

# Olfactory processing in a changing brain

Pierre-Marie Lledo<sup>1,CA</sup> and Gilles Gheusi<sup>1,2</sup>

<sup>1</sup>Laboratory of Perception and Memory, Centre National de la Recherche Scientifique, Unité de Recherche Associée 2182, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris; <sup>2</sup>LEEC, Université Paris 13, Avenue J. B. Clément, 93430 Villetaneuse, France

<sup>CA</sup>Corresponding Author

DOI: 10.1097/01.wnr.0000092070.64779.76

The perception of odorant molecules provides the essential information that allows animals to explore their surrounding. We describe here how the external world of scents may sculpt the activity of the first central relay of the olfactory system, i.e., the olfactory bulb. This structure is one of the few brain areas to continuously replace one of its neuronal populations: the local GABAergic interneurons. How the newly generated neurons integrate into a pre-existing neural network and how basic olfactory functions are maintained when a large percentage of neurons are subjected to continuous renewal, are important questions that have recently received new insights. Furthermore, we shall see how the adult neurogenesis is specifically subjected to experience-dependent modulation. In particular, we shall describe the

sensitivity of the bulbar neurogenesis to the activity level of sensory inputs from the olfactory epithelium and, in turn, how this neurogenesis may adjust the neural network functioning to optimize odor information processing. Finally, we shall discuss the behavioral consequences of the bulbar neurogenesis and how it may be appropriate for the sense of smell. By maintaining a constitutive turnover of bulbar interneurons subjected to modulation by environmental cues, we propose that adult ongoing neurogenesis in the olfactory bulb is associated with improved olfactory memory. These recent findings not only provide new fuel for the molecular and cellular bases of sensory perception but should also shed light onto cellular bases of learning and memory. *NeuroReport* 14:000–000 © 2003 Lippincott Williams & Wilkins.

**Keywords:** GABA; Granule Cells; Memory; Neurogenesis; Odor Discrimination; Synchronization.

## INTRODUCTION

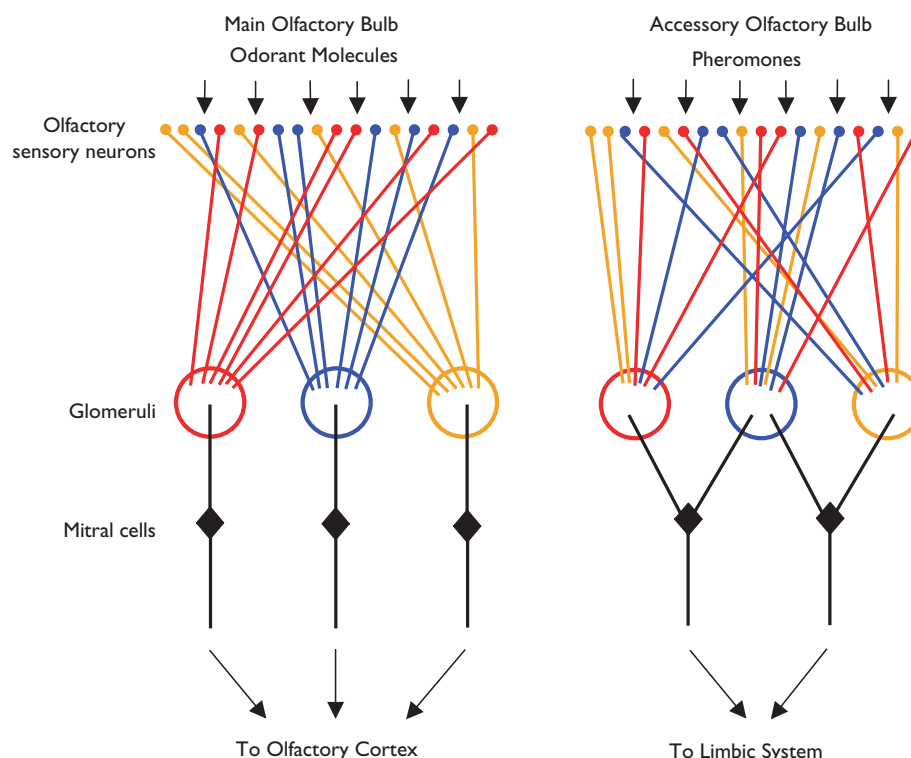
Progress in recent years in the field of sensory function has risen from the combined application of genetic, biochemical, cellular, and neurophysiological methods to understanding how external signals are processed as they move from the periphery to the higher areas of the brain. In the last decade the sensory system that has received the most insight from these research activities is the sense of smell. This recent interest has led several researchers to highlight the parallels in processing of odor information with other sensory modalities [1–4]. However, contrasts rather than similarities should be researched since several characteristics make olfaction unique compared to other sensory systems. First, odorant molecules must be recognized against a background of confounding chemical compounds always present in the natural environment, and this perception is context dependent, varying with the time of the day, the developmental stage or the motivational state [5]. Second, in contrast to other senses, odorant molecules produce immediate recall of the emotional valence and personal experiences related to the source of the smell [6,7]. Third, olfactory memories are of longer duration than memories involving other sensory modalities [8–10]. Finally, the first central relay of the olfactory system, named the main olfactory bulb, is one of the few structures in the mammalian CNS in which there is a continuous supply of newly generated interneurons [11], which make the extreme

stability of olfactory recognition and memory an exciting issue.

All these features allow the mammalian olfactory system to discriminate, memorize and recognize odorant chemicals that bring information from the external world. Odor molecules are processed by two anatomically and functionally distinct sensory organs in mammals, the olfactory epithelium and the vomeronasal organ [12,13]. The former is connected with the main olfactory bulb (referred to below as the olfactory bulb) and is thought to respond to more than a thousand odor molecules (called odorants). In contrast, the latter has traditionally been implicated in the recognition of a smaller number of odorants (called pheromones) that provide information about the social and sexual status of other individuals within the species. The vomeronasal organ projects to the accessory olfactory bulb [14] where convergence of sensory entry is less strict than the olfactory bulb, as similar vomeronasal receptor neurons converge onto multiple neighboring glomeruli (Fig. 1).

Despite the progress made at the early stages of the first contact between odor molecules and receptor proteins [15,16], how the brain 'smells' is still a mystery. To address this question, a better understanding of the cellular process that occur at the first relay of sensory-information processing (i.e. the olfactory bulb) is critical [17–22]. Nevertheless, studying how the mammalian olfactory system processes a large variety of odorant chemicals first requires asking





**Fig. 1.** Principles underlying the initial processing of odor information in olfactory systems. The main olfactory bulb (MOB) and the accessory olfactory bulb (AOB) diverge regarding the synaptic organization of their afferent inputs from the olfactory sensory neurons (OSN) and mitral cell connections with glomeruli. In the MOB (left), OSNs express only one type of olfactory receptor that recognize several odorant molecules and the sensory neurons, while scattered in the olfactory epithelium (colored code), project axons that converge onto few glomeruli. In the AOB (right), OSNs express one type of receptor belonging to one of three families (V1R, V2R or V3R) that recognize specifically one molecule (pheromone). Individual glomeruli receive projections from multiple types of sensory neurons and a mitral cell is always connected to more than one glomerulus.

specific questions about the nature of the coding that underlie information processing.

