A Bio-Electrical Tornado in The Hippocampus: Mechanisms of Temporal Lobe Epilepsy

H. Ümit Sayin

ABSTRACT

This review summarizes the structural and electrophysiological changes in the epileptic hippocampus in various animal models of epilepsy. Tornado hypothesis of epileptic hippocampus, states that there are many progressive detrimental changes in the hippocampus of the epileptic rats. PTZ, PTZ kindling, pilocarpine, electrical kindling, kainic acid, hyperthermia and in vitro models are taken as primary animal epilepsy models in this review. Paired pulse inhibition and GABAergic transmission is decreased in dentate gyrus (DG), CA3 and CA1 regions of the epileptic hippocampus. There is interneuron loss in the hilus, DG and CA3, while excitatory input from perforant path (PP) is increased and this is fed into the CA3 area by mossy fibers (MF) which have excessive sprouting that forms novel recurrent synapses with the pyramidal cells of CA3. Gate function of DG is either impaired or lost. CA3 area of hippocampus becomes an epileptic focus and sends ictal discharges. These ictal discharges are carried into CA1 by Schaffer collaterals and then into entorhinal cortex (EC). The input from EC is amplified in the hippocampal circuitry which is fed into hippocampus from EC into DG again successively. Thus, a weak electrical input into the hippocampal formation results in an amplified signal back into EC. Since the basic ultrastructural and electrophysiological feed-back control mechanisms are impaired, this electrical tornado cannot be compensated for and an epileptic amplified ictal discharge spreads to the limbic system and other adjacent structures of the brain. Eventually the hippocampal circuitry, that has developed a vicious circle, becomes a bio-electrical amplifier which triggers an electrical tornado, under certain bio-chemical conditions.

Key Words: Animal models of epilepsy, tornado hypothesis of epilepsy, CA3, CA1, Dentate gyrus, PPI, kindling, kainic acid, PTZ, epileptic hippocampus, pilocarpine, febrile convulsion, GABAergic inhibition, interneuron, mossy fiber sprouting, in vitro model of epilepsy, perforant path, tonic clonic seizures, intractable temporal lobe epilepsy.

DOI Number: 10.14704/nq.2017.15.1.1014 **NeuroQuantology 2017; 1: 92-103**

Introduction

Temporal lobe epilepsy is a status of the temporal structures of the brain (such as hippocampus, parahippocampal gyrus, amygdala, enthorinal cortex) in which neurons, interneurons, receptors, overall circuitry malfunction or have a hyper-function that results in generalized seizures. The prevalence of epilepsy is 0.5-1 % on the globe and nearly half of the epilepsies have the temporal lobe origin. Thus, temporal lobe structures, particularly hippocampus, are an important area to study to understand the mechanisms of epilepsy (Jefferys, 2010).

Since the establishment of animal models of epilepsy since 1950's, we have learned a lot about the basic mechanisms of epilepsy and today depending on such scientifically proven findings, we are able to treat most of the cases of epileptic seizures, if not all.

Corresponding author: H. Ümit Sayin, M.D., Ph.D.
Address: Institute of Forensic and Legal Sciences, İstanbul University, Cerrahpaşa Medical Campus, Cerrahpaşa-Aksaray, Istanbul, Adjunct Faculty, Institute of Advanced Study of Human Sexuality (IASHS) San Fransisco-USA
Phone: + 90-553 365 53 49
e-mail ✉ humitsayin@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 23 March2016; **Accepted:** 2 August 2016

Animal models of epilepsy, most of the time, try to mimic generalized convulsions, tonic clonic seizures, such as in the cases of pentetrazole, pilocarpine, focal or systemic kainic acid injection (Reddy, 2013; Loscher, 2011; Sayin, 2003). Kindling is a good established model for temporal lobe epilepsy (Sayin, 2003). Hippocampal slice model also gives invaluable clues about the increased excitation or decreased inhibition, by interneurons, in various hippocampal areas such as dentate gyrus, CA3 and CA1, as well as the altered cellular properties of dentate granule cells and CA3 pyramidal cells (Sayin 1995, 1997, 1999, 2001, 2003, 2004, 2015; Hadar, 2002; Rutecki, 2002; Westmark, 2005; Lynch, 2000; Sutula, 1998). Most of the novel anti-epileptics are designed according to the data obtained from such models and their anti-epileptic potencies are also tested by means of using these models. In this review, we will discuss about the outcome findings from some of these models to better understand what happens in an epileptic brain.

Animal Models of Epilepsy

There are many different models for acute or chronic tonic convulsions. A brief explanation of some of the models is essential to mention to have a vision of how these models can/could work.

Pentylenetetrazole (PTZ) model: Pentylenetetrazole (pentazol, cardiazol, pentetrazol, metrazol), a $GABA_A$ antagonist on picrotoxin site of $GABA_A$ receptor is injected i.p. (mice & rats). Onset of tonic clonic convulsions is 10-20 minutes, the tonic-clonic generalized seizures last for 2 to 5 minutes. Lethality may be high depending upon the species and the dose. Most of the anti-epileptic drugs are tested by pentylenetetrazole model. PTZ can also be used for PTZ-kindling, mimicking the effects of electrical kindling (Sayin, 2015; Westmark, 2005; Sloviter, 1987; Tilelli, 2005; Lothman, 1981).

Pilocarpine (Pilo) model: Pilocarpine, a non-selective muscarinic receptor agonist, is injected i.p. (mice & rats). Very severe *status epilepticus* may occur depending upon the dose. Lethality is very high. Pilo is also used in the hippocampal slice models, inducing epileptiform bursts or ictal patterns in the extracellular recordings of CA3 pyramidal cells (Sayin 1997, 2003; Hadar, 2002; Rutecki, 2005; Turski, 1984; Cavalheiro, 1987; Clifford, 1987).

Maximal Electroshock Seizures (MES): Electro-convulsive direct currents are passed onto the ears of mice or rats through the ear clips. Tonic clonic seizures which last for 2-3 minutes occur immediately. Many anti-epileptic drugs are tested with MES model before they are tested in other models (Jefferys, 2010; Reddy, 2013; Loscher, 2011).

Kainic Acid (KA): Kainic acid, a neurotoxin which is an agonist for kainate receptors or glutamate ionotropic receptors, and which may also -in the due course- activate the NMDA receptors, which takes part in the synaptic plasticity. KA is injected either i.p. (rats & mice-pups), or it may be injected at focal areas in the brain (such as cortex, amygdala, perforant path, hippocampus etc.). The subject animal has an immediate seizure for 2-5 min., and sometimes a status epilepticus lasting for longer. The effect is long term, some repeated injections of KA may result in spontaneous seizures after a while (Sayin 2004, 2015; Sperk 1983, 1985; Sutula 1983, 1998; Lynch, 2000; Ben-Ari, 1981).

Kindling model of temporal lobe epilepsy: Kindling model is a progressive electrical stimulation model (mostly rats or cats). The rats are implanted stimulation and recording electrodes into the amygdala, or hippocampus, or most of the time into the perforant path (PP). They are stimulated by square pulses of micro-ampere direct currents every day until an after-discharge EEG recording is observed; the stimulus intensities are determined according to a protocol depending upon the EEG recordings. They are continued being stimulated until they have a Class-V, short tonic clonic seizures which last for 0.5-1 minute. According to the course of the study and research design, the intensities which had not induced any seizure in the beginning will induce tonic clonic seizures eventually. The lethality is very low; the seizures are not as severe as the chemical models. Decreasing the current, animals may be continued to be stimulated to observe the effects of drugs or behavioral changes. In our studies, we were able to establish PP-kindling as a model to induce *spontaneous seizures* after 100-120 Class-V seizures (Sayin, 2003) (some of our rats had experienced 150-200 Class-V tonic clonic seizures before becoming spontaneously convulsing rats (SP-rat)). For this reason, this is a very striking, unique and useful model for investigating temporal lobe epilepsy. In the hippocampal slices of SP-rats, we performed electrophysiological studies, such as extracellular

recording, paired pulse inhibition recordings, current clamp and voltage clamp in dentate granule cells and CA3 pyramidal cells, and histology (Sayin 1999, 2003, 2015; Lynch, 2000; Tuff 1983; Gilbert, 1991).

