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Understanding the Regulation and Function of Adult Neurogenesis: Contribution from an Insect Model, the House Cricket

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Abstract

Since the discovery of adult neurogenesis, a major issue is the role of newborn neurons and the function-dependent regulation of adult neurogenesis. We decided to use an animal model with a relatively simple brain to address these questions. In the adult cricket brain as in mammals, new neurons are produced throughout life. This neurogenesis occurs in the main integrative centers of the insect brain, the mushroom bodies (MBs), where the neuroblasts responsible for their formation persist after the imaginal molt. The rate of production of new neurons is controlled not only by internal cues such as morphogenetic hormones but also by external environmental cues. Adult crickets reared in an enriched sensory environment experienced an increase in neuroblast proliferation as compared with crickets reared in an impoverished environment. In addition, unilateral sensory deprivation led to reduced neurogenesis in the MB ipsilateral to the lesion. In search of a functional role for the new cells, we specifically ablated MB neuroblasts in young adults using brain-focused gamma ray irradiation. We developed a learning paradigm adapted to the cricket, which we call the "escape paradigm." Using this operant associative learning test, we showed that crickets lacking neurogenesis exhibited delayed learning and reduced memory retention of the task when olfactory cues were used. Our results suggest that environmental cues are able to influence adult neurogenesis and that, in turn, newly generated neurons participate in olfactory integration, optimizing learning abilities of the animal, and thus its adaptation to its environment. Nevertheless, odor learning in adult insects cannot always be attributed to newly born neurons because neurogenesis is completed earlier in development in many insect species. In addition, many of the irradiated crickets performed significantly better than chance on the operant learning task.

Key words: *Acheta domesticus*, insect, learning and memory, mushroom body, neurogenesis, olfaction

Introduction

Neurogenesis, that is, the production of new neurons (including progenitor cell proliferation, neuronal differentiation, and newborn neuron survival), represents a fundamental process to allow brain morphogenesis during development, but its persistence in adulthood had been for a long time a much debated question. Although brain plasticity was recognized as a necessity for an animal's adaptation to and survival in a constantly changing environment, the dogma of the fixity of the adult central nervous system largely prevailed among neuroscientists, and neuronal plasticity in the adult brain was considered to be limited to dendritic and synaptic remodeling. However, the development of new labeling methods in the 1980s and 1990s definitively overturned this dogma (for review, see Gross 2000). It is now clearly established that new neurons are generated throughout life in the brain of many different animal species including insects (Cayre et al. 1994), crustaceans (Schmidt 1997; Harzsch et al. 1999; Beltz and Sandeman 2003; see Schmidt 2007), fishes (Zupanc and Horschke 1995), amphibians (Chetverukhin and Polenov 1993), reptiles (Perez-Canellas and Garcia-Verdugo 1996), birds (Goldman and Nottebohm 1983), and mammals (Kaplan and Hinds 1977; Ming and Song 2005; see Gheusi and Lledo 2007) including humans (Eriksson et al. 1998; Curtis et al. 2007). The conservation of this process during evolution argues for a biological significance of adult neurogenesis. Interestingly, only very specific and specialized structures undergo neurogenesis in the adult brain. In mammals, mainly 2 structures are concerned: the subventricular zone (SVZ) giving rise to new olfactory interneurons (Corotto et al. 1993; Lois and Alvarez-Buylla 1994) and the dentate gyrus of the hippocampus (Bayer et al. 1982). These structures play a role in sensory integration, learning, and memory (Vianna et al.

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2000; Gheusi and Rochefort 2002; Gheusi and Lledo 2007). Invertebrates also display such characteristic of a neuronal production restrained to some structures in adulthood. In crustaceans, 2 populations of interneurons innervating the olfactory lobe and the accessory lobe (a second-order multimodal structure) undergo adult neurogenesis (Beltz and Sandeman 2003; Sullivan et al. 2006; Schmidt 2007). In adult insects, neurogenesis occurs in the mushroom bodies (MBs), the main multimodal integrative center of the insect brain, often considered as the ''intelligence center'' (Dujardin 1850; Cayre et al. 1994). Considering the common features of these different structures, the hypothesis that adult-born neurons may play specific roles in olfactory information processing and in learning and memory appears reasonable. Indeed, a number of studies demonstrate strong correlations between neurogenesis and learning abilities. In birds, seasonal variations in the incorporation of new neurons in the high vocal center (HVC) are temporally correlated to song learning (Kirn et al. 1994). Moreover, the deterioration of learned song induced by the targeted cell death of the replaceable population that controls song production in the HVC is followed by the restoration of neuronal numbers and song recovery, suggesting that the compensatory neuronal replacement can restore a learned behavior (Scharff et al. 2000). In rodents, factors identified as increasing the production rate of neurons in the dentate gyrus, such as environmental enrichment, voluntary exercise, or estrogens, also improve learning performances of the animals (Kempermann et al. 1997; Tanapat et al. 1999; van Praag et al. 1999). Conversely, stress, aging, and glucocorticoids that negatively regulate adult neurogenesis also reduce learning abilities (Bodnoff et al. 1995; Krugers et al. 1997). Similarly, in the olfactory bulb, the reduction of new interneuron integration is correlated with deficits in odor discrimination (Gheusi et al. 2000), whereas exposure to odor enrichment leads to improved olfactory memory (Rochefort et al. 2002). However, in spite of all these correlative data, strong evidence of causal links between newborn neurons and learning and memory performances is still lacking, and the question of the functional significance of adult neurogenesis remains open.

