nature neuroscience

Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment

Dar Meshi[1,6], Michael R Drew[2,3,6], Michael Saxe[2], Mark S Ansorge[4], Denis David[2,5], Luca Santarelli[2], Chariklia Malapani[3], Holly Moore[4] & René Hen[2,4]

Environmental enrichment increases adult hippocampal neurogenesis and alters hippocampal-dependent behavior in rodents. To investigate a causal link between these two observations, we analyzed the effect of enrichment on spatial learning and anxiety-like behavior while blocking adult hippocampal neurogenesis. We report that environmental enrichment alters behavior in mice regardless of their hippocampal neurogenic capability, providing evidence that the newborn cells do not mediate these effects of enrichment.

Within the hippocampus, the subgranular zone of the dentate gyrus harbors progenitor cells that continuously divide and give birth to new neurons and glia in the adult brain[1]. The function of these new neurons is still unclear and highly debated. Over the last few years, data have been generated linking changes in the rate of adult hippocampal neurogenesis to changes in behavior[2–7]. A manipulation providing substantial evidence for this link is environmental enrichment[2], which is the housing of rodents in larger, more complex cages, usually including toys and running wheels.

Animals exposed to environmental enrichment show numerous differences when compared to animals living in standard housing[2], including an upregulation of adult neurogenesis specific to the hippocampus[8], a brain structure critical for learning and possibly affective processes[9]. Reported behavioral effects of enrichment include improvements in learning and memory, particularly in tests of spatial learning such as the Morris water maze[3,10]. Enrichment also affects emotional reactivity, resulting in decreased anxiety-like behaviors in certain tests[11]. This correlational evidence has led to speculation that upregulated adult hippocampal neurogenesis is a mediator of the behavioral effects of enrichment[3].

Investigation into a causal link between changes in the rate of neurogenesis and changes in behavior is underway. The systemic administration of the cytostatic agent methylazoxymethanol (MAM)[4,5] and whole-head irradiation have been used to block cell division[6]. Although these manipulations do not specifically target hippocampal neurogenesis, they cause impairments in some forms of hippocampal-dependent learning and have thus implicated adult hippocampal neurogenesis in the regulation of hippocampal-dependent behavior.

To test the hypothesis that the upregulation of hippocampal neurogenesis mediates the effects of environmental enrichment, we exposed mice to focal X-irradiation directly above the hippocampus in order to block hippocampal neurogenesis while leaving other neurogenic regions intact[7]. Because irradiation induces inflammation[12], mice were given 2 months to recover, a period after which we no longer detected markers of inflammation (**Supplementary Fig. 1** online). After the recovery period, mice were housed in an enriched environment, a large multicompartment housing unit including toys and running wheels. After 6 weeks, the mice were tested in mouse models of anxiety-like behavior and spatial learning. One week after the conclusion of behavior testing, mice were injected with 5-bromo-2′-deoxyuridine (BrdU) to label the dividing cell population, and killed (**Fig. 1** and **Supplementary Methods** online).

To determine if our enrichment and irradiation manipulations had the desired effects on hippocampal neurogenesis, we first examined doublecortin (DCX) and BrdU immunoreactivity in the dentate gyrus. DCX is an intermediate filament protein expressed in young, postmitotic neurons (less than 1 month old)[13]. Environmental enrichment increased the number of DCX- and BrdU-positive cells, whereas irradiation eliminated both DCX and BrdU immunoreactivity (**Fig. 2a**,**b** and **Supplementary Fig. 2** online). To confirm that enrichment increased neurogenesis, we performed a double labeling experiment using antibodies to BrdU and DCX. Enriched, sham-irradiated mice showed a significant increase in double-labeled (BrdU + DCX) cells ($P < 0.001$), confirming an increased number of adult-born neurons (**Fig. 2c**,**d**).

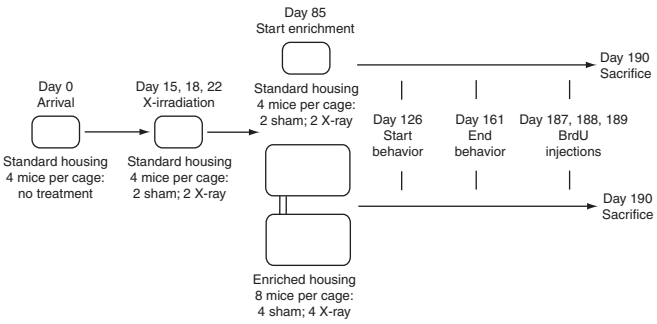

Figure 1 Timeline of experimental procedures. Mice arrived from supplier (day 0) at 8 weeks of age. All procedures conformed to US National Institutes of Health regulations and were approved by the Institutional Animal Care and Use Committees of Columbia University and the New York State Psychiatric Institute.

