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Electroconvulsive seizures increase hippocampal neurogenesis after chronic corticosterone treatment

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Keywords: adult neurogenesis, electroconvulsive treatment, glucocorticoids, hippocampus, rat

Abstract

Major depression is often associated with elevated glucocorticoid levels. High levels of glucocorticoids reduce neurogenesis in the adult rat hippocampus. Electroconvulsive seizures (ECS) can enhance neurogenesis, and we investigated the effects of ECS in rats where glucocorticoid levels were elevated in order to mimic conditions seen in depression. Rats given injections of corticosterone or vehicle for 21 days were at the end of this period treated with either a single or five daily ECSs. Proliferating cells were labelled with bromodeoxyuridine (BrdU). After 3 weeks, BrdU-positive cells in the dentate gyrus were quantified and analyzed for co-labelling with the neuronal marker neuron-specific nuclear protein (NeuN). In corticosterone-treated rats, neurogenesis was decreased by 75%. This was counteracted by a single ECS. Multiple ECS further increased neurogenesis and no significant differences in BrdU/NeuN positive cells were detected between corticosterone- and vehicle-treated rats given five ECS. Approximately 80% of the cells within the granule cell layer and 10% of the hilar cells were double-labelled with BrdU and NeuN.

We therefore conclude that electroconvulsive seizures can increase hippocampal neurogenesis even in the presence of elevated levels of glucocorticoids. This further supports the hypothesis that induction of neurogenesis is an important event in the action of antidepressant treatment.

Introduction

The dentate gyrus of the hippocampus is one of the few areas where there is an ongoing neurogenesis in the adult mammalian brain (Altman and Das, 1965; Eriksson *et al*., 1998). The hippocampus is also a vulnerable region prone to damage during ageing and stress (Stein-Behrens and Sapolsky, 1992).

In animal experiments, stress and glucocorticoids (GCs) (i.e. corticosterone; CORT) have been shown to both induce reversible dendritic atrophies in the hippocampal subfield CA3 and to decrease proliferation and neurogenesis in the dentate gyrus (Gould *et al*., 1992; Sapolsky, 1992; Cameron and Gould, 1994; McEwen and Sapolsky, 1995; Kuhn *et al*., 1996; Tanapat *et al*., 1998; Gould and Tanapat, 1999; McEwen, 1999; Alonso, 2000, 2001).

Patients suffering from major depression often have a disturbed hypothalamic-pituitary-adrenal axis with elevated levels of the glucocorticoid cortisol, and MRI-studies reveal that repeated episodes of major depression are associated with smaller hippocampal and amygdala volumes (Sheline *et al*., 1996; Sheline *et al*., 1998; Sheline *et al*., 1999; Bremner *et al*., 2000).

Whether elevated levels of GCs and subsequent atrophies and/or reduced proliferation of neurons and other cell types could account for the observed hippocampal volume reductions in the mentioned clinical materials is yet to be determined.

Electroconvulsive therapy (ECT) is a widely used and effective treatment for depression, however, the mechanisms of action remain unclear. We and others have recently shown that electroconvulsive seizures (ECS), an animal model of ECT, are associated with dramatically increased hippocampal neurogenesis in the adult rat (Madsen *et al*., 2000; Malberg *et al*., 2000; Scott *et al*., 2000). Furthermore, Malberg *et al*. (2000) reported that several antidepressant drugs are capable of inducing hippocampal neurogenesis. The mood stabilizing agent lithium has a similar effect on neurogenesis (Chen *et al*., 2000).

In this paper we were interested in extending our findings on ECS-induced neurogenesis by investigating the effects of acute and chronic ECS on hippocampal neurogenesis in adult rats pretreated with CORT, in order to mimic the elevated levels of GCs associated with major depression, which presumably reduce normal hippocampal neurogenesis.

Materials and methods

Animals and design of study

Adult male Wistar rats (Møllegaard breeding centre, Denmark), weighing 180 g at the beginning of the study were used. Rats were housed three per cage and kept on a 12-h 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. The rats (n = 36) were divided into the following groups: (i) 21 days of corticosterone (CORT) injections (n = 6); (ii) 21 days of CORT-injections and a single ECS-trial (n = 6); (iii) 21 days of CORT-injections and five ECS-trials (n = 6); (iv) 21 days of vehicle

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injections (n = 6); (v) 21 days of vehicle injections and a single ECS-trial (n = 6) and (vi) 21 days with vehicle injections and five ECS-trials (n = 6).

The weights of all rats were determined every third day during the entire experiment in order to monitor the effects of CORT- and vehicle-injections.

