Published in final edited form as:

Dev Neurobiol. 2012 June ; 72(6): 937–942. doi:10.1002/dneu.20986.

Electroconvulsive Seizure Promotes Spine Maturation in Newborn Dentate Granule Cells in Adult Rat

Chunmei Zhao^{1,*}, Jennifer Warner-Schmidt^{2,*†}, Ronald S. Duman², and Fred H. Gage¹

¹Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, California 92037

²Laboratory of Molecular Psychiatry, Center for Genes and Behavior, Departments of Psychiatry and Neurobiology, Yale University School of Medicine, 24 Park Street, New Haven, Connecticut 06508

Abstract

Neurogenesis continues in the dentate gyrus of the hippocampus throughout life in mammals. This process is influenced by daily activities such as exercise, learning, and stress and may contribute to certain forms of hippocampus-dependent learning and memory. Adult hippocampal neurogenesis is also subject to regulation by anti depressant treatment, including chronic treatment with antidepressant drugs or electroconvulsive seizure (ECS) therapy. Here we investigated how the connectivity of newborn and mature granule cells is influenced by ECS administration in rats. Specifically, we examined the dendritic spine morphology of newborn and mature granule cells in rats and found that ECS administration promoted the maturation of dendritic spines in newborn cells and increased spine density in mature cells. These changes could potentially lead to alteration in dentate circuitry and may partially contribute to the functional effects of ECS.

Keywords

adult neurogenesis; dendritic spines; electroconvulsive seizure therapy; depression; antidepressant

INTRODUCTION

Neurogenesis occurs in two discrete areas of the adult mammalian brain, the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus in the hippocampus (Gage, 2000). In the dentate gyrus, the majority of newborn cells become granule cells (GCs), the principal neurons in the dentate gyrus (Kempermann et al., 2004; Abrous et al., 2005; Ming and Song, 2005). These newborn GCs go through an extended period of morphological and functional maturation before they fully integrate into the existing circuitry (Esposito et al., 2005; Ge et al., 2006, 2007; Zhao et al., 2006; Deng et al., 2010). Adult neurogenesis in the DG contributes to certain forms of hippocampus-dependent learning and memory, such as spatial learning/memory and pattern separation (Shors et al., 2001; Snyder et al., 2005; Clelland et al., 2009; Deng et al., 2009; Aimone et al., 2010; Deng et al., 2010). Induction of DG neurogenesis also mediates, in part, the effects of antidepressant treatments (Santarelli et al., 2003; Sahay and Hen, 2007; Samuels and Hen, 2011).

© 2011 Wiley Periodicals, Inc.

Correspondence to: F.H. Gage (gage@salk.edu) or Ronald S. Duman (ronald.duman@yale.edu).

*Both the authors contributed equally for this work.

†Present address: Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Avenue, New York, NY 10065.

Neurogenesis in the DG is a highly regulated process (Zhao et al., 2008; Ma et al., 2009; Mu et al., 2010). The proliferation of hippocampal progenitors, the survival of newborn cells, and their integration into the DG are influenced in a positive or negative direction by various conditions (Kempermann et al., 1997; Gould et al., 1999; van Praag et al., 1999; Zhao et al., 2008; Deng et al., 2010). Among these manipulations, antidepressant treatments have pleiotropic effects on adult hippocampal neurogenesis (Warner-Schmidt and Duman, 2006; Samuels and Hen, 2011). Chronic administration of fluoxetine, a selective serotonin reuptake inhibitor (SSRI), promotes the proliferation of hippocampal progenitors and may promote the maturation of newborn granule cells (Malberg et al., 2000; Wang et al., 2008). Electroconvulsive seizure (ECS) therapy, one of the most effective treatments for depression, also increases the proliferation of hippocampal progenitors (Madsen et al., 2000; Malberg et al., 2000). Hippocampal neurogenesis has been identified as a substrate of antidepressant treatments, yet it is not clear how these treatments act through neurogenesis to impact the neural circuitry or whether they have more broad effects on DG function (Santarelli et al., 2003; Perera et al., 2011; Samuels and Hen, 2011). Here we used GFP retrovirus to label newborn granule cells and examined whether their integration is regulated by ECS treatment in rats. In addition, we used DiI to randomly label granule cells and assessed the effect of ECS treatment on the general population of neurons, which is mostly comprised of mature granule cells.

