Published in final edited form as:

Neuroscience. 2015 March 12; 288: 120–134. doi:10.1016/j.neuroscience.2014.12.017.

Age-dependent long-term structural and functional effects of early life seizures: evidence for a hippocampal critical period influencing plasticity in adulthood

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Abstract

Neural activity promotes circuit formation in developing systems and during critical periods permanently modifies circuit organization and functional properties. These observations suggest that excessive neural activity, as occurs during seizures, might influence developing neural circuitry with long-term outcomes that depend on age at the time of seizures. We systematically examined long-term structural and functional consequences of seizures induced in rats by kainic acid, pentylenetetrazol, and hyperthermia across postnatal ages from birth through postnatal day 90 in adulthood (P90). Magnetic resonance imaging (MRI), diffusion tensor imaging (DTI), and electrophysiological methods at P95 following seizures induced from P1 to P90 demonstrated consistent patterns of gross atrophy, microstructural abnormalities in the corpus callosum and hippocampus, and functional alterations in hippocampal circuitry at P95 that were independent of the method of seizure induction and varied systematically as a function of age at the time of seizures. Three distinct epochs were observed in which seizures resulted in distinct long-term structural and functional outcomes at P95. Seizures prior to P20 resulted in DTI abnormalities in

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Conflict of Interest:

T.S. has an equity interest in NeuroGenomeX, Inc.

corpus callosum and hippocampus in the absence of gross cerebral atrophy, and increased paired pulse inhibition (PPI) in the dentate gyrus at P95. Seizures after P30 induced a different pattern of DTI abnormalities in the fimbria and hippocampus accompanied by gross cerebral atrophy with increases in lateral ventricular volume, as well as increased PPI in the dentate gyrus at P95. In contrast, seizures between P20-P30 did not result in cerebral atrophy or significant imaging abnormalities in the hippocampus or white matter, but irreversibly decreased PPI in the dentate gyrus compared to normal adult controls. These age-specific long-term structural and functional outcomes identify P20-P30 as a potential critical period in hippocampal development defined by distinctive long-term structural and functional properties in adult hippocampal circuitry, including loss of capacity for seizure-induced plasticity in adulthood that could influence epileptogenesis and other hippocampal – dependent behaviors and functional properties.

Keywords

epilepsy; hippocampus; dentate gyrus; in vivo imaging; developmental plasticity; critical periods

INTRODUCTION

During postnatal brain development, electrical activity promotes synapse formation and organizes connectivity in immature circuits into networks supporting adult function and behaviors (Sur and Rubenstein, 2005). The developing brain also demonstrates age-specific susceptibility to seizure induction, presumably corresponding to age-specific differences in structure, physiology and metabolism influencing the synchronous electrical network activity that defines seizures (Moshe, 1993, Hauser, 1994, Swann et al., 1999, Sankar et al., 2002, Haut et al., 2004, Swann, 2005, Ben-Ari and Holmes, 2006, Holmes, 2009). These observations suggest that network synchronization during seizures in the developing brain may influence activity-dependent postnatal developmental processes such as formation and remodeling of neural connections and functional network properties (Crabtree et al., 1981, Ostrach et al., 1984, Swann et al., 1992, Swann et al., 1999, Bavelier and Neville, 2002, Pascual-Leone et al., 2005).

Although specific details about age-dependent seizure susceptibility and the consequences of developmental seizures are debated, experimental evidence demonstrates that early life seizures in rodents have distinctive effects which differ from seizures occurring during adulthood (Holmes and Ben-Ari, 1998, Lynch et al., 2000, Sankar et al., 2002, Ben-Ari and Holmes, 2006, Sankar and Rho, 2007). For example, seizure-induced cell loss and axonal reorganization are prominent consequences of recurring seizures in adulthood, but are much less evident after seizures occurring during early postnatal development (Holmes and Ben-Ari, 1998, Sankar et al., 2002, Haut et al., 2004, Ben-Ari and Holmes, 2006, Sankar and Rho, 2007). Seizures at specific time points during development induce abnormal network properties and structural differences at the cellular level (Cilio et al., 2003, Haut et al., 2004, Ben-Ari and Holmes, 2006, Holmes, 2009) and clinically relevant functional abnormalities including long-term diminished cognitive task performance (Lynch et al., 2000, Sayin et al., 2004, Karnam et al., 2009) suggesting that normal brain development is modified by seizures early in life.

Deprivation methods and modification of afferent activity in developing sensory and language systems provide substantial evidence of activity-dependent, age-specific developmental effects, including the existence of critical periods (Hubel and Wiesel, 1970, Hensch, 2004, 2005) in which deprivation of neural activity produces long-term structural and functional effects in cortical circuitry. The effects of deprivation or excessive neural activity during development are less clear in the hippocampus and limbic system. Induction of synchronous network events such as seizures during postnatal development provides an experimental opportunity to assess effects of excessive pathological electrical activity during development on adult hippocampal organization and function. Although it is known that the dentate gyrus undergoes substantial postnatal development (Schlessinger et al., 1975, Bayer, 1980), there have been few systematic comprehensive investigations into the effects of seizures induced across the broad range of ages from birth to adulthood on hippocampal development.

The purpose of this study was to systematically investigate the age-dependence of long-term consequences of seizures during postnatal development. Seizures were evoked across a range of ages (P1-P90) using three models of induction: status epilepticus (SE) by kainic acid (KA), repeated self-limited seizures evoked by pentylenetetrazol (PTZ), and repeated seizures evoked by hyperthermia (HT). Structural effects and functional circuit properties were then examined in adulthood (>P95) using *ex-vivo* diffusion tensor imaging (DTI) and magnetic resonance imaging (MRI) to identify microstructural brain abnormalities, paired pulse inhibition (PPI) as a measure of hippocampal network functional properties, and kindling to assess vulnerability to seizure-induced plasticity. The combination of these approaches demonstrated consistent age-dependent patterns of long-term structural and functional consequences of seizures during development, which varied systematically as a function of age at the time of seizures.

EXPERIMENTAL PROCEDURES

Study Design

The overall experimental design is illustrated in Fig. 1. Seizures were induced in Sprague-Dawley rats at different ages from P1 to P90 by KA, PTZ or HT. Long-term structural and functional consequences of these seizures were assessed in adulthood using *ex vivo* DTI and MRI, *in vitro* electrophysiological recordings in hippocampal slices, and *in vivo* assessment of vulnerability to kindling. All rats in this study (n=392) were housed and treated according to guidelines for the use of animals with institutional approval and oversight. Some of the data in this study have been previously presented in preliminary form (Sutula et al., 1992, Lynch et al., 2000, Hutchinson et al., 2012).

