

Inhibitory Interneurons in the Olfactory Bulb: From Development to Function

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Identifying and defining the characteristic features of the inhibitory neurons in the nervous system has become essential for achieving a cellular understanding of complex brain activities. For this, the olfactory bulb is ideally suited because it is readily accessible, it is a laminated structure where local interneurons can be easily distinguished from projecting neurons, and, more important, GABAergic interneurons are continuously replaced. How the newly generated neurons integrate into a preexisting neural network and how basic network functions are maintained when a large percentage of neurons are subjected to continuous renewal are important questions that have recently received new insights. Here, it is seen that the production of bulbar interneurons is specifically adapted to experience-dependent regulation of adult neural networks. In particular, the authors report the degree of sensitivity of the bulbar neurogenesis to the activity level of sensory inputs and, in turn, how the adult neurogenesis adjusts the neural network functioning to optimize information processing. By maintaining a constitutive neurogenesis sensitive to environmental cues, this neuronal recruitment leads to improving sensory abilities. This review brings together recently described properties and emerging principles of interneuron functions that may convey, into bulbar neuronal networks, a degree of circuit adaptation unmatched by synaptic plasticity alone. *NEUROSCIENTIST* 10(4):292–303, 2004. DOI: 10.1177/1073858404263460

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Communication between nerve cells is dominated by two modes of interaction that have opposite effects: excitation and inhibition. For a long time, excitation was assumed to play the dominant role in high brain functions (e.g., perception, learning, or memory), whereas inhibition was thought to serve mainly to eliminate unwanted, or excessive, activity. This traditional view inspired by the Yin and the Yang considers local inhibitory neurons as the regulators of excitatory neuron activity. According to this, the activity of the large network of principal excitatory neurons in the central nervous system is balanced by local-circuit neurons releasing inhibitory neurotransmitters. These interneurons occupy distinct positions within brain microcircuits where they form mainly local connections and perform their functions through a range of various processes. Because of these local connections, the term *interneuron* was originally quoted to refer to cells located at the interface between input and output neurons. Following pioneering

works leading to the concept of synaptic inhibition (Eccles 1964), this terminology then progressively conveyed the unifying principle that inhibitory cells were rather key players in regulating the local circuit excitability, in contrast to excitatory neurons that project information to distant brain areas. However, this view has recently been challenged by the continuous emergence of novel functional, biochemical, and anatomical characteristics clearly revealing much more complex functions for inhibitory local interneurons.

One of the most popular cell types studied today is the GABA-releasing neuron, also referred to as the local-circuit inhibitory interneuron. Recently, numerous data that would not strictly fit the definition given above have been generated. They have made the original concept of interneuron oversimplified, thus requiring readjustment to accommodate cellular types and functions. For instance, high-resolution immunocytochemical analysis for receptors, neurotransmitters, and enzymes involved in GABAergic synaptic transmission (Somogyi and others 1998) has provided compelling information about the molecular architecture required to interpret functional observations, in addition to numerous testable functional predictions. Likewise, combining anatomical, electrophysiological, and pharmacological approaches has allowed a better insight into the roles played by interneuron types at the network level, both in vitro and in vivo (Gulyas and others 1993; Buhl and others 1994;

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Klausberger and others 2003). At the cellular level, these functions range from governing action potential generation, firing pattern, membrane potential oscillations, and dendritic calcium spikes (Miles and others 1996). At the network level, inhibitory interneurons are considered to be important in controlling synaptic strength and plasticity and in the generation and pacing of large-scale, synchronous oscillatory activity (Bragin and others 1995).

Despite all this recent progress, one important problem that has constantly limited studies of interneuron function has been the tremendous heterogeneity in the interneurons' morphology and connectivity, as opposed to principal neurons, which are much more uniform in their appearance (Maccaferri and Lacaille 2003). Indeed, anatomically, interneurons represent one of the most diverse populations in the mammalian central nervous system, and classifications based solely on anatomical features tell us little about their function (McBain and Fisahn 2001). Due to this limitation, another approach to characterizing interneuron subtypes has been based on neurochemical content such as calcium-binding proteins (e.g., parvalbumin, calbindin, and calretinin) or neuromodulators (e.g., somatostatin, neuropeptide Y, and vasoactive intestinal peptide). However, neurochemically identical cells can possess surprisingly different functional properties, further complicating classification systems based only on neurochemical content (Freund 2003). Finally, a characterization based on functional properties has proven to be problematic as inhibitory interneurons exert pleiotropic actions (Fricker and Miles 2001). Therefore, any classification of inhibitory neurons, to be valid, has rather to take into account morphological, biochemical, and physiological characteristics together. Because in the olfactory bulb, all of these parameters have been thoroughly investigated, this first relay of olfactory information appears to be one of the best system models suitable to explore the functions of inhibitory interneurons.

Synaptic Organization of the Olfactory Bulb

The initial events in olfactory perception take place in sensory neurons located in the olfactory epithelium of the nasal cavity. These sensory neurons are embedded in a pseudostratified columnar epithelium. Odor molecules that dissolve in the nasal mucus bind to specific receptors on the cilia of olfactory sensory neurons (Firestein 2001). This epithelium contains three major cell types: sensory neurons, supporting cells, and olfactory stem cells. Sensory neurons are unusual in that they are short-lived cells that exist for only 30 to 60 days and are continually replaced by the stem cells (Murray and Calof 1999). From its basal pole, the sensory neuron sends a single axon to the olfactory bulb (Fig. 1). The unmyelinated sensory neuron axons merge to form the olfactory nerve, which transmits the electrical signals to the olfactory bulb. In this central relay, axons of sensory neurons form glutamatergic excitatory synapses in regions

known as glomeruli that are analogous to the multineuronal "barrels" and "columns" in the cerebral cortex (Shepherd and others 2004).

