LESION-INDUCED PROLIFERATION OF NEURONAL PROGENITORS IN THE DENTATE GYRUS OF THE ADULT RAT

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Abstract—In order to determine whether granule cell death stimulates the proliferation of granule cell precursors in the dentate gyrus of the adult rat, we performed both excitotoxic and mechanical lesions of the granule cell layer and examined the numbers of proliferating cells at different survival times. Using [3H]thymidine autoradiography, bromodeoxyuridine labelling and proliferating cell nuclear antigen immunohistochemistry, we observed an increase in proliferating cells on the lesioned side compared to the unlesioned side 24 h after surgery. A significant positive correlation between the extent of granule cell damage and the number of proliferating cells was observed. Combined [3H]thymidine autoradiography and immunohistochemistry for cell-specific markers revealed that the vast majority of proliferating cells had the morphological characteristics of granule cell precursors and were not immunoreactive for vimentin, a marker of immature glia. Combined [3H]thymidine autoradiography and terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling for degenerating cells showed that the proliferating cells did not rapidly degenerate. Three weeks after the lesion, most cells produced in response to the lesion had the morphological characteristics of mature granule neurons, were located in the granule cell layer and expressed markers of mature granule neurons, including neuron-specific enolase, the N-methyl-D-aspartate receptor subunit NRI and calbindin.

These findings suggest that granule cell death stimulates the proliferation of precursor cells, many of which survive and differentiate into mature granule neurons.

These cells are incorporated into the granule cell layer, extend axons into the mossy fibre pathway, form synaptic contacts and express neuronal markers.

The proliferation of granule cell precursors can be stimulated by removing the adrenal glands in adult rats, a treatment that paradoxically also results in massive death of granule neurons. Following adrenalectomy in adulthood, distinct populations of cells in the dentate gyrus either divide or die. An unusual association exists between cell proliferation and cell death in the developing dentate gyrus as well. Whereas most developing brain regions undergo a well-circumscribed period of cell production followed by a distinct period of cell death, the granule neuron population of the dentate gyrus undergoes periods of cell death coincident with or immediately preceding periods of increased cell production. Collectively, these findings raise the possibility that granule cell death stimulates proliferation of granule cell precursors and the eventual production of new granule neurons.

In order to determine whether granule cell death stimulates the proliferation of granule cell precursors, we performed both excitotoxic and mechanical lesions of the granule cell layer and examined the...
numbers of proliferating cells labelled with \([^{3}H]\) thymidine, bromodeoxyuridine (BrDU) or proliferating cell nuclear antigen (PCNA). To determine whether cells that proliferate after lesion survive and differentiate, we also examined the characteristics of \([^{3}H]\) thymidine-labelled cells at different survival times after surgery with cell-specific markers, including vimentin, a marker of immature glia, and neuron-specific enolase (NSE), calbindin and the glutamate receptor subtype N-methyl-D-aspartate (NMDA) receptor subunit NR1, markers of mature granule neurons.

**Experimental Procedures**

Animal treatments

A dult male Sprague-Dawley rats (Charles River, n=52, 200-300 g, 50-65 days old) were anaesthetized with nembutal (50 mg/kg) and stereotaxically injected using a Hamilton syringe with either 0.5 µl ibotenic acid (1% w/v in saline) or saline alone (2.0 µl or 5.0 µl) in the granule cell layer (gcl), stereotaxic coordinates AP, -3.8; LM, 1.8; DV, -3.0. Ibotenic acid destroys cells through an excitotoxic mechanism\(^8\) whereas saline destroys granule cells by mechanical disruption of neural connections. Twenty-four hours after surgery, 38 rats were injected with either 5.0 µCi/g body wt \([^{3}H]\) thymidine or 100 µg/g body weight 5-bromo-2'-deoxyuridine (BrDU; Sigma, St. Louis, MO) in saline with 0.007 N NaOH, markers of DNA synthesis that label proliferating cells and their progeny.\(^{4,27,28}\) Some animals were anaesthetized with nembutal (50 mg/kg) and perfused with 4.0% paraformaldehyde in 0.1 M phosphate buffer (PB) at survival times of 1 h or three weeks after injection and the brains were processed for combined \([^{3}H]\) thymidine autoradiography and immunohistochemistry for cell-specific markers or for BrDU immunohistochemistry alone. Additional rats were decapitated 1 h after \([^{3}H]\) thymidine injection and the brains were processed for terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labelling (TUNEL), a marker of degenerating cells, and for autoradiography and Nissl staining. The rats that did not receive injections of \([^{3}H]\) thymidine or BrDU were perfused 24 h after surgery and the brains were processed for PCNA immunohistochemistry. PCNA is an accessory protein for DNA polymerase expressed by cells in the G1, S, G2, but not M phases of the cell cycle.\(^{21,50}\) All animal procedures were in accordance with the guidelines of The Rockefeller University laboratory animal research committee. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques.

