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Electroconvulsive Seizures Induce Endothelial Cell Proliferation in Adult Rat Hippocampus

Johan Hellsten, Malin Wennström, Johan Bengzon, Paul Mohapel, and Anders Tingström

Background: Electroconvulsive seizures, an animal model for electroconvulsive treatment, induce a strong increase in neurogenesis in the dentate gyrus of adult rats. Hippocampal neurogenesis has previously been described as occurring in an angiogenic niche. This study examines the effect of electroconvulsive seizures on proliferation of vascular cells in rat hippocampus.

Methods: Rats were injected with bromodeoxyuridine to label proliferating cells in the dentate gyrus after single/multiple electroconvulsive seizures in a dose-response study and at various time points after single electroconvulsive seizures in a time-course study.

Results: A dose-response effect on the number of bromodeoxyuridine-labeled endothelial cells located in the granule cell layer, hilus, and molecular layer was noted, as was the case with the number of neural precursors in the subgranular zone. The time-course study revealed that endothelial cell and neural precursor proliferation occurred in concert in response to a single electroconvulsive seizure.

Conclusions: Our data suggest that in response to electroconvulsive seizures, endothelial cell and neural proliferation is coregulated. The increase in endothelial cell proliferation may act to support the increased neural proliferation and neuronal activity or vice versa, possibly leading to structural changes within the hippocampus of importance for the antidepressant effect of electroconvulsive seizures.

Key Words: ECT, seizures, neurogenesis, angiogenesis, hippocampus, major depression

The hippocampus is one of few areas of the mammalian brain where there is an ongoing neurogenesis in adulthood (Altman and Das 1965; Eriksson et al 1998). Dividing hippocampal precursor cells, residing in the subgranular zone (SGZ) on the border between the hilus and granule cell layer (GCL), express a variety of neuronal and/or glial progenitor markers and are often referred to as neural precursors (Palmer et al 2000).

We and others have shown that electroconvulsive seizures (ECSs), an animal model for the efficient antidepressant regimen electroconvulsive treatment (ECT), increase hippocampal neurogenesis in the adult rat (Hellsten et al 2002; Madsen et al 2000; Malberg et al 2000; Scott et al 2000). Neurons formed after ECS differentiated and survived for at least 3 months, and the number of neurons formed increased with the number of ECS trials administered. Also, antidepressant medication has been reported to increase neurogenesis (Malberg et al 2000), and it has been suggested that the formation of new hippocampal neurons can counteract degenerative processes (Czeh et al 2001; Jacobs et al 2000) associated with depressive disorder (Sheline et al 1996, 1999).

In the process of normal ongoing hippocampal neurogenesis, the dividing neural precursors in the SGZ initially form dense cell clusters in close proximity to hippocampal capillaries, and intermingled with the neural precursors are also proliferating cells expressing endothelial cell markers (Palmer et al 2000). This indicates that hippocampal neurogenesis occurs in an angiogenic niche that allows for close contact between neural cells and the vasculature.

Given the fact that ECS is strongly neurogenic and the seemingly close relationship between the vasculature and the neural precursors, we have in this study investigated the effects of ECS on proliferation of vascular cells in the dentate gyrus of the adult rat hippocampus.

Methods and Materials

Animals and Design of Study

The general layout of the experimental procedures performed is shown in Figure 1.

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. To assess the increase in endothelial cell and neural precursor proliferation after ECS, a dose-response study was designed where the rats were assigned to the following groups: Control ($n = 6$), one ECS ($n = 5$), three ECSs ($n = 6$), and five ECSs ($n = 6$). The time course of proliferation of endothelial and neural precursor cells after one ECS was investigated in a time-course study where the rats were assigned to the following groups: Control ($n = 4$), day 0 ($n = 6$), day 2 ($n = 6$), day 4 ($n = 6$), day 6 ($n = 5$), and day 8 ($n = 6$). A survival study was performed to determine the fate of the endothelial cells and clustered cells in the SGZ proliferating in response to ECSs. Rats were assigned to the following groups: Control ($n = 3$) and five ECSs ($n = 3$).