Hyperthermia or Febrile Seizures (FS): The pups of postnatal-1 (P-1) to postnatal day-30 (P-30) are exposed to hyperthermia (38-40 ° C) in a closed chamber while body temperature is monitored. They start to have tonic clonic convulsions in 10-15 minutes, lasting for 0.5-1 minute; the lethality is moderate. Their brains are then investigated after the seizure or when they are grown up (2-3 months old) (Sayin 2015; Dube 2000, 2006, 2010; Baram, 1997).

Hippocampal slice model: Hippocampal slices from normal or epileptic rats are prepared to sustain basic living condition in a chamber, they may stay alive for 15-20 hours, while consistent electrophysiological recordings can be made. Various stimulations and recordings can be made, such as investigating the paired pulse inhibition, current clamp or voltage clamp, for measuring the nature of action potentials and inward currents under the effects of certain drugs. Also, using chemicals, such as bicuculline, pilo, metabotropic glutamate receptor agonists, picrotoxin, opiate agonists etc., it's possible to detect the ictal and interictal patterns at the CA3 area, where pyramidal cells are very susceptible to become excitable and show ictal patterns to investigate some drugs. This is a very good and valid model of cellular epilepsy and spontaneous epileptiform discharges of CA3 (Sayin 1997, 2003; Hadar, 2002; Rutecki, 2002).

Here we will take some of our findings from our models we had utilized and some of other researchers' findings to establish some insights for the determinants of chronic tonic clonic seizures in the animal models of epilepsy, particularly temporal lobe epilepsy, as summarized in the table-1.

Determinants of Temporal Lobe Epilepsy and Chronic Seizures

As seen in Table-1, I have studied many of the animal models of epilepsy, some of the data is still unpublished; for the published part of our findings please refers to (Sayin 1995, 1997, 1999, 2003, 2004, 2015; Hadar, 2002; Lynch, 2000; Sutula, 1998; Rutecki, 2002; Westmark, 2005). Some of our observations were just recorded, some were presented in the meetings, and some were written as scientific articles. Between 1990 and 2015 my experiments, findings and research brought me to think in a holistic way, rather than following a reductionist pathway, and look at all the determinants of the causes of epilepsy as a whole, rather than focusing on only CA3 or Dentate Gyrus, because in the normal physiological conditions, the whole system is working together; to understand the "*gestalt"* of the system is more crucial. Reductionist approach is only a method to get some information about the parts or elements of a system. Using "*System Theory*" is very essential to understand the mechanisms of a disorder or a disease (here, *epilepsy*) (Sayin, 2016; Bertalanffy, 1969; Luhmann, 2013). Thus, in this review, I will summarize the possible important determinants which build up the system into a convulsion.

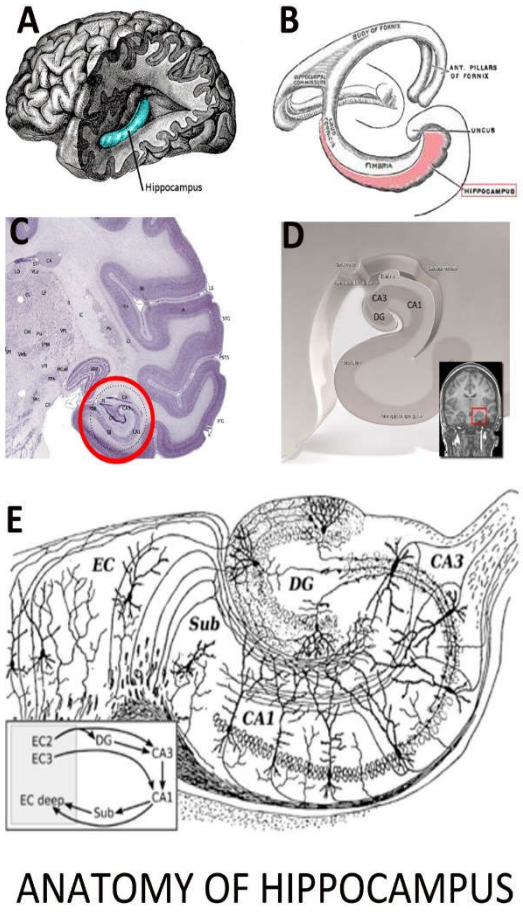


Figure 1. The Anatomy of hippocampus. A) Location of hippocampus in the brain B) Hippocampus with the adjacent structures of the limbic system C) Nissl-stained section of the macaque monkey, showing hippocampus (red circle) D) The hippocampal formation E) Basic circuits of hippocampus, as drawn by Ramon y Cajal. DG: Dentate gyrus; Sub: Subiculum; EC: Entorhinal cortex, CA3-CA1: Cornu ammonis (Source: Wikipedia and brainmaps.org)

Table 1. Classification of Animal Models of Epilepsy and Status Epilepticus.

Classification	Model	Spontaneous Seizures	Mossy Fiber Sprouting	References
ELECTRICAL	**Kindling (PP stimulation)**	**+++** (after 100-120 Cl-V seizures)	**++** or **+++**	(Sayin 1999,2003,2015; Lynch,2000; Tuff,1983; Gilbert,1991) This model studied in our lab
	MES (Maximal Electroshock Seizures)	**Ø** (acute short seizures)	**Ø**	(Loscher,2011) This model studied in my lab
CHEMICAL MODELS	**Kainic Acid**	**++++** (very severe)	**++++** (severe)	(Sayin 2004,2015, Lynch,2000; Sutula 1986,1998; Ben,1981; Sperk1983,1985) This model studied in our lab
	Pilocarpine	**++++** (very severe)	**++++** (severe)	(Sayin 1997,2003; Hadar,2002; Rutecki,2002; Turski,1984; Cavalheiro,1987) This model studied in our lab
	Cobalt	**++**	**+**	(Jefferys,2010; Reddy,2013; Loscher,2011)
	Pentetrazol	**+** (short acute seizures)	**+**	(Westmark,2005; Sayin,2015; Sloviter,1987; Tilelli,2005; Lothman,1981) This model studied in our lab
	Picrotoxin	**+** (short acute seizures)	**+**	This model studied in our lab
	Bicuculline (focal injection through a cannula)	**+** (short acute seizure)	**?**	This model studied in our lab
	Chemical kindling (PTZ, etc., continuous injection of PTZ at subconvulsive doses many times)	**+++**	**+++**	(Sayin,2015) This model studied in our lab
STATUS EPILEPTICUS MODELS	**Pilocarpine-lithium**	**++++**	**++++**	(Jefferys,2010; Reddy,2013; Loscher,2011; Sayin,1997 , Rutecki,2002) This model studied in our lab
	Kainic acid	**++++**	**++++**	This model studied in our lab
	DFP	**+++**	**++++**	(Jefferys,2010)
IN VITRO MODELS	**Low magnesium in hippocampal slices**	**++++** Ictal patterns in CA3, CA2		**(**Sayin1997,2003; Hadar,2002; Rutecki,2002) This model studied in our lab
	High Potassium in hippocampal slices	**++++** Ictal patterns in CA3, CA2		(Jefferys,2010; Reddy,2013; Loscher,2011; Sayin1997,2003; Hadar,2002; Rutecki,2002) This model studied in our lab
	4-Amino-pyridine in hippocampal slices	**+++**		(Jefferys,2010; Reddy,2013; Loscher,2011; Sayin1997,2003; Hadar,2002; Rutecki,2002) This model studied in our lab
	Bicuculline/picrotoxin in hippocampal slices	**++++**		(Jefferys,2010; Reddy,2013; Loscher,2011; Sayin1997,2003; Hadar,2002; Rutecki,2002) This model studied in our lab
	Pilocarpine in hippocampal slices	**++++** Ictal patterns in CA3		(Jefferys,2010; Reddy,2013; Loscher,2011; Sayin 1997,2003; Hadar,2002; Rutecki,2002; Turski,1984; Cavalheiro,1987; Clifford,1987) This model studied in our lab
	Glutamate receptor agonists (e.g. metabotropic M-GLU)	**+++** Ictal patterns in CA3		(Sayin,2003; Hadar,2002) This model studied in our lab
HYPERTHERMIA	**Febrile seizures in pups**	**+** (minimal)	**++** (little or moderate)	(Sayin,2015; Dube 2000,2006,2010; Baram,1997) This model studied in our lab