Seizure Induction

Kainic acid—Seizures were induced by subcutaneous (SC) or intraperitoneal (IP) injection of KA at doses of 1-2 mg/kg (P1, P3, P7), 2-4 mg/kg (P14), or 8-10 mg/kg (>P24) based on previous studies of doses of KA that evoke SE in these age groups (Tremblay et al., 1984). In a subset of adult rats, KA was administered by intracerebroventricular (ICV) injection of 0.5-0.75 µg KA in 0.05-0.75 µL of deionized H₂O in rats anesthetized with pentobarbital (60

mg/kg) during a 15 minute interval via a Hamilton syringe at 0.6 mm posterior, 2.0 mm lateral, and 3.5 mm ventral to bregma (Lynch et al., 2000). For all rats included in the study, behavioral seizures were classified by a modified Racine scale (Racine, 1972, Sutula and Steward, 1986) and were monitored for ~3-4 hours to confirm that KA induced SE as classified by combined behavioral and electrographic criteria as in previous studies (Lynch et al., 2000). In P1, P3, and P7 rats that received 1-2 mg/kg KA IP, recurring seizures consisted of hyperactive “bicycling” movements of all extremities with opisthotonic arching of the back, and tonic limb extension. Administration of kainic acid to P14 rats (2-4 mg/kg IP), and P20, P24, P31, P75 rats (8-10 mg/kg IP) reliably induced recurring behavioral seizures consisting of intermittent “freezing” or cessation of exploratory behavior, automatisms, salivation, tonic-clonic activities, and occasional loss of postural tone, which evolved into status epilepticus as reported previously. No significant differences in outcome measures were noted with systemic or ICV administration.

Pentylentetrazol—Seizures were induced by PTZ (30 mg/kg IP) in subsets of rats on each of the following days: (P1, P5, P10), (P21, P24, P30), or every other day $\times$ 3 after P95. The evoked seizures included irregular myoclonic, tonic extension of limbs, and arching movements which lasted several minutes and did not recur, as described previously (Golarai et al., 1992).

Hyperthermic seizures—Repeated brief seizures at intervals of 3 days were induced by hyperthermia as described in published methods (Baram et al., 1997, Jiang et al., 1999) in 3 groups of rats on the following days: (P4, P7, P10), (P11, P14, P17), or (P20, P23, P26). Seizures lasting ~1-5 min were evoked by a 4-6 °C increase of abdominal surface temperature from 34-36 °C to 39-41 °C and included clonic and tonic Class IV-V behaviors. The seizures were followed by a postictal phase with diminished motor activity lasting several hours, but recurring seizures were not observed.

Controls—The controls for electrophysiological studies in brain slices or kindling in adulthood were littermates injected with 0.9% (w/v) NaCl at the same age as KA or PTZ injections. The controls for pups with seizures induced by hyperthermia were littermates not exposed to hyperthermia. Approximately equal numbers of males and females were represented in all seizure groups except for the imaging experiments in which adult males that experienced status epilepticus induced by KA on P15, P25, P35, or P70 were compared to normal adult rats.

Hippocampal Electrophysiology

Hippocampal Slices—Long-term functional effects of seizures at different ages during development were examined in hippocampal slices obtained from adult rats (P95-115, n = 209). In the case of rats that received KA at P75-90, slices were obtained at least 3 weeks after SE for KA administered at P75-90. Transverse 450 μ m hippocampal slices cut with a tissue chopper from the dorsal hippocampus after decapitation under halothane or ether anesthesia were transferred into an interface chamber at 30-32°C with artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 124, KCl 4.5, NaH₂PO₄ 1.25, MgSO₄ 2.0, NaHCO₃ 26, CaCl₂ 2.0, glucose 10, saturated with 95%

O₂-5%CO₂. Extracellular field potentials consisting of population excitatory postsynaptic potentials (pEPSPs) and population spikes (PS) were recorded in the granule cell layer of the dentate gyrus (DG) with glass pipettes (5–10 MΩ, filled with 2M NaCl) in response to stimulus pulses delivered with a bipolar stainless steel electrode in the perforant path near the angular bundle external to the dentate gyrus. An input-output curve was determined for each slice with 0.05 mS duration constant-current pulses delivered at 0.03 Hz. The standard test stimulus intensity used for all subsequent stimulation was the lowest intensity evoking the maximum amplitude population spike. Slices with evoked pEPSPs < 1 mV were discarded. Population spike amplitude was calculated as the mean amplitude in mV of perpendicular lines from a tangent at the base of the spike to the maximum of the pEPSP at onset and end of the spike (see Figs. 2A1, 2A2).

Paired Pulse Inhibition—Paired pulses applied to perforant path fibers at 15 ms and 25 ms interpulse intervals (IPs) evoke a pair of pEPSPs with superimposed single population spikes. Using the standard test stimulus of the lowest stimulation intensity evoking the maximum amplitude PS delivered at every 30 sec (0.03 Hz), the second PS of each evoked pair is typically smaller than the first, which is referred to as paired pulse inhibition (PPI, see Fig. 2A1). PPI was calculated as the amplitude ratio (PS₂/PS₁) of the second population spike (P₂) to the first population spike (P₁) (Fig. 2A2). Smaller ratios relative to normal controls indicate increased PPI, larger ratios indicate reduced PPI, and ratios > 1 indicate loss of PPI or paired pulse facilitation.

Kindling—The rate of kindling in response to repeated electrical stimulation of the perforant path or repeated PTZ (30 mg/kg IP) was measured to assess susceptibility to seizure-induced plasticity in adulthood after seizures evoked during early development. For electrical kindling (n=140), an insulated stainless steel bipolar electrode was implanted in the perforant path under anesthesia with ketamine (80 mg/kg IM) and xylazine (10 mg/kg IM), or pentobarbital (60 mg/kg IP). After 2 week recovery, unrestrained awake rats received twice-daily kindling stimulation (five days per week) with a 1 sec train of 60-Hz biphasic constant current 1.0-msec square wave pulses according to published protocols (Sutula and Steward, 1986, Sayin et al., 2003). For PTZ kindling (n=31), seizures evoked by PTZ (30 mg/kg IP) administered 3 days/week were classified by standard criteria (Golarai et al., 1992, Sayin et al., 1995), including latency to onset and duration of behavioral seizures. Kindling rate for perforant path stimulation or repeated PTZ was the number of afterdischarges (ADs) required to evoke the first Class V seizure.

Ex-vivo Magnetic Resonance and Diffusion Tensor Imaging

Ex vivo imaging methods were used in male rats to examine structural effects of SE induced by IP KA at P15 (n=4), P25 (n=5), P35 (n=4), P70 (n=2) compared to normal controls (n=3). At ~P270 these rats were deeply anesthetized with pentobarbital 60 mg/kg IP and were transcardially perfused with fresh 4% paraformaldehyde (PFA). The fixed brains were removed and stored in 4% PFA for at least 1 month to standardize fixation, then were rinsed in 0.9% saline for 48 hours to minimize attenuating effects of fixative on the MRI signal. The brains were placed in a custom built holder and immersed in Fluorinert (FC-3283, 3M,

St. Paul, MN). The sample holder was designed to hold six brains for high throughput imaging and positioning of each brain consistently within the spatial frame of the scanner.