## INSIGHTS INTO THE VERTEBRATE OLFACTORY SYSTEM

The olfactory system encodes information through various process that take place in anatomically distinct structures. The first one occurs at the interaction between odorant molecules and their respective receptors in sensory neuron dendrites [12,13,20]. Here, odorant molecule receptors can bind a number of compounds with rather moderate affinity despite the overall high sensitivity of the system [23,24]. Since each individual receptor is substantially cross-reactive for different ligands, the receptor repertoire may evolve according to the concentration and the mixture of odorants [25]. Thus, the odor image manifests itself as combinatorial activation of odorant receptors, providing a practically unlimited coding capacity for the olfactory system.

The second important step occurs in the olfactory bulb that receives sensory neuron projections. At this level, the olfactory nerve contacts bulbar output neurons and, at least in mammals, makes the olfactory bulb the major site of integration for the olfactory information. The ten million, or more, olfactory sensory neurons project to discrete spherical modules (i.e. glomeruli) that lie in the superficial region of the olfactory bulb (Fig. 2). It is worth noting that axonal termini of olfactory sensory neurons synapse directly onto

second-order neurons within the forebrain [26,27]. They form glutamatergic synapses impinging onto both output neurons and local interneurons [28].

The topography of the sensory entry into the olfactory bulb has been the subject of extensive studies [29–33]. Odor-specific spatial activation patterns have been reported in mammals by analyzing immediate early gene expression such as *c-fos*, *c-jun*, *zif268* and *Arc* [34–41], 2-deoxyglucose mapping [42–52], and fMRI [53–55]. More recently, optical imaging based on intrinsic signals [56–60], calcium signaling [61,62] or voltage-sensitive dye [63,64] have been successfully applied to map individual glomeruli. These approaches revealed that the spatial pattern of activity in the olfactory bulb was extremely dynamic thus providing a picture of how odor identity and concentration could be represented by a combination of temporal and spatial coding.

## ODOR INFORMATION PROCESSING IN THE OLFACTORY BULB

To address how sensory information is processed, the rationality of the synaptic organization of the first relay in olfactory information processing has to be taken into consideration (Fig. 2). The intrabulbar circuit includes two classes of interneurons that participate in olfactory information processing: periglomerular and granule cells. Briefly, a principal neuron of the olfactory bulb (e.g. mitral or tufted

cell) receives glomerular synaptic input via the distal tuft of a primary (or apical) dendrite extending vertically from its soma. At this level, most of the periglomerular cells (GABAergic or dopaminergic interneurons) have dendrites restricted to one glomerulus and impinge onto olfactory nerve terminals or primary dendrites. The mitral soma also radiates secondary (or basal or lateral) dendrites which extend horizontally up to 1000  $\mu$ m across the external plexiform layer underlying the glomeruli [65,66]. These dendrites form reciprocal dendro-dendritic synapses with the dendrites of the larger population of bulbar interneurons: the granule cells [67–70]. Glutamate released by lateral dendrites of mitral (and tufted) cells, excites the dendrites of granule cells [71–73] which in turn, release GABA directly back onto mitral cells [72–75]. The extensive lateral dendrites of mitral cells and the possible spread of excitation through granule cell dendrites provide a mechanism for lateral inhibition between mitral cells that innervate different glomeruli [72,73,76–79] (Fig. 2).

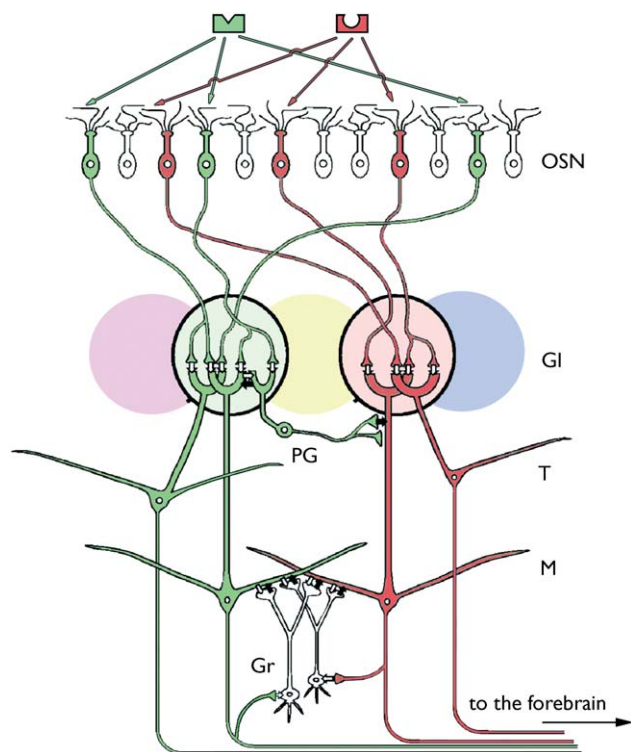
A number of studies have suggested that the lateral inhibition may sharpen odor tuning in a glomerulus as happens in the retina when a light shines on both the center and the surround. For instance, examination of the responses of individual mitral cells to inhalation of aliphatic aldehydes reveals that many individual cells are excited by one subset of these odorants, inhibited by another subset,

and unaffected by yet a third subset. Agents that block reciprocal synapses between mitral and granule cells suppress these inhibitory responses [19]. Thus, at the level of the olfactory bulb, the quality of the odor stimulus is encoded by a specific combination of activated mitral cells that depends on GABAergic inhibition. Finally, because a single granule cell is believed to contact a large number of mitral cells [21], this reciprocal inhibitory synaptic connection also contributes to synchronizing the mitral cell activity [80–82]. As a result, feedback inhibition has been proposed to be crucial for the complex dynamics of olfactory network responses [83,84] and for controlling action potential initiation either at the soma or the primary dendrite [85].

In addition to inhibitory inputs arising from granule cells, it has been reported that mitral cell lateral dendrites also receive large excitatory inputs when either inhibition was antagonized or magnesium was removed from the external medium [86–89]. In mammals, excitatory synapses onto mitral cells have been localized exclusively to the apical dendritic tufts that receive primary sensory afferents [69,70,90,91]. The origin of the excitatory inputs to the mitral cell lateral dendrites was therefore debated. It has been proposed that glutamate released from mitral cells also activates glutamate autoreceptors which can modulate burst firing [87,89,92]. However, it is unknown how the specificity for odor processing can be conserved if glutamate spillover alone governs excitatory transmission within the olfactory bulb. Using a combination of *in vitro* whole-cell recordings and immunogold detection of glutamate, we also demonstrated that ionotropic glutamate receptors on mitral cells could be activated by interneurons located in the granule cell layer [93]. Such feedback excitation would provide an effective mechanism for temporal and spatial coding in olfactory information processing.

