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Neurogenesis in the Adult Rat Dentate Gyrus Is Enhanced by Vitamin E Deficiency

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ABSTRACT

Neurogenesis occurs throughout adult life in rat dentate gyrus. Factors and mechanisms of adult neurogenesis regulation are not well known. Vitamin E deficiency has been found to deliver a neurogenetic potential in rat dorsal root ganglia. To determine whether the role of tocopherols in adult neurogenesis may be generalized to the central nervous system, changes in adult rat dentate gyrus neurogenesis were investigated in vitamin E deficiency. Neurogenesis was quantitatively studied by determination of the density of 5-bromo-2′-deoxyuridine (BrdU)-labeled cells and by determination of the total number of cells in the granule cell layer. The BrdU-labeled cells were immunocytochemically characterized by demonstration of neuronal marker calbindin D28K. The following results were found: (1) the volume of the granule layer increased in controls from 1 to 5 months of age, mainly due to cell density decrease; (2) the volume increased by a similar amount in vitamin E–deficient rats, mainly because of an increase in cell number; (3) BrdU-positive cells were more numerous in vitamin E–deficient rats in comparison to age-matched controls; (4) the increase in proliferated cells was located in the hilus and in the plexiform layer. This study confirms that neurogenesis occurs within adult dentate gyrus and demonstrates that this process is enhanced in vitamin E deficiency. This finding indicates that vitamin E may be an exogenous factor regulating adult neurogenesis. J. Comp. Neurol. 411:495–502, 1999.

Indexing terms: dentate gyrus; granule cells; postnatal cell birth; tocopherols

Production of new neurons had been demonstrated in adult mammalian dentate gyrus by several authors (e.g., Altman, 1962; Altman and Das, 1966; Kaplan and Hinds, 1977; Bayer, 1982; Kuhn et al., 1996). New neurons originate from proliferating, more or less committed, precursors or from multipotent stem cells (Kaplan and Bell, 1984; Gage et al., 1995, 1998; Palmer et al., 1997). Daughter cells in part migrate into the granule layer and then differentiate into cells expressing neuronal markers (Cameron et al., 1993; Suhonen et al., 1996), i.e., they receive synapses (Kaplan and Bell, 1984) and send axons through mossy fiber tract (Stanfield and Trice, 1988; Markakis and Gage, 1999). The finding that neurogenesis occurs within dentate gyrus throughout life is intriguing because it suggests a possible role of neuron turnover in establishing and consolidating spatial memory.

In some rat strains, postnatal neurogenesis causes an increase in the total number of granule neurons (Bayer, 1982), whereas in others their number does not change, despite neuron production (Boss et al., 1985). This suggests that, at least in some cases, the actual number of neurons is a dynamic result of precursor differentiation and naturally occurring cell death (Gould and McEwen, 1993).

Knowledge of factors and mechanisms underlying adult neurogenesis is important from both a theoretical and therapeutic point of view; in fact, it will help to make hypotheses about the regulation and physiologic significance of neurogenesis and to project restorative therapies for brain diseases. Mechanisms underlying postnatal neurogenesis in dentate gyrus and its regulation are not well-known. Several neurotransmitter receptors affect neurogenesis. Glutamate plays a double role: *N*-methyl-D-aspartate (NMDA) receptor activation inhibits neurogenesis (Gould et al., 1994; Cameron et al., 1995) and kainate enhances it (Gray and Sundstrom, 1998). The D_1-D_2 dopamine receptor antagonist haloperidol increases proliferation of granule cell progenitors (Dawirs et al., 1998). Moreover, adrenal steroids inhibit neuron precursor proliferation and neuron apoptosis, making neuron turnover slower, possibly through a pathway involving NMDA recep-

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tors (Cameron and Gould, 1994, 1996; Cameron et al., 1998). Interestingly, environmental enrichment (Kempermann et al., 1997b, 1998) or stress (Gould et al., 1997a, 1998) affect hippocampal neurogenesis, possibly acting through synaptic or endocrine mechanisms.

Vitamin E deficiency was found to allow neurogenetic potential expression in rat dorsal root ganglia (Cecchini et al., 1993, 1994, 1995). The obvious interest in identifying regulatory factors of adult neurogenesis spurred us on to verify if the role of vitamin E in neurogenesis regulation may be generalized to a classic neurogenetic region of an adult mammalian central nervous system.

To verify this hypothesis, the number of newborn cells was investigated in dentate gyrus both in normal and in vitamin E–deficient rats by using 5-bromo-2′-deoxyuridine (BrdU) labeling. Moreover, quantitative changes in the granule cell layer neuron population were investigated in both groups of rats to verify whether neurogenesis results in an increase in the actual neuron number.