Administration of electroconvulsive seizures

On day 15 of the injection regime, all rats were subjected to either a single ECS-trial or a sham treatment. ECS were delivered via silver electrode ear clips (Somedic Sales AB, Sweden) (50 mA, 0.5 s and 50 Hz unidirectional square wave pulses). The rats were monitored to ensure that clonic movements of the face and forelimbs occurred after ECS for 10–15 s (indicative of limbic motor seizures). Rats receiving five ECS-trials were given the remaining treatments once daily on days 16 to19. Sham treated rats were handled identically to the ECS-treated rats except no current was passed.

Administration of corticosterone

A stock emulsion of corticosterone (C2505; Sigma-Aldrich, St Louis, MO, USA) at a concentration of 33.3 mg/mL was prepared daily by vortexing corticosterone in sesame oil (Sigma-Aldrich) for 10 min, followed by 60 min of sonication. Prior to every injection, the emulsion was vortexed briefly and injections were made subcutaneously in the neck region (40 mg/kg) every 24 h. This dose is adequate to elevate blood levels of corticosterone over a 24-h period (Sapolsky *et al*., 1985). Control rats received only sesame oil injections.

Administration of BrdU

Bromodeoxyuridine (B5002; Sigma-Aldrich, St Louis, MO, USA) was dissolved in phosphate buffered saline (PBS) and administered intraperitoneally. All rats received ten injections of BrdU (50 mg/kg) in 12-h intervals, during day 17–21 of the CORT/vehicle injection regime.

Tissue preparation

Three weeks after the last injection of BrdU, the rats were anaesthetized with sodium pentobarbital, 60 mg/mL. In the absence of nociceptive reflexes, the rats were transcardially perfused with 0.9% saline for 2 min, followed by 4% ice-cold paraformaldehyde for 13 min. Following decapitation, the brain was removed from the skull and postfixated in 4% paraformaldehyde at 4 °C overnight. The adrenal glands were dissected and weighed in order to assess the degree of atrophy as a measurement of the efficacy of the CORT-treatment. Prior to sectioning on a freezing microtome, the brains were left in 30% sucrose in PBS until they sunk. Coronal sections, 40 μm thick, were cut through the mid-dorsal 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/neuron-specific nuclear protein immunohistochemistry

Brain sections were rinsed three times in 0.02 M potassium phosphate-buffered saline (KPBS) and then incubated in 1 M HCl at 65 °C for 30 min Following rinsing in KPBS (3 × 10 min) the sections were incubated in blocking solution (KPBS + 5% normal donkey serum (NDS) (Harlan Sera-Laboratory, Belton, UK) + 5% normal horse serum (NHS) (Sigma-Aldrich, St Louis, MO, USA) + 0.25% Triton X-100) for 1 h at room temperature.

Sections to be visualized with fluorescence were subsequently exposed to the primary antibody solution (blocking solution + 1 : 100 rat anti-BrdU (Harlan Sera-Laboratory, MAS 250p) + 1 : 100 mouse antineuron-specific nuclear protein (NeuN) (MAB 377, Chemicon, Temecula, CA, USA)) for 40 h at 4 °C and slow shaking. After washing with KPBS + 0.25% Triton-X (2 × 10 min) and KPBS + 0.25% Triton X-100 + 2% NDS + 2% NHS (2 × 10 min), the sections were incubated with the secondary antibodies in modified blocking solution (KPBS + 0.25% Triton X-100 + 2% NDS + 2% NHS + 1 : 200 Cy-3 donkey-anti-rat (Jackson 172-165-153, Jackson Immuno Research, West Grove, PA, USA) + 1 : 200 biotin horse-anti-mouse (Vector BA-2001, Vector Laboratories Inc, Burlingame, CA, USA)), for 2 h in darkness, at room temperature. Sections were then rinsed (3 × 10 min) in KPBS + 0.25% Triton X-100 before incubation with 1 : 200 Alexa 488 (Molecular Probes, Eugene, OR, USA) in KPBS + 0.25% Triton X-100 for 2 h in darkness, at room temperature. Sections were rinsed again in KPBS (3 × 10 min), and subsequently mounted on poly L-lysine coated slides, air dried, rinsed briefly (10 s) in H_2O and coverslipped with glycerol-based mounting medium.

Sections to be visualized with diaminobenzidine (DAB) were rinsed three times in 0.02 M KPBS and incubated in 1 M HCl at 65 °C for 30 min Following rinsing in KPBS (3 × 10 min) the sections were incubated in blocking solution (KPBS + 5% NHS + 0.25% Triton X-100) for 1 h at room temperature. Sections were subsequently exposed to the primary antibody solution (blocking solution + 1 : 25 mouse anti-BrdU (CAT 347580, Becton Dickinson, Franklin Lakes, NJ, USA)) for 40 h at 4 °C and slow shaking. After washing with KPBS + 0.25% Triton-X (3 × 10 min), the sections were incubated with the secondary antibody in blocking solution (KPBS + 5% NHS + 0.25% Triton X-100 + 1 : 200 biotin horse-anti-mouse (Vector BA-2001, Vector Laboratories Inc.)) for 2 h at room temperature. Sections were then rinsed (3 × 10 min) in KPBS + 0.25% Triton X-100 before incubation with avidin-biotin-peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories Inc.) in KPBS for 1 h, at room temperature. After rinsing in KPBS (3 × 10 min) peroxidase detection was performed for 3.5 min (0.5 mg/mL DAB, 0.5 mg/mL NiCl, 0.01% H_2O_2), followed by three rinses in KPBS, mounting, dehydration and coverslipping.

