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Atrophy of pyramidal neurons and increased stress-induced glutamate levels in CA3 following chronic suppression of adult neurogenesis

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Abstract

Following their birth in the adult hippocampal dentate gyrus, newborn progenitor cells migrate into the granule cell layer where they differentiate, mature, and functionally integrate into existing circuitry. The hypothesis that adult hippocampal neurogenesis is physiologically important has gained traction, but the precise role of new-born neurons in hippocampal function remains unclear. We investigated whether loss of new neurons impacts dendrite morphology and glutamate levels in area CA3 of the hippocampus by utilizing a human *GFAP* promoter-driven thymidine kinase genetic mouse model to conditionally suppress adult neurogenesis. We found that chronic ablation of new neurons induces remodeling in CA3 pyramidal cells and increases stress-induced release of the neurotransmitter glutamate. The ability of persistent impairment of adult neurogenesis to influence hippocampal dendrite morphology and excitatory amino acid neurotransmission has important implications for elucidating newborn neuron function, and in particular, understanding the role of these cells in stress-related excitotoxicity.

Keywords

Neurogenesis; Dentate gyrus; Excitotoxicity; Atrophy; Dendrite; CA3

Introduction

Adult neurogenesis encompasses proliferation of adult neural progenitor cells (NPCs) as well as their differentiation, maturation, and integration into the existing circuitry. In the hippocampal dentate gyrus (DG), dendrites of adult-generated granule cells (GCs) extend into the molecular layer while their axons project to the hilus and CA3. These cells receive morphologically mature, functional synapses; gamma-aminobutyric acid-ergic inputs are received within 8 days after birth, followed closely by glutamatergic inputs (van Praag et al.

2002; Schmidt-Hieber et al. 2004; Ge et al. 2006, 2007; Laplagne et al. 2006). New neurons exhibit unique physiological properties including a lower threshold for long-term potentiation and enhanced synaptic plasticity (Schmidt-Hieber et al. 2004; Ge et al. 2007). Adult-born GCs establish functional synapses with post-synaptic targets making contact with hilar interneurons, mossy cells, and CA3 pyramidal cells onto which they release the neurotransmitter glutamate (Toni et al. 2008).

Exposure to chronic stress and injection of corticosteroids induces atrophy in CA3 pyramidal cell dendrites (Magarinos and McEwen 1995; McEwen et al. 2002). These effects are blocked by phenytoin and NMDA-receptor antagonists, implicating toxicity from excitatory amino acid transmission in stress-induced pathology (Magarinos and McEwen 1995; Watanabe et al. 1992a). This is consistent with evidence that stress and glucocorticoids lead to potentiated glutamate release in the hippocampus (Lowy et al. 1993; Moghaddam 1993; Moghaddam et al. 1994; Stein-Behrens et al. 1994). Since the birth of new neurons is adversely affected by stress and glucocorticoids (Gould et al. 1997; Mirescu and Gould 2006), we explored whether new neurons play a role in the hyperactive excitatory input from GCs to CA3 that is responsible for stress-induced hippocampal pathology.

Based on computational models of the neuroanatomical and circuit properties of the DG-CA3 synapse, we hypothesized that chronic suppression of new neurons in the DG would affect CA3 excitability, leading to cellular alterations and changes in excitatory amino acid transmission in CA3. Indeed, after chronic suppression of adult neurogenesis, we found evidence of dendritic remodeling in pyramidal cells and increased stress-induced glutamate release in CA3. These data suggest that new neurons play a contributory role in regulating mossy fiber-CA3 excitation. This is significant because excessive excitation at this synapse is implicated in hippocampal atrophy observed in stress-related neuropsychiatric and neurological disorders (McEwen and Magarinos 1997; Pittenger and Duman 2008).

Materials and methods

Animals

Male hGFAPtk animals (Schloesser et al. 2009, 2010; Snyder et al. 2011) were maintained on a C57Bl6/J background and derived from female hGFAPtk heterozygote $\times$ male C57Bl6/J (baseline and microdialysis studies) or female hGFAPtk heterozygote $\times$ male homozygous Thy1M-eGFP (Stock #007788, Jackson Laboratories; 3D neuron reconstruction). Procedures were conducted according to NIH guidelines and approved by NIMH Institutional Animal Care and Use Committee.