Another important feature is the generation of synchronized network oscillations that emerge from activity of different parts of the olfactory pathway [94,95]. 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 gamma frequency band (30–80 Hz) in mammalian brains [96–98]. These fast oscillations that emerge from coordinated electrical activity across large groups of neurons [99] are thought to play an important role in sensory perception, information processing and memory formation [100,101] (recently reviewed in [102,103]). In the olfactory system, the inhalation of odor molecules has been reported to trigger oscillations with different frequency ranges. Whereas  $\gamma$ -waves are induced by odor inputs, the  $\theta$ -frequency band (3–12 Hz) is phase-locked with respiration [94,104–108]. It is remarkable that both rhythms have been observed in the olfactory bulb of mammals as well as in its functional equivalent in invertebrates and are thought to encode stimuli [109] as well as participate in the fine discrimination of close stimuli [110].

How might neural synchronization function in encoding olfactory information? It has been proposed that neural synchronization across glomerular outputs may enhance the representation of a complex olfactory stimulus by integrating the different signal streams activated by the odor into a unified olfactory image at the level of the sensory cortex



**Fig. 2.** The synaptic organization of the main olfactory bulb. The olfactory bulb receives sensory inputs from the olfactory sensory neurons (OSN) located in the olfactory epithelium. Both mitral (M) and tufted (T) cells represent the principal neurons of the olfactory bulb that receive glomerular (GL) inputs. Principal cells form reciprocal dendro-dendritic synapses with two classes of bulbar interneurons: periglomerular neurons (PG) and granule cells (Gr). Modified with permission from [19].

[19,82]. Despite evidence supporting the use of time as a coding dimension in olfaction, much of the current thought on mammalian olfactory coding focuses on the importance of spatial patterns of activity (reviewed in [20,29,111]). However, it seems probable that olfactory information is coded both spatially and in time. The combination of spatial coding and correlated spike activity may, during odor stimulation, synchronize spike responses in the mitral cells associated with glomeruli tuned to the odorant.

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 [112–114]. Theoretical models have proposed that oscillatory synchronization in the mammalian olfactory cortex [115] and the olfactory bulb [21,116–118] depends on fast synaptic inhibitory interactions, but experimental data in support of this were still lacking until very recently.

We have addressed the question of whether a specific bulbar neuronal population generates coherent inhibitory interplay with mitral cells, thereby providing a synaptic substrate to induce gamma frequency oscillations. Using an *in vitro* preparation, we found that olfactory nerve stimulation triggered fast local field potential (LFP) oscillations in the mitral cell body layer (Fig. 3). We took advantage of the segregated distribution of the bulbar interneuron populations to ascertain the identity of the local neurons responsible for generating these rhythms. We found that granule cells mediate synaptic inputs onto secondary dendrites and provide rhythmic feedback that generates gamma frequency oscillations of output neurons in the olfactory bulb. Thus, evidence from both intracellular and neural-ensemble recording studies indicate that the chemical identity of an odor is encoded spatially, according to which glomeruli are activated by the stimulus. Other key features of the stimulus including odor intensity, dynamics and the quality of specific odorant blends, are encoded in specific temporal patterns of activity superimposed on the spatial ensemble.

Two lessons can thus be learned from examination of information processes in the olfactory bulb. First, coding strategies may be much more dynamic than previously assumed. Second, even paleocortical structures such as the olfactory bulb may play an integral part in higher cognitive processes rather than acting simply as relay stations whose sole functions are the improvement of signal-to-noise ratio

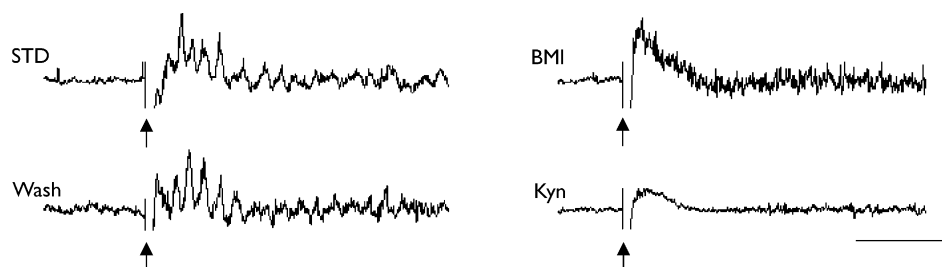
and contrast enhancement. The olfactory bulb neurons that relay activity from the odor receptors to the brain interact extensively with each other, both directly and through a network of coupled inhibitory and excitatory interneurons. As above described, these relay neurons can engage in highly synchronous oscillatory activity, thereby forming assemblies that, as a whole, convey information about a particular odor blend.

## CHALLENGING THE RECRUITMENT OF BULBAR INTERNEURONS

Neurogenesis persists in two regions of the adult mammalian brain: the hippocampus and the olfactory bulb [119–123]. In the hippocampus, several factors including age, genetic background, hormonal and activity levels influence the proliferation, differentiation and survival of progeny of adult stem cells [121,124–126]. In contrast, the molecular mechanisms regulating neurogenesis and the survival of progeny cells in the olfactory bulb have been poorly investigated and the physiological contexts in which such a regulation may occur remain to be settled. Though adult olfactory bulb neurogenesis has been mostly investigated in the rodent, there is evidence suggesting that it may also occur in humans [127–133].

The adult-generated neurons of the olfactory bulb are born in the subventricular zone and migrate along an intricate path of migration to reach their final position [134,135]. To populate the core of the olfactory bulb, the neuroblasts first undergo a tangential chain migration through the rostral migratory stream. Once in the bulb, the precursor cells turn radially away from the migratory stream to invade different layers, where they differentiate into local interneurons [136]. Importantly, most of the bulbar granule cells are generated postnatally and continue to be added in adulthood [120,137].

Bulbar neurogenesis in adulthood implies three different processes that could be independently regulated: (i) cell proliferation, (ii) neuroblast migration from the subventricular zone to the olfactory bulb, and (iii) neuronal differentiation and survival. As recent examples for proliferation, Ephrin molecules were shown to specifically control cell proliferation of neuroblasts in the subventricular zone [138] and mCD24, a glycosylated molecule expressed in the subventricular zone, to negatively regulate cell



**Fig. 3.** Local field potential (LFP) oscillations are reversibly blocked by GABA<sub>A</sub> receptor (bicuculline) and ionotropic glutamate receptors (kynurenate) antagonists. Bath application of bicuculline and kynurenate abolished LFP gamma frequency oscillations triggered by olfactory nerve stimulation (arrow-head). The four traces illustrate LFP recorded in standard medium (STD), in the presence of 20  $\mu$ M bicuculline methiodide (BMI), after 5 minutes of drug washout (Wash), and in the presence of 5 mM kynurenate (Kyn). Application of kynurenate demonstrates that oscillation depend only on olfactory nerve stimulation. Vertical bar = 100 mV; horizontal bar = 100 ms.



proliferation [139]. With respect to migration, two members from the Slit family of soluble proteins are good candidates for guiding neuroblasts towards the olfactory bulb [140,141]. *In vitro* experiments have shown that caudal septum explants secrete a diffusible factor, possibly the molecule Slit [142] that repels olfactory bulb neural precursors [143]. The polysialylated form of neural cell-adhesion molecules (PSA-NCAM) has also been identified as a key factor in assuring efficient migration of neuroblasts in the rostral migratory stream [144–148]. Finally, it has been shown that reelin acts on tangential migrating neuroblasts as a detachment signal [149].

