

**THE EFFECT OF PHARMACOLOGICALLY-
INDUCED EPILEPTIFORM-LIKE ACTIVITY ON
INTER-HEMISPHERIC HIPPOCAMPAL
FUNCTIONAL CONNECTIVITY IN THE
ANAESTHETISED RATS**

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ABSTRACT

Medial temporal lobe epilepsy (mTLE) is one of the most common epilepsies in the human population, yet it is difficult to control. Neuronal activity in a seizure reflects increased firing of individual neurones and alterations in the connectivity of neural networks.

In vivo electrophysiological recordings of neuronal ensemble single-unit and local field potential (LFP) activity were performed in both left and right hippocampi of isoflurane-anaesthetised rats with the aim of establishing a model of acute epileptiform-like activity induced by intrahippocampal kainic acid (KA) administration. Secondly, the main objective of this study was to examine the effects of the anticonvulsants valproate (VPA, 20 mg/kg administered intravenously), WIN 55,212-2 (WIN, 1.2 mg/kg, administered intravenously) and flurazepam (FLU, 2 mg/kg, administered intravenously) on acute epileptiform-like activity induced by KA. The effects of the selected drugs were evaluated according to normalised mean firing rate; normalised mean synchrony index, cross-correlations (mean K-index) and LFP frequency bands distribution.

We evaluated two concentrations of KA (1 mM and 10 mM administered locally) to establish the acute mTLE rat model. Epileptiform-like activity was induced by both KA 1 mM and 10 mM in the ipsilateral hippocampus. Although there was no direct administration of KA to the contralateral hippocampus (only injected in the ipsilateral hippocampus), the neuronal hyper-excitability effect was also propagated to the contralateral hippocampus. The inter-hemispheric hippocampal connectivity and the connectivity between neurones in the hippocampus (intra-hippocampal) during epileptiform activity in the mTLE model were evaluated according to four parameters studied: normalised mean firing rate, normalised mean synchrony index, cross-correlation (K-index) and LFP frequency band distribution. Our study has shown that administering intrahippocampal KA 1 mM is effective to induce epileptiform-like activity through significant increases in normalised mean firing rate in ipsilateral-CA1 and contralateral-CA3 hippocampus ($p<0.05$ and $p<0.0001$ when compared to basal, respectively). Increased normalised mean synchrony index in ipsilateral-CA1 ($p<0.001$) and increased normalised mean power in the LFP

frequency band of delta (1-4 Hz) and theta (4-8 Hz) in the ipsilateral and contralateral hippocampus were found in both CA1 and CA3, respectively. Therefore, we chose to administer KA 1 mM locally for our mTLE model.

Based on our findings, 20 mg/kg of VPA administered intravenously 20 minutes before KA prevented the neuronal hyper-excitability induced by KA in the ipsilateral-CA1 hippocampus, but there were no changes in the ipsilateral-CA3 and contralateral-CA1 hippocampus when compared to basal. However, 20 mg/kg VPA reduced the neuronal hyper-excitability in contralateral-CA3 by 55% compared to the experiment without VPA (mTLE model). VPA administered 20 min after KA administration was observed to attenuate the neuronal hyper-excitability induced by KA, since there was no neuronal hyper-excitability in any brain regions. These results indicated that VPA prevents KA inducing further neuronal hyper-excitability. Due to these effects, we proposed that VPA acts to prevent, reduce, and attenuate epileptiform-like activity. A dose of 1.2 mg/kg WIN administered before KA prevented neuronal hyper-excitability induced by KA, while the administration of 20 mg/kg WIN 20 min after KA showed that WIN attenuated the firing induced by KA. A 1.2 mg/kg intravenous administration of FLU - lower than the dosage used in the literature (30-60 mg/kg) - attenuated epileptiform-like activity induced by KA.

In conclusion, the data presented in this thesis have addressed the aims and hypothesis of this study by providing further evidence supporting the use of VPA, WIN and FLU in controlling hyper-excitability in the hippocampi. This thesis generates three main contributions; the mTLE model for anaesthetised rats is proposed; VPA administered after KA is the most effective treatment to attenuate the effect of KA than WIN and FLU; WIN and FLU are proposed as potential anti-epileptic drugs (AEDs) in mTLE model. Our study has highlighted the significance of using an electrophysiological approach in an animal model of mTLE using anaesthetised rats, and functional connectivity in the hippocampi, which are lacking in the present literature.