Corticosterone levels

Trunk blood was collected, and radioimmunoassay performed according to manufacturer (MP Biomedicals).

Drugs

Valganciclovir (VGCV) (Roche), the L-valyl ester of ganciclovir, was administered for 16 weeks (weeks 8–24) in the chow (15 mg/kg/day). Both WT and hGFAP^{tk} mice received VGCV chow.

Quantitative reverse-transcriptase PCR

0.5 µg of DNase-treated RNA was reverse-transcribed with Superscript iii (Invitrogen). 20 ng cDNA was used for each reaction with a Prime Time Probe Kit for HSVtk or Gapdh (500 nM primers/250 nM probe) (Integrated DNA Technologies) in 1× Gene Expression Master Mix (Applied Biosystems). Triplicate reactions were performed on a 4S Realplex Mastercycler (Eppendorf). PCR efficiencies were between 96 and 102 %, and data analysis was carried out by interpolation to standard curves derived for HSVtk and Gapdh in each tissue.

Tissue preparation

Animals were anesthetized with isoflurane and perfused transcardially with 4 % paraformaldehyde. Brains were post-fixed for 16 h and transferred to 30 % sucrose. 150 (3D reconstruction) or 50 (histology) µm sections were cut coronally, mounted in consecutive order onto glass slides, and coverslipped using a glycerin-based medium (Immunount, Thermo Scientific).

Immunohistochemistry

Sections were systematically sampled 480 µm apart into 12 wells of a 24-well plate. Free-floating sections were washed, blocked, and incubated with primary antibody (Doublecortin; sc-8066, 1:1,000, SCBT and c-fos; sc-52, 1:500, SCBT) overnight. Tissue was then washed and incubated for 2 h at room temperature with Alexa fluorescent secondary antibodies (Invitrogen) for doublecortin staining or with biotinylated goat anti-rabbit secondary antibody (Invitrogen) for c-fos staining. For c-fos staining, endogenous peroxidase activity was blocked using 0.3 % hydrogen peroxide for 30 min at room temperature. The HRP-DAB reaction was carried out using an avidin/biotin peroxidase complex (VectaStain ABC Kit, Vector Laboratories). Sections were incubated in ABC for 1 h and DAB (Sigma) for 3 min. Every 12th section through the hippocampal DG was identified and all doublecortin- or c-fospositive cells bodies were manually counted by a blinded investigator.

3D neuron reconstruction

Throughout neuron selection and 3D reconstruction, researchers were blind to genotype. Neurons were imaged with an Eclipse E800 fluorescent microscope (Nikon) with an Optronics Microfire camera. Image stacks were captured in the *x*, *y*, and *z*-axes using SRS Image Stack in NeuroLucida 9.0 (MBF Bioscience) on 20× magnification. Neurons were selected using the following criteria: (1) spine-bearing; (2) cells were relatively isolated; and (3) cell bodies were in the middle-third (*z*-axis) of the slice. Sampling was conducted blinded, sampling from all CA3 sub-regions. Locations of analyzed cells are plotted in Fig. 1c.