DTI acquisition and post-processing—For *ex-vivo* DTI acquisition, 3 brains were simultaneously imaged for ~ 7hrs using a 4.7 T Agilent MRI system and 3.5cm diameter quadrature volume RF coil. A series of multi-slice, diffusion-weighted, spin echo images were acquired with three non-weighted ($b \sim 0$) and 30 diffusion weighted ($b \sim 1200 \text{ s/mm}^2$), using non-collinear weighting directions. Other imaging parameters were TE/TR=24.17/2000ms, FOV = $30 \times 30 \text{ mm}^2$, matrix = 192×192 reconstructed to 256×256 , slice thickness = 0.5mm, number of slices = 35 and 2 signal averages.

DTI maps were created offline using a combination of FSL software and custom Matlab code. Raw images were corrected for eddy currents by rigid body co-registration and the diffusion tensor was determined at each voxel using a non-linear least squares fitting algorithm (Koay et al., 2006). DTI maps for fractional anisotropy (FA), mean diffusion (MD) and axial and radial diffusivity ($D_{\parallel}$ and $D_{\perp}$) were generated as well as images containing the eigenvector information for the principal eigenvalue used for orientation analysis. Offline registration of the non-weighted image to a template was performed to correct any misalignment of the measured orientation vectors.

Region of interest (ROI) analysis—All ROI analysis was performed in the native imaging space. In order to determine DTI values within the corpus callosum (CC), fimbria of the fornix, and hippocampus including the hilus and molecular layer (ML) of the DG, binary masks (see Fig. 7) were manually created in the native image space of each sample based on anatomical structures and landmarks identified with reference to a rat brain atlas (Watson C., 1982) by individuals who were blind to the experimental groups. ROIs for white matter structures such as the CC, fimbria, and for the ML of the DG were restricted to lines of single voxel width along the axis of the structure to minimize partial volume effects. A semi-automated approach was used to generate ROI masks for the lateral ventricle (LV) in which “rough” ROIs including the LV and surrounding brain tissue were manually created followed by thresholding based on MD value $> 0.0015 \text{ mm}^2/\text{s}$ to include only voxels corresponding to cerebrospinal fluid.

Structural orientation analysis—Based on the qualitative observation of disrupted layer-specific orientation patterns in DTI color maps of the dorsal hippocampus for adult KA-treated animals (e.g. Fig. 7a,b), the principle eigenvector orientation properties were investigated using Fisher statistics according to published methods (Hutchinson et al., 2012). ROIs in the hilus and ML of the dorsal DG were used to determine a mean direction vector for each brain sample ROI, and group comparisons were made by calculation of the F-statistic with subsequent determination of a p-value for the significance of group differences. The directional information was plotted on a unit sphere as a circle characterizing the 95% confidence angle of the control group and mean direction vectors for samples within all groups to visualize and inspect the orientation of brain tissue within the ROIs (see Fig.7c,d).

Statistical Procedures

PPI and kindling data reported as mean $\pm$ S.E.M were normalized across groups by expressing the results as a percent relative to age-matched adult controls. Group differences were evaluated by ANOVA or Students t-test. When data were not normally distributed, analyses were performed using nonparametric ANOVA by ranks (Kruskal-Wallis one-way, or Friedman two-way), or the Mann-Whitney rank sum test. MRI and DTI values for each ROI were compared using ANOVA. Two-tailed T-tests and Pearson's correlation were also performed where applicable.

RESULTS

Age-dependent effects of early life seizures on paired pulse inhibition (PPI) in the adult dentate gyrus reveal three distinct epochs: P1-20, P20-30, and >P30

There were long-term effects of seizures during development on network functional properties as measured by PPI in adulthood (P95) that were independent of the method of seizure induction and varied systematically as a function of age at the time of seizures. Representative examples of the long-term effects of seizures evoked by HT, PTZ, or KA during P1-10 and P20-30 on pairs of field potentials evoked in the adult DG by perforant path pulses at 15 mS IPIs are illustrated in Fig.2.

Epoch P1-20: Seizures during P1-20 result in increased PPI in the DG in adulthood—In hippocampal slices from adult rats (P95) that experienced prolonged seizures induced by KA on P1 (Fig. 2B1), 3 PTZ-induced seizures during P1-10 (Fig. 2C1), or 3 brief seizures evoked by HT during P3-10 (Fig. 2D1), PPI measured at 15 mS interpulse intervals was increased as indicated by reduced amplitude of the second population spike relative to the first spike compared to normal adult controls (compare Figs. 2B1, 2C1, 2D1 to 2A1). In some rats that experienced seizures evoked by hyperthermia at P4-10 or PTZ during P1-10, the increase in PPI was particularly large as a second population spike was not evoked at the standardized stimulus intensity (Figs. 2C1, 2D1). As reported previously after kindling of the perforant path and other hippocampal pathways (Tuff et al., 1983a, Oliver and Miller, 1985, Lynch et al., 2000, Sayin et al., 2003), PPI was also increased after 3 Class V seizures evoked by kindling of the perforant path in adulthood (Fig. 2E1 vs. 2A1). Increases in PPI at 25 mS interpulse intervals were also observed in the adult DG after seizures evoked by KA, PTZ or HT during P1-P20 (data not shown). Plots of PPI at 15 mS and 25 mS IPIs compared to normal adult controls (P90) demonstrated a consistent pattern of increased PPI in the adult DG after seizures induced by KA from P1-P20, PTZ during P1-10, and HT during P4-10 or P11-17 (Fig 3A,B,C).

Epoch P20-30: Seizures during P20-30 result in reduced or lost PPI in adulthood—In contrast to the long-term increases in PPI after seizures during P1-20, PPI was reduced or lost in adult rats (P95) that experienced seizures induced by KA, PTZ, or HT during the interval of P20-30 compared to normal adult controls (compare Figs. 2B2, 2C2, 2D2 vs. 2A1, and vs. 2B1, 2C1, 2D1). In some cases PPI was not only reduced but was lost as indicated by mean amplitudes of the second spike exceeding the amplitude of the first population spike (eg., Fig. 2C2), which is also referred to as paired pulse facilitation.

Increases in PPI at 25 mS interpulse intervals were also observed in the adult DG after seizures evoked by KA, PTZ or HT during P20-P30 (data not shown). Plots of PPI at 15 mS and 25 mS IPIs compared to normal adult controls (P90) demonstrated a consistent pattern of reduced or lost PPI in the adult DG after seizures induced by KA on P24, PTZ during P21-30, and HT during P20-26 (Fig 3A,B,C).

Epoch >P30: Seizures at ages >P30 result in increased PPI in adulthood—In contrast to the effects of seizures induced by KA, PTZ, or HT during P20-30 which reduced PPI, adult rats (P95) that experienced seizures induced by KA on P31 or P75 demonstrated an increase in PPI at both 15 ms and 25 ms IPIs compared to normal adult controls (P90) (Fig. 3A,B,C). Consistent with the observation that SE induced by KA at P75 increases PPI in the DG, PPI was also increased in the DG of adult rats that experienced 3 Class V seizures evoked by electrical kindling of the perforant path (Figs. 2E1,8) or repeated PTZ administered during adulthood (P95) (Fig. 8), confirming previous observations (Tuff et al., 1983a, Otis et al., 1994, Buhl et al., 1996, Sayin et al., 2003). The observation that SE induced by KA increased PPI at P31 suggests that the developmental transition from effects of seizures during P20-30 to adult-like patterns at P75 may be relatively abrupt at the age of ~P30.

