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A Glial Role in the Action of Electroconvulsive Therapy

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Electroconvulsive Seizures Induce Proliferation of NG2-Expressing Glial Cells in Adult Rat Hippocampus

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Background: *Analysis of postmortem tissue from patients with major depression and bipolar disorder has revealed structural changes in several brain regions. We have shown that electroconvulsive seizure (ECS), used for the treatment of severe depression, induces proliferation of both neuronal and nonneuronal cells in the adult rat hippocampus.*

Methods: *Male Wistar rats were subjected to one or several ECS treatments, then injected with bromode-oxyuridine (BrdU) to detect cell proliferation. Animals were perfused either 1 day or 3 weeks following the last BrdU injection. Cells were double stained for BrdU and the cell type markers chondroitin sulfate proteoglycan (NG2), complement 3-receptor OX-42, 2', 3'-cyclic nucle-otide 3'-phosphodiesterase (CNPase), Ca^+ binding protein S100-β, or neuron-specific nuclear protein (NeuN).*

Results: *We identified NG2-expressing cells as a major cell type proliferating in the rat dentate gyrus in response to ECS. A sharp increase in NG2-positive cell proliferation was seen 2 days after ECS, and a large number of NG2-expressing cells persisted at 3 weeks.*

Conclusions: *Our results show that antidepressant treatment can induce a strong proliferation of glial progenitor cells in the adult rat hippocampus. We propose that this may counteract degenerative changes found in depression and be an important neurobiological event underlying the clinical effect of electroconvulsive seizures.* Biol Psychiatry 2003;54:1015–1024 © 2003 Society of Biological Psychiatry

Key Words: Depressive disorder, electroconvulsive treatment, hippocampus, gliogenesis, NG2, BrdU

Introduction

Major depression is a common and severe illness affecting a large number of individuals at some point during their lifetime. Researchers within both clinical and preclinical fields have searched for the biological key components linked to this disease. Several studies have indicated that hippocampus is one of the brain regions affected by major depression, and brain imaging studies have revealed a hippocampal volume reduction in depressed patients (MacQueen et al 2003; Posener et al 2003; Sheline et al 1996; Starkman et al 1992). In animal studies, hippocampus has been shown to be a vulnerable brain region. It is well established that the hippocampal subfield CA3 undergoes morphologic changes with atrophies of dendrites and pyramidal cell loss in response to psychosocial stress or treatment with glucocorticoids (McEwen 1999; Stein-Behrens et al 1994; Watanabe et al 1992).

Although the hippocampus is a sensitive brain region, it possesses a substantial capacity for regeneration. There is now convincing evidence that formation of new neurons takes place in the hippocampal dentate gyrus not only during development but also during adulthood (Altman and Das 1965; Eriksson et al 1998; Gould et al 1997). The germinative subgranular zone is located in the dentate gyrus at the border between hilus and the granule cell layer (GCL). Here neural progenitors (expressing early neuronal or glial markers) divide and form small clusters. A large fraction of these cells then migrates into the GCL where they differentiate into mature granule neurons with dendrites extending out into the molecular layer (ML) and with axons (mossy fibers) projecting to the hippocampal subfield CA3 (Markakis and Gage 1999; Stanfield and Trice 1988). The formation of new granule neurons is suppressed by psychosocial stress and chronic glucocorticoid administration (Gould et al 1998; Tanapat et al 1998).

We have previously shown that electroconvulsive seizures (ECS), a rat model for the effective and fast-acting antidepressant treatment electroconvulsive therapy (ECT), can modulate the regenerating capacity of the adult rat dentate gyrus by increasing the neurogenesis (Madsen et al 2000). Furthermore, others have shown that mood stabilizing and antidepressant drugs increase hippocampal neurogenesis (Chen et al 2000; Malberg et al 2000; Manev et al 2001).
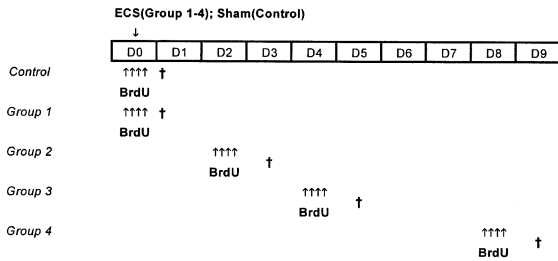
From our early studies, it has become clear that apart from neurogenesis there is a prominent proliferation of other nonneuronal cells in the dentate gyrus in response to

Dose response study
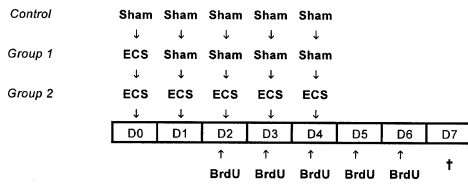
Time course study with short survival

