Published in final edited form as:

Neuroscience. 2011 September 29; 192: 699–707. doi:10.1016/j.neuroscience.2011.05.022.

Lithium pilocarpine-induced status epilepticus in postnatal day 20 rats results in greater neuronal injury in ventral versus dorsal hippocampus

Jeffrey J. Ekstrand¹, Wendy Pouliot², Peter Scheerlinck², and F. Edward Dudek²

¹Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah.

²Department of Physiology, University of Utah School of Medicine, Salt Lake City, Utah.

Abstract

Many quantitative animal studies examining the possible relationship between hippocampal neuronal loss and the development of epilepsy have examined only the dorsal hippocampus. The ventral hippocampus, however, represents the more homologous structure to the anterior hippocampus in humans which is the area associated with the maximal damage in patients with temporal lobe epilepsy. This study tested the hypothesis that the ventral hippocampus has greater neuronal injury than the dorsal hippocampus in an animal model of chemoconvulsant-status epilepticus at postnatal day 20. Status epilepticus was induced in postnatal day 20 Sprague Dawley rat pups with the chemoconvulsant lithium-pilocarpine and brain tissue was examined with Fluoro-Jade B. Horizontal sections (n=7) favoring a visualization of the ventral hippocampus showed marked Fluoro-Jade B staining in CA1, CA3, and hilar region. Coronal sections favoring a visualization of the dorsal hippocampus did not consistently show as robust a staining pattern in these regions. In coronal sections where both the dorsal and ventral hippocampus could be viewed, greater staining was always seen in ventral versus dorsal hippocampus. Quantitative analysis of cell counts demonstrated a significant difference between ventral and dorsal hippocampus in CA1 and CA3, but not hilus. These results demonstrate that in ventral hippocampus, lithium pilocarpine-induced status epilepticus consistently results in hippocampal neuronal injury in postnatal day 20 rats. This study shows the importance of including the ventral hippocampus in any analysis of seizure-induced hippocampal neuronal injury, and raises concerns about the accuracy of studies quantifying hippocampal neuronal loss when only the dorsal hippocampus is examined.

Keywords

Fluoro-jade B; lithium pilocarpine; status epilepticus; immature brain

The classic anatomical work by Margerison and Corsellis (1966) characterized the neuropathological lesions found in patients with temporal lobe epilepsy and helped initiate a plethora of epilepsy research designed to elucidate the types of atrophy, sclerosis, and

© 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Correspondence to: Dr. Jeffrey J. Ekstrand, Dept. of Pediatrics, School of Medicine, University of Utah, 420 Chipeta Way, Suite 1700, Salt Lake City, Utah 84108, jeffrey.ekstrand@hsc.utah.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

neuronal loss observed in the hippocampal formation of epileptic patients. Several animal models that induce status epilepticus using chemoconvulsants such as pilocarpine (either alone or following pretreatment with lithium) (Cavalheiro et al., 1991; Mello et al., 1993; Obenaus et al., 1993; Roch et al. 2002) or kainate (Pollard et al., 1994; Buckmaster and Dudek, 1997) have been developed that recapitulate both the phenomena of epilepsy (i.e. recurrent spontaneous seizures) and similar pathologic lesions seen in these early human pathology studies. One aim of these models is to better understand what role seizure-induced injury and neuronal death may have in the development of epilepsy. However, when systematically studied, model-specific differences, developmental differences, and (even in the same model) inter-animal variability has often prevented researchers from being able to consistently correlate a specific neuronal lesion to the development of epilepsy. Particularly with studies in the immature brain where, in general, younger animals are more resistant to both neuronal injury (Cavalheiro et al., 1987; Sperber et al., 1991; Stafstrom et al., 1992; Lado et al., 2002;) and the development of epilepsy (Sankar et al., 1998; Kubova et al., 2004), there have been conflicting reports on whether neuronal injury with neuronal death is present after status epilepticus. Several researchers have provided evidence that lithium pilocarpine-induced status epilepticus in immature animals does result in hippocampal neuronal death (Rose Priel et al., 1996; Sankar et al., 1997; Sankar et al., 1998; Nairismagi et al., 2006). However others using similar ages and inducing status epilepticus with lithium pilocarpine (Raol et al., 2003), hyperthermia (Baram, 1997), or hypoxia (Sanchez et al., 2001) have claimed that epilepsy can develop without hippocampal neuronal death. This controversy has been summarized in recent reviews (Dudek et al., 2010; Baram et al., 2011), and underscores the need for further studies to address this issue.