Histological procedures

Combined immunohistochemistry and \([^{3}H]\) thymidine autoradiography. In order to positively identify the types of cells that proliferate in response to lesion, immunohistochemistry for vimentin, a marker of immature glia,\(^4\) and for NSE, calbindin and NR1, markers of mature granule neurons,\(^9,35,49\) was combined with \([^{3}H]\) thymidine autoradiography. The brains of 25 lesioned animals were dissected, postfixed overnight and cut (40 µm) with an oscillating tissue slicer in a bath of PB. After incubating for 30 min in a solution of 0.3% hydrogen peroxide in phosphate-buffered saline (PBS), the sections were incubated overnight in a solution containing one of the following: (i) monoclonal anti-vimentin (diluted 1:50 in PB, clone V9, Boehringer Mannheim, Indianapolis, IN), (ii) polyclonal anti-NSE (diluted 1:1000 in PB, Polysciences, Warrington, PA), (iii) polyclonal anti-calbindin (diluted 1:5000 in PB, Sigma), or, (iv) monoclonal anti-NR1 (diluted 1:250 in PB, Pharmingen, San Diego, CA). Following several rinses in PB, the sections were processed immunohistochemically with the avidin-biotin-horseradish peroxidase method (ABC kit, Vector Labs, Burlingame, CA). The sections were incubated in biotinylated secondary antisera (anti-mouse for vimentin and NR1, anti-rabbit for calbindin and NSE) with normal serum in PB for 1 h, rinsed in PB, incubated in ABC in PB for 1 h, rinsed in PB and then reacted in diaminobenzidine and hydrogen peroxide in PB for 15 min. The sections were mounted onto gelatinized glass slides and dried. The slides were then dipped in NTB-2 photographic emulsion (Eastman Kodak Co., Rochester, NY), stored in the dark at 4°C for two weeks and then developed in Dektol (Eastman Kodak), fixed in Polymax T (Eastman Kodak), counterstained for Nissl using Cresyl Violet and coverslipped under Permount. Control sections were treated as described above without primary antisera and revealed no non-specific staining.

Bromodeoxyuridine and proliferating cell nuclear antigen immunohistochemistry. In order to corroborate findings obtained with \([^{3}H]\) thymidine autoradiography, the brains of 13 rats subjected to unilateral lesion of the gcl were processed for BrDU or PCNA immunohistochemistry. For BrDU immunohistochemistry, brain sections from five rats were placed in 0.3% hydrogen peroxide in PB for 30 min and then mounted onto 3-aminoethyl-triethoxysilane (3-AAS, Aldrich, Milwaukee, WI)-coated slides and dried. The slides were then incubated in 0.1% trypsin in 0.1 M Tris buffer for 10 min, rinsed twice in PBS (pH 7.4), incubated for 30 min in 2N HCl, rinsed twice in PBS (pH 6.0), incubated for 20 min in 3% normal horse serum in PBS, incubated in anti-BrDU (diluted 1:100 in PBS with 0.005% Tween-20; Novocastra Laboratories, Newcastle upon Tyne, U.K.), rinsed in PBS (pH 7.4) and then processed immunohistochemically with the ABC method (ABC kit, Vector Labs) as described above.

For PCNA immunohistochemistry, brain sections from eight rats not injected with \([^{3}H]\) thymidine or BrDU were incubated overnight in monoclonal anti-PCNA (Novocastra Laboratories, 1:50 in PB). The brains sections were then processed immunohistochemically with the ABC method (ABC kit, Vector Labs) and for Nissl staining as described above.