Fluoro-Jade staining

The staining was performed according to the protocol originally developed by Schmued *et al*. (1997). Brain sections were rinsed three times in 0.02 M KPBS, mounted on poly L-lysine coated slides and air dried overnight. The mounted sections were then immersed in 100% ethanol for 3 min, 70% ethanol for 1 min and distilled water for 1 min. Pre-treatment in 0.06% potassium permanganate for 15 min was followed by rinsing in distilled water for 1 min and subsequent staining with Fluoro-Jade working solution (Histo-Chem, Jefferson, AR, USA) for 30 min on a rotatory shaker. Following rinsing in distilled water (3 × 1 min) the mounted sections were air dried, immersed in xylene and coverslipped.

Silver staining

The staining was performed according to the protocol by Nadler and Evenson (1983). Brain sections were washed in 0.1 M Tris buffer (pH 7.6) followed by rinsing three times in H_2O.

After pretreatment in 4.5% NaOH and 8% NH_4NO_3, the sections were incubated in impregnation solution (5.4% NaOH : 6.4% NH_4NO_3 : 0.2% $AgNO_3$ in H_2O) for 10 min and then washed in 31.6% ethanol : 0.5% $NaCO_3$: 0.12% NH_4NO_3. The staining was developed in 0.05% citric acid : 0.55% formaldehyde : 9.5% ethanol : 0.12% NH_4NO_3 for 5 min. Sections were then rinsed in

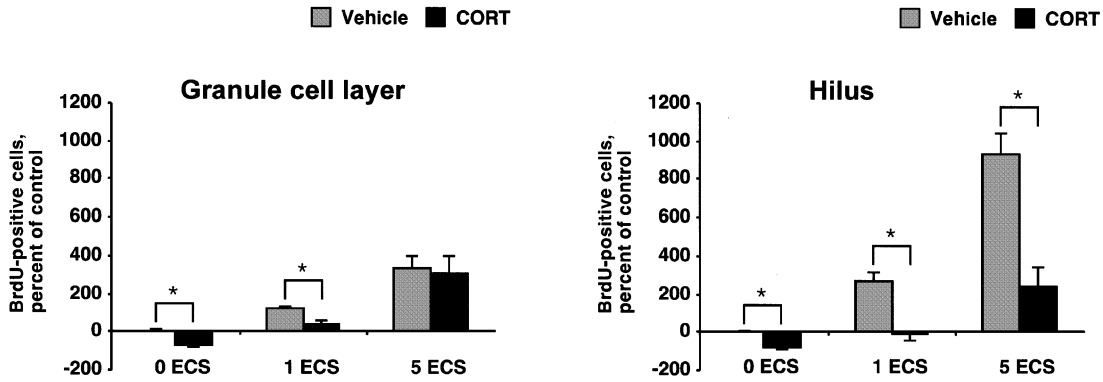

FIG 1. Effects of corticosterone (CORT) and electroconvulsive seizures (ECS) alone and/or combined on the number of BrdU-labelled cells in the granule cell layer and hilus. Increase/decrease in the number of cells is described as percentage of control (vehicle).

TABLE 1. The effect of daily treatment with CORT on adrenal weight, body weight and the adrenal weight : body weight ratio

	Non-CORT (n = 18)	CORT (n = 18)
Adrenal weight (mg)	30.0 ± 0.7	18.6 ± 1.0***
Body weight (g)	334.6 ± 5.8	289.0 ± 0.6***
Ratio (adrenal weight : body weight)	$8.9 \times 10^{-5} \pm 0.2 \times 10^{-5}$	$6.5 \times 10^{-5} \pm 0.4 \times 10^{-5}$***

Values represent means ± SEM. All rats treated with vehicle injections were pooled into one group (non-CORT) and all CORT-treated rats into another (CORT). ***$P < 0.001$, compared with non-CORT-treated rats (one-way ANOVA.).

0.1 M Tris buffer (pH 7.6), mounted, air-dried, dehydrated and coverslipped.

Cresyl violet staining

Brain sections to be stained were rinsed three times in 0.02 M KPBS, mounted on poly L-lysine coated slides and air dried overnight. The sections were briefly rinsed in H_2O twice and then subsequently dipped in 0.5% cresyl violet solution for 5 s followed by rinsing in H_2O, dehydration and coverslipping.

