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Neuronal regeneration: Lessons from the olfactory system

Richard C. Murray and Anne L. Calof

Neuronal regeneration takes place in the primary relay of the olfactory system, the olfactory epithelium (OE); however, its synaptic target in the central nervous system, the olfactory bulb (OB), undergoes continual neurogenesis but not true regeneration. In this review, cell interactions and growth factors regulating neurogenesis in both OE and OB are discussed. In addition, regulation of regeneration in the OE and regulation of neurogenesis in the OB are compared, with the goal of identifying characteristics that may account for the different abilities of these two tissues to regenerate.

Key words: growth factors / neurogenesis / neuronal progenitor cells / olfactory epithelium / olfactory bulb

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Introduction

MANY TISSUES in adult mammals are able to regenerate their characteristic differentiated cell types throughout life, but this does not take place in most of the central nervous system (CNS). Indeed, the inability of the CNS to generate new neurons in order to replace those that have been lost is a formidable obstacle to recovery from neuronal damage caused by injury or neurodegenerative disease, rendering such insults to the CNS devastating in their impact. However, there are parts of the CNS where long-term production of neurons takes place. These are the hippocampus and olfactory bulb (OB) of the brain, which continue to add new neurons throughout life.^{1,2} This ongoing neurogenesis requires that neuronal progenitor cells exist in the adult brain,

offering hope that, by learning how proliferation and differentiation of progenitor cells is regulated, it may be possible to identify conditions that promote frank neuronal regeneration in the CNS. Such information would be of considerable medical, as well as scientific, significance.

Interestingly, true neuronal regeneration is known to occur in adult mammals in the primary relay of odor detection, the olfactory epithelium (OE). The OE contains undifferentiated progenitor cells that generate new neurons throughout life.^{3,4} While the cell bodies of these neurons, the olfactory receptor neurons (ORNs), remain in the periphery, they send axons to the OB and their interactions with the bulb are important in regulating OE structure and function. Because of its peripheral location, the OE is readily accessible for study, both in tissue culture and in living animals, and much is known about the cell interactions and molecular factors that regulate neurogenesis and neuron replacement in this tissue (reviewed in refs 5,6). Several principles have emerged from such studies, especially with regard to the nature of the signals that initiate neurogenesis and cause it to halt. In this review, we highlight some of these principles and compare them with ideas emerging from studies of neurogenesis in the OB. Our goal is to identify parallels in how these processes are regulated between the OE, a peripheral neural tissue, and the OB, which is in the CNS.

Structure of the primary olfactory pathway

The primary olfactory pathway consists of the OE and its CNS target tissue, the OB (Figure 1A). [In addition to this main olfactory system, many vertebrates possess a separate system for the detection of pheromones called the vomeronasal system (reviewed in ref 7), which will not be discussed in this review.] Odor detection initiates in the OE, a sensory epithelium that lines the nasal cavity. The OE consists of three

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Figure 1. A schematic drawing of the primary olfactory pathway. (A): Sagittal view of the caudal nasal cavity and rostral forebrain of a mouse. The olfactory epithelium (OE) lines the nasal cavity and contains olfactory receptor neurons (ORN), which project through the cribriform plate (CP) to glomeruli (G) in the olfactory bulb (OB) within the central nervous system (CNS). The subventricular zone (SVZ) of the lateral ventricle (LV) is the source of progenitor cells that migrate in the rostral migratory stream (RMS) to the olfactory bulb (OB). (B): A section through the OE illustrating the relative positions of cell types. From apical to basal, these include supporting cells (SUP), olfactory receptor neurons (ORN), and basal cells (BC). The round or globose basal cells consist of at least 3 types of neuronal progenitors: stem cells (black), MASH1⁺ progenitors (gray), and Immediate Neuronal Precursors (white). The OE is separated from the underlying lamina propria (LP) by its basement membrane (BM). (C): A section through the OB illustrating the various layers. These include the olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (ML), internal plexiform layer (IPL), granule cell layer (GCL), and subependymal layer (SEL); composition of these layers is discussed in the text. Black stars indicate interneurons in the granule cell layer and periglomerular regions that are derived from the SVZ.