When assessing this extensive body of research, the hippocampus is often regarded as a homogeneous structure despite evidence showing significant functional, anatomical, and electrophysiological differences between the dorsal and ventral regions (Stevens and Cowey, 1973; Seress and Pokorny, 1981, Cavazos et al., 1992, Moser et al., 1993, Jung et al., 1994; Hunsaker et al., 2008). Regional differences within the hippocampus have also been found to relate to susceptibility to epilepsy (Elul, 1964; Mouritzen-Dam, 1980; Babb et al., 1984; Gilbert et al., 1985; Bragdon et al., 1986) and, when studied in the adult animals, researchers have shown that the dorsal hippocampus, although often the primary area examined, is more resistant to seizure-induced injury and neuronal death than the ventral hippocampus (McIntyre et al., 1982; Williams et al., 2002).

The goal of this study is to test the hypothesis that the immature brain has regional differences in susceptibility to seizure-induced neuronal injury between the ventral and dorsal hippocampus similar to what has been observed in adults. We utilized Fluoro-jade B, a histochemical marker for neuronal injury, to assess the degree of injury in the hippocampal formation in an animal model of lithium pilocarpine-induced status epilepticus. We demonstrate that at postnatal day 20 (P20), there is a marked difference between the two hippocampal regions in susceptibility to seizure-induced neuronal injury.

EXPERIMENTAL PROCEDURES

Surgical procedure for implantation of electrodes

Sprague-Dawley (Harlan) rat pups were housed as a litter with their dam, and kept in a standard light/dark cycle (12/12 h). At P16, pups were anesthetized with 2.0–2.5% isoflurane and placed into a stereotaxic frame. Using sterile surgical techniques, an incision was made midline on the scalp and the skull exposed. With a high-speed dental drill, a craniotomy was performed over the left parietal cortex (centered around a point 2 mm rostral and 2 mm lateral to bregma) and two burr holes were drilled for placement of the electrode wires. The dura and bone flap was left in place. The recording electrode was

secured to the skull with cold-curing dental cement, and the incision site was closed with suture. Animals were injected with 1.0 mL lactated ringers, and post operative pain control included 0.1 mL marcaine and 0.1 mL buprenorphine. The rats were then allowed to recover in a temperature-controlled environment, prior to returning to their dam and litter mates.

Induction of status epilepticus

On P19 animals were pretreated with intraperitoneal lithium (LiCl 3 mEq/kg). Status epilepticus was induced on P20 with intraperitoneal pilocarpine (60 mg/kg), with scopolamine (1 mg/kg) given 30 min prior to injection. Treated animals were compared to control animals receiving sham saline injection. EEG ictal discharges were defined as repetitive spiking (amplitude ≥ 3 times baseline) lasting longer than 15 sec. The EEG signals were stored on a computer (Acqknowledge software, BIOPAC systems, Inc), which allowed simultaneous recordings from 8 rats with individual EEG electrodes. Video data was analyzed to identify behavioral correlates of ictal EEG activity. Video monitoring was performed using EZWatchPro, Version 3.1HD, a standard video surveillance system with infrared cameras. Behavioral seizure activity was rated using a modified Racine scale (Racine 1972; stage 1, mouth and facial movements; stage 2, head nodding; stage 3, forelimb clonus; stage 4, rearing; stage 5, rearing and falling). Following the SE treatment, animals were allowed to recover in a temperature controlled environment and then returned as a litter to their dam.

Fluoro-jade B staining

Seventy-two hours after initiation of status epilepticus, animals were anesthetized with isoflurane and underwent cardiac perfusion-fixation with 4% paraformaldehyde. Brain sections 35 μm thick were cut in both coronal and horizontal sections in order to optimize sections through the dorsal and ventral hippocampus, respectively. Every sixth section was examined with Fluoro-jade B (F-JB, Histo-chem Inc., Jefferson, AR, U.S.A), a histological marker for early cell damage. Sections were incubated with 0.06% DMnO₄ followed by 0.001% Fluoro-Jade B according to the protocol described by Schmued et al. (1997). Evidence for florescent positive neuron cells was examined in the dentate gyrus (hilus region), and CA1 and CA3 hippocampal region. The hippocampal formation subdivisions (CA1, CA3, hilus) and sub-regions (ventral and dorsal) nomenclature were defined based on Amaral and Witter (1989). For assessing regions to analyze, using Paxinos and Watson (1986), the dorsal hippocampus was defined by coronal sections corresponding to atlas coordinates (-2.12 to -3.8 Bregma); the ventral hippocampus was defined by horizontal sections corresponding to coordinates (-4.74 to -7.10 Bregma).