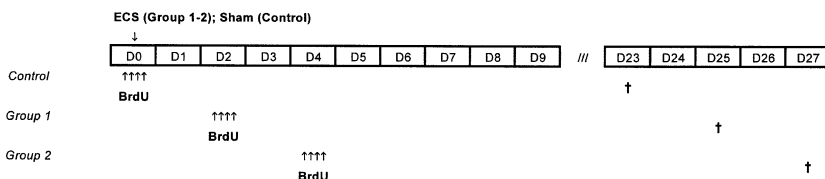## Time course study with long survival

Figure 1. Experimental design and group assignment. On the first day of the dose-response study (day 0), all rats were subjected to either a single electroconvulsive shock (ECS) trial or a sham treatment. Rats receiving five ECS trials were given the remaining treatments once daily on days 0–4. Control rats were sham treated at this time. All rats received bromodeoxyuridine (BrdU) injections twice daily at days 2–6. In the time course study, all rats were given either sham treatment (control) or a single ECS at day 0. Four injections of BrdU were given at 2-hour intervals on day 0, 2, 4, or 8. The rats were transcardially perfused by paraformaldehyde 20 hours after the last BrdU injection. The time course study with a long survival period was conducted as the time course study with regard to the administration of the single ECS at day 0 and the four BrdU injections at day 2 or 4. The rats were transcardially perfused by paraformaldehyde 21 days after the last BrdU injection. D0–27, days 0–27.

ECS (Hellsten et al 2002; Madsen et al 2000). These other proliferating cells are mainly found in the ML and in the hilus. Whereas the neuronal progenitors in the subgranular zone form small clusters, the nonneuronal proliferating cells in the ML and in the hilus are either lined up in stripes or are situated in pairs. We have previously identified the cells lined up in rows as RECA-1 positive endothelial cells (Hellsten et al, unpublished data). In this study, we used immunofluorescent staining in an attempt to determine the phenotypic characteristics of the remaining nonneuronal proliferating cells.

Methods and Materials

Animals

Adult male Wistar rats (Møllegaard breeding center, Denmark), weighing 180 g at the beginning of the study, were used. Rats were housed three per cage and kept on a 12-hour light–dark cycle with ad libitum access to food and water. Experimental procedures were carried out according to the guidelines set by the Malmö-Lund Ethical Committee for the use and care of laboratory animals.

Quantification of Cell Proliferation

The thymidin-analog 5-bromo-2′-deoxyuridin (bromodeoxyuridine, or BrdU) was used as a proliferation marker. Using similar protocols, others have shown that this stain detects cell proliferation rather then DNA repair (Cooper-Kuhn and Kuhn 2002; Palmer et al 2000).

Dose-Response Study

To assess the increase in cell proliferation after ECS, a dose-response study (Figure 1) was designed in which the rats were assigned to the following groups: control ($n = 6$), one ECS trial ($n = 6$), and five ECS trials ($n = 6$). On the first day of the dose-response study (day 0), all rats were subjected to either a single ECS trial or a sham treatment. The ECS was delivered via silver electrode ear clips (Somedic Sales AB, Sweden; 50 mA, .5 sec, 50-Hz unidirectional square wave pulses). The rats were monitored after ECS to ensure that clonic movements of the face and forelimbs occurred for 20–30 sec. Seizures engaging the face and forelimbs are taken as an indication of limbic seizures. Rats receiving five ECS trials were given the treatments once daily on days 0–4. Control rats were sham treated (i.e., handled identically to the ECS-treated rats except no current was passed). Bromodeoxyuridine (B5002; Sigma-Aldrich, St. Louis, Missouri) was dissolved in phosphate buffered saline (PBS) and

Table 1. Double Immunofluorescense Staining Protocols

	HCl	Block				PFA	HCl	Block		
NG2 BrdU		NGS	Rabbit-α-NG2 1:500	Biotin-goat-α-rabbit 1:200	Avidin-Alexa 488 1:250	X	X	NDS	Rat-α-BrdU 1:200	Cy3-donkey-α-rat 1:200
Ox42 BrdU		NGS	Mouse-α-Ox42 1:100	Cy3-goat-α-mouse 1:200		X	X	NDS	Rat-α-BrdU 1:200	FITC-donkey-α-rat 1:200
NeuN BrdU	X	NHS	Mouse-α-NeuN 1:100	Biotin-horse-α-mouse 1:200	Avidin-Alexa 488 1:250					
		NDS	Rat-α-BrdU 1:100	Cy3-donkey-α-rat 1:200	Avidin-Alexa 488 1:250					
S-100β BrdU		NGS	Rabbit-α-S-100β 1:1000	Biotin-goat-α-rabbit 1:200	Avidin-Alexa 488 1:250	X	X	NDS	Rat-α-BrdU 1:200	Cy3-donkey-α-rat 1:200
CNPase BrdU		NGS	Mouse-α-CNPase 1:100	Cy3-goat-α-mouse 1:200		X	X	NDS	Rat-α-BrdU 1:200	FITC-donkey-α-rat 1:200

Serum: Normal donkey serum (NDS; Harlan Sera, Lab, Belton, UK), Normal Horse serum (NHS; Sigma Aldrich, St. Louis, Missouri), normal goat serum (NGS; S26 Chemicon, Temecula, California).

Primary antibodies: Rabbit anti NG2 (NG2; gift from D. Stallcup), Mouse anti-OX -42 (OX-42; MCA275 Serotec, Oxford, UK), Mouse anti-neuron-specific nuclear protein (NeuN), (MAB 377, Chemicon, Temecula, California), Rabbit anti-S100β (S100β; SWANT [Swiss Antibodies, Bellinzona, Switzerland]); Mouse anti-CNPase (CNPase; MAB326, Chemicon, Temecula, California), Rat anti-BrdU (Oxford Biotechnology, OBT 0030, Kidlington, UK).