METHODS

Animals

Male Sprague Dawley rats weighed approximately 175 g (7 weeks of age) at the beginning of the experiment and were housed under standard conditions with a 12-h light/dark cycle. Animal use and procedures were approved by the Yale University School of Medicine Institutional Animal Care and Use Committees.

ECS Treatment and Virus Injection

ECS was delivered via bilateral ear clip electrodes using a pulse generator (ECT Unit 57800, Ugo Basile; 55 mA, 0.5 s duration, 100 Hz frequency) to generate a grand mal seizure, characterized by stereotypic tonic and clonic convulsions. Seizures lasted less than 15 s. Sham (control) groups were handled identically, but received no shock (Warner-Schmidt et al., 2008). Each rat received 2 ECS or sham treatments prior to surgery. Rats were anesthetized and underwent hippocampal injection of the NIT-GFP retrovirus (van Praag et al., 2002). The coordinates for stereotaxic injection were -4.3 anteroposterior, 2.5 mediolateral, -3.3 dorsoventral from Bregma. They then received a total of eight ECS/sham treatments (twice per week) (Warner-Schmidt et al., 2008). All rats were killed 2 days after the last seizure/sham handling, which was 30 days after viral infection [Fig. 1(A)]. Two groups of rats were used in this study. The first group underwent unilateral injection of the retrovirus into the hippocampus of the right hemisphere. The left hemisphere of this group was used for DiI analyses of mature neurons. Due to the insufficient labeling of newborn GCs with retrovirus in the first group, a second group of rats went through bilateral injection of the retrovirus and both hemispheres were used for analysis of newborn granule cells [Fig. 1(A)].

Sample Processing and Imaging

For DiI labeling, rat brains were stored in 4% PFA at 4°C and coronal sections ($100\ \mu\text{m}$) were obtained with a vibratome. Brain sections were then washed with PBS, and DiI crystals were placed in the middle of the granule cell layer. DiI-labeled brain sections were mounted on glass slide with ProLong Antifade mounting medium (Invitrogen, Eugene, OR) 16–24 h

after dye placement and imaged immediately. DiI-labeled dendritic processes in the outer half of the molecular layer were imaged for data analysis.

For imaging of GFP+ dendritic processes, rat brains were equilibrated in cold 30% sucrose/PBS for 2–3 days and sections of 40 μm thickness were prepared. Brain slices were rinsed with TBS and mounted on slide with DABCO mounting media. Dendritic processes in the outer molecular layer were imaged. Areas close to injection sites were avoided.

Both DiI and GFP localize to dendritic shafts and spines evenly, allowing for detection of all spines. However, due to the difference in processing of the samples, we cannot directly compare spine data between mature and newborn cells within the same treatment condition.

All samples were imaged with a Bio-Rad R2100 confocal system with a 40 $\times$ oil lens and a digital zoom of 6 (numerical aperture, 1.3; Olympus, Tokyo, Japan). Stacks of images were obtained at 0.5 μm intervals. Projection images of Z-series were used for data analysis.

Spine Analysis

For each sample, 10 or more dendritic segments in the outer molecular layer were used for spine analysis. These dendritic segments were from five or more individual cells with each cell contributing no more than two dendritic processes. All images were deconvolved using the AutoQuant Auto-Deblur Deconvolution Program (five iterations) and maximum intensity projections were created using the Confocal Assistance Program (CAS 40). For spine density analysis, a line was drawn following the dendritic process to obtain the length of the process using the IGL TRACE program. Spine density was calculated by dividing the length (μm) by the total number of spines along the process. Mushroom spines were identified if the estimated area of the spine head ($1/4 \times \pi \times d_x \times d_y$, d_x is the length of short axis of spine head, and d_y the length of long axis of spine head), is greater than 0.4 μm^2 (Zhao et al., 2006).

Statistics and Data Presentation

Un-paired two-tailed student *t*-test was used for all comparisons. Data were presented as mean $\pm$ standard error.

RESULTS

ECS is among the most efficient treatments for depression, yet the mechanism of its action is largely unknown. In this study, we examined the effect of ECS on spine morphology of mature and newborn granule cells in the adult rat hippocampus. Briefly, rats were given two ECS treatments prior to retrovirus labeling of newborn granule cells followed by eight ECS treatments throughout the rest of the experiments [Fig. 1(A)]. They were killed 48 hours after the last ECS administration and 30 days after retrovirus labeling.

