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TROPOMYOSIN RECEPTOR KINASE B (TRKB) BLOCKADE WITH LESTAURTINIB (CEP-701) TO ABORT STATUS EPILEPTICUS AND PREVENT ITS DETRIMENTAL BEHAVIORAL CONSEQUENCES

by
REEM MEHDI EL-JAMMAL

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submitted in partial fulfillment of the requirements
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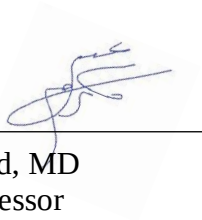

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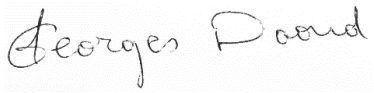

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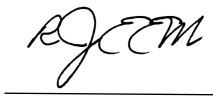

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ABSTRACT
OF THE THESIS OF

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Major: Neuroscience

Title: Tropomyosin Receptor Kinase B (TRKB) blockade with Lestaurtinib (CEP-701) to abort Status Epilepticus and prevent its detrimental behavioral consequences

Background: Convulsive status epilepticus (CSE), the most common pediatric neurological emergency is associated with long-term cognitive and psychiatric deficits. To date, no drug has been shown to be effective in preventing these detrimental consequences. In addition, the timely abortion of CSE is critical for the attenuation of its sequelae; however, this condition is resistant to anti-seizure drugs in up to 30% of cases. According to preliminary data in our laboratory, Lestaurtinib (CEP-701) modifies Tropomyosin receptor kinase B (TrkB) activity, increases phenobarbital's anticonvulsant efficacy against Kainic acid (KA)-induced CSE abortion, and inhibits CSE-induced depressive-like behaviors in the KA rat model of temporal lobe epilepsy (TLE). CEP-701 was tested as an adjuvant in a clinical therapy scenario for CSE in children, with the aim of aborting CSE and preventing its detrimental sequelae, namely the cognitive and emotional-behavioral abnormalities, as well as CSE-induced hippocampus neuronal damage.

Methods: In the short-term paradigm, CSE was induced with 0.5µg of intra-amygdalar (i.a.) KA in postnatal day 40 (P40) rats under electroencephalogram (EEG) monitoring. Diazepam (25 mg/kg) was administered 15 minutes post-CSE with either CEP-701 (15 mg/kg) or vehicle (DMSO) intra-peritoneally (i.p.). Another same dose of i.p. diazepam dose was injected 30 minutes post-CSE. A single dose of i.p. levetiracetam (750 mg/kg) was given 45 minutes post-CSE. Controls received intra-amygdalar saline, followed by CEP-701 or vehicle, and all received diazepam and levetiracetam. Rats were sacrificed 24 hors post-CSE, and brains were used for histological and molecular analyses. In the long-term paradigm, CSE was also induced with 0.5µg of intra-amygdalar KA in postnatal day 40 (P40) EEG-monitored rats. 15 minutes post-CSE onset, rats received either CEP-701 (15 mg/kg) or vehicle (DMSO) intraperitoneally (i.p.). Diazepam (50 mg/kg, i.p.) was given 2 hours post-CSE onset. The rats were subjected to 2 days of EEG monitoring post CSE induction and then sequentially subjected to the light dark test followed by open field test then the forced swim test was performed. The Morris water maze was then performed and then finally the modified active avoidance test. Post-behavioral testing, rats were subjected to continuous long-term EEG monitoring for 1 month and then were sacrificed at postnatal day P100 for further histological analyses.

Results: All rats experienced electroclinical seizures reaching Racine stages 4 following KA injections**.** Short-term outcomes revealed that the CEP-701 treated rats (SKCEP) had a statistically significant shorter seizure duration compared to the vehicle treated rats (SKV) ($p<0.05$) (SKV: 15.88±1.331 hours versus SKCEP: 8.644 ±1.413 hours). The SKV and SKCEP groups had comparable hippocampal neuronal densities and both were lower than their respective controls. In the long-term paradigm, the small number of rats per group was a limitation for performing statistical analyses, but compared to their control group (LSV), there were trends for the vehicle treated rats (LKV) post-CSE to have increased immobility in the FST test, increased time spent in the periphery in the OFT, and contextual deficits in the MWM and MAAV tests. The CEP-701 treated rats (LKCEP) that underwent CSE, had lower immobility than vehicle treated rats in the FST, and were comparable to controls. CEP-701 treated rats post-CSE were comparable to vehicle treated rats in the rest of the tests.

Conclusion: We show that CEP-701 enhances the anti-seizure effect of standard medications to abort CSE, but does not alleviate CSE-induced hippocampal damage. As far as CSE-induced behavioral sequelae, CEP-701 may reverse the depressive-like behaviors. Further work remains to be done to fully investigate the outcomes of the long-term experimental paradigms by increasing the number of rats per group. Given its human safety profile, CEP-701 is a promising drug to the standard clinical paradigms and can pave the way for future clinical trials, and thus plays a role as an adjuvant anti-seizure medication.

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ABBREVIATIONS

SE	Status Epilepticus
TLE	Temporal Lobe Epilepsy
CSE	Convulsive Status Epilepticus
CEP-701	Lestaurtinib
BBB	Blood Brain Barrier
EEG	Electroencephalography
E/I	Excitation/Inhibition
ILAE	Institutional League Against Epilepsy
NCSE	Non-Convulsive Status Epilepticus
AED	Anti-Epileptic Drug
DZP	Diazepam
IV	Intra-venous
BLa	Basolateral Nucleus
LTP	Long-Term Potentiation
DG	Dentate Gyrus
ERC	Entorhinal Cortex
MTLMS	Medial Temporal Lobe Memory System
KA	Kainic Acid
FST	Forced Swim Test
LDT	Light-Dark Test
OFT	Open Field Test
MWM	Morris Water Maze
MAAV	Modified Active Avoidance Test

CS	Conditioned Stimulus
BDNF	Brain-Derived Neurotrophic Factor
AMPA	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid
CREB	cAMP Response Element Binding Protein
ICER	Inducible cAMP Early Repressor
Egr3	Early Growth Response Factor 3
EPSPs	Excitatory Synaptic Potentials
PLC-γ	Phospholipase C- γ
PI3K	Phosphatidylinositol 3-kinase
PDS	Paroxysmal Depolarizing Shift
CCCs	Cation-Chloride Cotransporters
WNK	With-no-lysine kinase
OSR1	Oxidative stress-responsive kinase1
FRS	FGF Receptor Substrate
HS	Hippocampal Sclerosis
GCD	Granule Cell Dispersion
MF	Mossy Fiber
MFS	Mossy Fiber Sprouting
ES	Electroshock-induced seizures
AML	Acute Myeloid Leukemia
PKC	Protein Kinase C
JAK	Janus Kinase
STAT	Signal Transducer and Transcription Activators
MAPK	Mitogen-Activated Protein Kinase

CHAPTER 1
INTRODUCTION

The brain is an obscured structure with complex neuronal networks. The human brain's functioning, as well as its implications for brain architecture, remains a mystery: the non-trivial link between structure and function. Because of the accelerating pace of research, scientists are becoming able to discover the secrets and pathways of the brain. It is considered as a controller of the behavior, interpreter of the senses, initiator of body movements and a seat for intelligence (1).

The healthy brain is a dynamic system that works in its physiological multidimensional zone, most of the time, within certain limits. To maintain the neuronal network activity within physiological boundaries; neuronal excitability and synaptic strength are homeostatically controlled (2). Homeostatic plasticity can function on a number of different sub-cellular signaling cascades that also evolve in order to comply with the regulatory requirements of the network with minimal disruption to its functions (3). The failure of the homeostatic plasticity leading to hyperexcitability and excessive abnormal activity of the neuronal network is a key pathophysiological feature in many neurological diseases, one of which is Epilepsy (4,5).

Epilepsy is a chronic condition affecting the central nervous system that exhibit repetitive seizures, which are transient irregular brain synchronizations that interfere with the normal activity pattern of the brain (electrographic seizure) and may manifest with a number of signs and symptoms (electroclinical seizure) such as subjective sensations or involuntary muscle activity, resulting in rhythmic-clonic or tonic muscle stiffening in certain instances, known as convulsions (6). When the recurrent seizure

lasts more than 5 minutes without going back to the baseline or with continuous clinical and/or electrographic seizure activity, the condition is known to be as a status epilepticus condition (7).

Status epilepticus (SE) is an urgent neurological condition and a life-threatening emergency that may become refractory and causes neuronal death after 30 minutes (8). Epilepsy is associated with a disturbance of brain function, mostly in the brain area from which it originates, in addition to potential severe physical injury and an overall poor quality of life. The most common type of epilepsy in adults is the Temporal Lobe Epilepsy (TLE) which is a condition of persistent neuronal hyperexcitability and hypersynchrony, expressed as repeated unprovoked complex focal seizures within the temporal lobe origin (9).

In fact, the medial temporal lobe structures as the amygdala and hippocampus interact together to form the amygdalohippocampal circuit and play a role in emotion-associated memory and affect various cognitive processes as learning (10). Thus, any disruption in this circuit will lead to seizures as well as memory loss, behavioral abnormalities, cognitive deficits especially in spatial learning and psychiatric disorders with up to 40% risk of anxiety and depression (11,12). Patients with TLE experience these comorbidities the most.

There are no known effective therapeutic agents that can prevent seizures occurring after the Convulsive Status Epilepticus (CSE). In addition, in 35% of cases, existing medications fail to abort CSE in the emergency room (13). TLE is known to be the most common pharmacoresistant type of epilepsy (14). Thus, new medications are urgently needed to strengthen CSE abortion and potentially avoid its long-term

sequelae. Hence, there is an interest in an alternative approach that could become a possible therapy.

Lestaurtinib (CEP-701) is an FDA approved chemotherapeutic drug, which crosses the Blood Brain Barrier (BBB) and function as a tyrosine kinase inhibitor (15). CEP-701 reverses SE-induced depressive-like behavior, according to published and ongoing research in our lab, which supports the growing connection between the roles of tropomyosin receptor kinase B (TrkB) in seizure models (16). Therefore, we tested whether CEP-701 could reduce the effects of CSE in a kainic acid (KA)-induced TLE peri-adolescent model by blocking TrkB. Many of the electrophysiological, cognitive, psychiatric, and pathological changes seen in TLE patients may be recapitulated using this model (11).

1.1. Epilepsy: Recurrent seizures and neurodevelopmental deficiencies

A healthy brain is a dynamic structure controlled by the homeostatic plasticity to maintain the neuronal network activity within the physiological boundaries (2). This plasticity evolves in order to comply with the regulatory requirements of the network with minimal disruption to its functions (3). The failure of the homeostatic plasticity to maintain the network's activity within the target physiological limit due to the failure of cellular and/or molecular mechanisms, leads to hyperexcitability and excessive abnormal activity of the neuronal network that is a key pathophysiological feature in many neurological diseases, one of which is Epilepsy (4,5).

Epilepsy is often a progressive brain condition that occurs due to the perturbation of the neuronal activity which causes recurrent and unprovoked seizures and affects people of all ages (17). There is still controversy about the exact mechanism

by which seizures occur, but it is generally speculated that some circuits become hyperactive (18). Seizures are excessive or synchronous neuronal firings in the brain due to the disruption of the balance between excitatory and inhibitory neurotransmission accompanied by abnormal involuntary movements or sensations (19). Worldwide, about 50 million people have epilepsy, making it one of the world's most common neurological disorders. Up to 70% of patients living with epilepsy are estimated to be able to live free of seizures if properly diagnosed and treated. With conventional medical care, the remaining 30% are intractable and often need surgical intervention (14).

Clinical manifestations, change in the electroencephalography (EEG) and disturbance in the mechanisms of electrophysiological homeostasis are common (20). Many modifications occur at the cellular or synaptic levels as epileptogenesis progresses, eventually contributing to the development of dysfunctional neuronal circuits with increased excitability that include loss of neuronal cells, gliosis, neurogenesis, synaptogenesis, excitatory and inhibitory cell signaling alterations (21).

The main excitatory neurotransmitter is glutamate while the main inhibitory neurotransmitter is GABA. In the development of epilepsy, the shift in the balance between glutamatergic and GABAergic neuronal functions plays an important role (14). If any physiological condition (i.e. seizure-induced sprouting or increased the connectivity between excitatory pyramidal neurons) increases the glutamatergic synaptic activity or ion currents mediating membrane depolarization, the excitation/inhibition (E/I) balance will tip towards hyperexcitation. Glutamate works on the Kainate, AMPA and NMDA ionotropic glutamate receptors. By allowing the influx of monovalent cations such as Na+ and K+, Kainate and AMPA receptors induce rapid

depolarization of neurons, while NMDA receptors effect slower depolarization by allowing the influx of divalent cations, mainly Ca2++ (22).

The partial-onset seizures typical of TLE, regardless of their origin, are reported as focal interictal epileptiform spikes or sharp waves originating at or near the seizure focus. Prolonged calcium-dependent conductance causes neuronal membrane depolarization during paroxysmal depolarization shift (PDS), resulting in threshold voltage and activation of sodium voltage-gated channels. Sodium influx decreases the resting potential and induces several sodium-dependent action potentials to be fired, accompanied by hyperpolarization with active sodium ion transport from the neurons. Synchronous PDS discharges fired by millions of neurons produce a focal interictal epileptiform spike that is electrophysiologically observable and manifests as partial seizures (23-25) (Figure 1). Moreover, any condition (i.e. seizure-induced loss of inhibitory interneurons) in which GABAergic synaptic inhibitory activity is diminished or ion currents mediating membrane hyperpolarization are strongly stimulated (i.e. outward K+ or inward Cl-flux) will tip the balance (26).

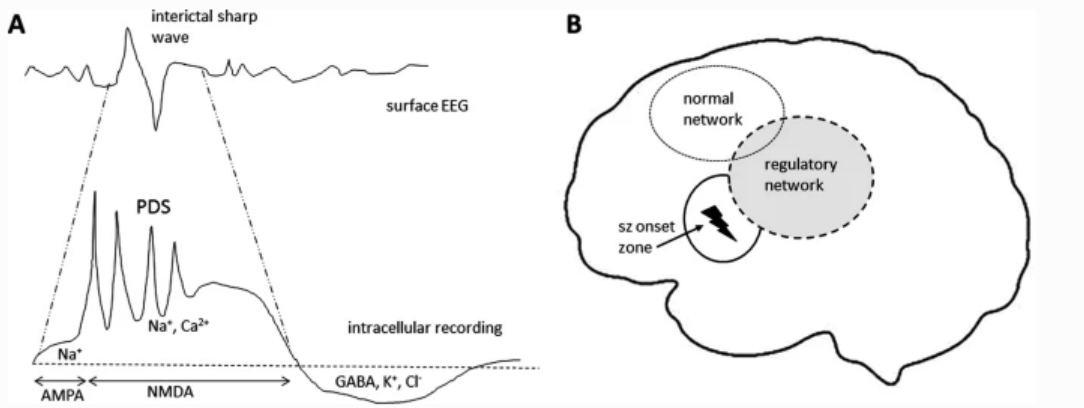


Figure 1. Focal seizure generation and regulation. Panel A. Shown is a surface EEG with interictal sharp wave and the paroxysmal depolarizing shift (PDS). The activation of AMPA receptors initiate the PDS, and this PDS plateau is maintained by NMDA receptors activation in addition to the conductance of sodium and calcium. PDS

termination occurs upon the activation of GABA-mediated inhibition and the conductance of potassium and chloride, resulting in membrane hyperpolarization. When a large number of neurons in a given area of the brain produce PDSs, the local inhibition can be overridden, resulting in a focal seizure. To prevent or stop a focal seizure, various antiseizure medications (ASDs) target each of these pathways. **Panel B.** According to the network theory of focal seizures, abnormal epileptic activity might start in the seizure onset zone or elsewhere, even in normal cortex. Regulatory networks regulate both the focus and normal cortex. To influence seizure initiation and propagation, these networks interact in complicated ways. Till now, no ASD has been created to target network function (217)

According to the Institutional League Against Epilepsy (ILAE), when the diagnosis by the clinician is determined to be Epilepsy, it is categorized according to multilevel classifications (27) (Figure 2). Starting with the seizure type, the classification of seizure begins with the determination of whether the seizure's initial manifestations are focal (partial) or generalized (Figure 3). It is possible to miss or obscure the onset, in which the seizure has an unknown onset. Focal seizures arise from one cerebral hemisphere and are subclassified as simple or complex based on the level of awareness of the individual's consciousness of himself and the environment during seizure. Simple focal seizures can be associated with symptoms that are sensory, somatosensory, autonomic, or psychological, but do not last more than one minute and do not cause loss of consciousness. Complex focal seizures, by comparison, are often associated with loss of consciousness and can last for a couple of minutes. Simple and complex partial seizures can spread and affect the other hemisphere also and eventually develop into secondary generalized seizures lasting little more than few minutes (28).

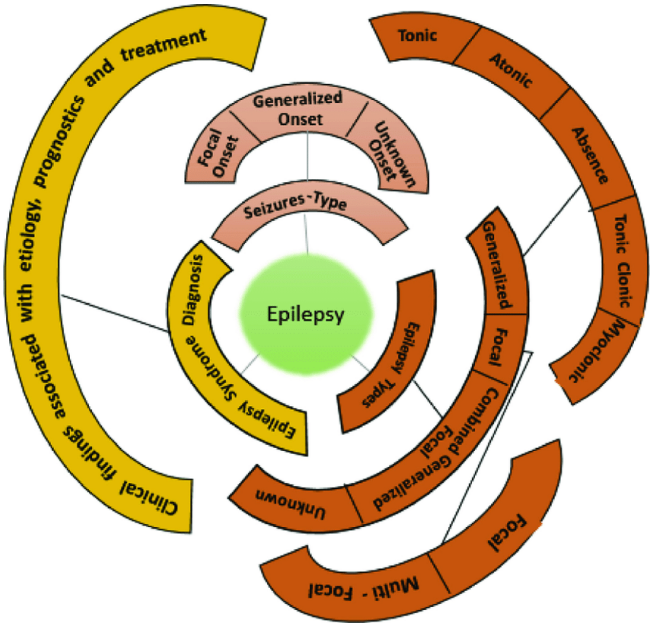


Figure 2. Epilepsy Classification. Multilevel classification of epilepsy according to the ILAE based on the seizure type, epilepsy type and epilepsy syndrome (212)

Furthermore, focal seizures are subgrouped at the onset as those with motor and non-motor signs and symptoms. If both motor and non-motor signs are present at the start of the seizure, unless non-motor (e.g. sensory) symptoms and signs are prominent, the motor signs will usually dominate. Focal to bilateral tonic-clonic reflects a seizure propagation pattern instead of a unitary type of seizure. Generalized seizures involve bilateral networks from the beginning. They split into seizures that are motor and non-motor (absence) (27). Primary generalized seizures arise from paroxysmal discharges in both hemispheres that can manifest as tonic-clonic seizures with loss of consciousness (grand mal seizures) or generalized tonic or clonic seizures with or without loss of consciousness (22).

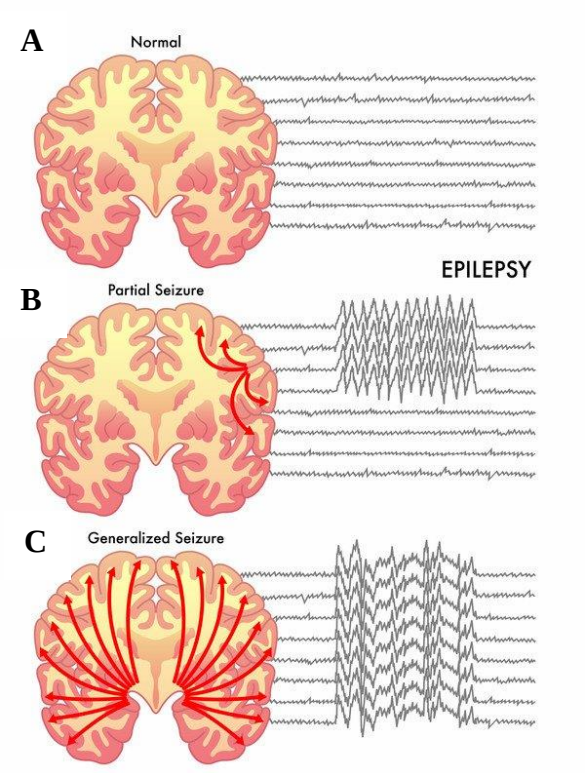


Figure 3. Schematic illustration of the healthy brain and seizure types with the corresponding electrographic pattern. Panel A. Normal healthy brain with baseline activity. **Panel B.** Focal seizures arising in one hemisphere and showing fast rhythmic spikes. **Panel C**. Generalized seizures are characterized by an abnormal neuronal firing that simultaneously recruits networks in both hemispheres at seizure onset and show fast bilateral spike and wave patterns. If seizure onset is focal (Panel B) and then spreads to the contralateral hemisphere (Panel C), it is classified as a secondary generalized seizure. However, when both hemispheres are affected at onset, the phenomenon is termed a primary generalized seizure.

Once the clinician has thoroughly diagnosed the type of seizure, his next step is to classify the epilepsy type. Types can be classified to focal, generalized, combined focal and generalized, or unknown. Focal epilepsy may be subdivided into unifocal or multifocal disorders. Generalized epilepsy shows spike-wave forms on the EEG. Patients with combined epilepsy have both focal and generalized seizure. But when epilepsy type cannot be determined, the epilepsy is of unknown type. Then epilepsy syndrome determination forms the third step of classification. It is used to describe a

series of signs and symptoms that characterize a particular diagnosis of epilepsy and integrates EEG recordings, seizure types and imaging features that occur together (28).

While research has led to effective drugs for the treatment of various forms of seizure disorders, one of the most common types of focal acquired seizures that is resistant to antiseizure medications and necessitates surgical intervention is the TLE in adult patients (14).

1.2. Status Epilepticus

By triggering inhibitory pathways, seizures are usually self-terminated. The failure of these mechanisms will lead to prolonged and frequent seizures known as Status Epilepticus (SE) that will exacerbate TLE and require emergent and potent anesthetics (29). SE is characterized by progressive internalization of GABAA receptors, and benzodiazepine resistance in refractory models (30,31). The ILAE describes SE as a seizure that in most patients shows no clinical signs of arrest after a period that involves the vast majority of seizures of that type or repeated seizures, without interictal restart of the activity of the baseline CNS. Most studies provide a 30-minute period that can demarcate the transition point at which neuronal seizure-induced injury occurs, although operational descriptions with a duration of as little as 5 minutes have been proposed (32). According to the recommendations of the clinical trial, care should immediately start if seizures last for ~ 5 minutes (33).

1.2.1. Status epilepticus with the emphasis on CSE

Status epilepticus is divided according to the semiology into the **Convulsive Status Epilepticus (CSE)** which lead to long-term injury with convulsions (motor

symptoms) that may involve jerking motions, grunting sounds, drooling, rapid eye movements, tonic muscle stiffening or clonic rhythmic activity and the **Nonconvulsive Status Epilepticus (NCSE)** where subjects appear confused or look like they're daydreaming without obvious tonic-clonic activity. This may affect memory and language.

The CSE is a disorder arising either from the failure of the seizure termination mechanisms or from the initiation of mechanisms leading to abnormally prolonged seizures (after 5 minutes). It is a condition that, depending on the type and length of the seizures, may have long-term effects (after 30 minutes), including neuronal death, neuronal damage, and alteration of neuronal networks (34). Via clinical trials and animal model studies, the ILAE determined these 2 timepoints. The establishment of such timepoints relies on 2 characteristics, where seizures lasting more than 5 minutes are less likely to resolve on their own (35) and become more pharmaco-resistance as CSE continues with the lack of treatment (36). Thus, adequate and immediate CSE management is important during this critical period.