Besides relaying sensory information to the olfactory cortex, the olfactory bulb also actively takes part in information processing through a series of steps that occur in anatomically distinct levels (Laurent 2002; Lledo and Gheusi 2003; Schoppa and Urban 2003). Sensory information is processed and refined within the olfactory bulb through neural circuits that include two classes of interneurons: periglomerular and granule cell (Fig. 1). These unusual interneurons lack an axon and instead release their neurotransmitter from dendritic spines at specialized reciprocal synaptic contacts with dendrites of principal neurons of the olfactory bulb (i.e., mitral or tufted cell; Shepherd and others 2004). The periglomerular cells (GABAergic or dopaminergic interneurons), which are restricted to one glomerulus, impinge onto olfactory nerve terminals and/or primary dendrites of principal neurons (Kosaka and others 1998). They exert mainly two types of inhibition. First, GABAergic periglomerular cells inhibit principal neurons via dendrodendritic synapses. This type of inhibition is mediated by postsynaptic GABA_A receptors. Second, GABAergic and dopaminergic periglomerular cells regulate the olfactory nerve–olfactory bulb synaptic transmission via presynaptic GABA_B and D₂ receptors expressed on olfactory sensory axons.

The second locus where information processing depends also on local interneurons lies in the external plexiform layer of the olfactory bulb, where other reciprocal synapses are heavily distributed (Price and Powell 1970b, 1970c). Individual principal neurons extend secondary dendrites over long distances (up to 1000 μ m) (Mori and others 1983; Orona and others 1984) to form reciprocal dendrodendritic synapses with the dendrites of granule cells (Rall and others 1966; Jackowski and others 1978), the main group of bulbar interneurons (Fig. 2). Granule cell dendrites are depolarized by glutamate released from activated principal neurons and in turn inhibit these neurons by releasing GABA. At dendrodendritic synapses between principal neurons and granule cells, the reciprocal circuit forms the basis of a reliable, spatially localized, recurrent inhibition. Thus, synaptic transmission between dendrites represents the major neuronal interaction in the olfactory bulb, providing a tight balance between inhibitory and excitatory signaling.

Finally, it should be mentioned that a model of olfactory memory based on altering lateral inhibition in the olfactory bulb has been proposed (Linster and Masson 1996), and several studies have indeed demonstrated the degree of plasticity of dendrodendritic reciprocal synaptic connections. In line with this, it is noteworthy that the two classes of local interneurons integrate information not only from the intrabulbar circuit but also from numerous centrifugal systems, including structures such as the piriform cortex, telencephalic basal ganglion, magnocellular basal forebrain nuclei, and brain stem locus coeruleus (Price and Powell 1970a; Shepherd and

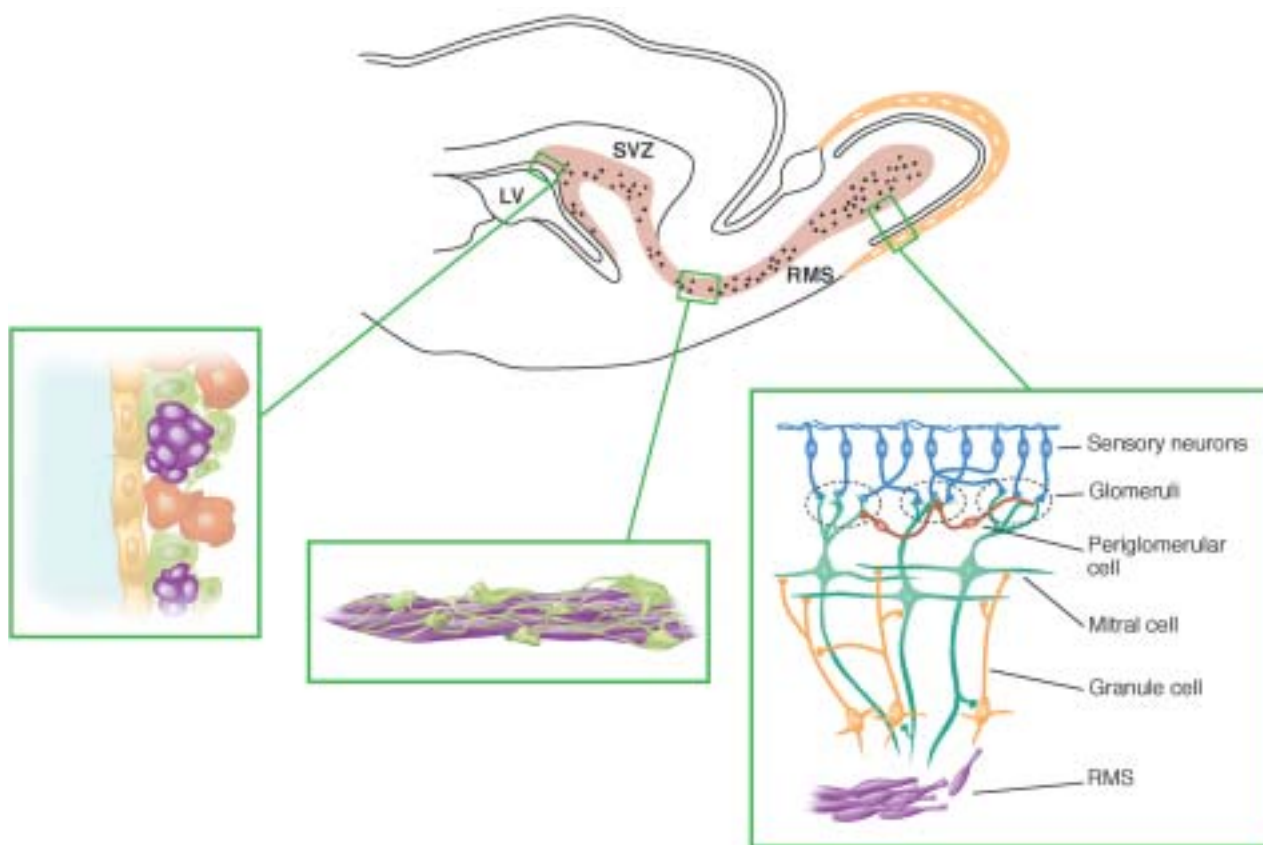


Fig. 1. Maturation events of olfactory interneurons. Ultrastructural analysis of the adult subventricular zone (SVZ) reveals that four major cell types constitute this neurogenic zone. A monolayer of ependymal cells lines the ventricle (orange cells in the left inset). Adjacent to the ependymal layer are clusters of neuroblasts (purple cells in the left, middle, and right insets). Astrocytes (green cells in the left and middle insets) surround the neuroblast chains and transitory amplifying progenitors (brown cells in the left inset). To reach their target, neuroblasts travel tangentially in the rostral migratory stream (RMS). Once in the bulb, these neuronal precursors turn radially away from the migratory stream to integrate the preexisting bulbar network. They invade different overlying layers, where they differentiate into two types of local GABAergic interneurons: the periglomerular and granule cells. LV: lateral ventricle.

others 2004). These central feedbacks might be relevant to the high degree of plasticity reported at dendrodendritic reciprocal synapses (Kendrick and others 1992; Wilson and Sullivan 1994; Hendin and others 1997). Thus, the level of synchronization among specific subsets of output neurons might change during the learning process, resulting in changes in the strength of temporal binding of signals originating from different odorant receptors.

