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Prenatal choline deficiency does not enhance hippocampal vulnerability after kainic acid-induced seizures in adulthood

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Abstract

Choline is a vital nutrient needed during early development for both humans and rodents. Severe dietary choline deficiency during pregnancy leads to birth defects, while more limited deficiency during mid- to late pregnancy causes deficits in hippocampal plasticity in adult rodent offspring that are accompanied by cognitive deficits only when task demands are high. Because prenatal choline supplementation confers neuroprotection of the adult hippocampus against a variety of neural insults and aids memory, we hypothesized that prenatal choline deficiency may enhance vulnerability to neural injury. To examine this, adult offspring of rat dams either fed a control diet (CON) or one deficient in choline (DEF) during embryonic days 12–17 were given multiple injections (i.p.) of saline (control) or kainic acid to induce seizures and were euthanized 16 days later. Perhaps somewhat surprisingly, DEF rats were not more susceptible to seizure induction and showed similar levels of seizure-induced hippocampal histopathology, GAD expression loss, upregulated hippocampal GFAP and growth factor expression, and increased dentate cell and neuronal proliferation as that seen in CON rats. Although prenatal choline deficiency compromises adult hippocampal plasticity in the intact brain, it does not appear to exacerbate the neuropathological response to seizures in the adult hippocampus at least shortly after excitotoxic injury.

Keywords

choline; glial fibrillary acidic protein; glutamic acid decarboxylase; growth factor; hippocampus; seizure

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2. Introduction

Choline is an essential nutrient, needed for the structural integrity and signaling functions of cell membranes. This nutrient is also the major source of methyl-groups in the diet and it directly affects cholinergic neurotransmission, transmembrane signaling and lipid transport/metabolism (Zeisel and Blusztajn, 1994). Pregnancy and lactation are times when demand for choline is especially high because transport of choline from mother to fetus depletes maternal choline stores (McMahon and Farrell, 1985; Zeisel et al., 1995). For this reason, mothers must consume large amounts of choline to ensure an adequate supply to the fetus. The Institute of Medicine of the National Academy of Sciences, USA (1998), set the adequate intake level for choline, but in the US fewer than 15% of pregnant women reportedly eat the recommended amount (Jensen et al., 2007). In fact, women vary greatly in dietary choline intake (from < 300 mg/d to > 500 mg/d) and at least 25% of women eat so little choline during pregnancy that they have an elevated risk of having a baby with a birth defect (Shaw et al., 2004; Shaw et al., 2006; Shaw et al., 2009). Using a rat model that limited choline availability to developing fetuses, numerous studies have shown that prenatal choline deficiency leads to long-lasting alterations in brain and behavior. When compared to rats whose mothers were fed a control diet, adult offspring of dams fed a choline-deficient diet on embryonic days (ED) 12–17 show decrements in spatial memory performance, but only when task demands are high. For example, prenatal choline deficient rats do poorly on massed, but not spaced trials on a radial arm maze (Meck and Williams, 1999); on temporal processing tasks, but only when they require divided attention or increments in attention following a change in the predictiveness of a stimulus (Buhusi et al., 2008; Lamoureux et al., 2008; Meck and Williams, 1997b; Meck and Williams, 1997c), and in sensory inhibition (Stevens et al., 2008). Adult offspring of choline deficient mothers also display a higher threshold to induce long-term potentiation (LTP) (Jones et al., 1999), show reduced mitogen-activated protein kinase (MAPK) and cAMP-response element binding (CREB) activation (Mellott et al., 2004), and fail to upregulate dentate neurogenesis in response to an environmental enriching experience (Glenn et al., 2007). Alterations in the fetal hippocampus can be detected as early as ED 18 following maternal choline deprivation during ED12–17. ED 18 fetuses of choline deficient mothers have decreased cell division and angiogenesis, increased apoptotic cell death, and altered methylation of genes that regulate neurogenesis in the developing hippocampus (Albright et al., 1999; Craciunescu et al., 2003; Mehedint et al., 2010a; Mehedint et al., 2010b). These data support our hypothesis that early choline availability may cause changes in neural development that confer long-lasting vulnerability of the adult brain and behavior.

The reported effects of prenatal choline deficiency are in striking contrast to findings showing that supplementation with choline during early development provide long-term benefits for brain and behavior. For example, a 4-fold increase in dietary intake of choline (over standard control levels seen in most laboratory chow) during ED 12–17 of rat pregnancy leads to improved memory precision and capacity of the adult offspring (Meck et al., 1988; Meck et al., 1989; Meck and Williams, 1997a; Meck and Williams, 1999; Meck and Williams, 2003; Meck et al., 2008; Wong-Goodrich et al., 2008a). These improvements in adult memory are accompanied by enhanced features of adult hippocampal plasticity known to support learning and memory function, such as a reduced threshold to induce LTP (Jones et al., 1999; Pyapali et al., 1998), increased dendritic spine density in the CA1 and dentate gyrus (Meck et al., 2008), increased depolarization-induced phosphorylation of MAPK and CREB activation (Mellott et al., 2004), elevated hippocampal expression of brain-derived neurotrophic factor (BDNF) (Glenn et al., 2007; Wong-Goodrich et al., 2008b; Wong-Goodrich et al., 2011), nerve growth factor (NGF) (Sandstrom et al., 2002), and insulin-like growth factors- 1 and 2 (IGF-1 and -2) (Napoli et al., 2008; Wong-Goodrich et al., 2008a), and increased levels of adult hippocampal neurogenesis (Glenn et al., 2007;

Glenn et al., 2008; Wong-Goodrich et al., 2008b; Wong-Goodrich et al., 2011). Enhanced hippocampal plasticity and trophic support may also underlie prenatal choline supplementation's protection against cognitive deficits and amount of neural damage in adulthood as a result of various pathological insults, such as excitotoxin-induced seizures (Holmes et al., 2002; Wong-Goodrich et al., 2011; Yang et al., 2000), neurotoxicity (Guo-Ross et al., 2002; Guo-Ross et al., 2003), and neonatal alcohol exposure (Thomas et al., 2004; Thomas et al., 2007; Wagner and Hunt, 2006).

Given that prenatal choline supplementation is neuroprotective, one hypothesis is that prenatal choline deficiency may, conversely, enhance vulnerability to neural injury. However, little is known about the effects of prenatal choline deficiency on the adult brain's response to injury. In terms of neuronal cell loss, prior work has found no additional effect of prenatal choline deficiency on hippocampal histopathology shortly following seizures in adulthood (Holmes et al., 2002), or neurotoxicity-induced neuronal cell death in the neocortex (Guo-Ross et al., 2003). Little is known about whether prenatal choline deficiency alters other markers of neuropathology following injury in adulthood. We have previously demonstrated that prenatal choline supplementation attenuates a number of hippocampal neuropathophysiological measures (including cell loss) at 16 days after kainic acid (KA)-induced status epilepticus (SE) (Wong-Goodrich et al., 2008b). Thus, one goal of the current study was to examine whether prenatal choline *deficiency* alters indices of hippocampal pathophysiology in the adult brain following KA-induced seizures.