Quantitative neuronal cell counting

Horizontal and coronal sections that included the hippocampal formation cut perpendicular to the septo-temporal axis as previously defined (corresponding to ventral and dorsal regions) were chosen for quantitative analysis. Three sections from each animal were randomly chosen within each defined region. A 300 μm $\times$ 400 μm area of interest was delineated as shown in Fig 5 for CA1, CA3, and hilar region. The area of interest was the same for each section examined. Images were obtained using a 25 $\times$ objective (Zeiss Apotome), and Fluoro-jade B positive cell bodies were counted for each area using Axiovision software. The examiner was blind to treatment condition and sub-region. Statistical differences between regions were determined using 2 sided student t-test assuming non-equal variances.

RESULTS

Lithium pilocarpine induced status epilepticus in immature rat pups

Sprague Dawley rat pups were implanted with surface EEG electrodes, and status epilepticus was induced at P20 with the chemoconvulsant lithium pilocarpine. After approximately 40–60 min, we successfully recorded lithium pilocarpine-induced status epilepticus (Fig 1). All animals (14/14) used for later histological studies showed electrographic and behavioral evidence of status epilepticus. The seizure activity had the characteristic gradual increase in amplitude with spike and spike-wave activity typical of lithium pilocarpine-induced status epilepticus recorded in adult rats and lasted for several hours. In this age group, the animals also had a clinical correlate of seizure activity that showed generalized tonic-clonic activity in all limbs with loss of stability (Racine class 5; Racine 1972).

Neuronal injury in the ventral versus dorsal hippocampus

The orientation of the rat hippocampal formation is shown in Figure 2 as a curved semi-circle shaped structure that can be divided into two sub-regions along its septo-temporal axis. The dorsal hippocampus comprises the septal end of this structure. Coronal sections through this region allow visualization of the typical “interlocking c’s” orientation of the different cellular layers. This orientation becomes less discernable in each coronal section as one moves along the septo-temporal axis toward the temporal region (Fig 2B). The most temporal region of the hippocampus consists of the ventral hippocampus, which can be best seen in horizontal sections (Fig 2C). The ventral hippocampus (not dorsal) is homologous to the anterior hippocampus of humans.

Seventy-two hours after lithium pilocarpine treatment, brain tissue from 14 animals was examined with Fluoro-Jade B and compared to control animals that did not receive lithium pilocarpine. Both coronal (n=7) and horizontal (n=7) sections were obtained in order to optimize visualization of the dorsal or ventral hippocampal formation, respectively. All treated animals demonstrated Fluoro-Jade B staining in the hippocampal formation (Fig 3). Horizontal sections favoring a view of the ventral hippocampus showed marked Fluoro-Jade B staining in CA1, CA3, and hilus (Fig 3A, E, G). In contrast, coronal sections favoring a view of the dorsal hippocampus showed a much less robust staining pattern in these regions (Fig 3B, F, H). Sections of the dorsal hippocampus also appeared to have more “patchy” distributions of staining corresponding to discrete areas of staining compared to sections in the ventral hippocampus (compare Fig 3A to 3B). Control animals did not demonstrate Fluoro-jade B staining of neurons (Fig 3C, D). Although later quantitative analysis comparing the two sub-regions was performed in sections that maintained an optimal orientation perpendicular to the septo-temporal axis, in coronal sections that included distorted orientations of both the dorsal and ventral hippocampus (Fig 4), Fluoro-jade B staining was consistently seen in the ventral hippocampus but not in the dorsal hippocampus. This supports the conclusion that in sections where both regions can be observed, neuronal injury is greater in the ventral hippocampus compared to dorsal hippocampus.

In order to determine both the degree of neuronal injury between the ventral and dorsal hippocampal regions as well as ascertain the potential variability between animals and tissue sections, we performed a non-stereological quantitative analysis of Fluoro-jade B positive neuronal somata within predefined regions of interest in the CA1, CA3, and hilus. Three sections from each animal were chosen and the area of interest for each hippocampal area is shown in Fig 5 A and B. There was marked variability between both individual sections as well as between animals. However, a significant difference was observed between the

ventral and dorsal hippocampus in CA1 and CA3 regions (both $p < 0.0001$). No significant difference between ventral and hippocampal regions was demonstrated in the hilar region.