Stereotaxic surgeries, microdialysis, and capillary electrophoresis

Surgeries and microdialysis were carried out essentially as described (Martinowich et al. 2012). Briefly, animals were anesthetized and a unilateral guide shaft was placed into right ventral CA3 (AP -2.70 , ML 3.25 , DV -0.5). Two weeks after surgeries, microdialysis probes (3.0 mm) were inserted 12 h prior to collection and perfused with artificial cerebrospinal fluid (ACSF); flow rate $0.2 \mu\text{l}/\text{min}$ for 9 h and $1.0 \mu\text{l}/\text{min}$ 3 h before and during sample collection. Samples were collected every 15 min into refrigerated tubes and frozen at -80°C . Three baseline samples were collected before placing animals on an elevated platform ($1 \text{ m} \times 10 \text{ cm} \times 10 \text{ cm}$; h, w, l) for 35 min. Nissl staining was used to evaluate probe placement; locations are plotted in Fig. 2e. Animals with placements outside the target area were removed. Analysis was carried out as previously described (Hernandez et al. 1993a, b). Briefly, samples were mixed with 20 mM carbonate buffer and 2.57 mM fluorescein isothiocyanate isomer I in acetone. A blank solution (ACSF) and glutamate standard solution ($5 \times 10^{-6} \text{ M}$) were derivatized, and mixtures placed in a light-protected, water-saturated chamber for 24 h. Mixtures were diluted tenfold and injected into a capillary zone electrophoresis with laser-induced fluorescence detection instrument. An argon ion 100 mW laser (Ion Laser Technology) was tuned to 488 nm and reflected by a dichroic mirror centered at 510 nm (Carl Zeiss). The laser was focused with a 0.85 NA objective (Zeiss) on the capillary window (Polimicro Technologies), which was located 20 cm from the anodic end of a 30 cm long, $20 \mu\text{m}$ bore fused-silica capillary filled with carbonate buffer. Fluorescence was measured, and stray radiation attenuated by a high-pass filter at 520 nm (Zeiss) and a notch filter at 488 nm (Andover). Fluorescence was focused on a R1477 multi alkali photomultiplier (PMT) (Hamatsu). PMT current was converted to voltage and electropherograms analyzed with Maxima (Waters). The electrophoretic run consisted of injecting solutions (blank, standard, and sample) hydrodynamically. A -19 psi suction was applied for 0.3 s at the capillary cathode while the anodic end was immersed in the mixture reservoir. The anodic end was transferred to the buffer reservoir and high voltage applied using a cathode and a platinum–iridium, wire anode.

Results

Physiological effects following suppression of neurogenesis

To suppress neurogenesis, we utilized mice expressing herpes simplex thymidine kinase (HSV-tk) under control of the human glial fibrillary acidic protein (*GFAP*) gene promoter (hGFAPtk mice) (Schloesser et al. 2009, 2010). In valgancyclovir (VGCV)-treated hGFAPtk animals (NG $-$ mice) HSV-tk phosphorylates ganciclovir into toxic metabolites that ablate actively dividing, *GFAP*-positive NPCs. We previously demonstrated that even after extended periods of VGCV treatment, there is no inflammation due to death of *GFAP*-positive NPCs, no changes in cell proliferation in brain regions other than the sub-granular zone of the DG, and the sub-ventricular zone of the olfactory bulb, no unwanted side effects compromising general health, gross pathology appeared normal, and baseline behaviors of locomotion and exploration, anxiety, and hedonic drive were not affected (Schloesser et al. 2010). Moreover, among several tissues examined by quantitative reverse-transcriptase PCR analysis, the thymidine kinase transgene is predominantly expressed in brain tissues (Fig. 3a).

We and others have reported that ablating neurogenesis results in a mildly hyperactive hypothalamic pituitary adrenal (HPA) axis response to acute stress (Schloesser et al. 2009; Snyder et al. 2011). Characteristic hallmarks of sustained HPA axis stress, including decreased total body weights and alterations in stress-sensitive organ weight, are reliably observed in animal models of chronic stress exposure. We examined whether prolonged suppression of neurogenesis results in a phenotype similar to chronic stress exposure. We found no effects of prolonged suppression of neurogenesis on baseline AM (WT: 45.24 ± 5.46 ng/ml and NG⁻: 38.09 ± 4.78 ng/ml; $n = 6$ each genotype; $p = 0.348$; t test, two-tailed; Fig. 3b) or PM (WT: 131.2 ± 7.4 ng/ml and NG⁻: 136.6 ± 7.83 ng/ml; $n = 6$ each genotype; $p = 0.624$; t test, two-tailed; Fig. 3b) corticosterone levels. Furthermore, total body weights (WT: 31.82 ± 0.71 g and NG⁻: 30.88 ± 0.43 g; $n = 9$ each genotype; $p = 0.275$; t test, two-tailed; Fig. 3c), weights of the adrenal glands (WT: 0.0056 ± 0.00033 g and NG⁻: 0.0051 ± 0.00036 g; $n = 9$ each genotype; $p = 0.296$; t test, two-tailed; Fig. 3d), thymi (WT: 0.052 ± 0.0055 g and NG⁻: 0.0552 ± 0.0046 g; $n = 9$ each genotype; $p = 0.666$; t test, two-tailed; Fig. 3e) and testes (WT: 0.0548 ± 0.0025 g and NG⁻: 0.0544 ± 0.0015 g; $n = 9$ each genotype; $p = 0.915$; t test, two-tailed; Fig. 3f) were not changed. These data do not support the view that NG⁻ mice are in a state of chronic HPA axis stress. Collectively, our data confirm the ability to selectively suppress adult neurogenesis without undesirable side effects.