Administration of Electroconvulsive Seizures

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 at 1400. Electroconvulsive seizures were delivered via silver electrode ear clips (Somedic Sales AB, Sweden) (50 mA, .5 second, 50 Hz unidirectional square wave pulses). The rats were monitored after ECS to ensure that clonic movements of the face and forelimbs (indicative of limbic motor seizures) occurred for a minimum of 20 to 30 seconds. Rats receiving three ECS and five ECS trials were given the remaining treatments once daily on days 1 and 2 or 1 through 4, respectively. Control rats were sham-treated (i.e., han-

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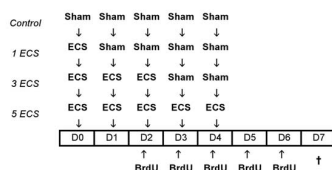
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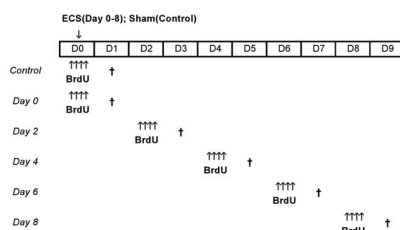
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Dose response study



Time course study



Survival study

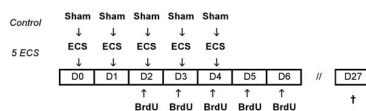


Figure 1. Experimental design and group assignment. On the first day of the dose-response study (day 0), all rats were subjected to either a single ECS trial or a sham treatment. Rats receiving three ECS or five ECS trials were given the remaining treatments once daily on days 1 and 2 or 1 through 4. Control rats were sham-treated at this time. All rats received BrdU injections (50 mg/kg) twice daily at days 2 through 6. The rats were transcardially perfused by paraformaldehyde 12 hours after the last BrdU injection. In the time course study, all rats were given either sham treatment (control) or a single ECS on day 0. Four injections of BrdU (100 mg/kg) were given in 2-hour intervals either on day 0, 2, 4, 6, or 8. The rats were transcardially perfused by paraformaldehyde 20 hours after the last BrdU injection. The survival study was conducted as the dose-response study with regard to the administration of the five ECS and the BrdU injections. The rats were transcardially perfused by paraformaldehyde 21 days after the last BrdU injection. D0–27 represent day 0–27. ECS, electroconvulsive seizure; BrdU, bromodeoxyuridine.

dled identically to the ECS-treated rats except that no current was passed).

For the time-course study, all rats were given a single electroconvulsive seizure on day 0 at 1000. The control rats were given sham ECS treatment at this time point. The survival study was conducted as the dose-response study with regard to the administration of the five ECSs.

Administration of Bromodeoxyuridine

Bromodeoxyuridine (BrdU) (B5002; Sigma-Aldrich, St. Louis, Missouri) was dissolved in phosphate buffered saline and administered intraperitoneally. All rats in the dose-response and survival study received 10 injections of BrdU (50 mg/kg) in 12-hour intervals (900 and 2100), during days 2 through 6 after the initial ECS treatment.

The rats in the time-course study were given four injections of BrdU (100 mg/kg) in 2-hour intervals (900, 1100, 1300, and 1500), either the same day or 2, 4, 6, or 8 days after the single ECS trial.

Tissue Preparation

Twenty hours (in the time-course study), 3 days (in the dose-response study), or 21 days (in the survival study) after the last injection of BrdU, the rats were anesthetized with sodium pentobarbital (60 mg/mL). In the absence of nociceptive reflexes, the rats were transcardially perfused with .9% saline for 2 minutes, followed by 4% ice-cold paraformaldehyde for 10 minutes. 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 (PBS) until they sank. Coronal sections, 40 μ m thick, were cut through the middorsal hippocampus (−3.30 mm to −4.52 mm relative to bregma) (Paxinos and Watson 1986) and stored in antifreeze cryoprotectant solution at −20°C until the immunohistochemical procedure.

BrdU/Rat Endothelial Cell Antigen-1/Laminin and BrdU/Neuron-Specific Nuclear Protein Immunofluorescence Stainings

Brain sections were rinsed (3 \times 10 minutes) in .02 mol/L potassium phosphate-buffered saline (KPBS) and then incubated in blocking solution (KPBS + 5% normal donkey serum [NDS] [Harlan Sera-Lab, Belton, United Kingdom] + .25% Triton X-100 [Sigma-Aldrich]) for 1 hour at room temperature. Sections were then incubated with 1:25 mouse anti-rat endothelial cell antigen-1 (RECA-1) (Serotec MCA 970, Oxford, United Kingdom) in blocking solution for 48 hours at 4°C.

