Neuroplasticity in Old Age: Sustained Fivefold Induction of Hippocampal Neurogenesis by Long-term Environmental Enrichment

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Neurons are continually born from endogenous stem cells and added to the dentate gyrus throughout life, but adult hippocampal neurogenesis declines precipitously with age. Short-term exposure to an enriched environment leads to a striking increase in new neurons, along with a substantial improvement in behavioral performance. Could this plastic response be relevant for explaining the beneficial effects of leading "an active life" on brain function and pathology? Adult hippocampal neurogenesis in mice living in an enriched environment from the age of 10 to 20 months was fivefold higher than in controls. Relatively, the increase in neuronal phenotypes was entirely at the expense of newly generated astrocytes. This cellular plasticity occurred in the context of significant improvements of learning parameters, exploratory behavior, and locomotor activity. Enriched living mice also had a reduced lipofuscin load in the dentate gyrus, indicating decreased nonspecific age-dependent degeneration. Therefore, in mice signs of neuronal aging can be diminished by a sustained active and challenging life, even if this stimulation started only at medium age. Activity exerts not only an acute but also a sustained effect on brain plasticity.

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It is common sense and backed by epidemiological data that to lead an "active" life is beneficial for mind and brain.[1–3] Both physical and intellectual activity have a positive influence on the incidence of neurodegenerative disorders and cognitive decline.[2,4] Knowledge about the cellular basis of this effect, however, is scarce, despite a vast literature on experience-dependent changes in the brain on a subcellular, biochemical, and molecular level.[5–7] The hippocampus is of particular interest in this context because of its importance for higher cognitive functions, especially learning and memory, and its pivotal involvement in degenerative disorders of the aging brain.

The current state of knowledge indicates that adult neurogenesis contributes to neuroplasticity in the hippocampal dentate gyrus throughout life.[8–14] In older age, baseline levels of adult hippocampal neurogenesis are very low[11–13]; in relative terms, however, exposure to a challenging environment evokes a much larger upregulation of adult neurogenesis in old animals than in younger ones.[11] Whereas these data show that the old brain still has the ability to acutely react to functional challenges with a neurogenic response, it remained unclear whether this type of cellular plasticity could be linked to the known positive effects of continued sensory stimulation and activity on the aging brain.

As more studies support the view that adult hippocampal neurogenesis is involved in hippocampal function, theories arise that link a failure of neurogenic regulation to hippocampal pathology, most notably major depression[15–17] and temporal lobe epilepsy with ammons horn sclerosis.[18,19] Suggestive links also exist for Alzheimer's disease.[20,21] All of these conditions share symptoms of hippocampal dysfunction and cognitive alterations, most prominently dementia or pseudodementia. If these theories hold, it will be reasonable to assume that adult hippocampal neurogenesis is a key factor in guarantying functional hippocampal integrity in old age. As such, diminished regulation of adult hippocampal neurogenesis might help to explain aspects of "normal" and disease-related cognitive decline with increasing age.

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Given the preventive effects of an intellectually and physically active life to protect from cognitive impairment and neurodegenerative disorders,[2,3] we asked to what extent hippocampal neuroplasticity and its activity-dependent regulation might be part of the cellular mechanisms induced by this continued activity and leading to the observed benefits. If merely changes in the environment, rather than sustained exposure to a certain level of environmental complexity, were responsible for the increase in new neurons, the changes in morphology and behavior would be transient with longer exposure to the changed environment, due to adaptation.

Ten-month-old mice were assigned randomly to either regular laboratory housing conditions or an enriched environment, "a complex combination of inanimate and social stimulation."[6,7,22] Behavioral tests were performed to assess functional effects of the procedure after 10 more months.