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ABBREVIATIONS

AD	after-discharge
AEDs	anti-epileptic drugs
α	alpha
AMPA	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid
AMPAR	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid receptor
AP	anterior posterior
β	beta
BOLD	blood oxygenation-level-dependent
CA	cornu ammonis
Ca^{2+}	calcium
CB	cannabinoid
CB1R	cannabinoid type 1 receptor
CB2R	cannabinoid type 2 receptor
Cl^-	chloride
δ	delta
DG	dentate gyrus
EC	entorhinal cortex
EEG	electroencephalogram
FLU	flurazepam
fMRI	functional magnetic resonance imaging
FPI	fluid percussion injury
γ	gamma
GABA	gamma amino butyric acid
HS	hippocampal sclerosis
i.p	intraperitoneal
i.v	intravenous
ILAE	International League Against Epilepsy
K^+	potassium
KA	kainic Acid / Kainate
KAR	kainate receptor
LEC	lateral entorhinal cortex
LFPs	local field potentials
LPP	lateral perforant path
lTLE	lateral temporal lobe epilepsy
MAP	multichannel acquisition processor
MEA	microwire electrode arrays
MEC	medial entorhinal cortex
MES	maximal electroshock seizure
ML	medial-lateral

min	minute or minutes
MPP	medial perforant path
MRI	magnetic resonance imaging
mTLE	medial temporal lobe epilepsy
Na ⁺	sodium
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
PP	perforant path
PTE	posttraumatic epilepsy
RASPUTIN	Real-Time Acquisition System Programs for Unit Timing
ROI	region of interest
S.E.M	standard error means
Sb	subiculum
SE	status epilepticus
SI	synchrony index
SWS	slow wave sleep
θ	theta
TDEM	t-distribution expectation maximisation
TLE	temporal lobe epilepsy
VD	dorsal-ventral
VPA	sodium valproate / valproic acid
WIN	WIN 55,212-2
Δ ⁹ -THC	Δ ⁹ -tetrahydrocannabinol

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Chapter 1

INTRODUCTION

1.1 Summary

Initially, the prevalence, incidence and definition of epilepsy will be specified, along with the pathophysiology, aetiology and comorbidities of seizures (Section 1.2). Types of epilepsies will then be described (Section 1.3) followed by an overview of temporal lobe epilepsy, with an emphasis on medial temporal lobe epilepsy and its associated underlying pathology (Section 1.4). The anatomic description of the hippocampus, and its function and physiology in relation to epilepsy is explained in Section 1.5. Techniques for the study of neuronal function in epilepsy will then be described, including electrophysiology and the synchronisation approach (Section 1.6). A brief introduction to the treatment with antiepileptic drugs will be described, followed by a description of the drugs used in this study, which are valproate, WIN 55,212-2, and flurazepam (Section 1.7). Some of the different types of animal models used in discovering antiepileptic drugs will be discussed in terms of their benefits and limitations, leading to the justification for using kainic acid model in our study (Section 1.8). Finally, previous studies in the existing literature will be discussed, leading to the contribution and significance of this research (Section 1.9), before a brief introduction to the aims and hypotheses of the whole study (Section 1.10).

1.2 Epilepsy

The prevalence of epilepsy in developed countries ranges from 4 to 10 cases per 1,000 persons. On the other hand, studies in developing and tropical countries have reported a higher prevalence rate of epilepsy, ranging from 14 to 57 cases per 1,000 persons (Téllez-Zenteno and Hernández-Ronquillo 2012). Approximately 50 per 100 000 people per year develop epilepsy in developed countries (Duncan et al., 2006), the most vulnerable groups being infants and elderly people (Ngugi et al., 2010; Newton and Garcia 2012). Their higher risk of brain infections could contribute to the higher incidence, which is usually above 100 per 100,000 people per year in countries (Duncan et al., 2006) with poor sanitation and inadequate healthcare delivery systems (Espinosa-Jovel et al., 2018).

Epileptogenesis refers to the development of a neuronal network in which spontaneous seizures occur (Giblin and Blumenfeld, 2010; Pitkänen et al., 2015). Previous work on epileptogenesis has focused on acquired forms of epilepsy due to brain injury or prolonged seizure (Webster et al., 2017), followed by a clinically latent period during which the brain reorganises into a hyper-excitabile state (Giblin and Blumenfeld, 2010; Clossen and Reddy, 2017; Huntsman et al., 2019).

Epilepsy has traditionally been referred to as a brain disorder, or family of disorders, characterised by recurrent seizures associated with excessive discharge in a population of hyper-excitabile neurones (Avanzini and Franceschetti, 2003; Koshal et al., 2017) and excessive synchronisation of large neuronal populations leading to a hypersynchronous condition (Jiruska et al., 2013; Jefferys 2015; Huntsman et al., 2019). According to the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy, epilepsy is considered to be a disease (Fisher et al., 2014) based on the following conditions:

1. At least two unprovoked (or reflex) seizures occurring more than 24 hours apart;
2. One unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years;
3. Diagnosis of an epilepsy syndrome.