Functions of Inhibitory Interneurons in the Olfactory Bulb

We have described above how a single principal neuron can be self-inhibited through dendrodendritic interactions. However, the activity of a principal neuron connected to a particular glomerular unit is also influenced by the activity of neighboring principal cells associated with other glomeruli. Thus, in addition to the feedback inhibition, principal neurons also receive lateral inhibition from granule cells activated independently by other principal neurons (Rall and others 1966; Isaacson and

Stowbridge 1998; Margrie and others 2001). The mechanisms underlying lateral inhibition of principal cells by granule cells are thought to be similar to self-inhibition, as described above. Interestingly, lateral inhibition is highly sensitive to blockade of N-methyl-D-aspartate (NMDA) receptors and can be observed in the presence of TTX, indicating that it does not require Na^+ action potential in granule cells (Isaacson 1999). However, the significance of the action potential-independent GABA release is still unclear because granule cells do fire action potentials in response to odor stimulation (Mori and Takagi 1977; Wellis and Scott 1990; Luo and Katz 2001; Margrie and Schaefer 2003).

According to the synaptic organization of the olfactory bulb circuit, GABAergic events impinging onto principal neurons originate from four distinct release mechanisms. First, with a moderate activation of a principal neuron, the synaptic activation of local domains of the granule cell dendritic tree might lead to GABA release from small subsets of granule cell spines (Woolf and others 1991). This provides the basis for the so-called self-inhibition. Second, if the activation of local areas of

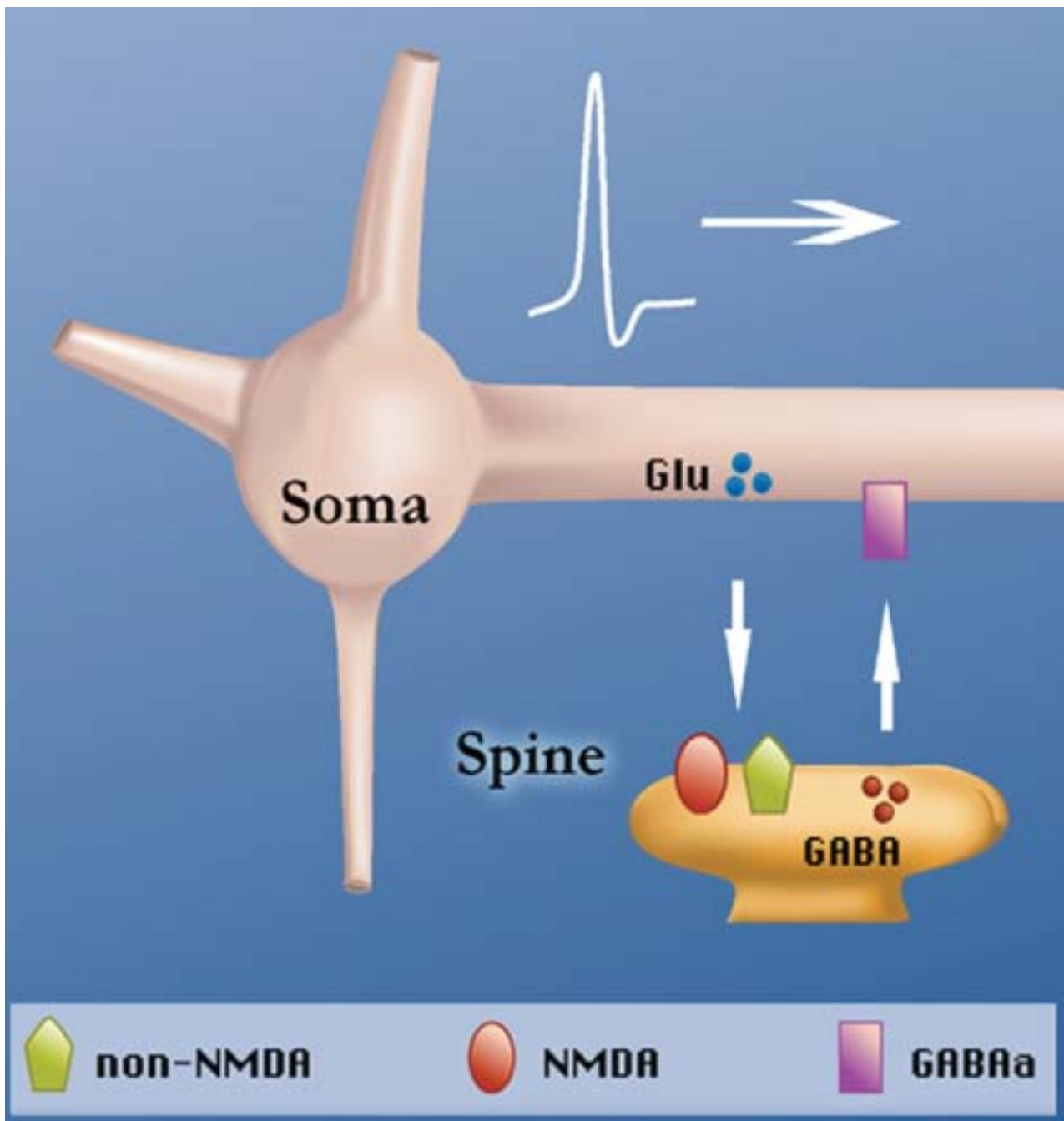


Fig. 2. Schematic of the reciprocal synaptic arrangement between the dendrites of principal and granule cells. Principal cells receive a large number of inhibitory synaptic contacts primarily on their lateral dendrites, which extend for large distances across the olfactory bulb. These inhibitory contacts arise from the large spines of axonless local interneurons, the granule cells. The term *reciprocal* implies that a principal cell releases glutamate that activates both N-methyl-D-aspartate (NMDA) and non-NMDA receptors, which in turn trigger neurotransmitter release by granule cells at the same synapse (arrows). Electron microscopic evidence indicates that principal cell dendrites contain synaptic vesicles clustered around active zones. Granule cells contact principal cells via large, vesicle-containing spines (also called gemmules) that are both presynaptic and postsynaptic to principal cell dendrites.