Proliferating cells in the subgranular zone of the aging dentate gyrus were labeled by injection of thymidine analog bromodeoxyuridine (BrdU) that is incorporated into the DNA of cells in S-phase[23,24] and consecutively visualized by immunofluorescence and confocal microscopy. The phenotypes of newly generated neurons were assessed by detecting colocalization of BrdU and neuronal nuclear marker NeuN.[25]

We designed the experiment to determine whether long-term environmental enrichment would have sustained effects on brain morphology and adult hippocampal neurogenesis or whether the activity- and experience-dependent regulation would wear off after continued stimulation. We reasoned that if the latter were the case it would be unlikely that baseline levels of adult hippocampal neurogenesis would constitute an integral part of activity-dependent benefits on the aging brain. If long-term stimulation had sustained beneficial effects, how would such a finding relate to general morphological signs of aging such as lipofuscin content in hippocampal neurons? How would it relate to functional and behavioral parameters? Behind these questions lies the fundamental issue, whether or not the aging brain continues to make use of the plastic potential inherent to adult hippocampal neurogenesis to cope with environmental challenges.

Materials and Methods

Animals and Experimental Design

Thirty female C57BL/6 mice, 10 months old, were obtained from the aging colony at the National Institute of Aging. Fifteen animals were housed in four standard cages (Ctr); 15 were placed into an enriched environment (Enr) as described previously.[11] Briefly, the enriched environment consisted of a large, specially designed cage with approximately $1m^2$ floor area. This cage was equipped with a rearrangeable set of plastic tubes, a small running wheel, nesting material, and toys. All mice had access to food and water ad libitum. No special diet was part of the enrichment protocol.

Because male mice show territorial behavior with stressful consequences for the subordinate males, and severe stress is known to decrease adult hippocampal neurogenesis,[26] this experiment was conducted using females.

Animals lived in their respective experimental conditions for 10 months. Three animals (one Ctr, two Enr) died over the experimental period. During the last 12 days of these 10 months, all mice received one daily intraperitoneal injection of 10mg/ml BrdU (5-bromo-2-deoxyuridine; Sigma, St. Louis, MO) in sterile 0.9% NaCl solution (daily dose, 50μg/gm body weight). On day 1 after the last injection, five animals from both groups were killed as described below. The remaining animals continued to live under their respective experimental conditions for 28 more days. During the last week, they were examined on behavioral tests (see below).

Serum Corticosterone

One day after the last injection of BrdU and under brief chloroform anesthesia, blood samples approximately equal to 50μl were taken retroorbitally. Samples were analyzed with a corticosterone radioimmunoassay (ICN Biomedicals, Costa Mesa, CA), following the protocol provided by the manufacturer.

Behavioral Tests

ACTIVITY CHAMBER. Overall activity and habituation to a new environment were examined using an automated activity recording system (San Diego Instruments, San Diego, CA) of approximately $1m^2$ floor area. Animals were placed in the dark and empty chamber for 1 hour, and interruptions in a grid of infrared beams caused by movements of the animal were recorded and analyzed in 5-minute intervals.

ROTAROD. To test aspects of motor coordination, physical fitness, and procedural learning, we tested animals on a rotarod (Columbus Instruments, Columbus, OH) for 4 consecutive days for three trials per day. Each mouse was placed on the rotating rod at a start speed of 5rpm. When the animal had found balance, the trial was started and the rod accelerated with $20rpm^2$ to a maximum of 65rpm (equal to 3 minutes). The length of time the animal could hold itself on the rotating rod was recorded.

WATER MAZE. Spatial learning was examined in a water maze task with hidden platform. The platform was hidden 1cm below the surface of the water, which had been made opaque with nontoxic white paint and remained at a constant position. The test was conducted over 6 consecutive days with four trials per day and an intertrial interval of 10 seconds on the platform. The four starting points were varied daily. Time (latency) and swim path needed to navigate to the platform were recorded by an automated video tracking

system (San Diego Instruments). If the animal had not successfully navigated to the platform after a maximum of 40 seconds, it was set on the platform.

Tissue Preparation

The mice were killed with an overdose of anesthetics (acepromazine, xylazine, and ketamine) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in phosphate buffer (0.1M). The brains were stored in the fixative overnight and then transferred into 30% sucrose. Coronal sections of 40μm thickness were cut from a dry ice-cooled block on a sliding microtome (SM 2000 R; Leica, Bensheim, Germany). The sections were stored at −20° C in cryoprotectant solution containing 25% ethylene glycol, 25% glycerin, and 0.05M phosphate buffer.