In animal models, CSE has been described as the period from the onset of seizures until it stops on its own or due to AED interference, most often diazepam (DZP). The CSE is not always constant in animal models, unlike in humans, and seizures fluctuate between stage 1 and 5 (Racine's scale) (37). CSE causes epileptogenesis which is the production and extension of tissues that are capable of generating spontaneous seizures, including epileptic disease development. Epileptogenesis occurs and progresses in humans or rodents over many years, and can interfere with normal neuronal growth and differentiation, and causes impairments in patients with epilepsy (14). In humans, a life-threatening medical emergency could be

induced if CSE lasts more than 30 minutes. Thus, quantitative examination of behavioral CSE and EEG in animal models can help to explain the effect of CSE severity on epileptogenesis (37).

CSE is correlated to both acute mortality and high morbidity rates. It is also correlated to psychological and cognitive issues, and the longer it lasts, the more dangerous the consequences become (38). As a result, the administration of medications that can reduce the severity of seizures and their possible manifestations could change the direction of CSE treatment.

1.2.2. Standard clinical treatment

The convulsive status epilepticus is a neurologic and medical emergency that needs urgent assessment and management by initiating successful therapy to stop an episode from worsening and prevent significant morbidity or mortality (39,40). 35-50 % of patients have partial or complete resistance to traditional medications, a disorder known as refractory CSE (38).

Assuming optimal treatment, three other key mortality determinants affect CSE: age, duration and etiology. Bimodal mortality is estimated by age at CSE time, with peak rates for very young and very old (41). CSE duration is the only theoretically modifiable mortality determinant that may be specifically modified with previous diagnosis as well as timely initiation and completion of therapy. Unfortunately, issues that are highly unique to a particular patient, such as transportation, intravenous (IV) access, availability of EEG, hemodynamic instability, and control of the airway may add crucial minutes, even hours, to the timing of initiation and efficacy of care. The most evident yet complex determinant of SE mortality is probably etiology.

SE activity will change and must be treated rapidly in a coordinated manner by administering the suitable medication (Figure 4), with simultaneous management of the airway, breathing and circulation upon monitoring the respiratory and heart rate, oxygen saturation and blood pressure (39,42). The main treatment method for patients with epilepsy arriving to the medical emergency is the AED, and it has been stated that around two thirds of epileptic seizures have been controlled by AEDs (43). AEDs do not cure or prevent epilepsy, but suppress seizures by targeting three main mechanisms: enhance GABA-mediated inhibitory neurotransmission, modulate voltage-gated ion channels and attenuate glutamate-mediated excitatory neurotransmission.

Figure 4. ED management of the Convulsive Status Epilepticus

Benzodiazepines (as diazepam and lorazepam) are the first-line AEDs of choice for emergent control (44) that can be given efficiently and rapidly. These drugs act at

the level of GABAA receptors by binding at the specific benzodiazepine active site, ensuring the entry of Cl- and hence membrane hyperpolarization. If seizures don't resolve after the first dose, a repeated dose may be administered after 5 minutes. IV administration is preferred, but if vascular access is not available, benzodiazepines may be administered via intramuscular, rectal, nasal or buccal routes (39).

Second-line therapy (as phenytoin, levetiracetam or valproate) is required for repetitive and continuous seizures which do not respond to benzodiazepines (39,45). The effectiveness and safety of these drugs do not vary significantly (46). Levetiracetam (trade name: Keppra®) pharmacological mechanism varies from other AEDs (47). It has a special binding receptor site, fast absorption, no known clinical cardiac effects, and is potentially a suitable candidate for use in drug-induced seizures (45,48). Its mechanisms of action are not fully understood, but it is thought to have inhibitory action by binding to the synaptic vesicle glycoprotein SV2a, that controls the release of neurotransmitters and the transport of vesicles in neurons. N-type calcium channels in hippocampal pyramidal cells and potassium-gated channels may also be inhibited (49,50). It is suggested that another recorded effect of levetiracetam works against GABA receptors negative modulators without binding to the receptors directly (51). In adults and children, phenytoin has a well-established efficacy by blocking the sodium voltage gated-channels (50). The pharmacological effects of valproic acid are seen in a variety of ways, such as by acting on CNS levels of GABA, blocking voltage-gated ion channels, and also by inhibiting histone deacetylase (52). Although Levetiracetam can be administered rapidly by IV infusion (5 min) compared to phenytoin (20 min), it has higher compatibility with the common IV fluids, and the risk of serious adverse events (cardiac arrhythmias, extravasation and hypotension) to occur is low (53). The EcLiPSE

and the ConSEPT trial did not show that Levetiracetam was faster than phenytoin in terminating CSE but suggested that it can be used as a first-choice drug in the second-line treatment (54,55).

The majority of CSE treatment protocols reported are based on a time schedule designed to handle seizures aggressively within the first hour in order to decide if continuous IV infusion control needs to be implemented in the second hour (40). Up to 30% of CSE patients fail to respond to two anti-seizure medications and are associated with a risk of neuronal death (13) which causes epilepticus to be treated as refractory status and requires more sedative medications to be given. The patient will be put in a medically induced coma upon the treatment with continuous infusion of an AED such as IV infusion of midazolam, pentobarbital, thiopental, or propofol (Due to the risk of propofol infusion syndrome, propofol infusions should not be used in children) (39).

The treatment of epilepsy has generally been far from satisfactory to date since most existing medications primarily counteract membrane proteins that control neuronal excitability and mediate epilepsy end-stage symptoms, i.e. the seizures themselves instead of addressing the primary signaling pathways that initially activate the various cellular and molecular downstream mechanisms mediating epilepsy (35).

1.2.3. CSE etiology

Regarding the etiology of seizures, there are several acquired and innate causes of the epileptic disorder. If the etiology is known, epilepsy is classified as symptomatic or of structural etiology that can be either due to structural abnormalities, CNS infections (meningitis and encephalitis), head trauma, metabolic abnormalities (hypoglycemia, hyponatremia), autoimmune disorders, hypoxia, genetics, vascular

diseases, drug toxicity or either traumatic brain injury (28,56). When the etiology is unknown, epilepsy is known to be as cryptogenic.

1.3. The TLE Syndrome: A pharmacoresistant focal epilepsy type

1.3.1. Refractory seizures

The temporal lobe is the brain region that processes memories and sounds, interprets vision, creates speech, understands language, and regulates certain unconscious/automatic reactions such as hunger, thirst, fight-or-flight, emotions, and sexual excitement (57). TLE is a disorder characterized by frequent, unprovoked seizures emanating from the medial or lateral temporal lobe. It is the most common type of the focal acquired seizures, and is often refractory to the used AED. Two-thirds of the patients with intractable TLE require surgical interventions to become seizure free (58).

Another distinctive attribute of TLE is its semiology. Typical auras such as rising epigastric sensations, déjà vu, affective phenomena (fear or sadness), or experiential phenomena accompanied by unilateral motor signs (often ipsilateral face or mouth contraction, head deviation) and bilateral motor phenomena in the face or axial muscles are the main semiological characteristics of TLE. In addition, oral automatisms and behavioral arrests are common. The bitemporal spread signals modifications in consciousness, amnesia, autonomic phenomena (change in heart and respiratory rates), and prominent motor automatisms (tonic and dystonic posturing) (59).

1.3.2. Psychiatric and cognitive detrimental consequences

In spite of treatment with AEDs that regulate behavioral convulsions, but not EEG convulsions, sustained CSE for more than 30 minutes typically causes high mortality or results in widespread brain damage that leads to devastating cognitive deficiency, deficits in neurodevelopment, cerebral palsy, psychiatric and emotional comorbidities and increased risk of epilepsy (37,38). Cognitive and emotional behavioral disturbances are the most common comorbidities, in addition to pharmacoresistant seizures, of both acquired and genetic epileptic disorders where patients with epilepsy are at a high 60% risk of these complications (60).

The most frequent psychiatric comorbidities in TLE patients include depression, panic attacks and anxiety disorders (61,62) where patients present with a high 40% risk of depression (12). The quality of life of patients with epilepsy can be more affected than the seizures themselves and they are at a great risk of psychological disorders such as suicide. Stress can also increase the risk of the development of epilepsy, particularly when encountered chronically or early in life (63).

In TLE, cognitive output, learning and long-term memory are frequently impacted as the epileptogenic focus is situated in the vicinity of one of the major memory-related brain regions (66). Learning disabilities are most frequently in the form of reading disabilities that occur in up to 30% in children with TLE (67). These cognitive impairments are affected by age and duration of illness in such a way that patients with childhood onset of TLE are characterized with poorer cognitive performance and children with seizure onset before 5 years of age are associated with lower IQ (14). Adults with severe TLE show hippocampal sclerosis that is linked to memory problems. Infant-onset TLE patients have a decreased overall amount of white matter associated

with lower cognitive status and learning disabilities (14,68,69). Other cognitive disabilities include poor performance in arithmetic, word recognition and spelling in addition to problems in attention and coordination (67,70,71). Language disturbances associated with temporal lobe seizures can lead to both dyslexia and aphasia (expressive, receptive and global) (72). KA-induced temporal lobe epileptogenic lesions are associated with behavioral problems, including aggression and hyperactivity, that indicate how active epileptogenic mechanisms can lead to the behavioral deficits found in patients with epilepsy, along with the impact of seizures and antiepileptic medications (73).

In order to assess the severity of CSE, continuous Video-EEG recording and/or careful direct observation allows it easier to measure the exact period of various phases of convulsive seizures (37).

1.4. Amygdalohippocampal Circuitry: main regulator in cognitive and emotional behaviors

The limbic system key components involved in emotion, learning, memory and complex behaviors are the amygdalar nuclear complex and the hippocampal region. The interconnections between these two components are complex and robust. While glutamatergic pyramidal cells in the amygdala and hippocampal region are well known as the primary mediators of interconnections between these regions, recent research suggests that long-range GABAergic projection neurons are also involved (74).

1.4.1. Amygdala

The amygdalar nuclear complex and hippocampal region are juxtaposed in the anterior part of the medial temporal lobe with the amygdala located just in front of the hippocampus in humans and nonhuman primates (74). The amygdalar nuclear complex is made up of over a dozen nuclei, each with its own set of extrinsic and intra-amygdalar connections (75). It contains three groups of nuclei: the cortical amygdalar nuclei (anterior, posterolateral, and posteromedial cortical nuclei, as well as the nucleus of the lateral olfactory tract), the basolateral amygdalar nuclear complex (BLC) (lateral, basolateral, and basomedial nuclei, as well as the amygdalohippocampal area) and the centromedial group (includes the medial and central nuclei) (Figure 5). The projection neurons of the cortical and basolateral nuclei, which make up about 85% of neurons, are glutamatergic pyramidal or pyramidal-like neurons with spiny dendrites, much like those in the cortex. GABAergic non-pyramidal neurons with spine-sparse dendrites make up the remaining neurons in the cortical and basolateral nuclei (76,77).

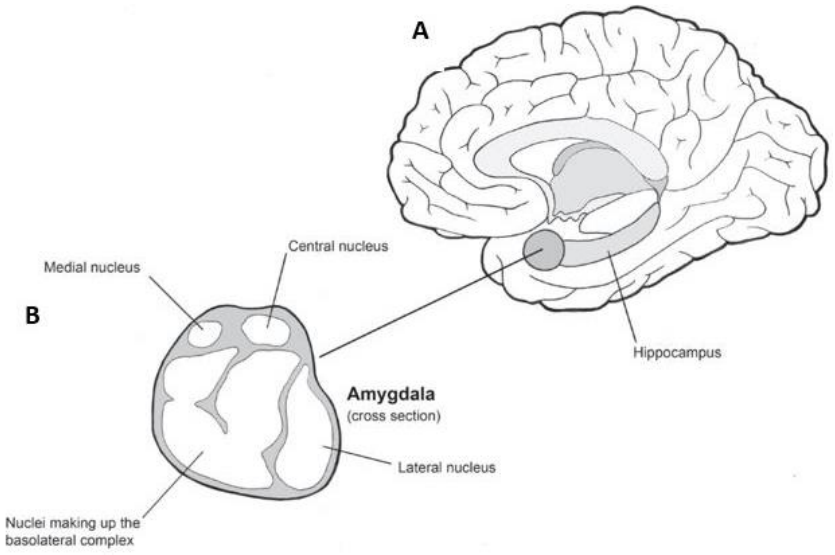


Figure 5.Schematic Midsagittal Section of the brain. Panel A. Shown is the amygdala and the hippocampus in the anterior part of the medial temporal lobe. **Panel B.** Cross section of the amygdala with its different nuclei (lateral nucleus, central nucleus, medial nucleus and the nuclei making up the basolateral complex) (214)

Since each amygdalar nucleus receives convergent inputs from a distinct set of sensory interaction areas, thalamic nuclei, as well as of intra-amygdalar connections, it appears that each amygdalar nucleus transmits distinct information to its hippocampal targets. This knowledge will pertain to the emotional salience of sensory stimuli in terms of emotional learning and memory, based on the connection of these stimuli with aversive stimuli or reward in the amygdala (74).

The basolateral amygdala, particularly the anterior portion of the basolateral nucleus (BLa), is well known for modulating the consolidation of memories of emotionally arousing experiences through projections to other brain regions, including the hippocampal area (78). Long-term potentiation (LTP) is a candidate memory formation process, and there is compelling evidence that amygdalohippocampal projections are involved in hippocampal LTP modulation in rats (79). BLa also plays a critical role in fear conditioning which is a form of behavioral associative learning in which participants learn to equate a conditioned stimulus with an unconditioned (painful) stimulus. It receives input from the lateral amygdala and sends it to subcortical sites that drive conditioned aversive behaviors (80).

1.4.2. Hippocampus

The human brain consists of 2 curved sea-horse shaped structures each known to be as a hippocampus. The hippocampal structure made up of dentate gyrus (DG), cornu ammonis (CA) fields CA1–CA4, and subiculum as well as the adjacent parahippocampal cortices that are made up of entorhinal cortex (ERC), perirhinal

cortex, postrhinal cortex, parasubiculum, and presubiculum are referred to as the hippocampal area (74).

The ERC, which is the "gateway to the hippocampal formation", receives input from the latter regions. The perforant pathway, which includes the efferents of the ERC to the hippocampal structure, targets the hippocampus, including the DG (Figure 6).

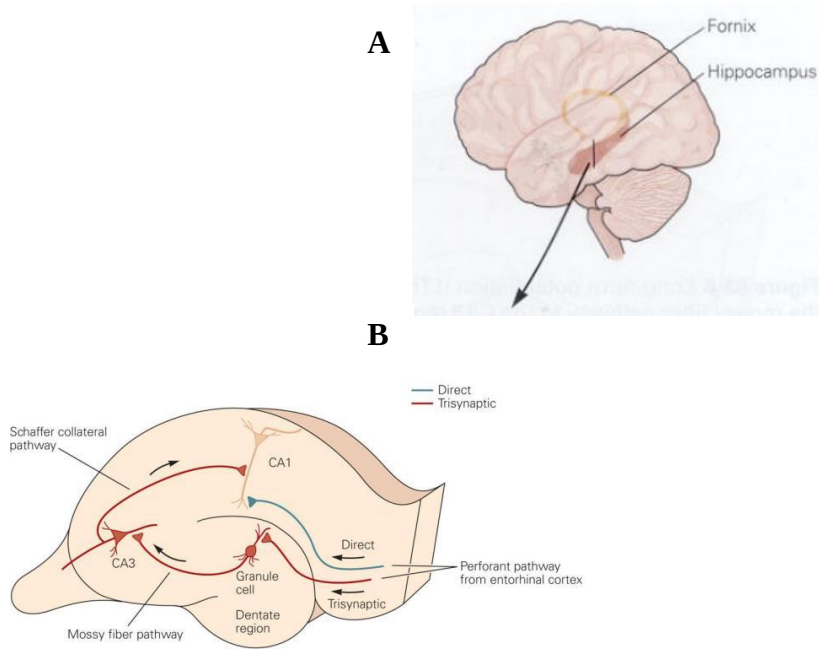


Figure 6. Illustration of the hippocampal neuronal circuitry. **Panel A.** A midsagittal section of the brain showing the location of the hippocampus in the medial region of the temporal lobe**. Panel B.** Pyramidal neurons of the CA1 region receive excitatory stimulations from the entorhinal cortex (direct) and from the Schaffer collaterals of the CA3 neurons that already received stimulations from the mossy fibers of the DG granule cells. The DG cells receive their stimulations from the entorhinal cortex (213).

The CA region has the pyramidal neurons while the DG has a granular cell layer. The CA4 layer is considered a part of the dentate gyrus and often referred to as the hilar region. The CA region connect to the DG and other brain areas through the Papez circuit that has a role in memory and function (81). Information is transmitted through the hippocampal region of all mammals through a network of connections that

includes the hippocampus's so-called trisynaptic circuit that involves three different groups of neurons, which are the granule cells of the DG and pyramidal cells of the CA1 and CA3 (82).

Dentate granule cells have polarized morphologies, with dendrites extending into the dentate molecular layer, and axons, called mossy fibers, projecting into the dentate hilus and CA3 pyramidal cell layer. Schaffer collaterals, CA3 axons, loop and extend towards the CA1 area and then extend to the subiculum that projects back to the ERC after information is processed by the hippocampus's classic trisynaptic circuit.

The hippocampal region's structures and circuits constitute the medial temporal lobe memory system (MTLMS) which is crucial for memory formation (77). The MTLMS and the amygdala have extensive, complex interconnections that are important for emotional memory. Polymodal association areas in the hippocampal region incorporate highly processed sensory input from all sensory modalities into complex configurational representations like context. CA1 and the subiculum, are the only sources of projections from the hippocampal structure to the amygdala (75) that receive outputs from the trisynaptic circuit. The ventral hippocampus, which corresponds to the anterior part of the primate hippocampus, is primarily responsible for stress and emotion whereas the dorsal hippocampus, which corresponds to the posterior part of the primate hippocampus, is primarily responsible for cognitive functions and spatial navigation (83,84). In the regulation of entorhinal-hippocampal circuitry in health and disease, dentate granule cells that are needed for normal learning and memory play a critical role and control the flow of information into the hippocampus (85).

Epochs of rhythmic and coordinated firing of large populations of neurons in both the amygdala and the hippocampus, result in currents that make up the EEG.

Synaptic interactions, like synaptic plasticity, are facilitated by synchronous oscillations in the amygdalohippocampal network, which generate recurrent "time windows." Rhythmic synchronous amygdalohippocampal oscillations have been shown to be essential for the consolidation of emotional memories, as well as fear conditioning and extinction (86,87).

1.4.3. TLE affecting hippocampal region

In adults, the most common form of seizures are the partial onset epilepsies and TLE is the most common form of partial epilepsy (88). Since partial resection of the temporal lobe, including the hippocampus and amygdala, virtually removes seizures in more than 80% of patients, it appears that most cases of TLE include dysregulation of the amygdalohippocampal activity (89). In addition to contributing to seizures, dysfunction of these systems in TLE patients is responsible for cognitive deficits and deficits in declarative and spatial memory (90,91). Deficits in emotional enhancement of declarative memory is due to the specific deficits in the emotion-driven encoding enhancement mediated by the amygdala-hippocampus loop (92). Seizure activity in one area quickly attracts seizure activity in the other. Neuronal loss in the hippocampus and amygdala is linked to this abnormal neuronal activity (93). Added to that, altered connectivity of granule cells can contribute to limbic epilepsy hyperexcitability (85).

1.4.4. Common behavioral tests for the assessment of the function of this circuit

Cooperation between the BLa and the hippocampus is essential for contextual emotional memory. The amygdala and dorsal hippocampal circuits are thought to handle the emotional and spatial aspects of an experience, respectively (94-96).

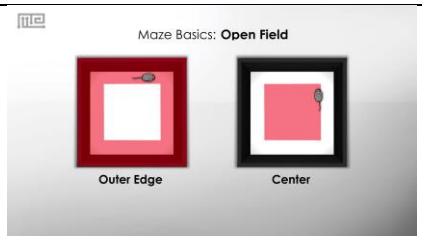
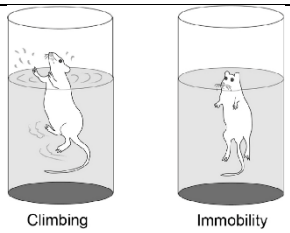
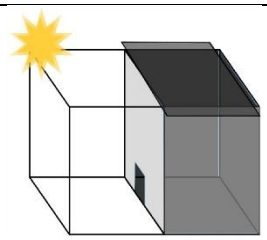
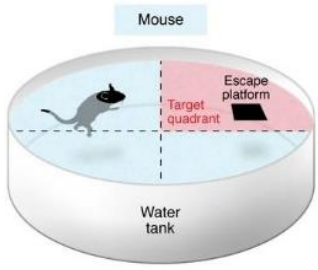
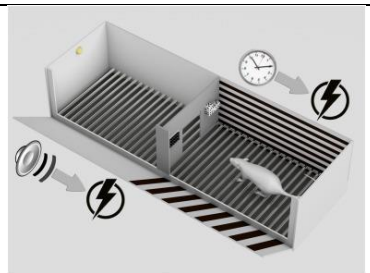
Memory loss and impaired-emotionally relevant learning are neurobehavioral deficits that can be studied in a controlled environment, where the effect and contribution of various factors to emotional and cognitive issues can be isolated and analyzed using various molecular and histological techniques.

Comprehensive studies of hippocampus and amygdalar injury on long-term cognitive and emotional-behavioral features of CSE caused by KA or any other chemoconvulsant have been examined in TLE models by utilizing various behavioral tests that mimic human psychiatric and cognitive disorders which helped researchers understand the basis of such interactions. In the absence of the ability to examine feelings directly, researchers have concentrated on their non-subjective elements, creating experimental paradigms that enable measurable emotional behaviors in rodents for emotionally-relevant learning and memory, as well as adaptive behavior in new situations (97). Although there are certain variations between the brain circuitry of rodents and humans that cause innate versus learned responses to threat, a behavioral parallelism has been drawn (98). Behavioral tests are inherently complicated, and all of their components must be considered, including the interaction between the experimenter and the subject, the cost, the required effort, and, most crucially, the test's validity. There are hundreds of behavioral tests, some of which have been proven, and others which have not.

In the following are some of the behavioral tests that are valid and assess anxiety, depression, and emotionally-relevant learning and memory, which enable the appropriate translation of preclinical data to clinical scenarios (Table 1). Anxiety-like, exploratory, and hyperactive behaviors are assessed using the light-dark box test (LDT) and open field test (OFT). The forced swim test (FST) is used to investigate learned

despair, struggle behaviors, and depressive-like behaviors. The Morris water maze (MWM) is a visual-spatial navigational test. The modified active avoidance (MAAV) tests the ability to recognize auditory emotional cues as well as hippocampal-dependent contextual emotional cues, and the acquisition of learned adaptive shock-avoidance behaviors. MAAV concept is based on classical conditioning, which was first established in 1927 by Ivan Pavlov (99,100) and then expanded by B.F. Skinner to include instrumental (operant) avoidance conditioning (101). A conditioned stimulus (CS) such as contextual visual cues, lights, or more often a tone, and an aversive unconditioned stimulus (US) such as a painful electrical foot shock are used in the classical Pavlovian fear conditioning studies (101). The rodent often performs an innate defensive freezing response when re-exposed to the same CS, as it identifies the CS with the aversive stimuli (102). During the two-way active avoidance paradigm, the rodent learns to associate the CS with the US and shuttle between the chambers of the two-way shuttle box to prevent an incoming foot shock (avoid) or terminate an ongoing one (escape) (103). A generalized immobility of the subject's musculature, excluding those engaged in respiration, is characterized as freezing (102). Active avoidance is a sort of instrumental conditioning in which the rodent develops an anticipatory learnt adaptive behavioral response and learn to associate between the CS and the US to avoid or terminate an aversive stimulus such as walking over a platform, jumping over a barrier, or shuttling between compartments (98). Its importance lies from the fact that fear of painful foot-shock is an innate survival response (104).

