷ and Barde³⁸).

Neurotrophic factors, polypeptide growth factors that support neuronal survival, appear to promote survival by inhibiting an intrinsic genetic program of cell death, known alternatively as programmed cell death or apoptosis (see reviews by Johnson and Deckwerth³⁹ and Raff et al.⁴⁰). Evidence for this includes the ability of inhibitors of transcription and translation to prevent death of growth factor-deprived neurons in culture.^{39,41,42} Although a great deal of progress toward identification of the "cell death" genes and their regulation has been made in invertebrates (reviewed in detail by Driscoll⁴³), such strides are just beginning in vertebrates. It is already apparent, however, that proteins similar to those involved in regulation of apoptosis in invertebrates are also functional in vertebrates.⁴⁴⁻⁴⁷

Mammalian cells undergoing apoptosis exhibit characteristic changes: e.g., condensation of chromatin (pyknosis), dissolution of the nucleus, contraction of cell volume, and formation of many small membrane-bound fragments that contain remnants of the nucleus and cytoplasm.⁴⁸ These membrane-bound fragments, called apoptotic bodies, eventually break away from the plasma membrane, and are ultimately phagocytosed, either by nearby cells or by macrophages.⁴⁹ In addition to characteristic morphologic changes, most cells undergoing apoptosis also undergo fragmentation of their nuclear DNA, a characteristic increasingly exploited as a marker for apoptotic cells since the development of methods for its detection *in situ*.^{8,50-52}

The first study to directly quantify cell death in normal OE employed histological techniques to detect cells with nuclear pyknosis, a morphological criterion for cell death. In that study, pyknotic cells were observed in all regions of the OE.²⁷ More recently, we have used *in situ* detection of DNA fragmentation (using terminal transferase-mediated dUTP-biotin nick end-labeling or TUNEL⁵⁰ to quantify apoptotic cells in normal OE).⁸ Using DNA fragmentation as a criterion for apoptosis, in combination with immunohistochemistry for cell type-specific markers, we have classified apoptotic cells in adult mouse OE and found that cells

at all stages of the neuronal lineage—neuronal precursors, immature ORNs, and mature ORNs—undergo apoptosis in normal, unoperated OE.⁸ DNA fragmentation was not observed in horizontal basal cells or sustentacular cells.⁸ Thus, there is a constitutive (normal, ongoing) level of apoptosis in all types of cells of the ORN lineage. This constitutive apoptosis occurs only at a low level; however, the number of apoptotic cells in the OE increases by as much as 20-fold following surgical ablation of the olfactory bulb (see below).

An example of *in situ* detection of DNA fragmentation in combination with cell type-specific immunohistochemistry in adult mouse OE is shown in Figure 3. As shown in 3D, few cells have fragmented DNA (are TUNEL-positive) in normal, unoperated OE. However, if one olfactory bulb is surgically removed (unilateral olfactory "bulbectomy"), then a striking increase in the number of TUNEL-positive cells is seen in ipsilateral OE as soon as 24 hours postsurgery (Fig. 3A). The anti-NCAM immunohistochemistry performed on these same sections (Fig. 3B,D) demonstrates that the vast majority of these apoptotic cells are within the neuronal (NCAM-positive) layer of the OE. Others have also observed a dramatic increase in DNA fragmentation in DNA extracted from the OE following bulbectomy.⁵³

We have used these techniques to quantify the timecourse and extent of apoptosis in different types of cells of the ORN lineage following unilateral bulbectomy in adult mice, and the results of this analysis are shown in Figure 4. Compared with unoperated OE, all cells in the neuronal lineage of ipsilateral OE undergo a marked increase in DNA fragmentation 24 hours after unilateral bulbectomy. When animals are allowed to survive for long periods, e.g., 2 months, following bulbectomy, DNA fragmentation remains elevated in ipsilateral OE exclusively in mature ORNs. These findings suggest that only mature ORNs are dependent upon the presence of the olfactory bulb for survival, whereas immature ORNs and neuronal precursors apparently do not depend upon the olfactory bulb for survival. It is possible that these cells obtain trophic support from other cell types within the OE, or, in the case of immature ORNs, from ensheathing cells of the olfactory nerve. These results also imply that immature ORNs undergo a developmental change in trophic factor requirements, such that at some point, perhaps coincident with reaching biochemical maturity, they become dependent upon their target tissue.⁸ We are currently testing, via administration of exogenous growth factors to mice that have undergone unilateral bulbectomy, whether polypeptide growth factors, e.g., neurotrophins, are able to prevent bulbectomy-induced PCD in neuronal cells of the OE *in vivo*.