The function of the brain is mediated through oscillations for which neuronal communication of inhibitory neurones (e.g., synaptic transmission), and intrinsic neuronal properties (e.g., the ability of a neurone to maintain burst firing) are crucial (Pernelle et al., 2018). Changes from normal to epileptiform behaviour are caused by multiple factors, such as greater spread and neuronal recruitment secondary to a combination of enhanced connectivity, enhanced excitatory transmission, failure of inhibitory mechanisms, and changes in intrinsic neuronal properties (Duncan et al., 2006; Pernelle et al., 2018).

A seizure is triggered by the excessive firing of the neurones and the fast spreading of these impulses over the brain (Stafstrom and Carmant, 2015; Fisher et al., 2017). The activation of the seizure depends on its site of origin within the brain, time duration and the electrical signal discharge. The changes in neuronal excitability induce abnormal activity in individual neurones and also recruit a critical mass of hyper-excitable cells in highly synchronised activities that are propagated through normal pathways (Avanzini and Franceschetti, 2003; Pitkänen et al., 2017). Thus, there are two phenomena in the pathophysiology of a seizure: either hyper-excitability of a neurone, or hyper-synchronisation. Brief synchronous activity of a group of neurones leads to an interictal spike, which is less than 250 milliseconds and is distinct from a seizure (Staley and Dudek, 2006). Hyper-synchronisation means that a hyper-excitable neurone leads to excessive excitability in a large group of surrounding neurones (Bromfield, 2006; Jefferys, 2015). This means that when a large electrical impulse is generated in one part of the brain, millions of neurones in the brain fire excessively triggering a seizure (Avanzini and Franceschetti, 2003).

Neuronal messages are transmitted by electrical impulses called the action potential. This is actually a net positive influx of ions that leads to depolarisation or voltage change in the neuronal membrane (Li and Chen, 2018). The ions involved are sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and chloride (Cl^-). Brain tissues prevent hyper-excitability by several inhibitory mechanisms involving negative ions such as Cl^- ions (Avanzini and Franceschetti, 2003; O'Dell et al., 2012). Disturbance of this normal excitability leads to hyper-excitability. In this state, the excitatory transmission of impulses will increase, and inhibitory transmission will decrease. The voltage gated ionic channels are open when the voltage difference across the neuronal membrane is

changed (Moran et al., 2015; Maljevic and Lerche, 2019). Once activated, the impulses flow through the neuronal circuits along the axons of the nerves (Avanzini and Franceschetti, 2003). An action potential travels down the axon to the terminal buttons and then releases neurotransmitters in the synaptic cleft. This carries the action potential from one nerve to another (O'Dell et al., 2012).

Co-morbidities of epilepsy are associated with neuropsychiatric (depression and anxiety) and neurologic disorders such as migraine (Sriviska and Shah, 2017). There is a bidirectional relationship between epilepsy and psychiatric disorders, implying that either of them can occur before or after the other, and the modification of one increases the risk for the development of the other (Berg et al., 2017; Sriviska and Shah, 2017). The increase risk of depression in epilepsy patients is associated with the existence of non-lesional partial epilepsy, increased frequency and duration of seizures (Berg et al., 2017), and the negative psychotropic effects of some anti-epileptic drugs (AEDs). The pathogenesis of depression in epilepsy may relate to dysfunction in the prefrontal, inferior frontal, striatal, and mesial temporal regions (Rudzinski and Meador, 2013). The relationship between patients with epilepsy, depression, and anxiety is also associated with the stigma of epilepsy, lack of control over the occurrence of seizures, lack of education regarding seizures and epilepsy, and the frequency of reported antiepileptic drug-related adverse events (Rudzinski and Meador, 2013). Migraine frequently occurs in those with epilepsy caused by head trauma (Rogawski et al., 2012). The increased risk of both migraine and epilepsy is proposed to be produced by genetic factors or can be acquired (such as in head injury) when the brain is in a state of hyperexcitability. Attacks begin with hypersynchronous activity. The activation of ionotropic glutamate receptor triggers the hyperexcitability in both epilepsy and migrain (Rogawski et al., 2012).

1.3 Types of Epilepsies

Epilepsies can be divided into generalised and localisation-related categories (Table 1.1) depending on whether the seizures begin simultaneously on both sides of the brain (generalised seizure) or in a part of one hemisphere (partial seizure). Generalised seizure is caused by an initial abnormal firing of neurones throughout both left and right hemispheres (Sanabria et al., 2002). Genetic factors are important in this type of seizures.

Generalised epilepsies can be divided into idiopathic, cryptogenic and symptomatic (Dixit et al., 2017). Idiopathic epilepsy is caused by genetic-related factors, which is associated with electroencephalogram (EEG) pattern of generalised spike and wave discharges (Kandratavicius et al., 2014; Maljevic and Lerche, 2019). Idiopathic epilepsy includes benign neonatal familial convulsions, an inheritable ‘channelopathy’ affecting K^+ channel kinetics (Baulac, 2010). Another type of idiopathic epilepsy is childhood and juvenile absence epilepsy, which are characterised by brief (5-10 seconds) non-convulsive episodes of apparent unconsciousness (Bayne, 2011), other types are juvenile myoclonic epilepsy, and epilepsies with seizures precipitated by specific modes of activation (Table 1.1).