Table 1. Common behavioral panels performed in our laboratory to study the role of the amygdalohippocampal circuit in epilepsy rodent models

Testing Panels	Test Illustrartion	Aim of the Test
Open Field Test (OFT)		To check for hyperactivity, exploratory and anxiety-like behaviors
Forced Swim Test (FST)		To check for depressive-like behaviors
Light-Dark Box Test (LDT)		To check for anxiety-like behaviors
Morris Water Maze (MWM)		To check for the visuospatial learning
Modified Active Avoidance Test (MAAV)		To check for the emotionally relevant learning of auditory and contextual cues and adaptive shock-avoidance behaviors

1.5. The potential culprit mechanisms underlying CSE pharmacoresistance and long-term sequelae

The molecular process underlying the production of TLE remains largely unknown. Increased excitability in epilepsy models is often followed by both increased excitatory synapse function and impaired inhibitory synapse function. The early changes are accompanied by changes in several factors essential to hippocampal synaptogenesis, learning and memory.

1.5.1. GABAA receptors structure and function

For proper cell membrane stability and neurologic function, an exquisite balance between the inhibitory neuronal transmission via GABA and excitatory neuronal transmission via glutamate is important. The main inhibitory control is provided by GABAergic neurons (105,106). It has been suggested that the spread of acute seizures, development and chronic manifestations of epileptiform activity are mostly due to alterations in GABAergic signaling, which include changes in the properties of interneurons and cells that become depolarized with reduced levels of inhibition in the cerebral cortex (106,108,109).

GABA acts on one of two forms of receptors: GABAA, that is a ligand-gated ion channel and GABAB, that is a G-protein-coupled receptor (110,111). Many researchers observed loss in subsets of hippocampal GABA neurons in animal epilepsy models and in the tissue of patients with TLE. The main sites of fast synaptic inhibition in the brain are GABAAR (112). Different subunits derived from different gene families (α1- α6, β1-β3, γ1-γ3, δ, ε, θ, π, ρ1- ρ3) form the GABA receptors. Five subunits enclosing the chloride channel constitute GABAA receptors with different

pharmacological and kinetic properties. The most preferred combination of these subunits contains two alpha, two β, and one γ. Benzodiazepines, that are sedative and antiseizure drugs, and GABA can bind on the modulatory domains of these subunits (113). CSE results in changes in the expression and membrane localization of several subunits of GABAAR (α1, α4, γ2) in the hippocampal dentate granule neurons that alter the pharmacological and physiological properties of the receptor (110,114,115). During CSE, the number of functional receptors on the post-synaptic membrane in the hippocampus decreases due to the decrease in the expression of specific subunits of the GABAAR that cause the gradual internalization and consequent erosion of inhibition of postsynaptic GABAARs (31,116). This explain the resistance to benzodiazepines that won't have a place to bind to which causes CSE to become more refractory.

BDNF is known to influence the trafficking of GABAAR and cell surface expression (117). GABAAR surface stability is maintained through its phosphorylation by protein kinases. The binding of BDNF on TrkB receptors, activates the TrkB/PI3K/ PKC pathway. PKC phosphorylates the serine residues 408 and 409 on the β3 and enhances the receptor function (119). In CSE, the phosphorylation of β3 subunit decreases. β3 subunit interaction with AP2 complex of clathrin-endocytotic machinery rises as a result which facilitate internalization (112). Also, NMDA receptor activation that occurs during SE and causes increased neuronal activity associated with seizures will cause an increase in the γ2 subunit internalization (120,121).

One of the pathways activated post- CSE and that alters the role of GABAR is the increased BDNF synthesis and TRKB activation which controls a variety of downstream pathways, including JAK/STAT, PKC, and MAPK. Transcriptional pathway activation sensors such as cAMP response element binding protein (CREB),

inducible cAMP early repressor (ICER), and early growth response factor 3 (Egr3) control the expression of α1 and α4 subunit genes in parallel (122). The activation of JAK causes its transphosphorylation leading to STAT protein phosphorylation which in turn increases ICER due to the presence of STAT-recognition element in the ICER promoter (123,124). ICER heterodimerizes with CREB which block CREB-induced transcription leading to decrease in α1 subunit (125,126). The increase in Egr3 expression due to activation of PKC and MAPK signaling pathways after SE induces the overexpression of α4 (127,128). In addition, the glia derived proinflammatory cytokine tumor necrosis factor alpha (TNF-alpha) induces GABAA receptor endocytosis and at the same time AMPA receptors exocytosis influencing the balance (129). Calcineurin mediates the dephosphorylation of GABAARs results in endocytosis of these receptors also (130).

1.5.2. AMPA and NMDA receptors structure and function

In the presence of chronic changes in synaptic inhibition, which are hypothesized to play a major pathological mechanism in epilepsy (131), excitatory connections in the hippocampus and other regions of the brain are crucial for epileptic activity generation. Fast excitatory synaptic potentials (EPSPs) are produced upon glutamate interaction with ionotropic glutamate receptors and are responsible for excitatory connectivity between pyramidal neurons and between these neurons and interneurons. EPSPs arriving at individual neurons add up to activate action potentials, and epileptic field potentials are formed due to synchronous EPSPs in groups of adjacent neurons.

Several forms of ionotropic receptors localized at the postsynaptic membrane mediate fast glutamatergic neurotransmission. At most excitatory synapses, AMPA and NMDA ionotropic receptors are the predominant type (132). AMPA receptors are formed from the combinations of the protein subunits GluA1, GluA2, GluA3, and GluA4 as tetramers. A particular function is played by the GluA2 subunit that makes AMPA receptors formed from this subunit impermeable for calcium (133). Most AMPA receptors are permeable to sodium and potassium. Functional NMDA receptors are made up of two GluN1 subunits along with either two GluN2 subunits or a combination of GluN2 and GluN3 subunits (134). NMDA receptors are permeable not only to sodium and potassium but also to calcium (135). Standard synaptic signaling and fast synaptic excitation is mediated by AMPA receptors.

Excitotoxicity due to excessive stimulation of glutamate receptors, results in pathologically increased concentrations of Ca2+ (136). After a seizure, the calcium influx has the ability to trigger a number of signaling cascades that have an influence on increasing the synaptic efficiency (135). Calcium activates phosphatases and kinases that modify the function of the ion channel and neurotransmitter receptor. The activity of PKC and calcium-calmodulin independent protein kinase II activity is increased within minutes after seizures, leading to an increase in phosphorylation on ser831 of GluR1 and ser880 on GluR2. The phosphorylation of ser845 of GluR1 is also increased by protein kinase A. This phosphorylation increases the channel conductance that lead to enhanced AMPAR mediated potentiation after seizures, and GluR2 subunit endocytosis (137). The endocytosis will enhance Ca2+ permeability through AMPAR furthermore and increase the excitotoxic vulnerability and cognitive impairments (136).

1.5.3. Maladaptive role of TRKB receptors

In the development of epilepsy, disturbances in the communication of dentate granule cells are thought to play a key role. Essential regulators of granule cell morphology are the BDNF and its TrkB receptor. The activation of the TrkB/BDNF receptor facilitates epileptogenesis due to status epilepticus and induce TLE (138,139). BDNF and TrkB receptor are involved in the control of neuronal growth, neuronal plasticity, learning, synaptic transmission and epilepsy development. BDNF depolarizes the neurons as fast as glutamate not only by activating TrkB (140), but also by improving the glutamatergic synaptic transmission (141). BDNF protein levels are increased in granule cells following seizures, and TrkB receptors are triggered in several models of limbic epileptogenesis in the mossy fiber pathway that are in direct relation to the severity of the seizure and supports the contribution of TrkB to the structural synaptic reorganization since changes in the mossy fiber pathway have been implicated in the maintenance of the hyperexcitable circuitry (142-144).

BDNF interaction with TrkB causes receptor dimerization, intracellular tyrosine residue transphosphorylation, and subsequent activation of the 3 major signaling pathways involving Ras/MAPK, phosphatidylinositol 3-kinase (PI3K), and C-γ phospholipase (PLCγ) (146). TrkB kinase activity involves the formation of LTP of excitatory synapses formed by mossy fiber axons of DG cells with CA3 cells (147). LTP contributes to limbic epileptogenesis and promotes the propagation of seizure activity across synaptic-coupled neuronal populations within and beyond the limbic system (148).

In the pathophysiology of several mood disorders, including drug addiction, depression and anxiety, BDNF and its receptor TrkB have emerged as key mediators

(146,149). The development of emotional-behavioral disturbances is linked to a disruption in TrkB signaling and abnormal synaptic plasticity (150). The light-dark box test shows that reversing TrkB activation after KA-induced CSE not only suppresses seizures but also reduces anxiety-like behaviors (138). Whether and how TrkB controls the structure of granule cells, however, is not fully known and is still under study.

This suggests that CSE-induced TrkB activation can play a role in behavioral and cognitive deficits in seizure models. These results corroborate the function of the TrkB pathway in anxiety and depression in animal models that also match our lab's published research on how CSE-induced chronic behavioral deficiencies are reversed with CEP-701-mediated TrkB blockade (16).

1.5.4. NKCC1 and KCC2 cotransporters structure and function

GABA activates chloride-permeable GABAAR and generates chloride ion (Cl-) flow, which is dependent on the postsynaptic neuron's intracellular ([Cl-]i). NKCC1 and KCC2 are two key regulators of GABA receptor activity in neurons, maintaining [Cl-]i homeostasis (151). Unknown reasons may disturb the balance between these two main secondary active transporters, NKCC1 and KCC2, resulting in inefficient GABA inhibition (152).

GABA can become depolarizing during epileptogenesis due to the persistent interictal-like activity in adult hippocampus slices that downregulates KCC2 and impair chloride extrusion necessary for GABAergic hyperpolarization. NKCC1 facilitates the uptake of Cl- into the cell, while KCC2 pumps Cl− across the plasma membrane out of the cell (151). 12 membrane-spanning segments, 6 extracellular loops, and intracellular

N- and C-terminals make up NKCC1 and KCC2. The positions of regulatory sequences, phosphorylation sites, and lengthy extracellular loops differ amongst them (153,154).

In mature adult neurons, higher KCC2 expression and lower NKCC1 expression result in a net Cl- influx. The disruption of this balance causes an increase in NKCC1 expression and a downregulation of KCC2 receptors that facilitate the entry of more Cl- into the cell resulting in a net Cl- outflow and consequent depolarization when GABA stimulates GABAARs (155,156) and remodeling of the excitatory glutamatergic neuronal circuits (157). This downregulation of KCC2 may play a crucial role in the pathogenesis of TLE (Figure 7).

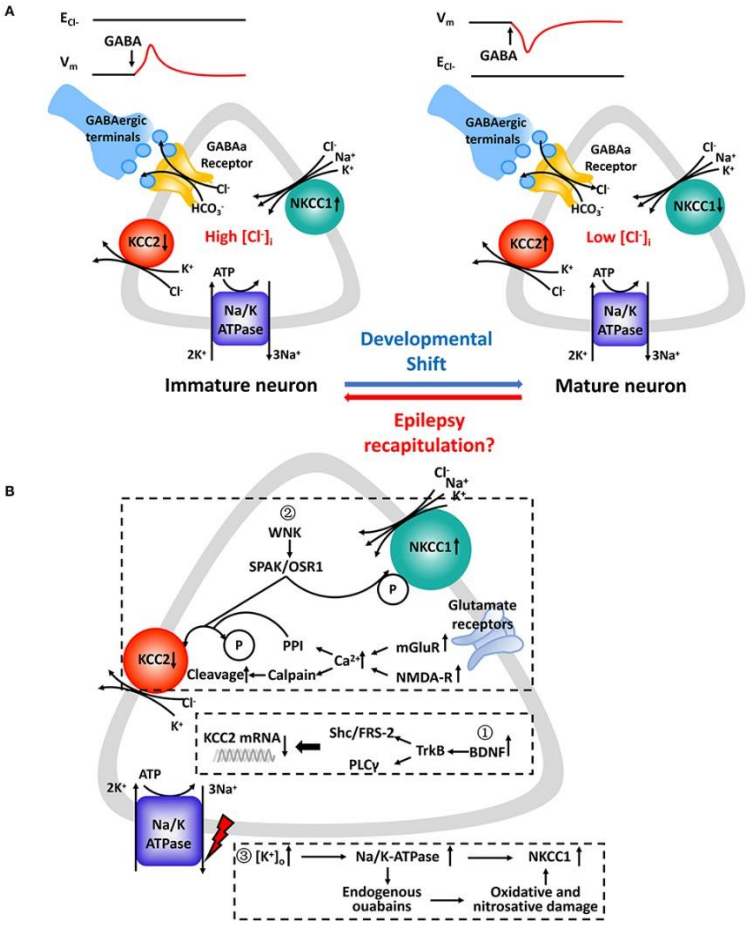


Figure 7. GABAA receptor-mediated responses in immature and mature adult CNS neurons are governed by chloride concentration regulating systems. Panel A. (Left): In parallel with the downregulation of KCC2 or in the absence of it, the upregulation of NKCC1 forms the primary regulator that mediates Cl^- uptake in

immature CNS neurons. (Right) In mature CNS neurons, KCC2 is the primary K-Cl cotransporter, with NKCC1 being subsequently downregulated. The Na+/K+-ATPase creates an electrochemical gradient of Na+ and K+, which helps NKCC1 and KCC2 transport Cl-. The value of ECl^- in relation to the membrane potential (Vm) is determined by the relative activity of NKCC1 and KCC2, as well as their opposing effects on $[Cl^-]i$. Upon the binding of GABA to GABAA receptors, ligand-gated Cl^- channels open that are permeable to HCO3−. At different phases of development, the expression profiles of NKCC1 and KCC2 change. **Panel B.** Neurons undergo "recapitulation" and "dedifferentiation" to some crucial and specific phases of early neural development in a variety of pathophysiologic disorders, such as epilepsy. Expression and functional abnormalities in NKCC1 and KCC2 are caused by molecular cascades. ① NKCC1 and KCC2 mRNA expression levels, ② protein levels, and③ identified regulatory pathways have all been found to be altered (218)

The molecular pathways that lead to unbalanced Cation-Chloride Cotransporters (CCCs) are still being investigated. During seizures, the hippocampus subiculum functions as a hub for synchronized firing to extend to other parts of the temporal lobe (158). First, endogenous BDNF and TrkB signaling pathways produce more BDNF, which binds to TrkB receptors and activates src homology domains containing transforming protein/FGF Receptor Substrate 2 (Shc/FRS-2) and PLC- γ that lowers the expression of KCC2 mRNA (151,159). KCC2 is downregulated also by Ca2+-dependent processes triggered by the buildup of Ca2+ from glutamate receptors. It also corresponds to the dephosphorylation of S940 by protein phosphatase 1 and the activation of the calcium-activated protease calpain, which is implicated in the cleavage of KCC2 (160-162).

Furthermore, phosphorylation of the KCC2 protein at Tyr 903/1087 causes enhanced lysosomal degradation of KCC2 (163). The phosphorylation of threonine residues T906 and T1007 occurs when the with-no-lysine kinase (WNK), SPS1-related proline/alanine-rich kinases (SPAK), or SPAK homolog oxidative stress-responsive kinase1 (OSR1) pathways are activated, resulting in decreased KCC2 activity and [Cl-]i

accumulation and EGABA change in a depolarizing direction. (164). NKCC1 is also phosphorylated by the WNK-SPAK/OSR1 pathway, which enhances NKCC1 activity (165).

1.5.5. Mossy fiber sprouting

Hippocampal Sclerosis (HS), which is the neuronal death of CA1, CA3 and CA4 pyramidal cells, is the most common pathological entity in TLE. DG cells undergo neuronal death to a smaller extent than pyramidal cells, but they do show two different phenomena: cell body migration, known as granule cell dispersion (GCD), and the growth and extension of granule cell axon, known as mossy fiber sprouting (MFS) (166). Mossy fibers (MF) usually extend to the hilus to project to the excitatory interneurons and inhibitory interneurons before synapsing onto CA3 pyramidal neurons through a tiny area called the stratum lucidum (167). In epileptic patients, due to the BDNF upregulation and the increased excitability of the DG cells, MF were observed to perform abnormal and extensive innervation within the dentate hilus to the inner molecular layer of the DG and lose their target cells in CA3 and CA4 (168,169) (Figure 8).

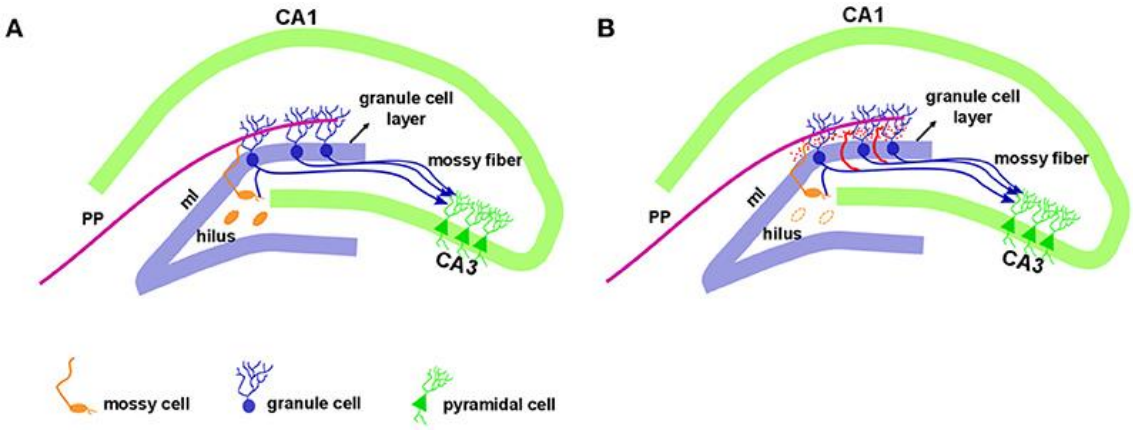


Figure 8. Hippocampal formation in a normal brain versus in an epileptic brain. **Panel A.** The dentate molecular layer (ml) of the hippocampus lies above the granule cell layer and is considered as a cell-free layer made up of the apical dendrites of granule cells. The entorhinal cortex sends information to the outer molecular layer via the perforant pathway (PP). In the normal brain, the mossy fibers of the granule cells extend to the hilus with projection to the mossy cells and CA3 neurons. The mossy cell axons project to the contralateral granule cell dendrites in the inner molecular layer. **Panel B.** In an epileptic brain, the mossy fibers lose their target in the hilus and sprout extensively to innervate the dentate inner molecular layer of the hippocampus, a phenomenon known to be as mossy fiber sprouting (illustrated in red) (167)

The vacancy of synaptic sites in granule cell proximal dendrites (170), induced by hilar cell loss after injury (171), and the downregulation of chemorepellents, such as Sema3A, have both been postulated to contribute to the extension of mossy fiber sprouts to the inner molecular layer (172). Sema3A, which is typically released by entorhinal axons projecting to the dentate molecular layer, interacts with a receptor complex made up of neuropinlin-1 and plexinA found in the dendrites of adult granular, hilar, and pyramidal cells, implying that this signaling pathway is active in the hippocampus formation but is lost after status epilepticus (173).

The prevalence and intensity of MFS are strongly connected with the number of spontaneous seizures, and the degree of cell loss (174). Although it is one of the most common TLE alterations, the extent of sprouting varies greatly ranging from extensive

to undetectable, which shows that it is not required for the development of this disorder (175).

1.6. Preclinical animal models

The relationship between the cognitive and emotional behavioral disturbances, psychiatric comorbidities and academic performance, intellectual ability and anti-seizure medication burden of the TLE has received considerable attention from researchers. Via well-established preclinical animal models, comprehensive studies have tested the electrographic, behavioral and pathological effects of CSE.

Animal models with proven face validity have been used to study the impact of hippocampal seizures on learning, memory and emotions as they recapitulate the clinicopathological findings seen in patients with temporal lobe seizures in a controlled environment (11,176,177). Post-CSE models earned the greatest acceptance of the animal models produced to investigate the pathogenesis of TLE since they are distinguished by a latency period, the development of recurrent spontaneous seizures, and a variety of lesions such as those of TLE (22). The choice of the model depends on practicality, availability, and efficacy.

1.6.1. Chemoconvulsant animal model

Seizures may be triggered by many chemoconvulsants that boost glutamatergic neurotransmission or block GABAergic inhibition, while improving cholinergic neurotransmission that can cause seizures by cholinergic hyperactivation and induce CSE. Injected rodents exhibit recurrent seizures with extraordinary histopathological

correlates of hippocampal sclerosis, normally secondarily generalized and of variable frequency (178).

The most commonly used chemicals are the kainic acid and pilocarpine that mimic some phenomenological characteristics of human TLE (179). In both models, neuropathological changes such as neuronal loss in many hippocampal subfields (180) and reorganization of mossy fibers into the fascia dentate molecular layer are observed and function as an anatomical substrate for epileptogenesis (181). However, as a muscarinic acetylcholine receptor agonist, systemic application of both kainic acid and pilocarpine often causes damage in neocortical regions and may represent human TLE pathology trends that extend to regions adjacent to the hippocampal and amygdaloid regions (22,182). Due to time constraints and cost, animal models of chronic epilepsy are not commonly used (183).

1.6.1.1. Kainic Acid

To cause prolonged neuronal depolarization and epileptic attacks in rodents with a damage focus inside the hippocampal structure, KA, an L-glutamate analogue that acts by activating ionotropic kainate receptors and glutamate AMPA receptors, is usually administered systemically, intra-hippocampal or intra-amygdalar (184). KA cause sustained neuronal depolarization that leads to the generation of seizures clinically characterized by changes in physical activity, stereotypic grooming, ‘wet dog’ shakes and continuous clonus that helped in the understanding of the pharmacological, molecular and cellular processes underlying epileptogenesis and ictogenesis (22). Adult rats were also reported to develop severe extra-hippocampal damage after systemic acid-induced CSE (185). Intra-amygdalar KA injections are not only appropriate for

practical purposes, but also for localizing the insult directly to the amygdala. Therefore, it is important to examine whether the changes are attributable to epilepsy rather than the chemo-convulsive agent in other brain areas. The contralateral side of the injection may be viewed as 'epileptic' regulation since it lacks the pattern of ipsilateral damage and represents a possible onset of seizures at the same time (186). KA models are simple and easily reproducible, and the resulting CSE is electrographically similar to that seen in humans (187). However, one of the constraints of KA is that it is variably responsive to rats of various strains, weights, sex and age (188).

1.6.1.2. Pilocarpine

Pilocarpine is an agonist of the muscarinic acetylcholine receptor and like KA, particularly in combination with lithium, represents many clinical and morphological aspects of TLE in rodents (22). Lithium administration subcutaneously before pilocarpine injection reduces the pilocarpine dose needed and epilepsy will be produced more reliably (189). Pilocarpine can be administered locally either intracerebroventricularly or directly into the hippocampus. Sprouting of mossy fibers and spontaneous recurrent seizures are also observed in rats injected with intrahippocampal-pilocarpine with near zero mortality (190). It can generate interictal activity in the subiculum and upregulate the neurotrophins in the hippocampus and neocortex of treated rats (191-193). Cognitive and memory deficits and aggression typically seen in TLE patients can be also observed in this model (194,195).

1.6.2. Electrical model

One of the first paradigms performed to study seizures and reproduce epileptogenic characteristics in the intact brain with low mortality and high reproducibility was electrical stimulation models. In addition, postictal changes from electrical stimulation can be studied when the epileptogenic cause is no longer present, unlike chemical-induced seizures (196,197). CSE induction could be done using low or high-intensity electrical stimulations applied to specific areas of the brain. The left basolateral amygdala is one of the triggered regions, whereby sustained pulsed-train electrical stimulation of high intensity induces CSE with subsequent neuronal harm (198). On the other hand, hippocampal electrical stimulation, in particular the stimulation of the perforating pathway for seizure induction, is of great concern (199). Among the most studied models of electrical stimulation are electroshock-induced seizures (ES). A single time electroshock is easily applied and does not require a stereotaxic electrode implant that involves whole-brain stimulation protocols (200). When used for chronic research, electrical stimulation protocols can be expensive and laborious (196).