MATERIALS AND METHODS

Animals

Sprague-Dawley male rats were used in all experiments. All use of the animals was conducted in accordance with the European Community guidelines and Italian laws and was approved by the Animal Experiments Committee of the University of Urbino. The rats were fed on a standard diet from weaning to 1 month of age. At this time, some animals were killed (group C1, young control rats). The others were divided into two groups: group C5, adult control rats, which continued to eat the same standard diet; and group D5, vitamin E–deficient rats, which ate a diet lacking in vitamin E, as described in Bruno et al. (1990), until death. After 2 months of this treatment in rats of the same strain, a decrease in the plasmatic level of tocopherols and a corresponding decrease in the brain, spinal cord, and nerves was found (Goss-Sampson et al., 1988; Ninfali et al., 1991). Some animals of groups C5 (n = 3) and D5 (n = 3) were injected intraperitoneally with BrdU (50 mg/kg), starting from 3 to 5 months of age, three times per week (Monday, Wednesday, and Friday), and four times a day over the last 2 days before death (8.30 AM, 12.00 AM, 3.30 PM, and 7.00 PM); therefore, the injection total number was 32. This administration period was chosen to observe neurogenesis during the entire period of vitamin E deficiency. The animals of the groups C5 and D5 were killed at 5 months of age at 9.30 AM (for the importance of fixing the exact time of rat death because of the existence of a circadian rhythm in cell death in dentate gyrus, see Gould et al., 1990). All the rats used were anesthetized with sodium thiopental (45 mg/kg body weight, intraperitoneally), and killed with an intracardiac injection of the same anesthetic; afterward, the brain was removed, fixed by Carnoy for 48 hours, and embedded in Histovax (Histo-lab, Ltd., Goteborg, Sweden; melting point = 56–58°C).

From the animals of groups C1, C5, and D5 serial coronal sections of 8 µm thickness were obtained from each brain and stained with cresyl-violet for neuron counting and granule cell measure. From animals of groups C5 and D5 that received BrdU, serial sections of 8 µm were also obtained for labeled cell detection; moreover some sections from these animals were stained with cresyl-violet to check tissue morphology.

Granule cell layer neuron counting

Quantitative analysis was carried out on the dentate gyrus, by using a Leitz microscope and camera lucida drawing tube, where external and internal blades are joined at the crest and the dentate gyrus is oriented horizontally beneath the corpus callosum. One section every 20 slices, i.e., every 160 µm, was analyzed to estimate volume. The outlines of granule cell layer were drawn at a magnification of 62.5×, and the area was measured by means of OPTILAB software for image analysis. These measurements provided the basis for the final estimation of volume, supposing that any change in the sectional area between the consecutive sections was linear. Therefore, the volume was calculated by multiplying the sum of the areas drawn by the thickness of dentate gyrus included between analyzed sections.

Cell density estimates were made from the same sections by counting neuronal nuclei every forth section in a standardized sampling field (five to six fields were randomly chosen and sampled for each section examined). The granule cell density per millimeter squared was estimated by using the formula:

$$d = (n/a) \times K^2,$$

where n is the total number of plotted nuclei, a is the total area of sampling field, and K is the linear magnification of the camera lucida projection (985×). The granule cell density per millimeter cubed was:

$$D = d \times 125,$$

where 125 is the number of sections included in 1 mm of thickness.

Total number of granule cells were estimated as follows:

$$N = \Sigma_i (V_i \times D_i),$$

where V_i is the volume of a 320-µm-wide zone of dentate gyrus included between two sections where the area of surface was calculated, and D_i is the granule cell density per millimeter cubed determined in the same zone. Because no significant difference in the size of granule cell nuclei was found between the groups (see below), no stereologic correction for sampling errors was applied.

Granule cell nucleus size measuring

At least 150 neurons for each animal were used for morphometric analysis. In the same sections chosen for counting, granule cells containing a clear nuclear profile with at least one nucleolus were selected and their nuclei were drawn with a drawing tube at a final magnification of 985×. The microscopic stage was advanced laterally in each blade in steps until the final number of profiles was reached. From the drawing obtained, the mean nucleus area was measured by means of OPTILAB software for image analysis.