Atrophy of CA3 pyramidal cells

To visualize and image individual CA3 pyramidal cells we crossed hGFAP^{tk} mice to the Thy1M-eGFP line, which label hippocampal principle cells in a Golgi-like pattern (Feng et al. 2000). eGFP expression allowed for unambiguous identification of CA3 pyramidal cells, 3D reconstruction, and analysis of dendrite morphology. To ensure effective suppression in double transgenics (hGFAP^{tk}/Thy1M-eGFP), we examined DCX immunoreactivity after VGCV-administration and confirmed loss of DCX-positive cells in NG⁻Thy1 mice (WT^{Thy1}: $2,219 \pm 138.1$ cells and NG⁻Thy1: 6.67 ± 2.94 cells ($n = 6$ each genotype; $p < 0.0001$; t test, two-tailed; Fig. 3g, h).

Compared to WT^{Thy1}, NG⁻Thy1 hippocampus showed significant remodeling of CA3 apical dendrites after 16 weeks of suppression (Fig. 1a–f), but no difference after 4 weeks of suppression (WT^{Thy1}: 11.83 ± 0.54 branch points; NG⁻Thy1: 12.33 ± 0.84 branch points, $p = 0.63$; t test, two-tailed and WT^{Thy1}: $1,810 \pm 123.3$ μ m total apical dendrite length; NG⁻Thy1: $2,032 \pm 233.0$ μ m total apical dendrite length; $p = 0.4196$, t test, two-tailed). After chronic suppression, sholl analysis revealed a significant main effect of genotype in apical dendrite intersections (WT^{Thy1}: $n = 5$ mice, 4–6 cells/mouse, 23 total cells and NG⁻Thy1: $n = 9$ mice, 4–6 cells/mouse, 38 total cells; $F_{1,543} = 70.77$, $p < 0.0001$; two-way ANOVA; 210 μ m: $t = 3.52$, $p < 0.05$; 220 μ m: $t = 3.56$, $p < 0.05$; 230 μ m: $t = 3.61$, $p < 0.05$; 240 μ m: $t = 3.52$, $p < 0.05$; 250 μ m: $t = 3.44$, $p < 0.05$; Bonferroni post hoc multiple-comparison test; Fig. 1d). Both the number of apical branch points (WT^{Thy1}: 12.69 ± 1.93 branch points and NG⁻Thy1: 8.90 ± 0.63 branch points; $p = 0.039$; t test, two-tailed; Fig. 1e) and total apical dendrite length (WT^{Thy1}: $1,896 \pm 251.7$ μ m and NG⁻Thy1: $1,233 \pm 92.23$ μ m; $p = 0.011$; t test, two-tailed; Fig. 1f) were significantly decreased in NG⁻Thy1 hippocampus. Sholl analysis of CA3 basal dendrites revealed no difference between WT^{Thy1} and NG⁻Thy1 (WT^{Thy1}: $n = 5$