1.6.3. Febrile seizure model

Thermal models consist primarily of increasing core body temperature or evoking febrile seizures. In children under 5 years of age, febrile seizures are the most common where fever provokes febrile seizures. Many complex methods have been used to model rodent fever. In one model, rats were treated with lipopolysaccharide, a bacterial endotoxin, that induces an immune response and raises the core temperature of immature rodents by 1°C (38). Another most widely used technique was to increase the

core temperature by heating the animal. A number of heating techniques have been used over the years, including hot water, infrared heat lamps, and warmed air streams. In these models, the core temperature of the animal increases and hence the brain temperature also increases, leading to hyperthermic seizures that enhance the limbic excitability and develop epilepsy (201,202). Such models are reproducible, with little to no neuronal loss observations.

1.7. CEP-701 as a promising drug for treatment

Lestaurtinib (CEP-701) is an oral indolocarbazole drug that functions as a nonspecific inhibitor to several receptor tyrosine kinases and inhibits ATP binding to the TRK kinase domain. Due to its favorable safety profile, it was used as a chemotherapeutic adjuvant in several cancer types mainly in acute myeloid leukemia (AML) (203,204). It is actively absorbed from the gastrointestinal tract and processed in the liver's P450 enzyme system with a half-life of 6.8 to 9.2 hours (203). Based on previous work done, CEP-701 reduces the risk of HS-induced seizures, most likely via inhibiting TrkB and attenuates short-term hyperexcitability (15).

Transient TrkB blockage with CEP-701 changes the inhibitory potency of GABAAR by preventing its internalization, which suppresses hyperactivity, according to previous work done in our lab. Because CEP-701 targets the TrkB pathway, if it proves to be protective against CSE, this FDA-approved drug might be easily repurposed for use in clinical trials on SE patients in addition to cancer patients.

CHAPTER 2

HYPOTHESES AND AIMS

CSE is a neurologic and medical emergency disorder associated with psychological and cognitive detrimental consequences and correlated to both high morbidity rates and acute mortality. Developing a novel adjuvant can enhance the standard clinical paradigm and improve prognosis. We propose to investigate CEP-701 as a potential treatment to terminate the consequences of CSE in the KA-induced temporal lobe epilepsy periadolescent rat model to pave the way for a clinical study that repurposes CEP-701 for use in epilepsy as delineated below.

Aim 1: To check the efficacy of CEP-701 in enhancing the timely abortion of CSE with standard medications and avert short-term hippocampal neuronal damage.

Hypothesis 1: Transient TrkB blockade post-KA-induced CSE with CEP-701 will promote the early abortion of SE with standard medications (One of the standard protocols used: Diazepam) and prevent the associated hippocampal neuronal loss.

Aim 2: To check the effects of blocking TrkB receptor with CEP-701 post KA-induced CSE (10 days post-CSE) on the emotional-behavioral deficits using testing paradigms for anxiety-like behavior (Light-dark box, Open field), and depressive-like behavior (Forced Swim Test).

Hypothesis 2: Transient TrkB blockade post-KA-induced CSE with CEP-701, will preserve normal brain plasticity resulting in appropriate adaptability to new environments, and therefore in normal emotional behaviors.

Aim 3: To check the effects of blocking TrkB receptor with CEP-701 post-KA-induced CSE (3 weeks post-CSE) on the cognitive deficits using testing paradigms for

amygdalohippocampal learning and memory (Morris Water Maze, Modified Active Avoidance).

Hypothesis 3: Transient TrkB blockade following KA-induced CSE with CEP-701, will prevent the recruitment of neuronal networks into abnormal epileptogenic brain circuitry, thus preserves normal brain plasticity resulting in improved learning and memory.

CHAPTER 3

MATERIALS AND METHODS

3.1. Animals and experimental paradigm

The Institutional Animal Care and Use Committee (IACUC) at the American University of Beirut (AUB) approved the animal care and behavioral studies. Postnatal day (P35) male Sprague Dawley rats housed in a temperature-controlled room (22°C) and maintained on a 12-hour light-dark cycle with permanent access to food and water were used in this study.

Two clinically-relevant experimental paradigms were applied, an acute and a long-term clinical paradigm, with four different groups used in each paradigm (KV: Vehicle administered following KA-induced CSE, KCEP: CEP-701 administered following KA-induced CSE, SV: Vehicle administered following saline, SCEP: CEP-701 administered following saline). Intra-amygdalar cannula implantation surgery was performed for these rats who subsequently rested for 5-6 days before CSE induction in both paradigms. Since TLE is associated with CSE (205), which is thought to begin in adolescence, KA inductions were carried out in the periadolescent stage (P40). CSE was elicited by injecting 0.5 μg of KA dissolved in 0.6 μl of normal saline (0.9%) into the amygdala (i.a). Using EEG and clinically based on Racine's scale (Racine, 1972), which is used to assess seizure intensity, CSE onset was determined. Rats that had progressed to the third stage of Racine's scale (forelimb clonus and rearing) were included in the study.

For the acute paradigm, following surgery and KA induction, EEG and clinically proven CSE onsets were recorded. Rats were progressively treated with a

CEP-701 dose (15 mg/kg) or DMSO and a diazepam dose (25 mg/kg) intraperitoneally (i.p.) 15 minutes post-CSE onset, followed by a second dose of diazepam 30 minutes post-CSE. Then, 45 minutes post-CSE, a dose of levetiracetam also known as Keppra (750 mg/kg i.p.) was administered (Figure 9).

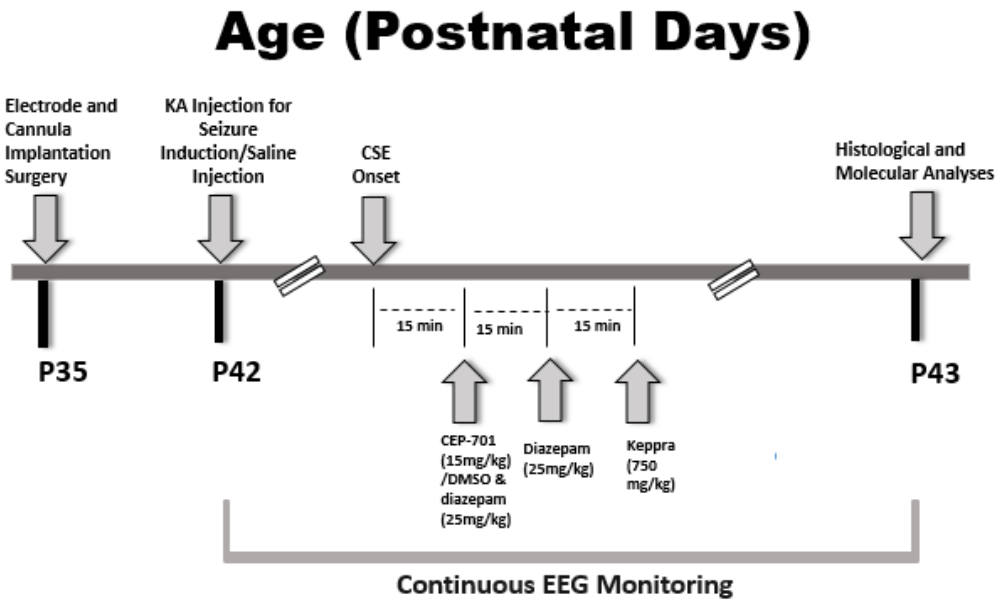


Figure 9. Short-Term Experimental Paradigm. Following the electrode and intra-amygdalar implantation surgery, P35 rats rested for 5 days before being connected to the EEG for KA-induced CSE administration or saline injection. Diazepam (25 mg/kg) first dosage was injected with CEP-701 (15 mg/kg) or with DMSO (vehicle) 15 min post KA induced-CSE onset then injected once again 30 min post-CSE. Keppra (750 mg/kg, i.p.) was then administered 45 minutes post-CSE. Rats were kept on the EEG for 24 hours and then were sacrificed for histological and molecular analysis.

CEP-701 was given 15 minutes after the CSE onset because, in a clinical scenario, 15 minutes after the onset of clinical symptoms that lead patients to the emergency room is a plausible estimate of the earliest opportunity to treat. Prior work in our lab was done on different doses of CEP-701 to finally choose the suitable dose (15 mg/kg) to be given.

Post CSE induction, rats were monitored on the EEG for 24 hours to determine the seizure duration. The identification of two-time points, CSE onset and CSE offset,

helped in determining the seizure duration. When spikes and abnormal fast activity occupied 50% of the tracing with the reemergence of the posterior dominant rhythm and intermixed slow waves, CSE offset was reached. Then perfusion was performed for histological and molecular analyses.

Rats were divided into four groups, respectively:

1-SKCEP group (Short Term Kainic Acid CEP): CEP-701 was given (i.p.) following KA-induced CSE (i.a.)

2-SKV group (Short Term Kainic Acid Vehicle): Vehicle (DMSO) was given (i.p.) following KA-induced CSE (i.a.)

3-SSCEP group (Short Term Saline CEP): CEP-701 was given (i.p.) following saline (i.a.)

4-SSV group (Short Term Saline Vehicle): Vehicle (DMSO) was given (i.p.) following saline (i.a.)

CEP-701 or DMSO injections for the control saline groups (SSCEP and SSV) were matched for the kainic acid groups (SKCEP and SKV) respectively. Because KA was dissolved in saline, and in order to be sure that saline by itself doesn't cause hyperexcitability or damage, saline was chosen to be injected for the control groups that don't undergo CSE.

For the long-term paradigm, following surgery and KA induction, EEG and clinically proven CSE onsets were recorded. Rats were treated with a CEP-701 dose (15 mg/kg) or DMSO intraperitoneally 15 minutes post-CSE onset, followed by a diazepam dose (50 mg/kg, i.p) 2 hours post-CSE onset. Due to high mortality rate in the first batch of rats because of the CSE severity, diazepam was then used in 35 mg/kg dose in the second batch. EEG monitoring continued for 2 days' post CSE induction and then

after 1 week, rats were subjected to different behavioral testing panels. Post-behavior, rats were then monitored at the EEG to check for any recurrent seizures and then perfusion was done for histological analyses at P100 (Figure 10).

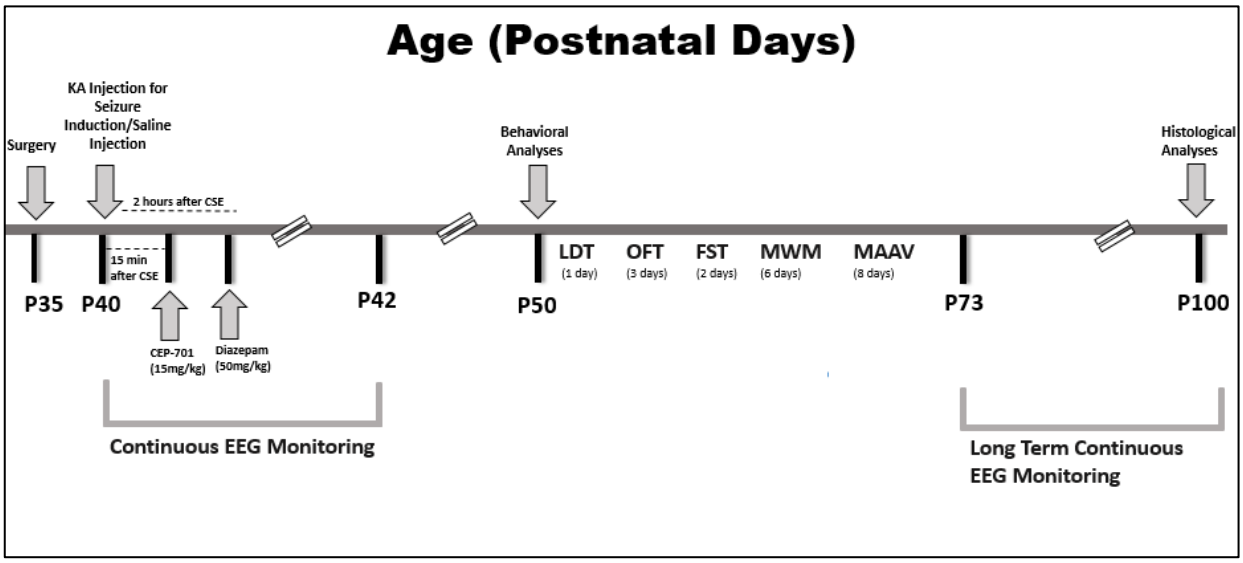


Figure 10. Long-Term Experimental Paradigm. Following the electrode and intra-amygdalar implantation surgery, P35 rats rested for 5 days before being connected to the EEG for KA-induced CSE administration or saline injection. CEP-701 (15 mg/kg) was administered 15 min after CSE onset. 2 hours post-CSE, diazepam (50 mg/kg) was injected and rats were kept on the EEG for monitoring for 2 consecutive days. At P50, rats were subjected for a series of behavioral tests and then were connected on the EEG for 1 month monitoring to be sacrificed then at P10 for histological analyses

Rats were also divided into four groups, respectively:

1-<u>LKCEP group (Long Term Kainic Acid CEP):</u> CEP-701 was given (i.p.) following KA-induced CSE (i.a.)

2-<u>LKV group (Long Term Kainic Acid Vehicle):</u> Vehicle (DMSO) was given (i.p.) following KA-induced CSE (i.a.)

3-<u>LSCEP group (Long Term Saline CEP):</u> CEP-701 was given (i.p.) following saline (i.a.)

4-<u>LSV group (Long Term Saline Vehicle):</u> Vehicle (DMSO) was given (i.p.) following saline (i.a.).

3.2. Stereotaxic Implantation of Electrodes with an Intra-amygdalar Cannula and EEG Recording

In order to efficiently monitor seizures and record continuous high-quality wired EEG simultaneously on multiple rats for 3 months, epidural electrode surgical implantation and wiring is employed. Once anesthesia (mixture of ketamine (60 mg/kg) and xylazine (6 mg/kg)) was administered intramuscularly and achieved completely (lack of signs of pain in response to toe pinching), the rat's head was shaved by a trimmer and then tightly secured with two ear bars on the stereotaxic frame (Figure 11.A). Then, a 2 cm single midline incision was done by a sterile surgical blade to be able to expose the skull by a retractor and scrap the soft tissue. Cauterization was then performed to control bleeding and the calvarium surface was cleaned and dried with few drops of 3% hydrogen peroxide to locate the bregma which is the intersection between the coronal and sagittal sutures (Figure 11.B). Using a high-speed drill, five small 1.4 mm holes were done to place five epidural electrodes in the skull. These electrodes include the left and right frontal electrodes that were 2 mm anterior to, and 3 mm lateral to the bregma, the left and right parietal electrodes that were 5 mm posterior to, and 3 mm lateral to the bregma and one anterior midline reference electrode that was 6 mm anterior to the bregma based on the Paxinos and Watson adult rat brain atlas. For the intra-amygdalar cannula (7.7 mm in length) implantation, an extra hole was formed into the left basolateral amygdala (Figure 11.C). The five sockets of the electrodes and the socket of the ground wire that was placed under the skin at the base of the neck were

connected then to the sixth-channel pedestal that was anchored with acrylic dental cement (Figure 11.D). The rats were then transferred to specialized single animal cages for recovery and post-operative observation, and Panadol was administered for 3 days for pain relief (1mg/ml in drinking water).

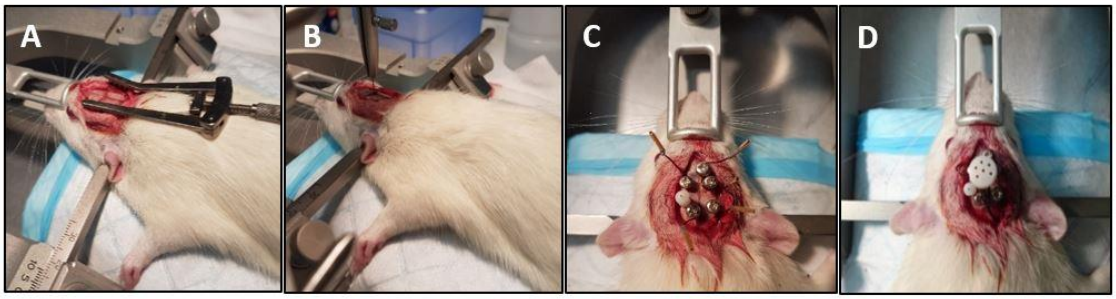


Figure 11. Stereotaxic Electrode Implantation Surgery. Panel A. The exposed skull is cleaned and dried after the head has been tightly secured. **Panel B.** The bregma is identified using the stereotaxic arm, and its respective coordinates are specified (Antero-posterolateral and lateral) to help in the calculation of the coordinates for the respective holes to be drilled for the 5 electrodes and intra-amygdalar cannula. **Panel C.** The electrodes placed with the inserted intra-amygdalar cannula. **Panel D.** The pedestal with the electrodes assembled within it and prepped to be adhered using the dental acrylic.

After 5 days, each rat was transferred to an EEG cage and the pedestal was connected to the EEG cable of the EEG recording system (Xltek, Natus Medical, USA) attached to the commutator that accommodates the movement of the rats (206) to monitor the baseline brain activity few hours before KA induction (Figure 12)

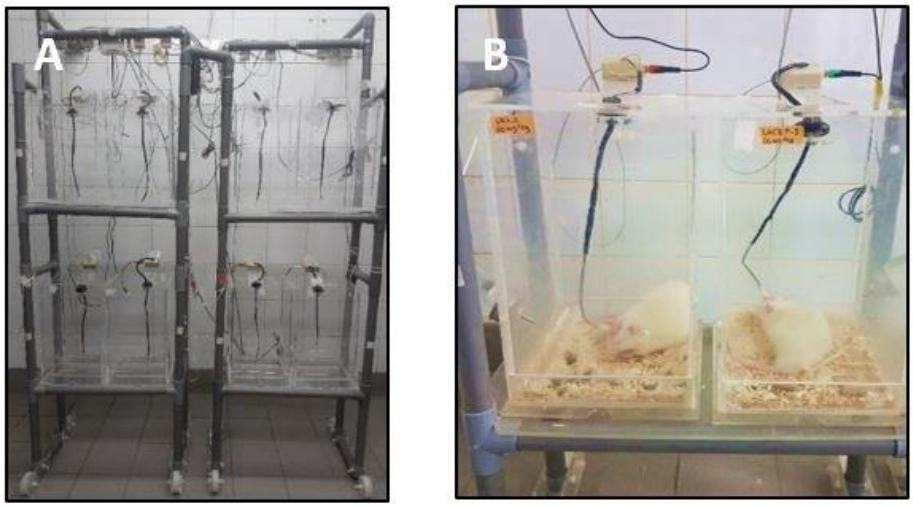


Figure 12. EEG Recording Cages. Panel A. The EEG setup consisting of customized EEG cages with the specialized commutators. **Panel B.** Each rat is placed in its respective EEG cage and connected to its commutator for EEG monitoring. The rats are able to move and rotate when connected to the commutators without any effect on the recording that help in long-term EEG monitoring.

The EEG recordings were read by two readers blinded to the treatment groups. The seizure durations were compared between the different groups in the acute paradigm, while rats with the same seizure duration were included in the study and subjected to behavioral experiments in the long-term paradigm. Post-behavior, EEG recordings were reviewed also to check for any spontaneous recurrent seizures and abnormal brain wave localization that are displayed in the form of spikes (very fast waves), poly-spikes (a quick series of spikes), spike waves (spikes followed by a slow wave), and sharp waves.

3.3. Cognitive and behavioral tests

To check for the chronic cognitive and emotional behavioral disturbances, five main behavioral testing panels where performed between P50 and P73 on the long-term

treated peri-adolescent rats. The tests were done in the sequence from the least aversive to the most aversive test to minimize the interference between the tests. The light dark test was done followed by the open field test then the forced swim test, then the Morris water maze test and finally the modified active avoidance test.

3.3.1. Light Dark Test (LDT)

The light dark test is one of the most commonly used tests to study the anxiety-like and exploratory behaviors in the epilepsy rodent models. It is conducted in a shuttling box (Coulbourn Instruments, Harvard apparatus, USA) that is divided into two equal compartments (H: 34 cm, W: 27 cm, L: 27 cm), linked by a 9 × 9 cm door located in the middle of a metallic partition wall (Figure 13). The dark chamber, covered with black foam panels, is the right side while the lit chamber, covered with white foam panels and visual cues (dices and beads), is the left side. The LDT is a 1-day test conducted over a 5 min session for each rat. The rat was placed in the right chamber and allowed to freely move in the box. The Graphic State 4 software (Coulbourn Instruments, Harvard Apparatus, USA) protocol was used calculate the time spent by the rat in each compartment and the number of shuttling between the two compartments. Between each rat, the box was cleaned via an odorless detergent and 70% ethanol (207,208).

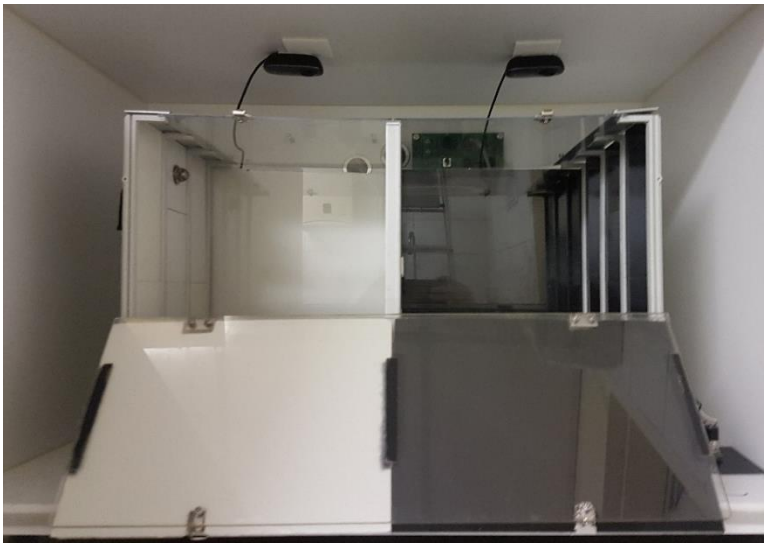


Figure 13. The Light Dark Box Test Setup (LDT). The right dark chamber is covered with black foam panels, while the left chamber is illuminated and covered with white foam panels (207)

3.3.2. Open Field Test (OFT)

In order to monitor the locomotor activity (hyperactivity), exploratory and anxiety-like behaviors, the open field test was conducted. It was performed over 3 consecutive days, with a 5 minutes' session each day in an opaque plexiglass squarefield (W: 80 cm, L: 80 cm, H: 40 cm) with the ceiling light turned off, and the customized LED wall light strips and circular neon lamp turned on. On the first day of testing, a single small object (cube) was placed in the center of the field's floor, and then a new object (ball then bottle) was introduced on each of the following two days (Figure 14). On each given day, rats were placed individually in the corner closest to the most recently added novel object after starting the video recorder on the monitoring laptop. Between each rat, the floor surfaces and walls of the apparatus were cleaned with odorless detergent and then a 70% alcohol solution. The rats' movements (distance traveled, time spent in each zone (central versus peripheral), time spent exploring

central objects) can be separately measured and analyzed using the SMART video tracking 3.0 software (Panlab, Harvard apparatus, USA) (207,208).