Relationships between age at the time of seizures (P1-P75) induced by various methods of seizure induction (KA, PTZ, or HT) and long-term effects on PPI in adulthood (P95) expressed relative to normal adult controls (P90) without seizures are summarized in Fig. 4. The compiled results demonstrate that seizures evoked by a variety of methods during developmental periods of P1-P20, P20-P30, and after P30 have consistent long-term effects on hippocampal network excitability as measured by PPI in the DG in adulthood. The abrupt transitions from increased PPI after seizures prior to P20 and after P30 with a discrete period of reduced or lost PPI after seizures during P20-P30 suggests the possibility that P20-P30 may be a developmental critical period in the hippocampus influencing network function in adulthood.

Early life seizures have age-dependent effects on brain structural measures in adulthood

As developmental critical periods are defined as intervals in early life when electrical or environmental activity influences selection and shaping of neural connections and are accompanied by permanent structural consolidation of these circuits into adulthood (Hensch, 2004, 2005), MRI and DTI methods were used to examine effects of seizures induced by KA across ages spanning P15-P70 on structural brain features including lateral ventricular volume (LVV) and microstructural organization of the CC, fimbria, hippocampus, and DG in adulthood (~P270).

Seizures after P30, but not before resulted in enlargement of ventricles—

Increasing LVV is a measure of generalized cerebral atrophy with increasing ventricle size corresponding to generalized reduction of brain parenchymal volume. A significant main effect for age at the time of seizures on LVV in adulthood was observed (Fig. 5, $p < 0.004$, ANOVA). There was no difference in LVV in adulthood between treatment groups and controls after SE at P15 or P25, but LVV was significantly increased in adulthood following

SE at P35 and P70 ($p < 0.005$), and there was a strong correlation between postnatal day at seizure induction and ventricle volume in adulthood ($R = 0.81$, $p < 0.005$). These observations are consistent with previous histological studies indicating that SE induced by KA in immature (P15) rodents typically induces more subtle neuronal damage compared to adult rats (Sperber et al., 1991, Lynch et al., 2000, Haas et al., 2001, Cilio et al., 2003, Ben-Ari and Holmes, 2006), and damage after SE induced by KA in P24 rats is also typically less severe compared to adult rats (Lynch et al., 2000).

White matter abnormalities follow early seizures (P15) and late seizures (P>30), but not seizures at P25—DTI measures in the white matter regions of the CC and fimbria are provided in Table 1. FA of the CC was reduced after seizures evoked by KA on P15 compared to controls ($p < 0.01$) and $D_{||}$ was also reduced in this group ($p < 0.01$). Diffusion metrics of the CC were not abnormal after seizures at P25 or after P35.

Fimbria FA was significantly reduced after seizures induced by KA at P35 and P70 compared to controls and furthermore $D_{\perp}$ and MD were significantly increased ($p < 0.05$ for all measures, see Table 1). There were no differences in diffusion metrics of the fimbria after seizures induced by KA at P15 or P25.

Abnormalities in anisotropy and orientation in the adult hippocampus depend on age at the time of seizures—Alterations in FA of the dorsal and ventral adult hippocampus as well as specific hippocampal subregions including the DG, CA1 and CA3 areas were examined following SE induced by KA at different ages (Fig. 6). There was a significant main effect of age at the time of seizures on FA in the dorsal adult hippocampus ($p = 0.016$, one-way ANOVA, Fig. 6A), but not in the ventral hippocampus ($p = 0.745$, data not shown). There were significant main effects of age at the time of seizures observed in the subregions CA1 ($p = 0.010$, Fig. 6B), CA3 ($p = 0.044$, Fig. 6C) and in the DG ($p = 0.033$, Fig. 6D). FA in the dorsal adult DG and molecular layer of the DG was reduced after KA at P15 compared to controls ($p < 0.05$, post-hoc t-tests, Fig. 6D,E) but not in the dorsal hilus of the DG (Fig. 6F). FA in the dorsal hippocampus and within the CA1 and CA3 subregions revealed trends toward increases after KA at P35 or P70 compared to controls ($p = 0.1$, post-hoc t-tests, Figs. 6A,B,C). Post-hoc analysis combining FA in the dorsal hippocampus at P35 and P70 and dorsal CA3 at P35 and P70 demonstrated significant increases compared to controls ($p < 0.05$, post-hoc t-tests, Fig. 6A,C). There was no significant difference from controls for any hippocampal region following seizures at P25.

Directional alterations within the tissue structure of the hilus in the adult dorsal DG after seizures during early life were also observed, and are illustrated in directionally encoded color (DEC) maps and line fields for the primary eigenvector orientation in Fig. 7. Visual inspection of the DEC maps suggests orientation changes in the hilus of the DG in adulthood following seizures after P35 (Fig. 7A,B). Spherical plots of the mean direction vectors for each group also demonstrated alterations in orientation in the hilus (Fig. 7C,D) which were confirmed to be significant by application of Fisher statistics (Hutchinson et al., 2012). In the hilus, a significant difference in primary tissue orientation was observed bilaterally following seizures at P35 and P70 ($p < 0.005$ and $p < 0.001$ respectively) but no difference was found for the other groups (Fig. 7D). Within the right ML, a group difference was found

following seizures at P15 ($p < 0.05$, Fig. 7C), however no other groups were found to have abnormal ML orientation. No group differences in tissue orientation were observed for the CC or fimbria.

Effects of early life seizures on seizure-induced plasticity in the adult dentate gyrus also depend upon epoch

Irreversibility of PPI loss after seizures during P20-30—In normal adult rats seizures evoked by kindling or KA increase PPI in the DG (Fig. 2E1, 3A) which is regarded as form of seizure-induced plasticity (Tuff et al., 1983a, Oliver and Miller, 1985, Sayin et al., 2003). It was of interest to assess the effects of seizures in adult rats (P95) manifesting long-term reduction or loss of PPI as a consequence of seizures during P20-30. In contrast to normal adult rats that experience increases in PPI in the DG in response to seizures evoked by electrical kindling of the perforant path (Fig. 2E1), PTZ (Fig. 8), or KA (Fig. 3A), there was no increase in PPI in response to seizures evoked by electrical stimulation of the perforant path or by repeated administration of PTZ in adult rats that experienced 3 brief seizures evoked by HT during P20-30 (compare Fig. 2E2 vs. 2E1, and see also Fig. 8). Seizures during P20-30 not only modified hippocampal network properties as reflected by long-term reduction or loss of PPI in the adult DG (Figs. 2B2, 2C2, 2D2, 3A,B,C, 4), but in addition modified the capacity for seizure-induced plasticity in response to additional seizures in adulthood that was independent of the method of seizure induction (Fig. 8).