In this context, the house cricket stands as an interesting model to study the regulation and function of adult neurogenesis as it shares many common aspects with the mammal adult neurogenesis but exhibits a simpler nervous system. In this review, we describe the main characteristics of neuronal production throughout the preadult stages and the adult life that lasts about 2 months in the cricket. In addition, the impact of environment and experience on adult neurogenesis and brain morphogenesis led us to postulate that there must be specific functions of newborn neurons during adult life.

The MBs: sites of neurogenesis in adult insects

MBs are paired structures located in the protocerebrum of the insect brain (Figure 1A). They are formed of interneurons called Kenyon cells, grouped in a cortex (Figures 1B and 2A), and of a neuropil that includes the projections of these intrinsic neurons and their synaptic contacts with afferent and efferent neurons. This neuropil is constituted of a calyx formed by Kenyon cell dendrites receiving sensory inputs and of a peduncle dividing into vertical (α) and medial (β, γ) lobes contacting efferent fibers (Figure 1B).

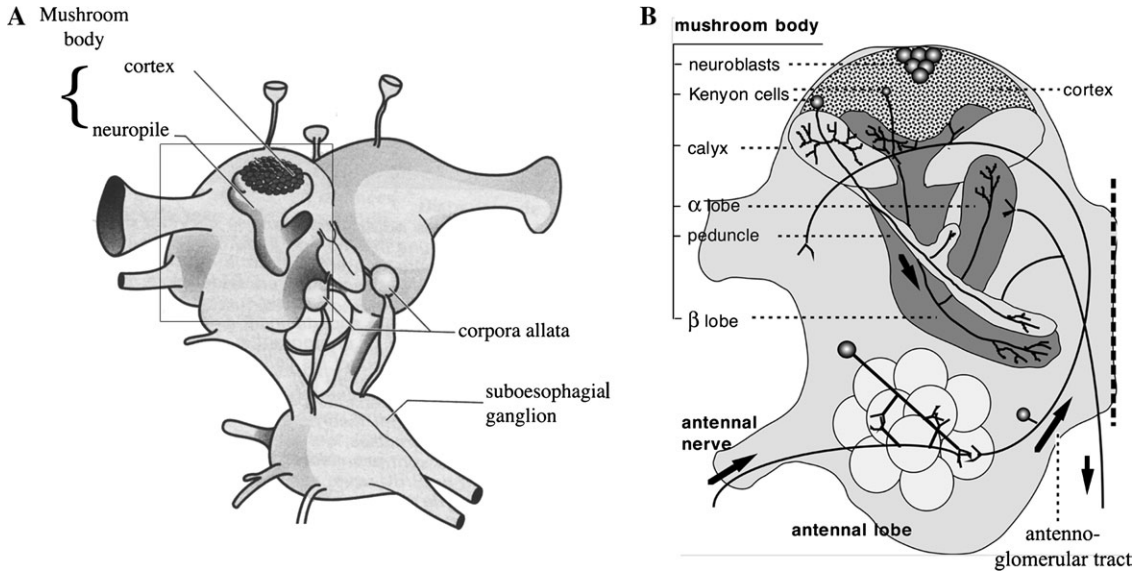

Figure 1 **(A)** Schematic representation of an insect brain (posterior view) showing the localization of the MBs. The structures inside the box are detailed in **(B)**. (B) Structure and organization of a cricket MB (frontal view). Fifty thousand interneurons called Kenyon cells are packed in a cortex and send their dendrites into the calyx and their axon through the peduncle into the lobes. At the apex of the cortex, a cluster of neuroblasts gives rise to new Kenyon cells throughout the insect's life. Kenyon cells receive olfactory and visual (not shown) inputs in the calyx and contact efferent fibers projecting to various brain structures. (Reprinted from *European Journal of Neuroscience* 2005; 21(11):2893–2902, with permission from Blackwell Publishing).

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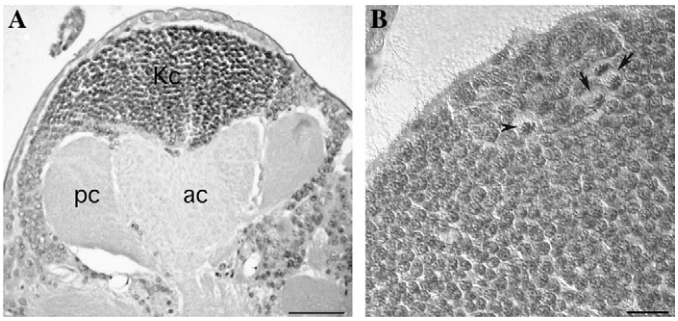

Figure 2 **(A)** Feulgen–Rossenbeck staining of a frontal section of an adult cricket brain showing the cortex and calyx of the MB. Interneuron cell bodies are small, round, and densely packed. **(B)** Enlarged view of the apex of the MB cortex. A cluster of larger cells (neuroblasts) is apparent; some of these cells are undergoing mitosis and patterns of metaphase and telophase are clearly visible (arrows). Kc: Kenyon cells; ac: anterior calyx; pc: posterior calyx; Nb: neuroblasts. Scale bars: 50 μm in (A); 10 μm in (B).

