

Neurogenesis in the Dentate Gyrus of the Rat Following Electroconvulsive Shock Seizures

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Electroconvulsive shock (ECS) seizures provide an animal model of electroconvulsive therapy (ECT) in humans. Recent evidence indicates that repeated ECS seizures can induce long-term structural and functional changes in the brain, similar to those found in other seizure models. We have examined the effects of ECS on neurogenesis in the dentate gyrus of the adult rat using bromodeoxyuridine (BrdU) immunohistochemistry, which identifies newly generated cells. Cells have also been labeled for neuronal nuclear protein (NeuN) to identify neurons. One month following eight ECS seizures, ECS-treated rats had approximately twice as many BrdU-positive cells as sham-treated controls. Eighty-eight percent of newly generated cells colabeled with NeuN in ECS-treated subjects, compared to 83% in sham-treated controls. These data suggest that there is a net increase in neurogenesis within the hippocampal dentate gyrus following ECS treatment. Similar increases have been reported following kindling and kainic acid- or pilocarpine-induced status epilepticus. Increased neurogenesis appears to be a general response to seizure activity and may play a role in the therapeutic effects of ECT.

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INTRODUCTION

Electroconvulsive therapy (ECT) has been used since the late 1930s as an effective and fast-acting treatment for a variety of psychiatric disorders (1). Most commonly, ECT is used in the treatment of depression and particularly in the treatment of drug-resistant depression (1). The procedure involves a series of electrically induced generalized convulsive seizures. Typically, eight convulsions are administered at 48-h intervals over the course of 2½ weeks (1).

Although ECT has been proven to be highly effective at alleviating the symptoms of depression, the treatment remains controversial due to fears of long-term

detrimental effects (15). Changes in learning ability, memory, and cognition occur for hours or days following ECT (1, 7). In addition, anterograde and retrograde amnesia occur for events immediately surrounding the treatment. These may persist for weeks or months (1, 12).

Extensive research has failed to find gross brain damage resulting from ECT (12). Studies of electroconvulsive shock (ECS), an animal model of ECT, have also failed to show gross structural damage following repeated treatments (12). Recent work using the electrical kindling seizure model, however, has shown that seizures can cause functionally significant and long-lasting changes in the brain without gross structural damage. In the kindling model, brief and temporally spaced seizure episodes result in long-lasting changes in intracranial evoked potentials (35), axonal sprouting (41), and cell loss (2, 11, 34, 45).

These discoveries using the kindling seizure model have prompted a reexamination of the effects of ECT/ECS on the brain. Recent work from our own and other laboratories has demonstrated ECS-induced structural and functional changes similar to those seen in the kindling model. Long-term increases in the amplitude of the evoked potentials in the entorhinal-dentate pathway have been found, as well as aberrant sprouting of dentate granule cell mossy fibers (6, 40, 42).

Seizures cause cell birth as well as cell loss. Production of new dentate granule neurons occurs in adulthood in several mammalian species and may be common to all mammals, including humans (10, 14). Kindling, kainic acid, and pilocarpine-induced status epilepticus have all been found to increase the rate of neurogenesis in the dentate gyrus (24, 32, 37). The present study, therefore, was undertaken to examine the effects of ECS on neurogenesis. During a course of eight ECS seizures, animals were injected with the thymidine analog bromodeoxyuridine (BrdU). Bromodeoxyuridine is incorporated into the DNA of dividing cells during mitosis and therefore provides a label for newly born cells and their offspring (30). One month following ECS, counting of BrdU-positive nuclei was

performed in the granule cell layer of the dentate gyrus using an optical disector technique and fractionator sampling scheme modified from West *et al.* (44). The proportion of cells double labeled for BrdU and the neuronal marker neuronal nucleus (NeuN) was also determined.

MATERIALS AND METHODS

Subjects

Male, albino Wistar rats (320–400 g, Charles River, St. Constant, Quebec, Canada) were used as subjects. Subjects were individually housed on a 12-h light–dark cycle (lights on at 7:00 AM) with food and water continuously available.