TUNEL staining. In order to determine whether the degenerating cells and proliferating cells represent distinct populations, the brains of 10 animals subjected to unilateral gcl lesion were examined with TUNEL staining combined with \([^{3}H]\) thymidine autoradiography. The brains of decapitated animals were dissected and immediately frozen on dry ice. Brain sections (18 µm) were cut with a cryostat and thaw-mounted onto slides. The sections were fixed for 30 min in 4.0% paraformaldehyde in PB, blocked in 0.3% hydrogen peroxide in methanol for 20 min and permeabilized in 0.1% Triton X-100 in 0.1% sodium citrate for 5 min. The sections were then incubated in TdT and fluorescein-labelled dUTP (Boehringer Mannheim) for 1 h at 37°C, incubated with anti-fluorescin conjugated with horseradish peroxidase (Boehringer Mannheim) for 1 h at 37°C and reacted in diaminobenzidine with hydrogen peroxide in PB for 10 min. The slides were then processed for \([^{3}H]\) thymidine autoradiography and Nissl staining. Control sections were treated as described above without TdT and revealed no non-specific staining.

Data analysis

For each brain processed for \([^{3}H]\) thymidine autoradiography, the number of \([^{3}H]\) thymidine-labelled cells that were immunoreactive or non-immunoreactive for vimentin, calbindin, NSE or NR1 were counted in the dentate gyrus on the lesioned and unlesioned sides. At least four sections
where the lesion was present (designated as "proximal to the lesion") and four sections where the lesion was absent (designated as "distal to the lesion") were analysed per brain. Sections that were considered distal to the lesion were located rostral to the lesion. A cell was considered \[3\text{H}\]thymidine-labelled when at least five grains were detected over the nucleus. This value is greater than 20 times the background level. Means were determined for the lesioned and unlesioned sides for each animal and the data were subjected to paired two-tailed Student's t-tests.

The cross-sectional area of the lesion, i.e. the portion of the gcl containing degenerating cells, was determined for each section where the lesion was present with a Zeiss Interactive Digitizing Analysis System. Means were determined for this variable and correlations were performed with \[3\text{H}\]thymidine-labelled cell counts from the same sections.

For each brain stained for either PCNA or BrdU, the number of labelled cells was determined in the dentate gyrus on the lesioned and unlesioned sides. At least six sections where the lesion was present were analysed per brain. Means were determined for each side for each animal and the data were subjected to paired two-tailed Student's t-tests.

**RESULTS**

Ibotenic acid or saline lesion: general observations

Stereotaxic injection of 2 µl saline did not produce a noticeable lesion despite the location of the cannula tract into the gcl. However, unilateral lesion with either ibotenic acid or 5 µl of saline resulted in substantial degeneration of the gcl 24 h later (Fig. 1). The extent of the lesion was verified by TUNEL and Nissl staining, which revealed degenerating cells that were small, round and darkly stained compared to unaffected granule neurons (Figs 1 and 2). The lesion extent was typically similar for ibotenic acid and saline, and usually involved either the suprapyramidal blade or the crest of the gcl (Figs 1–3). By three weeks after surgery, the lesion was typically larger than observed at 24 h post-lesion. The persistence of pyknotic or dying cells in or around the damaged gcl at three weeks suggested that active degeneration continues at least until this time-point.

Proliferating cells 24 h after lesion

Within 24 hours after lesion of the gcl, a dramatic increase in the number of cells labelled with either \[3\text{H}\]thymidine, BrdU or PCNA was observed in the dentate gyrus on the lesioned side (Fig. 1, Tables 1, 2). Most of these proliferating cells were located in close proximity to the lesion, in the hilus, although many \[3\text{H}\]thymidine-labelled cells were also detected on the lesioned side at areas that were not close to the region of degeneration. The proliferating cells were considerably larger than the degenerating cells and no evidence of \[3\text{H}\]thymidine-labelled, BrdU-labelled or PCNA-labelled pyknotic cells was observed. In animals where the lesion was not in the gcl (either within the CA1 region or the subiculum, n=6), no evidence of an increase in proliferating cells relative
to the unlesioned side was observed near the lesion or in the dentate gyrus (Fig. 3). Moreover, in animals where the cannula tract extended into the gcl but the injection (2 µl of saline) did not produce a noticeable lesion (n=4), the number of proliferating cells did not increase.

Fig. 2. Photomicrograph showing a representative lesion of the suprapyramidal blade of the granule cell layer produced by ibotenic acid 24 h before perfusion. The degenerating granule cells (arrows) can be clearly distinguished from the undamaged portion of the granule cell layer (arrowheads) by the presence of darkly-stained, small and rounded cell bodies. Scale bar=200 µm.