mice, 4–6 cells/mouse, 23 total cells and NG-^{Thy1}; $n = 9$ mice, 4–6 cells/mouse, 38 total cells; $p = 0.153$ for genotype and $p = 0.277$ for interaction between genotypes; two-way ANOVA; Fig. 3g). Total basal branch points (WT^{Thy1}: 8.41 ± 0.71 and NG-^{Thy1}: 7.38 ± 0.85 branch points; $p = 0.433$; t test, two-tailed; Fig. 3h) and total basal dendritic length (WT^{Thy1}: $910.6 \pm 106.7 \mu\text{m}$ and NG-^{Thy1}: $777.4 \pm 96.83 \mu\text{m}$; $p = 0.401$; t test, two-tailed; Fig. 3i) were not significantly different between WT^{Thy1} and NG-^{Thy1}.

Elevation of stress-induced glutamate levels in CA3

We used in vivo microdialysis to monitor extracellular glutamate levels (corrected; recovery probe: $21.65 \pm 2.28\text{SD } \mu\text{M}$) in WT and NG- CA3 at baseline and in response to stress (Fig. 2a–d). Exposure to acute elevation stress elicits a ~44 % increase in extracellular concentrations of glutamate in WT CA3 and a ~61 % increase in NG- CA3. There was a significant increase in glutamate levels 15, 30, 45, and 60 min after initiation of platform stress in both WT ($n = 5$; $p < 0.0001$; repeated measures ANOVA; 15 min: $t = 9.68$, $p < 0.0001$; 30 min: $t = 6.71$, $p < 0.0001$; 45 min: $t = 5.15$, $p < 0.0001$; 60 min: $t = 4.17$, $p < 0.001$; Bonferroni post hoc multiple-comparison test; Fig. 2a) and NG- ($n = 9$; $p < 0.0001$; repeated measures ANOVA; 15 min: $t = 12.54$, $p < 0.0001$; 30 min: $t = 11.27$, $p < 0.0001$; 45 min: $t = 10.10$, $p < 0.0001$; 60 min: $t = 5.47$, $p < 0.0001$; Bonferroni post hoc multiple-comparison test; Fig. 2a) hippocampus. Glutamate levels were higher in NG- hippocampus with a significant main effect of genotype ($F_{1,120} = 43.77$, $p < 0.001$; two-way ANOVA; 45 min: $t = 3.17$, $p < 0.05$; 60 min: $t = 4.66$, $p < 0.001$; 75 min: $t = 5.02$, $p < 0.001$; post hoc multiple-comparison test; Fig. 2a). Averaging values for baseline collections (WT: $0.390 \pm 0.0026 \mu\text{M}$ and NG-: $0.416 \pm 0.0086 \mu\text{M}$; $p = 0.046$, t test, two-tailed; Fig. 2b) stress collections (WT: $0.560 \pm 0.019 \mu\text{M}$ and NG-: $0.62 \pm 0.015 \mu\text{M}$; $p = 0.007$, t test, two-tailed; Fig. 2c), and recovery collections (WT: $0.455 \pm 0.014 \mu\text{M}$ and NG-: $0.493 \pm 0.0058 \mu\text{M}$; $p = 0.012$, t test, two-tailed; Fig. 2d) revealed significant increases in extracellular levels of glutamate in NG- CA3 at each phase of the experiment.

We used c-fos expression mapping to investigate neuronal activation patterns in the hippocampus following chronic suppression of neurogenesis at baseline and in response to elevation stress. Consistent with our previous results we found that the pattern of c-fos expression at baseline was extremely sparse in WT animals (Martinowich et al. 2012); a similar pattern of expression was observed in NG- animals. As expected, we found a substantial increase in labeling following exposure to elevation stress in WT animals. However, when compared to their WT counterparts, NG- animals showed significantly increased levels of c-fos expression in the DG and in CA3 ($n = 6$ both genotypes and conditions, DG: $F_{1,20} = 27.85$, $p < 0.0001$ for elevation stress, $F_{1,20} = 4.70$, $p = 0.042$ for genotype; two-way ANOVA; Bonferroni post hoc multiple-comparison test, WT vs. NG-, $p > 0.05$ for baseline and $p < 0.05$ for elevation stress, CA3: $F_{1,20} = 25.75$, $p < 0.0001$ for elevation stress, $F_{1,20} = 5.74$, $p = 0.023$ for genotype; two-way ANOVA; Bonferroni post hoc multiple-comparison test, WT vs. NG-, $p > 0.05$ for baseline and $p < 0.01$ for elevation stress; Fig. 2f, g).