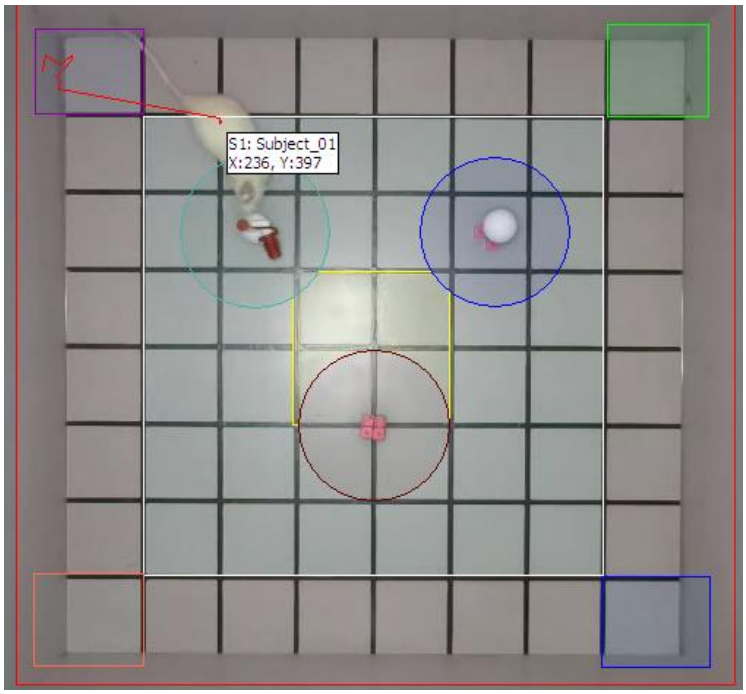


Figure 14. The Open Field Test (OFT). Shown is the setup of session 3 where the rat is being tracked for analysis as it roams within the box for 5 minutes and interacts with the 3 different objects placed in the center.

3.3.3. Forced Swim Test (FST)

This test was tailored to study the depressive-like behaviors in rats that can be measured by the immobility percentage and lack of struggling which are considered as the behavioral surrogates. It is a 2-day test consisting of 10 min session each day. It is made up of three transparent plexiglass cylinders (20 cm in diameter and 50 cm in height (Coulbourn Instruments, USA)) that are filled with water to a depth of 35 cm and the temperature adjusted to 25°C (Figure 15). Three rats were consecutively placed in each cylinder and were video recorded in order for their swimming behavior (Immobility and struggling) to be then analyzed using the SMART software. The

activity detection mode that allows the automated detection of the rat motor activity level has been adjusted in the software such that the lack of movement except for the minimal limb movement to stay afloat defines immobility. When the session ends, rats were removed and allowed to dry for several minutes on dry towels in a cage under the heating lamp and the water in the cylinders is changed (207,208).

Figure 15. The Forced Swim Test Setup (FST). Each rat was placed inside a water-filled cylinder, and kept for 10-minutes session. The rat's behavior was video recorded by a camera placed in front of the cylinders (red arrow) for analysis.

3.3.4. Morris Ware Maze Test (MWM)

Hippocampal-dependent visuospatial navigation is assessed through the Morris water maze test that consists of 7 days with habituation on day 1 followed by 5 days of spatial acquisition testing and a probe trial and visible platform subtest on day 7. A dark-blue circular plastic pool, 150 cm in diameter and 80 cm in height (Coulbourn Instruments, USA) was filled to a depth of 30 cm with 25°C temperature (Figure 16). The pool was virtually divided into four quadrants (NE, NW, SE, SW) and surrounded by visual cues adhered to the room's walls. On the habituation day, rats were allowed to freely swim for 2 min. During the spatial acquisition trials on the 5 days, an "invisible platform" (transparent plexiglass cylinder) was placed 2 cm below the water surface in

the NE quadrant. Each rat is carefully placed in the water facing the pool's wall and allowed to swim for 2 min to reach the platform. If the rat does not find the platform, it is placed on it for 30 seconds. Four daily trials were conducted for every rat, each with a 30-second rest time in between, and four equidistant immersion landmarks from the platform with their sequence changed each day. In order to assess the retention of the spatial learning, the probe trial was performed on day 7 where the rat was allowed to freely swim for 2 minutes without the platform and was immersed in the quadrant opposite to the quadrant that previously had the platform. Then, on the same day, the rats were allowed to swim to a visible platform (gray opaque plastic cylinder) placed in the SE quadrant, with four attempts per rat and four different immersion positions in the NW quadrant, in order to demonstrate that motor and visual functions are intact and not variables in the experiment. Following the daily trials, each rat was placed under a heating lamp to dry, and the pool was cleaned periodically. Every trial was video recorded and analyzed using the SMART video tracking 3.0 software (Panlab, Harvard Apparatus, USA) in order to measure the escape latency period from immersion in the pool until reaching the platform and the time spent in each quadrant (207,208).

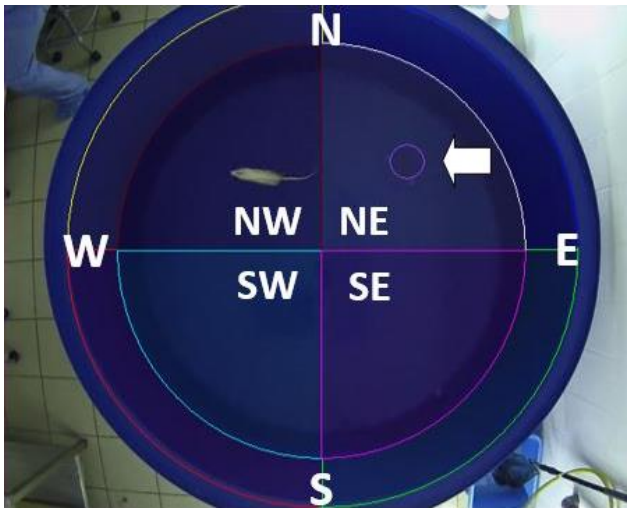


Figure 16. The Morris Water Maze Setup during the Acquisition Days. The invisible platform (white arrow) was placed under the water surface, and the rat was

placed in the filled pool for four trials with each trial from a different immersion landmark (S, W, SWW, SWE) that were equidistant to the platform and their sequence was changed every day (N: North; W: West; E: East; S: South; NW: Northwest; NE: Northeast; SW: Southwest; SE: Southeast).

3.3.5. Modified Active Avoidance Test (MAAV)

The MAAV test, which is designed in our lab, is a modified two-way active avoidance (TAA) test in which the contextual Pavlovian conditioning was incorporated in the standard two-compartment rat shuttle box. This helps in the assessment of active tone-signaled electrical foot shock avoidance in the left compartment and active context-cued shock avoidance in the right compartment of the box (Figure 17). The test consists of 7 days including 1-day habituation, followed by 5 training days and 1 retention testing day. The Graphic State 4.0 (GS4) software (Coulbourn Instruments, Allentown, PA, United States) was used to program the MAA experimental procedure, which monitors the transitions between the left and right chambers and delivers tone signals and electrical foot shocks via the modular Habistest system. During habituation, plain white foam panels cover the anterior and posterior plexiglass walls of each compartment and the rats were allowed to freely roam for 5 minutes without any tone or shock. During the training days, the left compartment was kept as before while in the right compartment, the anterior and posterior plexiglass walls were covered with black and white stripped foam panels and contextual cues made up of dices and beads were added on the interchamber partition wall. Each training day consists of 30 trials delivered. In the contextually modified right chamber, every rat entry is a context exposure trial and 10 seconds following the entry of the rat, an electrical foot shock (0.5 mA,15 sec) will be delivered. While in the plain left chamber, the trial consists of 15 seconds tone (CS) followed by an immediate 0.5-mA electrical foot shock for 15

seconds also with a 40 seconds inter-trial period. When shuttling between the chambers, the rat either avoid an incoming shock or terminate an ongoing one (escape). Following these training days for the rat to associate the unconditioned stimulus (shock) with the CS stimulus (tone in the left compartment and cues in the right compartment) the two-part retention test is performed. During the first part, no tone or electrical shock was delivered and the rat was allowed to freely move for 2 minutes in the shuttle box in order to check for retention of contextual learning. During the second part, the left compartment was made identical to the right one with the anterior and posterior walls of the left covered with white foam panels and cues were removed. After a 5-minute habituation period, 30 trials of foot shocks (0.5 mA, 15 s) are delivered in either the right or left chamber signaled by a preceding 15 second tone with a 30-second inter-trial period. Shuttling through the inter-chamber door during tone delivery will prevent an incoming electrical shock and is referred to as shock "avoidance", while shuttling during shock delivery is referred to as "escape" (103).

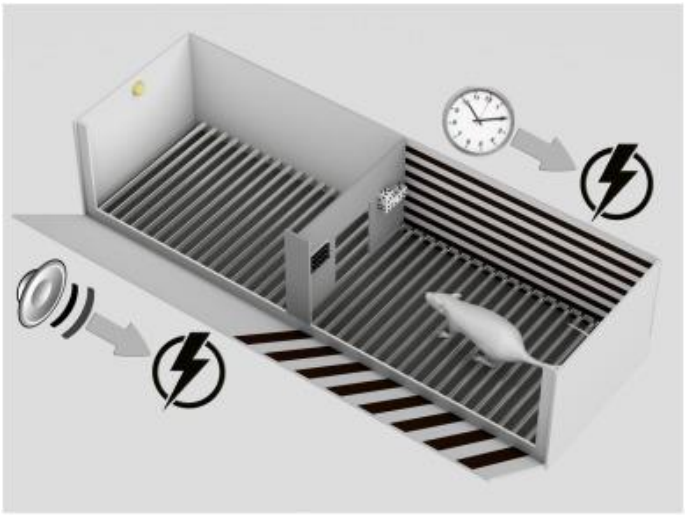


Figure 17. Schematic Design of the Modified Active Avoidance Test (MAAV). This test was developed by modifying the shuttling box in order to assess the emotionally-relevant auditory and contextual learning simultaneously. In the right compartment that

tests the contextual learning and contains visual cues, the shock is administered every 10 seconds the rat spends in the chamber. In the left compartment that tests for the auditory learning, the electrical foot shocks are signaled with a tone. This test assesses learning of adaptive shock-avoiding shuttling responses that replace innate fear responses (freezing).

3.4. Euthanasia and Cardiac Perfusion Surgery for Histological Studies

In order to perform various microscopic examinations, the rat's brain was removed at P100 through the trans-cardiac perfusion-fixation non-survival procedure. After achieving anesthesia and the rat become unresponsive to noxious stimuli, it was placed on its back and its limbs were restrained via pins to prevent accidental or fluid-induced shifting during the procedure. A cut in the abdomen was done just below the diaphragm in order to remove the diaphragm to access the rib cage. Along the side of the rib cage, two vertical incisions were done to help in lifting the rib cage and exposing the heart. In the apex of the left ventricle, a needle was inserted and a cut in the right atrium was done simultaneously to allow the fluid to drain. Then PBS (1X) solution was pumped slowly into the animal followed by the fixative solution: PFA (4%).
Once the animal's blood supply has been depleted, the brain was taken and stored in 4% PFA at 4°C for further histological analyses.

3.5. Paraffin embedding and sectioning

The process of embedding started with a 10% formalin fixation, followed by a 0.9% saline wash to remove any debris. Then, using a series of ethanol solutions (75%, 95%, then 100%; 2 hours each), dehydration was done followed by clearing with xylol. The brain was flattened, and paraffin was infiltrated in the whole tissue.

In order to obtain 8 µm thick sections, the microtome was used for sectioning of the paraffin blocks and then sections were attached to slides for staining and analyses.

3.6. Brain dissection and hippocampal microdissection

A Guillotine was used to decapitate the rat's head, which was then fixed for skull removal. After removing the cerebellum, the remaining structure was divided between the hemispheres. Both hemispheres were dissected at a microscopic level. The hippocampus, a seahorse-shaped structure found in each hemisphere, was gently scraped off during the microdissection process. After that, the hippocampus and cortex were kept at -80° C for molecular analysis.

3.7. Histological analysis (Hematoxylin and Eosin)

Hematoxylin and eosin are two dyes that are widely used to assess the hippocampal neuronal loss. Hematoxylin is a basic dye that colors the acidic nucleus (RNA and DNA) purple, while eosin is an acidic dye that stains the acidophilic cytoplasm red or pink. Following sectioning, the slides were de-paraffinized with two changes of xylol for 5 minutes each, followed by rehydration with a series of ethanol solutions (100% (4 min) – 95% (3 min) – 75% (3 min)) to replace the ethanol with water. Then, to remove any excess alcohol, the slides were rinsed in distilled water for 3 minutes. The nucleus was then stained with hematoxylin for 1 minute before being washed with tap water for 2 minutes to eliminate any excess hematoxylin. Eosin was used to counterstain the cytoplasm for 30 seconds. The sections were subsequently dehydrated with a series of ethanol solutions (75% (1 minute), 95% (3 minutes), and 100% (5 minutes)), before being cleared with two changes of xylol (4 minutes followed

by 5 minutes). The slides were then mounted with permount, topped with a coverslip, and allowed to dry overnight before being imaged with the uScope navigator 4.3 program (uScope MXII, USA).

An investigator who was blinded to the treatment groups counted the CA1-CA3 hippocampal areas using ImageJ software (NIH, USA). Three brains were chosen from each group, and four sections from each brain were counted.

3.8. Immunostaining (NeuN)

For the long-term neuronal damage assessment, NeuN staining was performed. NeuN is a biomarker for mature neurons that is found in the nuclei and perinuclear cytoplasm of the majority of CNS neurons (209).

Sections were de-paraffinized in 3 changes of xylene for 5 min each, followed by rehydration in 100% ethanol for 2 changes (3 min each) and then transferred to series of ethanol solutions (95%, 70% and 50% ethanol respectively) for 3 min each to be washed after that for 5 min with distilled water. After that, slides were incubated for 60 min in citrate buffer (pH=6) at 90 ^{o}C for antigen retrieval and then rinsed with distilled water for 5 min. Further steps were performed using the Novolink Polymer Detection Kit- 500 Tests (Leica Biosystems, Germany). The peroxidase block solution was added for 5 min to block the endogenous peroxidase activity followed by washing for 10 min. The protein block solution was then used for 5 min to reduce the nonspecific binding followed also by washing with distilled water for 10 min. Sections were then incubated overnight at 4 ^{o}C with the primary mouse anti-NeuN antibody (A60-MAB377;1/100; Sigma-Aldrich), where the antibody was diluted in a solution containing normal goat serum (NGS), phosphate buffer saline (1X), Triton (1X), and

bovine serum albumin (BSA). On the second day, the slides were washed in PBS 2 times for 10 min each, then incubated with a post primary (rabbit anti- mouse) to detect the mouse antibodies and then followed by the addition of a Novolink TM polymer solution (anti rabbit) for 30 min that detects rabbit immunoglobulins. Sections were further incubated with the DAB solution (DAB chromogen with DAB substrate Buffer) for 2 minutes whose reaction with the peroxidase produces a visible brown precipitate at the antigen site followed by washing. Counterstaining was done via Hematoxylin for 5 min and then dehydration with the series of ethanol solutions (75%, 95% and 100%) 5 min each took place. Sections were then cover-slipped with a mounting solution and kept to dry before being imaged on the uScope navigator 4.3 program (uScope MXII, USA) to check for the hippocampal neuronal loss.

3.9. Statistical analysis

Statistical analyses were performed using the GraphPad Prism 7 Software (USA) to analyze the neuronal damage. One-way analysis of variance (ANOVA) in conjunction with the post hoc Fisher least significant difference (LSD) test was used.

Independent sample t-test was performed to assess the CSE durations.

For the behavioral test (LDT, FST, OFT, MWM, AND MAAV), statistical analysis was not performed because of the small number of rats per group.

CHAPTER 4

RESULTS

4.1. Short term paradigm results

4.1.1. Patterns and durations of the CSE

In the short-term paradigm, rats were subjected to KA-induced CSE. Rats were connected to an EEG prior to the induction to measure baseline brain activity. At baseline, rats had normal slow synchronous theta waves of about 6 Hertz with thalamocortical characteristics that mimicked the human posterior dominant rhythm (Figure 18.A). All rats met the inclusion criteria both electrically and clinically after receiving KA (at least reaching stage three of Racine's scale). Electrically, CSE onset was expressed as recurrent paroxysmal rhythmic spike and wave patterns that evolved in space and time. The seizure started in the left hemisphere and then spread to both hemispheres, resulting in bilateral rhythmic spikes in both (Figure 18.B). All rats that received KA had comparable average latencies to the electrographic onset (17.5±2.751 minutes for SKCEP and 19.92±3.14 minutes for SKV, p=0.5692). Behaviorally, rats gradually displayed decreased locomotion, salivation, repetitive chewing, staring, wet dog shakes, and forelimb clonus with rearing.

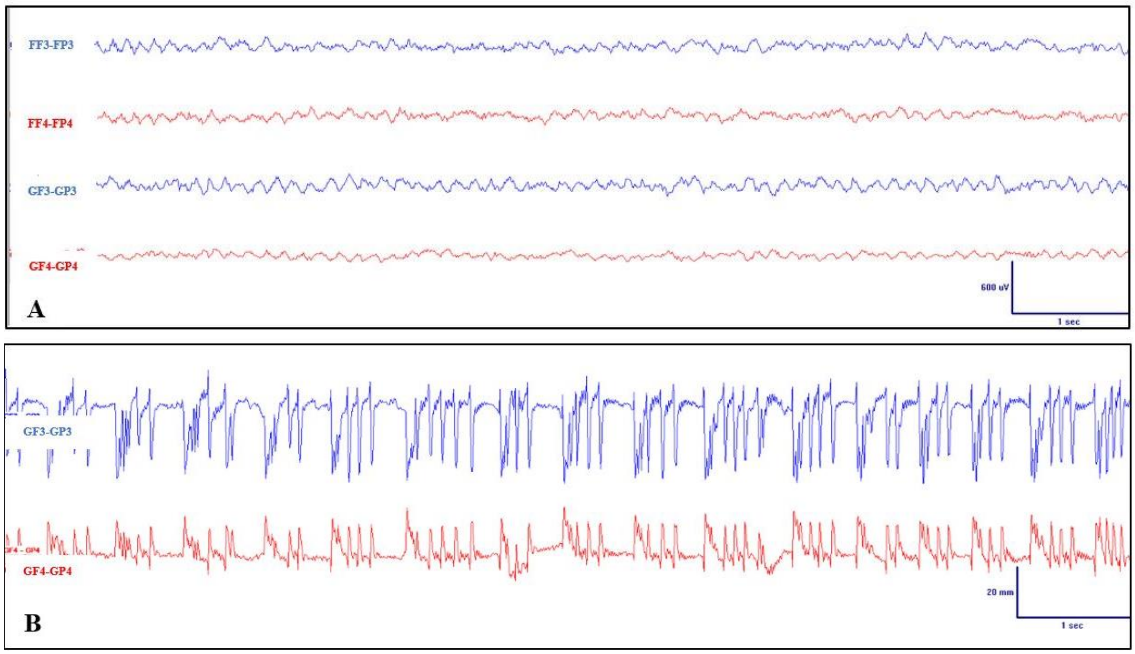


Figure 18. EEG Recordings. Panel A. Shown is the baseline activity in two rats (rat F and rat G) prior to KA-induced CSE each with four sampling electrodes (F3: Left Frontal; F4: Right Frontal; P3: Left Parietal; P4: Right Parietal). **Panel B.** The progression to a secondary generalization of CSE in rat G with repetitive rhythmic paroxysmal spikes. Recordings were done using the longitudinal bipolar montage.

We assessed the difference in CSE duration between the two injured groups (SKCEP and SKV). The group receiving CEP-701 (SKCEP) underwent seizures for a significantly less duration compared to the vehicle receiving group (SKV) ($p<0.01$ Independent sample t-Test; Mean± Standard Error of Mean: SKV: 15.88±1.331 hours, n=13 /SKCEP: 8.644± 1.413 hours, n=12). Saline control groups (SSV and SSCEP) showed no sign of seizure manifestation, an indication that the saline had no role in seizure induction.

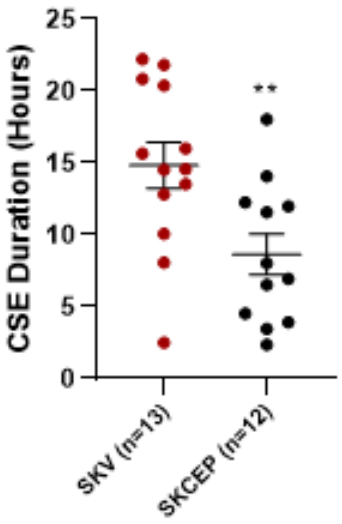


Figure 19. CSE Duration quantified during 24 hours post-CSE onset. Initially convulsive in the first 15 minutes prior to the administration of the first diazepam dose, then converted into a subclinical electrographic form. TrkB blockade with CEP-701 lead to a significant decrease in the duration of CSE in the CEP-701-treated rats compared to the vehicle treated group (SKV: 15.88± 1.331 hours, n=12; SKCEP: 8.644 ±1.413 hours, n=12; independent sample t-test, p = 0.0012) (CSE: Convulsive Status Epilepticus, SKCEP: Short Term Kainic Acid CEP, SKV: Short Term Kainic Acid Vehicle) (Saline groups didn't have seizures)

We assessed the difference in CSE duration between the two injured groups (SKCEP and SKV) (Figure 19). The group receiving CEP-701 (SKCEP) underwent seizures for a lesser duration compared to the vehicle receiving group (SKV) without reaching statistical significance ($p>0.05$) showing that there is a trend in seizure termination by CEP-701. Saline control groups (SSV and SSCEP) showed no sign of seizure manifestation, an indication that the saline had no role in seizure induction.

4.1.2. Neuronal damage assessment using H&E staining

CSE is known to induce neuronal damage. Therefore, we assessed the level of hippocampal neuronal loss 24 hours post-KA-induced CSE using hematoxylin and

eosin staining. The number of neurons of both the left and right hippocampi was quantified and normalized by the surface area (Figure 20). Both groups of rats that underwent seizures (SKCEP and SKV) were comparable to each other in the number of hippocampal neurons ($p > 0.05$; One Way ANOVA) but both had a significantly lower number of neurons compared to their saline groups (SSCEP and SSV) respectively ($p<0.05$). The saline groups showed no difference between each other as well.

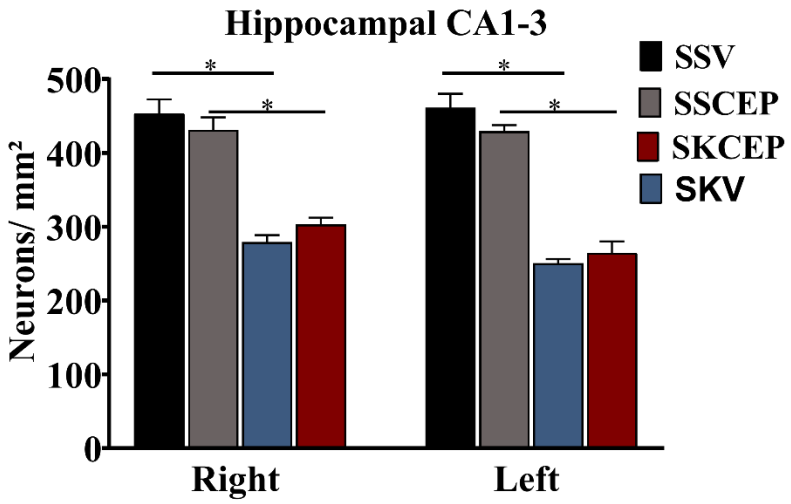


Figure 20. Hippocampal Neuronal Loss. One-way ANOVA with post hoc Fisher LSD revealed that the number of hippocampal neurons per mm² in the right and left hippocampi of the injured groups (SKCEP, SKV) were comparable to each other but statistically significant with their control groups (SSCEP, SSV) respectively ($p<0.05$) (SKV: Short Term Kainic Acid Vehicle, SKCEP: Short Term Kainic Acid CEP, SSCEP: Short Term Saline CEP, SSV: Short Term Saline Vehicle)

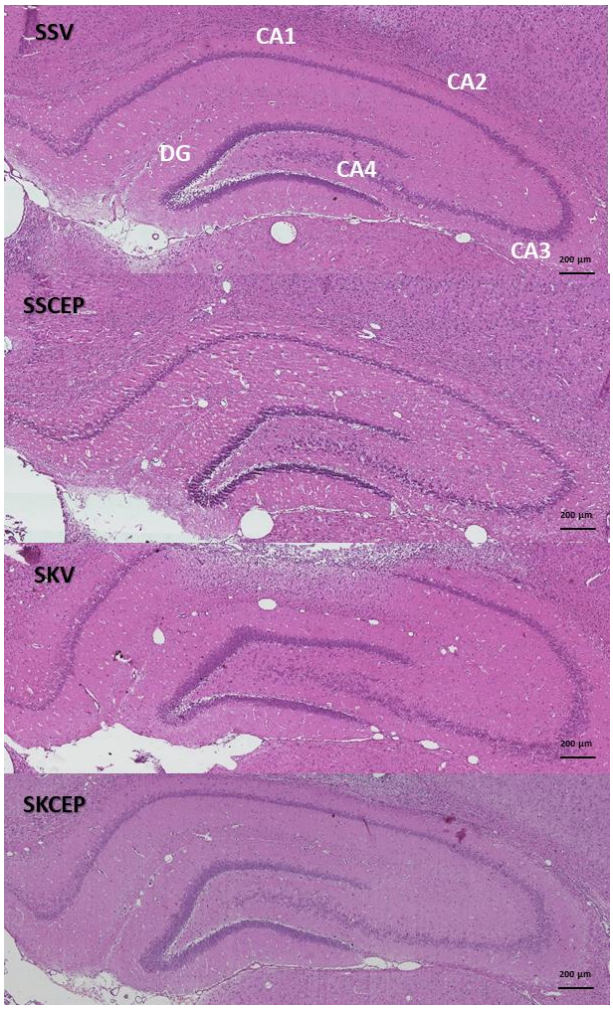


Figure 21. Hematoxylin and Eosin Staining of the Ipsilateral Left Hippocampi. Illustrative images of the four groups show that the injured groups (SKCEP and SKV) had a lower number of neurons compared to the control groups (SSCEP and SSV) (SKCEP: Short Term Kainic Acid CEP, SKV: Short Term Kainic Acid Vehicle, SSCEP: Short Term Saline CEP, SSV: Short Term Saline Vehicle, CA: Cornu Ammonis, DG: Dentate Gyrus). (Scale: 200µm).