main cell compartments: the apical compartment contains a single layer of supporting (or sustentacular) cells; olfactory receptor neurons (ORNs) are

found in the middle, largest compartment of the epithelium; and the basal compartment (approximately two cell layers thick), located adjacent to the basal lamina that separates the epithelium from the underlying lamina propria, contains a mixture of cell types often referred to collectively as basal cells (Figure 1B). ORNs can be further subdivided based on their position in the OE, markers they express, and their level of maturity. Mature ORNs are located more apically and express both the neural cell adhesion molecule, NCAM, and olfactory marker protein (OMP), a cytoplasmic protein whose expression appears to be limited to mature ORNs.^{8,9} Mature ORNs send a dendritic process to the apical surface of the epithelium, and this apical dendrite expands into a knob which projects a number of cilia into the overlying mucus. The axons of ORNs project into the lamina propria subjacent to the epithelium, through the cribriform plate of the ethmoid bone, and directly into the CNS where synapse with second order neurons in the OB. Immature ORNs are located underneath mature ORNs in the epithelium, and express both NCAM and the growth associated protein GAP-43, but not OMP.^{8,10} Immature ORNs have dendrites that do not reach the epithelial surface, and they appear to require sustained contact with the OB in order to mature and survive.¹¹

Basal cells of the OE can be subdivided into two broad morphological subtypes. The horizontal (or flat) basal cells lie adjacent to the basal lamina and express keratin intermediate filaments;⁸ these cells are not part of the neuronal lineage of the OE.¹² The so-called 'globose' basal cells (GBCs) lie above horizontal basal cells and have been shown to be the progenitor cells that divide and give rise to ORNs *in vivo*.^{12–14} There are at least three types of ORN progenitor cells among the GBCs, which can be distinguished on the basis of molecular markers and proliferative dynamics: these include neuronal colony-forming cells (putative neuronal stem cells of the OE); MASH1-expressing progenitors; and the Immediate Neuronal Precursors (INPs) (Figure 1B; see below and ref 6).

The second relay in the olfactory system, the OB, is also a layered structure (Figure 1C; reviewed in ref 15). The outermost layer, the olfactory nerve layer, consists of axons of ORNs and their associated (glial) ensheathing cells. ORN axons extend into the glomerular layer, which contains numerous spherical structures (glomeruli) consisting of axon terminals and the dendritic trees of the second-order neurons upon which ORNs synapse, the mitral and tufted

cells. Surrounding the glomeruli are interneurons known as periglomerular cells, which also receive synaptic input from ORNs. Interestingly, the organization of glomeruli within the OB is thought to form a sensory map for odorant detection. Odors are detected by G protein coupled, seven-transmembrane receptors present on the cilia of ORNs.^{16,17} Individual odorant receptors are expressed in one of four broad zones spanning the OE.^{18,19} Although ORNs expressing a single odorant receptor are widespread throughout a given zone in the OE, their axons all converge on one or a few glomeruli in the OB (Figure 1A; refs 20–22). In this fashion each glomerulus in the OB may be tuned to detect a specific set of odors, forming a spatial odorant map.

Beneath the glomerular layer, the external plexiform layer contains dendrites of tufted, mitral, and granule cells, as well as tufted cell somata; internal to this is the mitral cell layer. The axons of both mitral and tufted cells project to higher order structures in the olfactory cortex which are involved in odor discrimination and memory. The next layer, the internal plexiform layer, is relatively cell-free, and contains mostly mitral/tufted axons and granule cell dendrites; internal to this is the granule cell layer, which contains the cell bodies of granule cells, the second type of OB interneuron. Finally, in the center of the OB is the subependymal layer, the anterior extension of the rostral migratory stream, which contains neural progenitor cells derived from the subventricular zone (SVZ) of the lateral ventricles (see below). The progenitor cells in the subependymal layer give rise to the interneurons of the OB, the granule cells and periglomerular cells.²³

Ongoing neurogenesis and cell death in the OE and OB

Generation of new neurons and cell death are ongoing processes, throughout life, in both the OE and the OB. In the OE, ORNs are continually replaced by progenitor cells in the basal compartment of the epithelium.^{3,4,24,25} Initially, investigators interpreted the approximate 4-week turnover time of ORNs labeled with [³H]thymidine *in vivo*, to indicate that this short life span was an intrinsic property of these neurons.⁴ However, more recent experiments indicate that mature ORNs can live at least 90 days,²⁶ and if mice are reared in a laminar flow hood to prevent rhinitis, ORNs can survive as long as 12 months, close to the lifespan of the animal.²⁷ Results such as these

have been interpreted to suggest that turnover of ORNs, and by extension the rate of neurogenesis in the OE, is normally regulated by environmental factors. Thus, toxins or viruses are thought to cause death of ORNs, which are then replaced by the progenitor cells present in the basal compartment. The idea that ORNs are constantly undergoing cell death is supported by the observation that, in the OE of normal adult mice, apoptosis can be detected in cells at all stages in the ORN differentiation pathway, with the highest level occurring in mature ORNs.²⁸ As discussed below, experiments in which levels of ORN death are manipulated have led to the conclusion that the level of cell death and the level of neurogenesis are intimately related in the OE.