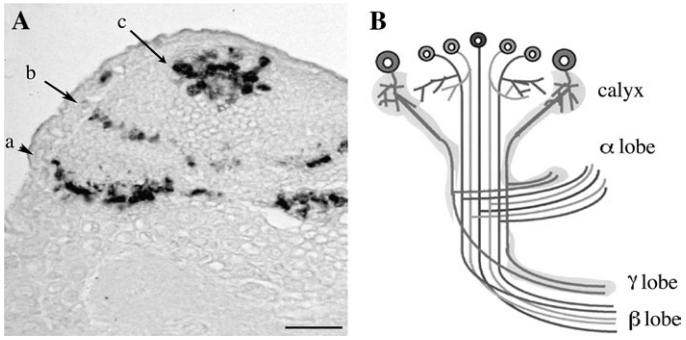

Figure 3 **(A)** BrdU immunolabeling on a frontal section of an adult cricket brain. The insect received 3 successive pulses of the S phase marker BrdU: the first during embryonic development (a), the second during larval life (b), and the third during adulthood (c). The animal was sacrificed 2 h after the last BrdU injection. Kenyon cells born early during development have somata occupying peripheral layers of the cortex, whereas interneurons born in late larval stages and during adult life fill more central areas. The cluster of neuroblasts is heavily labeled by the injection performed just before sacrifice. Scale bar: 50 μm. **(B)** Schematic representation of the age-dependent projection pattern of Kenyon cells. The large Kenyon cells generated during embryogenesis send their dendrites into the posterior calyx and their axons will form the γ lobe. The smaller Kenyon cells produced during larval development and adulthood are constantly pushed by the newborn cells to more peripheral layers and present specific projection patterns in the α and β lobes. (Adapted from *Journal Comparative Neurology* 2002; 452:215–227.)

MBs represent the main sensory integration center of the insect brain. The antenno-glomerular tract conveys olfactory information from the antennal lobe to the calyx, which also receives visual and tactile inputs from the optic lobe and the palpae (Mobbs 1982; Li and Strausfeld 1997). These different sensory modalities are integrated, allowing the production of an adapted motor response. Numerous studies using mechanical, chemical, or genetic approaches underlined the involvement of MBs in learning and memory processes in various insect species (Mizunami et al. 1993, 1998; Liu et al. 1999; for reviews, see Davis and Han 1996; Heisenberg 1998).

MBs are the unique sites of neurogenesis in the adult protocerebrum. Production of new Kenyon cells has been demonstrated in a variety of adult insects including some Orthoptera, Coleoptera, the praying mantis, the milkweed bug (Cayre M, Strambi A, Strambi C, unpublished observations), the lepidopteran *Agrotis ipsilon* (Dufour and Gadenne 2006), and the cockroach *Diploptera punctata* (Gu et al. 1999). In contrast, adult neurogenesis was looked for but not found in the honeybee (Fahrbach et al. 1995), *Drosophila* (Ito and Hotta 1992), and the migratory locust (Cayre et al. 1996). In these species, neuroblast proliferation proceeds during embryonic and larval stages, allowing MB morphogenesis, but stops just before the imaginal molt (Ito and Hotta 1992; Ganeshina et al. 2000).

It is in the cricket *Acheta domesticus* that adult neurogenesis has been most extensively studied. A cluster of neuroblasts located at the apex of the Kenyon cell cortex persists throughout the insect life (Figure 2B) and keeps producing new interneurons. Therefore, MB morphogenesis continues beyond the embryonic and larval periods of development. Kenyon cells are pushed by successive waves of newly born cells contributing to form concentric layers in the cortex, the outer layers being constituted of the oldest cells and the inner layers of the younger ones (Figure 3A; Malaterre et al. 2002). Therefore, the large Kenyon cells lining the calyx lips stem from embryonic origin and specifically project into the γ lobe, whereas small adult-born Kenyon cells stand in the core of the cortex and participate in the formation of α and β lobes (Figure 3B; Malaterre et al. 2002). Adult neurogenesis is a quantitatively important process in the MB because it has been estimated that adult-born neurons represent approximately 20% of the total cortex volume in a 40-day-old cricket (Malaterre et al. 2002). It should be emphasized that contrary to the neuronal replacement observed in crustaceans (Harzsch et al. 1999), birds (Kirn and Nottebohm 1993), and mammals (Biebl et al. 2000), production of new Kenyon cells is not accompanied by concomitant cell death in the cricket MBs. Interestingly, this continuous cell addition does not lead to an enlargement of the MB cortex but rather to an increased cell density (Cayre M, unpublished observations). This implies a constant reorganization of the MBs during adult life, associated with a high degree of structural neuronal plasticity. During differentiation and migration, newborn interneurons express lachesin, a protein of the immunoglobulin family (Karlstrom et al. 1993; Malaterre et al. 2002) related to polysialic acid neural cell adhesion molecule, which is, in adult vertebrates, considered as a potential marker of plasticity (Durbec and Cremer 2001; Kiss and Muller 2001). Therefore, MBs could be considered as brain structures exhibiting remarkable plasticity in the adult. Until recently, MB cortex was considered as a pack of homogenous Kenyon cells achieving identical functions, but the heterogeneity and specificity of different subtypes of Kenyon cells is now well documented. Subdivisions of the Kenyon cell population have

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been described on the basis of morphology of arborizations, patterns of immunoreactivity and gene expression, or of the relative diameter of the somata (Fahrbach 2006). Interestingly, in *Drosophila*, α, β, and γ lobes have been ascribed specific roles in learning and memory processes. Indeed, recent studies demonstrated that α/β neurons are required for long-term memory (Pascual and Preat 2001), whereas γ neurons are specifically necessary for short-term memory (Zars et al. 2000). Our data on continuous neurogenesis during adulthood suggest that Kenyon cells could present different properties during cell maturation, thus underlying specific functional roles.