4.2. Long term paradigm results

4.2.1. Induction and long-term EEG recordings

Rats were subjected to KA-induced CSE. Same as for the short-term paradigm, they were connected on the EEG for baseline brain activity recording prior to induction. In order to be included in the study, rats had to satisfy the same criteria applied to the rats of the short-term paradigm (Electrical and clinical manifestations). A total of 40

rats were subjected for KA induction, in which 15 rats died and the other 25 rats were included in the study. All convulsive seizures were aborted with diazepam 2 hours post-CSE onset, but they all continued to have seizures without convulsions (subclinical). EEG recording was maintained for all the rats for 2 days post-KA induction and then was resumed following the behavioral testing for 1 month. Recordings after the KA induction showed that there were spikes and polyspikes. The seizure duration for the injured groups is still under study. To check for seizure recurrences, the EEG recordings post-behavior were used. Preliminary results showed that no seizure recurrence was obtained during the 1 month recording period.

4.2.2. Anxiety-like behaviors, exploratory behaviors, and hyperactivity in the LDT and OFT

The effect of transient post-KA-induced CSE with CEP-701 on activity level, exploratory and anxiety-like behaviors was checked in closed (LDT) and open (OFT) environments, based on the natural aversion of rats to brightly illuminated areas and on their spontaneous exploratory behavior in response to novelty, such as environment, light, and novel objects. In the LDT, the vehicle injured group (LKV) spent comparable time in the lit chamber compared to the normal saline group (LSV), but less time than the CEP-701 treated group (LKCEP). The CEP-701 treated control group (LSCEP) spent comparable time in the lit chamber with the normal saline group (LSV) (Figure 22.A). The number of entries to the lit chamber was comparable between the different groups (Figure 22.B)

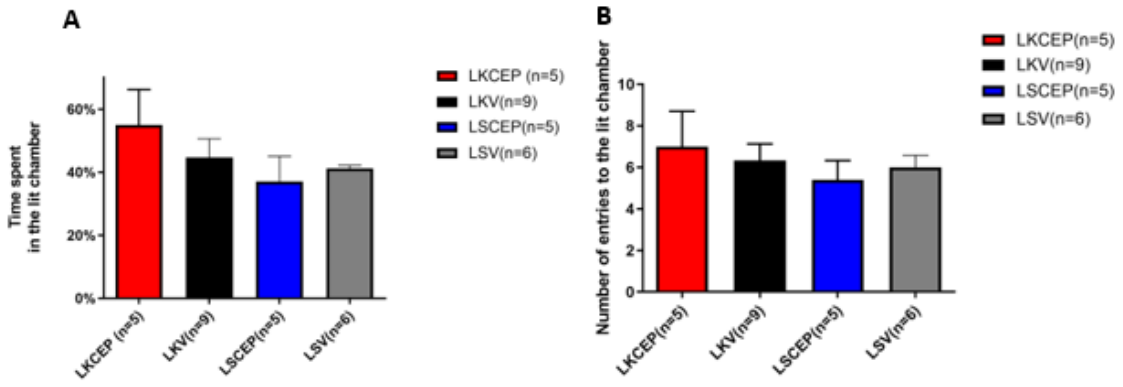


Figure 22. Light Dark Box Test. Panel A. The percentage of time spent in the lit chamber between all groups was comparable. **Panel B.** The number of entries to the lit chamber was similar between all groups. Mean± SEM are reported. (LKCEP: Long Term Kainic Acid with CEP, n=5; LKV: Long Term Kainic Acid with Vehicle, n=9; LSCEP: Long Term Saline with CEP, n=5; LSV: Long Term Saline with Vehicle, n=6)

In the OFT, there was no difference in the total distance travelled by the different groups in the cumulative distance for all the sessions. However, during sessions 1 and 2, the vehicle injured group (LKV) travelled a higher distance than the CEP-701 treated group (LKCEP). On session 2, the LKV group travelled a higher distance than the control group (LSV). During sessions 2 and 3, the saline treated group (LSCEP) travelled a distance less than the control saline group (LSV) (Figure 23.A).

The cumulative distance travelled in the periphery was comparable between the different groups over the 3 sessions. However, on session 1, the vehicle injured group (LKV) travelled a higher distance in the periphery compared to the CEP-701 treated group (LKCEP). In all sessions, the saline treated group (LSCEP) travelled a smaller distance compared to the control saline group (LSV) (Figure 23.B).

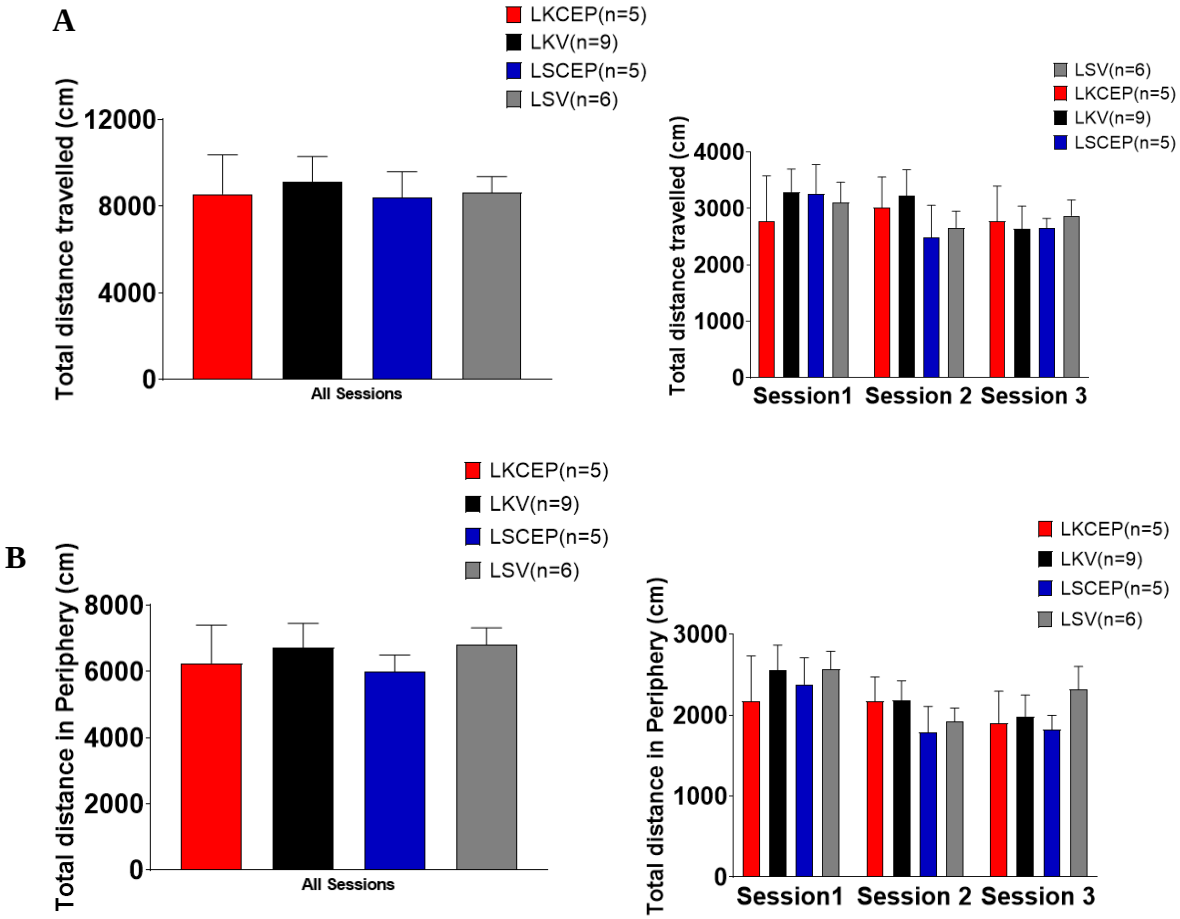


Figure 23. Open Field Test. Panel A. On the left side, the cumulative total distance travelled over the three testing sessions was comparable between the different groups. On the right side, LKV group travelled a total distance higher than the LKCEP group on sessions 1 and 2. **Panel B.** On the left side, the cumulative total distance travelled in the periphery was the same between the different groups. On the right side, the LSCEP group travelled a lower distance on all the sessions compared to LSV group. Mean ± SEM are reported. (LKCEP: Long Term Kainic Acid with CEP, n=5; LKV: Long Term Kainic Acid with Vehicle, n=9; LSCEP: Long Term Saline with CEP, n=5; LSV: Long Term Saline with Vehicle, n=6)

Most of the time spent by the rats was in the periphery which was similar between the different groups on any single day (Figure 24).

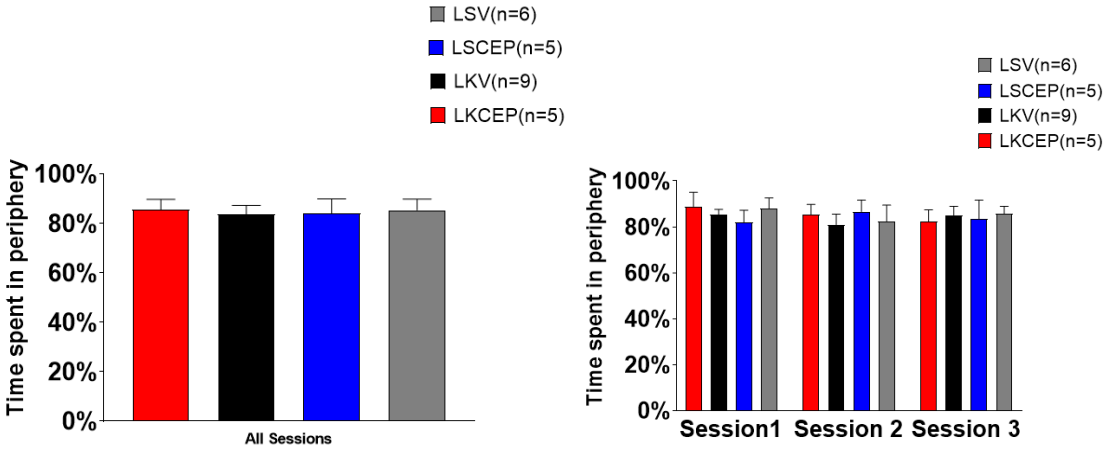


Figure 24. The Open Field Test. Percentage of time spent in the periphery was comparable between all the groups. Mean± SEM are reported. (LKCEP: Long Term Kainic Acid with CEP, n=5; LKV: Long Term Kainic Acid with Vehicle, n=9; LSCEP: Long Term Saline with CEP, n=5; LSV: Long Term Saline with Vehicle, n=6)

4.2.3. Depressive-Like Behavior in the FST

The effect of transient post-KA-induced CSE with CEP-701 on struggling and depressive-like behaviors were assessed in the FST. On the first testing day, the percentage of immobility steadily increased starting minute 2 of the test. The vehicle injured group (LKV) had a higher percentage of immobility compared to the control saline group (LSV) and the CEP-701 treated group (LKCEP). The saline treated group (LSCEP) showed a higher immobility percentage compared to the control saline group (LSV). However, the struggling behavior of the LKV and LSCEP groups diverge from the LKCEP and LSCEP groups at the 8th minute and showed a higher immobility percent at the last minute of the test. The CEP-701 treated group (LKCEP) showed comparable percentage of immobility with the control saline group (LSV) (Figure 25). On the second day of testing, all groups showed a similar behavior but with saline treated group (LSCEP) having the higher immobility percentage at the end of the test compared to the other groups.

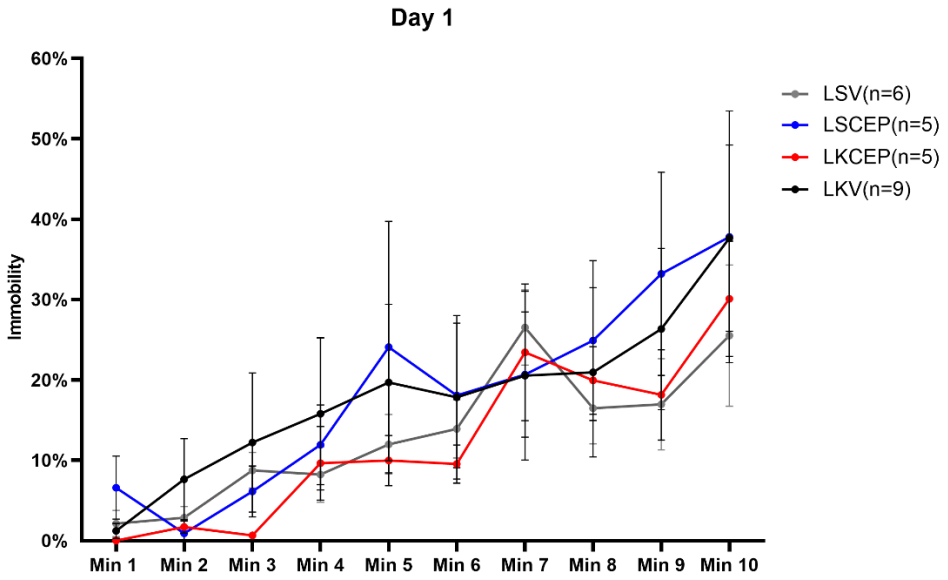


Figure 25. Percentage of immobility over the 10 min session of FST. The behavior of the LKV and LSCEP groups diverge from the other groups at minute 8 that persisted to the last minute of the test. Mean± SEM are reported. (LKCEP: Long Term Kainic Acid with CEP, n=5; LKV: Long Term Kainic Acid with Vehicle, n=9; LSCEP: Long Term Saline with CEP, n=5; LSV: Long Term Saline with Vehicle, n=6)

4.2.4. Visuospatial navigation in the MWM

In order to check the effect of transient post-KA-induced CSE with CEP-701 on the visuospatial learning and memory, the MWM test was performed. Over the 5 training days, preliminary data showed that the rats gradually learned to reach the escape platform. The vehicle injured group (LKV) took more time to reach the invisible platform (higher escape latency) compared to the control saline group (LSV) especially on days 1 and 3 but less time than to the CEP-701 treated group (LKCEP). The saline treated group (LSCEP) showed a higher escape latency compared to the control saline group (LSV). In the probe trial, the retention of learning was checked. All groups were comparable but LSV group had the highest preference in the NE quadrant (Figure 26).

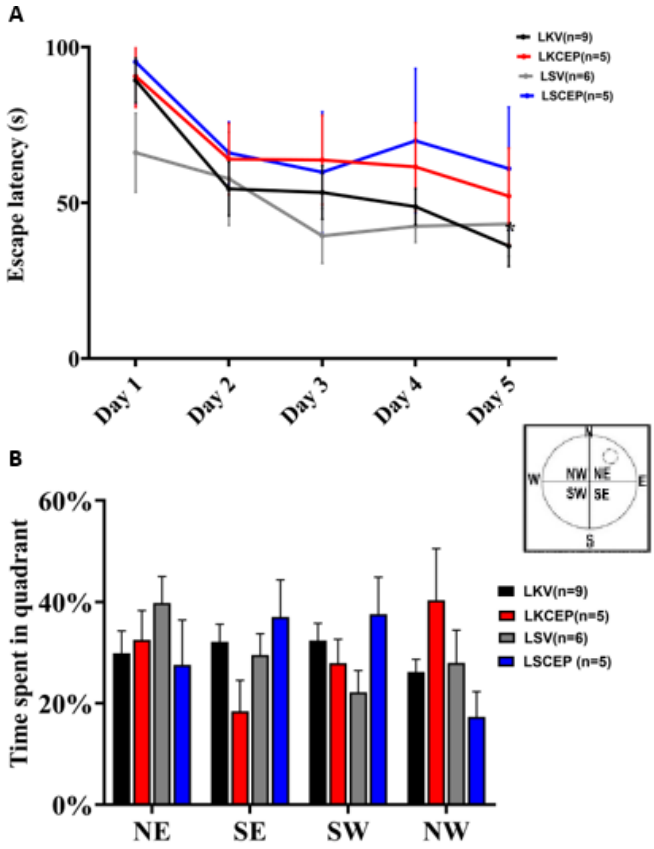


Figure 26. Morris Water Maze place learning and probe trial results. Panel A. Shown is the latency to reach the escape platform during the five training days. All groups had a similar performance in learning the place of the invisible platform during the five-day spatial acquisition training as measured by comparable average latencies. **Panel B**. In the probe trial test, the three groups (LKCEP, LKV and LSCEP) spent comparable time in the quadrant where the escape platform was previously located (NE) without any preference to this quadrant except for the LSV group that had the highest preference in the NE quadrant. The dashed circle in the water maze diagram corresponds to the previous location of the platform (N: north, E: east, S: south, W: west, NE: northeast, NW: northwest, SE: south east, SW: southwest). Mean± SEM are reported. (LKCEP: Long Term Kainic Acid with CEP, n=5; LKV: Long Term Kainic Acid with Vehicle, n=9; LSCEP: Long Term Saline with CEP, n=5; LSV: Long Term Saline with Vehicle, n=6)

The visual acuity of the rats post-KA-induced CSE with CEP-701 was also assessed in the MWM test. The escape latencies to the visible platform were comparable between the different groups (Figure 27).

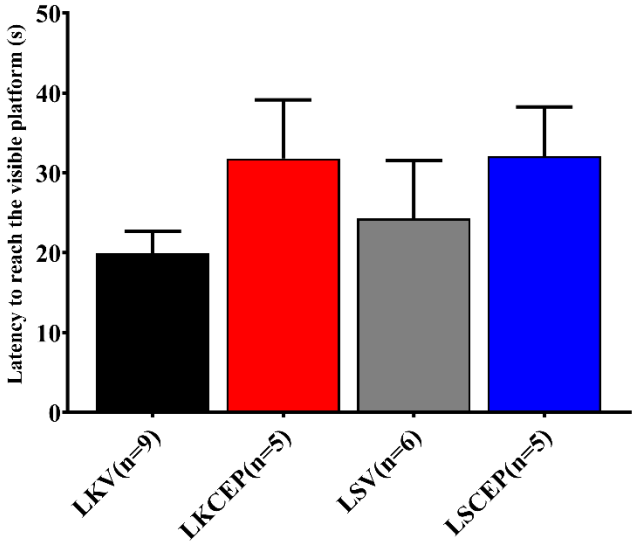


Figure 27. Visual Acuity Assessment. All the groups had comparable latency in reaching the visible platform indicating comparable visual and motor functions in all groups. Mean± SEM are reported. (LKCEP: Long Term Kainic Acid with CEP, n=5; LKV: Long Term Kainic Acid with Vehicle, n=9; LSCEP: Long Term Saline with CEP, n=5; LSV: Long Term Saline with Vehicle, n=6)

4.2.5. Auditory and contextual learning in MAAV

Learning of adaptive avoidance behaviors of tone-signaled or context-cured shocks were investigated in the modified active avoidance (MAAV) test. The percentage of avoiding the tone-signaled shocks in the left compartment was higher in the vehicle injured group (LKV) than the saline control group (LSV). CEP-701 treated group (LKCEP) showed a higher percentage in avoiding the tone-signaled shocks than the LKV group. The saline treated group (LSCEP) showed a higher percentage of avoidance than the saline normal group (LSV). During the retention of auditory learning, all groups were comparable in the percentage of tone-signaled shocks avoidance. However, in the contextual learning, there was a slow learning in the incremental acquisition of shock avoidance behavior. All groups showing comparable percentage of avoidance of context-cued shocks but with the saline control group (LSV) having a higher percentage. On the retention day of contextual learning, all groups

showed similar preference to the left compartment with comparable percentage of the contextual learning (Figure 28).

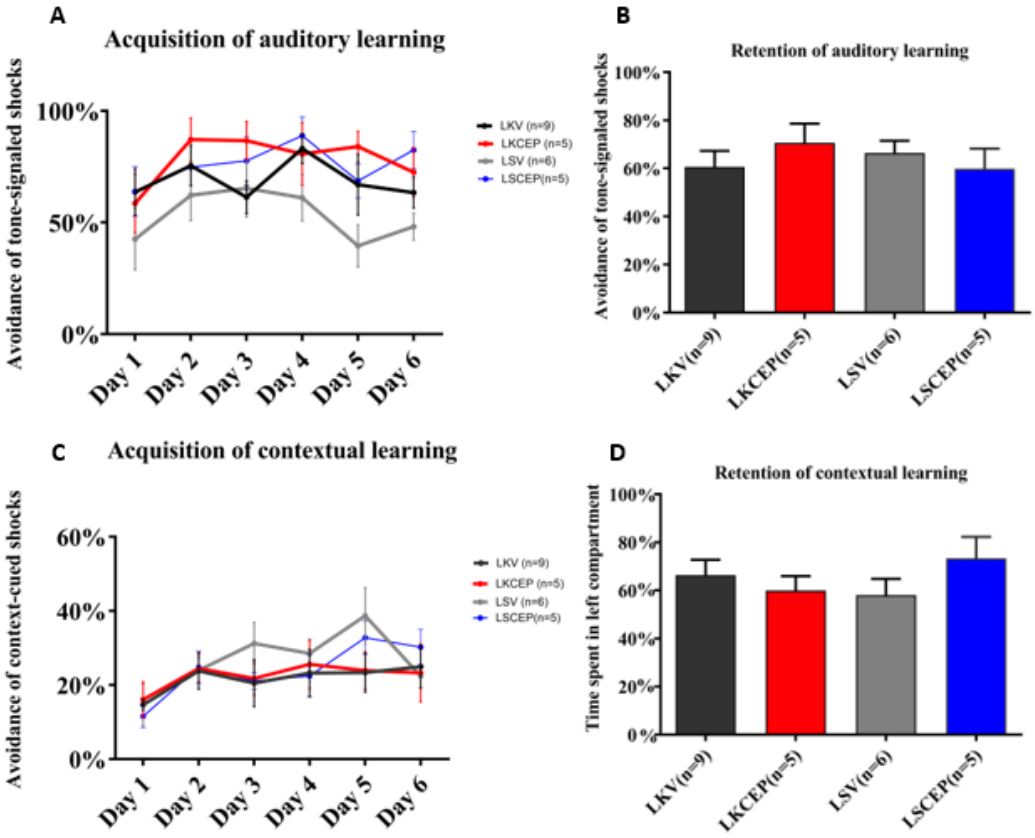


Figure 28. Learning deficits in the MAAV Test. Panel A. LKCEP group showed a higher percentage of avoidance of the tone-signaled shocks compared to LKV group. The LKV group showed a higher percentage compared to the LSV group. LSCEP group had a higher percentage of avoidance compared to LSV group. **Panel B.** In the auditory learning retention, all groups were comparable in avoiding the tone-signaled shocks. **Panel C.** In the acquisition of contextual learning subtest, all groups revealed comparable trends in learning to avoid the tone-signaled electrical foot-shocks in the right chamber but with a slow learning percentage and with LSV having the higher percentage compared to the other three groups. **Panel D.** In the retention of contextual learning, all groups had a similar preference to the left compartment when allowed to freely roam in the box with comparable percentage of time spent in the left side. Mean± SEM are reported. (LKCEP: Long Term Kainic Acid with CEP, n=5; LKV: Long Term Kainic Acid with Vehicle, n=9; LSCEP: Long Term Saline with CEP, n=5; LSV: Long Term Saline with Vehicle, n=6)

CHAPTER 5

DISCUSSION

In this study, we have shown that CEP-701 enhances the efficacy of the currently used standard drugs in aborting CSE in a timely manner and decreases the seizure duration. CEP-701 decreased the seizure duration as evidenced by the significant difference in seizure duration between the injured groups (SKCEP and SKV). This effect was likely mediated via TrkB inhibition. Given its known safety in humans, CEP-701, which is already an FDA-approved drug used for cancer in human, may be repurposed for the use in CSE studies in children to timely abort it. Nevertheless, there seems to be some side effects on the behavior that need to be confirmed with a higher number of rats per group.