The OB also undergoes continued cell addition in post-natal rodents, with the level of neurogenesis decreasing as animals age.^{2,29–31} OB interneurons that come to lie in the granule cell layer and periglomerular region are generated after birth from progenitors that are born in the SVZ, and migrate through the rostral migratory stream to the bulb.^{2,23,30,32} (Figure 1A). The addition of neurons to the granule cell layer continues throughout life and leads to a linear increase in the number of granule cells, with one analysis estimating that approximately 8800 new granule cells are added to the OB per day in adult rats.³¹ Interestingly, there also appears to be a high level of turnover of these newly-generated granule cells, with most of them dying within 12 months of their generation from proliferating progenitors.³¹ However, the question of whether the death of granule (and/or periglomerular) interneurons in the OB can influence production of new interneurons from the SVZ remains controversial, as discussed below.

Neuronal progenitors and their progeny in the OE and OB

The various stages of OE neuronal progenitor cells comprise the 'globose' basal cell (GBC) population in the epithelium *in vivo* (refs 8,12–14,33; reviewed in ref 6). The majority of GBCs are committed neuronal progenitors that undergo a limited number of divisions and give rise directly to ORNs.^{8,14,34} These cells are referred to as Immediate Neuronal Precursors (INPs). INPs are themselves the progeny of another committed amplifying progenitor cell type, which expresses the bHLH transcription factor, Mammalian Achaete Scute Homologue 1 (MASH 1; ref 13). Be-

cause the OE has the ability to renew its neuronal population throughout life, it is thought that it must harbor a neuronal stem cell. The current best candidate for this stem cell is referred to as the 'neuronal colony-forming cell' of the OE.³⁵ This cell, which occurs at a frequency of approximately 1 in 3600 OE neuronal progenitors, has only been studied *in vitro*, and has been shown to have the ability to divide continually and give rise to both undifferentiated neuronal progenitors and post-mitotic ORNs for at least 2 weeks (refs 35,36; reviewed in ref 6).

Neuronal progenitor cells that originate in the anterior part of the SVZ are responsible for postnatal neurogenesis in the OB.³⁷ These cells move through the rostral migratory stream to the subependymal layer of the OB, from which they disperse into the granule cell layer and periglomerular region and differentiate into interneurons.^{23,32} Interestingly, even though these progenitors express some markers characteristic of differentiated neurons while in the SVZ and rostral migratory stream, they still maintain their ability to divide.^{38,39} These cells have been referred to as neuroblasts (or 'Type A' cells; ref 40) to indicate their committed neuronal phenotype in combination with their ability to divide.⁴¹ Migrating neuroblasts are derived from a proliferating progenitor that is found only in the SVZ, the 'Type C' cell.⁴⁰ Lifelong production of neuronal progenitors and OB interneurons by the SVZ implies that, as in the OE, a stem cell must be present in this tissue. It has been reported that ependymal cells, which line the lateral ventricles and are in direct contact with the SVZ, can act as self-renewing stem cells that generate neurons, astrocytes and oligodendrocytes *in vitro*, suggesting that ependymal cells are the stem cells of the SVZ.⁴² However, others have been unable to detect cell division by ependymal cells *in vivo*,⁴³ or the production of neurons from isolated ependymal cells *in vitro*.⁴⁴ Most recently, Doetsch *et al* have provided evidence that an SVZ cell type with the immunological characteristics of an astrocyte (brain glial cell) can give rise to both neuroblasts and OB interneurons, strongly suggesting that this 'Type B astrocyte' of the SVZ is actually the stem cell.⁴³

Neurogenesis and neuronal regeneration in the perturbed olfactory system

Olfactory epithelium

Three main experimental models have been used to

study the regenerative properties of the OE. These are: (1) physically damaging the OE with chemical solutions or corrosive gases (zinc sulfate, Triton X-100, and methyl bromide); (2) disrupting synaptic contacts of ORNs by severing their axons (axotomy) or removing their target, the OB (bulbectomy); and (3) disrupting sensory input to ORNs by blocking the nasal opening (naris occlusion).