Regulation of adult neurogenesis: role of hormones and neuromediators

From the discovery of adult neurogenesis, the regulatory role of internal factors such as hormones, neurotransmitters, or growth factors has been extensively studied (Cayre et al. 2002; for review, Ming and Song 2005). In the invertebrates, the molting hormone, ecdysone, seems to have species-specific actions. In a cockroach, ecdysone displays a mitogenic effect on adult neurogenesis (Gu et al. 1999), whereas it inhibits the mitotic activity of neuronal precursors in the developing MBs of the honeybee (Malun et al. 2003), as well as in the adult cricket (Cayre et al. 1997a). Interestingly, ecdysone also affects neuronal differentiation as it induces in vitro a significant increase in the percentage of neurons growing neurites and a significant lengthening of their neurites (Cayre et al. 2000). By contrast, another morphogenetic hormone, the juvenile hormone (JH), has been shown to stimulate neuroblast proliferation in the MBs (Cayre et al. 1994). JH is also responsible for the ovarian maturation and the establishment of the oviposition behavior in adult females. As the MBs might contribute to adaptive behaviors, like the "egg-laying" behavior, a role of the new neurons in the ontogeny of this reproductive behavior was first suspected. Indeed, it has been shown in mammals that the hormone prolactin stimulates the production of neuronal progenitors in the SVZ of pregnant female mice, suggesting that the forebrain olfactory neurogenesis may contribute to adaptive behaviors in mating and pregnancy (Shingo et al. 2003). However, such hypothesis was refuted in crickets as the ablation of the neuroblasts by gamma irradiation does not affect the oviposition activity (Scotto-Lomassese et al. 2003).

The mechanisms of the mitogenic action of JH on the MB neuroblasts were further analyzed, and the specific involvement of a short-chain polyamine, the putrescine, has been clearly demonstrated (Cayre et al. 1997b). Long-chain polyamines, spermidine, and spermine are also involved in the regulation of adult neurogenesis, as they promote neurite outgrowth of new neurons in vitro and thus act on neuronal differentiation (Cayre et al. 2001).

Neurotransmitters and growth factors have been implicated as well in the regulation of adult neurogenesis in invertebrates. Experiments of serotonin depletion in crustaceans (Benton and Beltz 2001) or inhibition of serotonin synthesis in crickets (Strambi C, unpublished results) show that this neurotransmitter stimulates the proliferation of neuronal progenitors, as it does in mammals (Brezun and Daszuta 1999). In vitro studies also demonstrated that both insulin and insulin growth factor-I enhanced MB progenitor cell proliferation (Malaterre et al. 2003) underlying, as in vertebrates, the role of growth factors on adult neurogenesis (for review, Anderson et al. 2002).

Regulation of adult neurogenesis: role of sensory inputs

Considering the role of MBs in sensory integration, the following question can be asked: "can the quality of the sensory and social environment influence the neuronal production rate in the MBs of adult insects?" The potential effect of environmental enrichment or deprivation on neuroblast proliferation was therefore analyzed. For this purpose, female crickets were either reared in enriched conditions (with odors, hiding places, congeners, space, i.e., stimulations probably present in a normal cricket's life) or isolated in small dark cages. Neurogenesis was estimated by quantifying neuroblast proliferation by directly counting cells in the M phase of the cell cycle as evidenced by Feulgen–Rossenbeck nuclear coloration (Figure 2B) or using the S phase marker 5-bromo, 2′deoxyuridine (BrdU) (Figure 4A). Exposure of crickets to an enriched environment for 4–8 days led respectively to 19% and 35% increases in neuroblast proliferation in 4-day-old enriched crickets compared with deprived insects (Scotto-Lomassese S, Cayre M, unpublished results). The effect of environmental stimulation on neurogenesis decreased with age and was subject to habituation when the insects were reared for long periods in enriched conditions; however, it could be enhanced by a sudden change of environment (Scotto-Lomassese S, Cayre M, unpublished data). Interestingly, in rodents, enriched environment also triggers increased neurogenesis in the dendate gyrus (Kempermann et al. 1997; Nilsson et al. 1999) and in the olfactory bulb (Rochefort et al. 2002). However, these occur through different pathways: progenitor cell proliferation is unaffected by enrichment but newborn cell survival is significantly increased, leading to an augmentation of the total number of interneurons in these structures.