DISCUSSION

In this study, we investigated whether rat pups at P20 have different regional susceptibility to injury in the ventral versus dorsal hippocampus after lithium pilocarpine-induced status epilepticus. Status epilepticus was confirmed by both behavioral correlate and electrographic EEG monitoring. Examining horizontal and coronal sections separately, which maintained the hippocampal orientation perpendicular to the septo-temporal axis, demonstrated greater Fluoro-jade B staining in horizontal sections corresponding to the ventral hippocampus. In coronal sections where this perpendicular orientation was not maintained but both the ventral and dorsal hippocampus could be viewed, greater staining was always observed in the ventral hippocampus compared to the dorsal hippocampus. Finally, quantitative analysis of cell counts showed a significant difference between the ventral versus dorsal hippocampus in CA1 and CA3, but not hilus. This regional susceptibility difference between the dorsal and ventral hippocampus is consistent with previous animal model studies in the mature brain. In addition, these findings provide insight into how the immature brain may have different properties from the mature brain that influence an animal's susceptibility for neuronal death and epileptogenesis. Finally, these results have direct implications for previous studies of seizure-induced neuronal death in immature animals that based their results only on an analysis of the dorsal hippocampus.

Differences in ventral and dorsal hippocampus in epilepsy studies

The hippocampal formation is not a homogeneous structure. There is abundant evidence that the dorsal and ventral subregions have distinct functional, anatomical, and electrophysiological properties (Stevens and Cowey, 1973; Seress and Pokorny, 1981, Gilbert et al., 1985; Bragdon et al., 1986; Cavazos et al., 1992; Moser et al., 1993; Hunsaker et al., 2008). While our understanding of the specific functions of the dorsal and ventral subregions continues to evolve (for review see Fanselow and Dong, 2010), any of these properties may contribute to the observed differences in vulnerability to seizure-induced injury, neuronal loss, and the development of epilepsy. Clinical studies in humans have shown that the most common lesion found in the hippocampi of temporal lobe epilepsy patients is usually more severe in the anterior hippocampus (corresponding to ventral hippocampus in rodents) (Babb et al., 1984; Mouritzen Dam, 1980). In addition, surgical resection of the anterior portion of the hippocampus and adjacent temporal lobe structures, while leaving the posterior portion of the hippocampus, eliminates seizures in many temporal lobe epilepsy patients (Spencer et al., 1984). In these cases, presumably the anterior hippocampus or anterior temporal extra-hippocampal structures are more involved in epileptogenesis than the posterior portion of the hippocampus. There is also evidence from *in vitro* animal studies that slices taken from the ventral hippocampus have greater propensity for epileptiform activity compared to dorsal hippocampus (Gilbert et al., 1985; Bragdon et al., 1986). These studies support the idea that pathological changes in the ventral hippocampus may be more critical than those in the dorsal hippocampus in the development of epilepsy. Unfortunately, many previous studies have limited their analysis of the hippocampus to the dorsal subregion. A difference in vulnerability between dorsal and ventral subregions after seizure activity has previously been described in adult animal studies. McIntyre et al. (1982) showed that prolonged status epilepticus in previously kindled adult rats produced more extensive damage in ventral than dorsal hippocampal formation. Williams et al. (2002) demonstrated that after lithium pilocarpine-induced status epilepticus the maximal area of neuronal loss was in the ventral hippocampus. Our results are consistent with these studies, and demonstrate that this regional difference within the

hippocampus is also observed in the immature animal at least in P20 animals. As was observed in animal model studies in the mature brain, lithium pilocarpine-induced status epilepticus resulted in neuronal injury in the hippocampal formation in P20 rat pups, and the degree of injury varied depending on the sub-region along the septo-temporal axis. The ventral hippocampus consistently showed the maximal neuronal injury; the dorsal hippocampus showed marked variability compared to the ventral hippocampus, but in 6/7 animals the neuronal injury was quite minimal. Our results extend previously observed findings in the mature brain to at least postnatal day 20 and further studies in younger animals are needed to determine if this regional susceptibility difference persists at more immature ages.