Physical damage models have been useful for showing that the OE is capable of regenerating all of the cell types in the neuronal lineage, provided injury is not excessive. There does appear to be some limitation to the ability of the OE to regenerate with very harsh physical damage. For example, irrigation of the nasal cavity with zinc sulfate causes severe damage to all cell types in the OE, resulting in only limited regeneration, and that with significantly different temporal dynamics than what is seen in axotomy and bulbectomy models (see below).^{15,45} Following zinc sulfate treatment, the damaged nasal surface is first covered with respiratory epithelium, which is only partially replaced by sensory epithelium that can reinnervate the OB after long (> 150 days) periods of time.⁴⁵ A reasonable interpretation of these studies is that the limited ability of the OE to restore its neuronal population following zinc sulfate treatment is due to destruction of neuronal stem cells with this treatment regime.

Nasal irrigation with aqueous solutions of Triton X-100 or inhalation of methyl bromide (MeBr) gas also cause major physical damage to the OE, although regeneration following these methods of damage is usually much more robust than that following zinc sulfate treatment.^{46,47} Within 1 day following MeBr injury to the OE, most of the cells in the OE disappear, and the epithelium is only 1–2 cells thick.⁴⁷ This is followed by an increase in cell proliferation beginning 1–2 days following injury, which peaks at approximately 1 week post-lesion and remains at high levels for at least 4 weeks following exposure to the gas.⁴⁷ As would be expected in a situation in which most cells of the OE are destroyed, many cell types proliferate in response to MeBr treatment: supporting cells and horizontal basal cells, which are not in the neuronal lineage,^{8,12} as well as 'globose' basal cells (neuronal progenitors), incorporate BrdU in MeBr-treated OE.⁴⁷ Immature ORNs begin to appear at approximately 1 week post-lesion, and mature neurons start to appear approximately 1 week later. The epithelium is almost fully restored to the pre-lesion state by 6 weeks.⁴⁷

Axotomy and bulbectomy are both methods of

depriving ORNs of contact with their target cells (mitral, tufted, and periglomerular cells) in the OB. Severing the axons of ORNs results in their degeneration and subsequent regeneration from progenitor cells residing in the basal compartment of the epithelium.⁴⁸ It is difficult to perform complete axotomy of the olfactory nerve in rodents, however, and many studies have relied on removal of one OB (unilateral bulbectomy) to deprive ORNs in the ipsilateral OE of contact with their target. Following unilateral bulbectomy, most immature and mature ORNs on the ipsilateral side rapidly undergo apoptotic cell death—apoptosis is maximal approximately 2 days following bulbectomy.²⁸ Over the next few days, the OE degenerates,^{28,33,49} and cells in the basal compartment of the epithelium increase their rate of proliferation and generate new ORNs.^{13,33} However, the epithelium never fully regains its original thickness, as newly-generated ORNs do not mature in the absence of the OB and appear to turn over with a lifespan of 2 weeks or less.^{11,28,50} Initial recovery of the OE (i.e. reappearance of immature neurons) is much faster in the bulbectomy model than when the OE is damaged physically by irrigation with Triton X-100,⁴⁶ presumably because neuronal progenitor cells are not damaged by the bulbectomy procedure, whereas, all cell types are damaged indiscriminately by Triton X-100 irrigation. However, the OE fully recovers following Triton treatment, regaining its full complement of mature ORNs.⁴⁶ Recovery of the mature neurons is not seen following bulbectomy, since ORNs are deprived of the ability to make functional connections with the bulb, and such connection appears to be required for ORN maturation and survival.^{11,28,46}