Immunofluorescence

The detection of labeled cells was carried out by using an indirect immunohistochemical technique (Fig. 1). For DNA denaturation, rehydrated sections were treated with 0.1 M HCl at 4°C for 10 minutes, with 4 M HCl at 37°C for 30 minutes and rinsed with borate-buffered saline (0.1 M, pH

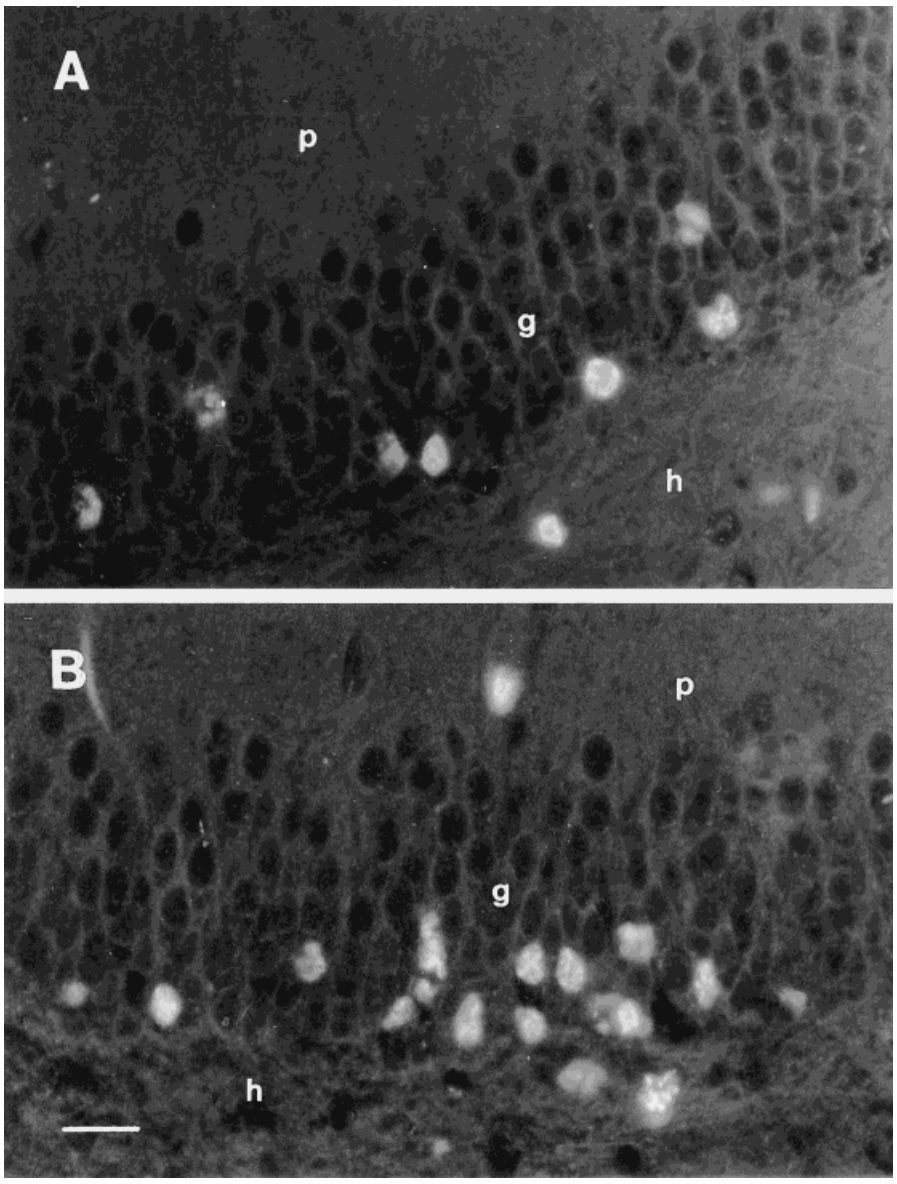

Fig. 1. Proliferation of granule cell precursors in the dentate gyrus of adult control (**A**) and of vitamin E–deficient (**B**) rat. Note the 5-bromo-2′-deoxyuridine–positive nuclei in the plexiform layer (p), in the granule cell layer (g), and at the border between the hilus (h) and the granule cell layer. Scale bar = 20 μm in B (applies to A,B).

8.5). Several rinses in 1% bovine albumin serum in phosphate-buffered saline (PBS; 0.1 M, pH 7.4) were then followed by incubation with normal horse serum 1:10 for 10 minutes. Subsequently, sections were incubated with a mouse anti-BrdU primary antibody (DBA; 1:300 in PBS) overnight at 4°C. After rinsing in PBS, sections were incubated with a fluorescein (isothiocyanate) (FITC)-conjugated horse anti-mouse secondary antibody (DBA, Vector; 1:50 in PBS). For viewing FITC fluorescence, a fluorescence microscope (Vanox, Olimpus, Italy S.R.L., MI) was used with a combination of BP 490 and EY 455 excitation filters.