Since previous studies with different stressors have reported increased circulating glucocorticoids, and increased corticosterone has been associated with increased

hippocampal glutamate levels we evaluated corticosterone levels after elevated platform stress. Corticosterone levels were higher in NG- mice with a significant main effect of genotype ($n = 6$ both genotypes at all time points, $F_{1,50} = 37.24$, $p < 0.0001$; two-way ANOVA; 60 min: $t = 3.662$, $p < 0.01$; 90 min: $t = 3.169$, $p < 0.05$; $t = 4.625$, $p < 0.001$; Bonferroni post hoc multiple-comparison test; Fig. 2h).

Discussion

It is recognized that stress is involved in the pathophysiology of mood and anxiety disorders (Joels and Baram 2009; Pittenger and Duman 2008; Post 1992; McEwen 2005; Schloesser et al. 2012). Volumetric changes have been identified in limbic areas of depressed patients, effects partially attributed to remodeling of dendritic arbors and loss of synaptic spines. Preclinical studies have observed increases in glutamatergic release and transmission in response to stress, and hence, it has been proposed that maladaptive increases in excitatory amino acid transmission may play crucial roles in morphological changes observed in depressive disorders (Carlson et al. 2006; Pittenger and Duman 2008; Schloesser et al. 2012). HPA axis disruptions are often found in patients with major depression, and a major role in stress-induced dendrite remodeling has been attributed to actions of glucocorticoid hormones, which are increased in response to stress (Sapolsky 2000; Watson et al. 2004). Adrenalectomy in the adult rat leads to increased apoptosis of existing dentate GCs (Gould et al. 1990; Sloviter et al. 1989); however, ongoing dentate neurogenesis is increased following adrenalectomy (Cameron and Gould 1994). Elevated glucocorticoid signaling as a result of aging and exposure to chronic stress leads to decreased levels of neurogenesis, and the stress-induced effects on neurogenesis and CA3 dendritic remodeling have been attributed to interactions of glucocorticoids with potentiated stress-induced glutamate release (McEwen and Magarinos 1997).

The human hippocampus is particularly sensitive to atrophy, and shows greater changes than other brain regions to a variety of insults as well as in normal aging, Cushing's disease, recurrent depressive illness, post-traumatic stress disorder, schizophrenia, and temporal lobe epilepsy (Jack 1994; Keller and Roberts 2008; Golomb et al. 1994; Convit et al. 1995; Sheline et al. 1996; Starkman et al. 1992; Bogerts et al. 1993; Fukuzako et al. 1996; Bremner et al. 1995; Gurvits et al. 1996). Moreover, the neurotoxic effects of both stress and seizures on CA3 pyramidal cells have been attributed to increased excitatory transmission caused by GC glutamate secretion (McEwen and Magarinos 1997; Musazzi et al. 2011). Stress-induced hippocampal atrophy can be reversed by phenytoin and NMDA receptor antagonists, suggesting that atrophy results from sustained hyperactivity of glutamatergic throughput at the mossy fiber-CA3 synapse (McEwen and Magarinos 1997; Sapolsky 2000; Watanabe et al. 1992a, b). Our data are consistent with previous findings that GCs participate in stress-induced accumulation of glutamate in CA3 (Lowy et al. 1993; Moghaddam 1993; Moghaddam et al. 1994; Stein-Behrens et al. 1994), and suggest that new neurons may play a contributory role in regulating GC excitation. A recent study demonstrating that new neurons can influence the activity of the downstream network via direct synaptic contact with mossy cells, CA3 pyramidal cells and hilar interneurons provides evidence that neurogenesis is capable of playing a direct role in regulating the balance of excitation/inhibition in hippocampal circuits (Toni et al. 2008).