Cryptogenic or symptomatic generalised epilepsies are: West syndrome, Lennox-Gastaut syndrome, epilepsy with myoclonic-astatic seizures and epilepsy with myoclonic absences (Table 1.1). West syndrome is a form of infantile spasms syndrome occurring in children younger than 1 year, characterised by developmental arrest or regression (Pavone et al., 2013; Stafstrom and Carmant, 2015). Lennox-Gastaut syndrome is classified among the age-related epileptic encephalopathies and consists of atypical absences (alteration of consciousness) with slow spike-and-wave complexes, tonic seizures that are mostly sleep-related and cognitive deterioration (Van Rijckevorsel, 2008; Stafstrom and Carmant, 2015).

Symptomatic epilepsies are those of non-specific aetiology in which the seizures are one manifestation of an underlying brain disorder (Schulze-Bonhage, 2019). This includes genetic disorders (e.g., tuberous sclerosis, phenylketonuria), hippocampal sclerosis (HS) (Dugladze et al., 2007; Shorvon, 2019), structural and metabolic disorders (infection, trauma or insult) tumours and congenital malformation (Pitkänen

et al., 2006). Some epilepsies and syndromes cause both generalised and focal seizures such as the Landau-Kleffner syndrome (Table 1.1). This syndrome is associated with language disturbances, word deafness and progressive neuropsychological impairment related to the appearance of paroxysmal electroencephalograph activity (Pearl et al., 2001).

Partial seizures develop when limited, confined populations of nerve cells develop an abnormal increase in electrical discharge activity in one limited area of the brain (Pitkänen and Sutula, 2002). In studies in human, the EEG becomes less chaotic within large areas of cortex before a seizure, suggesting that widespread synchronisation is taking place (Jefferys, 2015). In focal epilepsies, focal functional disruption results in seizures beginning in a localised fashion, and then spreading by recruitment of other brain areas (Fisher et al., 2017). The site of the focus and the speed and extent of spread determine the clinical manifestation of the seizure (Pitkänen and Sutula, 2002). Out of all types of epilepsy mentioned, temporal lobe epilepsy is of particular interest to us and will be discussed in detail in the next section.

Table 1.1 International classification of epilepsies, epileptic syndromes, and related seizure disorders (retrieved from Fisher et al., 2005; Fisher et al., 2014)

1. Localisation-related epilepsies and syndromes
1.1 Idiopathic
☐ Benign childhood epilepsy with centrotemporal spikes
☐ Childhood epilepsy with occipital paroxysms
☐ Primary reading epilepsy
1.2 Symptomatic
☐ Chronic progressive epilepsia partialis continua of childhood (Kojewnikow's syndrome)
☐ Syndromes characterised by seizures with specific modes of precipitation
☐ Temporal lobe epilepsies
☐ Frontal lobe epilepsies
☐ Parietal lobe epilepsies
☐ Occipital lobe epilepsies
1.3 Cryptogenic
2. Generalised epilepsies and syndromes
2.1 Idiopathic
☐ Benign neonatal familial convulsions
☐ Childhood absence epilepsy
☐ Juvenile absence epilepsy
☐ Juvenile myoclonic epilepsy
☐ Other generalised idiopathic epilepsies not defined above
☐ Epilepsies with seizures precipitated by specific modes of activation
2.2 Cryptogenic or symptomatic
☐ West syndrome
☐ Lennox-Gastaut syndrome
☐ Epilepsy with myoclonic-astatic seizures
☐ Epilepsy with myoclonic absences
2.3 Symptomatic
2.3.1 Non-specific aetiology
☐ Early myoclonic encephalopathy
☐ Early infantile epileptic encephalopathy with suppression burst
☐ Other symptomatic generalised epilepsies not defined above
2.3.2 Specific syndromes
☐ Diseases in which seizures are a presenting or predominant feature
3. Epilepsies and syndromes undetermined whether focal or generalised
3.1 With both generalised and focal seizures
☐ Neonatal seizures
☐ Severe myoclonic epilepsy in infancy
☐ Epilepsy with continuous spike-waves during slow wave sleep
☐ Acquired epileptic aphasia (Landau-Kleffner syndrome)
☐ Other undetermined epilepsies not defined above
3.2 Without unequivocal generalised or focal features
4. Special syndromes
4.1 Situation-related seizures
☐ Febrile convulsions
☐ Isolated seizures or isolated status epilepticus
☐ Seizures occurring only when there is an acute metabolic or toxic event (alcohol, drugs, eclampsia, nonketotic hyperglycaemia)