ECS Procedure

Subjects were randomly sorted into “ECS” and “control” groups. The ECS group received an ECS treatment every second day at 11:00 AM for a course of eight seizures. This schedule was designed to emulate the schedule of ECT treatments in clinical settings (1). The electrical stimulus was delivered through corneal electrodes for a duration of 0.2 s. It consisted of 60-Hz sine wave pulses, 150 mA in amplitude. Each stimulation resulted in a tonic–clonic seizure lasting 8 to 10 s. Sham-treated control animals received identical handling, but no electrical stimulation.

Bromodeoxyuridine Injections

Starting with seizure number 5, ECS-treated and sham-treated subjects received a single injection of BrdU (50 mg/kg in 0.9% saline and 0.01 N NaOH; Sigma-Aldrich) 2 to 3 h following each treatment. Injections were also given at the same time on the 3 days between treatments, resulting in a total of seven injections.

Tissue Preparation

To examine the effect of ECS on the net production of new neurons, rats were allowed to survive for 4 weeks after the last ECS seizure. This delay allows newly generated neurons to differentiate and presumably integrate into the granule cell layer of the dentate gyrus. Subjects were deeply anesthetized with sodium pentobarbital (65 mg/kg) and perfused transcardially with ice-cold phosphate-buffered saline (PBS, 100 ml, pH 7.2), followed by 4% phosphate-buffered paraformaldehyde (200 ml, pH 7.2). Brains were removed and post-fixed in 4% phosphate-buffered paraformaldehyde for 18 h. Hippocampi were dissected and placed in PBS containing 0.1% sodium azide and kept at 4°C until sectioning.

Before sectioning, left hippocampi were placed in 25% sucrose overnight and then exhaustively sectioned

in the transverse plane using a cryostat with a microtome setting of 40 μm . Every 20th section was selected for subsequent analysis in a systematic random fashion by randomly choosing the first section in the first interval of 20 sections (35). A total of 9 to 11 sections per animal was obtained for analysis.

Immunohistochemistry

Following DNA denaturing in 2 N HCl at 37°C for 40 min, free-floating sections were rinsed several times in PBS over a 15-min period and immunohistochemically stained for BrdU followed by NeuN. All antibody solutions contained 0.3% Triton X-100 in PBS and 1% normal goat serum (Sigma). Briefly, sections were first incubated overnight at 4°C in a pooled anti-BrdU and anti-NeuN primary antibody solution (rat anti-BrdU, 1:100 dilution, Accurate Chemical; mouse anti-NeuN, 1:1000, Chemicon). They were then washed at room temperature in PBS. To visualize BrdU immunoreactivity, sections were incubated for 1.5 h at room temperature in Cy2-conjugated goat anti-rat secondary antibody (1:200, Jackson ImmunoResearch), followed by a wash in PBS. To visualize NeuN immunoreactivity, sections were then incubated in the secondary antibody solution biotinylated donkey anti-mouse (1:50, Vector Laboratories), followed by a wash in PBS and streptavidin-conjugated Texas red (1:200, Gibco BRL) for 1.5 h each at room temperature. Sections were then washed in PBS, rinsed with distilled water, and mounted on slides with Permafluor antifade mounting medium (Lipshaw Immunon).

BrdU and NeuN Quantification

All tissue analysis was performed using a Zeiss LSM410 confocal laser scanning microscope with a 63 \times (1.4 N.A.) oil immersion objective lens. Analysis was done by an investigator “blind” to the treatment conditions. BrdU-positive nuclei in the entire granule cell layer (GCL), which included the subgranule zone (defined as two cell widths below the GCL) of each section, were counted using the optical disector technique with a fractionator sampling scheme described in detail by West *et al.* (44). Briefly, BrdU-positive nuclei were counted as they came into focus while scanning through the section. The disector height (h) was set at 10 μm and nuclei within the first 3 μm of the section was not counted. The section thickness was measured at roughly the middle of each of the two blades of the GCL and at the apex. The average of these measurements was used as the thickness of that section. The total number of BrdU-positive nuclei in each hippocampus was then estimated as

$$N = \sum Q^- \cdot t/h \cdot 1/asf \cdot 1/ssf, \quad (1)$$

where $\sum Q^-$ is the total number of counted BrdU-positive nuclei in each hippocampus, t is the average