the granule cell dendrites is stronger, then the local spread of depolarization (or calcium signals) through granule cell spines might mediate a local lateral inhibition among principal neurons. According to these two scenarios, GABA release, which does not require action potential, provides a graded synaptic transmission.

However, if synaptic activation of a granule cell spine is strong enough to elicit action potential, this might result in global elevation of calcium levels and the release of GABA from hundreds of granule cell spines. This in turn will support a global lateral inhibition and implies that the regenerative calcium spikes in granule cell dendrites

provide a long-lasting stimulus for GABA release. Finally, in granule cells, calcium influx through both voltage-dependent calcium channels (Isaacson and Strowbridge 1998; Isaacson 2001; Egger and others 2003) and NMDA receptors (Chen and others 2000; Halabisky and others 2000) can trigger GABA release. However, under physiological conditions, the NMDA receptors that control GABA release are tonically blocked by Mg^{2+} (Isaacson and Strowbridge 1998). Nevertheless, granule cells receive excitatory input onto their proximal dendrites (Kishi and others 1984; Orona and others 1984). One function of these proximal glutamatergic synapses may be to depolarize granule cell dendrites sufficiently to unblock NMDA receptors at GABA release sites. This mechanism would support a feed-forward inhibitory action of granule cells on principal neurons.

The effect of lateral inhibition on a principal neuron depends on the location and strength of the inhibitory input and its impact on local signaling processes. Previous anatomical studies have shown symmetric, presumably inhibitory synapses on the mitral cell membrane (Rall and others 1966; Price and Powell 1970b, 1970c; Toida and others 1994, 1996; Sassoe-Pognetto and Ottersen 2000; Crespo and others 2001). Reciprocal synapses are widely distributed over the soma, primary and secondary dendrites, and the axonal hillock and initial segment (Rall and others 1966; Price and Powell 1970b; Toida and others 1994, 1996). Furthermore, some granule cells seem to have specific targets. For instance, parvalbumin-immunoreactive interneurons (e.g., short axon cells) are known to synapse exclusively onto principal neurons (Toida and others 1994, 1996). This subtype of interneurons might act in concert with the axonal inhibitory input to control principal cell discharge. They might also act to shift the site of spike initiation up to the primary dendrite or completely block somatic spike output (Chen and others 1997). On the secondary (also called lateral) dendrites of principal neurons, the ability of GABAergic synapses to block action potential generation depends on their distance from the soma and the amount of shunting current that will control action potential propagation. Here, lateral inhibition relies on bidirectional signaling. The secondary dendrite transmits excitatory output from the soma in the form of centrifugally back-propagating action potentials, and they receive GABAergic events that are conducted centripetally to the soma.

A number of studies have suggested that the lateral inhibition provided by granule cells may sharpen odor tuning in a glomerulus as happens in the retina when a light shines on both the center and the surround (but see Laurent 1999). Consequently, it has been demonstrated that bulbar projecting neurons connected to different glomerular units and that respond to a wide range of related odor molecules also receive inhibitory inputs from neighboring glomerular units via lateral inhibition mediated by reciprocal connections (Mori and others 1999). By sharpening the receptive fields of individual

projecting neurons and weakening the activity of neighboring ones, lateral inhibition mediated by local interneurons enhances both the quantity and quality of information transferred to higher cortical areas. Altogether, the various functions mediated by GABAergic interneurons in the olfactory bulb revealed an unexpected degree of complexity that makes the bulbar local circuit central to major aspects of bulbar functions.

Oscillations and Inhibitory Functions

Synchronized oscillations of neuronal populations occur in diverse brain structures and are subjected to modulation by behavioral state and sensory inputs. Physiological studies have revealed large-scale synchronous and oscillatory activities in the γ frequency band (30–80 Hz) in mammalian brains (Engel and others 2001). The fast oscillations that emerge from coordinated electrical activity across large groups of neurons are thought to play important roles in information processing, memory formation, and sensory perception (Gilbert and others 2001; Varela and others 2001).

In sensory systems, evoked field potential oscillations are thought to encode stimuli and to participate in fine discrimination. They have been reported in neural assemblies of various sensory systems such as vision, audition, and olfaction. Pioneering studies from Lord Adrian have revealed that the most important feature of the olfactory system was the generation of synchronized network field oscillations that emerge from activity of different parts of the olfactory pathway. For instance, he was the first to report fast synchronous γ oscillations in the olfactory bulb following olfactory stimulations (Adrian 1942). For a given odor stimulus, the “induced rhythm” (as first quoted by Adrian) is synchronized transiently in time and only among a neural subpopulation that is selectively responsive to that particular odorant. Interestingly, all rhythms observed in the olfactory bulb of mammals have also been reported in its functional equivalent in invertebrates and are thought to encode stimuli (Laurent 1996). These local field potential oscillations result from synchronized spike discharges of principal cells in responses to odor presentation (Laurent 1996).