Epileptic Excitable CA3 Pyramidal Cells

Our lab's findings have pointed out that in the epileptic rats (kindled, kainic acid, PTZ) or in the rats that have experienced prolonged status epilepticus (pilocarpine or kainic acid), the CA3 pyramidal cells become more excitable and easily enter into ictal-like status as a response to lowered extracellular magnesium or increased extracellular potassium, compared to the controls. The population spikes from CA3 layer become multiple as an evoked potential, as well as the ones in CA1; no such change and spontaneous ictal patterns happened in dentate granule cells. In the hippocampal slices from the rats undergone status epilepticus, CA3 area goes into spontaneous bursting as a response to low bicuculline concentrations, compared to the controls; namely, the bicuculline concentrations that do not induce ictal or interictal patterns in the control CA3, *do* induce spontaneous interictal or ictal discharges in the CA3 areas of the hippocampi from the epileptic rats. The stimulus threshold to induce a bursting evoked multiple population spike in CA3 is also decreased in the hippocampal slices of epileptic rats.

It was documented that CA3 pyramidal neurons from spontaneously epileptic mutant rats had abnormal hyper-excitability; mossy fiber stimulation showed long lasting depolarizing shift accompanied by repetitive firing following a single stimulation in half of the CA3 pyramidal cells (Ishihara, 1993).

In a computer model which was based on the basic properties of hippocampal neurons, with 200 basket cells and 800 pyramidal cells; it was proven that, CA3 area may become hyper-excitable and prone to epileptic discharges as a response to a minute loss of dentritic inhibition (Sanjay,2015).

Rutecki et al. has shown numerous times the increased excitability of CA3 pyramidal cells in various models in the epileptic hippocampus (Sayin 1997, 2003; Hadar, 2002; Rutecki 1998, 2002).

Epileptic Excitable Dentate Granule Cells

The dentate gyrus is considered to function as an inhibitory gate limiting excitatory input to the hippocampus. Following status epilepticus (SE), this gating function is reduced and granule cells become hyper-excitable. In DG, the granule cells never act like pace maker bursting-ictal cells as in the case of CA3 pyramidal cells, however they spike more frequently and the spike numbers may be increased in the DG of hippocampal slices from pilocarpine and kainic acid treated rats (Lynch, 2000; Flynn, 2015). The granule cells become more excitable, generating epileptiform discharges in response to afferent stimulation in the kainic acid model, as a response to this new inhibitory interneurons are regenerated to compensate for this hyperexcitability by means of increasing GABAergic inhibition (Sloviter, 2006). Dentate gyrus is assumed to be a gate that filters the input from subiculum and enthorinal cortex, in chronic epilepsy or status epilepticus this gate function is impaired or lost.

Loss of Inhibitory Interneurons

In the kindled rats that undergo spontaneous seizures (after 100-120 Cl-V kindled evoked seizures), we have determined that there is a loss of paired pulse inhibition, mossy fiber sprouting loss of interneurons and decreased IPSC's (Sayin 2003) and also severe mossy fiber sprouting and axonal remodeling in kainic acid treated rats (Sutula 1998). Sloviter's group had also reported decreased hippocampal inhibition and loss of interneurons in experimental models long ago (Sloviter, 1987). In the pilocarpine model, it is reported that hilar somatostatin interneurons are lost (Hofmann, 2016). In a recent study, somatostatin (SS), neuropeptide Y (NPY), and parvalbumin (PV) in LiCl-pilocarpine-treated rats had some quantitative changes and axonal sprouting of GABAergic interneurons in the hippocampus, showing the compensation mechanisms of hippocampus as a response to the death of inhibitory interneurons in status epilepticus (Long, 2010). Also in other areas of hippocampus, there is loss of interneurons, such as subiculum, pyramidal cell layer, molecular in pilocapine treated rats and decrease of IPSCs, loss of presynaptic GABAergic input (Knopp, 2008). Many other studied point out the loss of inhibitory interneurons and formation of new recurrent excitatory circuits after mossy fiber sprouting (Dudek, 2007; Stief, 2007; Sun, 2007; Epsztein, 2006).

Mossy fiber sprouting and forming a more excitable new Dentate-CA3 circuitry

There are numerous reports stating that after status epilepticus, prolonged seizures, chronic tonic clonic convulsions in the animal models of epilepsy, such as kindling, PTZ, pilocarpine, kainic

acid, etc., there is mossy fiber sprouting and a new wiring and a more excitable circuitry is formed in the DG-CA3-CA1 axis by means of the following factors (Sayin 1999, 2003; Lynch 2000; Sutula 1998; Sloviter 2006; Hofmann 2016; Long 2010; Knopp 2008; Dudek 2007; Stief 2007; Sun 2007; Epstein 2006; Song 2015; Tian 2009; Holmes 1999) (See: Table-1 and Figure-2)

- Alterations in the neuronal properties of dentate granule cells, becoming more excitable
- CA3 pyramidal cells becoming hyper excitable, with ictal and interictal bursts
- Loss of GABAergic interneurons in DG, CA3, CA1, hilus, subiculum and enthorinal cortex
- Inhibitory post synaptic potential (IPSCs) decrement
- Paired pulse inhibition decrement
- Mossy fiber sprouting and formation of new excitable recurrent circuitry

Loss of Paired Pulse Inhibition in Dentate Gyrus and CA3

In our studies we have found that, although paired pulse inhibition (PPI) is increased in DG in the early phases of epileptogenesis, after a while this trend declines and after spontaneous seizures develops PPI decreases in DG. This finding is also consistent with our and other histological studies on the interneuron histology and immunohistochemistry of interneurons after status epilepticus and prolonged seizures (Sayin 1997, 2003; Westmark 2005; Sutula 1998; Sloviter 2006; Hofmann 2016; Long 2010; Knopp 2008; Dudek 2007; Sun 2007). In CA3, one of the reasons of hyper-excitability may also be the loss of hilar and CA3 inhibitory interneurons and loss of PPI. In the kindled rats that had spontaneous seizure (> 100-120 Cl-V evoked-kindled seizures) in the CA3 area of the hippocampal slices there was not only loss of PPI, but also facilitation. (See: Table-2 and Figure-3)

Other studies also found decreased PPI in CA1, CA3 and DG in various animal models of epilepsy, such as PTZ, PTZ kindling, Pilo and flurothyl (Holmes 1998, 1999; Fathollahi, 1997; Stringer, 1995; Psarropoulou, 1994). Febrile convulsions also have a detrimental effect on the inhibitory circuitry, particularly at certain periods; our results showed that febrile seizures at the postnatal day of 24 (P20-P30) had long term effects, such as decrease of PPI in dentate and CA3. Other labs also found confirming results, that febrile seizures decrease inhibition in CA1 and CA3, during adulthood (Boyce, 2013). Some researchers also found decreased PPI in DG by the perforant path stimulation (Naylor, 2005).