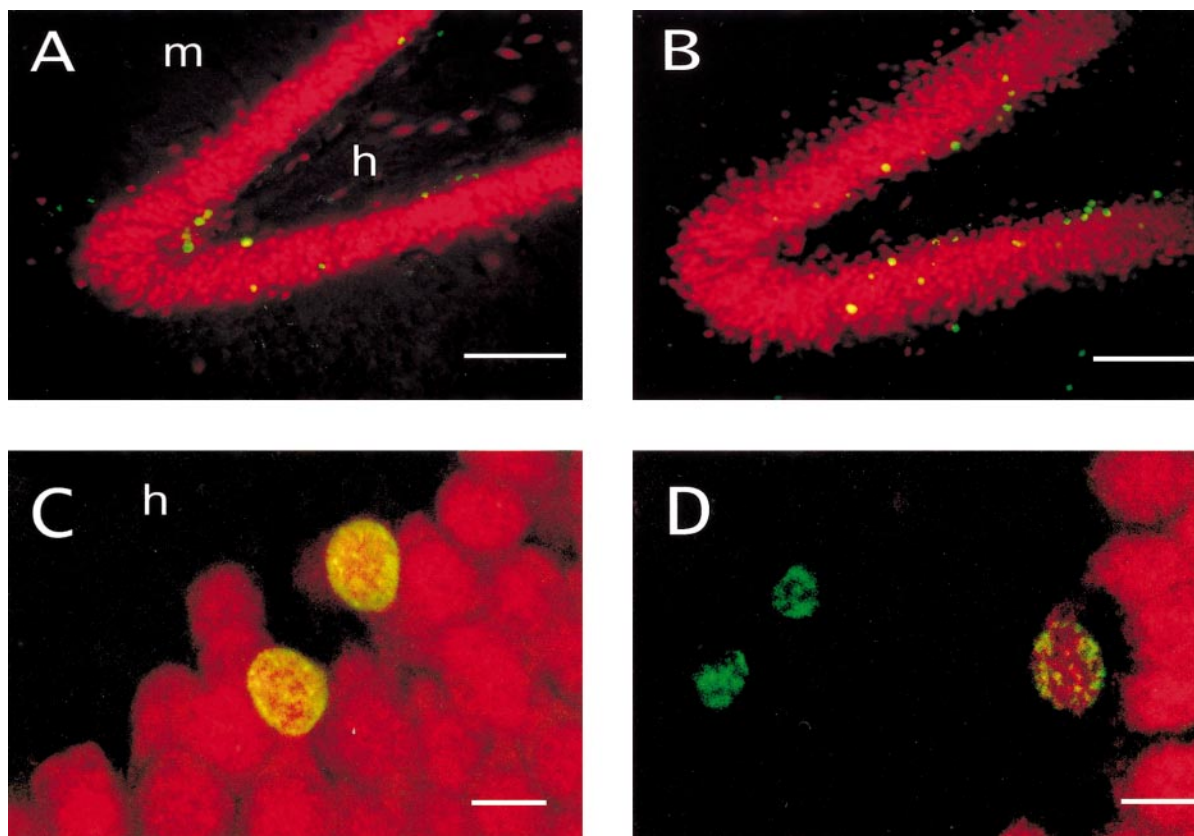


FIG. 1. Epifluorescence micrographs (A and B) and confocal images (C and D) of rat dentate gyrus double labeled for BrdU (green) and NeuN (red). More BrdU-positive nuclei were found within the granule cell layer of ECS-treated rats (B) than sham-treated controls (A). Cells colabeled for BrdU and NeuN (C and D, MES-treated rat) were found primarily near the hilar-granule cell layer border while smaller BrdU-only labeled nuclei were found mainly within the hilus (D) are presumed to be glial cells. (A and B) Bar, 100 μm . (C and D) Bar, 10 μm . m, molecular layer. h, hilus.

slice thickness, asf is the areal sampling fraction (equal to 1 since the entire GCL was sampled), h is the disector height, and ssf is the section sampling fraction equal to 1/20 (every 20th section was selected). Double labeling of each counted BrdU-positive nucleus for NeuN was confirmed by focusing through the nucleus with $5\times$ to $8\times$ magnification of the confocal image.