Another result of bulbectomy is that the levels of both ORN apoptosis and progenitor cell proliferation are permanently upregulated in the OE ipsilateral to the lesion.^{13,28,33,50} The increase in neuronal apoptosis is presumably a reflection of the increased turnover of ORNs unable to make synaptic connections with the olfactory bulb.^{11,28,50} The permanent upregulation of progenitor cell proliferation in the OE following bulbectomy suggests that ORN death may be involved in regulating proliferation of progenitor cells. Indeed, a tight temporal correlation between ORN loss, epithelial degeneration, and cell proliferation in the basal compartment of the OE is evident over the entire post-bulbectomy timecourse. Degeneration of the ORN cell layer in the OE, which can be quantified by measuring epithelial thickness, is maximum (i.e. epithelial thickness is at its minimum) approximately 5 days post-bulbectomy.^{28,33,49} Prolifer-

ation of neuronal progenitor cells reaches a peak at approximately this same time, 5–6 days post-bulbectomy.^{13,33} As new ORNs are generated by progenitor cell divisions, epithelial thickness increases again (to approx. 70% of its original value) and progenitor cell proliferation decreases, attaining a new steady state approximately 2 weeks post-surgery at a level that is somewhat elevated over that seen in the contralateral (control) OE.^{13,28,33}

The findings from bulbectomy studies suggest that somehow neuronal progenitors ‘read’ the number of differentiated neurons in their immediate environment, and regulate the production of new neurons accordingly.⁵ Evidence supporting this hypothesis has been provided by recent *in vitro* studies, which showed that when neuronal progenitors, purified from embryonic OE, were grown in the presence of a large excess of differentiated ORNs, their generation of new neurons was inhibited three- to four-fold.^{6,35} The results of these studies indicate that differentiated ORNs produce a signal that feeds back to inhibit neurogenesis by their own progenitors, an idea supported indirectly by anatomical studies showing an inverse relationship between the number of neurons in the epithelium and the rate of proliferation of cells in the basal compartment *in vivo*.^{51–53} A similar feedback inhibition mechanism for regulating neurogenesis has also been suggested for larval *Xenopus* retina, where ablation of certain types of cells *in vivo* by intraocular injection of specific neurotoxic agents results in preferential production of new cells of the appropriate type.^{54,55}

Naris occlusion, in which one nostril of a neonatal rodent is closed (e.g. by cautery), results in decreased airflow and sensory input to ORNs (reviewed in ref 56). The effects of naris occlusion on the OB are dramatic (see below), but only subtle changes are seen in the OE. Naris occlusion results in a 10–15% reduction in the thickness of the epithelium, depending on the length of time the naris is closed;⁵⁷ however, the number of mature ORNs, as measured by the density of dendritic knobs on the surface of the OE⁵⁸ or by cell counts and OMP staining,⁵⁷ does not appear to change. Since proliferation of progenitor cells in basal OE appears to be decreased following naris occlusion,^{57,59} it has been suggested that the decrease in OE thickness results from a reduction in the number of immature neurons, although no direct counts of immature ORNs have been performed to test this hypothesis. Also of interest, but untested, is the implication that the reduction in proliferation is the result of a decrease in ORN death, presumably

due to decreased environmental insult to the OE when the nostril is occluded. If proven to be correct, this would support the idea that neurogenesis is directly regulated by ORN death in the OE.

Recent studies using reversible naris occlusion indicate that the OE can rapidly recover from these changes. In both rabbits⁶⁰ and rats,⁶¹ reopening the occluded naris results in a rapid (6–10 days) return to normal thickness by the OE. Presumably this recovery involves increases in both ORN death and progenitor proliferation, but this has not been characterized.

Olfactory bulb

Does neural input from the OE influence ongoing neurogenesis in its target, the olfactory bulb? Certainly normal development of the OB appears to be dependent on ingrowth of axons from the OE (reviewed in ref 15). Indeed, studies in which olfactory placodes, which give rise to OE, have been extirpated or added during early development of *Xenopus* embryos have suggested that the number of ingrowing ORN axons can regulate the size of the OB,⁶² although the extent to which this occurs has been disputed.⁶³ In experimental models employing direct physical damage of ORNs, both functional and structural changes have been noted in the OB. Following nasal irrigation with zinc sulfate, the OB undergoes some atrophy, with its recovery in volume apparently governed by the extent to which ORNs are able to regenerate and re-innervate the tissue.^{45,64} For example, one study showed that the number of glomeruli with OMP immunoreactivity declined drastically (~77%) 5 days after the procedure, but recovered to within 23% of control after 20 days as ORNs regenerated and re-innervated the bulb.⁶⁵ Significantly, shrinkage of the OB appears to be due to loss of ORN axon terminals (which are engulfed by phagocytic cells after they degenerate), rather than degeneration of neurons intrinsic to the bulb occurring in response to loss of afferent input. Using electron microscopy, one study failed to observe degeneration of mitral/tufted and periglomerular cell processes in the bulb, even in regions where re-innervation fails to take place.⁴⁵ However, there does appear to be a relationship between afferent input and biochemical function in the OB. Levels of tyrosine hydroxylase, the rate limiting enzyme in biosynthesis of catecholamine neurotransmitters, decrease to 20–30% of control levels in the OB within 10 days following Triton X-100 irrigation of the nasal cavity, and then recover to control values over the course of the next