Fig. 3. Lesions located in the gcl, but not the CA1 region or subiculum, increased the number of proliferating cells in the dentate gyrus. These templates of coronal half brain sections (modified from Ref. 43a) depict representative lesions (stippled area) in the gcl (A, B), CA1 (C) and subiculum (D). Only lesions located in the gcl resulted in an increase in the number of [3H]thymidine-labelled cells in the dentate gyrus. c, crest; s, suprapyramidal blade.
Table 1. The number of $[^3H]$thymidine-labelled cells in the dentate gyrus increases after lesion of the granule cell layer with ibotenic acid or saline

<table>
<thead>
<tr>
<th>Number of $[^3H]$thymidine-labelled cells/section/side</th>
<th>Ibotenic acid lesion</th>
<th>Saline lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Lesion</td>
<td>Control</td>
</tr>
<tr>
<td>Proximal to the lesion</td>
<td>1.7±0.3</td>
<td>24.7±3.1*</td>
</tr>
<tr>
<td>Distal to the lesion</td>
<td>2.0±0.1</td>
<td>10.2±1.3*</td>
</tr>
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*Significant difference from control, P < 0.05.

The rats were injected with $[^3H]$thymidine 24 h after surgery and perfused 1 h later. Values are mean±S.E.M., n=6.

Table 2. The number of PCNA-immunoreactive or BrdU-immunoreactive cells in the dentate gyrus increases after lesion of the granule cell layer

<table>
<thead>
<tr>
<th>Number of proliferating cells/section/side</th>
<th>Control</th>
<th>Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCNA</td>
<td>6.0±1.2</td>
<td>35.3±3.2*</td>
</tr>
<tr>
<td>BrdU</td>
<td>2.5±0.9</td>
<td>20.0±1.5*</td>
</tr>
</tbody>
</table>

*Significant difference from control, P < 0.05. n=8 for PCNA, n=5 for BrdU.

The rats were injected with BrdU 24 h after surgery and perfused 1 h later. Values are mean±S.E.M. each obtained from at least six sections.

Following lesion of the gcl, the vast majority (~80%) of $[^3H]$thymidine-labelled cells in the dentate gyrus on the lesioned side had morphological characteristics of immature granule cell precursors, i.e. medium-sized, round or oval cell bodies (Fig. 1), and were non-immunoreactive for the glial marker vimentin (Fig. 4). The remaining $[^3H]$thymidine-labelled vimentin-immunoreactive cells had morphological characteristics of glial cells, including irregular or triangular-shaped small to medium-sized cell bodies. The $[^3H]$thymidine-labelled cells did not appear to be degenerating as determined by a lack of TUNEL staining and their location outside, but close to, the area of degeneration, i.e. in the hilus. A larger increase in the number of $[^3H]$thymidine-labelled cells was detected proximal (~10-fold), compared to distal (~four-fold), to the lesion 24 h after surgery (Table 1). No difference was observed in the number of silver grains per labelled cell on the lesioned side compared to the unlesioned side (mean number of silver grains/labelled cell=19.8±0.9 on the lesioned side, 22.3±1.8 on the unlesioned side, P > 0.05).

A positive relationship between the extent of granule cell damage and the number of $[^3H]$thymidine-labelled cells was observed; comparison of the cross-sectional area of the degenerating portion of the gcl to the number of $[^3H]$thymidine-labelled cells on the lesioned side revealed a strong positive correlation (r=0.9, P < 0.001). Brains that contained the largest lesions also demonstrated the greatest number of $[^3H]$thymidine-labelled cells. Moreover, individual sections that showed evidence of the greatest amount of degeneration also had the largest number of $[^3H]$thymidine-labelled cells on the lesion side.

Proliferating cells and their progeny three weeks after lesion

Despite the evidence of ongoing degeneration three weeks after the lesion, a five-fold increase in the number of $[^3H]$thymidine-labelled cells remained detectable in the dentate gyrus (Fig. 5). The majority of these cells resided in undamaged parts of the gcl, expressed markers of mature granule cells (~75% were NSE-immunoreactive, ~50% were calbindin-immunoreactive, ~65% were NR1-immunoreactive) and had the morphological characteristics of granule neurons (Fig. 4). Many of these $[^3H]$thymidine-labelled cells appeared to be morphologically identical to unlabelled granule neurons on both the lesioned and unlesioned sides. Three weeks after the lesion, a larger increase in $[^3H]$thymidine-labelled cells was observed distal compared to proximal to the lesion (Fig. 5).