Sections were rinsed with KPBS + .25% Triton X-100 (KPBS+) and subsequently incubated with 1:200 Biotin Donkey-anti-Mouse (Jackson 715-065-151, Jackson Immuno Research, West Grove, Pennsylvania) in blocking solution for 24 hours at 4°C. Sections were then rinsed 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, the sections were fixed in 4% paraformaldehyde for 10 minutes at room temperature, subsequently rinsed (2 \times 10 minutes) in KPBS, and then incubated in 1 mol/L hydrochloric acid (HCl) at 65°C for 30 minutes. After rinsing in KPBS (3 \times 10 minutes), the sections were then exposed to blocking solution (KPBS+, 5% normal donkey serum) for 1 hour in darkness at room temperature and then incubated with 1:100 rat anti-BrdU (Oxford Biotechnology, OBT 0030, Kidlington, United Kingdom) and 1:100 rabbit anti-laminin (Sigma-Aldrich, St. Louis, Missouri) in blocking solution for 40 hours in darkness at 4°C.

Sections were then rinsed in KPBS+ (2 \times 10 minutes) and KPBS+, 2% NDS (modified blocking solution) (2 \times 10 minutes) before being incubated with 1:200 Cy-3 Donkey-anti-Rat (Jackson 712-165-153, Jackson Immuno Research, West Grove, Pennsylvania) and 1:200 Cy-5 Donkey-anti-Rabbit (Jackson 711-175-152, Jackson Immuno Research, West Grove, Pennsylvania) in modified blocking solution for 24 hours in darkness at 4°C. After rinsing in KPBS,

sections were mounted on Poly-L-Lysine coated slides, air dried, and coverslipped with glycerol-based mounting medium.

The BrdU/Neuron-Specific Nuclear Protein (NeuN) staining was performed as described above with regard to origin of antibodies (except for NeuN [see below]), rinsing, DNA denaturation, and blocking. The sections were exposed to the primary antibodies in blocking solution (1:100 Rat anti-BrdU + 1:100 Mouse anti-neuron-specific nuclear protein (NeuN) [MAB 377, Chemicon, Temecula, California]) for 40 hours at 4°C. After washing, the sections were incubated with the secondary antibodies in modified blocking solution (1:200 Cy-3 Donkey-anti-Rat + 1:200 Biotin Donkey-anti-Mouse) for 2 hours in darkness, at room temperature. Sections were then rinsed (3 × 10 minutes) in KPBS+ before incubation with 1:200 Alexa 488 in KPBS+ for 2 hours in darkness, at room temperature. Sections were rinsed and mounted as previously described.

Endothelial Barrier Antigen Immunohistochemistry

Sections were rinsed in KPBS and incubated in blocking solution for 1 hour at room temperature. Sections were subsequently exposed to the primary antibody solution (blocking solution + 1:1000 Mouse anti-endothelial barrier antigen [EBA] [SMI 71, Sternberger Monoclonals, Lutherville, Maryland]) overnight at 4°C. After washing with KPBS+, the sections were incubated with the secondary antibody in modified blocking solution (1:200 Biotin Donkey-anti-Mouse) for 2 hours at room temperature. Sections were then rinsed (3 × 10 minutes) in KPBS+ before incubation with avidin-biotin-peroxidase complex (Vectastain Elite ABC kit; Vector Laboratories Inc., Burlingame, California) in KPBS for 1 hour, at room temperature. After rinsing in KPBS, peroxidase detection was performed for 7 minutes (5 mg/mL diaminobenzidine [DAB], 5 mg/mL NiCl₂, .01% hydrogen peroxide [H₂O₂]), followed by three rinses in KPBS, mounting, dehydration, and coverslipping.

Data Quantification and Statistical Analysis

Coronal 40 µm sections through the middorsal hippocampus (−3.30 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 SGZ), hilus, and molecular layer of the dentate gyrus, using an Olympus AX70 fluorescence microscope (Olympus Optical Co., Ltd., Tokyo, Japan) with a 40 × objective.

Proliferated endothelial cells were counted in the granule cell layer, hilus, and molecular layer. Cells lying within two cell diameters of the granule cell and hilar border were included in the granule cell layer count. Every fourth section throughout the middorsal hippocampus (averaging eight sections from each animal) was counted, and these values were averaged and expressed as means per section.