A series of six coronal sections (8 μm) of brain spaced 160 μm were selected for each animal. At least 18 non-consecutive sections per animal were analyzed for BrdU labeling in the entire granule cell layer (external and internal blades), in the hilus and plexiform layer. Hilus is defined as the region interposed between blades comprising the subgranular zone. To obtain the density of BrdU-positive cells/mm^2, the areas of granule cell layer, of hilus, and of plexiform layer were determined by using OPTILAB software for image analysis.

To characterize proliferated granule cells, calbindin D28K (Sloviter, 1989) and BrdU were shown together in the same section (Sloviter et al., 1989). Granule cells were labeled by using rabbit primary antibody against calbindin D28K (SWant; 1:1,000 in PBS) overnight at 4°C, and biotinylated goat secondary antibody anti-rabbit IgG (DBA, Vector; 1:150), followed by avidin-phycoerythrin complex (DBA, Vector; 1:100). For viewing phycoerythrin fluores-

TABLE 1. Quantitative Analysis of Granule Cell Layer in Young and Adult Control and Vitamin E–Deficient Rats

Rat[1]	Volume (mm^3)	Density (per mm^3)	Total cell number
1 (C1)	1.001	1,410,625	1,403,571
2 (C1)	1.021	1,409,143	1,437,326
3 (C1)	1.060	1,384,132	1,467,180
4 (C1)	1.071	1,421,000	1,520,470
Mean	1.040	1,406,225	1,457,136
SEM	0.020	7,822	24,788
1 (C5)	1.161	1,222,250	1,417,810
2 (C5)	1.170	1,247,747	1,459,863
3 (C5)	1.460	1,203,250	1,756,745
4 (C5)	1.240	1,287,500	1,596,500
5 (C5)[2]	—	1,036,373	—
Mean	1.260*	1,199,424**	1,557,726
SEM	0.070	43,135	6,520
1 (D5)	1.413	1,381,278	1,947,602
2 (D5)	1.148	1,366,146	1,557,406
3 (D5)	1.145	1,345,108	1,533,423
4 (D5)	1.314	1,353,125	1,772,529
5 (D5)[3]	1.253	—	—
Mean	1.254**	1,361,414**	1,702,756*
SEM	0.050	7,913	97,735

[1]C1, 1-month-old control rats; C5, 5-month-old control rats; D5, 5-month-old vitamin E–deficient rats.
[2]Several sections were lost, therefore, a careful volume computation was not possible for the rat C5.
[3]An exact cell counting was not possible due to nonperfect staining.
*$P < 0.05$, Mann-Whitney U test with respect to C1.
**$P < 0.02$, Mann-Whitney U test with respect to C1.

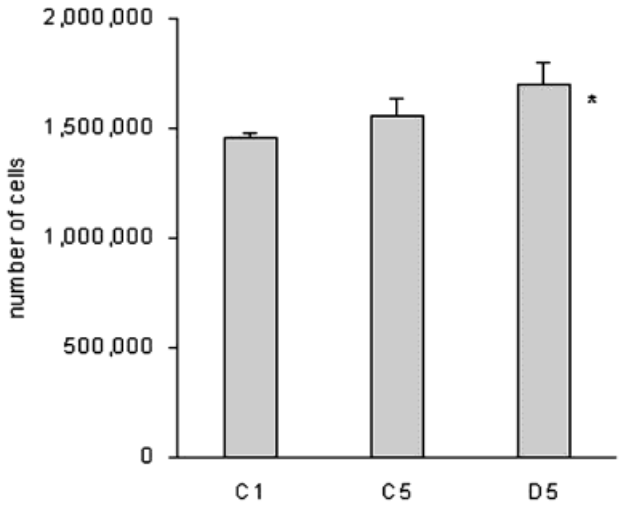

Fig. 2. Mean total cell number in the granule cell layer of C1 (1-month-old control rats), C5 (5-month-old control rats), and D5 (5-month-old vitamin E–deficient rats). The granule cell density and volume were determined in cresyl violet–stained sections and used to obtain the total cell number. Bars on the top of the columns indicate SEM. For N, see Table 1. Asterisk, Mann-Whitney U test: $P < 0.05$, with respect to C1.

cence, a combination of BP 545 and EO530 excitation filters was used.

