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Sensory Inputs Stimulate Progenitor Cell Proliferation in an Adult Insect Brain

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Summary

Although most brain neurons are produced during embryonic and early postnatal development, recent studies clearly demonstrated in a wide range of species from invertebrates to humans that new neurons are added to specific brain structures throughout adult life. Hormones, neurotransmitters, and growth factors as well as environmental conditions modulate this neurogenesis [1–9]. In this study, we address the role of sensory inputs in the regulation of adult neural progenitor cell proliferation in an insect model. In some insect species, adult neurogenesis occurs in the mushroom bodies [10], the main sensory integrative centers of the brain, receiving multimodal information [11, 12] and often considered as the analog of the vertebrate hippocampus. We recently showed that rearing adult crickets in enriched sensory and social conditions enhanced neuroblast proliferation in the mushroom bodies [13]. Here, by manipulating hormonal levels and affecting olfactory and/or visual inputs, we show that environmental regulation of neurogenesis is in direct response to olfactory and visual stimuli rather than being mediated via hormonal control. Experiments of unilateral sensory deprivation reveal that neuroblast proliferation can be inhibited in one brain hemisphere only. These results, obtained in a relatively simple brain, emphasize the role of sensory inputs on stem cell division.

Results and Discussion

Environment-Induced Neurogenesis Is Independent of Juvenile Hormone

It is known that environmental conditions can act on insect physiology and especially on hormone titers. For example, bumblebee reproduction may be affected by social environment, an effect mediated by juvenile hormone (JH) [14]. Considering the stimulating action of JH on neurogenesis [15], it was not clear whether the environmental cues directly affected neurogenesis via sensory inputs or whether they altered hormone levels which in turn acted on cell proliferation. To address this question, we performed hormonal manipulations: the corpora allata, glands secreting JH, were removed in order to obtain hormone-deprived adult insects. Allatectomized females were then reared in enriched and impoverished environments, and neuroblast proliferation was assessed using the thymidine analogue 5-bromo, 2′-deoxyuridine (BrdU). Allatectomized females (group A) had significantly fewer BrdU-labeled cells in their mushroom bodies than intact females in both enriched ($p = 0.01$) and impoverished ($p = 0.007$) conditions, emphasizing the stimulatory effect of JH on adult neurogenesis. However, even in the absence of JH, cell proliferation was still significantly higher in enriched than in impoverished females ($p = 0.046$) (Figure 1). The effect of environmental cues was less pronounced in CA− than in control females due to the low basal rate of neurogenesis induced by JH deprivation. These results provide evidence that although hormonal and sensory cues might both participate in environmental regulation of adult neurogenesis, external factors do not necessarily require JH to regulate neurogenesis.

Unilateral Sensory Deprivation Unilaterally Affects Mushroom Body Neurogenesis

To demonstrate that environment-induced neurogenesis was directly attributable to sensory inputs, we suppressed unilaterally both visual and olfactory inputs (group B). Despite the fact that the internal environment was the same for both mushroom bodies, unilaterally sensory-deprived insects in enriched housing conditions had significantly fewer BrdU-labeled cells in the mushroom body on the side deprived of sensory inputs (ipsilateral) compared with the side receiving external stimuli (contralateral) ($p = 0.001$) (Figure 2). Furthermore, such a difference was not observed in unilaterally sensory-deprived females reared in impoverished environment ($p = 0.851$) (Figure 2), stressing that, in conditions where stimuli are already minimal, further decrease in neurogenesis cannot be detected even after deafferentation. In control intact crickets, no difference was observed between the two halves of the brain, regardless of environmental conditions (data not shown). Thus, environment-induced neuroblast proliferation seems to be directly linked to neuronal activity of sensory inputs. Similarly, in rodents, unilateral nostril occlusion resulted in a net loss of granule cells in the ipsilateral olfactory bulb, due to reduced neurogenesis and reduced survival of the adult-generated neurons [16] together with increased cell death [17]. In the house cricket mushroom bodies, apoptosis does not seem to be a major component in cell number regulation, as TUNEL-positive interneurons have never been detected, and cell loss is very weak among newborn neurons at least between day 5 and day 20 [13].