Secondary antibodies: Cy3 Donkey-anti-Rat (Jackson 712-165-153, Jackson Immuno Research, West Grove, Pennsylvania), fluorescein isothiocyanate (FITC) Donkey anti-Rat (Jackson 712-095-153, Jackson Immuno Research, West Grove, Pennsylvania), Biotin Horse anti-Mouse (Vector BA -2001, Vector Laboratories, Burlingame, California), Biotin Goat anti-Rabbit (Vector BA -1000, Vector Laboratories, Burlingame, California).

Tertiary step: Alexa 488 (Molecular Probes, Eugene, Oregon).

CNPase, 2′-, 3′-cyclic nucleotide 3′-phosphodiesterase; NG2, neuron-glia antigen 2.

administered intraperitoneally. All rats in the study received 10 injections of BrdU (50 mg/kg) in 12-hour intervals, during days 2–6 after the initial ECS treatment. Rats were transcardially perfused by paraformaldehyde 12 hours after the last BrdU injection (Figure 1).

Time Course Study with Short Survival

The rate of cell proliferation after a single ECS was investigated in a time course study (Figure 1) in which the rats were assigned to the following groups: control (*n* = 6), day 0 (*n* = 6), day 2 (*n* = 6), day 4 (*n* = 6), and day 8 (*n* = 6). All rats were given a single electroconvulsive seizure at day 0. The control rats were given sham ECS treatment at this time point. Four injections of BrdU (100 mg/kg) were given at 2-hour intervals (8 AM, 10 AM, 12 PM, and 2 PM), on day 0, 2, 4, or 8. Rats were transcardially perfused by paraformaldehyde 12 hours after the last BrdU injection (Figure 1).

Time Course Study with Long Survival

A time course study with 3-week survival was performed to determine the fate of cells proliferating on days 2 and 4. The rats were assigned to the following groups: control (*n* = 6), day 2 (*n* = 6), and day 4 (*n* = 6). The study with a survival period was conducted as the time course study with regard to the administration of the single ECS and sham treatment at day 0 and the four BrdU injections at days 2 and 4. Rats were transcardially perfused by paraformaldehyde 3 weeks after the last BrdU injection (Figure 1).

Tissue Preparation

Rats were anesthetized with sodium pentobarbital. In the absence of nociceptive reflexes, the rats were transcardially perfused with .9% saline for 2 min, followed by 4% ice-cold paraformaldehyde for 13 min. Following decapitation, the brain was removed from the skull and postfixed in 4% paraformaldehyde at 4°C overnight. Before sectioning on a freezing microtome, the brains were left in 30% sucrose in phosphate buffered saline until they sunk. Coronal sections, 40 μm thick, were cut through the middorsal hippocampus, –2.80 mm to –4.52 mm, relative to bregma (Paxinos and Watson 1986) and stored in antifreeze cryoprotectant solution at −20°C until analyzed with immunohistochemistry.

Antibodies

The following antibodies were used: rabbit-anti-NG2 against the chondroitin sulfate proteoglycan NG2, expressed on glial progenitor cells (Stallcup 1981); mouse-anti-CD11b (OX42) against a β2 leukocyte integrin expressed on microglial cells (Milligan et al 1991); rabbit-anti-S100β against a Ca+ binding protein expressed on astrocyte cellbodies (Boyes et al 1986); mouse-anti-CNPase against a myeline-associated enzyme expressed by oligodendrocytes and Schwann cells (Sprinkle 1989); mouse-anti-NeuN against a transcription factor expressed in the nucleus and cytoplasm of mature neurons (Mullen et al 1992); for purchase information regarding these antibodies, see Table 1.

Double Fluorescence Immunostaining

Sections were stained for neuronal and glial markers according to the protocols outlined in Table 1. Brain sections were rinsed (3 × 10 min) in .02 mol/L potassium phosphate-buffered saline (KPBS) and then incubated in blocking solution (KPBS + 5% serum of appropriate species + .25% Triton X-100) for 1 hour at room temperature. Sections were then incubated with primary antibody in blocking solution for 24 hours at 4°C with slow shaking. Afterward the sections were rinsed (3 × 10 min) with
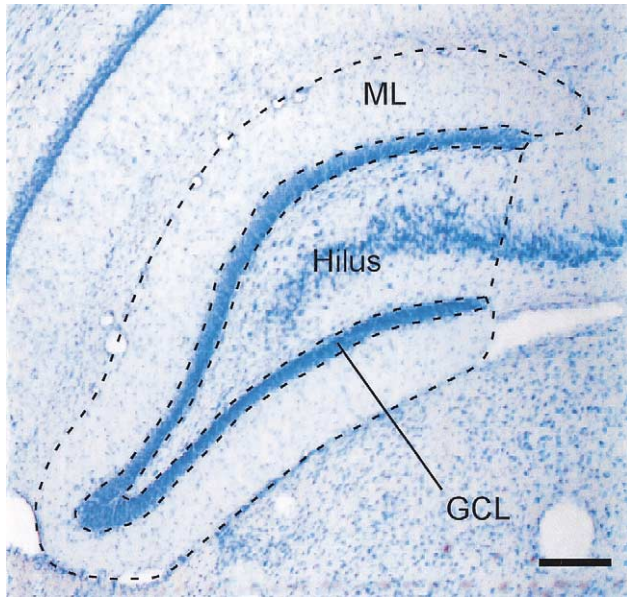
Figure 2. Image of a cresyl violet staining of rat dentate gyrus. Boundaries of the regions counted, the molecular layer (ML), the hilus, and the granular cell layer (GCL) are indicated by dashed lines. Scale bar = 200 μm.