[1]Department of Biological Sciences, Columbia University, New York, New York 10027, USA. [2]Center for Neurobiology and Behavior, Columbia University, 1051 Riverside Drive, Unit 87, New York, New York 10032, USA. [3]Biopsychology Unit, New York State Psychiatric Institute, 1051 Riverside Drive, Unit 50, New York, New York 10032, USA. [4]Department of Psychiatry, Columbia University, 1051 Riverside Drive, Unit 14, New York, New York 10032, USA. [5]Faculté de Pharmacie, Université Paris-Sud, Tour D1, 2e etage, EA3544, Chatenay-Malabry Cedex, F-92296, France. [6]These authors contributed equally to this work. Correspondence should be addressed to R.H. (rh95@columbia.edu).

Received 1 February; accepted 11 April; published online 30 April 2006; doi:10.1038/nn1696

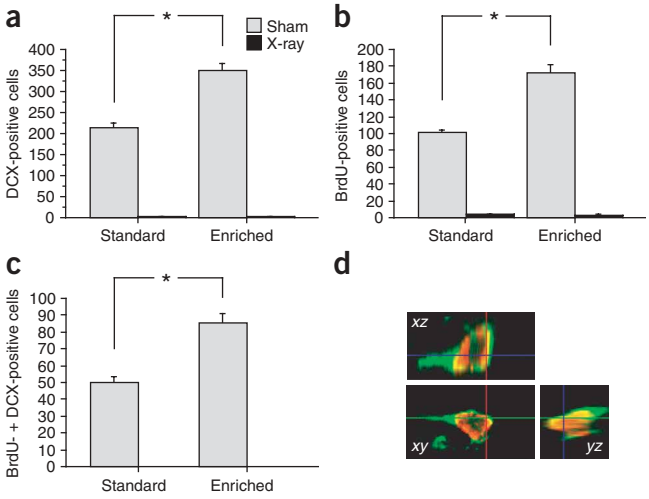

Figure 2 Effects of enrichment and hippocampal X-irradiation on DCX and BrdU immunostaining in the dentate gyrus. (**a**) DCX immunostaining was increased by enrichment ($F_{1,7} = 50.992$, $P < 0.001$) and decreased by irradiation ($F_{1,7} = 856.162$, $P < 0.001$), and there was a significant interaction between housing and irradiation treatment ($F_{1,7} = 50.496$, $P < 0.001$). (**b**) BrdU immunoreactivity was increased by enrichment ($F_{1,8} = 50.748$, $P < 0.001$), and decreased by irradiation ($F_{1,8} = 740.166$, $P < 0.001$), and there was a significant interaction between housing and irradiation treatment ($F_{1,8} = 53.689$, $P < 0.001$). (**c**) Fluorescent double staining for BrdU and DCX. Enrichment produced more double-labeled cells ($F_{1,8} = 27.189$, $P < 0.001$), irradiation reduced the number of double-labeled cells ($F_{1,8} = 380.457$, $P < 0.001$), and there was a significant interaction between housing and irradiation treatment ($F_{1,8} = 26.191$, $P < 0.001$). (**d**) Confocal photomicrographs showing a BRDU + DCX double-positive cell from orthogonal perspectives. Error bars represent s.e.m. * $P < 0.05$, Fisher's PLSD *post-hoc* analysis.

To assess anxiety-like behavior, we tested mice in the novelty-suppressed feeding protocol. Antidepressant drugs produce an anxiolytic effect in this protocol that is blocked by hippocampal X-irradiation[7]. Mice were food-deprived for 24 h and then placed in a brightly lit arena in which food pellets were anchored in the center. Enriched mice showed a decreased latency to feed, consistent with an anxiolytic effect of enrichment (**Fig. 3a**). Notably, irradiation did not moderate the effect of enrichment, indicating that hippocampal neurogenesis is not critical for the effect of enrichment in this protocol. Irradiation also did not alter performance in the nonenriched mice, as reported elsewhere[7]. To ensure that the effect of enrichment was not caused by differences in motivation to feed, we assayed both home-cage food consumption and weight loss due to food deprivation immediately after the novelty-suppressed feeding test. Experimental groups did not differ on either measure (**Fig. 3b** and **Supplementary Data** online). These results indicate that environmental enrichment produces an anxiolytic-like effect that does not require adult hippocampal neurogenesis.

Because enrichment has been reported to enhance habituation to an unfamiliar environment[14], we examined general activity in an enclosed new environment (**Supplementary Methods**). The activity of the enriched mice habituated significantly faster ($P < 0.001$) than that of standard-housed mice, and there were no effects of irradiation (**Supplementary Fig. 3** online and **Supplementary Data**), indicating that this effect of enrichment is also independent of hippocampal neurogenesis.