Estimation of total object number in a defined volume is based on counting objects in a known fraction of that volume. Estimates obtained using the optical disector approach with a fractionator sampling scheme are unbiased in that the technique involves no assumptions about the size, shape, or orientation of the objects under study and, as sample size increases, estimates approach the true value (39). Estimates are also unaffected by changes in tissue volume before or after tissue processing (44).

RESULTS

Figures 1A and 1B show epifluorescence micrographs of the dentate gyrus of representative sham-treated control (Fig. 1A) and ECS-treated (Fig. 1B)

animals labeled for both BrdU and NeuN. BrdU-positive nuclei in the GCL of control and ECS-treated animals were found primarily at the hilar border, but were also occasionally found deeper within the granule cell layer. Figures 1C and 1D also show confocal images of cells from an ECS-treated animal which were double labeled for BrdU and NeuN (Fig. 1C) and BrdU-positive only (Fig. 1D). Most nuclei of cells that were colabeled for BrdU and NeuN were large and round relative to the cell body, consistent with their being dentate granule neurons. Nuclei that were not colabeled tended to be smaller or elongated in shape and are presumed to have been glial or endothelial cells respectively. Nuclei which were labeled for BrdU, but not NeuN, were frequently seen within the hilus and much less frequently within the GCL.

Figure 2 shows the mean number (\pm SD) of BrdU-positive nuclei in the GCL of ECS and control subjects. ECS-treated animals had approximately twice as many BrdU-positive nuclei as control subjects ($P < 0.01$, two-tailed t test). ECS-treated animals were also found to have a significantly larger proportion (88.1%) of cells colabeled with BrdU and NeuN than untreated

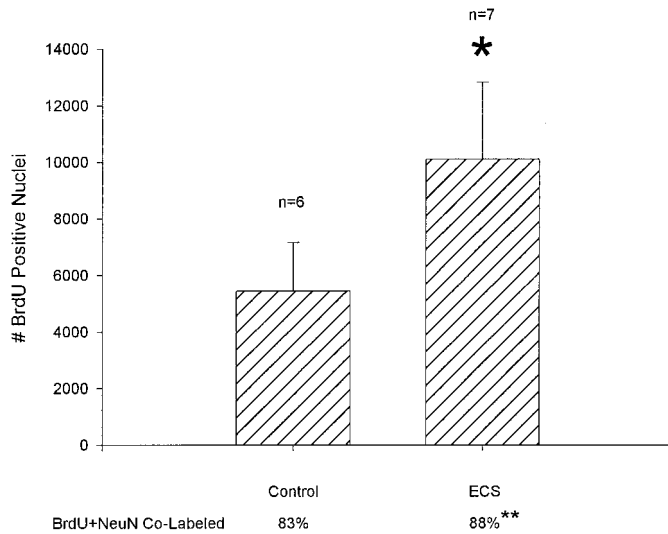


FIG. 2. Mean estimates (\pm SD) of total number of BrdU-positive nuclei within the granule cell layer of sham-treated control and ECS-treated rats. * $P < 0.01$ two-tailed t test. n, number of animals in each group. Percentages of BrdU-positive nuclei colabeled for NeuN are indicated. ** $P < 0.05$ two-tailed t test.

controls (83.1%, $P < 0.05$, two-tailed t test). This indicates a shift toward the production of neurons or an enhancement of neuronal survival relative to other cell types.