The neurotrophin family of polypeptide growth factors is one class of neurotrophic factor that may regulate neuronal survival in the OE. This idea was suggested by findings that neurotrophins are expressed in the olfactory bulb,^{54,55} and that appropriate receptors for some of these growth factors are expressed by ORNs.^{8,56} *In vitro* experiments indicate

Figure 3. Molecular, immunohistochemical, and structural analysis of mouse septal OE 24 hours after unilateral bullectomy, ipsilateral (A, B, C) and contralateral (D, E, F). DNA fragmentation in ipsilateral OE is markedly increased compared to contralateral OE (A, D). Cells with DNA fragmentation appear as brightly fluorescent dots in these photomicrographs of mouse OE, e.g. arrow in (A). Despite the marked difference in DNA fragmentation at 24 hours after target ablation, no obvious difference in NCAM expression (B, E) or in structural integrity of the OE (Nomarski optics; C, F). Reprinted with permission from Calof et al.⁹⁵

that ORNs from embryonic mouse OE undergo PCD, manifested by rapid DNA fragmentation and death, when cultured in the absence of survival factors.⁸ In addition, death of these cells in culture is inhibited by pharmacological agents known to inhibit PCD, e.g., aurintricarboxylic acid, cyclic AMP analogs, and inhibitors of transcription and translation.⁸ Certain neurotrophins, brain-derived neurotrophic factor,^{8,30} neurotrophin-3⁸ and neurotrophin-5,⁸ are also able to promote survival of ORNs to some extent *in vitro*. Whether neurotrophins, or other growth factors, are responsible for mediating neuronal survival in the OE *in vivo* is not known.

Pathological Causes of Neuronal Death in the OE

A variety of factors may cause or contribute to neuronal death in the OE—such factors likely vary depending on the type of cell within the ORN lineage. It is useful to classify these factors with regard to their etiology as extrinsic or intrinsic to the OE. It is also important to emphasize that neuronal cells of the OE are subject to both apoptotic

and non-apoptotic cell death (i.e., necrotic cell death), and that neither the magnitude nor the mechanism of death caused by most factors is known. Factors that account for a low, regular level of death may be viewed as contributing to constitutive (normal, ongoing) neuronal death in the OE. Factors that cause death of OE neurons on a much larger scale, then, would be viewed as pathologic. A summary of known and hypothesized factors, extrinsic and intrinsic, that may cause or contribute to constitutive and/or pathological death of cells of the ORN lineage is provided in Table 1.

Viral upper respiratory illness is associated with pathological damage, e.g., extensive neuronal cell loss, to OE of patients with subsequent anosmia or hyposmia.^{57–59} Destruction of neuronal cells of the OE may also result from infection with viruses trophic for these cells, but associated with nonrhinologic primary infection, e.g., herpes simplex encephalitis.⁶⁰ It is also possible that viruses may damage the OE in the absence of clinically apparent infection. Bacterial and fungal rhinitis may also cause local tissue destruction that may or may not spare the OE. To protect