Alterations in Voltage Gated Sodium Channels

It has been hypothesized that alterations in voltage-gated sodium channels (VGSCs) occur in chronic epilepsy, some anti-epileptic drugs are designed to target this deficiency (Mantegazza 2010). Recent data on changes of sodium channel expression, mutational changes in the receptor itself, molecular structure, and function has been associated with epilepsy, as well as on the interaction of new and established antiepileptic drugs with sodium currents (Köhling 2002; Mesiler 2002).

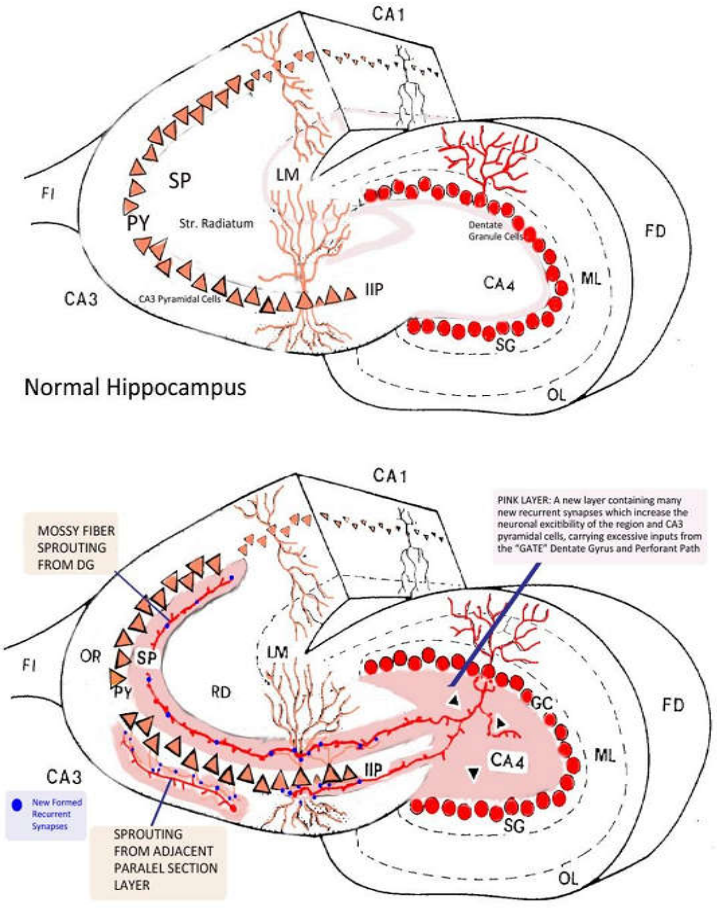


Figure 2. Normal and epileptic hippocampi. In the lower section there are recurrent mossy fiber sprouting and many new synapses carrying excessive and novel input into CA3 region of hippocampus. Pink layer depicts the area where there are many new recurrent synapses that results in excessive excitation fed from DG via mossy fibers.

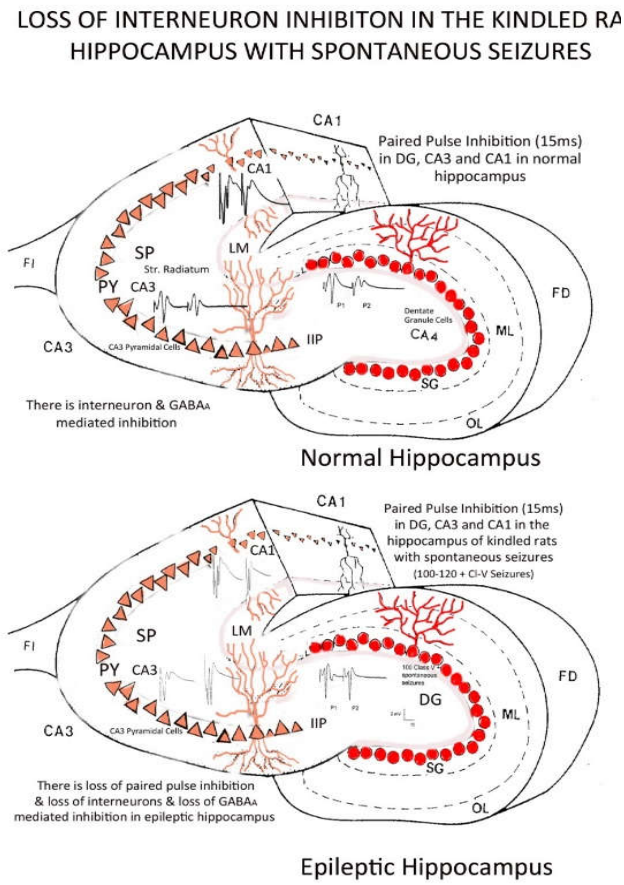


Figure 3. Paired pulse inhibition change in normal and epileptic hippocampus**.** In the rat hippocampus with many spontaneous seizures in the models of electrical kindling, pilocarpine and kainic acid, paired pulse inhibition is decreased in dentate gyrus (DG), CA3 and CA1. The traces of pulses are placed in the proper areas of the hippocampus in normal and epileptic rats. Paired pulse with an interval of 15 ms is shown. This finding shows that inhibitory circuitry and GABAergic inhibition are impaired either because of the loss of inhibitory interneurons or excessive excitation. DG: Dentate gyrus; ML: Molecular layer; FI: Fimbria; PY: pyramidal cell layer; SG: granule cell layer.

Lowered Threshold for the Generation of Action Potentials

We have observed hyperexcitability of granule cells and CA3 pyramidal cells after chronic seizures, chronic electrical and chemical kindling, and status epilepticus (pilo or kainic acid) (Sayin 1997, 1999, 2003; Lynch, 2000 and unpublished data). The threshold for generating an action potential is lowered for an evoked response and in CA3 spontaneous multiple population spikes occurred. With low magnesium, high potassium, and very minute concentrations of bicuculline, we observed interictal or ictal discharges in CA3 area; also in DG, evoked multiple spikes of granule were significantly increased in the hippocampal slices of epileptic rats compared to controls.

Some other studies also found neuronal excitability, lowered threshold for spiking action potentials in dentate and CA3, and easily occurring interictal patterns in CA3 (Yan,2012; Saly, 1993; Swartzwelder, 1988).

Hippocampus Becoming a Bio-Electric Amplifier

As schematized in Figure-4, the parameters and determinants we have mentioned eventually builds up into chronic temporal lobe epilepsy. For DG, these are: a) *Decreased PPI in DG* b) *Mossy fiber sprouting and excitable circuitry* c) *Decrement of IPSPs/IPSCs* d) *Inhibitory interneuron loss* e) *Granule cells becoming more excitable.* For CA3, these parameters are: a) *Decreased PPI in CA3* b) *Many new recurrent excitable input pathways* c) *Decreased IPSPs/IPSCs* d*) Inhibitory interneuron death and impaired GABAergic inhibition* e) *CA3 pyramidal cells becoming more excitable and easily inducing interictal and/or ictal discharges* f) *When there is even a weak stimulus or bio-electrical input via perforant path and then mossy fiber from DG into CA3, CA3 becoming like a pacemaker area and generating epileptiform ictal bursts and discharges which is carried to CA1 via Schafer collaterals, and then to enthorinal cortex.* (See: Figures 2-3-4-5-6)

Extra Temporal Lobe Circuits in Temporal Lobe Epilepsy

Hippocampal formation has many connections with the adjacent structures and extra-temporal lobe circuits (Hartley, 2014; Bertram, 2014). The weak bio-electrical input from entorhinal cortex can be amplified in an epileptic hippocampus within milliseconds and can be fed into the entorhinal cortex again under certain physiological and biochemical conditions, which may trigger a tonic-clonic convulsion. This circuitry can become a vicious loop such that, a chaotic *bio-electrical tornado effect* be carried into the adjacent structures, such as, parahippocampal gyrus, amygdala, hypothalamus and thalamus, piriform cortex, or olfactory cortex and other structures of the temporal lobe as an increasing and amplified signal (See Figures: 5-6). The mathematical modeling of this effect can be studied and designed using the Chaos Theory and also the Mandelbrot mathematics in a computer model of temporal lobe epilepsy.