1.4 Temporal Lobe Epilepsy (TLE)

Temporal lobe epilepsy (TLE) is a form of focal epilepsy, which is characterised by seizures affecting one part of the brain. It is also known as partial seizures. TLE can be lesional, mainly associated with HS, benign tumours, vascular malformations, cortical development malformations, and post-traumatic or post-infectious gliosis (Téllez-Zenteno et al., 2010; Clossen and Reddy, 2017; Huntsman et al., 2019). More than 80% of cases have related HS, the most common cause of TLE (Coan et al., 2015), which is a combination of atrophy and astrogliosis of the amygdala, hippocampus, parahippocampal gyrus, and the entorhinal cortex (Tatum, 2012; Boling, 2018). The majority of hippocampal specimens were also found to have granule cell dispersion within the dentate gyrus, associated with early seizure onset or status epilepticus (SE) at the early stage of the disease (Blümcke, 2008).

SE is a major medical and neurological emergency, it is characterised as a state of the continuous or recurrent seizures for duration greater than 30 min with incomplete recovery or full recovery between seizures (Karunakaran et al., 2012). The injury from SE has been shown to cause epileptogenesis, resulting in acquired epilepsy (AE). Long-term neuronal plasticity has been observed following SE, which may alter excitatory and inhibitory neurotransmission, produce changes in neurotrophic factors, and reorganise the hippocampal circuitry (O'Dell et al., 2012). Based on clinical and experimental observation, SE becomes progressively less responsive to anti-epileptic drugs even within a single episode, suggesting changes in the neuronal network during SE (Karunakaran et al., 2012). SE can cause widespread brain damage (Sharma et al., 2018) leading to cognitive impairment and increased risk of epilepsy (Reddy and Kuruba, 2013). SE-induced neuronal injury also triggers other negative effects such as inflammation, oxidation, apoptosis, neurogenesis and synaptic plasticity, which eventually leads to structural and functional changes in neurones (Reddy and Kuruba, 2013).

There are two types of TLE; medial temporal lobe epilepsy (mTLE) and lateral temporal lobe epilepsy (lTLE). MTLE is symptomatic focal epilepsy that can be subcategorised as limbic epilepsy and is one of the most common types of epilepsy referred for epilepsy surgery, often intractable to antiepileptic drugs (Bertram et al., 2001; Téllez-Zenteno and Hernández-Ronquillo 2012; Lévesque et al., 2016;

Muhlhofer et al., 2017). Seizures in mTLE arise from abnormalities within the hippocampus (Ben-Ari and Cossart, 2000), parahippocampal gyrus, and amygdala (Karunakaran et al., 2018). MTLE may follow prolonged febrile seizures or SE, head trauma, encephalitis, or be associated with tumours, vascular malformation or abnormalities of cortical development (O'Dell et al., 2012). In TLE in humans, electrophysiological recordings from the human brain during surgery and histology on resected tissues have contributed to our understanding of the disease. Nevertheless, a basic fundamental understanding of the chronology of TLE in humans is lacking because typically patients will have tried numerous drug treatments before surgery is conducted (Berg, 2008). The seizures triggered by lTLE, however, arises from the neocortex (Bertram et al., 2001; Téllez-Zenteno and Hernández-Ronquillo 2012).

Our study focused on TLE since it is the most common epilepsy in adults, hard to control and it is the consequence of recurrent excitation or inhibition of circuitry. Out of all animal models developed to investigate the pathogenesis of mTLE, post-SE models have received the greatest acceptance because they are characterised by a latency period, the development of spontaneous motor seizures and a spectrum of lesions, like those of mTLE. There are a few factors that have been discovered which regulate epileptogenesis in mTLE; neuronal degeneration and gliosis, mossy fibre sprouting, receptor regulation and ion channels (O'Dell et al., 2012).

1.4.1 Neuronal Degeneration and Gliosis

The most common underlying pathology is HS, also known as medial temporal sclerosis (Dugladze et al., 2007; Boling, 2018; Kelley et al., 2018). Initial characteristics of the disease include decreasing hippocampal size and hardening of the hippocampus (O'Dell et al., 2012). These neurobiological changes in HS include neuronal loss in CA1, CA3 and the hilus (CA4) of the hippocampus (Bertram et al., 2001, O'Dell et al 2012; Clossen and Reddy, 2017), axonal and dendritic plasticity of surviving neurones, abnormal neurogenesis, gliosis in the CA3 and CA1 regions of the hippocampus and molecular reorganisation in the cellular matrix and membrane (Jutila et al., 2002; Loewen et al., 2016). Extra-hippocampal neurone loss has also been observed in mTLE in the entorhinal cortex, pyriform cortex, and amygdala (Ben-Ari and Dudek, 2010). Gliosis is increased up to ten-fold in activated microglia in

human and animal epileptic tissue (Sharma et al., 2007). Gliosis is linked to excessive glutamate release, which may directly lead to hyper-excitability (O'Dell et al., 2012). Hyper-excitability was hypothesised to be the consequence of reactive astrocytes releasing trophic factors which leave a glial scar from epileptogenesis (O'Dell et al., 2012). Activated astrocytes result in sclerosis through astrogial activation by increasing inflammatory cytokine production and potentiate excessive synaptic activity (O'Dell et al., 2012).