Endothelial cells in the GCL not associated with clusters were counted in a regular fluorescence microscope as described above; however, for cells in the subgranular zone appearing in tight clusters, no distinction was made between endothelial cells and other cell types, such as neural precursors, while counting in the fluorescence microscope. The percentage of the cluster cells (endothelial plus neural) in the zero and five ECS groups from the dose-response study expressing endothelial cell markers was determined by confocal analysis using a Nikon confocal microscope (Nikon, Tokyo, Japan) with a 40 × objective and BioRad software (BioRad, Burlington, Massachusetts), in which 20 clus-

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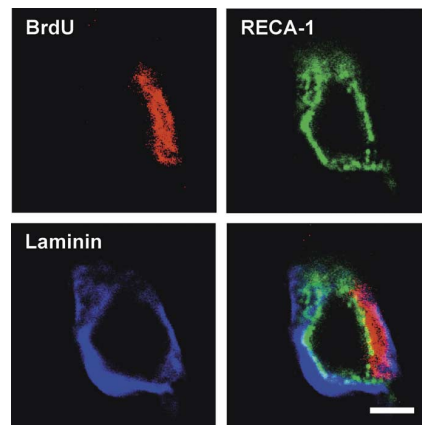


Figure 2. Histologic profile of a brain capillary. Confocal image depicting the appearance of a proliferated BrdU-labeled endothelial cell nucleus (red) situated between the RECA-1 positive luminal side of the vessel (green) and the laminin-labeled basal lamina (blue). Scale bar, 5 µm. BrdU, bromodeoxyuridine; RECA-1, rat endothelial cell antigen-1.

ters per animal (four animals from each group) were analyzed for possible BrdU/RECA-1 double-labeling.

Data are presented as means ± SEM and were analyzed with analysis of variance (ANOVA) and Bonferroni/Dunn post hoc test. Statistical significance was set at $p < .05$.

Results

Vascular Cells Display an Endothelial Phenotype

Rat endothelial cell antigen (RECA-1) immunoreactivity is expressed on the luminal side of endothelial cells (Duijvestijn et al 1992), while the basement membrane enclosing the entire vessel structure expresses laminin (Barsky et al 1983). Between these two layers lie endothelial cells and pericytes. The pericytes are completely surrounded by the basement membrane (Abbott et al 1989), while the endothelial cells are limited by the basement membrane at the abluminal side and RECA-1 positive structures at the luminal side. Utilizing fluorescence microscopy, cell nuclei were denoted as being of vascular origin by defining them by the following morphologic criteria: flattened and slightly cupped, somewhat elongated, and in immediate contact with the RECA-1 stained capillary luminal surface. Using confocal microscopy and analyzing a subset ($n = 100$) of vessel-associated BrdU-stained nuclei, we noted that all cells defined as vascular cells, as described above, were enclosed by RECA-1 and laminin positive structures, and we therefore concluded that this cell type is indeed of vascular origin and of an endothelial phenotype (Figure 2).

ECS Increase Endothelial Cell Proliferation

A single electroconvulsive seizure increased the proliferation of endothelial cells in the GCL, hilus, and molecular layer (ML). Proliferation was further enhanced by three and five electrocon-

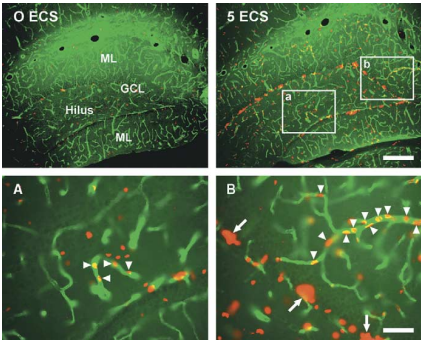


Figure 3. Distribution of proliferating cells in the dentate gyrus. Overview of the dentate gyrus of a rat having received no (upper left panel) and five ECSs (upper right panel). Proliferated BrdU-labeled cell nuclei are stained red, and brain vessel endothelium positive for RECA-1 appears green. In (A) an image of a vessel situated in the hilus is shown and in (B) a vessel emerging from the ML and extending into the GCL is depicted. Arrowheads indicate BrdU-labeled endothelial cell nuclei, while arrows indicate proliferative clusters in the subgranular zone. Scale bar in upper panels, 200 μ m and in A and B, 50 μ m. ECS, electroconvulsive seizure; BrdU, bromodeoxyuridine; RECA-1, rat endothelial cell antigen-1; GCL, granule cell layer; ML, molecular layer.

vulsive seizures (Figures 3 and 4). In agreement with an earlier study (Madsen et al 2000), this effect was also noted on clustered cells located in the SGZ (Figure 3 and 4), expressing either the early neuronal marker doublecortin (data not shown) or the endothelial marker RECA-1.