How could integration of newborn neurons lead to the observed effects on dendrite morphology and glutamate release in CA3? Observations from computational models of hippocampal circuitry may offer some insight. Hilar interneurons receive intense GC innervation, and are especially vulnerable to excessive excitation (Buckmaster and Dudek 1997; Sloviter 1987). Thus, the synaptic connection between new neurons and hilar interneurons may be particularly important because these cells provide robust inhibitory control over GC hyper-activity, which contributes to excitation at the mossy fiber-CA3 synapse and downstream atrophy. Our data showing that loss of new neurons results in increased glutamate levels and immediate early gene activation in response to an acute stress suggests upstream disinhibition. This disinhibition would be consistent with the possibility that innervation of hilar interneurons by newborn neurons could be important for providing homeostatic control over mossy fiber-CA3 excitation and protection from elevated excitatory neurotransmission. It has been proposed that adult-born neurons may decrease excitation of mature GCs via feedback inhibition (Sahay et al. 2011; Kheirbek et al. 2012), and several recent studies have supported the hypothesis that young neurons may exert an inhibitory influence on the DG: first, ablation of adult neurogenesis increases gamma-frequency bursting and the synchronization of existing dentate GC firing to this bursting (Lacefield et al. 2012); second, ablation of neurogenesis decreases inhibitory drive onto mature GCs (Singer et al. 2011); and third, ablation of neurogenesis leads to a reduction in immediate-early gene activation in the hippocampus following a behavioral conflict task (Burghardt et al. 2012). Our findings that suppression of neurogenesis leads to dendritic retraction in CA3, an increase in stress-induced immediate early gene activation and an increase in stress-induced glutamate release are in line with these findings and support the view that new neurons contribute to controlling inhibitory tone in the DG.

The precise relationship between enhanced glutamate transmission, circulating stress hormones and structural changes to the dendritic arbor is not clear. We hypothesized that adult-born hippocampal neurons are important for maintaining proper control over mossy fiber glutamatergic transmission and preventing downstream excitotoxic damage in CA3. We speculate that the observed changes in apical dendrite structure result from a response to increased excitatory transmission of mature dentate GCs arising from disinhibition at the mossy fiber-CA3 synapse, which could lead to long-term adaptive/maladaptive changes in the neural circuitry. The purpose of the CA3 apical dendrite atrophy is unclear and awaits future investigation; it could represent permanent excitotoxic damage, or it could serve as an adaptive mechanism to limit further damage from increases in excitatory input from entorhinal cortical inputs to the stratum lacunosum moleculare or from recurrent axon collaterals projecting from neighboring CA3 pyramidal cells (Yeckel and Berger 1990; Ishizuka et al. 1990; Li et al. 1994).

Adult neurogenesis has been implicated in the etiology of depressive disorders and in remediation of depressive-behavior by anti-depressants (David et al. 2010; DeCarolis and Eisch 2010; Santarelli et al. 2003). Physical activity, environmental enrichment, antidepressants, and learning increase proliferation and survival of new neurons while age and exposure to stress decrease neurogenesis (David et al. 2010; DeCarolis and Eisch 2010; Deng et al. 2010; Schoenfeld and Gould 2012). Such studies contributed to the “neurogenic hypothesis of depression”, which postulated that new neurons in the adult brain were

involved in the etiology of depression as well as in antidepressant treatment efficacy (Petrik et al. 2012; David et al. 2010). Additional and often contradictory research has complicated a simplistic understanding of the tenets of this hypothesis. In particular, ablation of neurogenesis, including in the hGFAP^{Cre} strain utilized here, does not cause depressive-like symptoms per se (Schloesser et al. 2010); stress does not always lead to decreases in neurogenesis; and at least some of the effects of antidepressants are not dependent on new neurons (Petrik et al. 2012).