DISCUSSION

The present study was designed to examine the effect of ECS on neurogenesis in the dentate gyrus of the adult rat. It was found that eight ECS seizures increased the number of newly generated neurons in the granule cell layer. To our knowledge, this is the first study to show that dentate granule cell neurogenesis can be increased by ECS. The prolonged seizures of pilocarpine and kainic acid-induced status epilepticus have also been reported to increase neurogenesis in the dentate gyrus of the rat (24, 32), as have the brief and temporally spaced seizures of the kindling model (2, 31, 37). In the kindling model, even a single afterdischarge appears to have a significant effect (2). Thus, in rats, increased neurogenesis seems to be a generalized response to seizure activity.

Proliferation vs Survival

Disruption of the blood-brain barrier has been reported to occur for a brief period immediately following both ECS and ECT treatments (1, 12). It is unlikely that the increase in the numbers of BrdU-labeled cells seen here is due entirely to changes in permeability of the blood-brain barrier to BrdU. If the rate of cell proliferation were unchanged following ECS treatment while the blood-brain barrier permeability to BrdU

increased, the proportion of cells colabeled with NeuN and BrdU to those labeled with BrdU only, would not be expected to change. We found a significantly larger proportion of cells colabeled with BrdU and NeuN, indicating that a greater number of neurons were in fact produced by increased division of neural progenitor cells and/or by the enhancement of neuronal survival.

Recent work by Malberg *et al.* (27) has shown that administration of a number of antidepressant drugs as well as ECS results in an increase in dividing cells in the dentate gyrus. The present study extends these findings to show that the vast majority of those new cells become new neurons in the dentate gyrus one month after the last seizure. Our experimental design does not allow us to determine whether the net increase was strictly due to a change in neuronal precursor proliferation or to enhanced survival of newly generated neurons as well. Work by Gould and colleagues has demonstrated that many newly generated neurons die and that there is a "sensitive period" where survival is determined (20). Seizures may enhance the likelihood of survival of some newly generated granule neurons which would otherwise be lost. The combined effects of ECS on both cell proliferation and survival might therefore result in the increased number of new neurons seen here.

Functional Integration

Newly generated neurons in the dentate gyrus send axonal projections to targets in CA3 (28, 38) and receive synaptic contacts (28). They have also been found to persist for up to 2 years in humans (14) and are very likely functionally integrated into hippocampal circuits. Following seizures, newly generated neurons which have been labeled for the early postmitotic neuronal marker collapsin response mediator protein-4 (CRMP-4; sometimes referred to as TOAD-64) also appear to project dendritic processes into the molecular layer as well as send axonal projections to CA3 (32, 37). Some newborn neurons may contribute to the aberrant mossy fiber sprouting seen in the pilocarpine status epilepticus model (32, 37, 43). Further experiments are required to determine if and exactly how the newly generated neurons following seizures are integrated into existing hippocampal circuitry and whether or not they are a permanent addition to the total number of neurons in the granule cell layer.

Recent evidence suggests that the younger population of dentate granule cells may have different morphological and physiological properties than the older population, including fewer dendritic spines and reduced GABAergic inhibition resulting in an enhanced capacity for long-term potentiation (43). Changes in the proportion of younger to older granule neurons may therefore alter the functioning of the dentate gyrus as

a whole, possibly contributing to the long-term increase in evoked potentials found there following ECS and altering the hippocampal regulation of the hypothalamic–pituitary–adrenal (HPA) axis (18).

Possible Factors Mediating Increased Neurogenesis

Seizures may produce their effects on neurogenesis by altering the levels of growth factors. Several growth factors are known to alter the proliferation, differentiation and survival of neuronal precursors *in vitro* and *in vivo* (9). For example, epidermal growth factor has been reported to increase cell division (22) as well as alter the ratio of neurons to glia produced in the dentate gyrus (25). ECS has been found to increase the expression of several growth factors including nerve growth factor (16), basic fibroblast growth factor (16), and brain-derived neurotrophic factor (29). The effects of these on dentate granule cell neurogenesis have not been well characterized as yet, but they provide a possible mechanism for the promotion of neurogenesis. Further work will be required to elucidate the possible role of growth factors on seizure-induced changes in neurogenesis.