Modulation of the adult neurogenesis may have important consequences for the bulbar activity. Indeed, using NCAM-mutant mice, we found that a reduction in the number of adult-generated granule cells was correlated with impaired odor discrimination, without changing odor detection or memory [150]. This suggests that a critical level of inhibition mediated by the activation of GABA<sub>A</sub> receptors localized on the secondary dendrites of mitral cells is crucial for olfactory processes [151].

Regarding survival of newborn neurons, it has been demonstrated that target structures are the major source for providing attractive and/or survival factors [152,153]. The maturation and survival of subventricular zone-generated neurons is partly under the control of neurotrophic factor (for instance, see [154]). Although the sources of these factors *in vivo* are not precisely known, olfactory bulb-derived factors are likely to influence the proliferation and/or survival of subventricular zone neuroblasts in the adult brain. In mice, neural activity may be important in regulating neuroblast survival since closure of one nostril affects the dynamics of neuronal death in the corresponding olfactory bulb [155]. Nevertheless, it has been reported that subventricular zone cells continue to divide and migrate after transection of the olfactory peduncle [156], following olfactory bulb removal [157] or unilateral nostril occlusion ([158] but see also [159]). This suggests that activity within the olfactory bulb is not essential for proliferation or the directional migration of newly generated interneurons although it is crucial for their survival.

It is widely accepted that the brain can respond to environmental and/or internal challenges inducing significant functional and anatomical modifications. These responses mainly occur at early stages, before the maturation of the developing brain is complete. However, the self-renewing capacity of the olfactory bulb inhibitory neuronal network leaves open the possibility that such a process may never end in the adult olfactory system. In fact, odor experiences have been reported to modulate adult olfactory bulb functions [160,161]. If activity-dependent recruitment of neurons is related to odor exposure, the question, then, is whether change in the number of newborn interneurons might be related to changes in olfactory behavioral function. We have recently reported that an odor-enriched environment enhances the bulbar interneuron population and improves olfactory memory [162]. Since bulbar neurogenesis is sensitive to sensory input, we propose that a production of newborn neurons may adjust the neuronal network activity to optimize the process of odor molecule information in the olfactory bulb.

Additional information is required to know how adult bulbar neurogenesis is regulated by physiological and pharmacological factors. A growing effort has already provided a list of some of the factors known to affect neurogenesis within the adult dentate gyrus. Hormones, neurotransmitters, growth factors and exogenous substances, such as drugs of abuse (opiates, nicotine, methamphetamine and alcohol) have demonstrated an influence on the rate of proliferation and/or survival of neural stem cells within the subgranular zone of the hippocampus. Unfortunately, at present, the regulation of adult bulbar neurogenesis by such treatments has not received the same attention. Several neurotransmitters (as well as drugs influencing their expression and release) may play a substantial role in affecting bulbar neurogenesis since the olfactory bulb is the target of massive cholinergic, serotonergic and catecholaminergic centrifugal projections.

### BEHAVIORAL CONSEQUENCES OF THE BULBAR NEUROGENESIS

Although recent estimates suggest that the quantity of new neurons produced in adulthood is much greater than previously thought, the rate of neuronal production in adulthood still remains lower than during development. As a result, if the adult-generated neurons bring similar functional properties as those brought by neurons generated early in life, then the adult neurogenesis should simply be considered as a neotenic process [163]. In contrast, if adult-generated neurons have unique properties that increase their impact relative to more mature neurons, then their constant integration into a functional circuitry would bring unique features. Recent investigations both in the adult hippocampus and the olfactory bulb support the second inference. Young granule cells in the adult dentate gyrus appear to exhibit robust plasticity that, in contrast to mature granule cells, cannot be inhibited by GABA [164,165]. These newborn neurons may respond preferentially to the modulation of stress hormones [166–168] and learning has been shown to increase the number of new neurons in the hippocampus [169]. Exercise increases both the number of new dentate gyrus cells and performance on a hippocampal-dependent task [170] whereas decreasing the number of new granule neurons was correlated with impairment on such a task [171]. Since new neurons are structurally plastic, they may be highly susceptible to changes according to different life experiences.

### THE OLFACTORY BULB: A LOCUS FOR MEMORY?

The olfactory bulb has been implicated in several types of olfactory learning and memory [172]. One theory proposes that the olfactory bulb plays a transient role in memory storage [173,174]. Support for the idea of temporary storage comes from electrophysiological studies [175,176]. The naturally occurring replacement of bulbar interneurons provides a rationale for the transfer of memories out of the olfactory bulb [174]. The loss of interneurons may be programmed to occur after the transfer of the memories held by these neurons to other parts of the brain. Alternatively, if the olfactory bulb is necessary for the

temporary processing of information that is sent elsewhere for storage, then a rejuvenating population of neurons capable of rapidly forming synaptic connections may be well suited to participate in such a function. It will be interesting to determine how learning, which leads to changes in the meaning of an odor, correlates with variation in the adult-generated interneuron population. In line with this, it is of interest to examine the electrophysiological properties of the newly generated neurons to know whether these neurons possess unusual characteristics supporting novel functions [177].

### ARE NEWBORN NEURONS FUNCTIONAL?

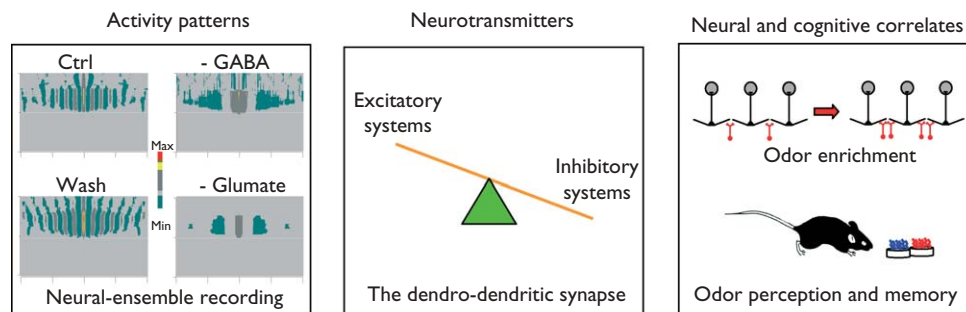
To date there are two lines of evidence showing that newly generated neurons which populate the adult olfactory bulb are functional. Huang and Bittman [178] reported that in male hamsters adult newborn cells localized in the accessory olfactory bulb and in the main olfactory bulb express *c-fos* in response to different stimuli, especially socio-sexual cues. In the olfactory bulb of estrous females, vaginal secretion as well as exposure to a male, elicited *c-fos* expression in BrdU-labeled cells and the higher percentage of immunoreactive *c-fos* newborn cells were localized into the mitral and periglomerular layers. Similarly, Carlén *et al.* [179] recently showed that neurons generated within the subventricular zone not only synapse into the olfactory bulb, establishing connections within the glomerular and granule cell layers, but also respond to a cocktail of odors. Furthermore, it has also been shown that new neurons generated in the dentate gyrus of the adult hippocampus displayed similar electrophysiological properties as mature dentate granule cells [180]. Altogether, these results demonstrate that newly generated neurons integrate functionally into the adult brain. Although showing that newborn neurons are functional is a critical step this does not indicate why and when adult neurogenesis has fundamental consequences for the animal.