Data quantification and statistical analysis

Coronal sections through the mid-dorsal hippocampus (–3.30 mm to –4.52 mm, relative to bregma) (Paxinos and Watson, 1986) were analyzed by observers blind to the treatments. Cells in the granule cell layer and hilus were counted separately. Cells lying within two cell diameters of the granule cell and hilar border were included in the granule cell count. Counting was performed using a conventional light microscope with a 20× objective. Every fourth section throughout the mid-dorsal hippocampus (averaging eight sections from each animal) was counted and these values were averaged and expressed as means per dentate gyrus. Confirmation of double-labelling was performed on a Nikon confocal microscope using a 40× objective and Bio-Rad software (Bio-Rad, Burlington, MA, USA). Twenty-five BrdU-positive cells per animal were analyzed for verification of colocalization within the granule cell layer and hilus, respectively. Cells were counted only in the top 15 µm of each section in order to account for differences in the penetration of the different antibodies.

By delineating the granule cell layer and the hilus on four randomly chosen cresyl violet stained sections from each animal, using CAST-GRID software (Olympus, Albertslund, Denmark) and an Olympus BH-2 microscope with a 10× objective and CCD-IRIS colour video camera, values of the cross-sectional areas of these regions were obtained. The total volume of the region of interest was estimated as the mean cross-sectional area multiplied with the length of the region sectioned, i.e. 1.22 mm. The relative differences between the groups investigated were of greater interest than absolute values of the volumes, and therefore the data was not corrected for shrinking associated with the histological processing of the material.

All data are presented as means ± SEM and were analyzed with ANOVA and Bonferroni/Dunn post-hoc test. Statistical significance was set at $P < 0.05$.

Results

Biological efficacy of the CORT-treatment

The corticosterone injections resulted in decreased body weight gain, decreased adrenal weight and lowered adrenal weight : body weight ratio (Table 1).

Corticosterone reduces proliferation and neurogenesis

Corticosterone- and vehicle-treated rats were given BrdU for five consecutive days (ten injections) and killed 3 weeks later. BrdU-positive cells were identified within the granule cell layer and hilus of the dentate gyrus. CORT-treated rats exhibited 75% fewer BrdU-positive cells in the granule cell layer relative to vehicle-injected rats (Figs 1 and 2; Table 2). A significant decrease of similar magnitude (~80%) was also seen in the number of BrdU-labelled cells located within the hilus (Figs 1 and 2; Table 2). No significant differences were detected between vehicle- and CORT-treated rats in the percentage of BrdU/NeuN double-labelled cells in the dentate gyrus or the hilus (79% and 8%, respectively) (Table 3).

A single ECS increase proliferation and neurogenesis back to baseline-levels in CORT-treated rats

A single ECS increased BrdU-labelled cells in the granule cell layer (Fig. 1; Table 2).

TABLE 2. Numbers of BrdU-positive cells detected in the granule cell layer and hilus of vehicle- and CORT-treated rats subjected to 0, 1 or 5 ECS-treatments

	BrdU-positive cells detected (*n*)					
	No ECS treatment		1ECS treatment		5ECS treatment	
	Vehicle	CORT	Vehicle	CORT	Vehicle	CORT
Granule cell layer	31.6 ± 4.4	8.1 ± 1.8*	69.6 ± 4.6*	42.8 ± 6.5	136.6 ± 21.9*	128.9 ± 28.6*
Hilus	6.4 ± 0.5	1.3 ± 0.7*	23.6 ± 3.1*	6.2 ± 2.4	66.2 ± 7.0*	21.6 ± 6.8*

Values represent means ± SEM. Data were analyzed with ANOVA and Bonferroni/Dunn post-hoc test. *$P < 0.05$, compared, in the same structure, with vehicle with no ECS treatment.

TABLE 3. The percentages of BrdU-labelled cells in the granule cell layer and hilus that are double-labelled with NeuN

	BrdU-labelled cells that are double-labelled with NeuN (%)					
	No ECS treatment		1ECS treatment		5ECS treatment	
	Vehicle	CORT	Vehicle	CORT	Vehicle	CORT
Granule cell layer	83.3 ± 4.8	74.8 ± 9.4	80.5 ± 7.4	72.2 ± 7.4	80.3 ± 7.6	69.4 ± 5.6
Hilus	5.6 ± 2.8	11.1 ± 2.8	13.8 ± 2.8	8.3 ± 4.8	11.1 ± 5.5	11.1 ± 2.8

Values represent means ± SEM. The percentages of BrdU/NeuN-positive cells were analyzed by confocal microscopy (25 cells per animal). Data were analyzed by ANOVA with Bonferroni/Dunn post-hoc test. No significant differences were detected with the different treatments ($P > 0.05$).