Another factor which may play a role in seizure-induced neurogenesis is seizure-induced cell death. Seizure activity has been shown to cause necrosis and apoptosis in the pilocarpine (17, 36), kainic acid (33), and kindling models (2, 11, 34, 45). ECS seizures may cause a subtle form of cell death, particularly apoptosis, although this has not been studied as yet. While no direct link between apoptosis and neurogenesis has been shown, several different manipulations that cause an increase in apoptosis in the dentate gyrus also cause an increase in neurogenesis. These include adrenalectomy (8), blockade of NMDA receptors (21), excitotoxic and mechanical lesions (23), and ischemia (26, 26). This suggests a possible relationship between the two processes. Conceivably, cell death may be a stimulus for increased neurogenesis which may then act as a compensatory mechanism (23).

Possible Relevance to Therapies for Depression

The functional significance of increased neurogenesis following ECS and whether it may play a role in the therapeutic actions of ECT is not known. There is now evidence of life-long neurogenesis in the dentate gyrus of human beings (14). If the process in humans is as sensitive to manipulation as it is in rats, seizures may have a significant effect on the composition of the dentate granule cell layer in patients subjected to ECT.

One theory of depression suggests that a dysregulation of the HPA axis may be responsible for some symptoms associated with depression (13). The hippocampus is known to provide negative feedback to the HPA axis (5). Neuronal atrophy and hippocampal dysfunction resulting from damage by elevated levels of corticosteroids may further aggravate HPA dysregula-

tion (5, 13). The increased production of new granule neurons following ECT in depressed patients may affect hippocampal function, which in turn may reduce HPA hyperactivity, alleviating symptoms of depression. The rapidity of the therapeutic action of ECT may argue against this possibility. As few as three ECT sessions over the course of a week may have antidepressant effects in some cases (1). Since relapse is much more likely to occur with fewer sessions, six to eight sessions are normally required to achieve lasting results. Neurogenesis may therefore be associated with the longer-lasting effects of further treatments.

Furthermore, increased production of new granule neurons may play a role in the therapeutic action of the antidepressant drugs which affect serotonin (5-hydroxytryptamine) reuptake. Drugs which block serotonin reuptake are potent antidepressants, yet have a delayed therapeutic effect of several weeks. This suggests that a form of adaptation to these drugs, not the rapid increase in synaptic serotonin levels, is responsible for their therapeutic effects (13). Serotonin has been shown to upregulate neurogenesis in the dentate gyrus (3, 4, 19). Blockade of serotonin reuptake may therefore increase neurogenesis in the dentate gyrus resulting in delayed effects on the HPA axis and ultimately on the symptoms of depression.

The present data add to a growing body of evidence suggesting that ECS causes long-lasting structural and functional changes in the brain (40, 42). Future research will be required to determine the extent to which similar changes occur after ECT and whether they play a role in the therapeutic effects of ECT or are possibly responsible for the undesirable amnesic effects of the procedure.

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REFERENCES

1. Abrams, R. 1992. *Electroconvulsive Therapy*. Oxford Univ. Press, New York.
2. Bengzon, J., Z. Kokaia, E. Elmer, A. Nanobashvili, M. Kokaia, and O. Lindvall. 1997. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. *Proc. Natl. Acad. Sci. USA* **94**: 10432–10437.
3. Brezun, J. M., and A. Daszuta. 1999. Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. *Neuroscience* **89**: 999–1002.
4. Brezun, J. M., and A. Daszuta. 2000. Serotonin may stimulate granule cell proliferation in the adult hippocampus, as observed in rats grafted with foetal raphe neurons. *Eur. J. Neurosci.* **12**: 391–396.
5. Brown, E. S., A. J. Rush, and B. S. McEwen. 1999. Hippocampal remodeling and damage by corticosteroids: Implications for mood disorders. *Neuropsychopharmacology* **21**: 474–484.