Staining the proximal part of the antennal nerve revealed a strong reduction of the innervation in the antennal lobe on the injured side (Figure 3). However, as neuroblast proliferation is identical in the two mushroom bodies of unilaterally injured crickets under impoverished environment, this suggests that the lesion per se did not alter neurogenesis. Similar conclusions have

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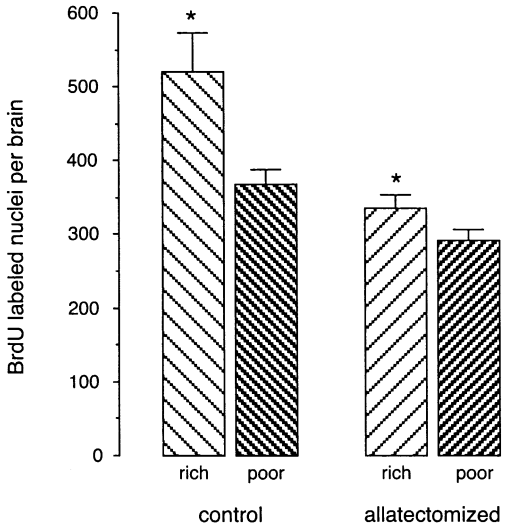

Figure 1. Quantitative Analysis of Cell Proliferation in Mushroom Bodies of Intact and Allatectomized Crickets Reared in Enriched or Impoverished Environmental Conditions

Mean number of BrdU-labeled cells ± SEM in females reared in rich or poor environment are shown for intact (519.5 ± 53.8 versus 367.9 ± 21.1; n = 8 and 7, respectively) and allatectomized (334.8 ± 18.4 versus 291.9 ± 14.3; n = 11 and 10, respectively) insects. *, significant difference ($p < 0.05$) from impoverished group by Mann-Whitney U test. Note that poor intact females are not significantly different from rich allatectomized females ($p = 0.149$).

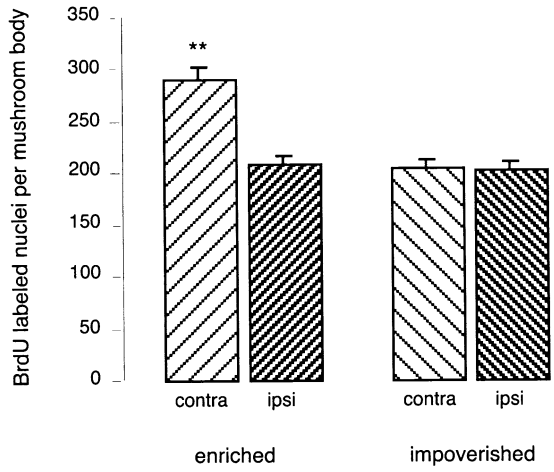

Figure 2. Quantitative Analysis of Cell Proliferation after Unilateral Deprivation of Visual and Olfactory Inputs in Females Reared in Enriched or Impoverished Environment

Mean number of BrdU-labeled cells ± SEM in contralateral ("contra") and ipsilateral ("ipsi") mushroom body cortices in enriched (290.4 ± 12.7 versus 209.2 ± 16.7; n = 10) and impoverished (203.9 ± 8.6 versus 202.1 ± 7.8; n = 11) females. **, significant difference ($p < 0.01$) from contralateral side by Student's t-test.

been drawn in lesion experiments on vertebrates [18]. Furthermore, sensory deprivation has been shown to induce dendritic and synaptic atrophy in different insect species [19–21]. Conversely, an increase in dendritic complexity of mushroom body neurons has been associated with increasing age and foraging experience in the honeybee [22]. Our results, together with these examples, strongly suggest that neuronal activity mediated by sensory inputs is able to shape adult brain structures by eliciting various plastic responses involving not only dendritic arborizations and synapses but also neurogenesis.

Within 6 days following neuron birth, no cell loss occurred in mushroom body of either the intact or sensory-deprived side (group C). Consequently, in contrast to neuroblast proliferation, cell survival was not affected by visual and olfactory deprivation (297.1 ± 10.6 BrdU-labeled cells in the mushroom body ipsilateral to deprivation versus 305.8 ± 13.1 in the contralateral side; $p = 0.385$). This result differs from data obtained in vertebrates where survival but not proliferation is affected by enriched housing conditions, but it corroborates what had already been observed and discussed with intact insects reared in impoverished environment [13].