1.4.2 Mossy Fiber Sprouting

Mossy fiber (MF) or granule cell axon sprouting is characterised by dentate granule cell axons forming synapses with cells in the granule cell layer and inner molecular layer (Sharma et al., 2007; Danzer, 2017). The mechanisms were proposed to be gliosis and the release of growth factors, cytokines, and adhesion molecules from activated astrocytes and microglia (O'Dell et al., 2012). Granule cells with basal dendrites are highly interconnected with each other and with other adult granule cells; they act as hubs for excitatory activity and enhance hyper-excitability and may restore inhibition in the dentate network (Noebels et al., 2010; Althaus et al., 2016). Kainite binding was found to exhibit aberrant mossy fiber terminal above the granule layers and the infra-pyramidal (CA3) zone compared to naïve animals (Ben-Ari et al., 2012). MF sprouting is commonly identified by Timm staining of the brain section at various intervals after induction of SE in the chronic and acute models of SE (Reddy and Kuruba, 2013). Rats with KA-induced epilepsy also show aberrant sprouting, and synaptic reorganisation that occur in many temporal lobe structures of animals and humans (Sharma et al., 2007). It was found that kainic acid receptors were increased in new synapses, whereas old synapses were dominated by AMPA receptors (Ben-Ari and Dudek, 2010). Neurones enriched with kainatergic synapses were found to degenerate the most in human and animal mTLE triggering deleterious effect on the neurones. Low and high concentrations of kainite inhibit mossy fiber synaptic inputs (Ben-Ari et al., 2012).

1.4.3 Receptor Regulation and Ion Channel

A nerve impulse is generated by excitatory transmission involving glutamate that is the principal excitatory neurotransmitter, and inhibitory gamma amino butyric acid (GABA) as the principal inhibitory neurotransmitter in the brain (Anand and Dhikav, 2012; Barker-Haliski and White, 2015; Hampe et al., 2017; Ye and Kaszuba, 2017; Huntsman et al., 2019). More explanation about GABA is explained in section 1.4.3.4. Neurotransmitters imbalances in the brain may cause hyper-excitability of the neurones and may lead to epileptogenesis (Huntsman et al., 2019). The ionotropic glutamate receptors (NMDA, KA and AMPA) modulate Ca^{2+} and Na^{+} channels and are responsible for fast synaptic transmission (O'Dell et al., 2012). Calcium sensitive K^{+} channels regulate neuronal excitability by regulating the firing frequency of action potentials (Diao et al., 2017). Defects in the channels cause epilepsy to occur. Na^{+} channels must be blocked to prevent seizure initiation in rat hippocampal neurones. Cl^{-} channels are associated with cell hyperpolarisation (O'Dell et al., 2012).

1.4.3.1 N-methyl-D-aspartate receptor (NMDAR)

NMDAR is an ionotropic neurotransmitter receptor and a ligand-gated ion-channel (McGinnity et al., 2015). It is an excitatory amino acid derivative acting as a specific agonist at the NMDA receptor (Wible, 2013). The receptor is a heteromeric complex that interacts with multiple intracellular proteins by three different subunits: NR1, NR2 and NR3. Upregulation of NMDAR observed in epileptic tissue was hypothesised due to NMDAR subunit NR1 contributing to neuronal hyper-excitability, synchronisation, and seizure generation (Bliss and Collingridge, 2019). Nevertheless, NMDAR abnormalities have also been proposed to play a major role in schizophrenia and other disorders (Javitt, 2009; McGinnity et al., 2015). NMDARs are widely distributed within the brain, mainly in the hippocampal CA1 area, and the rest are in other brain regions, such as the amygdala, frontal cortex, caudate nucleus, septum and nucleus accumbens. The hippocampus and, in particular, one subfield, the CA1 layer (output layer), has the highest concentration of NMDAR, which plays an essential role in lasting changes in synaptic efficacy (synaptic plasticity) and learning and memory processes, including both short term and long term memory (Anand and Dhikav, 2012; Wible, 2013). NMDAR is a type of glutamate receptor whose activity underlies long-term potentiation (LTP), a process that may underlie learning and

memory (Wible, 2013; Bliss and Collingridge, 2019). Activation of NMDARs stimulates the opening of an ion channel which is permeable to the three ions Ca^{2+} , Na^{+} and K^{+} (Yu et al., 2010). The mechanism underlying LTP relies on the influx of Ca^{2+} ions. The characteristics of NMDAR activation lead to the opening of the associated Ca^{2+} channel when occupied by glutamate and when the postsynaptic membrane is depolarised (Lynch, 2004).