There is a desperate need for novel drugs to treat CSE and enhance its abortion as 30% of patients with CSE do not respond to standard medications and require anesthetic and intensive care admissions. CEP-701 offers its role as a promising clinically translatable drug whose primary target in CSE is likely TrkB. We believe that this anti-seizure effect is mediated via TrkB given the literature on TrkB role in epilepsy (219) and especially that CEP did not protect against neuronal damage. CSE is known for inducing neuronal damage and accompanying hippocampal morphological abnormalities when it lasts more than 30 minutes (34). This neuronal loss may explain the behavioral problems that were not reversed by CEP-701.

In our study, we used a long-term experimental paradigm to check if administering CEP-701 post-CSE prevents the recognized long-term behavioral, cognitive and psychiatric detrimental consequences of CSE and TLE. Preliminary

results recapitulated the comorbidities of TLE with cognitive and psychiatric deficits. CEP-701 did not seem to attenuate the long-term behavioral consequences. Indeed, rats had contextual deficits in the MAAV and learning deficits in the MWM that were not reversed by CEP-701. For the auditory learning in the MAAV, the number of tone signaled trials was very low compared to the rats' entrance to the left compartment because rats were shuttling before hearing the tone which shows that the final results about CEP-701 could not be revealed and this test might not be used to assess the auditory learning. CEP-701 may reverse anxiety and depressive like-behaviors as seen from the preliminary results of the light dark box test and forced swim test, but due to the small number of rats, this cannot be confirmed. For the CSE long-term consequences, one of the main aims in the translational epilepsy research is to prevent the behavioral and psychiatric comorbidities that accompany them (211). However, drugs often potentiate these effects. In fact, there was a trend for CEP-701 to cause side effects in the normal rats where it worsened the learning in the saline treated group (LSCEP).

We did not detect, in our study, recurrent seizures and this is possibly due to the short period of recordings. Long-term recurrent seizures with spike and wave patterns, as well as polyspikes usually appears in KA-induced CSE models (22). In the literature, TrkB inhibition and blockade usually attenuate long-term recurrent seizures (220).

CHAPTER 6

CONCLUSION

In this study, CEP-701 has been shown to decrease the seizure duration. Although lacking any enhancement in decreasing neuronal damage as shown by hippocampal neuronal count, CEP-701 has a role as a potential anti-seizure medication. Despite the protective effect of CEP-701 and its previously established early anti-seizure effect, our long-term preliminary results showed that it did have a long-term negative effect on learning and memory and wasn't able to reverse these deficits. This negative effect is likely related to the drug multiple targets and non-specificity. This is not an uncommon side effect with anti-seizure medications including standard ones used in treatment such as phenobarbital.

CSE is a detrimental condition that necessitates the development of a new medicine that could abort seizures. Given that CEP-701's other targets have not been demonstrated to be involved in epilepsy, it is a promising clinically translatable medication whose major target in CSE is likely TrkB. Lestaurtinib (CEP-701) research in animal models can pave the way for future clinical trials through its potential blockade of TrkB receptor, and thus enhancing the efficacy of standard anti-seizure medications. Indeed, in current practice, there is a desperate need for interventions that abort CSE in a timely manner. Based on our long-term preliminary data, more studies are needed to further assess the effect of CEP-701 on the detrimental behavioral consequences. Ongoing work in our laboratory aims at confirming the long-term experimental paradigm by increasing the number of rats per group and further work is

to be done to check for seizure recurrence. We will also attempt to explore potential safe and more specific TrkB blocker.

BIBLIOGRAPHY

(1) Batista-García-Ramó K, Fernández-Verdecia CI. What we know about the brain structure–function relationship. Behavioral Sciences 2018;8(4):39.

(2) Abreu R, Leal A, Figueiredo P. Identification of epileptic brain states by dynamic functional connectivity analysis of simultaneous EEG-fMRI: a dictionary learning approach. Scientific reports 2019;9(1):1-18.

(3) Wefelmeyer W, Puhl CJ, Burrone J. Homeostatic plasticity of subcellular neuronal structures: from inputs to outputs. Trends Neurosci 2016;39(10):656-667.

(4) Khambhati AN, Bassett DS, Oommen BS, Chen SH, Lucas TH, Davis KA, et al. Recurring Functional Interactions Predict Network Architecture of Interictal and Ictal States in Neocortical Epilepsy. eNeuro 2017 Mar 8;4(1):10.1523/ENEURO.0091-16.2017. eCollection 2017 Jan-Feb.

(5) Chai Z, Ma C, Jin X. Homeostatic activity regulation as a mechanism underlying the effect of brain stimulation. Bioelectronic medicine 2019;5(1):1-9.

(6) Engel Jr J. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. Epilepsia 2001;42(6):796-803.

(7) Brophy GM, Bell R, Claassen J, Alldredge B, Bleck TP, Glauser T, et al. Guidelines for the evaluation and management of status epilepticus. Neurocritical care 2012;17(1):3-23.

(8) Abend NS, Loddenkemper T. Pediatric status epilepticus management. Curr Opin Pediatr 2014 Dec;26(6):668-674.

(9) Cavazos JE, Jones SM, Cross DJ. Sprouting and synaptic reorganization in the subiculum and CA1 region of the hippocampus in acute and chronic models of partial-onset epilepsy. Neuroscience 2004;126(3):677-688.

(10) Yang Y, Wang J. From Structure to Behavior in Basolateral Amygdala-Hippocampus Circuits. Frontiers in neural circuits 2017;11:86.

(11) Obeid M, Frank J, Medina M, Finckbone V, Bliss R, Bista B, et al. Neuroprotective effects of leptin following kainic acid-induced status epilepticus. Epilepsy & Behavior 2010;19(3):278-283.

(12) Kwon O, Park S. Depression and anxiety in people with epilepsy. Journal of clinical neurology 2014;10(3):175-188.

(13) Singh SP, Agarwal S, Faulkner M. Refractory status epilepticus. Ann Indian Acad Neurol 2014 Mar;17(Suppl 1):S32-6.

(14) Ono T, Galanopoulou AS. Epilepsy and epileptic syndrome. Neurodegenerative Diseases: Springer; 2012. p. 99-113.

(15) Obeid M, Rosenberg EC, Klein PM, Jensen FE. Lestaurtinib (CEP-701) attenuates “second hit” kainic acid-induced seizures following early life hypoxic seizures. Epilepsy Res 2014;108(4):806-810.

(16) Medlej Y, Salah H, Wadi L, Saad S, Bashir B, Allam J, et al. Lestaurtinib (CEP-701) modulates the effects of early life hypoxic seizures on cognitive and emotional behaviors in immature rats. Epilepsy & Behavior 2019 March 2019;92:332-340.

(17) Lignani G, Baldelli P, Marra V. Homeostatic plasticity in epilepsy. Frontiers in Cellular Neuroscience 2020;14:197.

(18) Devinsky O, Lai G. Spirituality and religion in epilepsy. Epilepsy & Behavior 2008;12(4):636-643.

(19) Fisher RS, Boas WVE, Blume W, Elger C, Genton P, Lee P, et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 2005;46(4):470-472.

(20) Kandel ER, Schwartz JH, Jessell TM, Siegelbaum SA, Hudspeth AJ. Principles of neural science. : McGraw-hill New York; 2000.

(21) Giblin KA, Blumenfeld H. Is epilepsy a preventable disorder? New evidence from animal models. Neuroscientist 2010;16(3):253-275.

(22) Sharma AK, Reams RY, Jordan WH, Miller MA, Thacker HL, Snyder PW. Mesial temporal lobe epilepsy: pathogenesis, induced rodent models and lesions. Toxicol Pathol 2007;35(7):984-999.

(23) Zhang N, Houser CR. Ultrastructural localization of dynorphin in the dentate gyrus in human temporal lobe epilepsy: a study of reorganized mossy fiber synapses. J Comp Neurol 1999;405(4):472-490.

(24) Masukawa L, O'Connor W, Lynott J, Burdette L, Uruno K, McGonigle P, et al. Longitudinal variation in cell density and mossy fiber reorganization in the dentate gyrus from temporal lobe epileptic patients. Brain Res 1995;678(1-2):65-75.

(25) Franck JE, Pokorny J, Kunkel DD, Schwartzkroin PA. Physiologic and morphologic characteristics of granule cell circuitry in human epileptic hippocampus. Epilepsia 1995;36(6):543-558.

(26) Shao L, Habela CW, Stafstrom CE. Pediatric epilepsy mechanisms: expanding the paradigm of excitation/inhibition imbalance. Children 2019;6(2):23.

(27) Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, et al. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58(4):522-530.

(28) Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58(4):512-521.

(29) Manno EM. Status epilepticus: current treatment strategies. The Neurohospitalist 2011;1(1):23-31.

(30) Goodkin HP, Kapur J. The impact of diazepam's discovery on the treatment and understanding of status epilepticus. Epilepsia 2009;50(9):2011-2018.

(31) Naylor DE, Liu H, Wasterlain CG. Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. J Neurosci 2005 Aug 24;25(34):7724-7733.

(32) Raspall-Chaure M, Chin RF, Neville BG, Scott RC. Outcome of paediatric convulsive status epilepticus: a systematic review. The Lancet Neurology 2006;5(9):769-779.

(33) Seinfeld S, Goodkin HP, Shinnar S. Status Epilepticus. Cold Spring Harb Perspect Med 2016 Mar 1;6(3):a022830.

(34) Trinka E, Cock H, Hesdorffer D, Rossetti AO, Scheffer IE, Shinnar S, et al. A definition and classification of status epilepticus–Report of the ILAE Task Force on Classification of Status Epilepticus. Epilepsia 2015;56(10):1515-1523.

(35) Beran RG. An alternative perspective on the management of status epilepticus. Epilepsy & Behavior 2008;12(3):349-353.

(36) Riviello JJ, Claassen J, LaRoche SM, Sperling MR, Alldredge B, Bleck TP, et al. Treatment of status epilepticus: an international survey of experts. Neurocritical care 2013;18(2):193-200.

(37) Sharma S, Puttachary S, Thippeswamy A, Kanthasamy AG, Thippeswamy T. Status epilepticus: Behavioral and electroencephalography Seizure Correlates in Kainate experimental Models. Frontiers in neurology 2018;9:7.

(38) Reddy D, Kuruba R. Experimental models of status epilepticus and neuronal injury for evaluation of therapeutic interventions. International journal of molecular sciences 2013;14(9):18284-18318.

(39) Wylie T, Sandhu DS, Goyal A, Murr N. Status Epilepticus. StatPearls Treasure Island (FL): StatPearls Publishing LLC; 2020.

(40) Boggs JG. Mortality associated with status epilepticus. Epilepsy currents 2004;4(1):25-27.

(41) Hauser WA. Status epilepticus: epidemiologic considerations. Neurology 1990;40(5 Suppl 2):9-13.

(42) Mullges W. Diagnosis and treatment of status epilepticus in the intensive care unit. Med Klin Intensivmed Notfmed 2019 Jun;114(5):475-484.

(43) Nunes VD, Neilson J, Sarri G, Cross JH. Diagnosis and management of the epilepsies in adults and children: summary of updated NICE guidance. BMJ 2012 Jan 26;344:e281.

(44) Joshi S, Kapur J. GABAA Receptor Plasticity During Status Epilepticus. In: 4th, Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. Jasper's

Basic Mechanisms of the Epilepsies Bethesda (MD): Michael A Rogawski, Antonio V Delgado-Escueta, Jeffrey L Noebels, Massimo Avoli and Richard W Olsen; 2012.

(45) Lee T, Warrick BJ, Sarangarm P, Alunday RL, Bussmann S, Smolinske SC, et al. Levetiracetam in toxic seizures. Clin Toxicol 2018;56(3):175-181.

(46) Kapur J, Elm J, Chamberlain JM, Barsan W, Cloyd J, Lowenstein D, et al. Randomized trial of three anticonvulsant medications for status epilepticus. N Engl J Med 2019;381(22):2103-2113.

(47) Yi ZM, -, Wen C, Cai T, Xu L, Zhong XL, et al. Levetiracetam for epilepsy: an evidence map of efficacy, safety and economic profiles. Neuropsychiatr Dis Treat 2018 Dec 17;15:1-19.

(48) Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A, et al. The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. Proc Natl Acad Sci U S A 2004 Jun 29;101(26):9861-9866.

(49) Deshpande LS, DeLorenzo RJ. Mechanisms of levetiracetam in the control of status epilepticus and epilepsy. Frontiers in neurology 2014;5:11.

(50) Ahrens S, Ream MA, Slaughter LA. Status epilepticus in the neonate: updates in treatment strategies. Current Treatment Options in Neurology 2019;21(2):8.

(51) Surges R, Volynski KE, Walker MC. Is levetiracetam different from other antiepileptic drugs? Levetiracetam and its cellular mechanism of action in epilepsy revisited. Therapeutic Advances in Neurological Disorders 2008;1(1):13-24.

(52) Rahman M, Nguyen H. Valproic Acid. StatPearls Treasure Island (FL): StatPearls Publishing LLC; 2020.

(53) Wheless JW, Clarke D, Hovinga CA, Ellis M, Durmeier M, McGregor A, et al. Rapid infusion of a loading dose of intravenous levetiracetam with minimal dilution: a safety study. J Child Neurol 2009;24(8):946-951.

(54) Lyttle MD, Rainford NE, Gamble C, Messahel S, Humphreys A, Hickey H, et al. Levetiracetam versus phenytoin for second-line treatment of paediatric convulsive status epilepticus (EcLiPSE): a multicentre, open-label, randomised trial. The Lancet 2019;393(10186):2125-2134.

(55) Dalziel SR, Borland ML, Furyk J, Bonisch M, Neutze J, Donath S, et al. Levetiracetam versus phenytoin for second-line treatment of convulsive status epilepticus in children (ConSEPT): an open-label, multicentre, randomised controlled trial. The Lancet 2019;393(10186):2135-2145.

(56) Langenbruch L, Krämer J, Güler S, Möddel G, Geßner S, Melzer N, et al. Seizures and epilepsy in multiple sclerosis: epidemiology and prognosis in a large tertiary referral center. J Neurol 2019;266(7):1789-1795.

(57) Engel Jr J. Introduction to temporal lobe epilepsy. Epilepsy Res 1996;26(1):141-150.

(58) Blair RD. Temporal lobe epilepsy semiology. Epilepsy research and treatment 2012;2012.

(59) Epilepsy A. Proposal for revised classification of epilepsies and epileptic syndromes. The treatment of epilepsy: principles & practice 2006;354.

(60) Ott D, Caplan R, Guthrie D, Siddarth P, Komo S, Shields WD, et al. Measures of psychopathology in children with complex partial seizures and primary generalized epilepsy with absence. Journal of the American Academy of Child & Adolescent Psychiatry 2001;40(8):907-914.

(61) Kalinin VV, Zemlyanaya AA, Krylov OE, Zheleznova EV. Handedness, alexithymia, and focus laterality as risk factors for psychiatric comorbidity in patients with epilepsy. Epilepsy & Behavior 2010;17(3):389-394.

(62) Obeid M, Wyllie E, Rahi AC, Mikati MA. Approach to pediatric epilepsy surgery: State of the art, Part I: General principles and presurgical workup. Eur J Paediatr Neurol 2009 Mar;13(2):102-114.

(63) Verrotti A, Carrozzino D, Milioni M, Minna M, Fulcheri M. Epilepsy and its main psychiatric comorbidities in adults and children. J Neurol Sci 2014;343(1):23-29.

(64) Helmstaedter C. Effects of chronic epilepsy on declarative memory systems. Progress in brain research: Elsevier; 2002. p. 439-453.

(65) Pitkänen A, Sutula TP. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. The Lancet Neurology 2002;1(3):173-181.

(66) Naegele JR. Neuroprotective strategies to avert seizure-induced neurodegeneration in epilepsy. Epilepsia 2007;48:107-117.

(67) Fastenau PS, Shen J, Dunn DW, Austin JK. Academic underachievement among children with epilepsy: proportion exceeding psychometric criteria for learning disability and associated risk factors. J Learn Disabil 2008;41(3):195-207.

(68) Motamedi G, Meador K. Epilepsy and cognition. Epilepsy & Behavior 2003;4:25-38.

(69) Hermann B, Seidenberg M, Bell B, Rutecki P, Sheth R, Ruggles K, et al. The neurodevelopmental impact of childhood-onset temporal lobe epilepsy on brain structure and function. Epilepsia 2002;43(9):1062-1071.

(70) Black KC, Hynd GW. Epilepsy in the school aged child: Cognitive-behavioral characteristics and effects on academic performance. School Psychology Quarterly 1995;10(4):345.

(71) Mitchell WG, Chavez JM, Lee H, Guzman BL. Academic underachievement in children with epilepsy. J Child Neurol 1991;6(1):65-72.

(72) Gabr M, Lüders H, Dinner D, Morris H, Wyllie E. Speech manifestations in lateralization of temporal lobe seizures. Ann Neurol 1989;25(1):82-87.

(73) Trimble MR, Thompson PJ. Memory, anticonvulsant drugs and seizures. Acta Neurol Scand 1981.

(74) McDonald AJ, Mott DD. Functional neuroanatomy of amygdalohippocampal interconnections and their role in learning and memory. J Neurosci Res 2017;95(3):797-820.

(75) Kemppainen S, Pitkänen A. Distribution of parvalbumin, calretinin, and calbindin-D28k immunoreactivity in the rat amygdaloid complex and colocalization with γ-aminobutyric acid. J Comp Neurol 2000;426(3):441-467.

(76) McDonald AJ. Projection neurons of the basolateral amygdala: a correlative Golgi and retrograde tract tracing study. Brain Res Bull 1992;28(2):179-185.

(77) Squire LR, Zola-Morgan S. The medial temporal lobe memory system. Science 1991 Sep 20;253(5026):1380-1386.

(78) MCGAUGH JL. The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci 2004;27(1):1-28.

(79) Abe K. Modulation of hippocampal long-term potentiation by the amygdala: a synaptic mechanism linking emotion and memory. The Japanese Journal of Pharmacology 2001;86(1):18-22.

(80) Lee S, Amir A, Headley DB, Haufler D, Pare D. Basolateral amygdala nucleus responses to appetitive conditioned stimuli correlate with variations in conditioned behaviour. Nature communications 2016;7:12275.

(81) Bhattacharyya KB. James Wenceslaus Papez, His Circuit, and Emotion. Ann Indian Acad Neurol 2017 Jul-Sep;20(3):207-210.

(82) Witter MP, Wouterlood FG, Naber PA, Van Haeften T. Anatomical organization of the parahippocampal-hippocampal network. Ann N Y Acad Sci 2000;911(1):1-24.

(83) Fanselow MS, Dong H. Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 2010;65(1):7-19.

(84) Strange BA, Witter MP, Lein ES, Moser EI. Functional organization of the hippocampal longitudinal axis. Nature Reviews Neuroscience 2014;15(10):655-669.

(85) Danzer SC, Kotloski RJ, Walter C, Hughes M, McNamara JO. Altered morphology of hippocampal dentate granule cell presynaptic and postsynaptic terminals following conditional deletion of TrkB. Hippocampus 2008;18(7):668-678.

(86) Paré D, Collins DR, Pelletier JG. Amygdala oscillations and the consolidation of emotional memories. Trends Cogn Sci (Regul Ed) 2002;6(7):306-314.

(87) Pape H, Pare D. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol Rev 2010;90(2):419-463.

(88) Tellez-Zenteno JF, Hernandez-Ronquillo L. A review of the epidemiology of temporal lobe epilepsy. Epilepsy Res Treat 2012;2012:630853.

(89) Walczak TS, Radtke RA, McNamara JO, Lewis DV, Luther JS, Thompson E, et al. Anterior temporal lobectomy for complex partial seizures: evaluation, results, and long-term follow-up in 100 cases. Neurology 1990 Mar;40(3 Pt 1):413-418.

(90) BAXENDALE S, HEANEY D, THOMPSON PJ, DUNCAN JS. Cognitive consequences of childhood-onset temporal lobe epilepsy across the adult lifespan. Neurology 2010;75(8):705-711.

(91) Guerreiro CA, Jones-Gotman M, Andermann F, Bastos A, Cendes F. Severe amnesia in epilepsy: causes, anatomopsychological considerations, and treatment. Epilepsy & Behavior 2001;2(3):224-246.

(92) Müller NG, Wohlrath B, Kopp UA, Lengler U. Emotional content does not interfere with verbal memory in patients with temporal lobe epilepsy. Epilepsy & Behavior 2009;15(3):367-371.

(93) Goldberg H, Weinstock A, Bergsland N, Dwyer MG, Farooq O, Sazgar M, et al. MRI segmentation analysis in temporal lobe and idiopathic generalized epilepsy. BMC neurology 2014;14(1):1-8.

(94) Xu C, Krabbe S, Gründemann J, Botta P, Fadok JP, Osakada F, et al. Distinct hippocampal pathways mediate dissociable roles of context in memory retrieval. Cell 2016;167(4):961-972. e16.

(95) Vazdarjanova A, McGaugh JL. Basolateral amygdala is involved in modulating consolidation of memory for classical fear conditioning. J Neurosci 1999 Aug 1;19(15):6615-6622.

(96) Redondo RL, Kim J, Arons AL, Ramirez S, Liu X, Tonegawa S. Bidirectional switch of the valence associated with a hippocampal contextual memory engram. Nature 2014;513(7518):426-430.

(97) LeDoux J, Phelps L, Alberini C. What we talk about when we talk about emotions. Cell 2016;167:1443-1445.

(98) Choi JS, Cain CK, LeDoux JE. The role of amygdala nuclei in the expression of auditory signaled two-way active avoidance in rats. Learn Mem 2010 Feb 26;17(3):139-147.

(99) LeDoux JE. Coming to terms with fear. Proc Natl Acad Sci U S A 2014 Feb 25;111(8):2871-2878.

(100) Pavlov PI. Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. Ann Neurosci 2010 Jul;17(3):136-141.

(101) McSweeney FK, Murphy ES. The Wiley Blackwell handbook of operant and classical conditioning. : John Wiley & Sons; 2014.