Age dependent susceptibility to neuronal injury, neuronal death, and epileptogenesis

An intriguing finding in many previous studies on animal models of epilepsy comparing the adult and immature brain is the observation that the immature brain is both more resistant to neuronal loss and less likely to develop epilepsy (for reviews see Stafstrom and Sutula, 2005; Lado et al., 2002). Most studies have not shown any evidence of hippocampal neuronal loss in animals less than 2 weeks of age, and when neuronal injury or neuronal loss is documented (either in this age group or in slightly older but still immature animals), it is always less than observed in adults (Cavalheiro et al., 1987; Sperber et al., 1991; Stafstrom et al., 1992). Also, the proportion of young animals that later develop epilepsy is also dramatically less compared to the proportion of adult animals (Rose Priel et al., 1996; Sankar et al., 1998; Kubova et al., 2004). Our results are consistent with previous studies in the immature brain and demonstrate that injury is less severe than observed in studies using similar methods in the mature brain (Cavalheiro et al., 1991; Rose Priel et al., 1996; Williams et al., 2002). Unlike in our study where neuronal injury is minimal in the dorsal hippocampus, neuronal loss could still be detected in this subregion with cell counting methods in adult animals, although it was still less than the loss observed in the ventral hippocampus. We interpret this as evidence that neuronal injury tends to be more restricted to the ventral hippocampus in immature animals while it extends across both sub-regions in mature animals.

Does epileptogenesis occur independent of hippocampal neuronal death in the immature brain?

The lithium pilocarpine model has been utilized in this age group to claim that hippocampal cell loss is not necessary for epileptogenesis (Raol et al., 2003). In the study by Raol and co-workers, 7 of 9 lithium pilocarpine-treated animals showed spontaneous recurrent seizures without apparent hippocampal neuron loss based on neuronal counts in histological nissl stained sections. While the authors did concede that the cell counting method performed in their study may not have detected subtle cell loss discernable with stereological methods, they concluded that hippocampal cell loss may not be necessary for subsequent epileptogenesis after status epilepticus at P20. However, Raol and colleagues based their conclusion on an analysis of the dorsal hippocampus only, an area which, based on our results, has minimal neuronal injury, and therefore is unlikely to be detected by cell counting methods. They did not assess neuronal loss in the ventral hippocampus which based on our study is the region where neuronal loss would be more easily detected. Cell counting methods are always inherently less sensitive than markers of neuronal injury at detecting limited neuronal loss because they rely on the number of lost (dead) cells being greater than the variability between animals or tissue sections. Our results suggest that the degree of neuronal injury in the ventral hippocampus, although clearly present in every animal, is in most cases too small to be detected by Nissl cell counting methods. Even if one assumes that every Fluoro-jade B neuron is lost, an assumption challenged by some researchers (Baram, 2002), in most cases it would be too small to be detected in the dorsal

hippocampus with cell counting methods. If some injured Fluoro-jade B neurons recovered, the amount of neuronal loss would be even lower. Other researchers that have used both markers for degenerating neurons and cell counting methods have found similar results and concluded that cell counting methods fail to detect minimal neuronal loss when there is evidence from markers of degenerating neurons suggesting injury has occurred (Nairismagi et al., 2006). When taking into account our study showing that maximal neuronal injury is consistently observed in the ventral hippocampus, a failure to detect measurable neuronal loss in the dorsal hippocampus using cell counting methods is weak evidence that neuronal death has not occurred.

Conclusions

Our results demonstrate that the area of maximal hippocampal neuronal injury after lithium pilocarpine-induced status epilepticus in the immature brain is the ventral hippocampus. This sub-region susceptibility difference is consistent with previous findings in the mature brain, although it appears that this difference is even more accentuated in the immature brain because the limited amount of neuronal loss in dorsal hippocampus is likely below detection using standard cell counting methods. Our findings challenge the previous conclusion by Raol and colleagues that claims epileptogenesis can occur in this age group independent of hippocampal cell death and suggest that further studies that examine the ventral hippocampus are warranted to address this issue.