Figure 4. Apoptosis in adult mouse OE. The relative number of apoptotic cells per mm OE is plotted against varied conditions. Constitutive apoptosis in adult mouse OE (Unop, 0d) affects all cells of the ORN lineage, mature ORNs (gray) > immature ORNs (stippled) > neuronal precursors (black). Compared with constitutive levels, unilateral target ablation (surgical removal of one olfactory bulb) in adult mice causes a dramatic increase in the magnitude of apoptosis of mature ORNs, immature ORNs, and neuronal precursors in ipsilateral OE at 24 hours postsurgery (OBX, 1d). When adult mice are allowed to survive for long times, e.g., 8 weeks, postsurgery, apoptosis is increased in ipsilateral OE (compared to constitutive levels) only in mature ORNs (OBX, > 56d). Compared to constitutive apoptosis, no significant difference in the magnitude of apoptosis among these cell types was seen in contralateral OE at 1d or > 56d postsurgery. Unop = unoperated. OBX = bulbectomized OE. Cont = contralateral OE. This figure is reprinted with permission from Holcomb et al.⁸

against diverse infectious insults, the OE uses various defenses, including both immune (secretory IgA) and nonimmune components (lactoferrin and lysozyme) secreted into mucus overlying the OE by Bowman's glands.⁶¹ Immune deficiency, e.g. isolated IgA deficiency, as well as poor nutritional status may partially compromise the defense barrier within the mucus of the OE.

As suggested by Mott and Leopold,⁶² chemical mediators of inflammation associated with various diseases of the nose and paranasal sinuses may also damage ORNs. Because cytokines have been shown to induce apoptotic death of some cultured neurons,⁶³ such a mechanism could conceivably operate to cause death of cells of the ORN lineage as well. Macrophages are a possible source of cytokines in the OE, since they are known to migrate into the OE to phago-

TABLE I

Neuronal Death in Mammalian Olfactory Epithelium

Extrinsic Factors

Exposure to toxic chemicals (not necessarily in high concentrations or for prolonged time periods)^{^74}

Hypothyroidism^{^73}

Infectious, e.g., viral rhinitis,^{^58-60} other (see text)

Loss of synaptic contact with the olfactory bulb, e.g., traumatic disruption of the fila olfactoria^{^,+4-11}

Limitation in amounts of target-derived trophic support (for mature ORNs)*

Intrinsic Factors

Action of absorbed chemicals bioactivated by phase I olfactory detoxifying enzymes

Age-related decrease in olfactory detoxifying enzymes and in regenerative capacity of the OE

Effects of inflammatory mediators, e.g., acute injury response following disruption of fila olfactoria or late phase allergic response

Failed acquisition of target-derived trophic support, i.e., failure by ORNs to synapse with appropriate glomerulus*

Impairment of secretory defense system of the OE

Inappropriate odorant receptor gene expression for a given odorant receptor expression zone*

Limitation in amounts of local trophic support (for immature ORNs and neuronal precursors)

*Italicized factors are hypothesized to cause neuronal death in the OE, while nonitalicized factors are known to cause neuronal death in the OE. ^ denotes factors known to cause anosmia or hyposmia. + denotes factors known to cause apoptotic neuronal death in the OE. * denotes factors hypothesized to occur during ongoing neuronal development in adult OE (and initial development of the OE).*

cytose cellular remnants and debris following axonal injury.⁴ Although speculative, the acute upregulation of apoptotic death of immature ORNs and neuronal precursors in ipsilateral OE of mice subjected to unilateral bulbectomy (see Fig. 4) may be the result of a local inflammatory response, e.g., effects of substances released by activated macrophages.⁸ A humoral response could not account for this unilateral up-regulation of apoptotic death of immature ORNs and neuronal precursors, while direct injury could not account for increased death of neuronal precursors, cells that do not have axons and that are not directly injured by the bulbectomy procedure.⁸

Although improvement in olfactory function may occur with administration of systemic corticosteroids to patients with olfactory loss associated with nonobstructive, noninfectious nasal/paranasal sinus disease, e.g., allergic rhinitis,⁶⁴ neither the effects that inflammatory mediators may exert on the OE nor the mechanism by which they may

cause olfactory loss is known. Although eosinophils may release several neurotoxic inflammatory mediators during the late phase allergic response, e.g., eosinophil-derived neurotoxic protein,⁶⁴ it is unlikely that such factors lead to functionally significant neuronal death in the OE, i.e., death of sufficient ORNs to cause anosmia, since patients with anosmia attributable to allergic rhinitis often experience transient spontaneous improvement in their ability to smell, or rapid regain of normosmia with short courses of systemic steroid therapy.⁶⁴