In CORT-treated rats receiving a single ECS 39% significantly fewer BrdU-labelled cells were detected in the granule cell layer relative to vehicle rats receiving a single ECS (Fig. 1). The number of BrdU-labelled proliferating cells within the granule cell layer in CORT-treated rats receiving a single ECS did not differ significantly from the number of proliferating cells in the granule cell layer of the vehicle-control rats that had not received ECS-treatment (Table 2). Thus, a single ECS was sufficient to increase proliferation back to baseline rates of proliferation in normal non-ECS treated rats. Approximately 76% of the BrdU-labelled cells in the granule cell layer in both vehicle- and CORT-treated rats co-labelled with the neuronal marker NeuN, with no significant differences between the groups (Table 3).

In the hilus, 75% fewer proliferating cells were detected in the CORT-group relative the vehicle-group, and no significant increase in proliferation was detected in response to a single ECS (Fig. 1; Table 2). Both groups displayed roughly the same percentage (11%) of double-labelling for BrdU and NeuN (Table 3).

Multiple ECS eliminate the inhibitory effect of CORT on proliferation and neurogenesis in the granule cell layer but not in the hilus

Multiple ECS further increased BrdU-labelling in the granule cell layer (Figs 1 and 2; Table 2). Both CORT- and vehicle-groups given five ECS displayed significantly elevated numbers of BrdU-positive cells relative to the single ECS groups. No significant differences in the number of BrdU-labelled cells within the granule cell layer were detected with either of the two treatment conditions (Fig. 1). Approximately 75% of the BrdU-labelled cells in the granule cell layer in CORT- and vehicle-treated rats co-labelled with the neuronal marker NeuN (Table 3, Fig. 3).

Multiple ECS also increased cell proliferation in the hilus (Figs 1 and 2; Table 2). Interestingly, unlike the granule cell layer proliferation, the number of BrdU-labelled cells within the hilus was still significantly lower in CORT-treated rats compared to vehicle-treated rats after multiple ECS (Fig. 1). Furthermore, only 11% of the newborn cells within the hilus in both treatment groups were double stained for BrdU and NeuN (Table 3).

No degenerating or dead cells are detected in the granule cell layer or hilus

None of the techniques utilized to detect cell death and/or degenerating cells in the hippocampal subfields granule cell layer and hilus were able to reveal any differences in these parameters in any of the treatment groups in the experiment. No cells with pyknotic appearance were detected with cresyl-violet staining. No argyrophilic cells were detected with the silver staining technique and also no Fluoro-Jade-positive cells were detected.

Chronic CORT-treatment does not induce detectable reductions in the volume of the granule cell layer or the hilus

The CORT-treatment did not induce any detectable reductions in the volume of the granule cell layer or the hilus. Multiple ECS-treatments did not increase the volume of either of these two subfields (Table 4).

Discussion

The present study was designed in order to examine the effects of ECS on hippocampal neurogenesis in adult rats with elevated levels of CORT. In this study we use the term neurogenesis to describe, not just neuronal proliferation, but the generation of new neurons, which is a process that includes proliferation of neuronal precursors, death of some of these newborn cells and finally differentiation of the surviving cells into mature neurons. We found that a single electroconvulsive seizure is able to restore the reduced number of BrdU-positive cells in the granule cell layer of rats treated with CORT back to normal levels. A series of multiple ECS further increased the generation of new cells to the point where no differences were detected between vehicle and CORT-treated rats. Approximately 80% of these BrdU-labelled cells were NeuN-positive both in CORT- and vehicle-treated rats, and we can therefore