How does the bulbar neuronal circuit synchronize the discharges of principal cells? In the mammalian brain, synchronizing mechanisms can depend in part on the ability of populations of GABAergic neurons to entrain the firing of principal neurons (Whittington and Traub 2003). Several studies using a modeling approach pointed to the importance of a rapid inhibitory feedback loop in principal neurons for generating γ oscillations in the olfactory bulb (Rall and Shepherd 1968; Freeman 1975; Li and Hopfield 1989; Bazhenov and others 2001; Davison and others 2003). Recent experimental findings have indicated that inhibitory interneurons are indeed instrumental in generating these fast oscillations. They demonstrated the particular role of granule cells in the emergence of the coherent activity. Among these studies

are the pharmacological manipulation of inhibition that impairs local field oscillations (Lagier and others 2004) and odor discrimination in honeybees (Stopfer and others 1997), the genetic manipulation of granule cell excitability that enhances field oscillations and alters discrimination in rodents (Nusser and others 2001), and the tetanic stimulation of the olfactory nerve that simultaneously enhanced both field oscillations and inhibitory synaptic events received by principal cells (Friedman and Strowbridge 2003). From all these studies, inhibitory interneurons appear to have a critical role in the temporal patterning of neuronal discharges, with respect to both the generation of oscillatory response patterns and the synchronization of distributed responses. These key functions conflict with the notion that inhibitory interneurons constitute a pool that mediates unspecific interactions. They support rather the growing evidence for an unexpected diversity of local interneuron actions in the olfactory bulb. For instance, immunohistochemical identification of GABAergic interneurons has revealed numerous morphologically distinct classes of inhibitory cells, many of which use a peptide neurotransmitter in addition to GABA, and the analysis of their connection patterns is beginning to show a surprisingly high degree of complexity. Although most bulbar interneurons release GABA as primary neurotransmitter (but see Didier and others 2001), the manifold effects of activation of GABA receptors could result from complex responses depending on the postsynaptic target domains, the pattern of presynaptic activity and dynamics of the postsynaptic targets, and the developmental stage considered.

It is also noteworthy that single neurons in the central nervous system are endowed with a large repertoire of voltage- and calcium-gated ion channels, distributed across the dendritic and somatic membrane. These channels can give rise to complex neuronal dynamics. In general, oscillation occurs in a single cell, when a strong fast positive feedback (generating the rising phase of membrane voltage) interacts with a slower negative feedback (producing the decay phase of the cycle). Activation of voltage-gated inward currents or activation of outward potassium currents can provide positive feedback within a cell. Hence, many neurons from the central nervous system exhibit intrinsic subthreshold fluctuations in membrane potential (Llinas 1988). In the olfactory bulb, we showed that subthreshold oscillations, ranging from 10 to 50 Hz, were generated from an inward conductance that interacts with GABAergic synaptic events (Desmaisons and others 1999). Such oscillations were generated by a TTX-sensitive sodium conductance that operates within a range of voltages above and below spike threshold and is crucial for spike timing as well as for filtering excitatory postsynaptic potentials. Therefore, GABAergic inputs on principal neurons could serve to phase-lock the subthreshold oscillations, thus providing a potential mechanism to integrate excitatory synaptic inputs into action potentials. As a result, this would lead to a widespread synchronization of prin-

cipal neuron action potentials during olfactory responses. This view is supported by results indicating that the odor-evoked spike discharge of principal neurons tended to be phase-locked with the oscillatory field potential in the γ frequency range (Kashiwadani and others 1999; Buonviso and others 2003; Lagier and others 2004).

Finally, the principal cell is not the only type of bulbar neuron to receive GABAergic synaptic inputs. It has been previously proposed that inhibition received by granule cells might also play an important role in γ field oscillations (Nusser and others 2001). Supporting this view is the recent work showing an implication of gap junctions between granule cells in the synchronization underlying γ field oscillations (Friedman and Strowbridge 2003). These electrical synapses might help to propagate signaling through the granule cell network, but their direct contribution in generating the γ rhythm remains unclear. For instance, anatomical data of electrical coupling between granule cells (Reyher and others 1991; Paternostro and others 1995) are still controversial (Kosaka T and Kosaka K 2003). In the near future, it may be fruitful to reexamine the role of the granule cell network with particular respect to the generation of γ oscillations.

Perinatal Production of Inhibitory Circuit of the Olfactory Bulb

Neural cell production, including both neurons and glial cells, is an imperative requirement for brain formation during fetal stages. Newborn cells, generated in germinal regions, move along precise pathways to the correct sites for their final differentiation and integration. This highly temporally and spatially orchestrated migration is essential for normal brain development and function. It is noteworthy that these cell movements occur following two modes, radial and tangential, that are distinguishable according to the orientation (Hatten 1999; Fig. 3). The radial mode is essentially characterized by cell migrations from the dorsal telencephalon toward the pial surface, along the processes of radial glia. The tangential mode applies to neuron migration from the ventral telencephalon orthogonal to the direction of radial migration (Marin and Rubenstein 2003).

Although most cells in the nervous system are born during the embryonic and early postnatal period, newborn neurons are still generated within two areas of the adult mammalian forebrain: the hippocampus and the olfactory bulb. As during the development of the immature brain, neurogenesis that occurs in the adult brain includes cell proliferation, migration, neuronal differentiation, and survival. However, the exact mechanisms involved in all these steps remain largely unknown in the adult brain. For instance, does the maturation process of newly generated neurons recapitulate the one described during embryogenesis?

In the olfactory bulb, interneurons are generated during a long period of time, from embryonic stages until the death of the animals, and are emanated from differ-

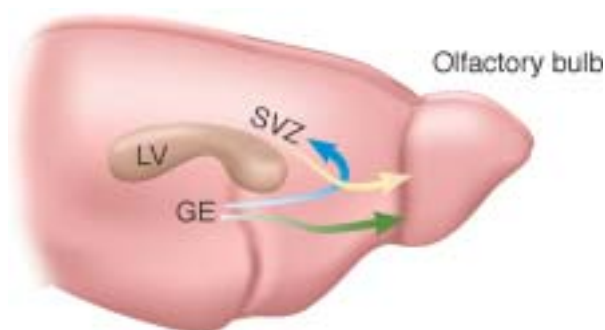


Fig. 3. Olfactory interneuron origins in embryonic and adult brains. During the embryonic stages, ganglionic eminence (GE) generates cells that ventrally and tangentially migrate into the olfactory bulb (green arrow). Some progenitor cells leave the GE and migrate and participate in the formation of a second germinal zone, the subventricular Zone (SVZ) (blue arrow). In early postnatal and adult stages, the anterior part of the SVZ constitutes the major source of olfactory interneurons. The yellow arrow indicates cell migration from the SVZ to the olfactory bulb during adulthood. LV = lateral ventricle.