RESULTS

Quantitative analysis in cresyl-violet stained sections

Quantitative analysis in cresyl-violet stained sections of dentate gyrus from control rats of 1 or 5 months of age and from vitamin E–deficient rats of 5 months of age was performed. No morphologic difference was detected in granule cells of different experimental groups. The mean granule cell nuclear area in 1-month-old animals was found to be 64.8 ± 4.4μm^2, in 5-month-old vitamin E–deficient rats 63.4 ± 1.3μm^2, and in age-matched controls 63.0 ± 2.7μm^2; the differences are not significant.

The volume of the granule cell layer increased with age by a similar amount in controls (+17%; Mann-Whitney U test: Z = -2.309, $P < 0.05$) and in vitamin E–deficient rats (+17.5%; Mann-Whitney U test: Z = −2.449, $P < 0.02$).

Granule cell density decreased in controls from 1 to 5 months of age (1,406,225 ± 7,822 cells/mm^3 and 1,199,424 ± 43,135 cells/mm^3, respectively; Mann-Whitney U test: Z = −2.449, $P < 0.02$), and less in vitamin E–deficient rats (1,361,414 ± 7,913; Mann-Whitney U test Z = −2.449, $P < 0.02$). The decrease in density is in agreement with increase and maturation of neuropil. The mean total number of granule cells increased in deficient rats with respect to young animals (Mann-Whitney U test: Z = −2.309, $P < 0.05$), whereas in control rats, no significant change occurred in the granule cell layer neuron number in the period considered, according to Boss et al. (1985) (Table 1, Fig. 2).

Quantitative analysis in BrdU-treated rats

Quantitative analysis in cresyl-violet stained sections of dentate gyrus in normal and vitamin E–deficient rats of 5 months of age injected with BrdU was performed. No change in the morphology of granule cells was found between the experimental groups. The results revealed a difference in the cell density in the granule cell layer between the two groups: 1,054,833 ± 14,267 and 1,174,589 ± 44,150 granule cells per mm^3 in controls and in vitamin E–deficient rats, respectively (Mann-Whitney U test: Z = −1.964, $P < 0.05$). The relative decrease in granule cell density in BrdU-treated rats with respect to nontreated ones (see above) was found to be similar in controls and in vitamin E–deficient rats.

BrdU-positive cells were found in the dentate gyrus of both vitamin E–deficient and control rats. Labeled cells were present in the granule cell layer, in the hilus, and in the plexiform layer. Substantially, the whole granule cell layer was found to express calbindin. Calbindin-positive cells were also found in the hilus. BrdU-labeled cells were frequently calbindin positive (Fig. 3) . About 25% of the BrdU-labeled cells were calbindin positive. Kempermann et al. (1997a) found a larger percentage of BrdU-positive cells colabeled with calbindin 4 weeks after BrdU injection. Our lower percentage is consistent with the administration protracted until death; therefore, the short period elapsing from labeling to rat death may be not sufficient for neuron specific protein expression for the last labeled cells.

Density of BrdU-positive cells was significantly higher in the entire dentate gyrus of vitamin E–deficient rats with respect to controls (Mann-Whitney U test: Z = −1.964, P U test: Z = −1.964, $P < 0.05$ for both) (Table 2, Fig. 4B). The proliferated cells were more numerous in vitamin E-deficient rats in the hilius, where neuron precursors are still located in adulthood (Cameron et al.), and in the plexiform layer, where most of the rare cells are known to be glial cells (Mann-Whitney U test: Z = −1.964, p < 0.05 for both; Table 2, Fig. 4B).

DISCUSSION

It is common knowledge that the bulk of granule cells of mammalian dentate gyrus is produced postnatally (Altman and Das, 1966; Schlessinger et al., 1975; Bayer, 1982);