KPBS + .25% Triton X-100 (KPBS+) and subsequently incubated with secondary antibody in blocking solution, for 24 hours at 4°C. Sections incubated with biotinylated secondary antibodies were then rinsed (3 × 10 min) in KPBS+ before incubation with 1:200 Alexa 488 (Molecular Probes, Eugene, Oregon) in KPBS+ for 24 hours in darkness at 4°C. After washing with KPBS (3 × 10 min), the sections were fixated in 4% paraformaldehyde for 10 min at room temperature, subsequently rinsed (3 × 10 min) in KPBS, and then incubated in 1 mol/L hydrochloric acid (HCl) at 65°C for 30 min. After rinsing in KPBS (3 × 10 min), the sections were then exposed to blocking solution (KPBS+ and 5% normal donkey serum; NDS) for 1

hour in darkness at room temperature and then incubated with primary antibody in blocking solution for 40 hours in darkness at 4°C. Sections were rinsed in KPBS+ (2 × 10 min), and KPBS+/2% NDS (2 × 10 min) before being incubated with secondary antibody blocking solution for 24 hours in darkness at 4°C. After rinsing in KPBS (3 × 10 min), the sections were mounted on poly L-lysine coated slides, air dried, rinsed briefly (10 sec) in water and cover slipped with glycerol-based mounting medium. Because of difficulties when staining for OX42 and CNPase, we used Cy3-conjugated secondary antibodies against the cell-type-specific primary antibody and FITC-conjugated secondary against BrdU. We performed BrdU/NeuN-staining according to a slightly different protocol, as outlined in Table 1.

Data Quantification and Statistical Analysis

Coronal sections through the middorsal hippocampus (−2.80 mm to −4.52 mm, relative to bregma; Paxinos and Watson 1986) were analyzed by observers blind to the treatments. Cell proliferation was assessed in the GCL (including the germinative subgranular zone), hilus, and molecular layer of the dentate gyrus, using an Olympus AX70 fluorescence microscope with a 40× objective. Proliferated BrdU-positive cells were counted in the granule cell layer, hilus, and molecular layer. Boundaries of the regions counted are as outlined in Figure 2. Cells lying within two cell diameters of the granule cell and hilar border were included in the granule cell layer count. Every sixth section throughout the middorsal hippocampus (six sections, 12 hippocampi from each animal) was counted in the dose response study and in the time course study with short survival. In the time course study with long survival, a larger number of antibodies were used and therefore every ninth section throughout the middorsal hippocampus (four sections, eight hippocampi from each animal) was counted. These values were averaged and expressed as mean number of cells per dentate gyrus. Cell number was determined using regular fluorescence microscopy in all stainings except in the BrdU/NeuN (21 days survival). Because granule cells are so densely packed, double-staining of these cells was assessed using confocal microscopy in which 50
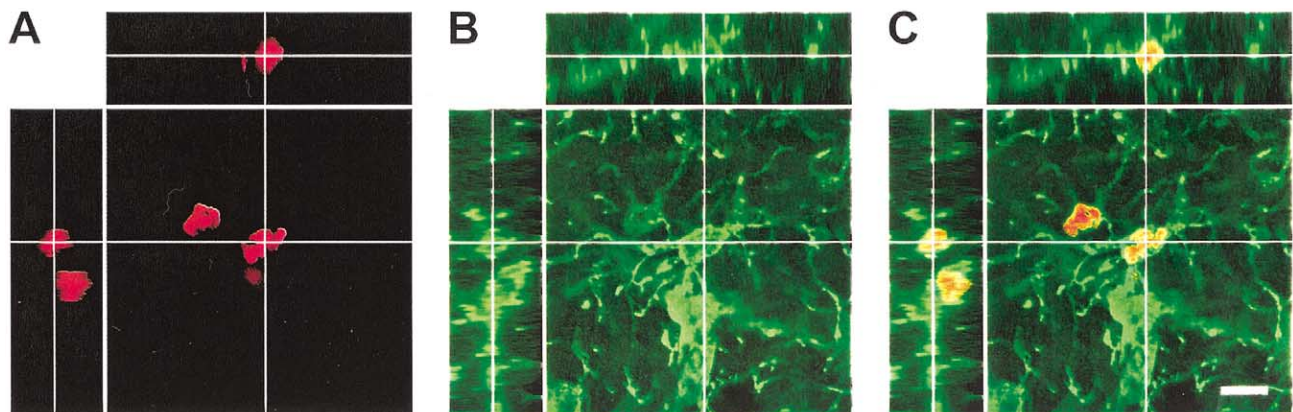

Figure 3. Confocal reconstruction of a cell in the hilus of dentate gyrus positive for bromodeoxyuridine (BrdU; red) and neuron-glia antigen 2 (NG2; green). Images **(A)** and **(B)** are superimposed in **(C)**. Three orthogonal (xy, xz, and yz) planes are shown. Scale bar = 5 μm **(C)**.

cells per animal (four animals from each group) were analyzed for possible BrdU/NeuN double labeling. Cells were randomly selected in the red fluorescence channel where only the BrdU staining is visible. To confirm double staining, a Nikon confocal microscope with a 40× objective and BioRad software (BioRad, Burlington, Massachusetts) was used. Cells were evaluated in z series with a minimum of six consecutive optical sections. Double immunofluorescense staining for BrdU and glial markers was confirmed using the same confocal microscopical technique. Data were analyzed with ANOVA and Scheffe post hoc test and are presented as means ± SEM. Statistical significance was set to $p < .05$.