Effects of seizures during development on kindling in adulthood—To further examine the effects of seizures during development on the capacity for seizure-plasticity in adulthood, the effect of early life seizures on the induction of kindling in response to stimulation of the perforant path was assessed in adult rats (P95) after seizures during P1-P20, P20-P30, or P31-P90. The rate of kindling expressed as the number of evoked ADs required to reach the milestone of the first evoked secondary generalized tonic-clonic seizure (Class V) varies systematically and reliably as a function of the pathway undergoing kindling stimulation (Goddard et al., 1969) such that increases or decreases in the rate of kindling are accurate measures, respectively, of resistance or susceptibility to seizure-induced plasticity. The kindling rate in adult rats (P95) in response to perforant path stimulation after KA-induced seizures during P1-14 or seizures evoked by HT during P4-17 was similar to normal adult controls (Fig. 9A,B). The rate of perforant path kindling was increased in adult rats (P95) that experienced seizures induced by KA at P24 or HT during P20-26 ($p = 0.005$, $p = 0.016$, respectively, Fig. 9A,B). Confirming previous studies (Sutula et al., 1992, Lynch et al., 2000), the rate of perforant path kindling was also increased after administration of KA at P75 - P90 (Fig. 9A).

As the age-dependent long-term effects of seizures on seizure-induced plasticity in adulthood were observed in response to seizures evoked by both KA and HT during development and were thus independent of the method of seizure induction, it was also of interest to determine if long-term effects on seizure-induced plasticity in adulthood were also observed in response to kindling evoked by other methods of induction during adulthood, such as repeated seizures evoked by PTZ. In adult rats that experienced 3 brief seizures evoked by HT during P21-30, the rate of development of Class V seizures in

response to repeated administration of PTZ (30 mg/kg) was faster than normal adult controls (6.0 ± 0.7 ADs vs. 11.4 ± 0.6 ADs, $p < 0.001$, ANOVA, and $p < 0.05$, Fig. 9C) and faster than rats that experienced seizures evoked by HT during P4-P17 ($p=0.003$, Fig. 9C). These results confirmed that the susceptibility to increases in seizure-induced plasticity in adulthood after seizures during P20-30 was independent of the method of seizure induction in adulthood.

Discussion

These electrophysiological and imaging experiments demonstrated that seizures during postnatal development induced distinct patterns of long-term functional and structural alterations in hippocampal circuitry which varied consistently as a function of the age at the time of seizures, appeared to be independent of the method of seizure induction, and were observed after both repeated brief seizures and prolonged SE. The analysis revealed 3 distinct postnatal epochs in the rat (P1-20, P20-30, and P31-90) during which seizures had consistent patterns of long-term structural and functional outcomes in adulthood. As the consistent patterns of long-term structural and functional alterations were observed after seizures that varied in duration from minutes to hours in response to the different induction methods, age at the time of seizures rather than seizure duration, severity, or induction method appeared to be a major influence on long-term outcomes.

Structural and functional consequences of seizures during P1-20

Seizures occurring during the postnatal period beginning at birth (P1) and ending at ~3 weeks (P20) in the rats, when weaning occurs, induced subtle DTI differences in the CC and hippocampus, and increased PPI in the DG in the absence of gross atrophy or changes in kindling rates in adulthood. This pattern of structural and functional abnormalities differed from the patterns of structural and functional outcomes observed following seizures in each of the subsequent development intervals of P20-P30 and P30-P90.

SE induced by KA at P15 did not result in ventricular enlargement in adulthood, confirming previous histological observations that SE in the early postnatal period does not induce the cell loss and damage as observed in adult rats (Sperber et al., 1991, Lynch et al., 2000, Haas et al., 2001, Cilio et al., 2003, Ben-Ari and Holmes, 2006). Despite the absence of evidence for gross atrophy or ventricular enlargement, more subtle DTI abnormalities including alterations in FA were detected in the CC and hippocampus.

Reduced FA in the CC of adults after seizures at P15 was accompanied by normal $D_{\perp}$ and reduced $D_{\parallel}$, a pattern distinct from that observed following SE in adulthood and potentially indicative of underdevelopment of axonal projections as opposed to direct structural damage or abnormal myelination (Beaulieu, 2002, Budde et al., 2007). FA was also reduced in the adult DG after SE at P15 but not in other hippocampal subregions, perhaps due to the later development of this subregion (Schlessinger et al., 1975, Bayer, 1980). The reduction in FA was most robust in the ML where anisotropy has been shown to be indicative of dendritic structure (Shepherd et al., 2006), thus suggesting that seizures early in development may affect number or morphology of dendrites. Reduced dendritic spine density has also been

observed histologically after tetanus toxin injection at P10 (Jiang et al., 1998, Swann et al., 2000, Benke and Swann, 2004, Casanova et al., 2012).

Seizures induced by different methods before P20 resulted in consistent long-lasting increases in PPI in the adult DG. While the long-term effects of seizures induced by different methods at ages <P20 were similar and thus enabled combined analysis of results using different induction methods during this interval, we did note that in the KA group with the most discrete days of seizure induction, there were suggestions that the earliest days of seizure induction (P1-P3) might differ from later days within the interval (eg, P7-P20). Future studies designed to further refine the effects of seizures on specific days during development might provide additional characterization of age-dependent effects.

PPI is a non-specific measure of network functional properties dependent on intrinsic neuronal properties, synaptic excitation, and synaptic inhibition, and varies as a function of stimulation and recording location, stimulus intensity, interpulse interval, and frequency of delivery of stimulus pairs (Waldbaum and Dudek, 2009). The use of PPI as a measure of network functional properties requires consistent stimulation and recording conditions. Interpretation of alterations in PPI in terms of specific cellular alterations is inherently limited without additional more direct measurements of physiological properties. Alterations in PPI evoked with the stimulation and recording parameters in these experiments have been correlated with alterations in GABA_A receptor dependent Cl⁻ inhibition (Otis et al., 1994, Titulaer et al., 1994, Titulaer et al., 1995, Buhl et al., 1996, Nusser et al., 1998, Sayin et al., 2003) and validated by direct measurements including evoked monosynaptic IPSCs recorded in granule cells by single electrode voltage clamp measures (Sayin et al., 2003), other direct cellular measurements (Otis et al., 1994, Buhl et al., 1996, Nusser et al., 1998), as well as biochemical measures of GABA_A function (Tuff et al., 1983b, Titulaer et al., 1994, Titulaer et al., 1995). While changes in PPI are not conclusively interpretable in terms of specific cellular alterations, the increase in PPI in the adult DG after seizures during P1-20 suggests that early life seizures may induce long-lasting increases in GABA_A inhibition in this region of hippocampal circuitry.

Structural and functional consequences of seizures during P20-30

The period of rat postnatal development from approximately P20 to P30 immediately follows weaning and precedes puberty and reproductive capability. The pattern of effects induced by seizures during this period was distinct from seizures during P1-20 and after P30. In contrast to the long-term structural and functional effects of seizures during P1-20 which included alterations in the CC and hippocampus, imaging measures after SE induced by KA at P25 were largely unremarkable. In contrast to the increases in PPI observed in the DG of adult rats after seizures during P1-20, PPI after seizures during P20-30 was reduced or lost. For reasons cited in the preceding section, this reduction or loss of PPI in the adult DG after seizures during P20-30 suggests that seizures during this period may induce long-lasting increases in GABA_A inhibition in this region of hippocampal circuitry. A particularly striking additional observation was that there was not only loss of PPI in adulthood, but additional seizures evoked in adulthood did not increase PPI as is observed in the DG of normal adult rats, indicating a long-term loss of capacity for seizure-induced plasticity after

seizures during P20-30. The long-term loss of capacity for seizure-induced plasticity in the circuitry of the DG is of interest as neural activity during early development in visual and other sensory systems such as the auditory-space localization pathway in barn owls not only influences the organization of adult circuitry, but also influences capacity for plasticity later in life (Knudsen, 1998, 2004).