### THE ADULT NEUROGENESIS AS AN ADAPTATIVE FUNCTION

It still remains necessary to assess adult bulbar neurogenesis in different ethologically relevant contexts. Because the

main events occurring during olfactory learning seem to occur at the mitral cell–granule cell synapses, this stresses the importance of investigating the plausible contribution of the permanent supply of interneurons to memory. It has been shown that a general physiological mechanism seems to operate within the olfactory bulb during olfactory learning in different contexts and different species [181]. Among the different neural modifications, change in the ratio of excitatory to inhibitory neurotransmitters has been extensively documented in the olfactory bulb as well as in the accessory olfactory bulb (memory of the mating male's pheromone, olfactory learning in neonatal rats, olfactory conditioning in adult mice; Fig. 4. Again, these neural changes emphasized the critical contribution of inhibition provided by granule cells. Because most newly generated neurons born in the subventricular zone differentiate into local interneurons, it is tempting to speculate that adult bulbar neurogenesis might contribute to regulate distinct olfactory processing [150,182].

Only very recent studies have brought new insights about the possibility of changes in bulbar neurogenesis following ethologically relevant events in rodents. Prairie voles are monogamous, and pair bond formation between partners occurs following mating. Since social affiliation in prairie voles involves olfactory learning (mate recognition), it is appealing to consider that an increase in the permanent supply of newly generated neurons within the olfactory bulb could partly support social attachment. Female prairie voles exposed to a male behind a wire mesh for 48 h show an increase of newly generated neurons in the subventricular zone and in the rostral migratory stream [183]. Because ovariectomized female prairie voles exposed to a male do not show an increase in cellular proliferation and estrogen treatment partly reinstates such a process, it seems likely that estrogen mediate the increase of neuronal proliferation following male exposure in this species. Since there is a gradual development of neuroblasts into mature neurons and because BrdU-labeled cells have been assessed in this study within the first 2 days following male exposure, it remains to define whether the increase in the newly generated neurons persists several weeks later. This was examined in a subsequent study in which the authors analyzed the survival rate of newborn neurons in female prairie voles housed with a male for 2 days or 3 weeks [184].



**Fig. 4.** Inhibition and odor information processing. Three main neural correlates that emphasize the contribution of interneurons and their permanent supply in the main olfactory bulb, at different levels during olfactory processing. Left, a critical level of inhibition mediated by GABA receptor activity, through the reciprocal synapse, has been shown to be necessary for odor coding at this first central relay. Middle, among different neural changes that occur during olfactory learning, changes in the ratio of excitatory to inhibitory neurotransmitters has been largely reported. Right, increasing the number of newly formed neurons in the adult olfactory bulb following enriched-odor exposure is associated with improved olfactory memory.

In contrast to previous experiment [183], they did not find any significant increase of newly generated neurons in females mated for 48 h with a male (a period sufficient to induce social attachment). Similarly, following 3 weeks of cohabitation, gestation, and 3 days of lactation, female prairie voles do not display significant change in the number of BrdU-labeled cells within the olfactory bulb [184]. The number of BrdU-positive cells increases both in the amygdala and the hypothalamus of the females but no quantification of double-labeled cells was reported and their origin remains unknown. Since in this study females have undergone mating, pregnancy and parturition, the contribution of different hormones other than estrogen [185], and the stimulation elicited by the pups during the first days postpartum cannot be ruled out in the regulation of neurogenesis.

Whatever the functional consequences of adult neurogenesis are, it is important to note that the functional benefit from bulbar neurogenesis should not be considered to be acute since it takes several weeks to generate a functionally integrated new interneuron. Thus, neurogenesis should rather be considered as a long-term adjustment of the olfactory bulb circuit to an experienced level of higher complexity governed by the sense of smell. Although there is to date no clear argument for the involvement of bulbar neurogenesis in the adult, social attachment constitutes a valuable context in which morphological and physiological events related to synaptic integration of new neurons should be more precisely examined. For instance, the selective maternal care of sheep toward their own lamb represents a suitable model to investigate the functional consequences of the adult neurogenesis. Once the bond between an ewe and its lamb has been established, infusion of an antagonist of GABA<sub>A</sub> receptors, in the olfactory bulb, prevents recognition of the lamb [186]. One can hypothesize that ablation of newborn neurons by injecting an antimitotic drug within the subventricular zone [171], after the bond has been established, should also impair the lamb recognition. In line with this, the neurobiology of attachment in ewes has largely progressed in identifying the role of different neurochemical systems. Investigating the contribution of bulbar neurogenesis in this attachment will also require specifying the role of neurotransmitters in regulating neurogenesis.

Even in non-selective mothers such as female rats, there is a clear behavioral shift associated with late pregnancy and delivery. At this time, female rats become progressively more attentive towards pups and initiate nurturing responses to them. As initiated by Weiss and colleagues [185], more studies are required to explore the adaptive consequences of adult bulbar neurogenesis across different life stages (e.g. weaning, mate choice, parental behavior, social status, aging).

We recently investigated the effects of an odor-enriched environment on the regulation of bulbar neurogenesis in adult male mice [162]. According to a probabilistic epigenesis of behavior [187], we assumed that bulbar activity may have an inductive, facilitative and/or maintenance function in the neurogenesis of interneurons which in turn may affect behavioral functions. In this respect, adult mice were daily exposure for 24 h to different aromatic fragrances for 40 days. No significant differences were found in the pro-

liferative activity of progenitor cells located in the subventricular zone but their survival in the olfactory bulb was dramatically increased. In parallel, behavioral analyses revealed that when submitted to different olfactory tasks odor-enriched mice displayed a longer and stronger (i.e. resistant to retroactive interference) short-term olfactory memory. Although there is no proof of causality, these results strongly support a correlation between the up-regulating effects of odor-enrichment on adult bulbar neurogenesis and the improvement of olfactory memory (Fig. 4).

As in the vision system, previous studies dedicated to investigating olfactory processing have postulated the convergence of information from deconstructed patterns in the olfactory bulb to 'cardinal cell assemblies' located at the top of a hierarchical perceptual system in higher brain centers. However, this notion of grandmother cells [188] has been revisited today by temporal coding of neural assemblies [189]. According to this latter view, oscillations and synchrony are attributes as relevant to neural identity and odor quality as the location of activated neurons. For this spatio-temporal coding, bulbar interneurons are known to be the key players since they extensively shape relay neuron responses to odor and they maximize differences in odor representations. The size modulation of their population by adult neurogenesis offers a unique way for the olfactory bulb circuit to optimize olfactory information processing in a changing environment.