>Lithium pilocarpine induces hippocampal neuronal injury in postnatal day 20 rats>Ventral hippocampus shows greater neuronal injury than dorsal hippocampus>Examining only dorsal hippocampus is insufficient for detecting hippocampal injury

Abbreviations

CA	cornu ammonis
DG	dentate gyrus
EEG	electroencephalogram
P	post natal day
Str Pyr	stratum pyramidale

REFERENCES

- Amaral DG, Witter MP. The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience*. 1989; 31:571–591. [PubMed: 2687721]
- Babb TL, Brown WJ, Pretorius J, Davenport C, Lieb JP, Crandall PH. Temporal lobe volumetric cell densities in temporal lobe epilepsy. *Epilepsia*. 1984; 25:729–740. [PubMed: 6510381]
- Baram TZ, Eghbal-Ahmadi M, Bender RA. Is neuronal death required for seizure-induced epileptogenesis in the immature brain? *Prog Brain Res*. 2002; 135:365–375. [PubMed: 12143355]
- Baram TZ, Gerth A, Schultz L. Febrile seizures: an appropriate-aged model suitable for long-term studies. *Brain Res Dev Brain Res*. 1997; 98:265–270.
- Baram TZ, Jensen FE, Brooks-Kayal A. Does acquired epileptogenesis in the immature brain require neuronal death. *Epilepsy Curr*. 2011; 11(1):21–26. [PubMed: 21461261]
- Bragdon AC, Taylor DM, Wilson WA. Potassium-induced epileptiform activity in area CA3 varies markedly along the septotemporal axis of the rat hippocampus. *Brain Res*. 1986; 378:169–173. [PubMed: 3742197]

- Buckmaster PS, Dudek FE. Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. *J Comp Neurol.* 1997; 385:385–404. [PubMed: 9300766]
- Cavalheiro EA, Silva DF, Turski WA, Calderazzo-Filho LS, Bortolotto ZA, Turski L. The susceptibility of rats to pilocarpine-induced seizures is age-dependent. *Brain Res.* 1987; 465:43–58. [PubMed: 3440212]
- Cavalheiro EA, Leite JP, Bortolotto ZA, Turski WA, Ikonomidou C, Turski L. Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures. *Epilepsia.* 1991; 32:778–782. [PubMed: 1743148]
- Cavazos JE, Golarai G, Sutula TP. Septotemporal variation of the supragranular projection of the mossy fiber pathway in the dentate gyrus of normal and kindled rats. *Hippocampus.* 1992; 2:363–372. [PubMed: 1308194]
- Covolan L, Mello LE. Temporal profile of neuronal injury following pilocarpine or kainic acid-induced status epilepticus. *Epilepsy Res.* 2000; 39:133–152. [PubMed: 10759302]
- Dudek FE, Ekstrand JJ, Staley KJ. Is neuronal death necessary for acquired epileptogenesis in the immature brain? *Epilepsy Curr.* 2010; 10(4):95–99. [PubMed: 20697505]
- Elul R. Regional differences in the hippocampus of the cat. I. Specific discharge patterns of the dorsal and ventral hippocampus and their role in generalized seizures. *Electroencephalogr Clin Neurophysiol.* 1964; 16:470–488. [PubMed: 14159001]
- Fanselow MS, Dong H. Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron.* 2010; 65:7–19. [PubMed: 20152109]
- Gilbert M, Racine RJ, Smith GK. Epileptiform burst responses in ventral vs dorsal hippocampal slices. *Brain Res.* 1985; 361:389–391. [PubMed: 4084805]
- Holmes GL, Sarkisian S, Ben-Ari Y, Cevassus-Au-Louis N. Mossy fiber sprouting after recurrent seizures during early development in rats. *J Comp Neuro.* 1999; 404:537–553.
- Hunsaker MR, Fieldsted PM, Rosenberg JS, Kesner RP. Dissociating the roles of dorsal and ventral CA1 for the temporal processing of spatial locations, visual objects, and odors. *Behav Neurosci.* 2008; 122:643–650. [PubMed: 18513134]
- Jung MW, Wiener SI, McNaughton BL. Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. *J Neurosci.* 1994; 14:7347–7356. [PubMed: 7996180]
- Kubova H, Druga R, Lukasiuk K, Suchomelova L, Haugvicova R, Jirmanova I, Pitkanen A. Status epilepticus causes necrotic damage in the mediodorsal nucleus of the thalamus in immature rats. *J Neurosci.* 2001; 21:3593–3599. [PubMed: 11331388]
- Kubova H, Mares P, Suchomelova L, Brozek G, Druga R, Pitkanen A. Status epilepticus in immature rats leads to behavioural and cognitive impairment and epileptogenesis. *Eur J Neurosci.* 2004; 19:3255–3265. [PubMed: 15217382]
- Lado FA, Laureta EC, Moshe SL. Seizure-induced hippocampal damage in the mature and immature brain. *Epileptic Disord.* 2002; 4:83–97. [PubMed: 12105072]
- Liu Z, Gatt A, Werner SJ, Mikati MA, Holmes GL. Long-term behavioral deficits following pilocarpine seizures in immature rats. *Epilepsy Res.* 1994; 19:191–204. [PubMed: 7698095]
- Margerison JH, Corsellis JA. Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. *Brain.* 1966; 89:499–530. [PubMed: 5922048]
- McIntyre DC, Nathanson D, Edson N. A new model of partial status epilepticus based on kindling. *Brain Res.* 1982; 250:53–63. [PubMed: 7139321]
- Mello LE, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, Finch DM. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. *Epilepsia.* 1993; 34:985–995. [PubMed: 7694849]
- Moser E, Moser MB, Andersen P. Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. *J Neurosci.* 1993; 13:3916–3925. [PubMed: 8366351]
- Mouritzen Dam AM. Epilepsy and neuron loss in the hippocampus. *Epilepsia.* 1980; 21:617–629. [PubMed: 6777154]