Of particular interest is the neurogenic response to seizures. A previous study reported that despite comparable levels of hippocampal histopathology and spatial learning performance in a standard Morris water maze at two weeks after KA-induced SE between control-fed and prenatally choline deficient adult rats, KA-treated deficient rats had significantly lower activity of hippocampal choline acetyltransferase (ChAT), which presumably leads to a reduction in the availability of acetylcholine (ACh) for synaptic transmission, compared to KA-treated control-fed rats (Holmes et al., 2002). Given that neurogenesis in the adult dentate gyrus is modulated by cholinergic transmission (Cooper-Kuhn et al., 2004; Kaneko et al., 2006), it is possible that decreased cholinergic neurotransmission in the hippocampus of prenatally choline deficient rats following seizures may alter both seizure-induced neurogenesis and perhaps other factors that contribute to the hippocampal response to seizures. Moreover, previous work from our laboratory has shown that compared to control-fed rats, prenatal choline supplemented and prenatal choline deficient rats do not upregulate dentate cell proliferation and neurogenesis in response to spatial exploration on a radial arm maze (Glenn et al., 2007), perhaps because supplemented rats are operating at a ceiling and deficient rats are unable to upregulate and/or respond to neurogenic factors that are crucial for reactive neurogenesis. However, we have also demonstrated that in response to seizures, prenatal choline supplemented rats exhibited a robust enhancement in dentate cell proliferation, though this seizure-induced increase was markedly attenuated compared to prenatally control-fed rats (Wong-Goodrich et al., 2008b). To determine whether the inability of prenatally choline deficient rats to upregulate neurogenesis in response to maze exploration also pertains to seizures, a second goal of the current study was to investigate whether prenatal choline deficiency alters the proliferative response observed in the dentate gyrus shortly after KA-induced seizures. Dissociating the effects of prenatal choline deficiency and supplementation on reactive dentate cell proliferation/neurogenesis may provide evidence that the effects of prenatal choline status on reactive neurogenesis may operate via different mechanisms depending upon the type of neurogenic stimuli.

To address these goals, we utilized a model of excitotoxic injury to examine whether prenatal choline supplementation modulates a variety of markers known to change shortly after KA-induced status epilepticus (SE), including hippocampal histopathology, glutamic

acid decarboxylase (GAD) expression, dentate cell/neuronal proliferation, astrogliosis, and growth factor content. To be consistent with our previous study (Wong-Goodrich et al., 2008b), we examined these measures at 16 days after the initial injury.

2. Results

2.1 Seizure activity and hippocampal histopathology

Male offspring of rat dams made deficient of choline (DEF) or given a standard control diet (CON) during ED 12–17 were reared on a standard control diet. At postnatal day (PD) 60, male CON and DEF offspring received multiple injections of either saline or KA to induce SE, as per our previous reports (Wong-Goodrich et al., 2008b; Wong-Goodrich et al., 2011). KA-treated rats were administered 2.5 mg/kg (i.p.) of KA every hour until rats displayed at least one Class IV/V seizure per hour for 3 consecutive hours. All rats treated with KA exhibited continuous Class III–V seizures for over 3 hours. Careful observation and analyses of behavioral activity of rats receiving KA revealed that the pattern of seizure activity (i.e., progression, number, and severity of seizures) was similar for both CON and DEF rats. There were no significant differences between CON and DEF rats in the number of Class III (CON = 1.00 ± 0.53 ; DEF = 1.40 ± 0.68), Class IV (CON = 4.37 ± 0.68 ; DEF = 5.00 ± 2.35), Class V (CON = 1.25 ± 0.62 ; DEF = 4.40 ± 1.81), or total number of Class III–V motor seizures (CON = 6.62 ± 1.02 ; DEF = 10.80 ± 4.63) observed within the first 1-hour period of observable seizure activity (all $ps > 0.05$). After exhibiting their first motor seizure, the majority of both CON and DEF rats either entered a continuous Class III–V seizure state or exhibited ≥ 10 Class IV/V seizures in the second 1-hour period, and by the third hour all rats entered a continuous Class III–V seizure state. No differences in seizure severity or duration occurred beyond the 3-hr time window used to define SE. All rats gradually dropped out of SE within 2–3 hours of the last KA injection. During this time window, continuous seizures subsided, followed by the emergence of 1–3 discrete Class III/IV/V seizures, and all seizure activity abated by 5 hours after the last injection. There were no significant differences between CON and DEF rats in the total amount of KA needed to induce SE (CON = 8.84 ± 1.11 mg/kg; DEF = 10.00 ± 1.31 mg/kg), or in the latency to the first motor seizure (CON = 172.88 ± 23.01 min; DEF = 201.00 ± 19.16 min). The variability in dose and latency to the first motor seizure in our KA-treated rats is consistent with our previous studies (Wong-Goodrich et al., 2008b; Wong-Goodrich et al., 2011) and previous reports showing that the Sprague Dawley rats show a more variable convulsant response to KA than other rat strains (Golden et al., 1991; Golden et al., 1995). Thus, while the dose of KA needed to induce SE varied across rats, the SE produced was quite comparable between our CON and DEF rats, which is consistent with previous work using a single universal dose of KA to induce SE in prenatal choline control-fed versus deficient rats (Holmes et al., 2002).

Five days after SE induction, all rats received one daily injection of BrdU (100 mg/kg, i.p.) for 10 consecutive days and were then euthanized at 16 days after SE-induction. KA-induced SE produced moderate to considerable cell loss, disruption of cellular architecture, and gliosis throughout the rostral-caudal extent of hippocampal areas CA1, CA3, and the hilus in both CON and DEF rats. The granule cell layer of the dentate gyrus remained relatively intact. There were no apparent differences in hippocampal lesion severity between KA-treated CON and DEF rats (Fig. 1). Histopathology scores, adapted from our previous studies (Wong-Goodrich et al., 2008b; Wong-Goodrich et al., 2011) and prior reports (Holmes et al., 2002; Yang et al., 2000), were made by two independent observers blind to the diet treatment condition of each rat where each rat was given a score of 0 (no lesion) to 3 (severe cell loss with considerable disruption of the general cellular architecture). KA treatment led to similar patterns of hippocampal histopathology between CON and DEF rats with comparable histopathology scores between KA-treated CON rats (2.13 ± 0.32) and

DEF rats (2.00 ± 0.27), which is consistent with a prior study showing statistically similar pathology scores between prenatal choline control and deficient rats at two weeks after KA-induced SE (Holmes et al., 2002).