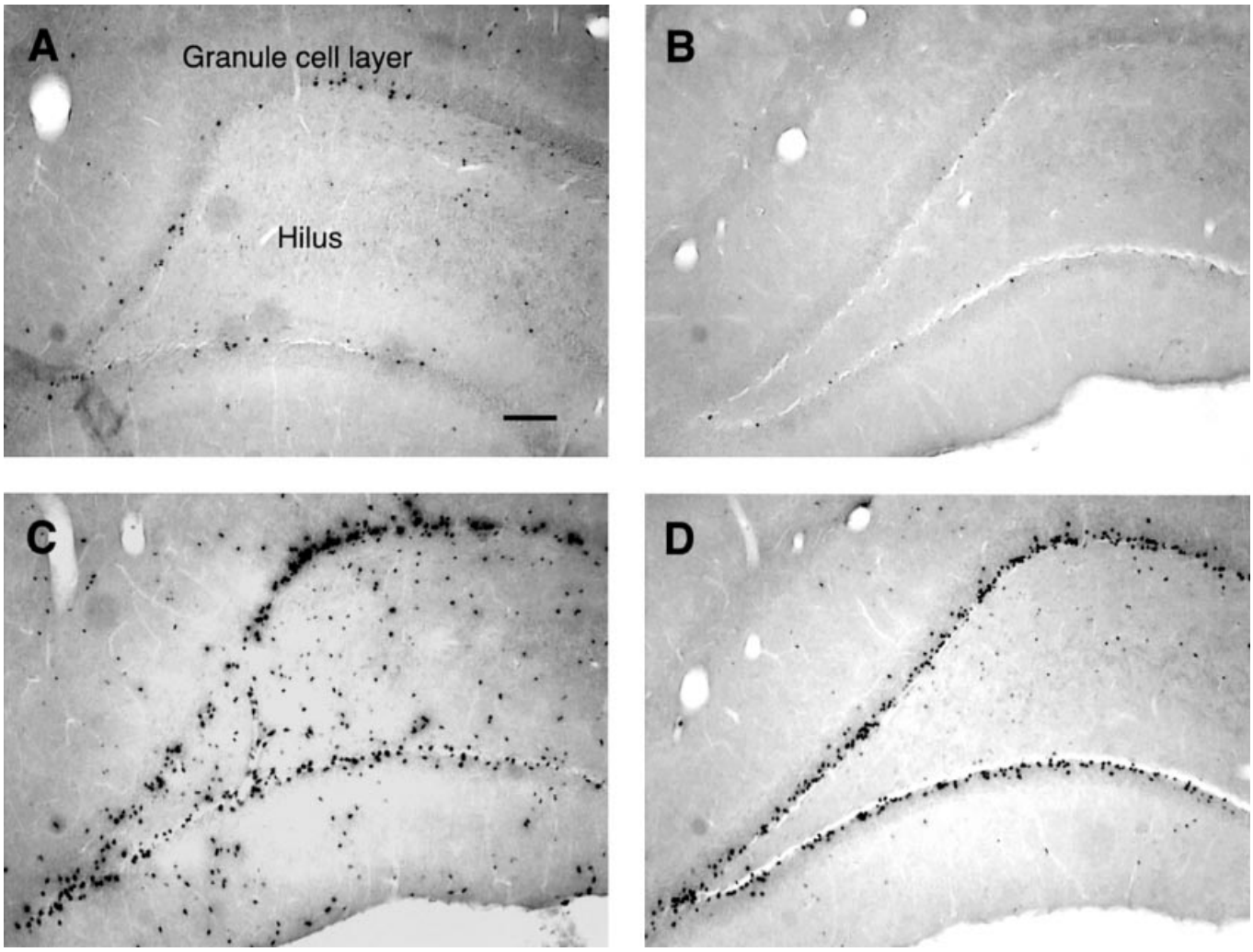

FIG 2. Chronic corticosterone (CORT)-treatment followed by a 3-week survival period resulted in reduced numbers of BrdU-positive cells detected in the dentate gyrus (B) compared to that seen in vehicle-injected rats (A). A series of five electroconvulsive seizures (ECS) increased the number of BrdU-positive cells in the granule cell layer of both CORT-treated (D) and vehicle-treated (C) rats. No significant difference in the number of BrdU-positive cells was detected in the granule cell layer between the two groups. In contrast, in the hilus fewer BrdU-positive cells were detected in the CORT-treated rats after five ECSs compared to vehicle-treated rats given 5 ECSc (D and C, respectively). Scale bar, 100 μm.

TABLE 4. Estimates of the volumes (mm^3) of the granule cell layer and the hilus in rats subjected to vehicle-/CORT-treatment and/or multiple ECSs

	Volumes of the granule cell layer and the hilus (mm^3)			
	Vehicle, no ECS	CORT, no ECS	Vehicle + 5ECS	CORT + 5ECS
Granule cell layer	$0.165 \pm 5.2 \times 10^{-3}$	$0.159 \pm 3.4 \times 10^{-3}$	$0.162 \pm 8.1 \times 10^{-3}$	$0.169 \pm 9.8 \times 10^{-3}$
Hilus	$0.364 \pm 6.8 \times 10^{-3}$	$0.353 \pm 20.6 \times 10^{-3}$	$0.401 \pm 14.9 \times 10^{-3}$	$0.391 \pm 12.6 \times 10^{-3}$

Values represent means ± SEM. Data were analyzed with ANOVA and Bonferroni/Dunn post-hoc test. No significant differences were detected with the different treatments ($P > 0.05$).

conclude that ECS can reverse the decrease in neurogenesis in the granule cell layer observed in rats three weeks after a period of chronic CORT-treatment. However, in the hilus, ECS could not normalize levels of newborn cells in CORT-treated rats. Only 10% of these BrdU-labelled hilar cells in both CORT- and vehicle-treated rats were positive for the neuronal marker NeuN. As the granule cell layer mainly consists of granule cell precursors and mature granule cell neurons, while the hilus, apart from different types of interneurons mainly contains other cell types such as glial cells, the differences in BrdU/NeuN double-labelling between these two regions are not surprising. Also, because the ratio of NeuN-positive cells in the hilus is conserved also after five ECSs, we can conclude