40 days.⁶⁶ Since tyrosine hydroxylase appears to be present only in neuronal processes intrinsic to the bulb, this suggests that synaptic function in the OB responds to loss of afferent input, even if cell numbers do not.⁶⁶

Using the naris occlusion model with juvenile rodents, significant changes are seen in the OB. When one nostril is closed on the first post-natal day (P1), this results in a 25% decrease in volume of the ipsilateral OB, compared to control (contralateral sides), by P30. This change in size appears to result from both a decrease in size of existing mitral cells and a decrease in the number of tufted, granule, and glial cells (reviewed in ref 56). Data on the time-course of OB growth in control versus occluded bulbs, when the procedure is performed on neonatal animals, suggest that the small size of occluded bulbs is due to retardation of their growth, compared to bulbs with normal sensory input.⁶⁷ However, even when the procedure is performed on adult animals, there is significant atrophy and loss of granule cells in deprived bulbs.⁶⁸ Deprived bulbs contain a larger number of apoptotic cells, including both periglomerular and granule cells, than control subjects, suggesting that the decrease in cell number seen in deprived bulbs is due to increased cell death.^{69,70}

Does the increase in cell death in occluded OBs result in an increase in neurogenesis in the bulb, the way death of ORNs results in increased neurogenesis in the OE? One investigation found that when naris occlusion, performed on neonatal rats, was reversed after 20 days, OB volume recovered and there was a significant increase, compared to contralateral non-deprived OBs, in the number of newly-generated neurons in the periglomerular layer—the OB cell layer that receives direct afferent input from ORNs.⁶¹ However, this same study found no increase in the number of newly-generated granule cells in OBs to which sensory input had been restored.⁶¹ An underlying assumption of this study was that progenitors of granule and periglomerular cells were equally likely to be labeled by a single injection of BrdU 24 h following re-opening of the nostril. Assuming this was the case, these findings suggest that there is a selective increase in generation and/or survival of one interneuron population (the periglomerular cells) but not another (the granule cells) in response to restoration of afferent input to the OB. What could be the difference between granule and periglomerular cells? It is not possible to resolve this question without further experiments, but one possibility is that there

is no increase in proliferation of SVZ progenitors giving rise to either periglomerular or granule cells, but instead the post-mitotic neurons have different requirements for their survival. For example, survival of periglomerular cells may be directly dependent on synaptic input from ORNs; when this input is suddenly increased following re-opening of the nostril, an abnormally high number of newly-generated periglomerular cells would then survive. According to this model, granule cells, which are not synapsed upon directly by ORNs, would not show increased survival following re-opening of the nostril, and since their rate of generation would be unchanged, no differences would be observed between control and experimental bulbs.

Do the progenitor cells responsible for generating neurons in the OB themselves respond to signals from the bulb, and by extension to neural input from the OE? In OE, progenitor cells respond to neuron loss by upregulating their proliferation and replacing lost neurons (reviewed in ref 5). This regenerative response has been postulated to result from loss of a neuron-derived inhibitory signal, which under normal conditions prevents OE progenitors from proliferating and generating new neurons, but is lost when ORNs degenerate, leaving progenitors free to respond with a surge of neurogenesis (reviewed in ref 6). OE neuronal progenitors lie in close proximity to ORNs, but progenitors responsible for ongoing production of OB interneurons are derived from the SVZ, which is located some distance away (6–8 mm caudally) in neonatal rodents (reviewed in ref 71). However, proliferating cells are observed throughout the rostral-caudal extent of the rostral migratory stream, through which SVZ-derived progenitors migrate (e.g. refs 32,72), so SVZ-derived progenitors at different levels in the stream might respond differentially to signals from the OB. The results of studies testing this idea are not in complete agreement. Indeed, one study, using the naris occlusion model, failed to detect differences in either the pattern or migration of labeled cells within the rostral migratory stream for any sample location or among groups with different occlusion periods, suggesting that neural progenitors from the SVZ are produced in normal numbers and take up their normal positions in odor-deprived bulbs.⁷² Another study using the naris occlusion model, however, reported reductions in the numbers of proliferating cells in the subependymal layer of the OB itself and in the rostral migratory stream just caudal to the OB.⁷⁰ In an intriguing set of experiments, Kirschenbaum *et al*⁷³ performed unilat-