Bromodeoxyuridine Immunohistochemistry and Immunofluorescence

BrdU immunohistochemistry and immunofluorescence were performed as described previously.[11,27] All antibodies were diluted in Tris-buffered saline containing 0.1% Triton X-100 and 3% donkey serum (Tris-buffered saline–plus). Primary antibodies were monoclonal rat anti-BrdU (Harlan Sera-Lab, Loughborough, England) 1 to 500; monoclonal mouse anti-NeuN (Chemicon, Temecula, CA) 1 to 100, and polyclonal rabbit anti-S100β (SWant, Bellinzona, Switzerland) 1 to 2,000. For indirect immunofluorescence, the following secondary antibodies were used (all from Jackson ImmunoResearch Laboratories, West Grove, PA; all 1:250): donkey anti–rabbit IgG conjugated with CY5; donkey anti–mouse IgG conjugated with fluorescein isothiocyanate, and donkey anti–rat IgG conjugated with rhodamine X.

For light microscopic quantification of BrdU-labeled cells, a series of every sixth 40μm section was used. Immunohistochemistry followed the peroxidase method with biotinylated donkey anti–rat IgG (Jackson ImmunoResearch Laboratories), 1 to 500, ABC Elite reagent (Vector Laboratories, Burlingame, CA), and diaminobenzedine (Sigma) as chromogen.[11,27]

The phenotypes of 50 BrdU-labeled cells per animal were determined with immunofluorescent triple labeling and spectral confocal microscopy (Leica TCS SP2).

Quantification

To determine the number of BrdU-labeled cells per dentate gyrus, we used a modified version of the fractionator principle, partly based on a method devised by Williams and Rakic.[28] Sampling of BrdU-positive cells was done throughout the extent of the granule cell layer in its rostrocaudal extension. Because BrdU-labeled cells are comparatively rare, no counting frames could be used. The stereological procedure was modified to exclude the uppermost focal plane only, but otherwise the granule cell layer was exhaustively counted. The resulting number of BrdU-positive cells then was related to the granule cell layer volume by multiplying the value by 6, because every sixth section had been used.

Cells with lipofuscin deposits were recognized by their characteristic appearance and fluorescence of similar intensity in all three channels investigated. Only heavily loaded cells, in which the deposits obscured more than half of the nucleus, were counted.

All cell counts were conducted in a blinded fashion.

Statistical Analyses

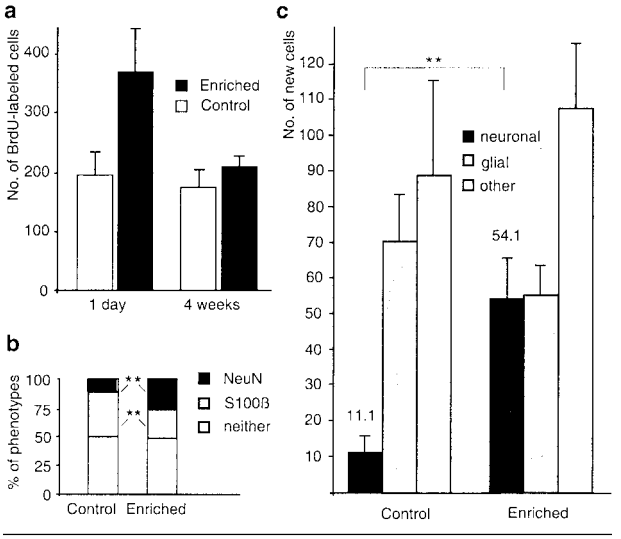
All statistical analyses were performed with Microsoft Excel (Office 98) and Statview 5.0 for Macintosh, and analysis of variance (ANOVA) or two-sided *t* tests were applied when appropriate.

Results

After long-term environmental enrichment of 10 months (and 1 day after the last injection of BrdU), on a level of descriptive statistics almost twice as many BrdU-labeled cells were found in the dentate gyrus of mice living under enriched conditions (Enr) as compared with controls (Ctr; Fig 1). However, this comparison was not statistically significant (all tests were ANOVA; $F = 4.149$; $p = 0.0760$), indicating no clear difference in proliferative activity in the subgranular zone.