There are significant outstanding questions surrounding the complex relationships between regulation of adult neurogenesis, modulation of the stress axis, hippocampal excitotoxicity, and stress-related structural remodeling in the hippocampus. While our studies raise additional questions about these relationships and the directionality of cause and effect, the data have important implications for understanding how neurogenesis contributes to hippocampal pathology associated with chronic stress and aging as well as the potentially protective effects afforded by treatments that increase neurogenesis. In conclusion, the ability of new neurons to influence dendrite morphology and excitatory amino acid transmission in DG target regions contributes to our knowledge base on the functional significance of adult neurogenesis, and in particular, the role of new neurons in influencing downstream hippocampal circuitry.

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Fig. 1. Atrophy of CA3 pyramidal cells after prolonged suppression of neurogenesis. **a** Representative images of eGFP-positive neurons in WT^{Thy1} and NG-^{Thy1} hippocampus. *Left panel* low magnification of hippocampus, *middle panel* individual CA3 pyramidal neuron and *right panel*; high magnification of CA3 pyramidal cell apical dendrites. **b** Representative camera lucida tracings of CA3 pyramidal cells from WT^{Thy1} and NG-^{Thy1}. **c** Diagram depicting locations of analyzed cells. **d** Sholl analysis reveals that CA3 pyramidal cells from NG-^{Thy1} hippocampus show significantly fewer apical dendrite intersections. **e**

Significantly decreased total CA3 pyramidal neuron apical branch points in NG^{-Thy1} hippocampus. **f** Significantly decreased apical CA3 pyramidal cell dendrite length in NG^{-Thy1} hippocampus. **g** Sholl analysis reveals no difference between WT^{Thy1} and NG^{-Thy1} CA3 pyramidal cell basal dendrite intersections. **h** No difference between WT^{Thy1} and NG^{-Thy1} in CA3 pyramidal cell total basal branch points. **i** No difference between WT^{Thy1} and NG^{-Thy1} in CA3 pyramidal cell total basal dendrite length. Values are mean $\pm$ SEM; * $p < 0.05$ two-way ANOVA and Bonferroni post hoc or t test

Fig. 2.

Elevation of stress-induced glutamate and corticosterone levels in CA3 after suppression of neurogenesis. **a** Stress-induced glutamate levels in hippocampal CA3 of freely moving WT and NG⁻ mice expressed as % of baseline values (mean $\pm$ SEM of three samples before $t = 0$, $n = 5$ WT, 9 NG⁻). Animals were acutely stressed (elevated platform) for 35 min. **b** Averaged values for all baseline collections. **c** All stress collections, and **d** all recovery collections. **e** Diagram depicting microdialysis probe placements. **f** Quantification of c-fos+ cells in WT and NG⁻ DG and CA3 at baseline and following exposure to elevation stress. **g** Representative photomicrographs of c-fos+ cells in the DG and CA3 of WT and NG⁻ following elevation stress. **h** Levels of corticosterone at baseline and in thirty minute increments following 35 min of acute elevation stress. * $p < 0.05$, ** $p < 0.001$, ANOVA and Bonferroni post hoc or t test

Fig. 3.

Effects of prolonged suppression of neurogenesis. **a** Relative HSV-tk expression in central and peripheral tissues in hGFAPtk animals assessed with quantitative PCR. Data is presented as fold change relative to HSV-tk levels in hippocampus ($n = 4$). **b** Morning (7 am) and evening (7 pm) corticosterone levels. No differences in diurnal corticosterone levels were observed between genotypes ($n = 6$, each time point, both genotypes). No differences were observed in **c** body weights, **d** adrenal weights, **e** thymus weights, **f** testes weights ($n = 9$, both genotypes). **g** Representative images of WT^{Thy1} and NG-^{Thy1} hippocampus. Thy1M transgene (green) is seen in both genotypes while DCX immunostaining (red) is absent in NG-^{Thy1} hippocampus. **h** Quantification of DCX-positive neuroblasts in WT^{Thy1} and NG-^{Thy1} hippocampus ($n = 6$, both genotypes). Values are mean $\pm$ SEM; *** $p < 0.0001$, t test