ECS Administration Promotes Spine Growth in Mature Granule Cells

To examine the effect of ECS administration on spine morphogenesis in mature granule cells, DiI crystals were placed in the granule cell layer of the DG in sections prepared from the hemisphere contralateral to the virus-injected side. Individual processes in the outer molecular layer were imaged using confocal scanning microscopy [Fig. 1(A,B)]. There was a small, but significant increase in total spine density in mature granule cells in response to ECS administration [Fig. 1(C), sham: 1.30 ± 0.03 , $n = 72$ from 6 rats; ECS: 1.41 ± 0.03 , $n = 79$ from 6 rats, $p < 0.02$). Mushroom spine density displayed a trend for an increase in response to ECS, but the difference was not statistically significant [Fig. 1(D), sham: 0.106 ± 0.008 , $n = 72$; ECS: 0.126 ± 0.008 , $n = 79$, $p > 0.07$). There is no difference in data

variance between groups. These data suggest that chronic ECS administration can potentially contribute to the remodeling of dentate circuitry by altering spine synapses in mature granule cells.

ECS Administration Promotes Spine Maturation in Newborn Granule Cells

To examine the effect of ECS administration on spine morphogenesis in newborn granule cells, GFP+ dendritic processes in the outer third of molecular layer were imaged using confocal scanning microscopy [Fig. 2(A,B)]. In contrast to mature granule cells, total spine density was not affected by ECS administration in newborn granule cells [Fig. 2(C), sham: 1.76 ± 0.07 , $n = 64$ from 6 rats; ECS: 1.65 ± 0.07 , $n = 62$ from 5 rats, $p > 0.2$). However, mushroom spine density was significantly increased in response to ECS administration [Fig. 2(D), sham: 0.037 ± 0.004 , $n = 64$; ECS: 0.053 ± 0.006 , $n = 62$, $p < 0.03$). These data suggest that ECS treatment exhibits a more profound effect on spine maturation in newborn granule cells compared to existing, mature neurons.

DISCUSSION

By comparing spine morphology of newborn and existing dentate granule cells, we show that ECS administration in rats promotes the formation of mushroom spines in newborn GCs, and to a lesser extent in existing mature GC neurons. ECS does not have a significant impact on total spine density in newborn cells but increases total spine density in mature GCs. These data are consistent with the notion that antidepressant treatments have pleiotropic effects on the central nervous system and that enhanced hippocampal neurogenesis is only one of the substrates of antidepressant treatments (Li et al., 2010; Samuels and Hen, 2011).

Dendritic spines are the major postsynaptic sites of excitatory synapses. The increase in total spine density in mature GCs suggests that ECS treatment promotes the formation of new spines, and consequently new synapses, onto mature GCs. Mushroom spines are considered to be sites of more mature and stable synapses due to the larger spine head size, postsynaptic densities, and insertion of glutamate receptors (Kessels and Malinow, 2009). The increase in mushroom spine density in newborn GC neurons in response to ECS treatment suggests that newborn granule cells form stronger synaptic connections within the dentate circuitry.

ECS administration does not appear to influence the total number of spines formed on the newborn GCs but did increase spine density in mature GCs. This can be explained by two possibilities. First, the rate of spine growth is at its peak when newborn GCs are within 4 weeks of age and may be activity dependent (Zhao et al., 2006). As young GCs are intrinsically more excitable (Schmidt-Hieber et al., 2004), the intermittent stimulation from ECS may not be sufficient to elicit a further enhancement in spine growth in young GCs. By comparison, mature GCs are less excitable and may be more susceptible to extrinsic stimulations. Second, spine growth may plateau as early as 4 weeks of age in newborn granule cells in rats and ECS is unable to further increase spine number. In contrast, mature GCs may have fewer spines due to spine pruning that occurred during development or maturation (Rakic et al., 1986) and this may allow addition of more spines in response to stimulation.