ent progenitor zones (Fig. 3). Hence, different studies have shown that the local circuit of the olfactory bulb is, in part, derived from several regions of the embryonic subpallium, particularly the dorsal region of the lateral ganglionic eminence (Marin and Rubenstein 2003). This embryonic area generates cells that tangentially migrate into the olfactory bulb via a ventral pathway. After birth, a different area produces the bulbar interneurons. They are generated in the anterior part of the subventricular zone (SVZ) of the lateral ventricle that is developed, in part, from residual progenitor cells issued from the lateral ganglionic eminence (Fig. 3). Thus, in a process that begins in the embryo (Wichterle and others 2001) and continues postnatally (Luskin 1993) and into adult life (Lois and Alvarez-Buylla 1994), SVZ stem cells give rise to neuroblasts that migrate tangentially along the rostral migratory stream into the olfactory bulb, where they migrate radially to complete their differentiation into neurons (Alvarez-Buylla and Garcia-Verdugo 2002). Several molecular mechanisms control the different steps of the birth, migration, maturation, and functional integration of future neurons. Because both intrinsic and extrinsic regulatory signals, as well as migratory modes and target structures, differ between germinal zones, it is conceivable that the mechanisms that orchestrate the bulbar neurogenesis in the developing brain are distinct from those operating during adulthood.

How immature neurons differentiate during development has been a subject of intense studies (Jessell and Sanes 2000), but little is known about the process governing neuronal maturation in the adult brain. Unlike the developing brain where neurogenesis usually precedes the maturation of neural circuits, in the adult, new neurons have to integrate into a preexisting neuronal network. Our recent work on neuronal maturation in the adult brain suggested that some, but not all, of the functional properties of immature neurons are distinct from

those described for the developing brain (Carleton and others 2003). This suggests that the rules governing the incorporation of adult-generated neurons into mature neuronal circuits may differ from those previously described in the developing brain.

Maturation and Integration of Newborn Neurons in the Adult Olfactory Bulb

The two germinative zones in the adult mammalian brain that have been extensively described are the SVZ (Temple and Alvarez-Buylla 1999) and the dentate gyrus of the hippocampus (Gage 2002). Within these two regions, the neural precursor cells are considered to be stem cells because they proliferate and give rise to several different cell types. In contrast to the function of neurons born during development, new neurons in the adult brain continually replace older ones within mature circuits (Gage 2002).

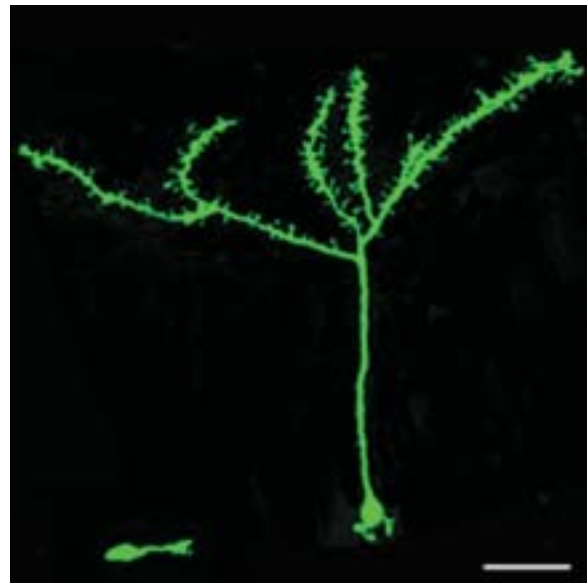
Although experimental and modeling studies have already provided arguments for a role of newborn GABAergic interneurons in olfactory discrimination (Gheusi and others 2000; Cecchi and others 2001), physiological data that would contribute to our understanding of the functional contribution of new neurons to the adult olfactory bulb are still sparse. For instance, whereas much work has been devoted to the understanding of the mechanisms of birth and migration of the new SVZ neuroblasts (Alvarez-Buylla and Garcia-Verdugo 2002), until recently no information was available concerning the temporal sequence of electrophysiological changes that accompany the maturation of the new cells. Such information is essential to understand how newly formed neurons become functionally integrated into adult neural networks. Filling this gap has been impeded by the inability to distinguish, in living brain tissues, newborn cells from older ones. Nevertheless, by using a retroviral labeling method, it has been recently possible to describe and time the development and maturation of adult-generated bulbar interneurons (Petreanu and Alvarez-Buylla 2002; Carleton and others 2003; Belluzzi and others 2003). Most of the adult-generated granule cells attain a fully mature morphology between 15 and 30 days after birth in the SVZ (Petreanu and Alvarez-Buylla 2002; Carleton and others 2003). The morphology of all the cells in the granule cell layer that originated in the SVZ, as determined by the focal viral injections, corresponded to that of granule neurons (see Box 1). This suggests that cells born in the SVZ that end up in the granule cell layer become only granule neurons. In addition, the sequence of morphological stages for the development of granule cells described using the viral labeling was similar to those observed in earlier postnatal animals using Golgi staining (Kishi 1987), suggesting that developing granule cells follow a similar maturation process in juvenile and adult animals, at least according to their morphological features. In agreement with previous studies in adult mice (Bayer 1983; Lois and Alvarez-Buylla 1994), a small proportion (about 1%) of periglomerular neurons was also observed.

Box 1: Maturation of Newborn Granule Cells of the Olfactory Bulb

The adult-generated neurons of the olfactory bulb are born in the subventricular zone (Luskin 1993; Lois and Alvarez-Buylla 1994) and migrate along a network of pathways into the rostral migratory stream and into the olfactory bulb, where they differentiate into granule and periglomerular cells. Most of the olfactory bulb granule neurons are generated postnatally and continue to be added during adulthood (Bayer 1983). Establishing the rate of neurogenesis is important for exploring the functional significance of the new cells as a percentage of the total population. It is also important for experiments trying to assess the role of various factors regulating neurogenesis. Quantitative studies have indicated a basal neurogenic rate of approximately 80,000 new granule neurons per bulb or 1% of the olfactory granule cell population per day in young adult rodent (Peterson 2002). In contrast, neurogenesis in the dentate gyrus of the hippocampus occurs at a considerably lower rate, with approximately 100 to 150 new granule cells per hippocampus, or 0.03% of total hippocampal dentate neuronal population per day (Kempermann and others 1997).