Population density is known to influence hormone titers in insects (Pener 1991). Therefore, to rule out a possible hormonal relay in the environment-induced neuroblast proliferation, experiments of environmental enrichment were repeated using allatectomized insects, that is, insects deprived of JH. Similar results were obtained with allatectomized and normal animals (Scotto-Lomassese et al. 2002) suggesting that although both hormonal and sensory cues affect adult neurogenesis, the environmental regulation of the neuroblast proliferation does not necessarily involve JH in insects. Furthermore, a series of experiments using either

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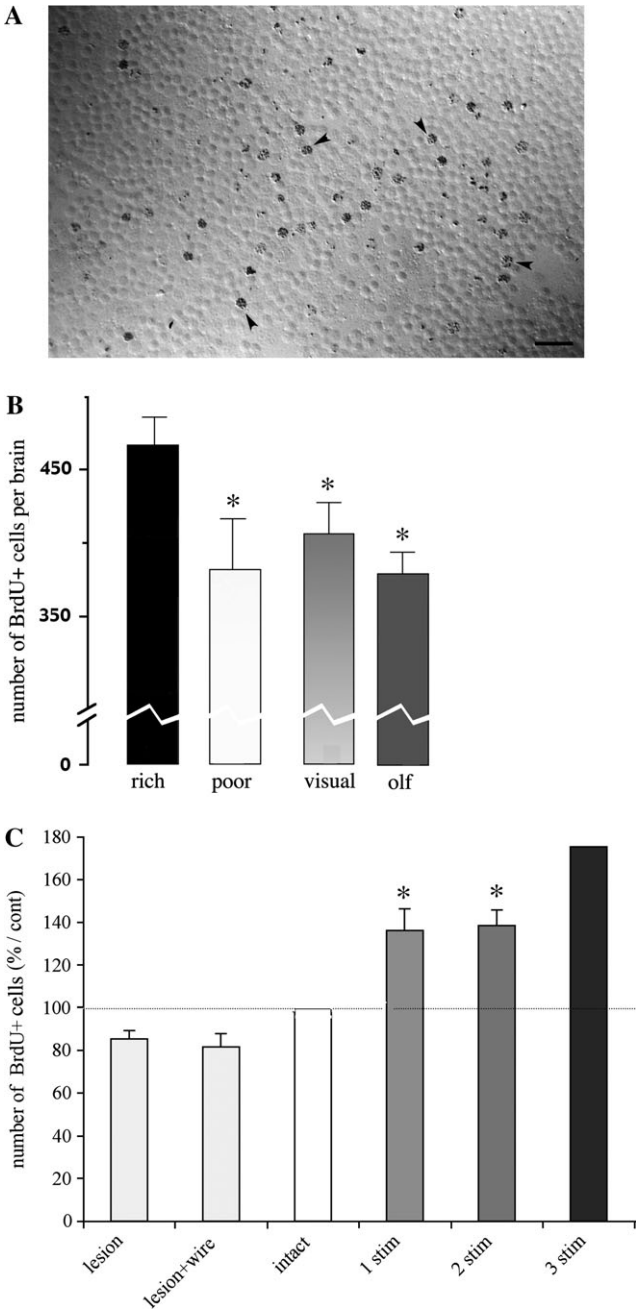

Figure 4 Regulation of proliferation of MB neuroblasts by environmental conditions **(B)** and antennal nerve stimulation **(C)**. **(A)** Monolayer spreading of a MB cortex labeled with BrdU. The insect received a BrdU injection one day before sacrifice and MB dissection. Among a stratum of nonlabeled Kenyon cells, some dark nuclei that have incorporated BrdU are clearly distinguishable (arrowheads). Counting the total number of BrdU-positive cells per MB gives an estimate of the neuroblast proliferation rate. Scale bar: 50 μm. (B) Enriched rearing conditions (rich) promote newborn neuron production, whereas deprivation of visual (visual) or olfactory (olf) inputs inhibits neuroblast proliferation. (C) After sectioning the antenna, insertion of an electric wire in the antennal nerve without applying any stimulation has no effect. In contrast, mimicking olfactory stimuli by unilateral physiological antennal nerve electric shock (30 min of 10 Hz monophasic square wave stimulation—0.2 ms per phase—at 10 V) results in increased neurogenesis. The nonstimulated MB is used as control.

electrical stimulation of the antennal nerve or sensory deprivation confirmed the direct effect of sensory stimuli in this regulation. Indeed, in crickets deprived of olfactory and/or visual inputs by sectioning of antennae and/or occlusion of eyes, neuroblast proliferation was significantly decreased (Figure 4B; Scotto-Lomassese et al. 2002). In addition, direct stimulation of the antennal nerve with electrical currents in physiological ranges resulted in increased number of BrdU-positive cells in the MB ipsilateral to stimulation (Cayre, Malaterre, Scotto-Lomassese, Holstein, et al. 2005). This increase appeared to be proportional to the number of stimulations applied (Figure 4C). Together these data demonstrate the direct influence of olfactory and, to a lesser degree, visual inputs on adult neurogenesis.

An increase in the number of BrdU-labeled cells does not differentiate between an acceleration of the cell cycle or a recruitment of quiescent neuroblasts into the cell cycle.

To address this question, double labeling using BrdU and proliferating cell nuclear antigen (PCNA) was performed on brain sections of adult crickets. Whereas BrdU incorporation occurs only during the S phase of the cell cycle, PCNA (a nonhistone protein associated with DNA polymerase delta) is expressed in all phases of the cell cycle. Using BrdU/PCNA labeling ratio, the upregulation of neurogenesis by sensory stimulation was shown to result from a shortening in the cell cycle (Cayre, Malaterre, Scotto-Lomassese, Aouane, et al. 2005). It is of note that regulation of proliferation of MB neuroblasts by JH proceeds by the alternative process of an increased recruitment of quiescent neuroblasts into the cell cycle (Cayre, Malaterre, Scotto-Lomassese, Aouane, et al. 2005). Thus, hormonal and sensory cues involve different pathways to stimulate adult neurogenesis in crickets.