2.2 Seizure-induced decreases in hippocampal GAD expression are not modulated by prenatal choline deficiency

Disruption of hippocampal GABAergic function is a consequence of seizures and likely contributes to hyperexcitability in the epileptic hippocampus (Brooks-Kayal et al., 2009; Morimoto et al., 2004; Obenaus et al., 1993). One measure of GABAergic disruption can be assessed via quantification of GAD65 and 67, enzymes important for local GABA synthesis at synaptic (GAD65) and cytoplasmic (GAD67) sites (Erlander and Tobin, 1991; Esclapez et al., 1994), and may play a role in the packaging and release of GABA (Namchuk et al., 1997; Tian et al., 1999). Hippocampal GAD65 and GAD67 protein (via Western blot) and mRNA levels in saline-treated and KA-treated CON and DEF rats at 16 days after treatment were quantified and these data, expressed as percent of control values, and are shown in Figure 2. Both CON and DEF rats exhibited a seizure-induced decrease in all GAD measures at 16 days after SE, which was confirmed by a main effect of Seizure for GAD65 protein, $F(1, 23) = 10.01$, $p < 0.01$, GAD67 mRNA, $F(1, 22) = 5.86$, $p < 0.05$, and GAD67 protein, $F(1, 20) = 24.41$, $p < 0.001$ (Figure 2). There was also a trend for a main effect of Seizure for GAD65 mRNA, $F(1, 22) = 3.34$, $p = 0.08$. These data are consistent with previous reports (Houser and Esclapez, 1996; Kobayashi and Buckmaster, 2003), as well as the seizure-induced reductions in hippocampal GAD65 and 67 content observed in KA-treated CON rats in our previous studies (Wong-Goodrich et al., 2008b; Wong-Goodrich et al., 2011). There were no effects of Diet or Diet $\times$ Seizure interactions for any GAD measure evaluated, and planned comparisons did not reveal any significant baseline differences in GAD levels between saline-treated CON and DEF rats. Thus, prenatal choline deficiency did not significantly alter hippocampal GAD levels or the reduction in hippocampal GAD65 and 67 mRNA and protein expression at 16 days after KA-induced SE.

2.3 Upregulation of cell division and number of immature neurons in the dentate gyrus shortly after SE is not modulated by prenatal choline deficiency

To examine changes in dentate cell proliferation following KA-induced SE, all rats were administered one daily injection of the mitotic marker, bromodeoxyuridine (BrdU; 100 mg/kg, i.p.) 5–15 days after saline or KA treatment and were then sacrificed 24 hours after the last injection. This time line and BrdU injection regimen was chosen to be consistent with our previous study, which revealed differences in BrdU labeling within the dentate gyrus after KA-induced SE between control-fed and prenatally choline supplemented rats (Wong-Goodrich et al., 2008b). Cells that were immunopositive for BrdU were visualized and quantified using the optical fractionator method. Figure 3 shows photomicrographs of BrdU labeling in the hippocampus of representative sections from CON and DEF saline- and KA-treated rats. The 10-day injection regimen of BrdU administration yielded a very high density of BrdU labeling in both saline- and KA-treated rats. BrdU+ cells were expressed throughout the rostral-caudal extent of the dentate gyrus and in both the suprapyramidal and infrapyramidal blades. BrdU+ cells were evident in the subgranular zone (SGZ) and granular cell layer (GCL), and a vast majority of BrdU+ cells displayed morphological characteristics of normal non-pyknotic cells (e.g., round or oval nuclei that did not appear highly condensed). In KA-treated rats, a considerable portion of BrdU+ cells was also present in the hilus. KA-treated rats had a much greater density of BrdU labeling than saline-treated rats, but there were no apparent differences between prenatal CON and DEF rats in either condition. This observation was confirmed by quantification of BrdU+ cells in the SGZ-GCL and hilus of each rat using unbiased stereology and these data are presented in Figure 4. Analyses of the number of BrdU+ cells revealed a main effect of Seizure for both SGZ-

GCL, $F(1, 21) = 13.96$, $p = 0.001$, and hilus, $F(1, 21) = 9.42$, $p < 0.01$, but no effect of Diet or significant Diet $\times$ Seizure interaction for either region (Fig. 4). Both CON and DEF rats exhibited a nearly threefold increase in the number of BrdU+ cells in the SGZ-GCL following seizures. Seizure-induced increases in hilar cell proliferation in CON and DEF rats were much more robust. KA-treated CON rats showed an ~9-fold increase in hilar cell proliferation and KA-treated DEF rats showed an ~6-fold increase, though this difference was not significantly different (Fig. 4). These data indicate that prenatal CON and DEF rats upregulated similar levels of dentate and hilar cell proliferation shortly after seizures.

To investigate whether prenatal choline deficiency altered the number of newborn neurons shortly after KA-induced seizures, cells that were immunopositive for the immature neuronal marker, doublecortin (DCX), were also quantified using unbiased stereology. DCX+ neurons in the hippocampus of CON and DEF rats were visible along the SGZ and GCL (Fig. 5). In KA-treated rats, DCX+ neurons were also present throughout the hilus region, and many DCX+ neurons in both KA-treated CON and KA-treated DEF rats appeared displaced and exhibited abnormal morphological features, such as horizontally oriented cell bodies and processes (Fig. 5C, 5D). Unbiased stereological estimates of the number of DCX+ neurons in the SGZ-GCL (all rats) and in the hilus (KA-treated rats) were generated and these data are shown in Figure 6. KA-induced SE significantly increased the number of DCX+ neurons in the SGZ-GCL in both CON and DEF rats, $F(1, 21) = 44.79$, $p < 0.001$, but this seizure-induced upregulation of immature neurons was not modified by prenatal choline deficiency, as there was no effect of Diet or Diet $\times$ Seizure interaction (Fig. 6). KA-induced SE elicited a near doubling of immature neurons in the SGZ-GCL in both CON and DEF rats (Fig. 6). KA-treated CON and KA-treated DEF rats also showed similar levels of DCX+ neurons in the hilus region ($p > 0.55$; Fig. 6). Thus, similar to the BrdU findings, prenatal choline deficiency does not appear to alter the seizure-induced upregulation in the number of immature neurons that are present in the dentate gyrus shortly after KA-induced SE.

To verify that the overall size of SGZ-GCL or hilus structure did not mask or account for any differences in numbers of new cells and immature neurons, the volumes of the SGZ-GCL and hilus regions in each rat were estimated based on the contours made during BrdU+ and DCX+ cell counting and using Cavalieri's principle (Mouton, 2002) and there were no significant differences in volumes between any treatment groups ($F_s < 1$).