We assessed spatial learning in the Morris water maze (**Fig. 3c**,**d**). There were two sessions of visible platform training, in which the location of the platform was marked by a salient visual cue, followed by five sessions of hidden platform training, in which the platform location could only be discerned using extra-maze spatial cues. There was no effect of environmental enrichment or irradiation in the visible platform phase. In hidden platform training, enriched mice used a shorter path to the hidden platform, consistent with enhanced hippocampal-dependent spatial learning. Irradiation did not attenuate this effect of enrichment, nor did it impair performance in standard

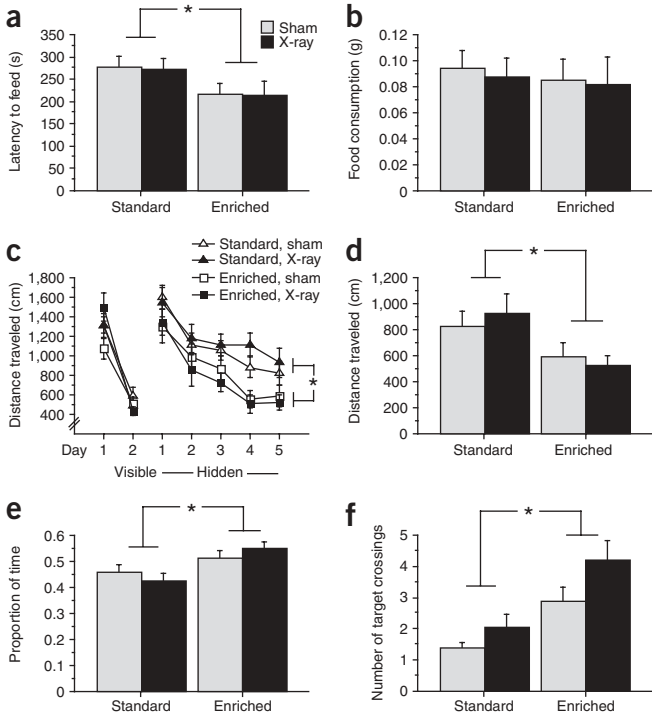

Figure 3 Behavioral effects of enrichment and hippocampal X-irradiation. (**a**) Enriched mice showed a significantly reduced latency to feed in the novelty-suppressed feeding test ($F_{1,50} = 5.236$, $P = 0.026$). There was no effect of irradiation ($F_{1,50} = 0.013$, $P = 0.908$) and no interaction between housing and irradiation treatment ($F_{1,50} = 0.002$, $P = 0.963$). (**b**) There was no effect of enrichment or irradiation on home-cage food consumption during the 5-min period immediately after the novelty-suppressed feeding protocol (**Supplementary Data** and **Methods**). (**c**) The groups performed similarly in the visible platform phase of the Morris water maze. In the hidden platform phase, enriched mice used a more direct route to the platform ($F_{1,60} = 16.395$, $P < 0.001$; note that groups did not differ on the first trial of day 1). There were no effects of irradiation ($F_{1,60} = 0.005$, $P = 0.944$) and no significant interactions. (**d**) On day 5 of the Morris water maze, enriched mice used a more direct route to the platform ($F_{1,60} = 7.401$, $P = 0.009$). There was no effect of irradiation ($F_{1,60} = 0.032$, $P = 0.858$) and no significant interaction ($F_{1,60} = 0.530$, $P = 0.470$). (**e**) Enriched mice spent a larger proportion of time in the target quadrant during the water maze probe trial ($F_{1,60} = 10.078$, $P = 0.002$). There was no effect of irradiation ($F_{1,60} = 0.001$, $P = 0.973$) and no interaction between housing and irradiation treatment ($F_{1,60} = 1.583$, $P = 0.213$). (**f**) During the water maze probe trial, enriched ($F_{1,60} = 16.523$, $P < 0.001$) and irradiated ($F_{1,60} = 5.029$, $P = 0.029$) mice crossed the target location more frequently, but there was no interaction between housing and irradiation treatment ($F_{1,60} = 0.491$, $P = 0.486$). Error bars represent s.e.m. Complete results in **Supplementary Data**.

housing conditions, verifying previous reports[5,6]. To confirm that mice were navigating their way to the platform using extra-maze visual cues, a probe trial was performed (**Fig. 3e,f**) in which the platform was removed and mice were placed in the pool for 60 s. The enriched mice spent a greater proportion of time in the target quadrant and made more target area crossings. Irradiation did not attenuate this enrichment effect. Notably, irradiated mice, regardless of their housing environment, made more target crossings in the probe trial, even though they did not spend more time in the target quadrant. Nevertheless, our results indicate that enrichment improved spatial learning in the water maze and that adult hippocampal neurogenesis was not required for this effect.