Endothelial and Cluster Cells Proliferate in Concert in Response to ECS

In response to a single electroconvulsive seizure, we detected a profound increase in endothelial cell proliferation in the GCL, hilus, and ML at day 2, with a very marked increase in the number of BrdU-labeled endothelial cells (Figure 5). No significant increases in endothelial cell proliferation relative to control could be detected at any of the other time points studied (Figure 5). In agreement with previously described results by Madsen et al (2000), the proliferation of cluster cells in the SGZ reached a maximum between days 3 and 5 (day 4) (Figure 5). At day 2, the rate of endothelial cell proliferation in all regions except the hilus was approximately 14 times higher than in the control animals, while the rate of proliferation of cluster cells in the SGZ at day 4 was approximately 3 times higher compared with control. We conclude that the nonclustered endothelial cells proliferate at a faster rate in response to a single ECS than the cluster cells located in the SGZ.

Increased Fraction of Endothelial Cluster Cells after ECS

The fraction of cells in the SGZ located in clusters, double-labeled with BrdU and RECA-1, was determined by confocal microscopy analysis. In four animals, each from the zero ECS and five ECS groups in the dose-response study, a total number of 87 ± 8.8 and 117 ± 4 cells, respectively, were analyzed per animal. The average cluster size in the zero ECS group was significantly smaller than in the five ECS group ($4.6 \pm .2$ and $5.8 \pm .2$ cells per cluster, respectively).

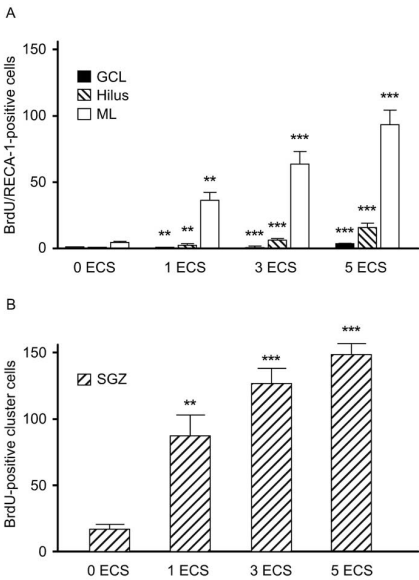


Figure 4. Electroconvulsive seizures increase cell proliferation. Treatment with one, three, or five ECSs increase the number of proliferating endothelial cells in the GCL, hilus, and ML of adult rats compared with rats receiving no ECS treatment (A). The same effect is also noted on the number of BrdU-labeled proliferating cluster cells (endothelial plus neural) in the SGZ (B). Asterisks indicate significant difference from the zero ECS group. Values represent mean \pm SEM number of cells detected per coronal section of the dentate gyrus. Data were analyzed with analysis of variance (ANOVA) and Bonferroni/Dunn post hoc test (* = $p < .05$; ** = $p < .01$; *** = $p < .0001$). Asterisk(s) indicate significant increase from the zero ECS group in the respective region analyzed. ECS, electroconvulsive seizure; GCL, granule cell layer; ML, molecular layer; BrdU, bromodeoxyuridine; SGZ, subgranular zone; ANOVA, analysis of variance.

In rats receiving no ECS-treatment, $8.7 \pm 1.4\%$ of the cluster cells were double-labeled with BrdU and RECA-1, while in rats receiving five ECS treatments, this fraction had increased significantly, with $18.9 \pm 1.8\%$ of the cluster cells being double-labeled for these two markers (Figure 6).

Cell Fate of the Proliferating Cells 3 Weeks after ECS

To determine the fate of the cells proliferating after zero or five ECSs, rats were given BrdU at days 2 through 6 after the first ECSs. Twenty-one days after a series of five ECSs, we detected significantly elevated numbers of BrdU-labeled cells in the SGZ and GCL (Figures 7 and 8), of which approximately 80% colabel with the neuronal marker neuron-specific nuclear protein (NeuN) (Figure 9). This finding is in direct agreement with what has previously been reported (Hellsten et al 2002; Madsen et al 2000). We also note that the number of BrdU/RECA-1-labeled cells in the GCL, hilus, and ML of rats receiving five ECSs are also still elevated compared to control (Figures 7 and 8).