Results

ECS Stimulates Proliferation of NG2-Positive Cells

To define the identity of the proliferating cells found in dentate gyrus after ECS, we double stained tissue sections with antibodies against the proliferation marker BrdU and various cell-type markers. Antibodies against the chondroitin sulfate proteoglycan NG2 stained a considerable number of the dividing cells in the GCL, the ML and in the hilus of the dentate gyrus. Other studies have implicated NG2 as a marker of oligodendrocyte progenitor cells, but it has recently been proposed as defining a distinct glial cell population (Nishiyama 2001; Ong and Levine 1999). To confirm that the BrdU-positive cells were double labeled with NG2 and not merely closely apposed to NG2-expressing cells, sections were analyzed using laser scanning confocal microscope. Orthogonal reconstructions in xy, xz, and yz planes of confocal Z stacks confirmed that the newly formed cells were NG2-positive (Figure 3). The cell bodies of the NG2 expressing cells were irregularly shaped and gave off radial-oriented primary processes that branched into thin secondary and tertiary processes (Figure 3). No morphologic changes of the NG2-positive cells were observed after ECS compared with sham treated control animals.

Proliferating NG2-Positive Cells Increase with the Number of ECS Trials Administered

A single ECS significantly increased the number of NG2/BrdU-positive cells with 134% in ML, 219% in hilus, and 255% in GCL relative to control animals. The proliferation of NG2-positive cells was further enhanced by five ECS trials and increased with 248%, 424% and 528% compared with control animals in ML, hilus, and GCL, respectively (Figure 4).

Note that although five ECS trials cause a sixfold increase of proliferating NG2-expressing cells in GCL, the absolute number of NG2/BrdU-positive cells in this dentate gyrus subfield is much lower than in hilus and in ML. Furthermore, not only proliferating NG2+ cells but also
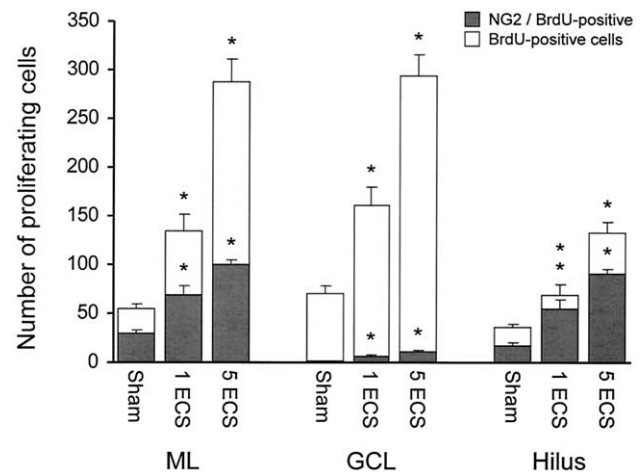

Figure 4. Electroconvulsive seizures (ECS) increase the proliferation of bromodeoxyuridine (BrdU)-labeled (open bars) and NG2-labeled (gray bars) cells in a dose-dependent manner in the molecular layer (ML), the granule cell layer (GCL), and the hilus. Asterisks indicate significant difference from control animals. NG2, neuron-glia antigen 2.

the total number of NG2+ cells increased in all dentate gyrus subregions in response to ECS. In ML, five ECS trials caused a 38% increase of NG2+ cells (control 138 ± 4; five ECS 191 ± 8), in GCL a 93% increase (control 27 ± 2; five ECS 52 ± 3), and in hilus a 33% increase (control 212 ± 6; five ECS 283 ± 8).