Table 2. The Effects of Epilepsy Models on PPI in Hippocampus.

Hippocampal Area	**3 Cl-V Seizures**	**30-35 Cl-V**	**70-90 Cl-V**	**>100-120 Cl-V** w. spontaneous s.	**COMMENTS**
Kindling	Electrical Kindling				
Dentate Gyrus	**PPI ↑↑↑**	**PPI ↑↑**	**PPI ↑**	**PPI ↓↓**	IPCS's measured and decreased [5] (after spontaneous seizures)
CA3	**PPI ↑↑↑**	**PPI ↑**	**PPI ↓**	**PPI ↓↓↓↓**	IPCS's in CA3 measured, decreased (unpublished)
CA1	**PPI ↑↑↑**	**PPI ↑↑**	**PPI ↑**	**PPI ↓↓**	Similar effects with CA3
Pilocarpine	**PILO**				
	In vivo (acute) Systemic injection	**In vivo (chronic)** After spontaneous s.		**In vitro** Acute Slice model	
Dentate Gyrus	**PPI ↑↑↑**	**PPI ↓↓**		**PPI ↓↓**	After Spon. S. IPSC's measured, decreased (unpublished)
CA3	**PPI ↑**	**PPI ↓↓↓**		**PPI ↓↓↓**	Spontaneous ictal bursts in CA3
CA1	**PPI ↑**	**PPI ↓↓**		**PPI ↓↓**	
Kainic Acid	**Kainic Acid**				
	In vivo (acute) Systemic injection	**In vivo (chronic)** After spontaneous s.		**In vitro** Acute Slice model	
Dentate Gyrus	**PPI ↑↑↑**	**PPI ↓↓**		**PPI ↓↓↓**	After spontaneous seizures in vivo, IPCS's decreased.
CA3	**PPI ↑**	**PPI ↓↓↓**		**PPI ↓↓↓**	Ictal and interictal patterns
CA1	**PPI ↑**	**PPI ↓↓**		**PPI ↓**	Interictal-like patterns, multiple population spikes
PTZ	**Pentetrazol (or picrotoxin in vitro)**				
	In vivo (acute) Systemic injection Slice after the seizure	**In vivo (chronic)** After PTZ chemical kindling (many seizures)		**In vitro** Acute Picrotoxin Slice model	
Dentate Gyrus	**PPI ↑↑**	**PPI ↓↓**		**PPI ↓↓↓**	After chemical kindling inhibition was impaired
CA3	**PPI ↑**	**PPI ↓↓↓**		**PPI ↓↓↓↓**	After chemical kindling inhibition was impaired
CA1	**PPI ↑**	**PPI ↓↓**		No data	

Hippocampal Area	P1-P10	P10-P20	P20-P30 Critical time: P24	Adult (of P24)	Febrile Seizure at the postnatal days P24-P30 were critical turning point
Febrile Seizures	**Hyperthermia-Febrile Seizures**				
Dentate Gyrus	**PPI ↑↑↑**	**PPI ↑↑↑**	**PPI ↓↓**	**PPI ↓**	Adults of other periods were not affected; PPI was decreased in the adult of P20-P30
CA3	**PPI ↑↑↑**	**PPI ↑**	**PPI ↓↓↓↓**	**PPI ↓↓↓**	Adults of other periods were not affected; PPI was decreased in the adult of P20-P30
CA1	**PPI ↑↑↑**	**PPI ↑**	**PPI ↑**	**PPI ↑**	Adults of other periods were not affected; PPI was decreased in the adult of P20-P30

Note: Some of the above data has not yet been published. All areas were tested in hippocampus in all models. In different models the results were very consistent: In the beginning of epileptogenesis, there is increased paired pulse inhibition as a reactive response to seizures. Gradually this GABAergic interneuron inhibition increase subsides, when the seizures are repeated or when there are spontaneous seizures, there is a loss of inhibition in DG and CA3 of hippocampus, as well as CA1. Arrow number also shows the intensity of inhibition or disinhibition. Number of arrows shows the approximate intensity of the increase or decrease of PPI.

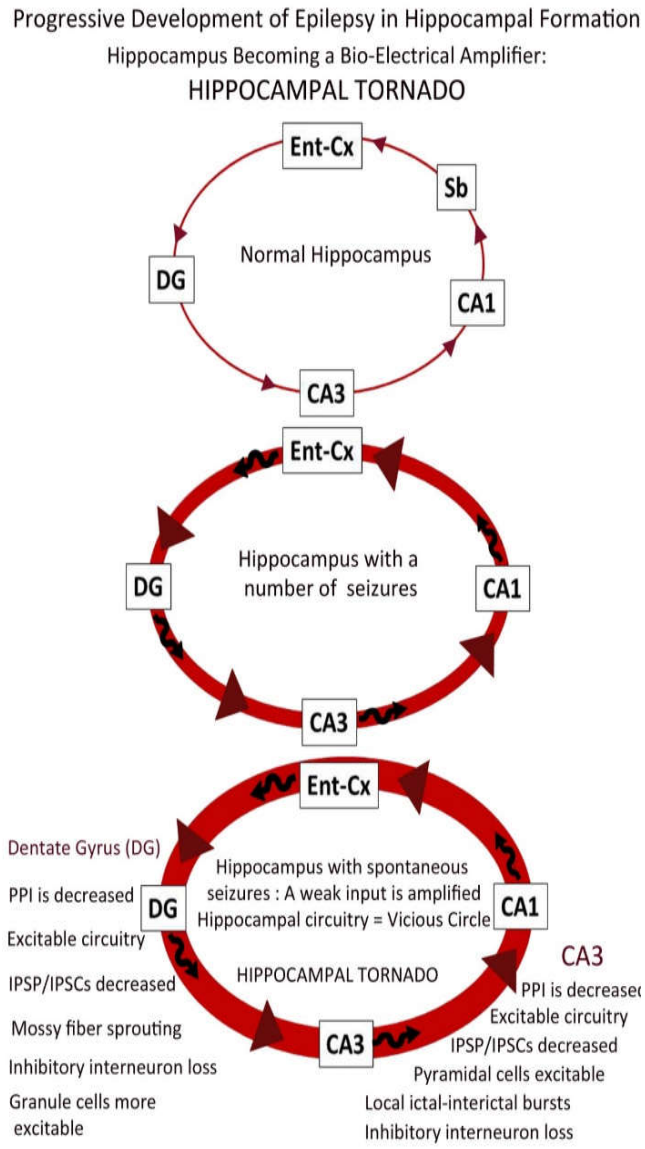
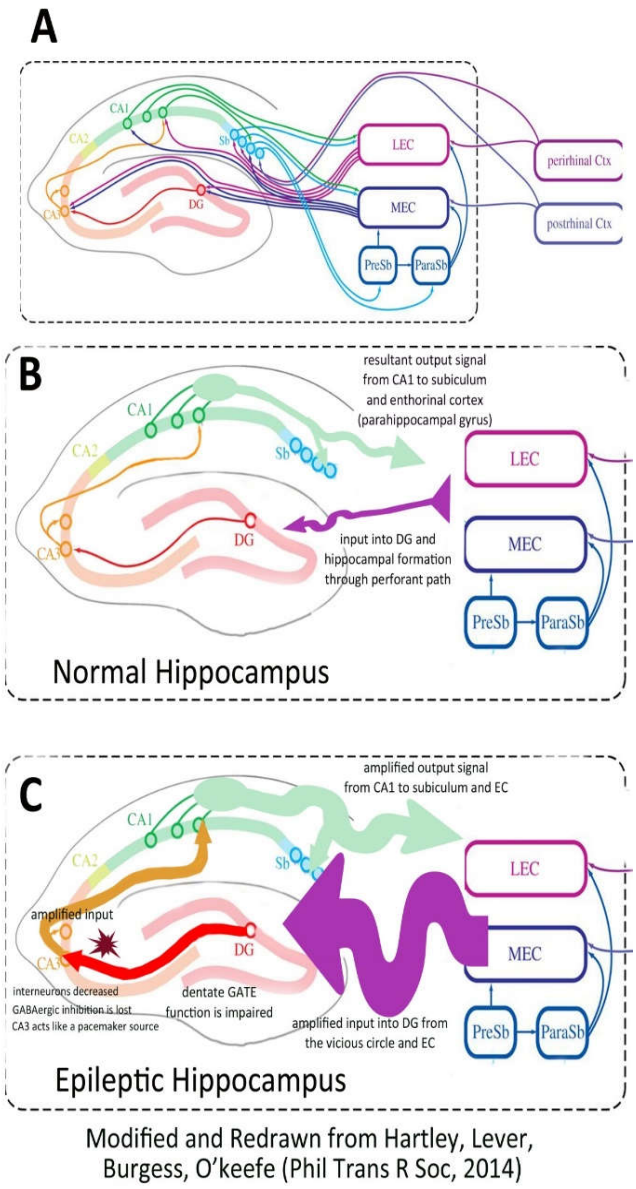