We were concerned that irradiation might alter mouse interactions with the enriched environment, thereby altering the behavioral effects of enrichment. Therefore, we performed both an observational assessment of mice in the enriched environment as well as a quantification of wheel-running distance (**Supplementary Methods**). Neither of these measures was altered by irradiation (**Supplementary Data**), suggesting that both sham and irradiated mice experienced equivalent amounts of environmental enrichment. We were also concerned that compensatory mechanisms might develop during the 2-month wait between irradiation and enrichment. To assess this possibility, a separate group of mice was irradiated and then immediately transferred to enriched housing. After 6 weeks of enrichment, mice were tested in the novelty-suppressed feeding protocol; the behavioral results were identical to those obtained in mice given the 2-month postirradiation recovery period (see **Supplementary Fig. 4** online).

Our results seem to conflict with a recent study showing that the antimitotic agent MAM blocks the effect of enrichment on novel-object recognition in rats, suggesting a role for neurogenesis in this behavioral task[4]. There are several possible explanations for this discrepancy. First, the behavioral assay is different from the assays presented here; hippocampal neurogenesis may be required for some behavioral effects of enrichment and not others. Second, there is a species difference. Enrichment and hippocampal neurogenesis may have different effects in mice than in rats. Last, unlike our irradiation procedure, the effects of MAM were not limited to the hippocampus. MAM reduces cell division throughout the body, and, although no side effects were observed at the low dose used in the previous study, slightly higher doses cause weight loss and reductions in locomotion[15].

In summary, the housing of adult mice in an enriched environment caused an increase in adult hippocampal neurogenesis, decreased anxiety-like behavior, faster habituation to an unfamiliar environment and improved spatial learning. Arrest of adult hippocampal neurogenesis did not markedly affect performance in these behavioral protocols, nor did it attenuate the effects of environmental enrichment. Therefore, the effects of enrichment on spatial learning, habituation to an unfamiliar environment, and conflict-based anxiety do not require adult hippocampal neurogenesis in our experimental conditions. This result is in contrast with recent data demonstrating that neurogenesis is required for the anxiolytic-like effects of antidepressants in mice[7]. We propose that anxiolytic effects can be achieved through multiple pathways, including neurogenesis-dependent and -independent mechanisms. The effects of environmental enrichment on anxiety-like behavior and spatial learning seem to be mediated by a neurogenesis-independent mechanism. Candidate mechanisms may include the upregulation of growth factors such as brain-derived neurotrophic factor, as well as morphological changes such as increased dendritic branching and synaptogenesis[2].

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We thank J.A. Gordon, C.O. Lacefield and A. Sahay for their comments on the manuscript. This work was supported by US National Institute of Mental Health grants 5R01MH068542-03, 5T32MH018264-22 and 5F31MH069089-02.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Published online at http://www.nature.com/natureneuroscience
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Gage, F.H. *Science* **287**, 1433–1438 (2000).
2. van Praag, H., Kempermann, G. & Gage, F.H. *Nat. Rev. Neurosci.* **1**, 191–198 (2000).
3. Kempermann, G., Kuhn, H.G. & Gage, F.H. *Nature* **386**, 493–495 (1997).
4. Bruel-Jungerman, E., Laroche, S. & Rampon, C. *Eur. J. Neurosci.* **21**, 513–521 (2005).
5. Shors, T.J., Townsend, D.A., Zhao, M., Kozorovitskiy, Y. & Gould, E. *Hippocampus* **12**, 578–584 (2002).
6. Snyder, J.S., Hong, N.S., McDonald, R.J. & Wojtowicz, J.M. *Neuroscience* **130**, 843–852 (2005).
7. Santarelli, L. *et al. Science* **301**, 805–809 (2003).
8. Brown, J. *et al. Eur. J. Neurosci.* **17**, 2042–2046 (2003).
9. Gray, J.A. & McNaughton, N. *The Neuropsychology of Anxiety* (Oxford Univ. Press, New York, 2000).
10. Nilsson, M., Perfilieva, E., Johansson, U., Orwar, O. & Eriksson, P.S. *J. Neurobiol.* **39**, 569–578 (1999).
11. Roy, V., Belzung, C., Delarue, C. & Chapillon, P. *Physiol. Behav.* **74**, 313–320 (2001).
12. Monje, M.L., Mizumatsu, S., Fike, J.R. & Palmer, T.D. *Nat. Med.* **8**, 955–962 (2002).
13. Couillard-Despres, S. *et al. Eur. J. Neurosci.* **21**, 1–14 (2005).
14. Elliott, B.M. & Grunberg, N.E. *Behav. Brain Res.* **165**, 187–196 (2005).
15. Dupret, D. *et al. Eur. J. Neurosci.* **22**, 778–783 (2005).