Survival of BrdU-labeled cells was assessed 4 weeks later. Again, no statistically significant difference was

*Fig 1. Increased hippocampal neurogenesis in aging mice, living in an enriched environment for 10 months. (a) Number of bromodeoxyuridine (BrdU)-labeled cells per dentate gyrus at 1 day and 4 weeks after the last of 12 injections of BrdU. Numbers are means ± SEM. (b) The distribution of phenotypes (see Fig 2) among the BrdU-labeled cells at 4 weeks after injection of BrdU. There is a relative phenotypic shift from glial cells (BrdU/S100β-positive) to neurons (BrdU/NeuN-positive). (c) Net neurogenesis, gliogenesis, and production of undetermined phenotypes were calculated by multiplying the numbers of BrdU-labeled cells at 4 weeks after injection by the ratio of phenotypes for each animal. **p < 0.01.*



found for the total number of BrdU-positive cells, regardless of their phenotype ($F = 0.920$; $p < 0.3510$; see Fig 1). Taken together, these data indicate that long-term enrichment and activity do not have a clear measurable effect on the number of BrdU-labeled cells early or late after BrdU administration.

However, analysis of phenotypes showed that 4 weeks after BrdU in Enr 26% of the BrdU-labeled cells were also NeuN-positive, but only 8% in Ctr (unpaired t test; df = 15; $p = 0.0085$; Figs 1 and 2). Conversely, the Ctr group produced relatively more astrocytes ($p = 0.0088$). There was no difference for the cells displaying neither phenotype.

Therefore, the net neurogenesis (surviving BrdU-labeled cells × ratio of NeuN/BrdU-positive cells) was five times higher in Enr than in Ctr mice ($F = 11.079$; $p = 0.0046$). Thus, the induction of adult neurogenesis occurred at the expense of gliogenesis, but in absolute numbers no significant decrease in hippocampal gliogenesis occurred. Taken together, these data can be interpreted as a sustained survival-promoting effect acting selectively on new neurons and induced by long-term exposure to an enriched environment.

Lipofuscin deposits in neurons are a prominent sign of aging in the brain and are considered to be relatively nonspecific.[29] Hippocampal granule cells are among the cell populations particularly vulnerable to lipofuscin accumulation. Lipofuscin pigments are remnants from lipid peroxidation and are thought to indicate chronic oxidative stress.[30] It appears that to some degree their quantity can be correlated with a decline in cognitive function, but epidemiological data are lacking, and no causal link is known. We examined lipofuscin in the dentate gyrus as a measure of "cellular health" in this region. Figure 3a displays the microscopic appearance of cells loaded with lipofuscin granules. When the number of lipofuscin-loaded granule cells was quantified, the granule cell layer of Ctr mice contained 50% more lipofuscin cells than Enr animals (see Fig 2; ANOVA; $F = 5.210$, $p = 0.0375$). This implies that the dentate gyrus of the Enr group appears "healthier" for a decrease in degeneration (lipofuscin deposits) and for an increase in regeneration (neurogenesis). It also indicates that, in the aging brain, neurogenesis in the dentate gyrus normally proceeds in a microenvironment of degeneration, highlighted by intracellular lipofuscin accumulation.

It has been speculated that lipofuscin load might be influenced by altered regulation along the hypothalamic-pituitary-adrenal axis. For example, there is evidence that increased adrenocorticotropic hormone levels can reduce the lipofuscin load in the adrenal cortex.[31] It also has been described that adrenalectomy maintains hippocampal neurogenesis in the old rat brain at an elevated