eral bulbectomies on adult mice, thereby removing the target for migratory progenitors derived from the SVZ. Following bulbectomy, the ipsilateral rostral migratory stream persisted, and increased twofold in volume in the 3 months following bulbectomy, indicating that many more cells survive when the bulb is absent. Furthermore, the proportion of BrdU-incorporating cells in the stream on the bulbectomized side was the same as that on the control side at 3 days and 3 weeks post-bulbectomy, suggesting that the rate of production of neural progenitors was unaffected by absence of the bulb, even though the fate of these cells was altered (i.e. many more survived on the bulbectomized side). These results argue strongly that factors regulating the rate of progenitor cell production and migration of cells are intrinsic to the SVZ and rostral migratory stream, and not controlled by the OB; however, as discussed above, the fate of these cells may ultimately be determined by factors in the OB.

Molecular factors affecting neurogenesis and regeneration in the OE and OB

In the OE, both stimulatory and inhibitory factors have been shown to influence the proliferation of neuronal progenitor cells in tissue culture studies. Stimulatory factors include the fibroblast growth factors (FGFs), which have been shown to promote neurogenesis in two ways: First, FGFs increase the number of cell divisions that INPs undergo, resulting in an increase in the number of neurons that are generated; second, FGFs support proliferation and/or survival of rare progenitors, possibly OE neuronal stem cells, which give rise to INPs.³⁴ It has been reported that FGF8 is expressed in the OE, making this growth factor a good candidate for an endogenous positive regulator of OE neurogenesis *in vivo*.⁷⁴ Other molecules that have been reported to stimulate proliferation of OE cells *in vitro* include epidermal growth factor (EGF),^{8,75} transforming growth factor- β 2 (TGF- β 2),⁷⁶ TGF- α ,⁷⁷ and olfactory marker protein (OMP),⁷⁸ but roles for these molecules in neurogenesis remain to be determined.

As mentioned previously, ORNs produce a signal that feeds back to inhibit neurogenesis by their own progenitor cells *in vitro*.³⁵ Recently, it has been shown that this anti-neurogenic effect can be mimicked by growth factors in the bone morphogenetic protein (BMP) family. In tissue culture studies, BMPs 2, 4, and 7 completely inhibited neurogenesis by progeni-

tor cells purified from embryonic OE.⁷⁹ Interestingly, BMPs appeared to act by targeting MASH1, a transcription factor known to be essential for ORN development *in vivo*, for degradation via the proteasome pathway.⁷⁹ The fact that BMPs are expressed in the OE *in vivo* suggests that they may be important endogenous regulators of neurogenesis in this tissue (ref 80; J. Shou and A.L. Calof, unpublished data), an idea currently being tested in a transgenic mouse model in which a BMP antagonist, Noggin, is ectopically expressed in the OE.⁸¹

Growth factors of the neurotrophin family have been shown to have effects on survival of cultured ORNs. In one study, the neurotrophins brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin 4/5 (NT-4/5), promoted survival of cultured ORNs derived from embryonic mouse OE; nerve growth factor (NGF), however, had no effect.²⁸ Similarly, BDNF and NT-3, but not NGF, were reported to increase ORN survival in cultures obtained from neonatal rats.⁸² Since the BDNF/NT-4/5 high affinity receptor, TrkB, and the NT-3 receptor, TrkC, are expressed by subsets of neurons *in vivo*,^{28,82} and several neurotrophins are expressed in the OB,⁸³ neurotrophins are good candidates for factors involved in ORN survival and/or maturation *in vivo*. However, it has been shown that none of these neurotrophins stimulate proliferation of OE neuronal progenitor cells *in vitro*, so a role for neurotrophins in replacing ORNs lost due to cell death appears unlikely.³⁴