Determining the mechanisms by which sensory enrichment or deprivation regulates neural progenitor cell proliferation and exploring the molecular cascade triggered by neuronal excitation leading to increased proliferation became an important goal. Among the putative molecular factors involved, nitric oxide (NO) was viewed as a likely candidate. Indeed, our recent results showing the expression of NO synthase in Kenyon cells (Cayre, Malaterre, Scotto-Lomassese, Holstein, et al. 2005) underline the importance of NO in olfaction processing, which is in agreement with other neuroanatomical (Bicker 2001) and functional (Wasserman and Itagaki 2003; Collmann et al. 2004) studies performed in insects. Moreover, regulation of neuroblast proliferation by NO in vivo as well as in vitro also suggests that NO could play a crucial role in environment-induced neurogenesis in the adult cricket (Cayre, Malaterre, Scotto-Lomassese, Holstein, et al. 2005). In mammals, although numerous studies suggest that NO regulates adult neurogenesis, contradictory results have been reported on the enhancing or inhibiting effect of NO on cell proliferation in normal conditions as well as after stroke injury (for reviews, see Contestabile and Ciani 2004; Cardenas et al. 2005). These studies suggest that cell-specific expression and differential distributions of NO synthase isoforms may lead

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to variability in the regulation of adult neurogenesis. Besides, if a variety of growth factors such as vascular endothelial growth factor, brain derived neurotrophic factor, and glial cell line-derived neurotrophic factor have been shown to participate in the environmental regulation of adult neurogenesis (Cao et al. 2004), it does not seem to be the case for NO. In insects, little is presently known about putative different isoforms of NO synthase, and the molecular mechanisms underlying the effect of NO on cell cycle progression remain unknown. In conclusion, the data obtained with the insect model *A. domesticus*, in accordance with studies performed in other invertebrates and vertebrates, emphasize the functional importance of NO in brain structures undergoing continuous neurogenesis and involved in learning and memory processes (Bicker 2001; Bon and Garthwaite 2003).

Toward a functional role of adult neurogenesis in olfactory learning

To study the functional role of newborn neurons, it was necessary to develop a technique to specifically block adult neurogenesis in the cricket brains. Hydroxyurea, an antimitotic drug previously used on larvae of honeybees (Malun 1998; Malun et al. 2002) and *Drosophila* (de Belle and Heisenberg 1994) to prevent MB development, was first tested. However, the efficiency of this treatment to suppress adult neurogenesis did not appear convincing enough, and the required doses were toxic and lethal (Cayre M, Strambi A, Strambi C, unpublished results). A new method was therefore developed using ionizing rays. Immature and dividing cells are particularly sensitive to ionizing rays, whereas differentiated cells are not or little affected. This strategy has been successfully used in rodents to induce cell death among the SVZ and dentate gyrus progenitor cells (Peissner et al. 1999; Amano et al. 2002; Mizumatsu et al. 2003; Wojtowicz 2006) with doses ranging from 2 to 10 Gy. Insects are considered to be much more resistant to irradiation than vertebrates. To determine the optimal irradiation dose for adult crickets, the insect's head was exposed to gamma ray irradiation for variable times in order to establish a curve dose–response. Forty gray produced a 90% reduction of the mitotic index without any side effects on ovarian development, oviposition behavior, locomotor activity, or survival rate (Scotto-Lomassese et al. 2003). Neuroblasts and ganglion mother cells (i.e., Kenyon cell precursors stemming from neuroblast asymmetric division) underwent massive apoptosis from day 1 to 4 following irradiation, and 3 weeks later virtually no BrdU-positive cells were detected in the MBs. In contrast, differentiated Kenyon cells did not exhibit any sign of cell death. Irradiation therefore appears useful to study the functional role of adult neurogenesis in the cricket because it generates adult insects lacking MB neuroblast proliferation.

Whereas the honeybee (Menzel and Giurfa 2001) and *Drosophila* (Davis 1993) are conventional insect models for learning and memory studies, no learning paradigm was known for the house cricket. A learning paradigm had to be developed and applied to crickets to examine the effect of adult neurogenesis suppression on mnesic abilities of these animals. Matsumoto and Mizunami (2000) described an operant discriminatory conditioning paradigm in which another cricket, *Gryllus bimaculatus*, was able to associate a particular odor with reward (water) or punishment (saline water). However, we failed to reproduce this test with *A. domesticus* because of an absence of aversion of this cricket for the saline, bitter, or spicy water that was used as a negative reinforcement in this paradigm. The Tennessee Williams paradigm (a terrestrial adaptation of the Morris water maze) used in cockroaches (Mizunami et al. 1993, 1998) did not produce better results. However, while observing the crickets during these tests, we noticed that the main motivation of the insects was to escape from the arena, running all around the walls. We decided to take advantage of this natural behavior to develop a new learning task: the "escape paradigm." The crickets were placed in a round arena placed under strong light (aversive stimulus) in which there are 2 holes, one leading to a trap (an assay tube) and the other allowing the cricket to escape into a large dark cage. The insects used olfactory and/or visual cues to discriminate between the 2 holes. Control animals were able to rapidly learn the task, confirming that this paradigm works well for crickets (Scotto-Lomassese et al. 2003). Moreover, we also verified that odor perception ability was unaffected by irradiation and thus showed that newly born MB neurons are not required for normal olfactory discrimination, a result opposite to what is observed in the rodent olfactory bulb (Gheusi et al. 2000). This difference is not surprising because the olfactory bulb is the first-order integration center, whereas the MB is a second-order integration center after the antennal lobe, where no adult neurogenesis occurs.