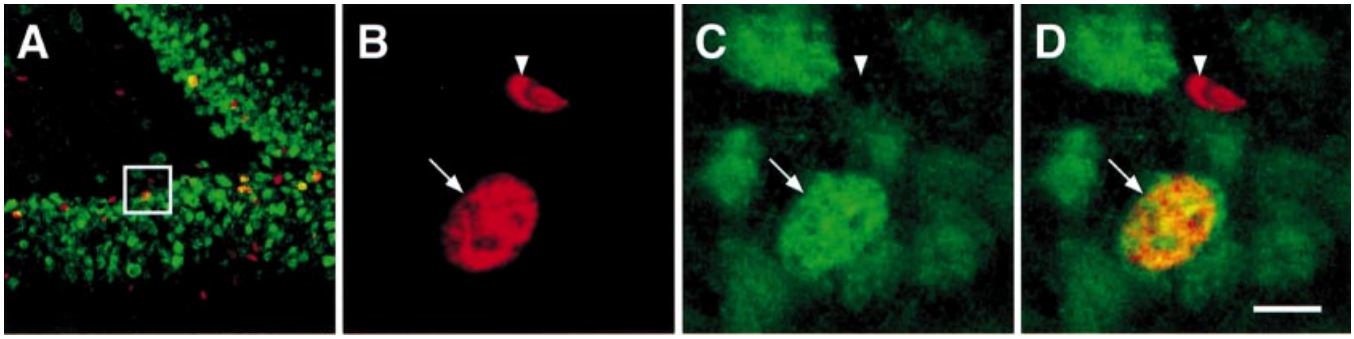

FIG 3. Confocal image of BrdU-labelled (red) and NeuN-positive (green) cells in the dentate gyrus of a rat receiving vehicle injections and five electroconvulsive seizures (A). The box indicated in A on the border between the granule cell layer and hilus is shown in higher magnification in B–D. (A) BrdU and NeuN-positive cell with granule cell nuclear morphology (arrow) is shown, as well as a non-neuronal BrdU-positive cell, lacking granule cell nuclear morphology (arrowhead) (B–D). Images B and C are merged in D. Scale bar, 5 μm (B–D)

that ECS not only promotes neurogenesis but also the generation of other non-neuronal cell types. Further studies will be required to determine the phenotype of these cells.

Effects of CORT on hippocampal neurogenesis

We found that neurogenesis within the granule cell layer was reduced by approximately 75% in rats that were injected with CORT for three weeks and then allowed to survive for an additional three weeks. This finding corresponds with other reports examining effects of elevated levels of CORT on hippocampal neurogenesis (Cameron and Gould, 1994; Gould *et al*., 1997; Pham *et al*., 1999; Alonso, 2000, 2001).

CORT is interacting with two types of adrenal steroid receptors (type I and type II) in a dose dependent manner (Reul and de Kloet, 1985), and selective activation of type II receptors induces hippocampal cell loss (Hassan *et al*., 1996; Sousa *et al*., 1999). The high CORT-concentrations in our experiment presumably activate both receptor types, and an overweight in the type II pathway could reduce the survival of the newly formed cells. The fact that ECS cannot reverse the CORT-mediated reduction in proliferating hilar cells may be explained by different expression of the two adrenal steroid receptors on these cells compared to the granular neuronal precursors.

In the absence of CORT and other adrenal steroids after adrenalectomy, granule cell death by apoptosis has been reported by several investigators (Sloviter *et al*., 1989; Gould *et al*., 1990; Cameron and Gould, 1994). A different interpretation of the reduced number of BrdU-labelled cells in the CORT-treated animals is thus that the discontinuation of CORT-administration would result in such low CORT-levels at the beginning of the three week survival period that cell death for this reason will be induced.

Three different techniques utilized for detecting degenerating cells (Fluoro-Jade staining, silver staining, cresyl violet staining) did, however, not reveal any evidence of dead or degenerating cells present in the dentate gyrus or hilus, but because the animals were allowed to survive for three weeks after the completed injection regime, we cannot rule out the possibility that some cell death could have occurred earlier. Also, we did not detect any differences in the volumes of the granule cell layer or the hilus in the treatment groups investigated (Table 4).

Cameron *et al*. (1993) showed that granular cell precursors do not express either of the two mentioned adrenal steroid receptors, suggesting that any effect by CORT on these cells must be indirect. Activation of *N*-methyl-D-aspartate (NMDA)-receptors appears to be one mechanism by which CORT exerts its effect (Cameron *et al*., 1998). Another mechanism could be regulation of factors necessary for cell growth and survival such as brain-derived neurotrophic factor (BDNF).