Contribution of Visual versus Olfactory Inputs in Environment-Induced Neurogenesis

In order to estimate the respective part of visual and olfactory inputs in the regulation of neuroblast proliferation, we performed bilateral sensory deprivation. The absence of either visual or olfactory stimuli (group D) resulted in a decrease of the number of BrdU-labeled cells compared with enriched controls, at levels similar to those seen in impoverished intact females (Figure 4). Interestingly, although we could not evidence a significant difference between olfactory- and visually-deprived females, the effect of antennal section was however more significant ($p = 0.006$) than that of visual deprivation ($p = 0.05$).

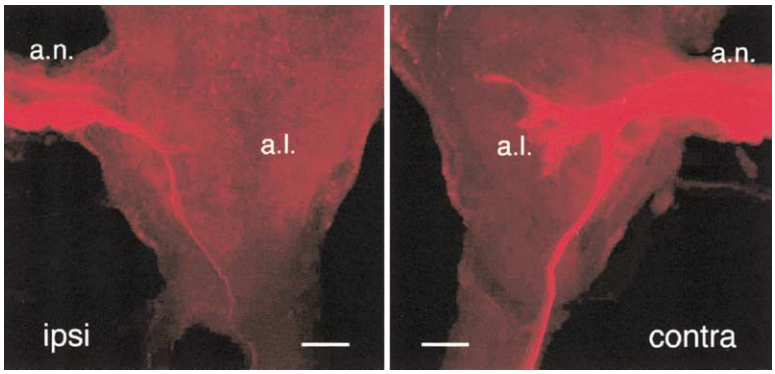

Figure 3. Labeling of Antennal Lobe Inputs by Texas Red Dextran

Four days after cutting one antenna, the side with the lesion shows a strong reduction in the number and branching of sensory afferent fibers entering the antennal lobe. a.n., antennal nerve; a.l., antennal lobe; ipsi, brain side ipsilateral to antenna ablation; contra, brain side contralateral to antenna ablation. Scale bars, 100 μm.

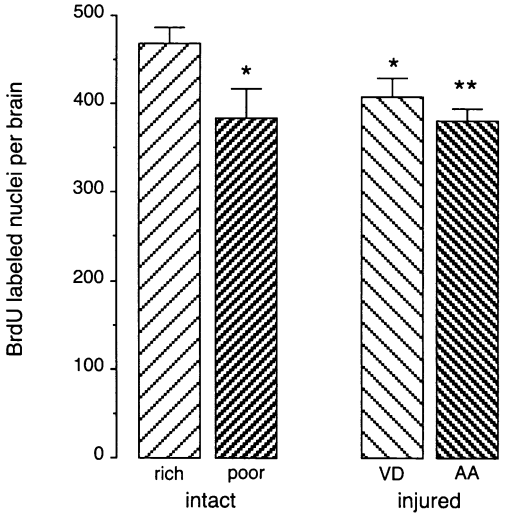

Figure 4. Respective Effects of Visual or Olfactory Deprivation on Cell Proliferation in Females Reared in Enriched Environmental Conditions

For comparison, the mean number of BrdU-labeled cells ± SEM are given for intact females reared either in rich or poor environment (468.1 ± 18.8 versus 383.2 ± 33.8; n = 14 and 8, respectively). VD, visually deprived females (407.6 ± 21.0; n = 7); AA, antennae-ablated females (380.5 ± 13.6; n = 9). VD and AA females had significantly fewer labeled cells (*$p < 0.05$; **$p < 0.01$) than intact females reared in enriched environment by Mann-Whitney U test.

The results of the present study provide evidence that environmental regulation of neurogenesis may be the direct effect of neural inputs associated with vision and odors, even though visual projections to mushroom bodies are scarce as compared to olfactory ones [23, 24]. It is noteworthy that, although most visual outputs stay in the same hemisphere, few fibers cross the midline to connect the contralateral side of the brain; by contrast, no olfactory outputs have been observed to project into the contralateral hemisphere ([23] and our unpublished data).

In the present study, we only examined the effects of olfaction and vision, yet the complex environment we used for enriched females also included tactile, social, and motor stimulation. It is not unreasonable to believe that these stimuli may also affect neuroblast proliferation, for in rodents, voluntary exercise could be responsible for most neural changes resulting from exposure to enriched environment [25]. However, in our model, locomotor activity does not seem to be a major factor controlling neuroblast proliferation, since we observe differential neurogenesis effects in the two halves of the same brain (cf., Figure 2, enriched crickets).