Jafek et al. and others have noted decreased numbers of ORNs in OE from patients with long-standing post-traumatic anosmia (PTA).^{59,65-67} In this respect, OE from patients with PTA resembles OE from mice with long-term survival following surgical ablation of the olfactory bulb. Jafek et al. also noted the abnormal finding of large numbers of axons within the OE of patients with PTA, and proposed that fibrotic healing of the cribriform plate prevents regenerating axons from reaching the olfactory bulb.⁶⁵ The abnormal finding of large numbers of axons within the OE of patients with PTA may be explained, however, by phenotypic analysis of cell death in mouse OE acutely following target ablation. The fila olfactoria contain bundles of axons, from both mature ORNs (already connected with target glomeruli in the olfactory bulb) and immature ORNs (many not yet having reached the olfactory bulb), that are surrounded by ensheathing cells. Recall that after surgical removal of the olfactory bulb in mice, a reasonable model for acute changes in OE of humans with shearing injuries of the fila olfactoria, all cell types of the ORN lineage undergo a dramatic increase in apoptotic death (see above). If all axons within a group of ensheathing cells degenerate, the physical space previously occupied by these axons is not likely to be maintained, and axons from newly-generated ORNs may be unable to enter.

Various endocrine disorders, e.g., diabetes mellitus,^{68,69} hypothyroidism,^{70,71} and pseudohypoparathyroidism,⁶² have been associated with decreased olfactory ability. Of these, hypothyroidism is of particular interest since inducing the hypothyroid state in adult mice leads to a reversible loss of the sense of smell.⁷² In adult mice with propylthiouracil-induced hypothyroidism, the majority of newly-generated ORNs undergo premature death (living >5, but <15 days).⁷³ This indicates that the hypothyroid state interferes with survival of newly-generated ORNs. Since synaptic contact with the olfactory bulb is necessary for prolonged survival of ORNs,¹⁹ it may be the case that hypothyroidism interferes with developmental processes necessary for this to occur. Alternatively, it may be that thyroid hormone is itself a survival factor for ORNs.

In the absence of pathogens, local inflammation, trauma and endocrinopathy, death of OE neuronal cells may result from insults of an environmental nature, e.g., exposure to airborne chemicals (not necessarily in high concentrations or for prolonged periods of time). It is known that exposure to a wide variety of chemicals may cause damage to the

OE⁷⁴ and variable loss of olfaction.⁶² That the OE is susceptible to such insults is supported by the finding that when OE is isolated from the environment, precluding exposure to airborne chemicals or pathogens, ORNs live considerably longer than under normal circumstances.³⁶ Also consistent with the idea that neuronal cells of the OE may be highly susceptible to various airborne chemicals, the sustentacular cells of the OE are known to contain extremely high levels of detoxifying enzymes.⁷⁵ Interestingly, levels of one cytochrome P-450 isoform in human OE decline in patients over 60 years of age.⁷⁶ Such detoxifying enzymes may be critical for maintaining neuronal cells of the OE, since a sharp decline in odor identification ability also occurs in patients in this age group.^{77,78} Chemical insults to the OE may also arise, in part, as a result of endogenous metabolism in the OE: several chemicals are known to undergo bioactivation to highly toxic metabolites by detoxification enzymes located in the olfactory epithelium, e.g., cytochrome P-450-catalyzed conversion of hexamethylphosphoramide (HMPA) to formaldehyde.⁷⁹ Detoxification enzymes other than cytochrome P-450-dependent monooxygenases (phase I biotransformation enzymes), e.g., olfactory UDP glucuronosyl transferase (UGT, a phase II biotransformation enzyme), may also be critical for protecting the OE from toxic chemicals.⁸⁰