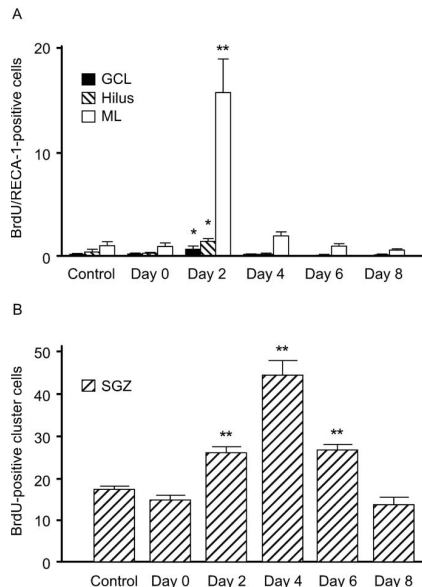


Figure 5. The proliferation of endothelial and neural cells occurs in concert. The numbers of BrdU-labeled endothelial cells detected in the GCL, hilus, and ML after a single ECS are significantly elevated compared with control at day 2 but at no other time points analyzed following the ECS trial (**A**). The number of BrdU-labeled proliferating cluster (endothelial plus neural) cells in the SGZ detected after a single ECS are elevated compared to control at days 2 through 6 and reaches a peak at day 4 (**B**). Values represent mean \pm SEM number of cells detected per coronal section of the dentate gyrus. Data were analyzed with analysis of variance (ANOVA) and Bonferroni/Dunn post hoc test (* = $p < .05$; ** = $p < .01$; *** = $p < .0001$). Asterisk(s) indicate significant increase from control in the respective region analyzed. BrdU, bromodeoxyuridine; GCL, granule cell layer; ML, molecular layer; ECS, electroconvulsive seizure; SGZ, subgranular zone; ANOVA, analysis of variance.

No Reduction of EBA-Expression after ECS

We detected no differences in staining intensity of EBA between control and ECS-treated animals in either the time-course study (control and days 0 through 4) (Figure 10) or the dose-response study (zero and five ECSs) (data not shown). As a positive control for the staining, we used brain sections from rats subjected to 2 hours of middle cerebral artery occlusion (MCAO). These animals display extensive and profound loss of EBA expression in regions of the brain ipsilateral to the insult (Figure 10).

Discussion

We show that electroconvulsive seizures, which previously have been shown to induce a marked increase in proliferation of neural precursors residing in the SGZ, also induce a very pronounced increase in proliferation of hippocampal endothelial cells. The seizure-induced proliferation of endothelial cells and

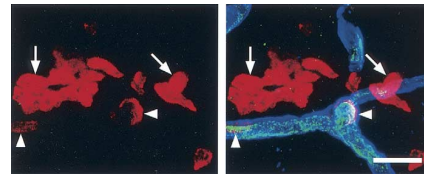


Figure 6. Confocal image of the relationship between proliferating cells in the subgranular zone and the vasculature. In the left panel, BrdU-labeled cells in a proliferative cluster from a rat having received five electroconvulsive seizures are depicted. In the right panel, an adjacent capillary immunoreactive for RECA-1 and laminin is also shown. Arrows indicate neural cell nuclei, while arrowheads indicate endothelial cell nuclei. Scale bar, 20 μ m. BrdU, bromodeoxyuridine; RECA-1, rat endothelial cell antigen-1.

neural precursors occurs in concert and the effect is dose-dependent, meaning that the number of proliferating endothelial cells increases with the number of electroconvulsive seizures administered.

Electroconvulsive seizures are associated with an intense activity in hippocampal neural networks. Despite extensive studies, cell death, as regularly seen after prolonged epileptic seizures (i.e., status epilepticus) (Kondratyev et al 2001) has not been observed after ECS. Hallmarks of physiologic hippocampal activation, such as neurotrophin regulation, mossy fiber sprouting, synaptic remodeling, and neurogenesis, have all been described after ECS (Duman and Vaidya 1998; Madsen et al 2000; Stewart and Reid 2000). The magnitude of these molecular, subcellular, and cellular changes is generally greater after ECS than during normal physiologic hippocampal activity.

The causes and consequences of the dramatic endothelial cell proliferation reported in this study are not known, but it is tempting to speculate that increased neural network activity and remodeling, in response to ECS, may lead to a concomitant angiogenic response. Whether the endothelial cell proliferation is followed by an expansion of the vascular tree is yet to be determined. Quantitative stereological analyses to assess possible ECS-induced topological changes in the hippocampal vasculature are currently underway.

It is, however, important to recognize that apart from the obvious role of angiogenesis in meeting an increased metabolic

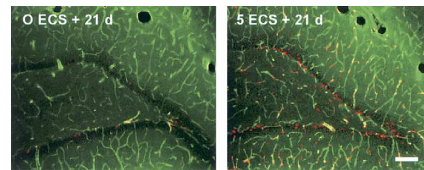


Figure 7. Distribution of proliferated cells 21 days after ECSs. Overview of the dentate gyrus of rats receiving no (left) and five ECSs (right), with a survival time of 21 days after ECS. Proliferated BrdU-labeled cell nuclei are stained red, and brain vessel endothelium positive for RECA-1 appears green. The BrdU-labeled cells in the granule cell layer have distributed evenly over the extent of the layer, while the BrdU-labeled endothelial cells in the granule cell layer, hilus, and molecular layer are still present. Scale bar, 100 μ m. ECS, electroconvulsive seizure; BrdU, bromodeoxyuridine; RECA-1, rat endothelial cell antigen-1.