- Nairismagi J, Pitkanen A, Kettunen MI, Kauppinen RA, Kubova H. Status epilepticus in 12-day-old rats leads to temporal lobe neurodegeneration and volume reduction: a histologic and MRI study. *Epilepsia*. 2006; 47:479–488. [PubMed: 16529609]
- Obenaus A, Esclapez M, Houser CR. Loss of glutamate decarboxylase mRNA-containing neurons in the rat dentate gyrus following pilocarpine-induced seizures. *J Neurosci*. 1993; 13:4470–4485. [PubMed: 8410199]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press; 1986.
- Pollard H, Charriaud-Marlangue C, Cantagrel S, Represa A, Robain O, Moreau J, Ben Ari Y. Kainate-induced apoptotic cell death in hippocampal neurons. *Neuroscience*. 1994; 63:7–18. [PubMed: 7898662]
- Priel MR, dos Santos NF, Cavaleiro EA. Developmental aspects of the pilocarpine model of epilepsy. *Epilepsy Res*. 1996; 26:115–121. [PubMed: 8985693]
- Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol*. 1972; 32:281–294. [PubMed: 4110397]
- Raol YS, Budreck EC, Brooks-Kayal AR. Epilepsy after early-life seizures can be independent of hippocampal injury. *Ann Neurol*. 2003; 53:503–511. [PubMed: 12666118]
- Rapp PR, Gallagher M. Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. *Proc Natl Acad Sci USA*. 1996; 93:9926–9930. [PubMed: 8790433]
- Roch C, Leroy C, Nehlig A, Namer IJ. Predictive value of cortical injury for the development of temporal lobe epilepsy in 21-day-old rats: an MRI approach using the lithium-pilocarpine model. *Epilepsia*. 2002; 43:1129–1136. [PubMed: 12366725]
- Roch C, Leroy C, Nehlig A, Namer IJ. Magnetic resonance imaging in the study of the lithium-pilocarpine model of temporal lobe epilepsy in adult rats. *Epilepsia*. 2002; 43:325–335. [PubMed: 11952761]
- Sanchez RM, Koh S, Rio C, Wang C, Lamperti ED, Sharma D, Corfas G, Jensen FE. Decreased glutamate receptor 2 expression and enhanced epileptogenesis in immature rat hippocampus after perinatal hypoxia-induced seizures. *J Neurosci*. 2001; 21:8154–8163. [PubMed: 11588188]
- Sankar R, Shin DH, Wasterlain CG. Serum neuron-specific enolase is a marker for neuronal damage following status epilepticus in the rat. *Epilepsy Res*. 1997; 28:129–136. [PubMed: 9267777]
- Sankar R, Shin DH, Liu H, Mazarati A, Pereira d V, Wasterlain CG. Patterns of status epilepticus-induced neuronal injury during development and long-term consequences. *J Neurosci*. 1998; 18:8382–8393. [PubMed: 9763481]
- Schmued LC, Albertson C, Slikker W Jr. Fluoro-Jade: a novel fluorochrome for the sensitive and reliable histochemical localization of neuronal degeneration. *Brain Res*. 1997; 751:37–46. [PubMed: 9098566]
- Seress L, Pokorny J. Structure of the granular layer of the rat dentate gyrus. A light microscopic and Golgi study. *J Anat*. 1981; 133:181–195. [PubMed: 7333948]
- Spencer DD, Spencer SS, Mattson RH, Williamson PD, Novelly RA. Access to the posterior medial temporal lobe structures in the surgical treatment of temporal lobe epilepsy. *Neurosurgery*. 1984; 15:667–671. [PubMed: 6504282]
- Sperber EF, Haas KZ, Stanton PK, Moshe SL. Resistance of the immature hippocampus to seizure-induced synaptic reorganization. *Brain Res Dev Brain Res*. 1991; 60:88–93.
- Stafstrom CE, Sutula TP. Models of epilepsy in the developing and adult brain: implications for neuroprotection. *Epilepsy Behav. Suppl*. 2005; 3:S18–S24.
- Stafstrom CE, Thompson JL, Holmes GL. Kainic acid seizures in the developing brain: status epilepticus and spontaneous recurrent seizures. *Brain Res Dev Brain Res*. 1992; 65:227–236.
- Stevens R, Cowey A. Effects of dorsal and ventral hippocampal lesions on spontaneous alternation, learned alternation and probability learning in rats. *Brain Res*. 1973; 52:203–224. [PubMed: 4700704]
- West MJ, Slomianka L, Gundersen HJG. Unbiased stereological estimation of the total number of neurons in the subdivision of the rat hippocampus using the optical fractionators. *Anat Rec*. 1991; 231:482–497. [PubMed: 1793176]