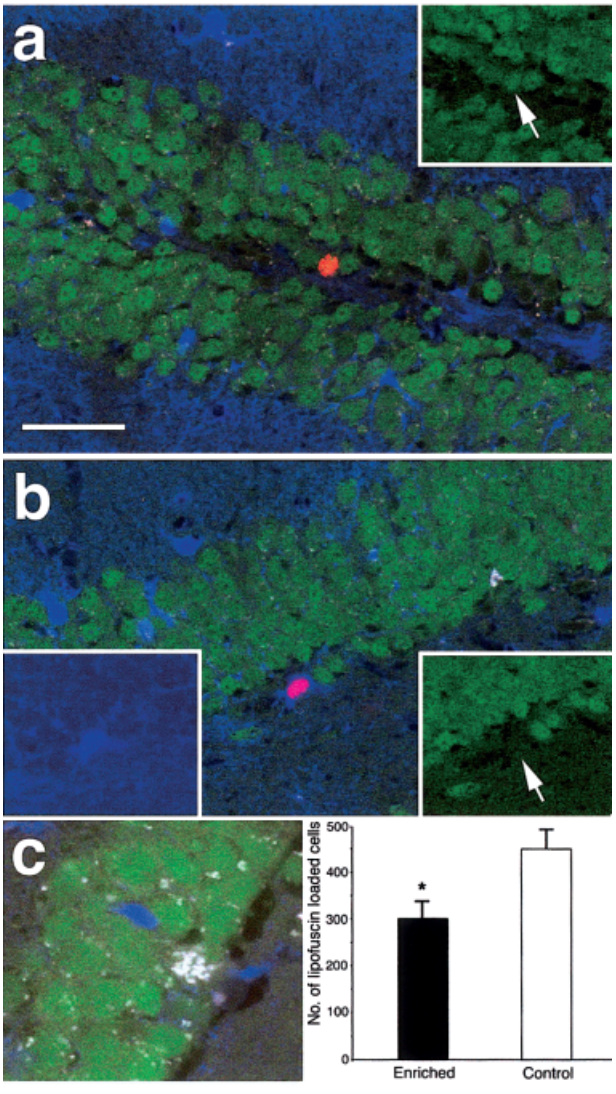


*Fig 2. Phenotypic differentiation of new generated cells in the dentate gyrus. (a) One cell showing colocalization for bromodeoxyuridine (BrdU; red) and neuronal marker NeuN (green), 4 weeks after the last injection of BrdU in a 20-month-old mouse from the enriched group (Enr). The arrow in the inset indicates the new neuron without the BrdU signal. (b) A new astrocyte, as identified by S100β immunoreactivity (blue) and BrdU colocalization (which appears pink) in a control animal. The left inset shows the new astrocyte without the BrdU signal; the arrow in the right insert indicates that at the location of the S100β immunoreactivity no NeuN signal can be detected. (c) Lipofuscin granules can be made visible because of their strong autofluorescence over a broad spectrum of emission wavelengths (appearing white). Although most lipofuscin is found evenly distributed, some cells are heavily loaded. If these cells were quantified (right), a significant reduction in the number of lipofuscin loaded cells was found in the Enr group compared with controls. Scale bar in a = 50μm for a and b, and 100μm for c. *$p \leq 0.01$.*

level.[13] To gain a first impression whether endogenous glucocorticoid levels might play a role in the regulation seen in this experiment and to establish one measure of

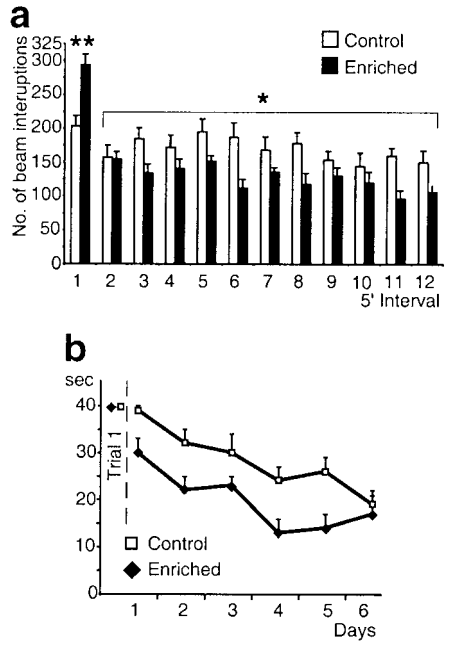


*Fig 3. Long-term enrichment affects performance in behavioral tests. (a) Animals were individually placed in an empty box. Locomotor activity was recorded automatically. When faced with the new environment, Enr mice showed significantly more exploratory behavior in the first 5-minute interval than controls (*p = *0.0009) but significantly less locomotor activity over the remaining test period (*p = *0.0133). This can be interpreted as a faster habituation by Enr mice to a new environment of low complexity. (b) The learning curve (latency to reach the platform in seconds) of Enr mice was significantly below Ctr mice (overall analysis of variance,* p = *0.0020). For the difference on day 1, note that there was no difference on the first trial (of four). Enr mice learned the task faster than controls; the recall of the learned spatial information, as assessed by a probe trial on day 7, was not different between the groups (not shown).* $^{*}p \leq 0.05$; $^{**}p \leq 0.01$.