In mature GC neurons, mushroom spine density showed a strong trend for an increase in response to ECS. It is not clear whether the new mushroom spines were derived from existing spines or from new spines formed in response to ECS. Our hypothesis is that ECS induces the formation and maturation of new synapses in existing GCs and guides the maturation of synapses in newborn GCs. A recent study demonstrates that ketamine, a glutamate-NMDA receptor antagonist that produces a rapid antidepressant response in

treatment resistant depressed patients (Berman et al., 2000; Zarate et al., 2006), induces new spine formation and spine maturation in medial prefrontal cortex (Li et al., 2010). We hypothesize that for antidepressant treatments that influence glutamate activity and are more efficacious than typical antidepressants, new synapse formation may be a common mechanism through which these treatments modify the connectivity in the CNS. Indeed, ECS also increases synapse number in CA1 area of the hippocampus (Chen et al., 2009). The new connections formed in response to ECS treatment within the hippocampal circuitry may reverse the atrophy of neurons that is caused by stress and depression and thereby contribute to the antidepressant effects of ECS.

Acknowledgments

Contract grant sponsor: James S. McDonnell Foundation.

Contract grant sponsor: National Institutes of Health; contract grant number: NS-05050217; contract grant number: NS-05052842.

We thank Ed Yip for technical assistance and Mary Lynne Gage for editorial comments.

References

- Abrous DN, Koehl M, Le Moal M. Adult neurogenesis: From precursors to network and physiology. *Physiol Rev.* 2005; 85:523–569. [PubMed: 15788705]
- Aimone JB, Deng W, Gage FH. Adult neurogenesis: Integrating theories and separating functions. *Trends Cogn Sci.* 2010; 14:325–337. [PubMed: 20471301]
- Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, Krystal JH. Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry.* 2000; 47:351–354. [PubMed: 10686270]
- Chen F, Madsen TM, Wegener G, Nyengaard JR. Repeated electroconvulsive seizures increase the total number of synapses in adult male rat hippocampus. *Eur Neuropsychopharmacol.* 2009; 19:329–338. [PubMed: 19176277]
- Clelland CD, Choi M, Romberg C, Clemenson GD Jr, Fragniere A, Tyers P, Jessberger S, et al. A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science.* 2009; 325:210–213. [PubMed: 19590004]
- Deng W, Aimone JB, Gage FH. New neurons and new memories: How does adult hippocampal neurogenesis affect learning and memory? *Nat Rev Neurosci.* 2010; 11:339–350. [PubMed: 20354534]
- Deng W, Saxe MD, Gallina IS, Gage FH. Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. *J Neurosci.* 2009; 29:13532–13542. [PubMed: 19864566]
- Esposito MS, Piatti VC, Laplagne DA, Morgenstern NA, Ferrari CC, Pitossi FJ, Schinder AF. Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *J Neurosci.* 2005; 25:10074–10086. [PubMed: 16267214]
- Gage FH. Mammalian neural stem cells. *Science.* 2000; 287:1433–1438. [PubMed: 10688783]
- Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H. GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature.* 2006; 439:589–593. [PubMed: 16341203]
- Ge S, Yang CH, Hsu KS, Ming GL, Song H. A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. *Neuron.* 2007; 54:559–566. [PubMed: 17521569]
- Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. Learning enhances adult neurogenesis in the hippocampal formation. *Nat Neurosci.* 1999; 2:260–265. [PubMed: 10195219]
- Kempermann G, Jessberger S, Steiner B, Kronenberg G. Milestones of neuronal development in the adult hippocampus. *Trends Neurosci.* 2004; 27:447–452. [PubMed: 15271491]
- Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. *Nature.* 1997; 386:493–495. [PubMed: 9087407]