Williams PA, Wuarin JP, Dou P, Ferraro DJ, Dudek FE. Reassessment of the effects of cycloheximide on mossy fiber sprouting and epileptogenesis in the pilocarpine model of temporal lobe epilepsy. *J Neurophysiol.* 2002; 88:2075–2087. [PubMed: 12364529]

Figure 1.

Lithium pilocarpine induces status epilepticus at P20. Representative EEG recording showing initiation of status epilepticus. Arrows indicate beginning of expanded time scale for tracings prior to seizure activity (A), at initiation of one early seizure (B), and during status epilepticus (C). Note the slowly increasing amplitude in trace B which is characteristic of electrographic seizure activity. D shows trace at point C of top trace at even more expanded time scale in order to visualize individual spike activity during status epilepticus.

Figure 2. Orientation of the rat hippocampus in relationship to surrounding cortical structures (A). Sections cut in coronal orientation allowed optimal visualization of the dorsal hippocampus, which becomes distorted as one progresses toward the temporal pole (B). Sections cut in horizontal orientation allowed optimal visualization of the ventral hippocampus, which maintains orientation perpendicular to septo-temporal axis in the temporal pole (C). Figure adapted from Amaral and Witter. In: Paxinos. The Rat Nervous System, 1995.

Figure 3.

Fluoro-jade B predominantly stains neuronal soma in ventral versus dorsal hippocampus. Horizontal (A, C, E, and G) and coronal (B, D, F, and H) sections allowed optimal visualization of the ventral and dorsal hippocampal formation, respectively. Ventral hippocampus (A) showed much more staining than dorsal hippocampus (B). Figures A and B are taken at approximate level of cross section of Figure 2C and first cross section of Figure 2B, respectively. Fluorescent staining could be seen predominantly in the stratum pyramidale layers CA1-CA3. The granule layer of the dentate gyrus was spared, although some mossy cells in the hilus were visualized. Dorsal hippocampus also demonstrated more “patches” of Fluoro-jade B staining (compare A and B). C and D show absence of staining

in untreated control animals. (E and F) Higher magnification of stratum pyramidale layer. At even higher magnification (G and H) individual pyramidal neuron soma and apical dendrites are visualized. DG, dentate gyrus; Str Pyr, stratum pyramidale layer.

Figure 4.

Representative coronal section that includes portions of both dorsal and ventral hippocampal formation (approximately at 2nd cross section in fig 2B). Sections with little to no staining in the dorsal hippocampus showed definite staining in the ventral hippocampus despite distortions in anatomical orientation.

Figure 5.

Quantitative analyses of Fluoro-jade B staining shows significant difference between ventral and dorsal hippocampus in CA1 and CA3 region. (A and B) Areas of interest for CA1, CA3, and hilus chosen for quantitative analysis. (C-K) Bar graphs of Fluoro-jade B positive cell bodies. Counts from three separate sections for each animal were assessed for ventral (C, F, I) and dorsal (D, G, J) hippocampus for CA1, CA3, and hilus in order to show variability between tissue sections and animals. (E, H, K) Mean counts for ventral and dorsal hippocampus for all tissue sections samples show significant difference between staining in ventral vs dorsal hippocampus in CA1 and CA3, but not hilus. Error bars represent SEM. * = significance ($p < 0.001$)