6. Burnham, W. M., G. A. Cottrell, D. Diosy, and R. J. Racine. 1995. Long-term changes in entorhinal-dentate evoked potentials induced by electroconvulsive shock seizures in rats. *Brain Res.* **698**: 180–184.
7. Calev, A. 1994. Neuropsychology of ECT: Past and future research trends. *Psychopharmacol. Bull.* **30**: 461–469.
8. Cameron, H. A., and E. Gould. 1996. Distinct populations of cells in the adult dentate gyrus undergo mitosis or apoptosis in response to adrenalectomy. *J. Comp. Neurol.* **369**: 56–63.
9. Cameron, H. A., T. G. Hazel, and R. D. McKay. 1998. Regulation of neurogenesis by growth factors and neurotransmitters. *J. Neurobiol.* **36**: 287–306.
10. Cameron, H. A., and R. McKay. 1998. Stem cells and neurogenesis in the adult brain. *Curr. Opin. Neurobiol.* **8**: 677–680.
11. Cavazos, J. E., I. Das, and T. P. Sutula. 1994. Neuronal loss induced in limbic pathways by kindling: Evidence for induction of hippocampal sclerosis by repeated brief seizures. *J. Neurosci.* **14**: 3106–3121.
12. Devanand, D. P., A. J. Dwork, E. R. Hutchinson, T. G. Bolwig, and H. A. Sackeim. 1994. Does ECT alter brain structure? *Am. J. Psychiatry* **151**: 957–970.
13. Duman, R. S., J. Malberg, and J. Thome. 1999. Neural plasticity to stress and antidepressant treatment. *Biol. Psychiatry* **46**: 1181–1191.
14. Eriksson, P. S., E. Perfilieva, T. Bjork-Eriksson, A. M. Alborn, C. Nordborg, D. A. Peterson, and F. H. Gage. 1998. Neurogenesis in the adult human hippocampus. *Nature Med.* **4**: 1313–1317.
15. Fink, M. 1988. Fifty years of electroconvulsive therapy. *Convulsive Ther.* **4**: 2–3.
16. Follsea, P., K. Gale, and I. Mocchetti. 1994. Regional and temporal pattern of expression of nerve growth factor and basic fibroblast growth factor mRNA in rat brain following electroconvulsive shock. *Exp. Neurol.* **127**: 37–44.
17. Fujikawa, D. G. 1996. The temporal evolution of neuronal damage from pilocarpine-induced status epilepticus. *Brain Res.* **725**: 11–22.
18. Gombos, Z., A. Mendonca, R. J. Racine, G. A. Cottrell, and W. M. Burnham. 1997. Long-term enhancement of entorhinal-dentate evoked potentials following 'modified' ECS in the rat. *Brain Res.* **766**: 168–172.
19. Gould, E. 1999. Serotonin and hippocampal neurogenesis. *Neuropsychopharmacology* **21**: 46S–51S.
20. Gould, E., A. Beylin, P. Tanapat, A. Reeves, and T. J. Shors. 1999. Learning enhances adult neurogenesis in the hippocampal formation. *Nature Neurosci.* **2**: 260–265.
21. Gould, E., H. A. Cameron, and B. S. McEwen. 1994. Blockade of NMDA receptors increases cell death and birth in the developing rat dentate gyrus. *J. Comp. Neurol.* **340**: 551–565.
22. Gould, E., and P. Tanapat. 1997. EGF stimulates proliferation of granule cell precursors in the dentate gyrus of adult rats. *Soc. Neurosci. Abstr.* **23**: 317.
23. Gould, E., and P. Tanapat. 1997. Lesion-induced proliferation of neuronal progenitors in the dentate gyrus of the adult rat. *Neuroscience* **80**: 427–436.
24. Gray, W. P., and L. E. Sundstrom. 1998. Kainic acid increases the proliferation of granule cell progenitors in the dentate gyrus of the adult rat. *Brain Res.* **790**: 52–59.
25. Kuhn, H. G., J. Winkler, G. Kempermann, L. J. Thal, and F. H. Gage. 1997. Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. *J. Neurosci.* **17**: 5820–5829.