hormonal status baseline, we determined serum corticosterone levels by a radioimmunoassay. The free hormone competes with radioactively labeled hormone for binding to a specific antiserum. The binding of the radioactive (exogenous) hormone was 80.13 ± 0.38% in Ctr and 55.58 ± 0.33% in Enr ($p = 0.0052$). Plotted onto the standard curve, these values correspond to a serum corticosterone level of approximately equal to 20ng/ml in Ctr and approximately equal to 60ng/ml in Enr. This single measurement does not allow final conclusions about a potential role of glucocorticoids in activity-dependent regulation of adult neurogenesis, but the result of an increased corticosterone level in the Enr group argues against a solitary role of glucocorticoids in promoting neurodegeneration.

The morphological changes were associated with significant behavioral alterations (see Fig 3). The behavioral data do not substantiate per se causal links to the morphological results, but they highlight the functional contexts in which the morphological effects occur. As a gross measure of bodily adaptation, body weight was determined in both groups. Controls weighed 26.07 ± 0.81gm, whereas Enr mice weighed 24.47 ± 0.24gm ($F = 8.854$; $p = 0.0600$). In this experiment, this difference was not statistically significant, but it fit the trend suggested by other studies including our own.[27,32–34]

When placed into a new environment, Enr mice showed significantly more exploratory behavior in the first 5-minute interval than controls (ANOVA; $F = 16.176$, $p = 0.0009$) but significantly less locomotor activity over the remaining test period (see Fig 3; $F = 7.748$, $p = 0.0133$). This can be interpreted as a faster habituation to a new environment of low complexity in the Enr group. In addition, the Enr group remained on the accelerating rotarod almost twice as long as the Ctr group (85.6 ± 3.8 seconds vs 47.4 ± 2.8 seconds; means over four trials; $F = 43.567$, $p < 0.0001$).

In the Morris water maze, the learning curve of the Enr group was significantly below the curve for the Ctr group (see Fig 3 for latency, overall $F = 13.278$, $p = 0.0020$; swim path, $F = 5.779$, $p = 0.0279$, not shown). Although this might reflect "faster learning," the general view to be drawn from these behavioral data is that Enr mice were more adaptive in exploration and had greater locomotor skills and endurance and showed measurable, but not necessarily specific, differences when tested in the water maze. When a probe trial was performed with the escape platform removed from the water maze pool, the difference during the acquisition phase was not followed by a difference for parameters assessing recall of the learned information (relative time and swim path attributed to the target quadrant, the ratios between the target and the opposite quadrant and the number of entries to the target quadrant; data not shown). The behavioral data confirm that the morphological findings occurred in the context of measurable changes in behavior, induced by long-term environmental enrichment.

Discussion

The findings of experience-induced increases in adult hippocampal neurogenesis on long-term challenge by environmental enrichment indicate that the stimulatory effect does not wear off on continued exposure to a complex environment. This has important consequences for understanding the role of adult hippocampal neurogenesis and its regulation for hippocampal function in health and disease. As in this experiment, complexity of the environment did not change besides

regular rearrangements, the finding of more adult hippocampal neurogenesis does not reflect an acute response to a novel stimulus, but rather a persistently elevated baseline level of neurogenesis. This can be interpreted as a significantly enhanced potential for neuroplasticity in aging Enr mice.

The selection of a 10-month period was arbitrary but was supposed to cover "the second half of life" and the entry to senescence. Menopause of a female C57BL/6 mouse is at roughly 10 to 13 months of age; the median life span is approximately 2 years.[35] From our data, we cannot conclude whether there are times in which the brain is particularly susceptible to the effects that we saw. Also, no exact dose–response relationship for effects of environmental enrichment has been established to date.