Discussion

These findings indicate that degeneration of granule neurons in the dentate gyrus of the adult rat results in a significant increase in the number of proliferating cells, labelled with $[^3H]$thymidine, BrdU or PCNA, in the hilus 24 h after lesion. Twenty-four hours after the lesion, the majority of proliferating cells had the morphological characteristics of granule cell precursors and did not stain for vimentin, a marker of immature glia. Many cells labelled with $[^3H]$thymidine 24 h after the lesion, as well as their progeny, survive at least three weeks and differentiate into mature granule neurons. These cells are incorporated into the granule cell layer, are morphologically indistinguishable from neighbouring granule neurons and express NSE, calbindin and NR1, markers of mature granule neurons.

Methodological considerations

Because several recent studies have demonstrated that cells undergoing apoptosis in other systems can express markers of proliferating cells, it is possible that the increase in PCNA-, BrdU- or
[\textsuperscript{3}H]thymidine-labelled cells we detected on the lesioned side represents degenerating cells. There are several indications, however, that this interpretation is incorrect. First, no [\textsuperscript{3}H]thymidine-, PCNA- or BrdU-labelled degenerating cells were observed on the lesioned side. Second, no examples of [\textsuperscript{3}H]thymidine-labelled TUNEL-stained cells were detected on the lesioned side. Finally, the increase in [\textsuperscript{3}H]thymidine-labelled cells remained detectable three weeks after the lesion, indicating that a large number of cells that incorporated [\textsuperscript{3}H]thymidine, and their progeny, did not degenerate.

The observation that similar results were obtained with ibotenic acid and saline, which destroy granule cells through different mechanisms, suggests that the increase in proliferating cells occurred as a result of granule cell death, and not as a result of a characteristic of the type of lesion performed, e.g., changes in excitation in the case of ibotenic acid. The observation that lesions outside of the dentate gyrus, i.e. in the subiculum and CA1 region, or a stereotaxic injection of saline (2 µl) into the granule cell layer, which did not induce granule cell death, did not increase cell proliferation in the dentate gyrus suggests that the increase in proliferating cells following gcl lesion was not a non-specific effect of the cannula or surgery.

It is possible that the lesion, by destroying the blood-brain barrier, increased the uptake of [\textsuperscript{3}H]thymidine into cells that normally would proliferate. This is unlikely, though, because the number of silver grains per cell is not higher on the lesioned side than the unlesioned side and these values are consistent with previously reported values of [\textsuperscript{3}H]thymidine-labelled cells in the intact dentate gyrus of the adult rat. Moreover, the observation that the numbers of cells labelled with other markers of proliferating cells, including PCNA which does not require uptake, increase following lesion supports the contention that the increase in the number of [\textsuperscript{3}H]thymidine-labelled cells is a result of an increase in the number of proliferating cells.

**Differentiation and survival of cells produced after the lesion**

Although lesion-induced gliogenesis is a common phenomenon, the production of neurons in response to cell death is not a typical feature of the mammalian brain. The results of the present study have indicated that most cells that proliferate after the lesion are neuronal progenitors, i.e. these cells have the morphological characteristics of granule neuron precursors and do not stain for vimentin, a marker of immature glia. Moreover, the results of the present study show that three weeks after the lesion and [\textsuperscript{3}H]thymidine injection, the majority of [\textsuperscript{3}H]thymidine-labelled cells on the lesioned side express markers of mature granule neurons, such as NSE, NR1 and calbindin. Our previous studies have shown that proliferating cells with these characteristics, in the intact adult rat brain, typically express...
Fig. 5. Lesion of the gcl stimulates the production of cells that express granule neuron markers. Three weeks post-lesion, the numbers of \(^{3}H\)thymidine-labelled cells that are NSE-immunoreactive (A), NR1-immunoreactive (B) or calbindin-immunoreactive (C) increased in the dentate gyrus on the lesioned side. Bars represent the mean number of \(^{3}H\)thymidine-labelled cells/section/side (+S.E.M.). Asterisks represent significant difference from control side, \(P<0.05\), n=5.
neuronal markers within three weeks of DNA synthesis.\(^{6,9}\) The location of these cells in the granule cell layer and their similar morphological and biochemical characteristics to neighbouring granule neurons suggest that many cells produced in response to the lesion differentiate into granule neurons.