2.4 Prenatal choline deficiency does not alter SE-induced upregulation of hippocampal GFAP expression

To examine whether prenatal choline deficiency alters reactive astrogliosis following seizures, hippocampal glial fibrillary acidic protein (GFAP) protein (via Western blot) and mRNA levels were quantified in saline- and KA-treated CON and DEF rats at 16 days after treatment and these data, expressed as percent of control levels, are presented in Figure 7. Consistent with our previous report (Wong-Goodrich et al., 2008b), KA-induced SE elicited a significant increase in hippocampal GFAP protein and mRNA levels in both CON and DEF rats. Analyses revealed a main effect of Seizure for both GFAP protein, $F(1, 20) = 89.93$, $p < 0.001$, and mRNA, $F(1, 23) = 8.33$, $p < 0.01$, but no effects of Diet or Diet $\times$ Seizure interactions, indicating that prenatal choline deficiency did not alter seizure-induced GFAP expression in the hippocampus.

2.5 Hippocampal growth factor expression as a function of KA-induced SE and prenatal choline deficiency

To examine whether prenatal choline deficiency alters seizure-induced changes in the adult hippocampal microenvironment, protein levels of various neurotrophic/growth factors that were measured in our prior study (Wong-Goodrich et al., 2008b) (BDNF, IGF-1, FGF-2,

NGF, and NT-3) were quantified via ELISA in the hippocampi of saline- and KA-treated CON and DEF rats at 16 days after treatment. The relative protein levels, expressed as percent of control values, are presented in Figure 8. Analyses revealed a main effect of Seizure for BDNF, $F(1, 23) = 18.44$, $p < 0.001$, and for FGF-2, $F(1, 22) = 41.42$, $p < 0.001$, but no effects of Diet or Diet $\times$ Seizure interactions for either growth factor. Both CON and DEF rats showed a large, significant increase in both BDNF and FGF-2 protein at 16 days after KA-induced SE ($ps < 0.05$; Fig. 8). Planned comparisons revealed that saline-treated DEF rats had significantly higher levels of hippocampal FGF-2 protein than saline-treated CON rats ($p = 0.05$; Fig. 8), which meant that the increase in FGF-2 in KA-treated DEF rats was not as large (~3-fold) compared to KA-treated CON rats (~5-fold). There was a strong trend for a main effect of Seizure for IGF-1, $F(1, 23) = 3.75$, $p = 0.06$, suggesting that KA-induced SE may have increased hippocampal IGF-1 as well in both CON and DEF rats. Increases in BDNF, FGF-2, and IGF-1 at 16 days after KA-induced SE is consistent with the large increases in these growth factors observed in KA-treated CON rats in our prior study (Wong-Goodrich et al., 2008b). There was also a main effect of Diet for IGF-1, $F(1, 23) = 3.75$, $p < 0.05$, where DEF rats expressed lower overall levels of IGF-1 than CON rats, and planned comparisons revealed that saline-treated DEF rats had significantly lower levels of hippocampal IGF-1 protein than saline-treated CON rats ($p = 0.01$; Fig. 8). There were no effects of Seizure, Diet, or Diet $\times$ Seizure interactions for levels of NGF or NT-3.

3. Discussion

The results of the current study reveal that choline deficiency during ED 12-17 did not enhance vulnerability to KA-induced seizures in adulthood, as revealed by comparable levels of hippocampal histopathology, loss of hippocampal GAD mRNA and protein expression, increases in hippocampal GFAP and dentate cell proliferation and neurogenesis, and increases in several hippocampal growth factors at 16 days after excitotoxic injury between prenatally choline deficient and control-fed rats. In addition, prenatally choline deficient rats were not more susceptible to seizure induction; both control rats and prenatally choline-deficient rats required the same total amount of KA to induce a seizure and displayed similar latencies to the first motor seizure. We did find that prenatal choline deficiency decreased IGF-1 protein levels, and increased FGF-2 protein levels in the intact hippocampus. Thus, while prenatal choline deficiency appears to compromise plasticity in the intact adult hippocampus under some conditions (Glenn et al., 2007; Jones et al., 1999; Mellott et al., 2004; Montoya et al., 2000), the current findings suggest that prenatal choline deficiency does not appear to enhance the vulnerability of the hippocampus to excitotoxic seizures.

These data add to the small number of reports examining the effects of prenatal choline deficiency versus supplementation on the adult brain's response to injury. For example, while prenatal choline supplementation has been shown to protect against NMDA receptor antagonist-induced neurotoxicity (i.e., neuronal cell death) in the adult posterior cingulate and retrosplenial cortices, prenatal choline deficiency had no effect on the severity of toxicity-induced neuronal cell death in these neocortical regions compared to control diet-fed rats (Guo-Ross et al., 2003). Similarly, while we have shown that prenatal choline supplementation attenuates a number of hippocampal neuropathophysiological markers at 16 days after KA-induced SE (Wong-Goodrich et al., 2008b), another study reported that at two weeks after SE (induced by administering a single injection of KA), prenatally choline-deficient adult rats exhibited similar levels of hippocampal cell loss as control-fed rats (Holmes et al., 2002), which is consistent with our current findings using a repeated low dose KA injection model to induce SE. This prior study also examined spatial learning on a standard reference memory water maze task and found that prenatally choline deficient rats were just as impaired as control rats at two weeks after SE (Holmes et al., 2002). The

current findings, along with these prior studies (Holmes et al., 2002; Wong-Goodrich et al., 2008b; Yang et al., 2000), support the view that prenatal choline availability does not appear to modulate the adult hippocampal response shortly after seizures in a dose-dependent manner. Future research is needed, however, to determine whether prenatal choline deficiency alters longer-term recovery from seizures and/or exacerbates seizure-induced cognitive deficits under conditions where task demands are increased, which is when intact prenatally choline deficient animals exhibit impairments (Buhusi et al., 2008; Lamoureux et al., 2008; Meck and Williams, 1997b; Meck and Williams, 1997c; Meck and Williams, 1999). It is also possible that both prenatal choline deficient and control rats that survived the KA-induced SE show maximal cognitive and neural responses to KA-induced seizures, and that vulnerability would only be revealed if damage could be titrated (which would potentially allow us to examine rats that would have otherwise died from the SE induction procedure).

Perhaps somewhat surprising was that DEF rats had similar levels of dentate cell proliferation and neurogenesis in the adult hippocampus shortly after KA-induced seizures as that of KA-treated CON rats. These data are of interest because prenatally choline deficient rats do not upregulate adult neurogenesis in the intact hippocampus in response to an enriching experience (Glenn et al., 2007), suggesting that the ability of prenatally choline deficient rats to upregulate neurogenesis in response to neurogenic stimuli appears to depend on the nature and/or magnitude of the stimuli. The dramatic seizure-induced increases in hippocampal BDNF (3-fold) and FGF-2 (5-fold) expression observed in DEF rats (comparable to that of control-fed rats) in the current study may have been sufficient to override the putative initial inability to upregulate dentate neurogenesis (Glenn et al., 2007), as the expression of various growth factors is thought to be a robust mediator of adult hippocampal neurogenesis (Anderson et al., 2002; Cameron et al., 1998; Frielingsdorf et al., 2007; Lee et al., 2002a; Lee et al., 2002b; Rai et al., 2007; Scharfman et al., 2005).