When only olfactory cues were available, 75% of the control crickets performed correctly after only 2 sessions, whereas only 15% of irradiated crickets did. At the end of the learning period, 94% of control crickets were able to discriminate properly between the 2 holes versus only 77% for the irradiated crickets (Figure 5A). Furthermore, not only learning but also retention performances 24 h after the last training session were affected by irradiation (Figure 5C). These results are consistent with studies showing that α/β neurons are required for long-term memory in *Drosophila* (Pascual and Preat 2001) because newly born Kenyon cells contribute to α/β lobe development (Malaterre et al. 2002).

In contrast, no significant difference was observed between control crickets and those lacking adult neurogenesis when visual cues were used instead of odors (Figure 5B,D). Another retention test performed 7 days after the last training session showed that control crickets failed to remember the task, whatever the sensory modality used. This result was surprising because in another cricket (*G. bimaculatus*), the retention of an elementary olfactory conditioning has been shown to last up to 10 weeks (Matsumoto and Mizunami

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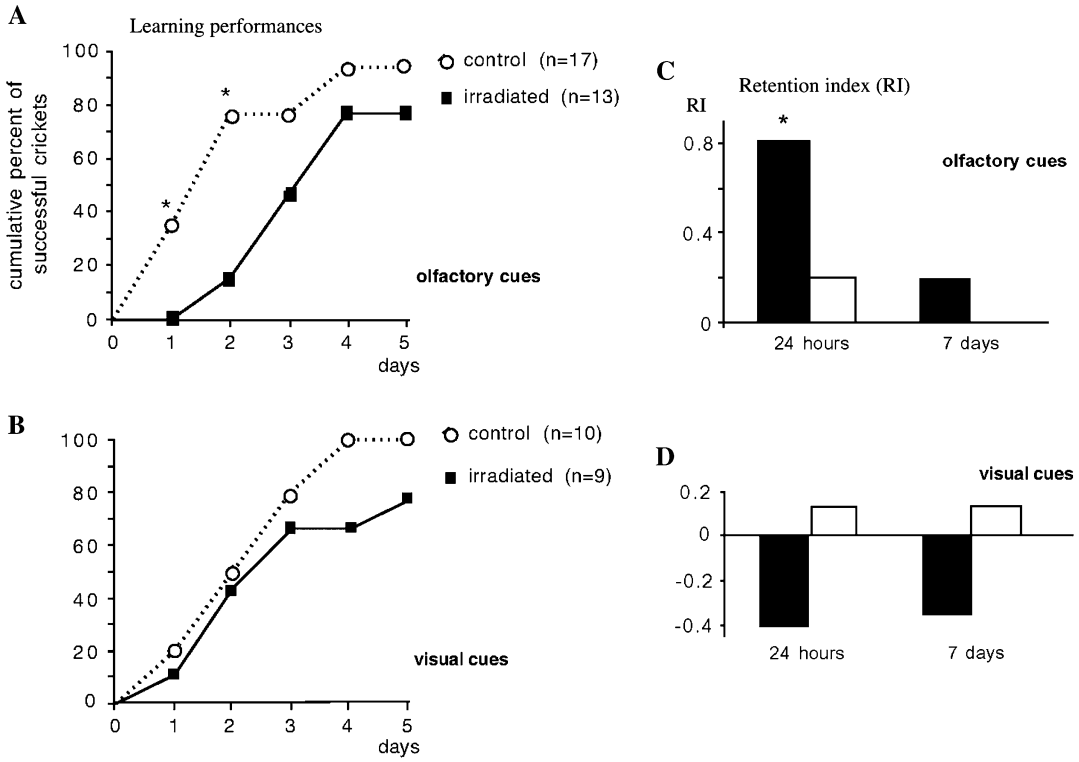

Figure 5 Effect of destruction of MB neuroblasts on mnesic abilities of adult crickets. Learning **(A, B)** and retention **(C, D)** performances of control and irradiated crickets were determined in the escape paradigm described in the text. Olfactory cues (A, C) or visual cues (B, D) were provided for discrimination between the 2 holes. Crickets lacking adult neurogenesis (i.e., irradiated crickets) have impaired acquisition and retention of the task only when olfactory cues are used. Adapted from *Journal of Neuroscience* 2003; 23:9289–9296. Copyright 2003 by the *Society for Neuroscience*.

2002). However, in contrast to the honeybee and *Drosophila* in which specific molecular mechanisms underlying short-, medium-, and long-term memory have been identified, nothing is known about the different phases of memory in Gryllidae. As the escape paradigm is developed over several days, the kinetics are completely different from those involved in simple associative paradigms (such as the proboscis extension reflex or pairing an odor with a footshock) used to study memory phases. It is therefore hazardous to establish parallels between these 2 models. For instance, using flies from 2 enhancer trap lines that express the temperature-sensitive shibire allele primarily in the MBs, Dubnau et al. (2001) concluded that MBs were required for the retrieval but not for the acquisition of odor memories. However, our studies suggest that newborn Kenyon cells are involved in the acquisition of our olfactory task. Considering the huge differences between the 2 experimental paradigms, it is very likely that what we refer to as the "acquisition" phase is not equivalent to what Dubnau et al. (2001) name acquisition.