Proliferation of NG2-Positive Cells Increases Two Days after ECS

To determine the time of proliferation in the three hippocampal subfields (GCL, hilus, and ML), we administered BrdU at various time points (days 0, 2, 4, 8) after a single ECS. Two days after ECS administration, a significant increase in numbers of BrdU-labeled cells was seen in GCL, hilus, and ML. At day 4, the proliferation in GCL was still increased, whereas the cell proliferation in hilus and ML had returned to baseline levels (Figure 5 and 6). No significant difference was observed between control animals and animals given BrdU at day 0 or day 8 (Figure 5). Staining against NG2 showed that NG2-expressing cells are represented in all dentate gyrus subregions but are most prominent in the ML and hilus with a more dense distribution in the hilus (Figure 6). At 2 days after ECS, the NG2-positive cells represented 71% of the dividing cell in hilus and 29% in ML. In GCL, where the neural progenitors reside and the majority of the dividing cells are known to become neurons, only 14% (day 2) and 1% (day 4) of the BrdU-labeled cells were NG2-positive (Figure 5 and 6). A small fraction (6%) of the proliferating
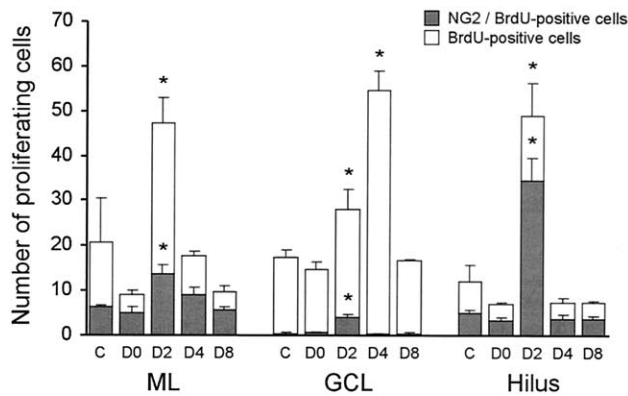
Figure 5. Time course study of NG2-positive cell proliferation after a single electroconvulsive seizure (ECS). The number of BrdU-labeled NG2-positive cells (gray bars) detected in the molecular layer (ML), the granule cell layer (GCL) and the hilus in response to ECS are significantly elevated compared with control animals (C) at day 2. The number of BrdU-labeled proliferating cells (open bars) in the granule cell layer detected after a single ECS are elevated compared with control animals at day 2 and day 4. D0–8, days 0–8. Asterisks indicate significant difference from control animals.

Expression of Cell Type Markers in BrdU-Labeled Cells Three Weeks after ECS Administration

To investigate the fate of the proliferating cells, rats were given BrdU at 2 or 4 days following a single ECS. After a 3-week survival period, cells labeled with BrdU 2 days after ECS still showed a high degree of colabeling with NG2 (Figure 7). In the hilus, 54% of the proliferating cells were NG2/BrdU-positive, whereas only 12% and 11% of the BrdU-positive cells expressed NG2 in ML and GCL, respectively. The numbers of NG2-positive cells labeled with BrdU 4 days after ECS did not, after a 3-week survival period, differ from that of control animals. The number of OX-42 positive cells in ML, which were BrdU-labeled 2 days after ECS, had after 3 weeks decreased to 3% of the BrdU-positive cells and was no longer significantly different compared with control animals. None of the BrdU-labeled cells was positive for the mature oligodendrocyte marker CNPase, and few (less than 1%) expressed the astrocyte antigen S100β. In agreement with previous reports a large fraction (82% and 89% of cells BrdU-labeled at day 2 and day 4, respectively) in the GCL were, after the 3-week survival period, colabeled with NeuN (Figure 7).

Discussion

This study demonstrates that ECS not only induces an increased proliferation of neuronal progenitors, but also strongly stimulates proliferation of cells expressing the chondroitin sulfate proteoglycan NG2 and to a lesser extent OX-42 expressing microglia. The proliferating NG2-expressing cells were mainly located in the hilus and

cells in ML, BrdU-labeled 2 days after ECS, expressed the microglial/macrophage marker OX-42. This increase was not detected in GCL or the hilus. The vast majority of the microglia had small cell bodies and extensive processes, indicating a nonactivated state (data not shown). None of the BrdU-labeled cells were positive for the mature oligodendrocyte marker CNPase, and (less than 1%) few expressed the astrocyte antigen S100β.
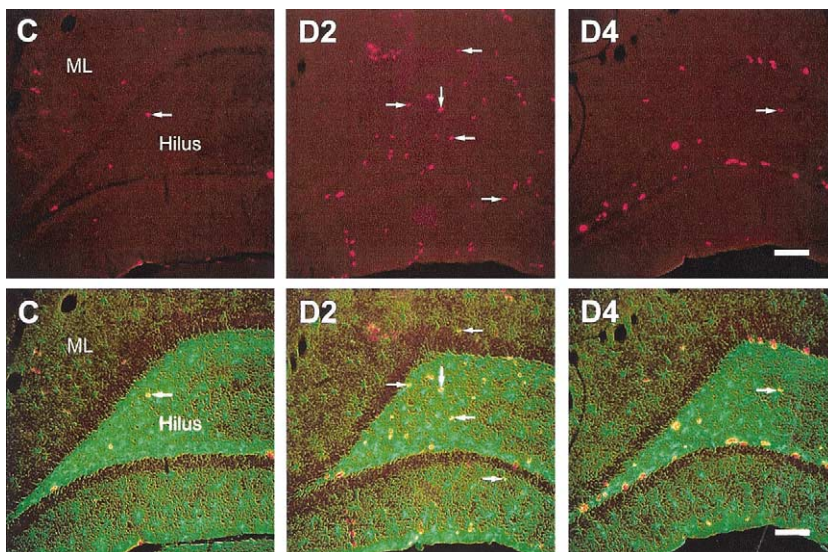