Moreover, compared to KA-treated control-fed rats, prenatally choline deficient rats show decreased hippocampal ChAT (an enzyme important for the ACh synthesis) activity two weeks after KA-induced SE, suggesting a reduction in cholinergic transmission in the hippocampus (Holmes et al., 2002). Although there is considerable evidence that adult neurogenesis is modulated by cholinergic activity (Cooper-Kuhn et al., 2004; Itou et al., 2010; Kaneko et al., 2006; Kotani et al., 2006; Kotani et al., 2008) and previous reports have demonstrated that prenatally choline deficient rats have reduced hippocampal ACh content and a relative inability to sustain depolarization-evoked ACh release in the intact adult brain (Meck et al., 2008), our DEF rats in the current study still exhibited robust seizure-induced increases in BrdU and DCX immunolabeling in the dentate gyrus that were comparable to that of control diet-fed rats. It is unknown whether cholinergic input is necessary for seizure-induced neurogenesis in the hippocampus, but given the significant concomitant increase in hippocampal neurotrophic/growth factor expression and astrogliosis in DEF rats in the current study, it is possible that these additional molecular signals overrode the effects of compromised hippocampal cholinergic function conferred by prenatal choline deficiency on the capacity for seizure-induced neurogenesis. This override in prenatally choline deficient animals may, however, occur with additional consequences. Given that newborn neurons in the adult hippocampus express various ACh receptor subtypes (Itou et al., 2010; Kaneko et al., 2006; Mohapel et al., 2005), it is possible that robust increases in adult neurogenesis due to seizures may increase the demand for cholinergic input to the hippocampus. One hypothesis is that perhaps because prenatally choline deficient rats already have lower hippocampal ACh content prior to seizures (Blusztajn et al., 1998; Cermak et al., 1998; Cermak et al., 1999; Meck et al., 2008), the reduced hippocampal ChAT activity following seizures (and presumed decreased availability of ACh for cholinergic transmission) may reflect a further depletion of the cholinergic system as a result of the seizure-induced

upregulation of neurogenesis. Future studies are needed to examine this possibility as well as the potential involvement of the cholinergic system in seizure-induced hippocampal neurogenesis in the adult brain.

The current study did not detect differences in numbers of newly generated cells or immature neurons in the adult dentate gyrus between saline-treated CON and DEF male rats, which extends the previous work with 8-month-old female rats (Glenn et al., 2007) to include males and younger (~2-month-old) rats and the notion that prenatal choline availability does not necessarily exert dose-dependent effects on all features of adult hippocampal plasticity. However, the results of the current experiment did reveal that prenatal choline deficiency reduced basal levels of hippocampal IGF-1 protein in young adult male rats, which is in concert with our previous study showing a similar reduction in hippocampal IGF-1 protein in prenatally choline deficient male rats at ~16 months of age (Wong-Goodrich et al., 2008a). Taken together, these findings suggest a persistent reduction in hippocampal IGF-1 levels by prenatal choline deficiency throughout life. While the precise role of IGF-1 in enrichment-induced hippocampal neurogenesis is not known, previous work has demonstrated that IGF-1 mediates exercise-induced enhancements in hippocampal neurogenesis and spatial memory (Ding et al., 2006; Trejo et al., 2001). Reductions in basal levels of IGF-1 in the intact hippocampus by prenatal choline deficiency may thus partially contribute to the inability to upregulate neurogenesis in response to environmental enrichment and/or physical activity, both of which were components in the maze exploration task in our previous study (Glenn et al., 2007).

Interestingly, basal levels of hippocampal FGF-2 were elevated in prenatally deficient rats compared to control-fed rats in the current experiment. Systemic or ICV administration of FGF-2 can increase adult hippocampal neurogenesis (Perez et al., 2009; Rai et al., 2007), and it has been suggested that FGF-2 makes an important contribution to the hippocampal trophic system that maintain levels of adult neurogenesis into old age (Mudo et al., 2009). However, elevated FGF-2 can also inhibit adult hippocampal neurogenesis, but this inhibition may depend on the availability of other neurotrophic/growth factors, including IGF-1 (Chen et al., 2007). Additional studies are needed to determine whether enhanced hippocampal FGF-2 (and perhaps accompanied by reductions in IGF-1) conferred by prenatal choline deficiency translates into positive or negative effects on adult hippocampal neurogenesis.

Importantly, while prenatal choline supplementation has been shown to be neuroprotective against a number of insults (including seizures) to the adult brain (Guo-Ross et al., 2002; Guo-Ross et al., 2003; Holmes et al., 2002; Thomas et al., 2004; Thomas et al., 2007; Wong-Goodrich et al., 2008b; Wong-Goodrich et al., 2011; Yang et al., 2000), prenatal choline deficiency does not necessarily appear to be a severe risk factor for exacerbated neuropathophysiology at least shortly after KA-induced prolonged seizures. A majority of pregnant women in the US do not receive the recommended amount of daily choline (Jensen et al., 2007), and menopausal status and some genetic polymorphisms of choline metabolism may further compromise choline availability in humans (da Costa et al., 2006), suggesting our findings may be clinically relevant to women who may be more vulnerable to choline deficiency in that a compromised dietary choline status during pregnancy may not necessarily reflect an increase in the adult offspring's vulnerability to diseased states.

4. Experimental Procedure

4.1 Animals and prenatal diet

Forty-four timed-pregnant Sprague-Dawley rats (CD strain, Charles River, Kingston, NY) were obtained on day 9 of gestation (ED 9). All dams were individually housed in clear

polycarbonate cages (27.9×27.9×17.8 cm) that were individually ventilated, and the colony was maintained at 21°C on a 12-h light/dark cycle with lights on at 7 a.m. Dams were fed a control diet *ad libitum* (AIN76-A from Dyets, American Institute of Nutrition, ICN, Nutritional Biochemical, Cleveland, Ohio; 1.1 g/kg choline chloride substituted for choline bitartrate). On the evening of ED11 to the morning of ED18 (ED 12-17), pregnant dams were either given *ad libitum* access to a control diet ($n = 29$) or a diet deficient in choline ($n = 15$). Control and deficient dams were also given water sweetened with 50 mM saccharine because these dams were treated alongside choline supplemented dams for a different experiment. On the morning of ED18, all dams were returned to normal drinking water. There were no significant differences in the amount of water intake, food consumed, or body weights on ED11-18 between control and deficient dams ($ps > 0.05$; data not shown). After birth, offspring from the control (CON) and choline deficient (DEF) dams were toe clipped for identification and then were selected randomly and cross-fostered to dams that consumed the control diet throughout pregnancy to yield 10 pups per litter (5 males and 5 females, with a equal representation of control, deficient, and supplemented offspring per litter). There were no significant differences between CON and DEF litter size or pup birth weights ($ps > 0.05$). On postnatal day (P) 25, pups were weaned and pair-housed with a rat of the same sex and prenatal diet condition. All offspring were given *ad libitum* access to the control diet through the duration of the study. Male offspring were used as subjects. All animal procedures were in compliance with the Institutional Animal Care and Use Committee of Duke University.