The stages of maturation of newly generated granule cells in adult rodents have been described using retroviral labeling of the newly born neurons in the subventricular zone (Petreanu and Alvarez-Buylla 2002; Carleton and others 2003). Once mature, granule cell bodies cluster to form sheets in the granule cell layer. They extend a few short neurites toward deeper parts of the granule cell layer and one large dendrite toward the external plexiform layer (Petreanu and Alvarez-Buylla 2002; Carleton and others 2003) where it ramifies extensively. Granule neurons have no axons, and their output is mediated by bidirection-

al dendrodendritic synapses located in spines (Woelf and others 1991). Granule cells are thought to modulate the activity of principal neurons (e.g., mitral and tufted cells) of the olfactory bulb, thus optimizing olfactory function through GABA_A-mediated inhibition from granule cell spines.



Different maturation stages of newly generated cells in the adult olfactory bulb. Representative photomicrographs of immature neuron at early stages of maturation (*left*) and at later stages, after developing dendritic spines (*right*). Scale bar = 50 μ m.

Whereas much work has been devoted to elucidating the mechanisms of birth and migration of new SVZ neuroblasts, no information was available concerning the temporal sequence of electrophysiological changes that accompany their maturation. Such information is essential to appreciate how newly formed neurons become functionally integrated into adult neural networks. To address this question, the electrophysiological properties of newborn cells during their migration and differentiation were characterized using a replication-defective retrovirus that carries GFP (Belluzzi and others 2003; Carleton and others 2003). Following different survival times after viral injection into the SVZ, GFP⁺-labeled neurons were recorded from acute olfactory bulb slices. It was found not only that SVZ precursor-derived neurons integrate the adult neuronal circuit but also that they receive synaptic inputs during their radial migration in the olfactory bulb. Interestingly, GABAergic and glutamatergic synapses impinging onto newborn interneurons

were established sequentially, GABAergic synapses being established first. In contrast to the sequential acquisition of synapses during embryogenesis, NMDA responses were found in neurons that were unable to fire and had started to receive GABA_A and AMPA inputs (Carleton and others 2003). As summarized in Figure 4, the sequential acquisition of synaptic receptors, in migrating adult-generated neuroblasts, largely differs from that observed during embryogenesis. For instance, radial migratory neurons in the adult olfactory bulb received massive synaptic inputs but still remained unable to fire. The spiking ability was observed only after integration in the functional olfactory circuit was completed. In other words, the rules that govern the incorporation of adult-generated neurons into mature neuronal networks differ from those previously described in the developing brain. The correlated maturation of intrinsic electrical properties with synaptic activity of newborn cells may influence both maturation