1.4.3.2 Kainate receptor (KAR)

KAR involvement in mTLE was associated with mossy fiber sprouting. Aberrant sprouts have been shown to have increased KAR expression compared with normal mossy fibers, suggesting that upregulation of KARs helps to propagate epileptic activity (Ben-Ari and Dudek, 2010). KA receptor subunits, GluK1 (GluR5) and GluK2 (GluR6) are the most widely studied in this case. GluK1 was involved in presynaptic regulation of glutamate release (Falcón-Moya et al., 2018). GluK1 was observed to be upregulated in mTLE patient tissue samples, implying its involvement in the generation of spontaneous seizures (Li et al., 2010). GluK2 has also been suggested as a potential player in postsynaptic response to glutamate release (Falcón-Moya et al., 2018), and involved in seizure propagation, cell death and also in GABA release modulation (Chamberlain et al., 2011).

1.4.3.3 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid receptor (AMPA)

Another receptor involved in CA1 hippocampal function is commonly called the AMPA receptor. This receptor belongs to the non-NMDA type ionotropic transmembrane receptor for glutamate that mediates fast excitatory synaptic transmission, activation and desensitisation (Cooper et al., 2003). The main function of AMPARs is to allow the permeability of Na^{+} , K^{+} and Ca^{2+} to pass into or out of a cell or organelle (Rozov et al., 2012). A rapid change in the membrane electrical potential difference occurs on the inside, and the outside of the cell as all Na^{+} ions begin flooding the cell. A brief, fast excitatory current is seen, indicating the membrane depolarisation process (Lynch, 2004).

1.4.3.4 Gamma-aminobutyric acid receptor (GABA)

GABA receptors in the brain can be categorised into two types, which are GABA_A, and GABA_B. GABA_A receptors are ligand-gated Cl⁻ channels (also known as ionotropic receptors). They mediate fast inhibition and have a wide distribution throughout the central nervous system. This receptor sits in the membrane of its neurone at a synapse. GABA_B are metabotropic transmembrane receptors, which are G-protein-coupled receptors that open ion channels via intermediaries, the G proteins (Zarrindast et al., 2001).

GABA_A receptors composed of five subunits that can belong to different subunit classes (two α , two β and one $\gamma 2$ subunits). GABA_A receptors act at different types of pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, neuroactive steroids, anaesthetics and convulsants (Sieghart et al., 2012). GABA acts at the two extracellular $\beta + \alpha^-$ and the extracellular $\alpha + \gamma 2^-$ interfaces. This $\alpha + \gamma 2^-$ interface is homologous to the benzodiazepine binding site and is also strongly influenced by the type of α subunit present in the receptor. Drugs interacting with this binding site cannot directly activate but only allosterically modulate GABA_A receptors (Sieghart et al., 2012). Changes in GABA_A receptors have long been implicated in epileptogenesis. However, the specific changes associated with epileptogenesis remain unclear.

The ligand GABA is the endogenous compound that causes GABA receptor to open, and in normal inhibitory transmission once bound to GABA, the protein receptor changes conformation within the membrane, resulting in the opening of the pore. GABA_A receptors are directly associated with Cl⁻ channels, thus the opening of the pore mediate Cl⁻ to pass down an electrochemical gradient (Zarrindast et al., 2001). The binding results in the opening of ion channels to allow the flow of negatively charged Cl⁻ into the cell or K⁺ out of the cell, resulting in the surrounding membrane becomes more negative (Cooper et al., 2003; Khazipov, 2016). Because of this process, the GABA_A channel opens rapidly and contributes to the early part of the inhibitory postsynaptic potential (IPSP) and decreases as part of the epileptogenic mechanism, which allows glutamate signalling to continue out of control (Cooper et al., 2003; Khazipov, 2016). Besides that, the GABA receptor also plays an active role

in processing information and controlling the balance of excitability and inhibitory states in the cortex, the hippocampus and the inter-neurons (Rezayof et al., 2007; Bonansco and Fuenzalida, 2016). GABA induces several pharmacological effects including sedation, analgesia and anticonvulsant effects.

1.5 Hippocampal Anatomy, Function and Physiology and its relation to Epilepsy

The hippocampus is a part of the brain located inside the temporal lobe, and is part of the limbic system, which is highly interconnected that plays a role in memory and learning processes (Azad et al., 2003; Wu et al., 2018). Therefore, memory deficits represent the major cognitive impairment in focal epilepsy (Dugladze et al., 2007). The hippocampus helps humans process and retrieve memories related to facts and events, which ultimately results in the storage of long-term memory in widespread cortical regions (Wible, 2013). Thus, abnormal activation of the hippocampus would affect perceptual cortical regions: in particular, regions showing high functional connectivity with the hippocampal system (Wu et al., 2018).