Age-Related Changes in Neuronal Death in the OE

Death of neuronal cells of the OE may reflect normal developmental events associated with continuing neurogenesis in the OE. For example, death of neuronal cells of the OE may reflect limitation in amounts of trophic substances for cells at particular stages in the ORN lineage, failure of immature ORNs to establish appropriate synaptic connections within the olfactory bulb or selection against cells based on phenotypes. Since a large portion of constitutive neuronal death in the OE affects immature ORNs and neuronal precursors,⁸ it is possible that these cells are constitutively overproduced. One possible means for controlling numbers of these cells would be limitation in amounts of survival factors, such that cells lacking sufficient survival factor(s) undergo PCD. The possibility of selection against cells based on phenotype is discussed below.

In mice and rats, the OE appears to be organized, along anteroposterior and cephalocaudal axes, into four discrete zones of odorant receptor expression.^{81,82} Since the number of odorant receptor genes is estimated to be ~1000 in mammals,⁸³⁻⁸⁶ and since little or no overlap appears to occur in odorant receptor expression between different zones,^{81,82} each odorant receptor expression zone may contain several hundred different odorant receptors.⁸² Although ORNs expressing the same odorant receptor appear to be spread out over anteroposterior and cephalocaudal extents of each zone,^{81,82} these cells appear to innervate relatively few (one to several) glomeruli within the olfactory bulb.^{87,88} The existence of odorant receptor expression zones and the specificity with which ORNs appear to converge upon in-

dividual glomeruli within the olfactory bulb has been proposed as a potential anatomical basis for spatial patterning of odorant responsiveness in the olfactory system,^{87,88} an idea also suggested by electrophysiological studies.^{89,90} If such an organization does indeed exist, it is conceivable that cells failing to reach appropriate target glomeruli or to express appropriate odorant receptors might undergo apoptosis. In support of a role for apoptosis during development and organization of the OE, we have observed substantial numbers of apoptotic neuronal cells, distributed in a bilaterally symmetric pattern, in early postnatal mouse OE (J.D. Holcomb and A.L. Calof, unpublished observation).

Death of neuronal cells of the OE also appears to increase with increasing age. In their analysis of sections of post-mortem samples of human OE, Getchell and Getchell noted that apoptotic bodies were present more frequently in fetal, neonatal and old adult subjects than in young to middle-aged adult subjects.⁹¹ Increased numbers of apoptotic bodies in OE of old adult subjects correlated with decreased numbers of mature ORNs in these subjects. The cause for this decrease in numbers of mature ORNs in elderly subjects is not known. In part, this may reflect an age-dependent decline in the ability of the OE for self-repair through neurogenesis.⁹² Whether age-related replacement of OE with respiratory epithelium that occurs in humans⁹³ results from discrete areas of the OE becoming senescent, with gradual physiologic loss of both ORNs and the ability to replace them, or from cumulative effects of environmental insults is not known.

CONCLUSIONS

Many factors, both genetic and epigenetic, are potentially involved in maintenance of neuronal homeostasis in mammalian OE. It is clear that all cell types of the neuronal lineage of the OE undergo constitutive death that is characteristic of programmed cell death or apoptosis, and that acute and prolonged target deprivation differentially affect apoptotic death of these cells. To some degree, the constitutive apoptosis of neuronal cells of the OE, and the marked sensitivity of ORNs to removal of their target tissue, may reflect normal developmental phenomena related to proliferation, differentiation, and maturation of neurons of the OE. Other factors, e.g., pathogens, local inflammation, thyroid function, levels of olfactory detoxifying enzymes, bioactivation of various airborne chemicals to highly toxic metabolites, and age, also influence neuronal death in the OE. Tissue culture experiments have been useful for identifying growth factors and pharmacological agents that can at least partially ameliorate neuronal death in the OE. If specific survival factors, or other pharmacological inhibitors of apoptosis, are able to inhibit apoptotic death of cells of the neuronal lineage of the OE *in vivo*, it will be of interest to determine whether such agents have any therapeutic benefit for specific anosmias in man.

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