Figure 4. Epileptic hippocampus becoming a bio-electric amplifier: Tornado Effect. Because of various parameters and reasons given in the bottom drawing, the input coming from perforant path into DG is carried as an amplified stimulus into the CA3. The gate function of DG is either impaired or lost. Mossy fiber sprouting and new recurrent synapses induce epileptiform bursts and ictal discharges in the CA3 region of hippocampus. These impulses are carried to CA1 by Schaffer collaterals and then into the entorhinal cortex. This vicious circle is repeated many times and many feedback mechanisms to control the excessive excitation are impaired. By time this hippocampal circuitry becomes a bio-electrical amplifier and the excessive ictal discharges are fed into the other structures of the cerebrum, such as adjacent structures of the limbic system, motor cortex and somatosensory cortex. Thus a minute bio-electrical impulse can be fed into an amplified ictal discharge under certain conditions, such as extracellular chemical or ionic changes, which increase the susceptibility to seizures. In the figure, the thickness of the red line in the circuitry depicts the amplified signals.



Modified and Redrawn from Hartley, Lever, Burgess, O'keefe (Phil Trans R Soc, 2014)

Figure 5. The hippocampal formation and signal input and output pathways. A) The pathways in normal hippocampus. DG: Dentate gyrus, Sb: Subiculum, PreSb: Presubiculum, ParaSb: Parasubiculum, LEC: Latereral entorhinal cortex, MEC: Medial entorhinal cortex. **B)** In a normal hippocampus input from entorhinal cortex (purple thin line) C) In an epileptic hippocampus the input from entorhinal cortex is amplified because of many factors, such as recurrent synapses, loss of GABAergic inhibition, the vicious circle of the hippocampal loop. Thickness of the arrow depicts the intensity of the bio-electrical input.

Conclusion

As a conclusion, in the epileptic hippocampus, because of various parameters and factors (see Figure-4), the input coming from perforant path into DG is carried as an amplified stimulus into the CA3. The gate function of DG is either impaired or lost (Figure-4). The harnessing role of GABAergic transmission and interneuron inhibition are impaired (Table-2; Figures-3-4). Mossy fiber sprouting and new recurrent

synapses induce epileptiform bursts and ictal discharges in the CA3 region of the hippocampus (Figure-2). These impulses are carried to CA1 by Schaffer collaterals and then into the entorhinal cortex. This vicious circle is repeated many times and many feedback and defense mechanisms to control the excessive excitation are impaired (Figures-3-4).

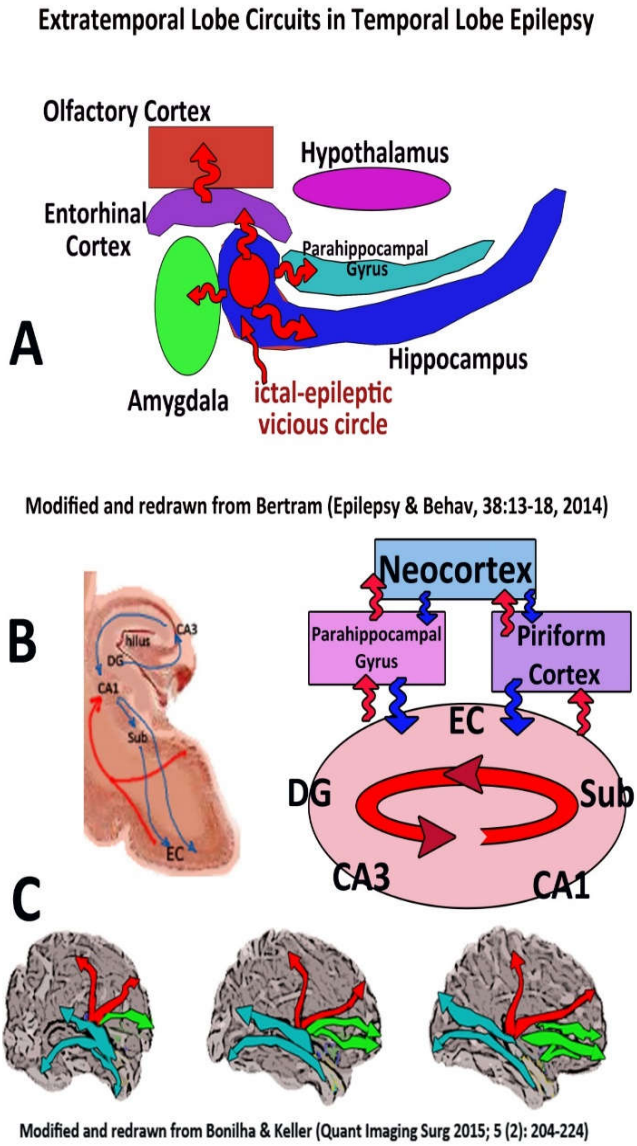


Figure-6. The spread of epileptic discharges into and through extra-hippocampal and extra-temporal structures. **A)** Epileptic focus in the vicious circle loop of the hippocampus spreads to amygdala, parahippocampal gyrus, entorhinal cortex, hypothalamus and thalamus, olfactory cortex. **B)** The vicious circle in the hippocampus and its direct relation with the adjacent extra-hippocampal structures. **C)** MRI imaging studies show the spread of epileptic discharges into other parts of the brain and causing the tonic clonic convulsions. The figure is modified and redrawn from the MRI images during the image recordings of temporal lobe epilepsy seizures (from Bonilha and Keller, 2015)

By time this hippocampal circuitry becomes a bio-electrical amplifier and the excessive ictal discharges are fed into the other structures of the cerebrum, such as adjacent structures of the limbic system, temporal lobe, motor cortex and somatosensory cortex (Figures 5-6). Thus a minute or weak bio-electrical impulse can be fed into an amplified ictal discharge under certain conditions, such as extracellular chemical or ionic changes, which increase the susceptibility to seizures. Hence, hippocampus becomes a bio-electric amplifier, in which a bio- electrical tornado originating from the temporal lobe occurs during an epileptic seizure. This hypothesis has been supported by many researchers and *tornado hypothesis* may give many insights for the medical or surgical treatment of intractable temporal lobe epilepsy, such as the resection or dissection of some pathways, or concentrating on the treatments to break this vicious circle at one of the causing factors, such as enhancing GABAergic inhibition, as in the case of vigabatrin therapy, which increases synaptic GABA levels nearly 10 times (Sayin, 1995) or a possible future implantation of cultured GABAergic interneurons into the hippocampus, particularly in the dentate gyrus, hilus and CA3. Taking the attributing factors of temporal lobe epilepsy as explained in this review, it may be possible to treat intractable temporal lobe epilepsy totally soon.