## REFERENCES

- DeVries SH and Baylor DA. *Cell* **72**, 139–149 (1993).
- Shepherd GM. *Neuron* **13**, 771–790 (1994).
- Reed R and Singer W. *Curr Opin Neurobiol* **5**, 429–433 (1995).
- Torre V, Ashmore JF, Lamb TD and Menini A. *J Neurosci* **15**, 7757–7768 (1995).
- Laurent G, Stopfer M, Friedrich RW *et al.* *Annu Rev Neurosci* **24**, 263–297 (2001).
- Otto T and Giardino ND. *Ann NY Acad Sci* **933**, 291–309 (2001).
- Wallace KJ and Rosen JB. *J Neurosci* **21**, 3619–3627 (2001).
- Koster EP, Degel J and Piper D. *Chem Senses* **27**, 191–206 (2002).
- Herz RS and Schooler JW. *Am J Psychol* **115**, 21–32 (2002).
- Dade LA, Zatorre RJ and Jones-Gotman M. *Brain* **125**, 86–101 (2002).
- Alvarez-Buylla A and Garcia-Verdugo JM. *J Neurosci* **22**, 629–634 (2002).
- Buck LB. *Cell* **100**, 611–618 (2000).
- Firestein S. *Nature* **413**, 211–218 (2001).
- Keverne EB. *Science* **286**, 716–720 (1999).
- Buck LB and Axel R. *Cell* **65**, 175–187 (1991).
- Buck LB. *Annu Rev Neurosci* **19**, 517–544 (1996).
- Sullivan SL and Dryer L. *J Neurobiol* **30**, 20–36 (1996).
- Shipley MT and Ennis M. *J Neurobiol* **30**, 123–176 (1996).
- Mori K, Nagao H and Yoshihara Y. *Science* **286**, 711–715 (1999).
- Mombaerts P. *Nature Neurosci* **4**, 1192–1198 (2001).
- Shepherd GM and Greer CA. Olfactory bulb. In: Shepherd GM, (ed). *The Synaptic Organization of the Brain*. New-York: Oxford University Press; 1998, pp. 159–203.
- Ebrahimi FA and Chess A. *Curr Opin Neurobiol* **8**, 453–457 (1998).
- Kauer JS. *Nature* **417**, 336–342 (2002).
- Sullivan S. *Neuroreport* **13**, 9–17 (2002).
- Duchamp-Viret P, Chaput M and Duchamp A. *Science* **284**, 2171–2174 (1999).
- Moulton DG. *Ann NY Acad Sci* **237**, 52–61 (1974).
- Costanzo RM and Morrison EE. *J Neurocytol* **18**, 381–391 (1989).
- Ennis M, Zimmer LA and Shipley MT. *Neuroreport* **7**, 989–992 (1996).
- Xu F, Greer CA and Shepherd GM. *J Comp Neurol* **422**, 489–495 (2000).