- Kessels HW, Malinow R. Synaptic AMPA receptor plasticity and behavior. *Neuron*. 2009; 61:340–350. [PubMed: 19217372]
- Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*. 2010; 329:959–964. [PubMed: 20724638]
- Ma DK, Kim WR, Ming GL, Song H. Activity-dependent extrinsic regulation of adult olfactory bulb and hippocampal neurogenesis. *Ann NY Acad Sci*. 2009; 1170:664–673. [PubMed: 19686209]
- Madsen TM, Treschow A, Bengzon J, Bolwig TG, Lindvall O, Tingstrom A. Increased neurogenesis in a model of electroconvulsive therapy. *Biol Psychiatry*. 2000; 47:1043–1049. [PubMed: 10862803]
- Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci*. 2000; 20:9104–9110. [PubMed: 11124987]
- Ming GL, Song H. Adult neurogenesis in the mammalian central nervous system. *Annu Rev Neurosci*. 2005; 28:223–250. [PubMed: 16022595]
- Mu Y, Lee SW, Gage FH. Signaling in adult neurogenesis. *Curr Opin Neurobiol*. 2010; 20:416–423. [PubMed: 20471243]
- Perera TD, Dwork AJ, Keegan KA, Thirumangalakudi L, Lipira CM, Joyce N, Lange C, et al. Necessity of hippocampal neurogenesis for the therapeutic action of antidepressants in adult nonhuman primates. *PLoS One*. 2011; 6:e17600. [PubMed: 21525974]
- Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science*. 1986; 232:232–235. [PubMed: 3952506]
- Sahay A, Hen R. Adult hippocampal neurogenesis in depression. *Nat Neurosci*. 2007; 10:1110–1115. [PubMed: 17726477]
- Samuels BA, Hen R. Neurogenesis and affective disorders. *Eur J Neurosci*. 2011; 33:1152–1159. [PubMed: 21395859]
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science*. 2003; 301:805–809. [PubMed: 12907793]
- Schmidt-Hieber C, Jonas P, Bischofberger J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature*. 2004; 429:184–187. [PubMed: 15107864]
- Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E. Neurogenesis in the adult is involved in the formation of trace memories. *Nature*. 2001; 410:372–376. [PubMed: 11268214]
- Snyder JS, Hong NS, McDonald RJ, Wojtowicz JM. A role for adult neurogenesis in spatial long-term memory. *Neuroscience*. 2005; 130:843–852. [PubMed: 15652983]
- van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci*. 1999; 2:266–270. [PubMed: 10195220]
- van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. *Nature*. 2002; 415:1030–1034. [PubMed: 11875571]
- Wang JW, David DJ, Monckton JE, Battaglia F, Hen R. Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. *J Neurosci*. 2008; 28:1374–1384. [PubMed: 18256257]
- Warner-Schmidt JL, Duman RS. Hippocampal neurogenesis: Opposing effects of stress and antidepressant treatment. *Hippocampus*. 2006; 16:239–249. [PubMed: 16425236]
- Warner-Schmidt JL, Madsen TM, Duman RS. Electroconvulsive seizure restores neurogenesis and hippocampus-dependent fear memory after disruption by irradiation. *Eur J Neurosci*. 2008; 27:1485–1493. [PubMed: 18336568]
- Zarate CA Jr, Singh J, Manji HK. Cellular plasticity cascades: Targets for the development of novel therapeutics for bipolar disorder. *Biol Psychiatry*. 2006; 59:1006–1020. [PubMed: 16487491]
- Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. *Cell*. 2008; 132:645–660. [PubMed: 18295581]

Zhao C, Teng EM, Summers RG Jr, Ming GL, Gage FH. Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci*. 2006; 26:3–11. [PubMed: 16399667]

Figure 1.

ECS treatment increased spine density in mature granule cells. (A) A cartoon of the experimental design. Two groups of rats were used in this study. Both underwent two ECS treatments before the injection of the retrovirus NIT-GFP. These rats then underwent a total of eight ECS treatments over the next 4 weeks and were killed 2 days after the last treatment. Rats in Group 1 received unilateral injection of retrovirus on the right hemisphere and the left hemisphere were processed for DiI placement and imaging. Rats in Group 2 received bilateral injection of retrovirus and GFP+ cells from both hemispheres were used for imaging and analyses. (B) Sample images of DiI-labeled dendritic processes in the outer molecular layer. (C) ECS treatment increased total spine density in mature granule cells ($p < 0.02$). (D) ECS treatment elicited a non-significant increase in mushroom spine density in mature granule cells ($p > 0.07$). Arrows indicate some of the mushroom spines. Scale bar: 5 μm .

Figure 2.

ECS treatment promoted spine maturation in newborn GCs in rats. (A) An example of imaging acquisition. Left panel is an example of GFP-labeled newborn GC. A continuous stretch of dendritic shaft was identified in the outer molecular layer and was imaged with a digital zoom (right panel). (B) Sample images of GFP+ dendritic processes in the outer molecular layer from both control and treated rats. (C) ECS treatment does not affect total spine density in newborn GCs ($p > 0.2$). (D) ECS treatment increased mushroom spine density in newborn GCs ($p < 0.03$). Arrows indicate some of the mushroom spines. Scale bars: 10 μm (A) and 5 μm (B).