Both EGF and FGF2 have been shown to stimulate proliferation *in vitro* of dissociated cells derived from adult rodent SVZ.^{84–87} In these experiments, most of the SVZ cells that were initially plated actually died after 1–2 days in culture, but a small number of cells survived in the presence of EGF or FGF2 and after 1–3 weeks produced ‘spheres’ containing cells expressing the neuroepithelial intermediate filament, nestin.^{84–86,88} These spheres could be dissociated, replated at low density, and would continue to generate new spheres for multiple passages, suggesting that they contained self-renewing cells. Moreover, when spheres were replated onto appropriate substrata, they gave rise to differentiated neurons, astrocytes, and oligodendrocytes. These findings have been interpreted to suggest that the SVZ-derived spheres contain multipotent neural stem cells, which are responsive to EGF and FGF and are capable of giving rise to both neurons and glia.^{84,86–88} Receptors for both EGF and FGF are expressed in the SVZ,^{87,89} and infusion of EGF or FGF into the lateral ventricle has

been shown to lead to increased proliferation of SVZ cells *in vivo*.^{90,91} Interestingly, one of these studies found an increase in the number of newly-formed neurons in animals infused with FGF2, but not EGF, when the OB was examined several weeks later (EGF infusion resulted in an increase in the number of newly-formed astrocytes in the OB).⁹¹

Neurotrophins have been shown to have effects on differentiation and/or survival of postmitotic neurons derived from SVZ progenitors, as observed for ORNs. The high affinity BDNF receptor, TrkB, is expressed by cells in EGF-generated spheres *in vitro*, and treatment of these spheres with BDNF leads to a twofold increase in the number of neurons generated by the spheres.⁹² In these experiments, BDNF appeared to enhance initial neuronal differentiation but not survival: proliferation of cells in the spheres was not affected by BDNF, and the BDNF-stimulated increase in neuron number did not persist at longer culture times.⁹² However, other investigators have reported that BDNF does promote survival of neurons in SVZ explant cultures isolated from adult rats,^{93,94} NGF and NT-3, however, do not appear to have this effect.⁹³ BDNF appears to have effects on SVZ-derived neurons *in vivo* as well. After 12 days of intraventricular infusion of BDNF, a significant increase in the number of newly-generated cells in the SVZ, rostral migratory stream, granule cell layer and periglomerular regions of the OB was observed in a recent study.⁹⁵ Moreover, the majority of these newly-generated cells expressed neuronal markers—greater than 90% of them expressed TuJ1, MAP-2 was expressed by more than 80%, and less than 10% expressed the astrocyte marker GFAP.⁹⁵ These results indicate that BDNF can promote the differentiation or survival (or both) of SVZ-derived neurons. Since BDNF is known to be expressed in the OB,⁸³ it could play a role in regulating the number of SVZ-derived neurons in this tissue *in vivo*.

Conclusions

Neurogenesis in the OE is clearly a regenerative process, in which the rate of proliferation of neuronal progenitor cells is increased to replace differentiated neurons that have been lost due to death or injury. Control of this process appears to be vested, at least in part, in a negative feedback system in which differentiated neurons produce an inhibitory signal that acts to prevent progenitor cells from dividing and generating new neurons as long as the normal

complement of neurons is intact. In contrast, ongoing proliferation by progenitors in the SVZ and rostral migratory stream, which give rise to periglomerular and granule cells of the OB, appears to be relatively insensitive to the turnover of these OB interneurons. Thus, neurogenesis appears to be a constitutive process, rather than a highly-regulated one, in the OB. Interestingly, however, despite these differences in regenerative behavior, progenitor cell proliferation and neuronal survival and maturation appear to be regulated by similar molecular factors (FGFs and neurotrophins, respectively) in the two tissues comprising the primary olfactory pathway.

Notably, two recent studies have provided data to suggest that, even though neuronal regeneration does not take place in the OB, the SVZ itself may be a regenerating tissue. If antimitotic agents are used to kill all rapidly-dividing cells in the SVZ and rostral migratory stream *in vivo*, the relatively quiescent stem cells of the SVZ apparently can regenerate the entire complement of progenitor cells within a few days, and normal cell migration and neurogenesis then resume.^{43,89} At present, it is not understood how this is regulated. For example, no factors have been described that inhibit proliferation of SVZ stem cells, but studies of OE regeneration suggest that such inhibitory factors may exist and may play an important role in controlling regeneration of the SVZ. Determining the molecules involved in this process will be crucial for understanding how neuronal regeneration could be made to occur in the mammalian CNS.

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