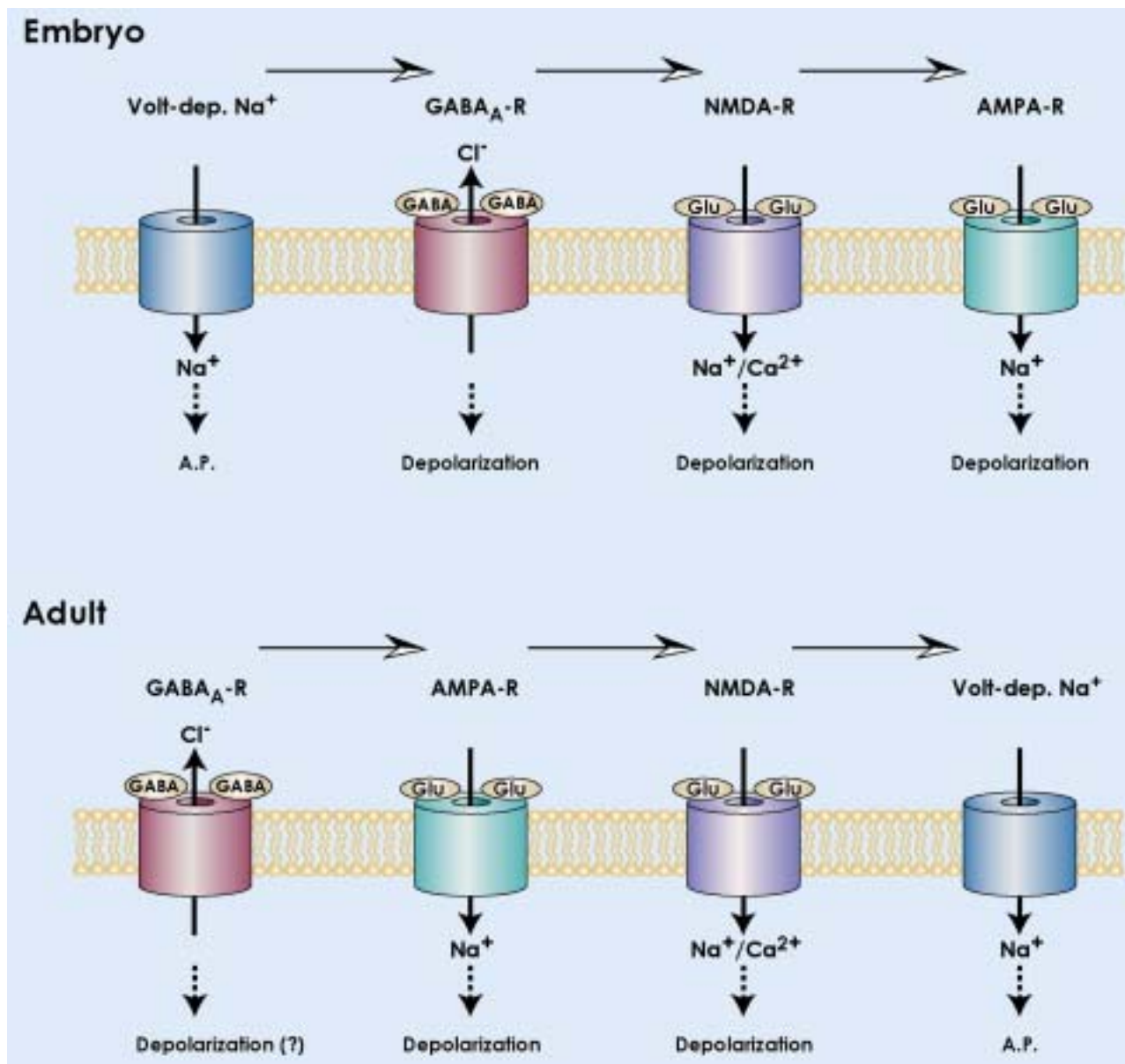


Fig. 4. Sequential acquisition of synaptic receptors in the embryo (*top*) and adult (*bottom*) neurons. During embryonic stages, AMPA ionotropic receptors become functional on mature neurons. In the adult, NMDA receptors are generated only after AMPA and GABA_A receptors. Note that apparition of sodium conductance constitutes one of the last events of neuronal maturation during adulthood in contrast to the embryogenesis. AP = action potential; Glu = glutamate.

and integration processes and therefore represents a mechanism by which neuronal activity may regulate neurogenesis. Interestingly, the sequence of the development of voltage-dependent ionic channels and synaptic connections revealed major differences between newly generated periglomerular and granule neurons (Belluzzi and others 2003; Carleton and others 2003). In periglomerular neurons, the maturation of voltage-dependent sodium current, and consequently the capacity of the newly generated cells to fire action potentials, preceded the appearance of synaptic contacts (Belluzzi and others 2003), whereas in granule cells, a full development of the sodium current was observed only after

the establishment of synaptic connections (Carleton and others 2003). This difference indicates that the pattern of functional integration of new neurons depends on the nature of the newborn neurons. Altogether, the unique set of electrophysiological properties of migrating cells allow them to integrate the mature brain without altering cognitive processes.

Adult Production of Bulbar Inhibitory Interneurons: Functional Consequences

Although the ongoing neurogenesis and migration have been extensively documented, their function remains

unknown. We postulated that if newborn interneurons generated throughout life were indeed necessary for bulbar function, then modifications of the processes of migration, leading to changes in the recruitment of newborn interneurons, would affect olfactory processing. We tested this hypothesis by quantifying the level of migration and the size of the GABAergic interneuron population in mutant mice that exhibit altered neuroblast migration. We found that the reduction (about 40%) in the number of newborn granule cells in adult mutants was accompanied by impaired odor discrimination (Gheusi and others 2000). This was further confirmed using a theoretical approach that showed the importance of newborn neurons for plasticity and olfactory discrimination (Cecchi and others 2001). This strongly supports a specific role for newborn interneurons in downstream coding of sensory information. A critical level of inhibitory activity by GABA_A receptor activation on the secondary dendrites of bulbar principal neurons seems therefore crucial for olfactory processing. To challenge this view, we investigated whether the number of newborn bulbar interneurons correlates with the memory strength. For this purpose, adult mice were exposed to an odor-enriched environment. By increasing the incoming activity, we found that the number of newly formed interneurons was enhanced by about 50% (Rocheffort and others 2002). Interestingly, such enriched mice retain learned olfactory information for longer periods of time than do controls. In particular, animals raised in an enriched environment were able to recognize familiar odors in a more durable and stronger way (i.e., they expressed a high resistance to retroactive interference) as compared to animals raised in standard conditions. It is likely that a similar activity-dependent mechanism may already be operating after birth. Importantly, 90% of the granule cells are incorporated postnatally, and their survival is also dependent on incoming activity (Rosselli-Austin and Williams 1990). An optimal olfactory bulb circuit can therefore be assembled according to the initial olfactory environment of the animal. The continuation of interneuron generation and replacement in adult life could reflect the necessity to readapt the olfactory bulb to ongoing environmental changes to maintain optimal discrimination.

Concluding Remarks

The basic circuit responsible for excitatory and inhibitory interactions in the olfactory bulb reveals a wide range of functions underlying synaptic transmission. This diversity amplifies the possibilities for differential pathways through the principal neurons and for the number and variety of microcircuit interactions with local inhibitory interneurons. In the olfactory bulb, information is encoded across neuron assemblies that cannot be extracted by averaging only the firing frequency. GABAergic inhibition is therefore important for olfactory coding but within a framework that differs from conventional inhibition rules described for other sensory systems. In addition, odor representation is dynamic and

highly complex, perhaps requiring a unique mechanism of plasticity. The persistence of a high level of inhibitory interneuron turnover within the olfactory bulb and its conservation throughout evolution suggest that adult neurogenesis likely constitutes part of this adaptive mechanism. A combination of both cell addition and cell elimination events controlling the population of GABAergic interneurons may create conditions under which olfactory abilities are up- or downregulated.

Finally, it should be kept in mind that it takes about 2 weeks for a new SVZ neuron to become part of the existing circuit in the olfactory bulb. During this time, the newborn neuron undergoes a series of unique maturational stages as described above. Each of these functional stages could contribute to particular features of bulbar plasticity. Presence of young neurons at different stages of differentiation in the active olfactory bulb may allow for adjustments in the way these cells become integrated (e.g., grow their dendrites, establish synapses, use neurotransmitters, or modify their pattern of gene expression). Because it takes several weeks for new neurons to extend neurites and become synaptically integrated, the bulbar neurogenesis may contribute to long-term adjustment of the olfactory abilities rather than to acute plastic changes. However, other mechanisms may also take advantage of new neurons. For instance, more than half of the new interneurons in the olfactory bulb die within a month after having reached their mature state (Petreanu and Alvarez-Buylla 2002). Selective elimination of neurons may allow for rapid modification of circuitry. Together, the ability to control how new neurons become integrated and to continually eliminate older cells without depleting the neuronal population may bring into neuronal networks a degree of circuit adaptation unmatched by synaptic plasticity alone.

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