Structural and functional consequences of seizures after P30

The pattern of structural effects induced by seizures after P30 was distinct from seizures during P1-20 and P20-P30. In contrast to the subtle alterations in FA of the CC and hippocampus after SE during P1-20 and the largely unremarkable imaging measures after SE during P20-30, there was gross cerebral atrophy and dramatic alterations hippocampal FA and tissue organization after SE in juvenile rats (P35) which resembled previously reported structural MRI and DTI measures of damage after SE in adult rats (>P70).

Ventricle enlargement caused by SE after P35 is consistent with the gross morphological changes found in both previous MRI and histological studies (Sperk et al., 1985, Wolf et al., 2002). Decreased white matter FA was significant in the fimbria and was accompanied by increased diffusivity, which differed from the white matter changes detected after SE at P15 (Table 1). White matter FA decreases were accompanied by increased $D_{\perp}$ and have been described in the WAG/Rij and GAERS rat models of epilepsy (Chahboune et al., 2009) and associated with alterations of the myelin sheath (Klawiter et al., 2011). The localization of decreased FA to the limbic white matter is also consistent with reduced fornix FA following SE by electrical stimulation (Parekh et al., 2010). Reduced FA in human fornix following surgical resection for temporal lobe epilepsy has been correlated with axonal circumference (Concha et al., 2010).

Following SE after P35, the structural orientation of the hilus in adult rats was altered as indicated by robust changes in eigenvector direction from anterior-posterior to primarily dorsal-ventral, confirming that epileptogenic processes may alter the highly laminar pattern of tissue orientation in the hippocampus (Shepherd et al., 2006). While additional studies are necessary to characterize the cellular basis for this directional structural abnormality, previous imaging studies have correlated alterations of hippocampal anisotropy with Timm histochemistry and mossy fiber sprouting, suggesting that FA may be an imaging marker of epileptogenic hippocampal mossy fiber plasticity (Kuo et al., 2008, Laitinen et al., 2010). The development of dorsal-ventral orientation in the hilus of the DG is geometrically consistent with intra- and inter-blade mossy fiber sprouting of axons in the hilus (Sutula et al., 1998), but seizure-induced cell loss in the hilus could also “unmask” underlying dorsal-ventral orientation. Increases in FA in the dorsal hippocampus including CA3 may also indicate more widespread axonal reorganization in the hippocampus, which has been observed in both the hilus and CA1 in addition to the molecular layer of the DG (Cavazos et al., 2004, Cross and Cavazos, 2007). The current results suggest that measurement of hippocampal subfield anisotropy and orientation may provide a sensitive measure of alterations in tissue orientation associated with epileptogenic processes during development which influence functional circuit properties in adulthood.

In contrast to the reduction or loss of PPI in the adult DG after SE or several repeated brief seizures evoked by PTZ or HT during P20-30, SE at P31 and P75 was followed by increased PPI in the adult DG. These findings confirmed numerous previous studies demonstrating that seizures evoked by a variety of methods increase PPI and measures of inhibition in the adult DG (Oliver and Miller, 1985, Milgram et al., 1991, Hellier et al., 1999, Lynch et al., 2000, Sayin et al., 2003). The significance of the increases in PPI after seizures during P1-20 or P31, or the decreases in PPI after seizures during P20-30, and the implications of these consistent patterns of network alterations possibly reflecting changes in GABA_A inhibition for the development of epilepsy in adulthood are uncertain. Although it is uncertain if increases in PPI after seizures during P1-20 or >P30 reflect common underlying network abnormalities, the structural difference in adulthood after seizures during these epochs suggests that the underlying mechanisms of the long-term functional abnormalities are also likely to be distinct.

P20-P30 as a hippocampal critical period

The abrupt age-dependent transitions from increased PPI after seizures occurring at < P20 to reduced or lost PPI after seizures during P20-30 followed by increased PPI after seizures at

P31 suggest that P20-30 could be a critical period in hippocampal development.

Developmental critical periods are defined by features that include evidence for influences of electrical or environmental activity on the selection and shaping of permanent neuronal connections and circuits, evidence for unique regulated timing of opening, duration, and closure of a window of neural activity which permanently influences circuit function, and structural consolidation (Hensch, 2004, 2005, Takesian and Hensch, 2013). With sharply delimited borders of opening and closure, episodic synchronous electrical activity influencing long-term functional properties, and temporally specific imaging features consistent with a structural consolidation process, the period of P20-P30 in the hippocampus appears to fulfill major criteria defining a developmental critical period. The observations of this study suggest that many of the activity-dependent critical period phenomena characterized in developing sensory systems including life-long, behaviorally significant functional effects and capacity for plasticity may have counterparts in hippocampal circuitry of the limbic system. While the above noted features of the long-term effects of seizures across early postnatal development in the hippocampus are consistent with a critical period, these experiments do not provide insight into the cellular and molecular processes that may contribute to the observed long-term effects, including the possibilities that age-dependent differences in how seizures activate the brain, age-dependent differences in seizure-induced metabolism, or other neural processes driven by environmental experience may be contributing influences. These questions are potentially interesting for consideration in future studies. Although handling and stress associated with injections during development might contribute to long-term effects (Adamec et al., 1991), this possibility seems less likely given that the long-term effects were not observed in age-matched saline-injected littermate controls.

Implications of seizure-induced developmental plasticity and critical periods for epilepsy

These experiments unexpectedly identified distinct postnatal periods when abnormal electrical activity during seizures was followed by consistent patterns of long-term structural

and functional effects in the CC, fornix, and hippocampus. As electrical activity driven by environmental experience in developing sensory systems promotes synapse formation and organization of synaptic connectivity influencing structural and functional properties of neural circuitry in adulthood, it is not surprising that synchronous network activity associated with seizures during postnatal development might also modify the development and functional properties of hippocampal circuitry, particularly in the DG which undergoes substantial postnatal development (Schlessinger et al., 1975, Bayer, 1980). With emerging evidence that the opening of critical periods depends on alterations in the balance of inhibitory and excitatory activity during development (Takesian and Hensch, 2013, Toyozumi et al., 2013), it is also not unexpected that seizures might influence opening of a critical period in the hippocampus with long-term effects on hippocampal circuit properties, as observed in these experiments. While induced seizures were the experimental manipulation that revealed age-dependent effects of abnormal electrical activity on long-term outcomes, the possibility that more subtle modulation of physiological activity such as stress could also have systematic effects cannot be excluded, and could be pursued in future studies.