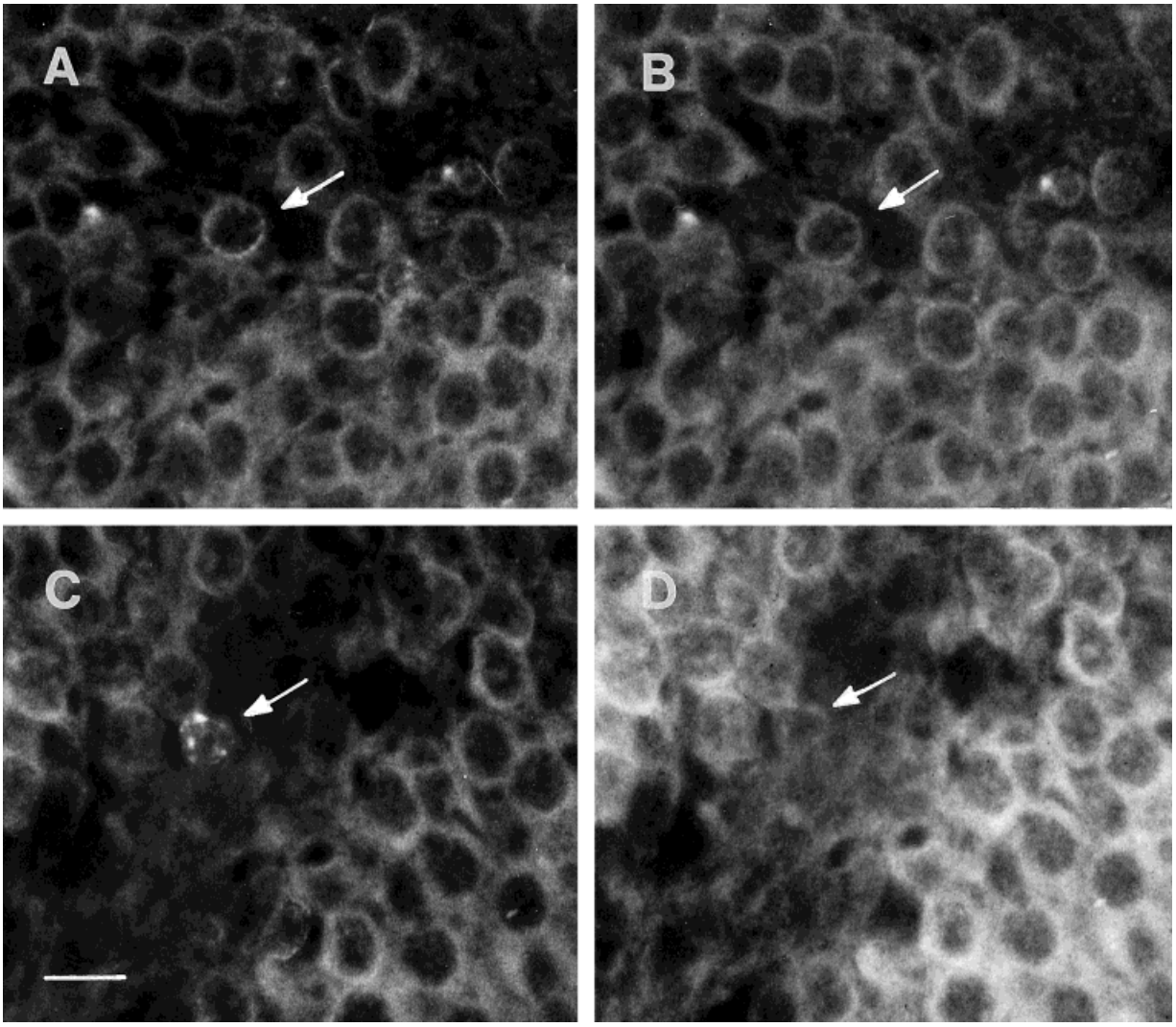

Fig. 3. Double labeling of granule cell layer in the dentate gyrus of control adult rats. Immunofluorescence labeling for 5-bromo-2′-deoxyuridine (BrdU) was combined with labeling for calbindin D28K. The arrows indicate two BrdU-positive cells (**A,C**); the same cells are shown to be calbindin D28K positive (**B,D**). The weak calbindin D28K expression in the newborn cell showed in D is probably related to its early phase of differentiation. Scale bar = 13μm in C (applies to A–D).

TABLE 2. 5-Bromo-2′-deoxyuridine–Labeled Cell Density (per mm²) in Dentate Gyrus of Control and Vitamin E–Deficient Rats

Rat[1]	Overall	Granule cell layer	Hilus	Plexiform layer
11 (C5)	75.17	179.92	53.09	33.22
13 (C5)	57.64	159.66	34.13	26.81
14 (C5)	75.04	208.91	44.57	32.11
Mean	69.28	182.79	43.93	30.71
SEM	5.82	14.29	5.48	1.97
12 (D5)	79.11	152.55	81.66	36.55
15 (D5)	77.50	169.57	61.37	42.84
16 (D5)	87.94	206.30	54.23	43.87
Mean	81.52*	176.14	65.75*	41.09*
SEM	3.24	15.86	8.22	2.29

[1]C1, 1-month-old control rats; C5, 5-month-old control rats; D5, 5-month-old vitamin E–deficient rats.
*$P < 0.05$, Mann-Whitney U test with respect to C5.

their production continues throughout adult life (Bayer, 1982; Boss et al., 1985; Stanfield and Trice, 1988) and is reduced in aged rats (Kuhn et al., 1996). In this work, we studied the numerical changes in granule cells in vitamin E–deficient rats and changes in neurogenesis in dentate gyrus throughout vitamin E deficiency period.