Figure 6. Immunofluorescence images showing rat dentate gyrus in sham-treated control animals (C) given BrdU at day 0 and electroconvulsive seizure (ECS)-treated rats given BrdU at day 2 (D2) or day 4 (D4). At D2, the bromodeoxyuridine (BrdU)-positive cells (red) are mainly located in the hilus and the molecular layer (ML). Most of the cells proliferating at D4 are distributed along the inner border of the granule cell layer. The NG2-positive cells (green; lower panel) are located mainly in the hilus and the molecular layer (ML), with a more dense distribution in the hilus. Arrows indicate newly formed NG2-positive cells. Scale bar = 90 μm.
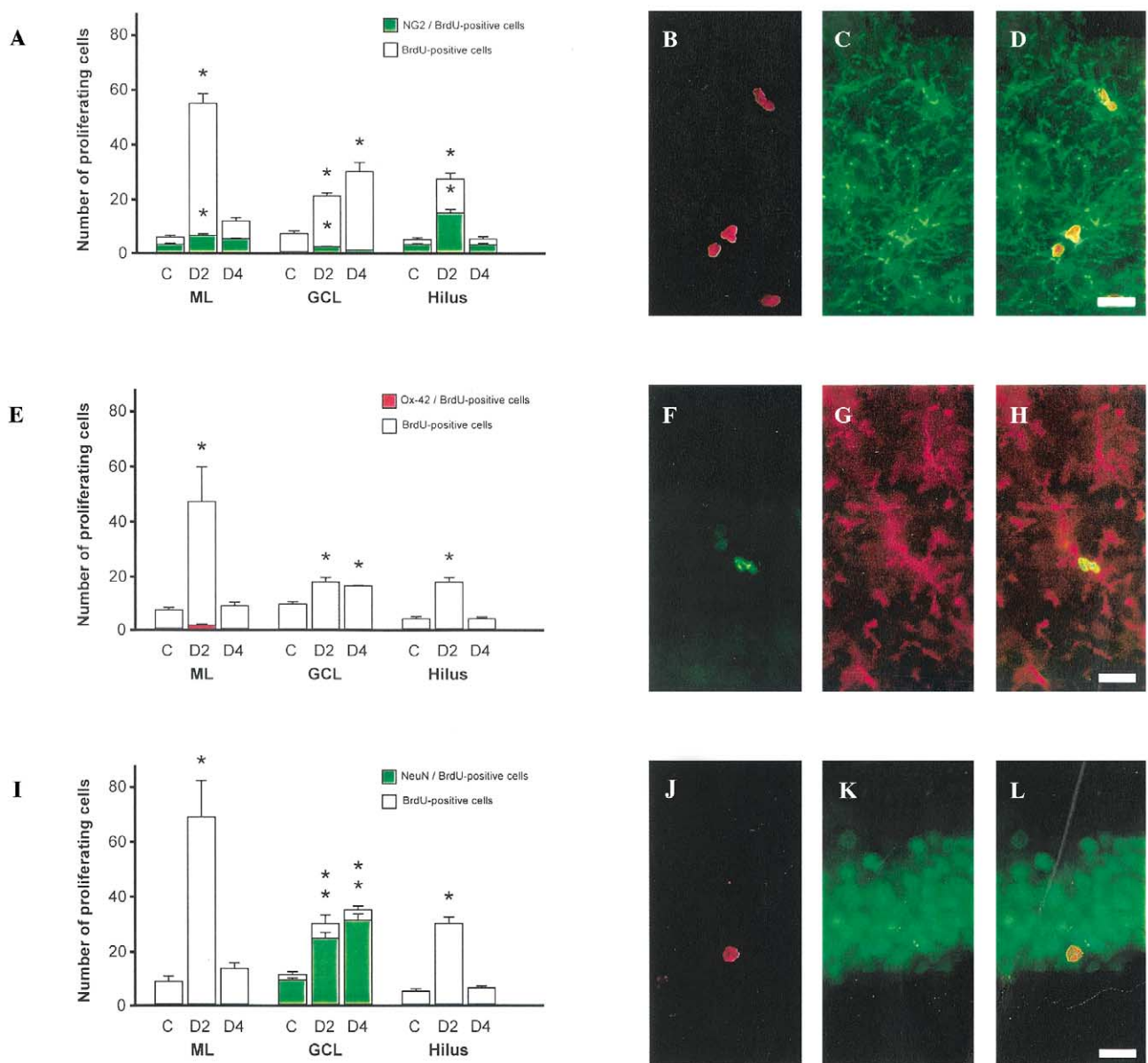
Figure 7. Time course study with a 3-week survival period after a single electroconvulsive seizure (ECS). Many of the cells proliferating 2 and 4 days after ECS in the molecular layer (ML), the granule cell layer (GCL), and the hilus still express NG2 after a 3-week survival period **(A)**. A few of the cells proliferating 2 days after ECS still express OX-42 after 3 weeks. However, the number did not differ significantly from control **(E)**. In the granule cell layer (GCL) NeuN-positive cells are the dominating cell type **(I)**. Asterisks indicate significant difference from control animals. Images of the various immunofluorescence stainings are shown in the right column. The cells are double labeled with rat anti-BrdU antibodies **(B, F, J)** and rabbit anti-NG2 **(C)**, mouse anti-OX-42 **(G)**, or mouse anti-NeuN antibodies **(K)**. Images are merged in **D, H, L**. The image of the NG2-positive cell is captured from the hilus, the OX-42 positive cell is from the ML, and the NeuN is from the GCL. Scale bar = 15 μm. The intensity of BrdU staining varies among stainings, hence the variation in total number of BrdU-cells. BrdU, bromodeoxyuridine; NeuN, neuron-specific nuclear protein; NG2, neuron-glia antigen 2.

the molecular layer, and many of them were still detectable after 3 weeks of survival.