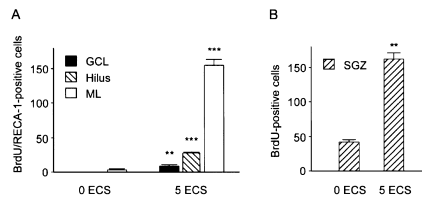


Figure 8. The number of BrdU-labeled endothelial cells detected in the GCL, hilus, and ML after five ECSs are still significantly elevated 21 days after the last BrdU injection. The number of BrdU-labeled cells in the SGZ is also still elevated. Values represent mean \pm SEM number of cells detected per coronal section of the dentate gyrus. Data were analyzed with unpaired t test (* = $p < .05$; ** = $p < .01$; *** = $p < .0001$). Asterisk(s) indicates significant increase from the zero ECS group in the respective region analyzed. BrdU, bromodeoxyuridine; GCL, granule cell layer; ML, molecular layer; ECS, electroconvulsive seizure; SGZ, subgranular zone.

demand, newly formed endothelial cells may have different properties than their mature counterparts, for example, producing a different set of growth factors or expressing other cell-cell or cell-matrix receptors, thereby contributing to an alteration in hippocampal cell-cell interactions. In line with this hypothesis, a recent investigation (Louissaint et al 2002) describes a close relationship between adult brain neurogenesis and angiogenesis in the higher vocal center (HVC) of female canaries. Testosterone is shown to induce a vascular endothelium growth factor (VEGF)-mediated increase in endothelial cell proliferation. This event is followed by expansion of the vascular tree and a subsequent production of brain-derived neurotrophic factor (BDNF) by the endothelial cells. The authors suggest a causal interaction between angiogenesis and neurogenesis where endothelium-derived BDNF promotes neuronal recruitment into the HVC from the HVC ventricular zone.

Similar interactions between endothelial cells and neurons may also occur in the mammalian brain. It is known that BDNF is secreted by endothelial cells (Leventhal et al 1999) and that it has both mitogenic and antiapoptotic effects on neuronal cells (Linnarsson et al 2000; Pencea et al 2001; Zigova et al 1998). The two angiogenic factors, VEGF and basic fibroblast growth factor (bFGF), can be produced by endothelial cells (Biro et al 1994; Hoehn et al 2002; Seghezzi et al 1998), and both are known to stimulate neurogenesis (Jin et al 2002; Wagner et al 1999). Furthermore VEGF stimulates axonal outgrowth in the peripheral nervous system (Sondell et al 1999), and bFGF can regulate synaptic function and has been described as a neurotransmitter

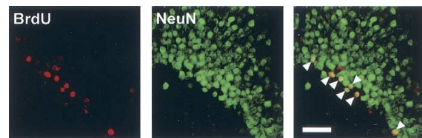


Figure 9. Many cluster cells become neurons. Confocal image of BrdU-labeled (red) and NeuN-positive (green) cells in the granule cell layer of a rat surviving for 21 days after receiving five electroconvulsive seizures. Bromodeoxyuridine and NeuN double-labeled cells indicating a mature neuronal phenotype are indicated by arrowheads. Scale bar, 40 μ m. BrdU, bromodeoxyuridine; NeuN, neuron-specific nuclear protein.

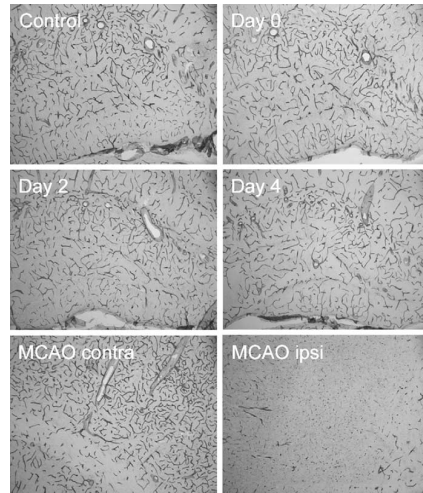


Figure 10. No reductions in staining intensity for EBA are detected at either day 0, 2, or 4 after a single ECS compared with control animals (upper and middle panels). As a positive control for the staining, brain sections from rats subjected to 2 hours of MCAO were used. These animals display extensive and profound loss of EBA expression in regions of the brain ipsilateral to the insult, in this case temporal-parietal cortex (lower panels). EBA, endothelial barrier antigen; ECS, electroconvulsive seizure; MCAO, middle cerebral artery occlusion.