26. Liu, J., K. Solway, R. O. Messing, and F. R. Sharp. 1998. Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. *J. Neurosci.* **18**: 7768–7778.
27. Malberg, J. E., A. J. Eisch, E. J. Nestler, and R. S. Duman. 1999. Chronic antidepressant administration increases granule cell genesis in the hippocampus of the adult male rat. *Soc. Neurosci. Abstr.* **25**: 1029.
28. Markakis, E. A., and F. H. Gage. 1999. Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. *J. Comp. Neurol.* **406**: 449–460.
29. Nibuya, M., S. Morinobu, and R. S. Duman. 1995. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J. Neurosci.* **15**: 7539–7547.
30. Nowakowski, R. S., S. B. Lewin, and M. W. Miller. 1989. Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an anatomically defined population. *J. Neurocytol.* **18**: 311–318.
31. Parent, J. M., S. Janumpalli, J. O. McNamara, and D. H. Lowenstein. 1998. Increased dentate granule cell neurogenesis following amygdala kindling in the adult rat. *Neurosci. Lett.* **247**: 9–12.
32. Parent, J. M., T. W. Yu, R. T. Leibowitz, D. H. Geschwind, R. S. Sloviter, and D. H. Lowenstein. 1997. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J. Neurosci.* **17**: 3727–3738.
33. Pollard, H., C. Charriaud-Marlangue, S. Cantagrel, A. Represa, O. Robain, J. Moreau, and Y. Ben-Ari. 1994. Kainate-induced apoptotic cell death in hippocampal neurons. *Neuroscience* **63**: 7–18.
34. Pretel, S., C. D. Applegate, and D. Piekut. 1997. Apoptotic and necrotic cell death following kindling induced seizures. *Acta Histochem.* **99**: 71–79.
35. Racine, R. J., J. G. Gartner, and W. M. Burnham. 1972. Epileptiform activity and neural plasticity in limbic structures. *Brain Res.* **47**: 262–268.
36. Roux, P. P., M. A. Colicos, P. A. Barker, and T. E. Kennedy. 1999. p75 neurotrophin receptor expression is induced in apoptotic neurons after seizure. *J. Neurosci.* **19**: 6887–6896.
37. Scott, B. W., S. Wang, W. M. Burnham, U. De Boni, and J. M. Wojtowicz. 1998. Kindling-induced neurogenesis in the dentate gyrus of the rat. *J. Neurosci. Lett.* **248**: 73–76.
38. Stanfield, B. B., and J. E. Trice. 1988. Evidence that granule cells generated in the dentate gyrus of adult rats extend axonal projections. *Exp. Brain Res.* **72**: 399–406.
39. Sterio, D. C. 1984. The unbiased estimation of number and sizes of arbitrary particles using the disector. *J. Microsc.* **134**: 127–136.
40. Stewart, C., K. Jeffery, and I. Reid. 1994. LTP-like synaptic efficacy changes following electroconvulsive stimulation. *NeuroReport* **5**: 1041–1044.
41. Sutula, T., X. X. He, J. Cavazos, and G. Scott. 1988. Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science* **239**: 1147–1150.
42. Vaidya, V. A., J. A. Siuciak, F. Du, and R. S. Duman. 1999. Hippocampal mossy fiber sprouting induced by chronic electroconvulsive seizures. *Neuroscience* **89**: 157–166.
43. Wang, S., B. W. Scott, and J. M. Wojtowicz. 2000. Heterogenous properties of dentate granule neurons in the adult rat. *J. Neurobiol.* **42**: 248–257.
44. West, M. J., L. Slomianka, and H. J. Gundersen. 1991. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anat. Rec.* **231**: 482–497.
45. Zhang, L. X., M. A. Smith, X. L. Li, S. R. Weiss, and R. M. Post. 1998. Apoptosis of hippocampal neurons after amygdala kindled seizures. *Brain Res. Mol. Brain Res.* **55**: 198–208.