This neurotrophic factor is essential for the survival of proliferating cells within the subventricular zone and granule cell layer of juvenile mice (Linnarsson *et al*., 2000), and infusion of BDNF into the ventricles has been reported to increase neurogenesis in the olfactory bulb, and to induce neurogenesis in striatum, septum, thalamus and hypothalamus (Zigova *et al*., 1998; Pencea *et al*., 2001). Furthermore, it is known that increased levels of endogenous CORT by means of restraint stress as well exogenously administered CORT lowers the expression of mRNA for BDNF in subfields of the adult rat hippocampus (Smith *et al*., 1995). It is thus tempting to speculate that CORT-induced reduction of hippocampal neurogenesis may in part be mediated by reduced expression of BDNF.

Effects of ECS on hippocampal neurogenesis

We have previously reported that electroconvulsive seizures strongly up-regulate neurogenesis in the dentate gyrus of the adult rat hippocampus (Madsen *et al*., 2000). This finding has since been confirmed by two other research groups (Malberg *et al*., 2000; Scott *et al*., 2000). The newly generated neurons display normal granule cell morphology and dendritic processes (Wennström *et al*. unpublished observation). The report by (Bengzon *et al*., 1997) on seizure-induced neurogenesis in the adult rat brain stated that the increase in neurogenesis is accompanied by increased apoptotic cell death. In contrast, our previous study (Madsen *et al*., 2000) revealed no evidence of increased cell death after ECS. In fact it has been reported that electroconvulsive seizures completely protect against adrenalectomy-induced apoptosis in the granule cell layer (Masco *et al*., 1999) as well as preventing neuronal apoptosis by kainic acid-evoked status epilepticus (Kondratyev *et al*., 2001). It is thus possible that ECS can increase the generation of new neurons partly by counteracting apoptosis.

As described elsewhere, CORT has profound effects on the expression of mRNA for BDNF. Electroconvulsive seizures also affect the expression of this important neurotrophic factor. Chronic ECS cause a sustained increase in mRNA for BDNF (Zetterstrom *et al*., 1998), and its receptor trkB (Nibuya *et al*., 1995). Furthermore, the latter report showed that chronic ECS blocks the down-regulation of BDNF mRNA in response to restraint stress. The effects of ECS on BDNF expression can be attenuated by NMDA-receptor block,

implying the role of the NMDA-system in the mechanism of action of ECS (Chen *et al*., 2001). However, as seizure duration is shortened by ketamine-treatment, other mechanisms attributable to this effect may also be involved in this reduction of BDNF expression. In conclusion, ECS-mediated increases of BDNF-expression could potentially add to the protective mechanisms against cell death discussed previously and may also promote cell proliferation.

Regulation of BDNF has also been suggested to be involved in the therapeutic action of antidepressants and direct infusion of BDNF into the dentate gyrus of adult rats produces antidepressant effects in two behavioural models for depression, the learned helplessness and forced swim test paradigms (Shirayama *et al*., 2002). The effect of the BDNF-infusions is similar to that achieved from treatments with regular antidepressants.

Just as ECS, antidepressants and the mood stabilizing drug lithium have been shown to induce neurogenesis in the adult rat hippocampus (Chen *et al*., 2000; Malberg *et al*., 2000). As additional support to the theory of increased neuronal resiliency in the dentate gyrus of the hippocampus as an important aspect of antidepressant therapy, Czeh *et al*. (2001) recently showed that simultaneous treatment with the antidepressant drug tianeptine prevents stress-induced decreases in the proliferation rate of granule cell precursors in the adult tree-shrew.

To summarize, neurogenesis is reduced in animals treated with high levels of exogenous CORT. ECS totally restores neurogenesis back to normal levels presumably by both stimulating neuronal proliferation and by counteracting cell death. These mechanisms may be mediated by neurotrophic factors. Electroconvulsive seizures thus appear to be able to normalize hippocampal neurogenesis in animals where the normal adrenal steroid feedback mechanisms are distorted. This investigation further adds to the growing body of knowledge concerning the role of hippocampus in stress and affective disorders, and the means whereby antidepressant treatment potentially attenuate or abolish stress-induced changes in the hippocampal formation.

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Abbreviations

BDNF, brain-derived neurotrophic factor; BrdU, bromodeoxyuridine; CORT, corticosterone; ECS, electroconvulsive seizure; ECT, electroconvulsive treatment; GC, glucocorticoid; KPBS, potassium phosphate-buffered saline; NDS, normal donkey serum; NHS, normal horse serum; NMDA, *N*-methyl-D-aspartate; NeuN, neuron-specific nuclear protein; PBS, phosphate buffered saline; RIA, radioimmunoassay.

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