How environmental cues activate neuroblast proliferation and/or neuron survival remains unclear, especially when proliferative centers are not directly exposed to sensory inputs. Neural activity results in altered gene expression via activation of inducible transcription factors as c-Fos and -Jun [26–28] and NGF, BDNF, and glucocorticoid expression is enhanced in the brain of animals reared in enriched conditions [29–31]. In canaries, singing has been shown to increase BDNF expression in the class of brain neurons that continue to be replaced in adulthood [32]. Recently, a study analyzed gene expression in the cortex of mice exposed to enriched environment using high-density oligonucleotide microarrays and found altered expression of numerous genes linked to neuronal structure, synaptic plasticity, and transmission [33]. Further studies will be required to determine if some of these genes are involved in the regulation of neuroblast proliferation and newly generated neuron survival.

The present data, obtained in a relatively simple brain, suggest that the regulation of neurogenesis by neural activity, in particular linked to sensory inputs, is a general property of nervous structures. The implications of environmental regulation on secondary neurogenesis are not clearly understood. Several studies have revealed striking correlations between the rate of neurogenesis in vertebrate dentate gyrus and the learning abilities, suggesting a functional role of these newly formed neurons in hippocampus-dependent tasks [34–38]. It is tempting to speculate that the richness and variability of environment stimulate neurogenesis and that in turn the newly generated neurons improve the capabilities of adult animals to exploit their habitat.

Experimental Procedures

Rearing Conditions and Animal Treatment

Housing Conditions

Enriched females were maintained in groups of 20 in large cages (50 × 30 × 30 cm) located in the rearing room, allowing them to hear male songs and to smell environmental odors. The cages were equipped with variable floor textures, hiding places, and aromatic plant branches. Food and water were distributed in such a way that females were forced to explore the three dimensions of their environment. Impoverished females were housed in small individual cages (9 × 6 × 6 cm) where water and food were easily available. Furthermore, they were maintained in continuous darkness and deprived of congeneric sounds and odors. In both conditions, the relative humidity was 55% and the temperature 29°C ± 1°C.

Allatectomy

Females were allatectomized as described elsewhere [39], during the last preimaginal instar, 5 days before adult emergence (group A). The next day, animals were reared either in enriched or in impoverished environment until sacrifice.

Sensory Deprivation

Unilateral deprivation was obtained by sectioning one antenna and painting the ipsilateral eye with black Kiriol paint (GMC, Aubagne, France) (groups B and C); bilateral deprivation was obtained by sectioning the two antennae or painting the two eyes (group D). Females from group B were reared either in enriched or in impoverished environment from emergence until sacrifice, whereas insects from groups C and D were only held under enriched conditions.

Estimation of Cell Proliferation and Cell Survival

The thymidine analogue 5-bromo, 2′-deoxyuridine (BrdU, Sigma Aldrich, France) was used as a marker of cell proliferation. Adults were injected 4 days after the imaginal molt with 10 μl BrdU (40 mg/ml). This age was chosen because at that time crickets are sexually mature and exhibit maximal cell proliferation in their mushroom bodies [15]. Proliferating activity of progenitor cells was estimated by sacrificing the animals 24 hr later (groups A, B, and D), whereas survival of newly generated cells was estimated by analysis 6 days after the BrdU injection (group C). The brain was dissected out, the neural sheath removed, and the mushroom body cortices excised and spread onto a polylysined glass slide (Polylabo, France). Immunocytochemical detection of BrdU was performed as described elsewhere [13]. The total number of BrdU-labeled cells per

cortex was counted under a microscope by an observer blind to the treatment conditions.

Fiber Tract Labeling

The degeneration of olfactory inputs was determined using lysine fixable MW 3000 dextran coupled with Texas red (Molecular Probes, the Netherlands). A drop of dye, diluted in saline, was applied onto the proximal part of the antennal nerve after section of the antenna. Migration of the dye was allowed for 4 hr, then the brain was dissected and fixed for 30 min in 4% paraformaldehyde, dehydrated through progressive alcohol washes, then cleared in methyl-salicylate before observation under a Zeiss confocal microscope.

Data Analysis

Number of BrdU-labeled cells were expressed either as mean ± SEM per brain (i.e., in the two mushroom bodies) or as mean ± SEM per mushroom body for unilaterally injured animals. A paired (one-tailed) Student's t test was used to compare cell proliferation between mushroom bodies of sensory-deprived and intact sides. For the other experiments, data were analyzed using the nonparametric Mann-Whitney U test.

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