Our results are in accordance with data indicating that even after discontinued environmental stimulation, effects on the population of proliferating stem or progenitor cells in the dentate gyrus can be detected.[27] The earlier study, however, had yielded ambiguous results, mainly because very high variances interfered with clear statistical analysis.

The finding that at 1 day after BrdU the number of labeled cells in Enr was almost twice as high as in Ctr (even though the comparison was not significant, but $p = 0.0760$) appears surprising but matches an earlier observation.[27] Generally, we have found that in C57BL/6 mice environmental enrichment had a survival promoting effect on newly generated cells in the dentate gyrus. In contrast, in 129/SvJ we had also seen a strong induction of stem or progenitor cell proliferation.[36] As voluntary running enhanced cell proliferation in C57BL/6, this finding might indicate that physical activity (and fitness) plays a more dominant role in environmental enrichment on long-term exposure than short-term exposure. The rotarod data confirm how much better the physical fitness of Enr mice was compared with the Ctr group. Alternatively, a potential difference in cell proliferation might reflect a change in the activation of genetic programs underlying the experience-dependent regulation of adult hippocampal neurogenesis. Last, as we have argued previously, the increased cell proliferation might reflect a preceding survival-promoting effect on the proliferating stem or progenitor cells themselves.[27] Further experiments are needed to answer this question. The finding of strain differences in adult neurogenesis[37,38] and in certain aspects of the neurogenic response to environmental enrichment[36] also will allow invention of strategies to search for key genes involved in the regulation of adult hippocampal neurogenesis.[39,40]

Remarkably, the net effect at 4 weeks after BrdU seen in this experiment was not recognizable on the level of BrdU-positive cells but became apparent only after phenotypic analysis. Relatively, the induction of adult neurogenesis occurred at the expenses of gliogenesis. In absolute numbers, no significant decrease in hippocampal gliogenesis occurred. The ratio of cells displaying neither phenotype remained unchanged. This is suggestive of a experience-induced phenotypic shift,[38] but it remains unclear whether the underlying mechanism is a selective survival-promoting effect (with increased survival of neurons and decreased survival of astrocytes) or an interaction with the fate choice decision on the level of a multipotent hippocampal stem or progenitor cells.[41]

An important aspect of this experiment was that the increase in baseline adult neurogenesis occurred on the background of signs of decreased degeneration. Lipofuscin is a product of lipid peroxidation that accumulates over time in neuronal and nonneuronal cells.[30] Lipofuscin accumulation is a slow process that is considered to be a sign of long-standing oxidative stress. Increased levels of lipofuscin deposits have been linked to cognitive decline. Despite a vast literature on effects of environmental enrichment on brain morphology, lipofuscin load never has been studied in these models. Our results indicate that lipofuscin deposits in neurons can be influenced by function, because long-term enrichment significantly reduced the number of lipofuscin-loaded granule cells in the dentate gyrus. This implies that the sustained induction of adult neurogenesis takes place in an cellular microenvironment, which is characterized by reduced neuronal damage. On a superficial level, this can be interpreted as the dentate gyrus of enriched living mice being generally more "healthy," both for degeneration and regeneration. Caloric restriction, which has robust effects on longevity, can induce adult hippocampal neurogenesis[42] and reduces hippocampal lipofuscin load.[43] In this context, it is not known whether the fact that mice in an enriched environment tend to be lighter than controls reflects some sort of natural self-administered caloric restriction or whether increased physical activity is responsible for the effect.