The results obtained in both control and vitamin E–deficient rats confirm that cell proliferation occurs in dentate gyrus of rat during adult life and that new granule neurons are produced. A substantial uniformity in the total number of control dentate gyrus granule cells between 1 and 5 months of age was found, agreeing with the data from the literature (Boss et al., 1985), despite neuron formation. The main finding is that vitamin E deficiency affects neurogenesis in dentate gyrus of rats, as is shown by the higher density of BrdU-labeled cells and by the increase in the total number of granule cells.

About the term "neurogenesis"

Before discussing the significance of the results, a semantic question has to be posed: what does the term "neurogenesis" mean, when referred to an adult animal? In general, neurogenesis in the adult refers to genesis of

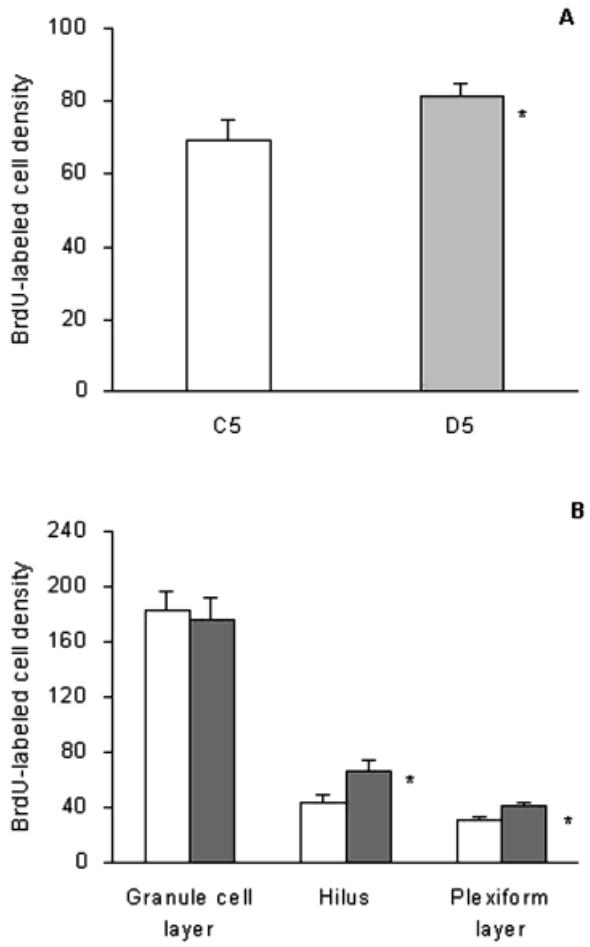

Fig. 4. Neurogenesis in vitamin E–deficient rats (D5, shaded columns) and in age-matched controls (C5, open columns). The dorsal hippocampus of three rats per group was analyzed on 18 coronal brain sections per animal. The amount of cell birth is expressed as the number of BrdU-positive cells/mm^2 in the overall dentate gyrus (**A**) and in the granule cell layer, hilus, and plexiform layer, separately (**B**). Bars on the top of the columns indicate SEM. Asterisk, Mann-Whitney *U* test: $P < 0.05$ with respect to corresponding controls.

neurons in animals in which development has concluded. Neurogenesis involves formation of neurons from proliferating or postmitotic precursors. Neurogenesis, so defined, does not necessarily cause an increase in the neuron number; the actual number of neurons, in general, depends on the balance between cell genesis and death, in the absence of migration. Therefore, neurogenesis may be associated with an increase, decrease, or steady state in neuron number, if death rate is lower or higher or if it is equal to the differentiation rate, whereas an increase in neuron number always suggests neurogenesis.

If neurogenesis is quantitatively evaluated by counting neurons that have incorporated labeling during the S phase, such as [^{3}H]thymidine or BrdU, a definition of neurogenesis as a formation of neurons derived from proliferating precursors is implicit. In this case, the actual number of labeled cells in each hippocampus results from precursor proliferation rate on one hand and from labeled cell death and under-threshold labeling dilution on the other. Factors affecting the number of BrdU-labeled cells might act changing each of these processes.