Altogether, these results suggest that newborn neurons participate in olfactory processing and enhance the performances of the insects in the context of an operant associative learning. Newly born Kenyon cells are immature interneurons that probably present higher plasticity in terms of synaptogenesis and modulation of synaptic strength in response to environmental cues. They may present different membrane and electrophysiological characteristics that could confer upon them specific functional properties. For instance, newly generated Kenyon cells express different neurotransmitters and exhibit specific arborization patterns (Strambi et al. 1998; Schürmann et al. 2000; Malaterre et al. 2002). In adult rodents, it has been demonstrated that newborn hippocampal neurons display enhanced synaptic plasticity and more robust long-term potentiation than mature interneurons (Wang et al. 2000; Schmidt-Hieber et al. 2004). Also, recently generated adult-born olfactory neurons and older, preexisting granule neurons undergo contrasting experience-dependent modifications in vivo (Magavi et al. 2005). Thus, the constant incorporation of immature neurons into the MB circuitry may be more important than the total increase in neuron number for improving the acquisition rate and underlying behavioral abilities.

Conclusion

Adult neurogenesis is remarkably similar in vertebrates and invertebrates. In both groups, adult neurogenesis takes place in important brain structures exhibiting a high degree of structural plasticity and displaying important functional analogies. For instance, MBs and hippocampus are regulated

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by networks of oscillatory interneurons (Laurent and Davidowitz 1994; Buzsaki 1997) and are the site of long-term potentiation or depression (Bliss and Collingridge 1993; Oleskevich et al. 1997). In vertebrates as in invertebrates, hormones, neurotransmitters, and environmental cues are able to modulate adult neurogenesis (for reviews, Cayre et al. 2002; Abrous et al. 2005). Although the mechanisms involved in environment-induced neurogenesis are slightly different (increased cell survival in mammals vs. increased cell proliferation in insects), the net result is an augmentation of the total number of interneurons in the structures where the new cells are produced or recruited. Using an insect model with a relatively simple brain, we were able to point out major regulation and functional characteristics of adult neurogenesis. Altogether, the results described in this review led us to propose the following scheme. Living in enriched sensory conditions favors brain plasticity and more specifically increases the production of new interneurons possibly via activation of NO synthesis. In turn, this increased neurogenesis in a brain structure involved in sensory integration and learning processes leads to improved mnesic performances, therefore enhancing the ability of the insect to benefit from its own experience. Consequently, adult neurogenesis could facilitate adaptive abilities of animals to a continuously changing environment. Reviewing studies concerning the functional role of adult neurogenesis in mammals, Lledo et al. (2006) concluded similarly and proposed that "adult neurogenesis could represent a form of metaplasticity: a change in the brain that facilitates further changes in the brain." In the moth *A. ipsilon*, the sensitivity of antennal lobe neurons for the sex pheromone increases with age and JH biosynthesis (Anton and Gadenne 1999); the authors suggested that persistent neurogenesis in the adult MB of this moth may be responsible for this olfactory-based neuronal plasticity (Dufour and Gadenne 2006). This hypothesis is conceptually interesting but still remains to be demonstrated. Examining the role of adult neurogenesis in other insect species such as cockroaches in which learning paradigms are well developed and memory phases beginning to be analyzed (Pinter et al. 2005) or coleopterans in which MB development differs according to specific different behaviors (Farris and Roberts 2005) might help deciphering the functional significance of such process.

Finally, it is important to remember that not all insects undergo adult neurogenesis. Until now, no clear indication could be found to clarify why some species do experience adult neurogenesis and others do not. Phylogenetic criteria, life span, and behavioral complexity failed to provide satisfactory explanations. Among species that do not, the honeybees present very complex social behaviors such as communication, navigation, and foraging, tasks requiring learning capacities and good memory. Strikingly, although honeybee MBs lack adult neurogenesis, they remain highly plastic during adult life, exhibiting experience-dependent volume variations due to longer and more branched dendrites (Withers et al. 1993; Farris et al. 2001). Therefore, it would be far too simplistic to conclude that adult neurogenesis is necessary to develop efficient learning and memory performances or even to believe that this is the only function of adult neurogenesis. Despite a number of convergent correlations linking adult neurogenesis and learning performances in several animal species, no direct evidence is really available, mainly due to methodological limitations, and many questions remain to be elucidated (Kempermann et al. 2004; Leuner et al. 2006). Although neurogenesis occurs in the adult human brain (Eriksson et al. 1998; Curtis et al. 2007), considering the relative reduction of the olfactory bulb volume and the weak olfactory abilities in human compared to rodents or nocturnal insects such as crickets, it is not clear whether adult neurogenesis participates in olfactory learning in humans. It is thus unlikely that adult neurogenesis participates in olfactory learning in humans. Besides, this observation is in agreement with the relative reduction of the olfactory bulb volume and the weak olfactory abilities in humans compared with rodents or to nocturnal insects such as crickets. Recently, it has been proposed that modifications of adult neurogenesis rate may play a role in stress and anxiety regulation (Dranovsky and Hen 2006) or may serve as a latent mechanism for brain repair (Mitchell et al. 2004). Indeed, the discovery of neural stem cells in adult human brain has raised hope for the development of new therapeutic strategies for neurodegenerative diseases (for review, see Lie et al. 2004). But so far, before adult neural stem cells can become of clinical use, a complete knowledge of mechanisms regulating their proliferation, migration, differentiation, and functional integration is necessary. In this context, all animal models with a high promise of providing useful information should be nurtured.

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