Acknowledgements

I would like to thank Prof. Thomas P. Sutula and Prof. Paul A. Rutecki for their support and help for the realization of these studies. The research in this article was supported by NIH grants (T. Sutula), Veterans Administration Hospital grants (P. Rutecki) and Epilepsy Foundation grant (Ü. Sayın). This review is also supported by İstanbul University and BAP (I.U) research grants (Ü. Sayin).

References

Baram TZ, Gerth A, Schultz, L. Febrile seizures: An appropriate-aged model suitable for long-term studies. Dev Brain Res 1997; 98: 265–270.

Ben-Ari Y, Tremblay E, Riche, D, Ghilini G, Naquet R. Electrographic, clinical and pathological alterations following systemic administration of kainic acid, bicuculline or pentetrazole: Metabolic mapping using the deoxyglucose method with special reference to the pathology of epilepsy. Neuroscience 1981; 6: 1361–1391.

Bertalanffy von L. General System Theory. New York: George Braziller, 1969.

Bertram EH. Extrahippocampal lobe circuits in the temporal lobe epilepsy. Epilepsy & Behav 2014; 38: 13-18.

Bonilha L, Keller SS. Quantitative refractory temporal lobe epilepsy: relationship with surgical outcomes. Quant Imaging Surg 2015; 5 (2): 2014-224.

Boyce R, Leung LS. Loss of dendritic inhibition in the hippocampus after repeated early-life hyperthermic seizures in rats. Epilepsy Res 2013; 103(1): 62-72. doi: 10.1016/j.

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.

Clifford DB, Olney JW, Maniotis, A, Collins RC, Zorumski CF. The functional anatomy and pathology of lithium-pilocarpine and high-dose pilocarpine seizures. Neuroscience 1987; 23: 953–968.

Dubé C, Chen K, Eghbal-Ahmadi M, Brunson, K, Soltesz I, Baram TZ Prolonged febrile seizures in the immature rat model enhance hippocampal excitability long term. Ann Neurol 2000; 47 336–344.

Dubé C, Richichi C, Bender RA, Chung G, Litt B, Baram TZ. Temporal lobe epilepsy after experimental prolonged febrile seizures: Prospective analysis. Brain 2006; 129: 911–922.

Dubé CM, Ravizza T, Hamamura M, Zha Q, Keebaugh A, Fok K, Andres AL, Nalcioglu O, Obenaus A, Vezzani A et al. Epileptogenesis provoked by prolonged experimental febrile seizures: Mechanisms and biomarkers. J Neurosci 2010; 30: 7484–7494.

Dudek FE, Sutula TP. Epileptogenesis in the dentate gyrus: a critical perspective. Prog Brain Res 2007; 163:755-73.

Epsztein J, Milh M, Bihi RI, Jorquera I, Ben-Ari Y, Represa A, Crépel V. Ongoing epileptiform activity in the post-ischemic hippocampus is associated with a permanent shift of the excitatory-inhibitory synaptic balance in CA3 pyramidal neurons. J Neurosci 2006; 26(26):7082-92.

Fathollahi Y, Motamedi F, Semnanian S, Zardoshti M. Examination of persistent effects of repeated administration of pentylenetetrazol on rat hippocampal CA1: evidence from in vitro study on hippocampal slices. Brain Res 1997; 758(1-2): 92-8.

Flynn SP, Barriere S, Scott RC, Lenck-Santini PP, Holmes GL. Status Epilepticus Induced Spontaneous Dentate Gyrus Spikes: In Vivo Current Source Density Analysis. PLoS One 2015; 6; 10 (7): e0132630. doi: 10.1371/journal.pone.0132630.

Gilbert ME. Potentiation of inhibition with perforant path kindling: an NMDA-receptor dependent process. Brain Res 1991; 564(1):109-16.

Hadar E, Yang Y, Sayin Ü, Rutecki PA. Suppression of Pilocarpine-induced Ictal Oscilations in the Hippocampal Slice, Epilepsy Res 2002; 49: 61-71.

Hadar E, Yang Y, Sayin Ü, Rutecki PA. Suppression of Pilocarpine-induced Ictal Oscilations in the Hippocampal Slice, Epilepsy Res 2002; 49: 61-71.

Hartley T, Lever C, Burgess N, O'Keefe J. Space in the brain: how the hippocampal formation supports spatial cognition. Phil Trans Soc B 2014; 369: 20120510.

Hofmann G, Balgooyen L, Mattis J, Deisseroth K, Buckmaster PS. Hilar somatostatin interneuron loss reduces dentate gyrus inhibition in a mouse model of temporal lobe epilepsy. Epilepsia 2016; 57 (6): 977-83. doi: 10.1111/epi.13376.

Holmes GL, Sarkisian M, Ben-Ari Y, Chevassus-Au-Louis N. Mossy fiber sprouting after recurrent seizures during early development in rats. J Comp Neurol 1999; 404(4): 537-53.

Holmes GL, Gairsa JL, Chevassus-Au-Louis N, Ben-Ari Y. Consequences of neonatal seizures in the rat: morphological and behavioral effects. Ann Neurol 1998; 44(6): 845-57.

Ishihara K, Sasa M, Momiyama T, Ujihara H, Nakamura J, Serikawa T, Yamada J, Takaori S. Abnormal excitability of hippocampal CA3 pyramidal neurons of spontaneously epileptic rats (SER), a double mutant. Exp Neurol 1993; 119 (2): 287-90.

Jefferys, JGR. Advances in understanding basic mechanisms of epilepsy and seizures. Seizure 2010; 19: 638-646.

Kandratavicius L, Balista PA, Lopes-Aguiar C, Ruggiero RN, et al. Animal models of epilepsy: use and limitations. Neuropsych Disease & Treat 2014; 10: 1693–1705.

Knopp A, Frahm C, Fidzinski P, Witte OW, Behr J. Loss of GABAergic neurons in the subiculum and its functional implications in temporal lobe epilepsy. Brain 2008; 131(Pt 6): 1516-27. doi: 10.1093/brain/awn095.

Köhling R. Voltage-gated sodium channels in epilepsy. Epilepsia 2002; 43(11): 1278-95.

Long L, Xiao B, Feng L, Yi F, Li G, Li S, Mutasem MA, Chen S, Bi F, Li Y. Selective loss and axonal sprouting of GABAergic interneurons in the sclerotic hippocampus induced by LiCl-pilocarpine. Int J Neurosci 2011; 121(2): 69-85. doi: 10.3109/00207454.2010.530007

Loscher W. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure 2011; 20: 359-368.

Lothman EW Collins RC. Kainic acid induced limbic seizures: Metabolic, behavioral, electroencephalographic and neuropathological correlates. Brain Res 1981; 218; 299–318.

Luhmann N. Systems Theory. Malden, Trans. By Petern Gilgen. MA: Polity Press, 2013.

Lynch M, Sayin Ü, Golarai G, Sutula TP. NMDA-Receptor Dependent Plasticity of Granule Cell Firing in the Dentate Gyrus of Normal and Epileptic Rats. J Neurophysiol 2000; 84: 2868-2879.

Lynch M, Sayin Ü, Bownds J, Janumpalli S, Sutula TP. Long Term Consequences of Early Postnatal Seizures on Hippocampal Learning and Plasticity. European. J Neurosci 2000; 12: 2252-2264.