30. Treloar HB, Feinstein P, Mombaerts P and Greer CA. *J Neurosci* **22**, 2469–2477 (2002).
31. Mombaerts P, Wang F, Dulac C *et al.* *Cell* **87**, 675–686 (1996).
32. Wang F, Nemes A, Mendelsohn M and Axel R. *Cell* **93**, 47–60 (1998).
33. Mombaerts P. *Annu Rev Neurosci* **22**, 487–509 (1999).
34. Guthrie KM, Anderson AJ, Leon M and Gall C. *Proc Natl Acad Sci USA* **90**, 3329–3333 (1993).
35. Guthrie K, Rayhanabad J, Kuhl D and Gall C. *Neuroreport* **11**, 1809–1813 (2000).
36. Guthrie KM and Gall CM. *Chem Senses* **20**, 271–282 (1995).
37. Guthrie KM and Gall CM. *Neuroreport* **6**, 2145–2149 (1995).
38. Johnson BA, Woo CC, Duong H, Nguyen V and Leon M. *Brain Res* **699**, 192–200 (1995).
39. Baba K, Ikeda M, Houtani T *et al.* *Brain Res* **774**, 142–148 (1997).
40. Schaefer ML, Young DA and Restrepo D. *J Neurosci* **21**, 2481–2487 (2001).
41. Inaki K, Takahashi YK, Nagayama S and Mori K. *Eur J Neurosci* **15**, 1563–1574 (2002).
42. Skeen LC. *Brain Res* **124**, 147–153 (1977).
43. Stewart WB, Kauer JS and Shepherd GM. *J Comp Neurol* **185**, 715–734 (1979).
44. Jourdan F, Duveau A, Astic L and Holley A. *Brain Res* **188**, 139–154 (1980).
45. Astic L and Saucier D. *Brain Res* **254**, 243–256 (1982).
46. Coopersmith R and Leon M. *Science* **225**, 849–851 (1984).
47. Royet JP, Sicard G, Souchier C and Jourdan F. *Brain Res* **417**, 1–11 (1987).
48. Sicard G, Royet JP and Jourdan F. *Brain Res* **481**, 325–334 (1989).
49. Johnson BA, Woo CC and Leon M. *J Comp Neurol* **393**, 457–471 (1998).
50. Johnson BA, Woo CC, Hingco EE *et al.* *J Comp Neurol* **409**, 529–548 (1999).
51. Johnson BA and Leon M. *J Comp Neurol* **422**, 496–509 (2000).
52. Johnson BA and Leon M. *J Comp Neurol* **426**, 330–338 (2000).
53. Sobel N, Prabhakaran V, Desmond JE *et al.* *J Neurosci Methods* **78**, 115–123 (1997).
54. Yang X, Renken R, Hyder F *et al.* *Proc Natl Acad Sci USA* **95**, 7715–7720 (1998).
55. Xu F, Kida I, Hyder F *et al.* *Proc Natl Acad Sci USA* **97**, 10601–10606 (2000).
56. Rubin BD and Katz LC. *Neuron* **23**, 499–511 (1999).
57. Uchida N, Takahashi YK, Tanifuji M and Mori K. *Nature Neurosci* **3**, 1035–1043 (2000).
58. Luo M and Katz LC. *Neuron* **32**, 1165–1179 (2001).
59. Belluscio L and Katz LC. *J Neurosci* **21**, 2113–2122 (2001).
60. Meister M and Bonhoeffer T. *J Neurosci* **21**, 1351–1360 (2001).
61. Bozza T and Kauer JS. *J Neurosci* **18**, 4560–4569 (1998).
62. Wachowiak M and Cohen LB. *Neuron* **32**, 723–735 (2001).
63. Keller A, Yagodin S, Aroniadou-Anderjaska V *et al.* *J Neurosci* **18**, 2602–2612 (1998).
64. Spors H and Grinvald A. *Neuron* **34**, 301–315 (2002).
65. Mori K, Kishi K and Ojima H. *J Comp Neurol* **219**, 339–355 (1983).
66. Orona E, Rainer EC and Scott JW. *J Comp Neurol* **226**, 346–356 (1984).
67. Rall W, Shepherd GW, Reese TS and Brightman MW. *Exp Neurol* **14**, 44–56 (1966).
68. Price JL and Powell TPS. *J Cell Sci* **7**, 91–123 (1970).
69. Pinching AJ and Powell TPS. *J Cell Sci* **9**, 347–377 (1971).
70. Shepherd GM. *Physiol Rev* **52**, 864–917 (1972).
71. Trombley P and Shepherd GM. *Curr Opin Neurobiol* **3**, 540–547 (1993).
72. Schoppa NE, Kinzie JM, Sahara Y *et al.* *J Neurosci* **18**, 6790–6802 (1998).
73. Isaacson JS and Strowbridge BW. *Neuron* **20**, 749–761 (1998).
74. Nowycky MC, Mori K and Shepherd GM. *J Neurophysiol* **46**, 639–648 (1981).
75. Jahr CE and Nicoll RA. *J Physiol* **326**, 213–234 (1982).
76. Scott JW, Wellis DP, Riggott MJ and Buonviso N. *Microsc Res Tech* **24**, 142–156 (1993).
77. Yokoi M, Mori K and Nakanishi S. *Proc Natl Acad Sci USA* **92**, 3371–3375 (1995).
78. Schoppa NE and Westbrook GL. *Nature Neurosci* **2**, 1106–1113 (1999).
79. Margrie TW, Sakmann B and Urban NN. *Proc Natl Acad Sci USA* **98**, 319–324 (2001).
80. Buonviso N, Chaput MA and Berthommier F. *J Neurophysiol* **68**, 417–424 (1992).
81. Desmaisons D, Vincent JD and Lledo PM. *J Neurosci* **19**, 10727–10737 (1999).
82. Kashiwadani H, Sasaki YF, Uchida N and Mori K. *J Neurophysiol* **82**, 1786–1792 (1999).
83. Wehr M and Laurent G. *Nature* **384**, 162–166 (1996).
84. Laurent G. *Science* **286**, 723–728 (1999).
85. Chen WR, Midtgard J and Shepherd GM. *Science* **278**, 463–467 (1997).
86. Nicoll RA and Jahr CE. *Nature* **296**, 441–444 (1982).
87. Isaacson JS. *Neuron* **23**, 377–384 (1999).
88. Aroniadou-Anderjaska V, Ennis M and Shipley MT. *J Neurophysiol* **82**, 489–494 (1999).
89. Friedman D and Strowbridge BW. *J Neurophysiol* **84**, 39–50 (2000).
90. White EL. *Brain Res* **37**, 69–80 (1972).
91. Kosaka K, Toida K, Margolis FL and Kosaka T. *Neurosci* **76**, 775–786 (1997).
92. Salin PA, Lledo PM, Vincent JD and Charpak S. *J Neurophysiol* **85**, 1275–1282 (2001).
93. Didier A, Carleton A, Bjaalie JG *et al.* *Proc Natl Acad Sci USA* **98**, 6441–6446 (2001).
94. Adrian ED. *Electroencephalogr Clin Neurophysiol* **2**, 377–388 (1950).
95. Freeman J. *Electroencephalogr Clin Neurophysiol* **44**, 586–605 (1978).
96. Singer W and Gray CM. *Annu Rev Neurosci* **18**, 555–586 (1995).
97. Traub RD, Spruston N, Soltesz I *et al.* *Prog Neurobiol* **55**, 563–575 (1998).
98. Engel AK, Fries P and Singer W. *Nature Rev Neurosci* **2**, 704–716 (2001).
99. Gray CM. *J Comput Neurosci* **1**, 11–38 (1994).
100. Gray CM and Singer W. *Proc Natl Acad Sci USA* **86**, 1698–1702 (1989).
101. Lisman JE and Idiart MA. *Science* **267**, 1512–1515 (1995).
102. Gilbert CD, Sigman M and Crist RE. *Neuron* **31**, 681–697 (2001).
103. Varela F, Lachaux JP, Rodriguez E and Martinerie J. *Nat Rev Neurosci* **2**, 229–239 (2001).
104. Bressler SL and Freeman WJ. *Electroencephalogr Clin Neurophysiol* **50**, 19–24 (1980).
105. Freeman WJ and Schneider W. *Psychophysiol* **19**, 44–56 (1982).
106. Bressler SL. *Electroencephalogr Clin Neurophysiol* **57**, 270–276 (1984).
107. Eeckman FH and Freeman WJ. *Brain Res* **528**, 238–244 (1990).
108. Kay LM and Freeman WJ. *Behav Neurosci* **112**, 541–553 (1998).
109. Laurent G, Stopfer M, Friedrich RW *et al.* *Annu Rev Neurosci* **24**, 263–297 (2001).
110. Stopfer M, Bhagavan S, Smith BH and Laurent G. *Nature* **390**, 70–74 (1997).
111. Kauer JS and White J. *Annu Rev Neurosci* **24**, 963–979 (2001).
112. von Krosigk M, Bal T and McCormick DA. *Science* **261**, 361–364 (1993).
113. Cobb SR, Buhl EH, Halasy K *et al.* *Nature* **378**, 75–78 (1995).
114. Whittington MA, Traub RD and Jefferys JGR. *Nature* **373**, 612–615 (1995).
115. Wilson M and Bower JM. *J Neurophysiol* **67**, 981–995 (1992).
116. Rall W and Shepherd GM. *J Neurophysiol* **31**, 884–915 (1968).
117. Freeman WJ. In: *Mass Action in the Nervous System*. New York: Academic Press; 1975.
118. Mori K and Takagi SF. Inhibition in the olfactory bulb: dendrodendritic interaction and their relation to the induced waves. In: Katsuki K, Sato M, Takagi SF and Oomura Y (eds). *Food Intake and Chemical Senses*. Tokyo: University of Tokyo Press; 1977, pp. 33–43.
119. Altman J and Das GD. *J Comp Neurol* **124**, 319–335 (1965).
120. Kaplan MS and Hinds JW. *Science* **197**, 1092–1094 (1977).
121. Gage FH. *Science* **287**, 1433–1443 (2000).
122. Gross CG. *Nature Rev Neurosci* **1**, 67–73 (2000).
123. Temple S. *Nature* **414**, 112–117 (2001).
124. van der Kooy D and Weiss S. *Science* **287**, 1439–1441 (2000).
125. Magavi SS and Macklis JD. *Neuropsychopharmacology* **25**, 816–835 (2001).
126. Gould E and Gross CG. *J Neurosci* **22**, 619–623 (2002).
127. Kirschenbaum B, Nedergaard M, Preuss A *et al.* *Cereb Cortex* **4**, 576–589 (1994).
128. Pincus DW, Harrison-Restelli C, Barry J *et al.* *Clin Neurosurg* **44**, 17–25 (1997).
129. Bernier PJ, Vinet J, Cossette M and Parent A. *Neurosci Res* **37**, 67–78 (2000).
130. Doetsch F and Scharff C. *Brain Behav Evol* **58**, 306–322 (2001).
131. Garcia-Verdugo JM, Ferron S, Flames N *et al.* *Brain Res Bull* **57**, 765–775 (2002).
132. Lim DA, Flames N, Collado L and Herrera DG. *Brain Res Bull* **57**, 759–764 (2002).
133. Rakic P. *Nature Rev Neurosci* **3**, 65–71 (2002).
134. Luskin MB. *Neuron* **11**, 173–189 (1993).