Effects of vitamin E deficiency on neurogenesis show regional differences

The increase in BrdU-positive cells found in vitamin E–deficient rats is located in the hilus, but not in the granule cell layer. This finding might suggest cell migration impairment or precocious death of newborn cells occurring before reaching the final destination (Cameron et al., 1993). However, this should be confirmed by a lower number of BrdU-labeled cells in granule layer in deficient rats than in age-matched controls, whereas a very similar number was found in both groups. Moreover, the increased total population of granule layer neurons in the period considered in deficient rats, rather suggests changes in proliferation, death rate, or both. Tocopherols may directly or indirectly affect both cell proliferation and death.

Direct mechanisms possibly involved in tocopherol effects on neurogenesis

Tocopherols play a pivotal role as a radical scavenger within the membranes. Oxidative stress causes neuronal death, and reactive oxygen species play a role in apoptosis induced by different apoptotic stimuli (Ceballos-Picot, 1997; Keller et al., 1998). Precursor proliferation may be induced by granule cell death in amount dependent on extent of death and daughter cells differentiate in mature granule cells (Gould and Tanapat, 1997). Therefore, the increase in density of BrdU-labeled cells in vitamin E–deficient rats might be an indirect effect of cell death caused by enhanced oxidative stress, possibly through an increased basic fibroblast growth factor (bFGF) availability (Ray et al., 1993).

On the other hand, superoxide and hydrogen peroxide in low concentrations play a role in promoting proliferation, by activating pathways common to growth factors. Pathways may involve oxidized glutathione, protein kinase C, and MAPK; activation of early growth response 1 transcription factor gene (*egr1*), *c-fos*, and *c-jun* is also involved (Burdon et al., 1994; Burdon, 1995).

Finally, α-tocopherol inhibits proliferation of smooth muscle and other transformed or nontransformed cells, possibly through mechanisms other than its action as a radical scavenger, involving protein kinase C activity and gene regulation (Chatelain et al., 1993; Clement et al., 1997; Fazzio et al., 1997).

Indirect mechanisms possibly involved in tocopherol effects on neurogenesis

In addition to these direct effects, tocopherols may indirectly affect neurogenesis or neuron death. Adrenal steroids affect neurogenesis and neuron survival. In fact, adrenalectomy causes an increase in neuron production (Cameron and Gould, 1994, 1996; McEwen, 1996), through a pathway involving regulation of NMDA receptor activation (Cameron et al., 1998). On the other hand, adrenalectomy induces massive death of older granule cells of dentate gyrus (Sloviter et al., 1989; Gould et al., 1990; Gould and McEwen, 1993; Cameron and Gould, 1996), indicating that adrenal steroids prolong the life of granule cells; this effect occurs through a direct action on type 2 receptors (Gould et al., 1997b). Then, a decrease in the adrenal steroid level increases cell birth and death and speeds up neuron turnover. Release of steroids by adrenal

cortex under acute stress is diminished by α-tocopherol (Shorin et al., 1985; Petrova et al., 1985; Doroshkevich et al., 1991). Therefore, an increase in BrdU-labeled neuron number in vitamin E–deficient rats might be consistent with a decreased neuron death rate caused by enhanced adrenal cortex responsiveness induced by vitamin E deficiency.

bFGF promotes postnatal precursor proliferation in the nervous system (Gritti et al., 1996; Tao et al., 1997; Kuhn et al., 1997). Vitamin E inhibits DNA synthesis induced by lysophosphatidylcholine by means of bFGF in smooth muscle cells, through inhibition of bFGF release (Chai et al., 1996). Therefore, tocopherol deficiency might enhance neuron precursor proliferation through increasing bFGF release; consistent with this idea, bFGF receptor mRNA (Wanaka et al., 1990) and bFGF (Pettmann et al., 1986) and its mRNA (Emoto et al., 1989) were found in hippocampus.

CONCLUSION

The increase in density of BrdU-labeled dentate gyrus neurons in vitamin E–deficient rats might be due to an increased production and/or decreased death of cells. Although our results do not permit to choose between these two alternatives, we prefer to believe that vitamin E deficiency causes an increase in cell proliferation rate rather than a decrease in cell death rate because most literature discussed above supports this interpretation. The well-known role of free radicals in cell death does not conflict with present findings, if we suppose vitamin E deficiency causes a faster turnover of neurons, and the increase in cell proliferation rate exceeds the increase in cell death rate. If this is the case, the increase in the number of BrdU-labeled cells should be accompanied by structural instability of dentate gyrus, which might impair its function. This should be consistent with the role of vitamin E in learning (Ichitani et al., 1992; Socci et al., 1995; La Rue et al., 1997; Joseph et al., 1998) possibly related to changes in long-term potentiation (Xie and Sastry, 1993). The present results indicate a class of exogenous molecules, tocopherols, as factors regulating hippocampal neurogenesis in adult mammalian.

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