4.2 Induction of status epilepticus

Status epilepticus (SE) induction procedures were performed as previously described (Wong-Goodrich et al., 2008b; Wong-Goodrich et al., 2011) and were based on a model of chronic temporal lobe epilepsy that yields a low mortality rate and mimics several aspects of human temporal lobe epilepsy (Hattiangady et al., 2004; Hellier et al., 1998; Hellier and Dudek, 1999; Hellier and Dudek, 2005; Pitkanen et al., 2007; Sharma et al., 2007). KA was obtained from Tocris Bioscience (Ellisville, MO) and was dissolved in 0.9% sterile saline (Sigma, St. Louis, MO). A group of PD 60 adult male CON and DEF offspring were injected with KA (2.5 mg/kg, i.p.) every hour and observations of seizure behavior were recorded according to Racine's scale (Racine, 1972). A separate group of 60-day old CON and DEF male offspring was similarly treated with hourly injections of 1 ml/kg saline. All KA-treated animals initially showed wet dog shakes and head-nodding, followed by Class III (unilateral forelimb clonus), Class IV (bilateral forelimb clonus with rearing), and Class V (bilateral forelimb clonus with rearing and falling over) motor seizures. KA treatment continued until rats displayed at least one Class IV/V seizure per hour for 3 consecutive hours, although most rats exhibited many more seizures during these 1-hour periods and all rats eventually entered a state of continuous Class III–V seizures for over 3 hours. If a rat displayed ≥ 10 Class IV/V seizures in an hour, administration of the next injection was delayed to the next hour. If a rat displayed bouncing seizures after reaching SE, it was given a low dose of diazepam (2.5 mg/kg) to reduce the risk of mortality. If a rat did not have a bouncing seizure, it was administered the low dose of diazepam at the conclusion of SE. We have observed and others have demonstrated (Pierce et al., 2005; Scharfman et al., 2000) that administering a low dose of diazepam following SE does not compromise the development of spontaneous epileptic motor seizures. The total KA dosage administered was titrated for each rat according to each rat's motor seizure activity. Our procedures were designed such that all KA-treated rats experienced similar duration and severity of seizure activity.

After KA treatment, rats were injected subcutaneously with 5 ml of lactated Ringer's solution (Abbott Laboratories, Chicago, IL) to prevent dehydration and were monitored

closely until all seizure activity subsided. Saline-treated rats were also given 5 ml of lactated Ringer's solution to equate their post-treatment experience to that of KA-treated rats. For the next 5 days, all rats continued to receive daily subcutaneous injections of 1–5 ml of lactated Ringer's solution and were provided with moistened chow and slices of fresh fruit to aid recovery. Although more DEF rats did not survive the KA treatment, mortality rates did not differ significantly between CON rats (4/12) and DEF rats (8/13), χ^2 (1, N = 25) = 1.99, p = 0.24. Only measures taken from KA-treated rats that survived were included in the analyses. Rats remained pair-housed throughout the duration of the experiment, yielding the following groups: saline-treated CON (n = 8), saline-treated DEF (n = 6), KA-treated CON (n = 8), and KA-treated DEF (n = 5).

4.3 Bromodeoxyuridine injections and tissue harvesting

Five days after being treated with KA or saline, all rats were administered one daily injection of 5-bromo-2-deoxyuridine (BrdU; 100mg/kg/day, i.p.; Sigma, St. Louis, MO) for 10 consecutive days (10 injections total) to label dividing cells. This injection regimen was based on our previous report and was designed to capture the period of time during which we have shown alterations in SE-induced dentate cell proliferation and hippocampal pathology as a result of prenatal choline supplementation (Wong-Goodrich et al., 2008b). Twenty-four hours after the last injection (16 days after KA-induced SE), rats were given an overdose of a ketamine/xylazine cocktail, decapitated, and brains were rapidly removed and midsagittally sectioned. The hippocampus from one half-brain was immediately dissected for mRNA and protein analyses and stored at -80°C until assayed. The other half brains were immediately post-fixed in 4% paraformaldehyde for 72 hours at 4°C and then cryoprotected in a 30% sucrose solution in 1M phosphate buffer (PB). Brains were then sectioned coronally at 60 μm on a freezing microtome through the rostral-caudal extent of the hippocampus and every fifth section was collected in 0.1% sodium azide in 1M PB to yield five series of 8–10 sections each. Separate series of sections were processed for BrdU or doublecortin (DCX) immunohistochemistry for subsequent cell counting.

4.4 BrdU and doublecortin immunohistochemistry

Immunohistochemical procedures were performed as previously described (Wong-Goodrich et al., 2008b; Wong-Goodrich et al., 2011). Briefly, free-floating sections were rinsed with tris-buffered saline (TBS; pH 7.3), treated for 30 minutes in 50% methanol, treated for 30 minutes in 0.6% hydrogen peroxide, and rinsed. Sections were then treated for 2 hours in 50% Formamide/2x SSC (0.3 M NaCl, 0.03 M sodium citrate) at 65°C , rinsed in 2x SSC for 10 minutes, incubated in 2 N HCl for 30 minutes at 37°C , and rinsed in 0.1 M boric acid (pH 8.5) for 15 minutes. Sections were rinsed in TBS, incubated in 0.1% Triton X-100 (TTX, Sigma) and 3% normal horse serum (Vector Laboratories) in TBS for 30 minutes, and then incubated with mouse anti-BrdU (1:400, Roche) or goat anti-DCX (1:200, Santa Cruz Biotechnology) for 24 hours at 4°C . Sections were then rinsed and incubated with a biotinylated secondary antibody (horse anti-mouse [BrdU] or horse anti-goat [DCX], 1:200, Vector Laboratories) for 2 hours, rinsed, incubated in an avidin-biotinylated peroxidase complex (ABC, Vector Laboratories) for 1 hour, rinsed, and treated for peroxidase detection with diaminobenzidine (BrdU; Vector Laboratories, nickel intensified) or Vector grey substrate (Vector Laboratories) for 5 minutes. Stained sections were mounted on gelatin-coated slides, dehydrated, counterstained with cresyl violet (BrdU), and coverslipped.