Observations about systematic long-term effects of seizures in the developing rodent brain are potentially relevant to the common human condition of recurring seizures during infancy and childhood (Berg and Shinnar, 1996). As the hippocampus and DG are prominently involved in epilepsy, the long-term network alterations after seizures corresponding to the period of P20-30 in the rat might have implications for human epileptogenesis. For example, febrile seizures occur in an estimated 4-5% of children (Berg and Shinnar, 1996, Lewis et al., 2013) and a subset of children later develop intractable temporal lobe epilepsy as adolescents and adults (Sagar and Oxbury, 1987, Berg and Shinnar, 1996). One possible variable influencing the emergence of intractable temporal lobe epilepsy after childhood febrile seizures may be the age at the time of the seizures. Identification of a possible similar period of vulnerability to long-term alterations in hippocampal properties and plasticity in human postnatal development would have prognostic and therapeutic implications.

As noted, interpretation of alterations in PPI in terms of specific cellular alterations is inherently limited without additional more direct measurements of physiological properties, and the relationship of possible underlying changes in GABA_A inhibition in circuitry of the DG as an age-dependent consequence of seizures during development are complicated and uncertain. While loss of the capacity for seizure-induced plasticity after seizures during P20-30 could be expected to result in increased vulnerability to epileptogenesis later in life, emergence of spontaneous seizures in adulthood may still require additional contributing factors such as environmental insults, subtle head trauma, other acquired processes, or underlying complex genetic predisposition, and might thus be consistent with a “two hit” hypothesis of epileptogenesis (Lewis, 2005). As activity-dependent critical period developmental phenomena affect a variety of sensory systems and behaviors, seizures in developing limbic circuitry may also induce other adverse long-term behavioral effects which were not assessed in this study.

Conclusions

The distinctive structural and functional effects after seizures in each of the identified developmental epochs of P1-20, P20-30, and P31-P75 confirmed that seizures across a broad range of postnatal ages modify developmental outcomes and hippocampal circuit organization in adults. These observations imply that abnormal synchronous neural activity during seizures influences activity-dependent developmental processes with functional consequences that vary systematically as a function of age at the time of seizures. The interval of P20-P30 was identified as a possible critical period in hippocampal development when abnormal neural activity during seizures had distinctive long-term structural and functional effects in adult hippocampal circuitry and reduced capacity for seizure-induced plasticity in adulthood.

Acknowledgements

The authors thank Michael Lynch, Craig Levenick, Jeff Ockuly, and Susan Osting for technical contributions to this work. This study was supported by NINDS R01 25020 (T.S.) and the Epilepsy Foundation (U.S.).

ABBREVIATIONS

MRI	magnetic resonance imaging
DTI	diffusion tensor imaging
PPI	paired pulse inhibition
SE	status epilepticus
KA	kainic acid
PTZ	pentylentetrazol
HT	hyperthermia
ICV	intracerebroventricular
IP	intraperitoneal
pEPSPs	population excitatory postsynaptic potentials
PS	population spike
IPIs	interpulse intervals
IM	intramuscular
AD	afterdischarge
FA	fractional anisotropy
MD	mean diffusion
D	axial diffusivity
D_⊥	radial diffusivity
ROI	region of interest

CC	corpus callosum
ML	molecular layer
DG	dentate gyrus
LV	lateral ventricle
DEC	directionally encoded color

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- age at time of postnatal seizures has distinctive effects in adult hippocampus
- early life seizures prominently modify hippocampal development
- postnatal days 20-30 identified as a critical period in hippocampal development

Figure 1.

Overview of the experimental design. Horizontal bars indicate method of seizure induction (KA = kainic acid, HT = hyperthermia, PTZ = pentylenetetrazol, see Methods for details of administration), age at the time of seizure induction (eg., P1-P90), and experimental outcome measurements in adulthood (paired pulse inhibition, *ex-vivo* DTI, and kindling, far right). Ages of seizure induction in parentheses indicate groups of rats that experienced seizures on each of the indicated days.

Figure 2.

Representative examples of paired potentials evoked in the dentate gyrus by stimulation of the perforant path at 15 mS interpulse intervals in hippocampal slices from normal adult rats (> P90) and rats that experienced seizures evoked by kainic acid (KA), pentylenetetrazol (PTZ), or hyperthermia (HT) during development.

(A1) Paired pulse inhibition (PPI) in from a normal rat at >P90 is indicated by reduction of the amplitude of the second evoked population spike (PS2) compared to the first population spike (PS1). (A2) Schematic of method of measurement of PPI. Smaller ratios of the amplitude of the second to the first population spike indicate increased PPI and larger ratios indicate reduction of PPI. See text for details. (B1) PPI in an adult rat that experienced seizures evoked by KA on postnatal day 1 was increased, as indicated by reduced amplitude of PS2 compared to PS1. (B2) PPI was reduced in an adult rat that experienced seizures evoked by KA on P24, as indicated by increased amplitude of PS2 relative to PS1. Note the ratio PS2/PS1 is greater than in B1 and A1. (C1) PPI was increased in an adult rat that experienced seizures evoked by PTZ during P1-10 compared to controls. In this example there was no spike evoked by the second pulse at the standardized stimulation pulse intensity. (C2) PPI was reduced or lost in an adult rat that experienced seizures evoked by PTZ during P21-30. (D1) PPI was increased in an adult rat that experienced seizures evoked by HT during P4-10 compared to controls. In this example there was no spike evoked by the second pulse at the standardized stimulation pulse intensity. (D2) PPI was reduced or lost in an adult rat that experienced seizures evoked by HT during P20-26. (E1,E2) PPI increased after 3 Class V seizures evoked by perforant path kindling in adult rats, but did not increase after seizures evoked in adulthood in rats that experienced seizures evoked by HT during P20-30. Calibration bars apply for A1-E2.

Figure 3.

Paired pulse inhibition (PPI) evoked in the dentate gyrus by stimulation of the perforant path at 15 mS and 25 mS interpulse intervals (IPIs) in hippocampal slices from adult rats that experienced seizures at different ages during development. PPI was expressed as a percentage of PPI in normal control adult rats (P90). Error bars are S.E.M.s. **(A)** PPI was increased at 15 mS and 25 mS IPIs in the adult dentate gyrus after status epilepticus induced by kainic acid during P1-20 and at P31 through P75, but was reduced after seizures on P24 (ANOVA, $p < 0.001$). **(B)** PPI at 15 mS and 25 mS IPIs was increased in the adult dentate gyrus after a series of 3 brief seizures evoked by pentylenetetrazol during P1-10 but was reduced after 3 brief seizures evoked by pentylenetetrazol during P21-30 (ANOVA, $p < 0.001$). **(C)** PPI at 15 mS and 25 mS IPIs was increased in the adult dentate gyrus after a series of 3 seizures evoked by hyperthermia during P4-10 and P11-17, but was reduced after 3 brief seizures evoked by hyperthermia during P20-26 (ANOVA, $p < 0.001$).

Figure 4.