(102) Shoji H, Takao K, Hattori S, Miyakawa T. Contextual and cued fear conditioning test using a video analyzing system in mice. J Vis Exp 2014 Mar 1;(85). doi(85):10.3791/50871.

(103) Salah H, Abdel Rassoul R, Medlej Y, Asdikian R, Hajjar H, Dagher S, et al. A Modified Two-Way Active Avoidance Test for Combined Contextual and Auditory Instrumental Conditioning. Front Behav Neurosci 2021 Jun 21;15:682927.

(104) Motta SC, Goto M, Gouveia FV, Baldo MV, Canteras NS, Swanson LW. Dissecting the brain's fear system reveals the hypothalamus is critical for responding in

subordinate conspecific intruders. Proc Natl Acad Sci U S A 2009 Mar 24;106(12):4870-4875.

(105) Moehler H. GABA A receptors in central nervous system disease: anxiety, epilepsy, and insomnia. Journal of Receptors and Signal Transduction 2006;26(5-6):731-740.

(106) Allen MJ, Sabir S, Sharma S. GABA Receptor. StatPearls Treasure Island (FL): StatPearls Publishing LLC; 2020.

(108) Kaila K, Ruusuvuori E, Seja P, Voipio J, Puskarjov M. GABA actions and ionic plasticity in epilepsy. Curr Opin Neurobiol 2014;26:34-41.

(109) Asada H, Kawamura Y, Maruyama K, Kume H, Ding R, Ji FY, et al. Mice lacking the 65 kDa isoform of glutamic acid decarboxylase (GAD65) maintain normal levels of GAD67 and GABA in their brains but are susceptible to seizures. Biochem Biophys Res Commun 1996;229(3):891-895.

(110) Sperk G, Furtinger S, Schwarzer C, Pirker S. GABA and its receptors in epilepsy. Recent advances in epilepsy research: Springer; 2004. p. 92-103.

(111) Treiman DM. GABAergic mechanisms in epilepsy. Epilepsia 2001;42:8-12.

(112) Terunuma M, Xu J, Vithlani M, Sieghart W, Kittler J, Pangalos M, et al. Deficits in phosphorylation of GABA(A) receptors by intimately associated protein kinase C activity underlie compromised synaptic inhibition during status epilepticus. J Neurosci 2008 Jan 9;28(2):376-384.

(113) Galanopoulou AS. GABAA receptors in normal development and seizures: friends or foes? Current neuropharmacology 2008;6(1):1-20.

(114) Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, et al. International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function. Pharmacol Rev 1998 Jun;50(2):291-313.

(115) Zhang N, Wei W, Mody I, Houser CR. Altered localization of GABA(A) receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. J Neurosci 2007 Jul 11;27(28):7520-7531.

(116) Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J. Subunit-specific trafficking of GABA(A) receptors during status epilepticus. J Neurosci 2008 Mar 5;28(10):2527-2538.

(117) Jovanovic JN, Thomas P, Kittler JT, Smart TG, Moss SJ. Brain-derived neurotrophic factor modulates fast synaptic inhibition by regulating GABA(A) receptor phosphorylation, activity, and cell-surface stability. J Neurosci 2004 Jan 14;24(2):522-530.

(119) Brandon NJ, Jovanovic JN, Colledge M, Kittler JT, Brandon JM, Scott JD, et al. A-kinase anchoring protein 79/150 facilitates the phosphorylation of GABAA receptors by cAMP-dependent protein kinase via selective interaction with receptor β subunits. Molecular and Cellular Neuroscience 2003;22(1):87-97.

(120) Goodkin HP, Yeh JL, Kapur J. Status epilepticus increases the intracellular accumulation of GABAA receptors. J Neurosci 2005 Jun 8;25(23):5511-5520.

(121) Mazarati AM, Baldwin RA, Sankar R, Wasterlain CG. Time-dependent decrease in the effectiveness of antiepileptic drugs during the course of self-sustaining status epilepticus. Brain Res 1998;814(1-2):179-185.

(122) Brooks-Kayal AR, Russek SJ. Regulation of GABAA receptor gene expression and epilepsy. Epilepsia 2010;51:51-51.

(123) Darnell JE,Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994 Jun 3;264(5164):1415-1421.

(124) Zhong Z, Wen Z, Darnell JE,Jr. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 1994 Apr 1;264(5155):95-98.

(125) Hu Y, Lund IV, Gravielle MC, Farb DH, Brooks-Kayal AR, Russek SJ. Surface expression of GABAA receptors is transcriptionally controlled by the interplay of cAMP-response element-binding protein and its binding partner inducible cAMP early repressor. J Biol Chem 2008 Apr 4;283(14):9328-9340.

(126) Jaworski J, Mioduszewska B, Sanchez-Capelo A, Figiel I, Habas A, Gozdz A, et al. Inducible cAMP early repressor, an endogenous antagonist of cAMP responsive element-binding protein, evokes neuronal apoptosis in vitro. J Neurosci 2003 Jun 1;23(11):4519-4526.

(127) Roberts DS, Raol YH, Bandyopadhyay S, Lund IV, Budreck EC, Passini MA, et al. Egr3 stimulation of GABRA4 promoter activity as a mechanism for seizure-induced up-regulation of GABA(A) receptor alpha4 subunit expression. Proc Natl Acad Sci U S A 2005 Aug 16;102(33):11894-11899.

(128) Roberts DS, Hu Y, Lund IV, Brooks-Kayal AR, Russek SJ. Brain-derived neurotrophic factor (BDNF)-induced synthesis of early growth response factor 3 (Egr3) controls the levels of type A GABA receptor alpha 4 subunits in hippocampal neurons. J Biol Chem 2006 Oct 6;281(40):29431-29435.

(129) Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. J Neurosci 2005 Mar 23;25(12):3219-3228.

(130) Sanchez RM, Dai W, Levada RE, Lippman JJ, Jensen FE. AMPA/kainate receptor-mediated downregulation of GABAergic synaptic transmission by calcineurin after seizures in the developing rat brain. J Neurosci 2005 Mar 30;25(13):3442-3451.

(131) Timofeev I, Bazhenov M, Seigneur J, Sejnowski T. Neuronal synchronization and thalamocortical rhythms in sleep, wake and epilepsy. Jasper's Basic Mechanisms of the Epilepsies [Internet]. 4th edition: National Center for Biotechnology Information (US); 2012.

(132) Rogawski MA. AMPA receptors as a molecular target in epilepsy therapy. Acta Neurol Scand 2013;127:9-18.

(133) Shepherd JD. Memory, plasticity and sleep-A role for calcium permeable AMPA receptors? Frontiers in molecular neuroscience 2012;5:49.

(134) Paoletti P. Molecular basis of NMDA receptor functional diversity. Eur J Neurosci 2011;33(8):1351-1365.

(135) Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H. Structural basis of long-term potentiation in single dendritic spines. Nature 2004;429(6993):761-766.

(136) Egbenya DL, Hussain S, Lai Y, Xia J, Anderson AE, Davanger S. Changes in synaptic AMPA receptor concentration and composition in chronic temporal lobe epilepsy. Molecular and Cellular Neuroscience 2018;92:93-103.

(137) Rakhade SN, Zhou C, Aujla PK, Fishman R, Sucher NJ, Jensen FE. Early alterations of AMPA receptors mediate synaptic potentiation induced by neonatal seizures. J Neurosci 2008 Aug 6;28(32):7979-7990.

(138) Liu G, Gu B, He X, Joshi RB, Wackerle HD, Rodriguiz RM, et al. Transient inhibition of TrkB kinase after status epilepticus prevents development of temporal lobe epilepsy. Neuron 2013;79(1):31-38.

(139) Boulle F, Kenis G, Cazorla M, Hamon M, Steinbusch HW, Lanfumey L, et al. TrkB inhibition as a therapeutic target for CNS-related disorders. Prog Neurobiol 2012;98(2):197-206.

(140) Kafitz KW, Rose CR, Thoenen H, Konnerth A. Neurotrophin-evoked rapid excitation through TrkB receptors. Nature 1999;401(6756):918-921.

(141) Levine ES, Dreyfus CF, Black IB, Plummer MR. Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. Proc Natl Acad Sci U S A 1995 Aug 15;92(17):8074-8077.

(142) Danzer SC, He X, McNamara JO. Ontogeny of seizure-induced increases in BDNF immunoreactivity and TrkB receptor activation in rat hippocampus. Hippocampus 2004;14(3):345-355.

(143) Danzer SC, McNamara JO. Localization of brain-derived neurotrophic factor to distinct terminals of mossy fiber axons implies regulation of both excitation and feedforward inhibition of CA3 pyramidal cells. Journal of Neuroscience 2004;24(50):11346-11355.

(144) Gall CM. Seizure-induced changes in neurotrophin expression: implications for epilepsy. Exp Neurol 1993;124(1):150-166.

(146) Cazorla M, Premont J, Mann A, Girard N, Kellendonk C, Rognan D. Identification of a low-molecular weight TrkB antagonist with anxiolytic and antidepressant activity in mice. J Clin Invest 2011 May;121(5):1846-1857.

(147) Huang YZ, Pan E, Xiong Z, McNamara JO. Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. Neuron 2008;57(4):546-558.

(148) Sutula T, Steward O. Facilitation of kindling by prior induction of long-term potentiation in the perforant path. Brain Res 1987;420(1):109-117.

(149) Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, et al. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 2006 Oct 6;314(5796):140-143.

(150) Arango-Lievano M, Lambert WM, Bath KG, Garabedian MJ, Chao MV, Jeanneteau F. Neurotrophic-priming of glucocorticoid receptor signaling is essential for neuronal plasticity to stress and antidepressant treatment. Proc Natl Acad Sci U S A 2015 Dec 22;112(51):15737-15742.

(151) Rahmati N, Hoebeek FE, Peter S, De Zeeuw CI. Chloride homeostasis in neurons with special emphasis on the olivocerebellar system: Differential roles for transporters and channels. Frontiers in cellular neuroscience 2018;12:101.

(152) Moore YE, Kelley MR, Brandon NJ, Deeb TZ, Moss SJ. Seizing control of KCC2: a new therapeutic target for epilepsy. Trends Neurosci 2017;40(9):555-571.

(153) Gamba G. Molecular physiology and pathophysiology of electroneutral cation-chloride cotransporters. Physiol Rev 2005;85(2):423-493.

(154) Hartmann A, Nothwang HG. Molecular and evolutionary insights into the structural organization of cation chloride cotransporters. Frontiers in cellular neuroscience 2015;8:470.

(155) Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, et al. NKCC1 transporter facilitates seizures in the developing brain. Nat Med 2005;11(11):1205-1213.

(156) Löscher W, Puskarjov M, Kaila K. Cation-chloride cotransporters NKCC1 and KCC2 as potential targets for novel antiepileptic and antiepileptogenic treatments. Neuropharmacology 2013;69:62-74.

(157) Wang Y, Wang Y, Chen Z. Double-edged GABAergic synaptic transmission in seizures: the importance of chloride plasticity. Brain Res 2018;1701:126-136.

(158) Cohen I, Navarro V, Le Duigou C, Miles R. Mesial temporal lobe epilepsy: a pathological replay of developmental mechanisms? Biology of the Cell 2003;95(6):329-333.

(159) Rivera C, Voipio J, Thomas-Crusells J, Li H, Emri Z, Sipila S, et al. Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. J Neurosci 2004 May 12;24(19):4683-4691.

(160) Lee HH, Walker JA, Williams JR, Goodier RJ, Payne JA, Moss SJ. Direct protein kinase C-dependent phosphorylation regulates the cell surface stability and activity of the potassium chloride cotransporter KCC2. J Biol Chem 2007 Oct 12;282(41):29777-29784.

(161) Puskarjov M, Ahmad F, Kaila K, Blaesse P. Activity-dependent cleavage of the K-Cl cotransporter KCC2 mediated by calcium-activated protease calpain. J Neurosci 2012 Aug 15;32(33):11356-11364.

(162) Lee HH, Deeb TZ, Walker JA, Davies PA, Moss SJ. NMDA receptor activity downregulates KCC2 resulting in depolarizing GABA A receptor–mediated currents. Nat Neurosci 2011;14(6):736-743.

(163) Lee HH, Jurd R, Moss SJ. Tyrosine phosphorylation regulates the membrane trafficking of the potassium chloride co-transporter KCC2. Molecular and cellular neuroscience 2010;45(2):173-179.

(164) Rinehart J, Maksimova YD, Tanis JE, Stone KL, Hodson CA, Zhang J, et al. Sites of regulated phosphorylation that control K-Cl cotransporter activity. Cell 2009;138(3):525-536.

(165) Begum G, Yuan H, Kahle KT, Li L, Wang S, Shi Y, et al. Inhibition of WNK3 kinase signaling reduces brain damage and accelerates neurological recovery after stroke. Stroke 2015;46(7):1956-1965.

(166) Thom M. Hippocampal sclerosis in epilepsy: a neuropathology review. Neuropathol Appl Neurobiol 2014;40(5):520-543.

(167) Cavarsan CF, Malheiros J, Hamani C, Najm I, Covolan L. Is mossy fiber sprouting a potential therapeutic target for epilepsy? Frontiers in neurology 2018;9:1023.

(168) Koyama R, Yamada MK, Fujisawa S, Katoh-Semba R, Matsuki N, Ikegaya Y. Brain-derived neurotrophic factor induces hyperexcitable reentrant circuits in the dentate gyrus. J Neurosci 2004 Aug 18;24(33):7215-7224.

(169) Freiman TM, Häussler U, Zentner J, Doostkam S, Beck J, Scheiwe C, et al. Mossy fiber sprouting into the hippocampal region CA2 in patients with temporal lobe epilepsy. Hippocampus 2021.

(170) Longo B, Covolan L, Chadi G, Mello LEA. Sprouting of mossy fibers and the vacating of postsynaptic targets in the inner molecular layer of the dentate gyrus. Exp Neurol 2003;181(1):57-67.

(171) Sloviter RS. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. Science 1987 Jan 2;235(4784):73-76.

(172) Holtmaat AJ, Gorter JA, De Wit J, Tolner EA, Spijker S, Giger RJ, et al. Transient downregulation of Sema3A mRNA in a rat model for temporal lobe epilepsy: a novel molecular event potentially contributing to mossy fiber sprouting. Exp Neurol 2003;182(1):142-150.

(173) Tamagnone L, Comoglio PM. Signalling by semaphorin receptors: cell guidance and beyond. Trends Cell Biol 2000;10(9):377-383.

(174) Polli RS, Malheiros JM, Dos Santos R, Hamani C, Longo BM, Tannús A, et al. Changes in hippocampal volume are correlated with cell loss but not with seizure frequency in two chronic models of temporal lobe epilepsy. Frontiers in neurology 2014;5:111.

(175) Proper E, Jansen G, Van Veelen C, Van Rijen P, Gispen W, De Graan P. A grading system for hippocampal sclerosis based on the degree of hippocampal mossy fiber sprouting. Acta Neuropathol 2001;101(4):405-409.

(176) Mikati MA, Choueri R, Khurana DS, Riviello J, Helmers S, Holmes G. Gabapentin in the treatment of refractory partial epilepsy in children with intellectual disability. J Intellect Disabil Res 1998 Dec;42 Suppl 1:57-62.

(177) Mikati MA, Holmes GL, Chronopoulos A, Hyde P, Thurber S, Gatt A, et al. Phenobarbital modifies seizure-related brain injury in the developing brain. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society 1994;36(3):425-433.

(178) Raedt R, Van Dycke A, Van Melkebeke D, De Smedt T, Claeys P, Wyckhuys T, et al. Seizures in the intrahippocampal kainic acid epilepsy model: characterization using long-term video-EEG monitoring in the rat. Acta Neurol Scand 2009;119(5):293-303.

(179) Lévesque M, Avoli M, Bernard C. Animal models of temporal lobe epilepsy following systemic chemoconvulsant administration. J Neurosci Methods 2016;260:45-52.

(180) Rao MS, Hattiangady B, Reddy DS, Shetty AK. Hippocampal neurodegeneration, spontaneous seizures, and mossy fiber sprouting in the F344 rat model of temporal lobe epilepsy. J Neurosci Res 2006;83(6):1088-1105.

(181) Sibbe M, Häussler U, Dieni S, Althof D, Haas CA, Frotscher M. Experimental epilepsy affects N otch1 signalling and the stem cell pool in the dentate gyrus. Eur J Neurosci 2012;36(12):3643-3652.

(182) Thom M, Eriksson S, Martinian L, Caboclo LO, McEvoy AW, Duncan JS, et al. Temporal lobe sclerosis associated with hippocampal sclerosis in temporal lobe epilepsy: neuropathological features. Journal of Neuropathology & Experimental Neurology 2009;68(8):928-938.

(183) Mathern GW, Adelson PD, Cahan LD, Leite JP. Hippocampal neuron damage in human epilepsy: Meyer's hypothesis revisited. Prog Brain Res 2002;135:237-252.

(184) Nadler JV, Perry BW, Cotman CW. Intraventricular kainic acid preferentially destroys hippocampal pyramidal cells. Nature 1978;271(5646):676.

(185) Chen S, Buckmaster PS. Stereological analysis of forebrain regions in kainate-treated epileptic rats. Brain Res 2005;1057(1-2):141-152.

(186) Jimenez-Mateos EM, Engel T, Merino-Serrais P, McKiernan RC, Tanaka K, Mouri G, et al. Silencing microRNA-134 produces neuroprotective and prolonged seizure-suppressive effects. Nat Med 2012;18(7):1087-1094.

(187) Treiman DM. Efficacy and safety of antiepileptic drugs: a review of controlled trials. Epilepsia 1987;28(s3).

(188) 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(7):1361-1391.

(189) Voutsinos-Porche B, Koning E, Kaplan H, Ferrandon A, Guenounou M, Nehlig A, et al. Temporal patterns of the cerebral inflammatory response in the rat lithium–pilocarpine model of temporal lobe epilepsy. Neurobiol Dis 2004;17(3):385-402.

(190) De A. Furtado M, Braga GK, Oliveira JA, Del Vecchio F, Garcia-Cairasco N. Behavioral, morphologic, and electroencephalographic evaluation of seizures induced by intrahippocampal microinjection of pilocarpine. Epilepsia 2002;43:37-39.

(191) Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science 2002 Nov 15;298(5597):1418-1421.

(192) Knopp A, Kivi A, Wozny C, Heinemann U, Behr J. Cellular and network properties of the subiculum in the pilocarpine model of temporal lobe epilepsy. J Comp Neurol 2005;483(4):476-488.

(193) Kandratavicius L, Monteiro MR, Assirati Jr JA, Carlotti Jr CG, Hallak JE, Leite JP. Neurotrophins in mesial temporal lobe epilepsy with and without psychiatric comorbidities. Journal of Neuropathology & Experimental Neurology 2013;72(11):1029-1042.

(194) Kandratavicius L, Monteiro MR, Hallak JE, Carlotti CG, Assirati JA, Leite JP. Microtubule-associated proteins in mesial temporal lobe epilepsy with and without psychiatric comorbidities and their relation with granular cell layer dispersion. BioMed research international 2013;2013.

(195) Pauli E, Hildebrandt M, Romstock J, Stefan H, Blumcke I. Deficient memory acquisition in temporal lobe epilepsy is predicted by hippocampal granule cell loss. Neurology 2006 Oct 24;67(8):1383-1389.

(196) Kandratavicius L, Balista PA, Lopes-Aguiar C, Ruggiero RN, Umeoka EH, Garcia-Cairasco N, et al. Animal models of epilepsy: use and limitations. Neuropsychiatr Dis Treat 2014 Sep 9;10:1693-1705.

(197) Pitkänen A, Buckmaster P, Galanopoulou AS, Moshé SL. Models of seizures and epilepsy. : Academic Press; 2017.

(198) Brandt C, Glien M, Potschka H, Volk H, Löscher W. Epileptogenesis and neuropathology after different types of status epilepticus induced by prolonged electrical stimulation of the basolateral amygdala in rats. Epilepsy Res 2003;55(1-2):83-103.

(199) Kowski AB, Holtkamp M. Electrically Induced Limbic Seizures: Preliminary Findings in a Rodent Model. Journal of experimental neuroscience 2015;9:JEN. S23759.

(200) Browning R, Nelson D. Variation in threshold and pattern of electroshock-induced seizures in rats depending on site of stimulation. Life Sci 1985;37(23):2205-2211.

(201) Dubé C, Vezzani A, Behrens M, Bartfai T, Baram TZ. Interleukin-1β contributes to the generation of experimental febrile seizures. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society 2005;57(1):152-155.

(202) Dube CM, Ravizza T, Hamamura M, Zha Q, Keebaugh A, Fok K, et al. Epileptogenesis provoked by prolonged experimental febrile seizures: mechanisms and biomarkers. J Neurosci 2010 Jun 2;30(22):7484-7494.

(203) Shabbir M, Stuart R. Lestaurtinib, a multitargeted tyrosinse kinase inhibitor: from bench to bedside. Expert Opin Investig Drugs 2010;19(3):427-436.

(204) Minturn JE, Evans AE, Villablanca JG, Yanik GA, Park JR, Shusterman S, et al. Phase I trial of lestaurtinib for children with refractory neuroblastoma: a new approaches to neuroblastoma therapy consortium study. Cancer Chemother Pharmacol 2011;68(4):1057-1065.

(205) Rzezak P, Valente KD, Duchowny MS. Temporal lobe epilepsy in children: executive and mnestic impairments. Epilepsy & Behavior 2014;31:117-122.

(206) Medlej Y, Salah H, Wadi L, Atoui Z, Fadlallah Y, Asdikian R, et al. Methods in electrode implantation and wiring for long-term continuous EEG monitoring in rodent models of epilepsy and behavioral disturbances. Psychiatric disorders: Springer; 2019. p. 429-439.

(207) Salah H, Medlej Y, Karnib N, Darwish N, Asdikian R, Wehbe S, et al. Methods in Emotional Behavioral Testing in Immature Epilepsy Rodent Models. Methods Mol Biol 2019;2011:413-427.

(208) Medlej Y, Salah H, Wadi L, Saad S, Asdikian R, Karnib N, et al. Overview on Emotional Behavioral Testing in Rodent Models of Pediatric Epilepsy. Methods Mol Biol 2019;2011:345-367.

(209) Gusel'Nikova V, Korzhevskiy D. NeuN as a neuronal nuclear antigen and neuron differentiation marker. Acta Naturae (англоязычная версия) 2015;7(2 (25)).

(210) Kuk R. Lestaurtinib (CEP-701) potentiates the anticonvulsant effect of phenobarbital against kainic acid-induced status epilepticus. 2018.

(211) Keezer MR, Sisodiya SM, Sander JW. Comorbidities of epilepsy: current concepts and future perspectives. The Lancet Neurology 2016;15(1):106-115.

(212) Iqbal MJ, Wasim M, Rashid U, Zeeshan N, Ali R, Nayyab S, et al. Mutational screening of GABRG2 gene in Pakistani population of Punjab with generalized tonic clonic seizures and children with childhood absence epilepsy. Journal of the Chinese Medical Association 2018;81(8):665-669.

(213) https://neupsykey.com/pre-frontal-cortex-hippocampus-and-the-biology-of-explicit-memory-storage/.

(214) Wenzel A. The sage encyclopedia of abnormal and clinical psychology. : SAGE Publications; 2017.

(215) Castelhano AS, Cassane GS, Scorza FA, Cysneiros RM. Altered anxiety-related and abnormal social behaviors in rats exposed to early life seizures. Frontiers in behavioral neuroscience 2013;7:36.

(216) Gröticke I, Hoffmann K, Löscher W. Behavioral alterations in a mouse model of temporal lobe epilepsy induced by intrahippocampal injection of kainate. Exp Neurol 2008;213(1):71-83.

(217) Stevens CE, Stafstrom CE. Pharmacotherapy for Focal Seizures in Children and Adolescents. Drugs 2018;78(13):1321-1337.

(218) Liu R, Wang J, Liang S, Zhang G, Yang X. Role of NKCC1 and KCC2 in Epilepsy: From Expression to Function. Frontiers in Neurology 2020;10:1407.