4.5 Quantification of BrdU+ and DCX+ cells

BrdU+ and DCX+ cells in each dentate gyrus were counted using the optical fractionator method (Mouton, 2002; West, 1993; West, 1999) and based on previous methods (Wong-Goodrich et al., 2008b). Every fifth section was sampled throughout the extent of the dentate gyrus in two sampling regions: one region (SGZ-GCL) included both the subgranular zone

(SGZ), which was designated as an approximately 2-cell thick zone between the inner rim of the granular cell layer (GCL) and the hilus, and the GCL that encompassed the suprapyramidal and infrapyramidal granule cell blades, and the other region included the hilus, which did not include the CA3c region. StereoInvestigator (Microbrightfield Inc., Williston, VT) was used to sample systematically through the designated region and count numbers of labeled cells in 8 sections per brain. For counting BrdU+ cells, a $40 \times 40 \mu\text{m}$ counting frame was used. For DCX+ cells, a larger counting frame ($80 \times 80 \mu\text{m}$) was used to compensate for the more sporadic distribution of DCX+ cells. These parameters ensured adequate sampling of BrdU- and DCX-labeled cells through the SGZ-GCL and the hilus. For analysis, an optical dissector height of $20 \mu\text{m}$ with a $4\text{-}\mu\text{m}$ guard zone was set and stained cells were counted in each frame using a 40x objective lens. Optical fractionator estimates were multiplied by 2 to account for both hemispheres. Finally, estimates of the volume of the regions of dentate gyrus that was sampled for BrdU and DCX estimates were made using Cavalieri's principle (Mouton, 2002). For each section examined, the area of the SGZ-GCL and hilus was calculated by the StereoInvestigator software and was based on the boundaries of the contour tracings. Volume estimates were obtained by multiplying the section area estimates with the spacing between sampled areas. Spacing was derived by multiplying the measured, post-histology thickness of each sample by the number of sections examined, which was constant for all sections for all rats (~50% shrinkage).

4.6 Reverse Transcriptase PCR, Western blot analyses, and ELISA

The entire hippocampus from one half-brain was immediately dissected out and divided into pieces of approximately equal length along the rostral-caudal extent of the hippocampus. Pieces for reverse transcriptase PCR assays were immediately homogenized in cold guanidine isothiocyanate solution, frozen on dry ice, and stored at -80°C . The remaining pieces were immediately stored at -80°C until assayed for protein. Reverse transcriptase (RT)-PCR procedures for GAD65, GAD67, and GFAP were performed according to our previous studies (Wong-Goodrich et al., 2008b; Wong-Goodrich et al., 2011). In brief, total RNA was extracted from tissues by phenol and chloroform method (Chomczynski and Sacchi, 1987; Sambrook, 1989) and precipitated. RNA was resuspended and its quantity was determined using Quant-iTTM RiboGreen[®] RNA assay kit (Molecular Probes) and the Victor³ multi-label plate reader (PerkinElmer Life Sciences). Hippocampal RNA was used for RT-PCR using Superscript OneStep RT-PCR with Platinum *Taq* (Invitrogen). PCR products were separated on a 10% TBE polyacrylamide gel and stained with ethidium bromide. PCR products were then visualized with the Kodak Image Station 440 (Rochester, NY) and product intensities were quantified using Kodak software. PCR product levels were normalized with β -actin values.

Western blot analysis procedures were also performed according to our earlier reports (Wong-Goodrich et al., 2008b; Wong-Goodrich et al., 2011). In brief, extracts were normalized for total protein and subjected to SDS-PAGE. After transfer of protein to an Immoblin P membrane (Millipore), the membrane was blocked with 5% nonfat dry milk in 1X Tris-buffered saline (TBS) containing 0.1% Tween 20 for 2 hours and then probed overnight with either anti-GFAP monoclonal antibody GA5 (1:1000) (Cell Signaling Technology), anti-GAD65 or 67 polyclonal antibody (1:1000) (Chemicon), or anti- β -actin monoclonal A5441 (1:5000; Sigma). The antibody/antigen complexes on the membranes were detected using a peroxidase-conjugated anti-mouse IgG for GFAP and β -actin (1:2000) or anti-rabbit IgG (1:5000) for GAD65/67 and visualized using the enhanced chemiluminescence method (Western Lightning, Perkin Elmer) and a Kodak Image Station 440. Digitized images of immunoblots were quantified using Kodak ID software. Protein levels were normalized with β -actin values.

ELISA assay procedures for brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF-1), fibroblast growth factor-2 (FGF-2), nerve growth factor (NGF), and neurotrophin-3 (NT-3) were performed according to our previous study (Wong-Goodrich et al., 2008b). All dissected samples were first weighed individually to get their wet weights. Whole tissue extracts were prepared by adding lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% Nonidet NP-40, 10% glycerol, 2 mM 4-(2-aminoethyl)- benzenesulfonyl fluoride, 1 µg/ml leupeptin, 2 µg/ml aprotinin, 2 µg/ml pepstatin), followed by gentle sonication, incubation on ice for 15 min, and a brief centrifugation to clear. The supernatant from each sample was diluted 5 times with Dulbecco's PBS and acidified to pH 2.6. After 15 min of incubation at room temperature, the diluted supernatants were neutralized to pH 7.6, aliquoted and frozen for subsequent measurement of BDNF, NGF, and IGF-1 using ELISA. The above procedure was performed for all samples because a previous study indicated that acidification and subsequent neutralization with base increase the amount of detectable neurotrophins in extracts of CNS tissues (Okragly and Haak-Frendscho, 1997). The ChemiKine™ BDNF sandwich ELISA kit (Chemicon Int., Inc.) was used to assay the BDNF levels in hippocampal lysates according to manufacturer's instructions. The Quantikine® sandwich ELISA kits (R & D Systems) were used to assay IGF-1 and FGF-2 levels in the samples according to manufacture's instructions. Finally, The Emax® immunoassay systems (Promega) were used to measure NGF and NT-3 in the samples and according to manufacturer's instructions. The optical density of each well was measured using the Victor³ microplate reader (PerkinElmer Life Sciences). The intensity of color was measured at a wavelength of 450 nm for all ELISAs. In order to correct for optical imperfections in the plate, readings at 540 nm were subtracted from readings at 450 nm. The standard curve was used to assess the validity of the protocol and to determine the relative concentrations of the growth factors. Values in all samples were normalized per gram of tissue assayed, and the average value for each sample was calculated separately before determining the group means.

4.7 Statistical analyses

Numbers of BrdU-labeled and DCX-labeled cells estimated with the optical fractionator, the volumes of dentate gyrus that were estimated using Cavalieri's principle, and protein and mRNA levels (expressed as percent of control levels) were all subjected to a 2 (Diet: CON vs. DEF) × 2 (Seizure: saline vs. KA) between-subjects ANOVA. Of particular interest were Diet × Seizure interactions. Where appropriate, *a priori* pairwise comparisons were used to evaluate differences between group means. A significance level of 0.05 was set for all statistical tests. Values are reported in the text as means ± SEM. Subjects contained within each experimental condition were randomly selected from different litters (*n* of 1/litter). Thus, necessary precautions were taken to be sure that the findings are not contaminated by a lack of within-litter variability.