Combined plot of PPI at 15 mS and 25 mS interpulse intervals in the dentate gyrus evoked by stimulation of the perforant path in hippocampal slices from adult (P95) rats that experienced seizures evoked by kainic acid (KA), pentylentetrazol (PTZ), or hyperthermia (HT) across a range of ages during development. PPI expressed as a percentage of normal adult controls (P90) was increased after seizures evoked by different induction methods during P1-20 and after P31, but was reduced after seizures during P20-30. Kainic acid-induced status epilepticus is indicated on each of the indicated days (black circles). Horizontal lines span days with brief seizures evoked by pentylentetrazol (green squares) or hyperthermia (red triangles). The long-term effects of seizures evoked by different methods across the range of ages from P1 through P75 varied as function of age (ANOVA $p < 0.001$). The transition from increased PPI during P1-20 to reduced PPI during P20-30 was followed by an abrupt transition to increased PPI after P31. Combined values of PPI at each age are expressed as means $\pm$ S.E.M.

Figure 5.

Lateral ventricle volume as a function of age of seizure induction by kainic acid. Error bars represent standard error of the mean. There was a significant main effect ($p<0.001$, ANOVA) and a direct correlation ($R = 0.81$, $p<0.0005$) of age at the time of seizures with LVV in adulthood ** denotes $p<0.01$ for post-hoc t-test comparison to the control group.

Figure 6.

Fractional anisotropy (FA, dimensionless) in adulthood in (A) the dorsal hippocampus, and dorsal subfields including (B) CA1, (C) CA3, (D) the dentate gyrus, (E) molecular layer and (F) hilus as a function of age at the time of seizures induced by kainic acid. Error bars indicate standard error or the means. By convention, measures of FA are a proportion and have no units. There were significant differences in FA in adulthood as a function of age at the time of seizures. Main effects of group are reported for each region (one way ANOVA). The asterisks denote significant differences in post-hoc analysis compared to control groups; # denotes trends ($p<0.1$).

Figure 7.

Tissue orientation in adulthood derived from measures of FA as a function of age at the time of seizures induced by kainic acid. **(A)** Coronal slices from directionally encoded color (DEC) maps for representative samples of a control rat and rats that experienced seizures at P15, P25, P35, and P70. In DEC maps, tissue directional properties are indicated by red = medial-lateral, blue = rostral-caudal, and green = dorsal-ventral. **(B)** DEC line maps representing the primary eigenvector orientation in the adult hippocampus and dentate gyrus demonstrate alterations in tissue orientation in the hilus of the dentate gyrus that vary as a function of age at the time of seizures. The inset at bottom left illustrates regions of interest (ROIs) that defined the location of the ML and hilus. **(C,D)** Spherical plots for direction vectors within the left and right ML, and left and right hilus of each brain sample (filled circles, color coded by group) compared to control mean direction vector (black line) and 95% confidence angle (black circle). The inset at bottom left indicates colors representing P15, P25, P35, and P70 groups in the spherical plots. Asterisks * denote $p < 0.05$ for group differences in orientation (Fisher's test).

Figure 8.

Effects of seizures evoked in normal adult (P90) rats and groups of adult (P95) rats that experienced seizures evoked by HT during P20-26. PPI was increased in normal adult rats (P90) after 3 secondary generalized tonic-clonic (Class V) seizures evoked by kindling of the perforant path or 3 repeated seizures evoked by PTZ (30 mg/kg every other day $\times$ 3 after

P95). Adult rats that experienced 3 seizures evoked by HT during P12-26 demonstrated reduced PPI compared to normal controls, and in contrast to normal rats did not demonstrate increases in PPI after 3 secondary generalized tonic-clonic (Class V) seizures evoked by kindling of the perforant path or 3 repeated seizures evoked by PTZ. Results are expressed as means $\pm$ S.E.M.

Figure 9.

Rates of kindling development in adulthood (P95) after seizures during early life varied systematically as function of age at the time of seizures but were independent of the methods of seizure or kindling induction. **(A)** After administration of kainic acid that induced status epilepticus, there were effects on rate of kindling development in adult rats in response to electrical stimulation of the perorant path that varied as a function of age at the time of seizures ($P < 0.001$, ANOVA). While there was no effect of status epilepticus induced by kainic acid administration during the period of P1-14, kindling development was faster after seizures induced by kainic acid on P24 ($p = 0.005$) or during P75-90 ($p = 0.017$). **(B)** There were effects on rate of kindling development in adult rats in response to electrical stimulation of the perorant path after a series of 3 brief seizures evoked by hyperthermia during P4-17 and P20-26 ($P = 0.042$, ANOVA). While there was no effect of seizures evoked by hyperthermia during P4-17, the rate of kindling development was faster in adult rats that experienced 3 seizures evoked by hyperthermia during P20-26 ($p = 0.016$). **(C)** The effects of seizures evoked by hyperthermia during development on kindling rate in adulthood were not dependent on the method of kindling induction at P95 ($p < 0.001$, ANOVA). The rate of kindling in response to repeated administration of subconvulsant doses of PTZ (30 mg/kg IP every other day) was increased in adult rats that experienced 3 seizures evoked by hyperthermia during P20-26 ($p < 0.001$) and rats that experienced 3 seizures during P4-17 ($p = 0.003$). Rats that experienced seizures evoked by hyperthermia during P20-26 had faster rates of kindling development in response to repeated PTZ at P95 than rats that experienced seizures evoked by hyperthermia during P4-17 ($p = 0.01$).

Table 1

		control	SE-P15	SE-P25	SE-P35	SE-P70
Corpus Callosum						
	FA	0.716 $\pm$ 0.004	0.691 $\pm$ 0.009 $\downarrow^{**}$	0.714 $\pm$ 0.011	0.668 $\pm$ 0.041	0.674 $\pm$ 0.046
$\times 10^3$ (mm ² /s)	D	0.614 $\pm$ 0.008	0.592 $\pm$ 0.006 $\downarrow^{**}$	0.610 $\pm$ 0.007	0.606 $\pm$ 0.015	0.604 $\pm$ 0.014
	D	0.157 $\pm$ 0.004	0.164 $\pm$ 0.006	0.156 $\pm$ 0.004	0.184 $\pm$ 0.027	0.176 $\pm$ 0.030
	MD	0.309 $\pm$ 0.005	0.306 $\pm$ 0.005	0.307 $\pm$ 0.004	0.324 $\pm$ 0.022	0.319 $\pm$ 0.024
Fornix						
	FA	0.861 $\pm$ 0.017	0.786 $\pm$ 0.102	0.840 $\pm$ 0.047	0.816 $\pm$ 0.014 $\downarrow^*$	0.806 $\pm$ 0.021 $\downarrow^*$
$\times 10^3$ (mm ² /s)	D	0.763 $\pm$ 0.003	0.726 $\pm$ 0.031	0.771 $\pm$ 0.037	0.774 $\pm$ 0.025	0.794 $\pm$ 0.011 $\uparrow^*$
	D	0.095 $\pm$ 0.011	0.137 $\pm$ 0.057	0.109 $\pm$ 0.026	0.131 $\pm$ 0.013 $\uparrow^*$	0.139 $\pm$ 0.018 $\uparrow^*$
	MD	0.318 $\pm$ 0.007	0.333 $\pm$ 0.028	0.329 $\pm$ 0.013	0.345 $\pm$ 0.015 $\uparrow^*$	0.358 $\pm$ 0.016 $\uparrow^*$