Although a dramatic increase in the number of \([^{3}H]\)thymidine-labelled cells was still detectable three weeks after the lesion, the increase proximal to the lesion was smaller compared to the increase at the 24 h time-point. Because the lesion itself appeared to be larger at three weeks compared to the lesion at 24 h after surgery, it is possible that many cells that proliferated in response to the lesion, i.e. \([^{3}H]\)thymidine-labelled cells, degenerated in the three week time-period. Another interpretation is that these cells were no longer detectable due to label dilution from multiple cell divisions. However, the observation that a substantial increase in the number of \([^{3}H]\)thymidine-labelled cells still can be detected in the dentate gyrus on the lesioned side at three weeks indicates that most cells generated in response to the lesion survive and do not undergo innumerable divisions.

Possible mechanisms underlying lesion-induced granule cell proliferation

The mechanisms that underlie lesion-induced proliferation of granule cell precursors are currently unknown. One possibility is that granule cell precursors are normally inhibited from proliferating by cues received from an intact granule cell layer. Degeneration of granule neurons would release this inhibition and allow neighbouring precursors to progress through the cell cycle. The observation that the majority of proliferating cells is located proximal to the lesion is consistent with this possibility. However, the finding that cell proliferation is also stimulated distal to the lesion, albeit to a lesser extent, argues against this hypothesis. Alternatively, degenerating cells or neighbouring cells could release a factor that stimulates precursor cells to divide. There are several, not mutually exclusive, possible sources of a lesion-induced mitogenic signal. First, dying granule neurons could release factors that directly stimulate the proliferation of neighbouring precursor cells. Second, glial cells could release a mitogenic factor in response to neuronal injury in the area. Third, undamaged granule cells could release a mitogenic factor in response to loss of neighbouring cells. However, this possibility seems less likely because smaller increases in \([^{3}H]\)thymidine-labelled cells were detected in portions of the dentate gyrus that were distal to the lesion.

Dying cells or undamaged neighbouring cells could release growth factors that would interact with receptors on granule cell precursors and stimulate mitosis. There are several putative candidates for factors that directly induce cell proliferation following lesion. Epidermal growth factor (EGF) has been shown to stimulate cell proliferation in a variety of systems\(^{13,25,36}\) and approximately 40% of granule cell precursors appear to express EGF receptors.\(^{32}\) mRNA for transforming growth factor (TGF)\(\alpha\), the endogenous ligand for the EGF receptor, is present in neurons of the dentate gyrus\(^{40,53}\) and could conceivably be released by neurons following lesion. Another possible candidate is TGF\(\beta\), a factor that appears to be present in microglia\(^{33,52}\) and has been shown to be increased under conditions of cell damage.\(^{24,33,52}\) TGF\(\beta\) acts as a mitogen on some glial cells in culture\(^{17}\) but its effects on granule cell precursors have not been investigated. However, the observations that the vast majority of granule cell precursors express TGF\(\beta\) receptors (unpublished observations) and that the levels of TGF\(\beta\) are increased in the dentate gyrus following adrenalectomy\(^{29}\) and entorhinal cortex lesion,\(^{30}\) treatments that also increase granule cell production,\(^{6,8}\) are consistent with the view that TGF\(\beta\) released from glial cells in the vicinity of the lesion could stimulate granule cell precursors to divide.

Relationship between dying cells and proliferating cells in the dentate gyrus

During development, an unusual relationship between cell proliferation and cell death exists in the dentate gyrus of the rat. In most other developing brain regions, cell proliferation occurs during a discrete period followed several days later by a period of naturally occurring cell death. In the dentate gyrus, however, cell death appears to coincide with and even immediately precede periods of cell proliferation. During development and in adulthood, experimental manipulations that alter cell death appear to alter cell proliferation in a similar manner. Treatment with the adrenal steroid corticosterone inhibits cell death and cell proliferation in the dentate gyrus both during development\(^{15}\) and in adulthood,\(^{6,17}\) whereas removal of adrenal steroids by adrenalectomy stimulates both of these processes.\(^{6,16}\) The results of the present study indicate that granule cell degeneration stimulates the proliferation of precursor cells in the dentate gyrus of the adult rat. Many of these cells and their progeny differentiate into neurons, become incorporated into the granule cell layer and survive for at least three weeks. Collectively, these results suggest that granule cell death can influence the rate of cell proliferation in the dentate gyrus throughout life under normal conditions and following experimental manipulations.

Acknowledgements—The authors gratefully acknowledge Dr Bruce S. McEwen and Dr Fernando Nottebohm for helpful comments on the manuscript. This work was supported by MH 52423 and a NARSAD Young Investigator Award (EG).
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