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Abbreviations

CON	prenatal choline control
DEF	prenatal choline deficient
BrdU	bromodeoxyuridine
DCX	doublecortin

GAD	glutamic acid decarboxylase
GFAP	glial fibrillary acidic protein
BDNF	brain derived neurotrophic factor
NGF	nerve growth factor
IGF-1	insulin-like growth factor-1
FGF-1	fibroblast growth factor-1
NT-3	Neurotrophin-3

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Research Highlights

- Prenatal choline deficiency can impair adult brain and cognitive function.
- We examined prenatal choline deficiency's effects on the adult response to seizures.
- Hippocampal cell loss, GAD reduction, and GFAP increase were not affected.
- Dentate proliferative response to seizures was not altered by prenatal diet.
- Prenatal choline deficiency does not enhance hippocampal vulnerability to seizures.

Figure 1.

Hippocampal Histopathology of CON and DEF Rats 16 Days After KA-Induced SE. Histopathology of the hippocampus in CON (*B*) and DEF (*C*) rats. Panel A depicts representative sections of CA1 (*A1*), CA3 (*A2*), and dentate gyrus (*A3*) regions from an intact hippocampus from a saline-treated CON rat. Saline-treated DEF rats also did not show any lesions (histology data not shown). Areas of damage (cell loss, disruption of cytoarchitecture, and gliosis) are indicated by arrows. KA-treated CON and KA-treated DEF rats exhibited similar severity of hippocampal neurodegeneration. Photomicrographs in each set were taken with a 10x objective. Bars indicate 50 μ m. GCL, granule cell layer. SGZ, subgranular zone. H, hilus.

Figure 2. Hippocampal GAD Expression 16 Days After Saline or KA Treatment in CON and DEF Rats. Comparison between CON (white bars) and DEF rats (grey bars) in GAD65 and GAD67 mRNA levels (A) and protein levels (B) (mean $\pm$ SEM percent of control levels) in the intact hippocampus (open bars) and 16 days following KA-induced SE (hatched bars). mRNA levels were quantified using RT-PCR and protein levels were quantified using Western blot analysis. An overall effect of Seizure was evident for GAD67 mRNA, GAD65 protein, and GAD67 protein ($p < 0.05$) with a strong trend for GAD65 mRNA ($p = 0.08$) where KA-induced SE similarly reduced hippocampal GAD expression in CON and DEF rats. # main effect of Seizure at $p < 0.05$. * statistically different from within-diet saline-treated group at $p < 0.05$.

Figure 3.

BrdU Immunolabeling 16 Days After Saline or KA Treatment in CON and DEF rats. BrdU-immunopositive cells (i.e., newly generated cells) in the SGZ-GCL and hilus 16 days following saline treatment (A, CON; B, DEF) or KA-induced SE (C, CON; D, DEF). The number of BrdU-labeled cells significantly increased 16 days after SE for both diet groups. Photomicrographs in the left column were taken with a 10x objective and images in the right column were taken with a 40x objective. Bars indicate 50 μ m. GCL, granule cell layer. SGZ, subgranular zone. H, hilus.

Figure 4.

Quantification of BrdU+ Cells in the Dentate Gyrus 16 Days After Saline or KA Treatment in CON and DEF Rats. Mean ($\pm$ SEM) numbers of BrdU+ cells detected in the SGZ-GCL and hilus of CON (white bars) and DEF (grey bars) rats 16 days following saline treatment (open bars) or KA-induced SE (hatched bars). SE significantly increased the number of BrdU+ cells in both CON and DEF rats ($p < 0.05$). * statistically different from within-diet saline-treated group.

Figure 5.

DCX Immunolabeling 16 Days After Saline or KA Treatment in CON and DEF Rats. DCX-immunopositive neurons (i.e., newly generated neurons) in the SGZ-GCL and hilus 16 days following saline treatment (A, CON; B, DEF) or KA-induced SE (C, CON; D, DEF). The number of DCX-labeled cells significantly increased 16 days after SE for both CON (A, C) and DEF (B, D) rats. Note that in KA-treated rats of both diet groups (C, D), DCX-positive neurons were aberrantly located in the hilus and exhibited abnormal morphological features, such as horizontally oriented cell bodies and processes (arrows). Photomicrographs in the left column were taken with a 10x objective and images in the right column were taken with a 40x objective. Bars indicate 50 μ m. GCL, granule cell layer. SGZ, subgranular zone. H, hilus.

Figure 6.

Quantification of DCX+ Cells in the Dentate Gyrus 16 Days After Saline or KA Treatment in CON and DEF Rats. Mean ($\pm$ SEM) numbers of DCX+ cells detected in the SGZ-GCL and hilus of CON (white bars) and DEF (grey bars) rats 16 days following saline treatment (open bars) or KA-induced SE (hatched bars). KA-induced SE significantly increased the number of DCX+ cells in both CON and DEF rats (p s < 0.05). * statistically different from within-diet saline-treated group.

Figure 7.

Hippocampal GFAP Expression 16 Days After Saline or KA Treatment in CON and DEF Rats. Comparison between CON (white bars) and DEF rats (grey bars) in GFAP mRNA levels (A) and protein levels (B) (mean $\pm$ SEM percent of control levels) in the intact hippocampus (open bars) and 16 days after KA-induced SE (hatched bars). mRNA levels were quantified using RT-PCR and protein levels were quantified using Western blot analysis. SE significantly similarly increased GFAP mRNA and protein levels in both CON and DEF rats ($p < 0.05$). # main effect of Seizure at $p < 0.05$. * statistically different from within-diet saline-treated group. + different from within-diet saline-treated group at $p = 0.07$.

Figure 8.

Hippocampal Growth Factor Expression 16 Days After Saline or KA Treatment in CON and DEF Rats. Comparison between CON (white bars) and DEF (grey bars) rats in growth factor protein levels (mean $\pm$ SEM percent of control levels) in the intact hippocampus (open bars) and 16 days following KA-induced SE (hatched bars). Protein levels were quantified using ELISA. A main effect of Diet was present for IGF-1, where DEF rats had lower levels of hippocampal IGF-1 protein than CON rats across both saline- and KA-treated groups ($p < 0.05$). An overall effect of Seizure was evident for BDNF and FGF-2 protein ($ps < 0.05$) with a strong trend for IGF-1 protein ($p = 0.06$) where KA-induced SE similarly increased hippocampal growth factor expression in CON and DEF rats. Saline-treated DEF rats had significantly lower expression levels of IGF-1 and significantly higher expression levels of FGF-2 compared to saline-treated CON rats. * statistically different from within-diet saline-treated group at $p < 0.05$. + statistically different from saline-treated CON rats at $p < 0.05$.