(for review, see Abe and Saito 2001). Conversely, the neurotrophin nerve growth factor (NGF) has been reported to induce postnatal angiogenesis in rat superior cervical ganglia (Calza et al 2001).

Recently, a close spatial relationship between endothelial cells and neural progenitors has been proposed. Palmer et al (2000) found that at days 2 through 7 after BrdU-labeling, a substantial amount of the proliferating cells in the SGZ express the endothelial cell marker RECA-1. When analyzing clustered, newly proliferated cells within the SGZ, we found that in control animals approximately 9% of these cells are BrdU/RECA-1 positive. This fraction increased to 19% after a series of five electroconvulsive seizures. The vast majority of these BrdU-labeled cluster cells are not positive for RECA-1 but are instead grouped around and in close proximity to capillaries in the SGZ, still allowing for close contact between the vasculature and the neural progenitors (Figure 6). We believe that the increase in the fraction of RECA-1 positive cells per cluster noted after five ECSs is a reflection of the fact that, as compared to neural precursors, endothelial cells proliferate at a higher rate in response to ECS.

It is important to emphasize that proliferating endothelial cells are not only seen in the SGZ but along the full extent of capillaries, originating from larger vessels bordering the ML and extending through ML, GCL, and into the hilus. Although most numerous in ML, proliferating endothelial cells are thus detected in all dentate gyrus subfields, including the neural proliferative zone SGZ, and we

detect a fifteenfold to fiftyfold increase in endothelial cell proliferation in the various layers of the dentate gyrus analyzed after a series of five ECSs. As described above, there is a potential role for endothelial cell proliferation in the process of neurogenesis but not less importantly, the increases in endothelial cell proliferation in the nonneurogenic zones could also affect neuronal function in these regions. The ML harbors the afferent synapses of the dentate gyrus and the dendrites of the granule cells, while the hilus contains axons from the granule cells (mossy fibers) connecting to pyramidal neurons in the CA3 region.

The increase in endothelial cell proliferation in the ML and hilus could thus possibly affect synaptogenesis, as well as dendritic and axonal outgrowth in the respective region.

Brain endothelium and neighboring cells, i.e., astrocytes, have been shown to be able to interact and are believed to thus affect both blood brain barrier permeability and neuronal energy supply (for review, see (Abbott 2002)). In this article, the author argues that while situations where the blood brain barrier is compromised are generally expected to be deleterious, transient and reversible barrier opening could also be beneficial by, for example, supplying blood-borne factors that could stimulate neuronal growth and sprouting. Proliferation of vascular cells has in ischemic models been associated with severe blood brain barrier breakdown (Kataoka et al 2000); however, when staining for endothelial barrier antigen, which is expressed on endothelial cells with uncompromised barrier function (Rosenstein et al 1992), we detect no differences between control and ECS-treated animals either at day 0, day 2, or day 4 in the time-course study (Figure 10) or after five ECSs in the dose-response study (data not shown). This implies that any such blood brain barrier breakdown, if present, is transient and reversible or maybe not severe enough to affect the expression of EBA.

Remodeling and growth are in various organs commonly accompanied by increased vascularization to meet the raised metabolic demands, and conversely, a reduced blood supply is followed by atrophy. Major depression has been associated with hippocampal volume reductions, as assessed in clinical materials with magnetic resonance imaging volumetry (Sheline et al 1996, 1999). Although conflicting results exist, studies using single photon emission computerized tomography point to decreased blood flow in limbic and frontal brain regions in patients with major depression (Bonne and Krausz 1997; Bonne et al 1996; Milo et al 2001). Interestingly, an increase in regional cerebral blood flow has been observed in the temporal lobe at approximately 1 week after clinically successful electroconvulsive therapy (Mervaala et al 2001; Vangu et al 2003). Whether this increase is caused by an increased vascularization or a change in the regulation of the regional blood flow is, at present, not known.

The findings reported here represent the first evidence that electroconvulsive seizures, commonly used to treat severe depression, induce proliferation of brain endothelial cells. It is tempting to speculate that the very profound increase in endothelial cell proliferation detected in response to ECSs leads to changes in the hippocampal cell-cell interactions and possibly also to an expansion of the vascular tree, alterations that could oppose negative effects on hippocampal structure and function associated with major depression.

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