135. Lois C and Alvarez-Buylla A. *Proc Natl Acad Sci USA* **90**, 2074–2077 (1993).
136. Alvarez-Buylla A, Garcia-Verdugo JM. *J Neurosci* **22**, 629–634 (2002).
137. Bayer SA. *Exp Brain Res* **50**, 329–340 (1983).
138. Conover JC, Doetsch F, Garcia-Verdugo JM *et al.* *Nature Neurosci* **3**, 1091–1097 (2000).
139. Belvindrah R, Rougon G and Chazal G. *J Neurosci* **22**, 3594–607 (2002).
140. Hu H. *Neuron* **23**, 703–711 (1999).
141. Wu W, Wong K, Chen J *et al.* *Nature* **400**, 331–336 (1999).
142. Mason HA, Ito S and Corfas G. *J Neurosci* **21**, 7654–7663 (2001).
143. Hu H and Rutishauser U. *Neuron* **16**, 933–940 (1996).
144. Bonfanti L and Theodosis DT. *Neuroscience* **62**, 291–305 (1994).
145. Rousselot P, Lois C and Alvarez-Buylla A. *J Comp Neurol* **351**, 51–61 (1995).
146. Doetsch F and Alvarez-Buylla A. *Proc Natl Acad Sci USA* **93**, 14895–14900 (1996).
147. Lois C, Garcia-Verdugo JM and Alvarez-Buylla A. *Science* **271**, 978–981 (1996).
148. Chazal G, Durbec P, Jankovski A, Rougon G and Cremer H. *J Neurosci* **20**, 1446–1457 (2000).
149. Hack I, Bancila M, Loulier K, Carroll P and Cremer H. *Nature Neurosci* **5**, 939–945 (2002).
150. Gheusi G, Cremer H, McLean H *et al.* *Proc Natl Acad Sci USA* **97**, 1823–1828 (2000).
151. Nusser Z, Kay LM, Laurent G *et al.* *J Neurophysiol* **86**, 2823–2833 (2001).
152. Kennedy TE and Tessier-Lavigne M. *Curr Opin Neurobiol* **5**, 83–90 (1995).
153. Svendsen CN and Sofroniew MV. *Perspect Dev Neurobiol* **3**, 133–142 (1996).
154. Kirschenbaum B and Goldman SA. *Proc Natl Acad Sci USA* **92**, 210–214 (1995).
155. Murray RC and Calof AL. *Semin Cell Dev Biol* **10**, 421–431 (1999).
156. Jankovski A, Garcia C, Soriano E and Sotelo C. *Eur J Neurosci* **10**, 3853–3868 (1998).
157. Kirschenbaum B, Doetsch F, Lois C and Alvarez-Buylla. *J Neurosci* **19**, 2171–2180 (1999).
158. Frazier-Cierpial L and Brunjes PC. *J Comp Neurol* **289**, 481–492 (1989).
159. Corotto FS, Henegar JR and Maruniak JA. *Neuroscience* **61**, 739–744 (1994).
160. Rabin MD. *Percept Psychophys* **44**, 532–540 (1988).
161. Woo CC and Leon M. *J Comp Neurol* **360**, 634–642 (1995).
162. Rochefort C, Gheusi G, Vincent JD and Lledo PM. *J Neurosci* **22**, 2679–2689 (2002).
163. Nottebohm F. *J Neurosci* **22**, 624–628 (2002).
164. Wang S, Scott BW and Wojtowicz JM. *J Neurobiol* **42**, 248–257 (2000).
165. Snyder JS, Kee N and Wojtowicz JM. *J Neurophysiol* **85**, 2423–2431 (2001).
166. Herman JP, Schafer MK, Young EA *et al.* *J Neurosci* **9**, 3072–3082 (1989).
167. Gould E and Tanapat P. *Biol Psychiatry* **46**, 1472–1479 (1999).
168. Lemaire V, Koehl M, LeMoal M and Abrous DN. *Proc Natl Acad Sci USA* **97**, 11032–11037 (2000).
169. Gould E, Beylin A, Tanapat P, Reeves A and Shors TJ. *Nature Neurosci* **2**, 260–265 (1999).
170. van Praag H, Christie BR, Sejnowski TJ and Gage FH. *Proc Natl Acad Sci USA* **96**, 13427–13431 (1999).
171. Shors TJ, Miesegaes G, Beylin A *et al.* *Nature* **410**, 372–376 (2001).
172. Brennan PA and Keverne EB. *Prog Neurobiol* **51**, 457–481 (1997).
173. Freeman WJ and Skarda CA. *Brain Res Rev* **10**, 147–175 (1985).
174. Lynch G and Granger R. In: *olfaction*, Eds Davis JL and Eichenbaum H (MIT Press, MA), pp. 141–165 (1991).
175. Gray C, Freeman WJ and Skinner JE. *Behav Neurosci* **100**, 585–596 (1986).
176. Gray C and Skinner JE. *Exp Brain Res* **73**, 189–197 (1988).
177. Carleton A, Petreanu LT, Lansford R, Alvarez-Buylla A and Lledo PM. *Nature Neurosci* **6**, X–X (2003).
178. Huang L and Bittman EL. *Hormones Behav* **41**, 343–350 (2002).
179. arlén M, Cassidy RM, Brismar H *et al.* *Curr Biol* **12**, 606–608 (2002).
180. van Praag AF, Schinder AF, Christie BR *et al.* *Nature* **415**, 1030–1034 (2002).
181. Brennan PA and Keverne EB. *Neuroscience* **51**, 457–481 (1997).
182. Cecchi GA, Petreanu LT, Alvarez-Buylla A and Magnasco MO. *J Comp Neurol* **11**, 175–182 (2001).
183. Smith MT, Pencea V, Wang Z *et al.* *Horm Behav* **39**, 11–21 (2001).
184. Fowler CD, Liu Y, Ouimet C and Wang Z. *J Neurobiol* **51**, 115–128 (2002).
185. Shingo T, Gregg C, Enwere E *et al.* *Science* **299**, 117–120 (2003).
186. Kendrick KM. *Behav Proc* **33**, 89–112 (1994).
187. Gottlieb G. *Synthesizing Nature–Nurture*. Prenatal Roots of Instinctive Behavior. Mahwah, NJ: Lawrence Erlbaum Associates; 1997.
188. Barlow HB. *Perception* **1**, 371–394 (1972).
189. Perez-Orive J, Mazor O, Turner GC *et al.* *Science* **297**, 359–365 (2002).

**Acknowledgements:** This work was supported by the Pasteur Institute, the Annette Gruner-Schlumberger Foundation, the CNRS, the Fondation pour la Recherche Médicale, and a grant from the French Ministry of Research and Education (ACI Biologie du Développement et Physiologie Intégrative 2000). We thank Kathleen McBride for carefully reading this manuscript.

AUTHOR QUERY FORM

**LIPPINCOTT  
WILLIAMS AND WILKINS**

**JOURNAL NAME**

**WNR**

**8/21/03**

**ARTICLE NO:**

**601**

**QUERIES AND / OR REMARKS**

<b>Query No</b>	<b>Details Required</b>	<b>Authors Response</b>
	No queries	