Cells expressing NG2 comprise a large population of glial cells in the adult mammalian brain (Alonso 2000; Nishiyama et al 1999). They are often referred to as oligodendrocyte progenitors, but because many fail to

M. Wennström et al

differentiate further and instead remain NG2-positive, without expression of markers found on differentiated astrocytes, oligodendrocytes, or microglia (Nishiyama et al 1997; Ong and Levine 1999; Reynolds and Hardy 1997), it has been suggested that these cells represent a distinct glial cell type. The functions of NG2-positive cells are not well established. The NG2 proteoglycan share certain structural features with cell adhesion molecules, and it may thus be involved in cell–cell recognition and binding (Stegmuller et al 2002). Indeed, direct synaptic connections between NG2-expressing cells and neurons have been described (Bergles et al 2000). In addition, NG2-positive cells can form close contacts with classical neuron–neuron synapses (Bergles et al 2000). Furthermore, NG2 and other proteoglycans, together with cell adhesion molecules such as neural-cell adhesion molecule (N-CAM), are thought to play an important role in regulating axonal growth and path finding (Margolis and Margolis 1997). The NG2-proteoglycan was recently reported to bind (via a link protein) to alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-class glutamate receptors (Stegmuller et al 2002), and it has previously been shown that NG2 cells can regulate synaptic diffusion of glutamate (Ong and Levine 1999). Thus, it is plausible that NG2-expressing cells can have an important role in regulating synaptic plasticity and function (Dou and Levine 1994).

In response to injury, such as viral infection, stab wound, or excitotoxin-induced epileptic seizures, NG2-positive cells proliferate and become reactive (Levine 1994; Levine et al 1998; Ong and Levine 1999). Reactive NG2-expressing cells have enlarged cell bodies with filopodiallike extensions, and the expression of NG2 is higher than on nonactivated cells (Ong and Levine 1999). In ECS-treated animals the NG2-expressing cells showed no morphologic changes compared with NG2-expressing cells in control animals. The absence of morphologically activated NG2-positive cells in ECS-treated animals as well as the low number of activated microglia correlate with earlier studies showing that ECS, in contrast to excitotoxin-induced epileptic seizures, does not cause neuronal cell death or other signs of cellular damage (Devanand et al 1994).

The fraction of NG2/BrdU-positive cells in the time course study with 3-week survival was reduced compared with the fraction of NG2/BrdU-positive cells in the time course study with 12-hour survival. The reason for this decline needs further investigation but several possible explanations exist. Either some proliferating NG2-positive cells die during the 3-week survival period or the fraction of NG2/BrdU-cells decreases as a result of proliferation of other cell types. A third possibility is that NG2-expressing cells differentiate and lose their NG2-expression. Al-

though it is known that NG2-cells in the cortex and spinal cord can differentiate into oligodendrocytes (Levine et al 2001; Watanabe et al 2002), it was recently reported that NG2-positive cells in hippocampus can differentiate into neurons (Belachew et al 2003). Our finding that none of the BrdU-positive cells in any of the hippocampal areas investigated were double-labeled with CNPase after 3-week survival supports the idea that NG2-positive cells in the hippocampal dentate gyrus might play a role different from that of oligodendrocyte progenitors.

The seizure-induced proliferation of NG2-positive cells in dentate gyrus overlaps in time with that of neural progenitors. A recent investigation has shown that astrocytes from adult hippocampus are capable of regulating neurogenesis by instructing stem cells to adopt a neuronal fate (Song et al 2002). Because NG2-expressing cells have certain properties in common with astrocytes, such as regulation of synaptic plasticity, it is tempting to speculate that also NG2-positive cells have an active regulatory role in the mature central nervous system and under certain circumstances may regulate the fate of newly formed neurons. ECS also induces a significant proliferation of OX-42 expressing microglia. Studies have shown that microglial cells are directly associated with NG2-positive cells. The two cell types are often closely apposed to each other, and cell–cell signaling seems to be conducted through microglial processes contacting NG2-positive cells (Nishiyama et al 1997).

In light of these findings, it is interesting to note that postmortem studies have revealed specific neuronal and glial alterations in certain brain regions (prefrontal cortex and amygdala) in individuals with a history of affective disorder. The morphologic changes include cell loss and cell atrophy and an overall reduction in tissue volume (Rajkowska 2000). In preclinical attempts to mimic certain aspects of depressive disorder, it has been shown that chronic stress treatment and repeated glucocorticoid administration give rise to cellular changes such as dendritic atrophy, shrinkage of the neuronal cell body, and nuclear pyknosis in CA1 and CA3 of the primate and rodent hippocampus (Sapolsky et al 1990; Watanabe et al 1992). Studies have also shown that chronic corticosterone treatment negatively affects the proliferation of NeuN and NG2-positive cells in hippocampus (Alonso 2000). It is thus tempting to speculate that the hippocampal volume reduction seen in the clinical material (Sheline et al 1996; Starkman et al 1992) might be at least in part attributable to a glial cell loss, just as the case in the prefrontal cortex and the amygdala. Assuming that NG2-positive cells take part in the regulation of synaptic function and affect the fate of newly formed neurons, we suggest that the clinical effects of ECT may in part depend on the increased proliferation of NG2-expressing cells.

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