Regarding "cellular health," however, the question arises of to what extent hippocampal cell death and birth are interdependent.[44] It is the new cells rather than the old ones that undergo apoptosis in the adult dentate gyrus,[45] suggesting a mechanism not unlike during embryogenesis.[46] There, a surplus of neurons is generated and consequently selected for function. Young and colleagues have found that in enriched-living mice the number of apoptotic cells in the dentate gyrus decreases.[47]

One of the suspected key regulators in hippocampal cell damage and also negative regulators of adult hippocampal neurogenesis are glucocorticoids. There is strong evidence of detrimental effects of elevated corti-

costerone levels on the hippocampus, particularly in the context of chronic stress.[48–50] The relationship between glucocorticoids and adult neurogenesis, however, appears to be complex. For example, voluntary running robustly induces adult hippocampal neurogenesis,[51] but running increases glucocorticoid levels both in humans[52] and in animals.[53] In this study, we found that long-term environmental enrichment and persistently elevated levels of adult hippocampal neurogenesis are associated with significantly higher levels of serum corticosterone than controls. However, only a single measurement was taken. Still, this result is in accordance with findings that animals in an enriched environment have elevated serum corticosterone levels.[54] It does not argue against an important role of glucocorticoids in regulating hippocampal plasticity[26] but supports a view that assigns a complex and presumably dose-dependent relationship. Coincidentally, there is evidence that increased corticotropin levels can reduce the lipofuscin load in the adrenal cortex.[31] Further studies specifically designed to address this point will have to clarify how corticoids interact with adult neurogenesis and neuronal plasticity in the aging brain. Regarding potential effects of other hormones, the situation is even less clear. Few studies have addressed the issue for example of gender differences directly, and although there appear to be moderate and transient effects on cell proliferation, no change in net neurogenesis has been observed.[55,56]

Regulation of adult neurogenesis is an important sign of neuroplasticity in the aging brain. Neuroplasticity receives and deserves widespread attention because of its relevance for understanding (and treating) neurodegenerative disease and age-related declines in cognitive functions, in particular memory. Using correlational data, we have speculated about the potential functional relevance of adult hippocampal neurogenesis.[57]

To date, no proof of the hypothesis that new neurons are necessary for hippocampal function has been discovered, although the link has been inferred and it has been shown that the newly generated granule cells show electrophysiological characteristics indistinguishable from the surrounding older granule cells.[58] As a coarse measure, increases in adult neurogenesis have been paralleled by improved performance on learning tasks in several studies.[11,51,59] Specific learning stimuli can elicit a survival-promoting effect on the progeny of neuronal stem or progenitor cells in the dentate gyrus,[60] and toxically reduced cell proliferation in the dentate gyrus was accompanied by diminished performance of a hippocampus-dependent conditioning task.[61] Physical activity had a strong upregulatory influence on adult hippocampal neurogenesis and induced hippocampal long-term potentiation as an important electrophysiological correlate of learning.[51] In contrast, one of the speculative reasons why preweaning enrichment did not influence adult neurogenesis later in life might lie in the fact that this experimental paradigm lacks physical activity.[34] Therefore, we have speculated that the contribution of adult hippocampal neurogenesis to hippocampal plasticity might require a physically active pursuit as opposed to a passive sensory stimulation. Our data support this view, although additional studies will have to prove this hypothesis.

Taken together, our data present first evidence of the neurogenic events that might underlie beneficial effects of an active and stimulated life on brain integrity and cognitive function as they have been repeatedly described for humans. The concept of environmental enrichment in studies with inbred rodents cannot be easily applied to the human condition. What is called "enriched" under laboratory conditions is arguably substantially impoverished against a rodent habitat in the wild (for a more detailed discussion, see, eg, van Praag and colleagues[6] and Cummins and colleagues[62]). However, the key point in this and other studies with such a paradigm is not to gain a precise knowledge of individual stimuli sufficient to elicit the effect of interest. These will certainly greatly differ between rodents and humans. However, our data show a more fundamental process: the aging hippocampus maintains the potential for cellular plasticity, and experience or activity influence the degree of this plasticity.

Earlier animal studies of experience-dependent hippocampal neuroplasticity on the level of single neurons had focused on acute effects and were not tailored to address the question, whether the beneficial effects on brain structure seen under acute exposure to an enriched environment would be identical to those evoked by long-term stimulation. In the context of the potential role of neurogenesis and hippocampal plasticity in chronic neurodegenerative disorders and their prevention and treatment, however, the new data allow the conclusion that baseline levels of adult hippocampal neurogenesis might be one parameter determining the activity-induced "health" of the aging brain.

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