Mantegazza M, Curia G, Biagini G, Ragsdale DS, Avoli M. Voltage-gated sodium channels as therapeutic targets in epilepsy and other neurological disorders. Lancet Neurol 2010; 9(4): 413-24. doi: 10.1016/S1474-4422(10)70059-4.

Meisler MH, Kearney JA, Sprunger LK, MacDonald BT, Buchner DA, Escayg A. Mutations of voltage-gated sodium channels in movement disorders and epilepsy. Novartis Found Symp 2002; 241:72-81; discussion 82-6, 226-32.

Naylor DE, Wasterlain CG. GABA synapses and the rapid loss of inhibition to dentate gyrus granule cells after brief perforant-path stimulation. Epilepsia 2005; 46 Suppl 5: 142-7.

Psarropoulou C, Matsokis N, Angelatou F, Kostopoulos G. Pentylenetetrazol-induced seizures decrease gamma-aminobutyric acid-mediated recurrent inhibition and enhance adenosine-mediated depression. Epilepsia 1994; 35(1): 12-9.

Reddy DS, Kuruba R. Experimental Models of Status Epilepticus and Neuronal Injury for Evaluation of Therapeutic Interventions. Int J Mol Sci 2013; 14: 18284-18318.

Rutecki PA, Sayin Ü, Yang Y, and Hadar E. Determinants of Ictal Epileptiform Patterns in the Hippocampal Slice, Epilepsia 2002; 43 (Suppl. 5): 179-183.

Rutecki PA, Yang Y. Ictal epileptiform activity in the CA3 region of hippocampal slices produced by pilocarpine. J Neurophysiol 1998; 79(6): 3019-29.

Saly V, Andrew RD. CA3 neuron excitation and epileptiform discharge are sensitive to osmolality. J Neurophysiol 1993; 69(6): 2200-8.

Sanjay M, Neymotin SA, Krothapalli SB. Impaired dendritic inhibition leads to epileptic activity in a computer model of CA3. Hippocampus 2015; 25(11):1336-50.

Sayin Ü, Osting S, Hagen J, Rutecki PA, Sutula T. Spontaneous Seizures and Loss of AxoAxonic and Axo-Somatic Inhibition Induced by Repeated Brief Seizures in Kindled Rats. J Neuroscience 2003; 23 (7): 2759-2768.

Sayin Ü and Rutecki PA. Effects of Pilocarpine on the Paired Pulse Inhibition in the CA3 Region of the Rat Hippocampus, Brain Res 1997; 758: 136-142.

Sayin Ü and Rutecki PA. Group I Metabotropic Glutamate Receptor Activation Produces Prolonged Epileptiform Neuronal Synchronization and Alters Evoked Population Responses in the Hippocampus, Epilepsy Res 2003; 53: 186-195.

Sayin Ü, Sutula TP, and Stafstrom C. Seizures in the Developing Brain Cause Adverse Long-Term Effects on Spatial Learning and Anxiety. Epilepsia 2004; 45 (12) 1539-1548.

Sayin Ü, Rutecki PA, Sutula TP. NMDA Dependent Currents in Granule Cells of the Dentate Gyrus Contribute to the Induction but not the Permanence of Kindling J Neurophysiol 1999; 81 (2): 564-574.

Sayin Ü, Timmerman W, Westerink BHC. The Significance of Extracellular GABA in the Substantia Nigra of the Rats During Seizures and Anticonvulsant Treatment. Brain Res 1995; 669: 67-72.

Sayin HÜ. A short introduction to system theory: Indispensible postulate systems and basic structures of the systems in quantum physics, biology and neuroscience (Review). NeuroQuantology 2016; 1:126-142.

Sloviter, R.S. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. Science 1987; 235: 73–76.

Sloviter RS, Zappone CA, Harvey BD, Frotscher M. Kainic acid-induced recurrent mossy fiber innervation of dentate gyrus inhibitory interneurons: possible anatomical substrate of granule cell hyper-inhibition in chronically epileptic rats. J Comp Neurol 2006; 494(6): 944-60.

Song MY, Tian FF, Wang YZ, Huang X, Guo JL, Ding DX. Potential roles of the RGMa-FAK-Ras pathway in hippocampal mossy fiber sprouting in the pentylenetetrazole kindling model. Mol Med Rep 2015; 11(3):1738-44.

Sperk, G.; Lassmann, H.; Baran, H.; Kish, S.J.; Seitelberger, F.; Hornykiewicz, O. Kainic acid induced seizures: Neurochemical and histopathological changes. Neuroscience 1983; 10: 1301–1315.

Sperk, G.; Lassmann, H.; Baran, H.; Seitelberger, F.; Hornykiewicz, O. Kainic acid-induced seizures: Dose-relationship of behavioural, neurochemical and histopathological changes. Brain Res 1985; 338: 289–295.

Stief F, Zuschratter W, Hartmann K, Schmitz D, Draguhn A. Enhanced synaptic excitation-inhibition ratio in hippocampal interneurons of rats with temporal lobe epilepsy. Eur J Neurosci 2007; 25(2):519-28.

Stringer JL. Pentylenetetrazol causes polysynaptic responses to appear in the dentate gyrus. Neuroscience 1995; 68(2): 407-13.

Sun C, Mtchedlishvili Z, Bertram EH, Erisir A, Kapur J. Selective loss of dentate hilar interneurons contributes to reduced synaptic inhibition of granule cells in an electrical stimulation-based animal model of temporal lobe epilepsy. J Comp Neurol 2007; 500(5):876-93.

Sutula T, Zhang P, Lynch M, Sayin Ü, Golarai G and Rod R. Synaptic and axonal remodeling of mossy fibers in the hilus and supragranular region of the dentate gyrus in kainate treated rats. J Comp Neurol 1998; 390 (4): 578-594.

Sutula T, Harrison C, Steward O. Chronic epileptogenesis induced by kindling of the entorhinal cortex: The role of the dentate gyrus. Brain Res 1986; 385: 291–299.

Swartzwelder HS, Anderson WW, Wilson WA. Mechanism of electrographic seizure generation in the hippocampal slice in Mg^{2+}-free medium: the role of $GABA_A$ inhibition. Epilepsy Res 1988; 2(4): 239-45.

Tian FF, Zeng C, Guo TH, Chen Y, Chen JM, Ma YF, Fang J, Cai XF, Li FR, Wang XH, Huang WJ, Fu JJ, Dang J. Mossy fiber sprouting, hippocampal damage and spontaneous recurrent seizures in pentylenetetrazole kindling rat model. Acta Neurol Belg 2009; 109(4): 298-304.

Tilelli, C.Q.; Del Vecchio, F.; Fernandes, A.; Garcia-Cairasco, N. Different types of status epilepticus lead to different levels of brain damage in rats. Epilepsy Behav 2005; 7: 401–410.

Tuff LP, Racine RJ, Adamec R. The effects of kindling on GABA-mediated inhibition in the dentate gyrus of the rat. I. Paired-pulse depression. Brain Res 1983; 277(1):79-90.

Turski, W.A.; Cavalheiro, E.A.; Bortolotto, Z.A.; Mello, L.M.; Schwarz, M.; Turski, L. Seizures produced by pilocarpine in mice: A behavioral, electroencephalographic and morphological analysis. Brain Res 1984; 321: 237–253.

Westmark CJ, Gourronc FA, Bartleson VA, Sayin Ü., Bhattacharya S, Sutula T and Malter JS, HuR mRNA Ligands Expressed After Seizure, J Neuropath & Exp Neurol 2005; 64 (12): 1037-1045.

Yan HD, Ishihara K, Seki T, Hanaya R, Kurisu K, Arita K, Serikawa T, Sasa M. Inhibitory effects of levetiracetam on the high-voltage-activated L-type Ca^{2+} channels in hippocampal CA3 neurons of spontaneously epileptic rat (SER). Brain Res Bull 2013; 90: 142-8. doi: 10.1016/j.brainresbull.2012.10.006.