The human hippocampus derives its name from the resemblance of its elongated, curled S-shaped to the shape of a seahorse (genus *Hippocampus*) (Deshmukh and Knierim, 2012; Dudek et al., 2016). The hippocampus comprises the dentate gyrus, the cornu ammonis (CA) fields and the subiculum (Anand and Dhikav 2012). The CA fields of the hippocampus consist of pyramidal cells and are usually subdivided into four regions (CA1–CA4) (Cherubini and Miles, 2015). CA1 functions primarily in matching and mismatching of information obtained from CA3 (Jonas and Lisman, 2014). CA2 is known for its high resistance to HS due to epileptic damage (Anand and Dhikav 2012; Dudek et al., 2016). The dentate gyrus is U, or V shaped granule cells in rats, and arranged in four to six cells thick. The dentate gyrus is an input region which receives input from the entorhinal cortex (Deshmukh and Knierim 2012). The granule cell dendrites are connected with the granule cell layer and molecular layer by synaptic connections. The axons of the granule cells are called mossy fibers, originating from the basal portion of the cell body and extending into the hilus (Squire, 2008).

The entorhinal cortex provides the major input to the hippocampus and also receives output from the CA1 layer via the subiculum (Deshmukh and Knierim, 2012). The entorhinal cortex functions primarily in pattern recognition and encoding of memories (Anand and Dhikav, 2012). The entorhinal cortex provides input to the hippocampus via two routes; one from the dentate gyrus and CA3 fields and the other from CA1 and the subiculum (Wible, 2013). The subiculum then sends a major input back to the entorhinal cortex (Figure 1.1). The parahippocampal regions, including the subiculum and the entorhinal cortex, play a major role in memory and navigation (Deshmukh and Knierim, 2012; Wible, 2013).

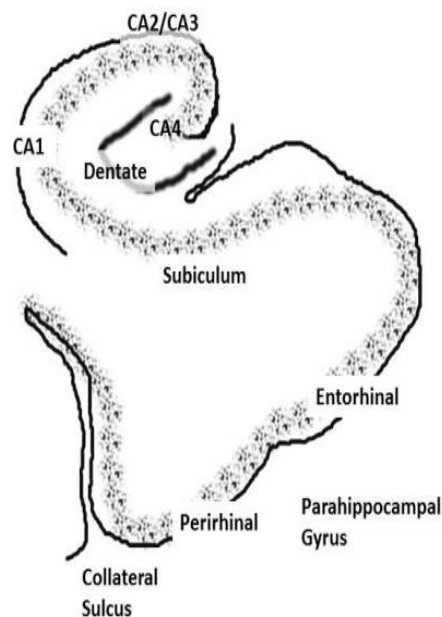


Figure 1.1 Anatomy of the hippocampus (retrieved from Wible, 2013)

The hippocampus involves three pathways which are perforant pathway; mossy fibre pathway and Schaffer collateral pathway (Figure 1.2). The perforant pathway conveys the main input of the hippocampus from the entorhinal cortex to the dentate gyrus. The second pathway, the mossy fibre pathway, then connects the granule cells of the dentate gyrus to the hippocampus CA3 region. Connections are continued from the hippocampus CA3 region to the hippocampus CA1 region through the Schaffer Collateral pathway. The information from the CA1 region travels to the subiculum, passing through the alveus, fimbria and fornix to other areas of the brain and then to the entorhinal cortex (Squire, 2008). The perforant pathway is observed to severely degenerate in patients with Alzheimer's disease, whereas the Schaffer collateral part plays an important role in memory formation and also in the emotional network (Anand and Dikhav, 2012).

The function of the hippocampus includes behavioural inhibition, memory and spatial memory (Lynch, 2004; Wu et al., 2018). The behavioural inhibition theory is based on observations of animals with hippocampal damage being hyperactive and experiencing difficulty in learning to inhibit previously learnt responses (Wu et al., 2018). The second function relates the hippocampus to memory, in particular, describing the synthesis of episodic and semantic memories. Scoville and Milner's research in 1957 revealed that bilateral hippocampal removal as a treatment for epilepsy suffered by patient caused severe anterograde and partial retrograde amnesia. This indicated the vital role of the hippocampus and temporal lobe structures in memory (Lynch, 2004; Wu et al., 2018). The most studied patient in medical science was Henry Gustav Molaison, who developed anterograde and partial retrograde amnesia after removal of hippocampus due to refractory epilepsy (Preilowski, 2009). He was unable to form new episodic memories following this surgery. A study by Markowitsch and Pritzel (1985) has shown that damage to the hippocampus also causes anterograde amnesia and often retrograde amnesia.

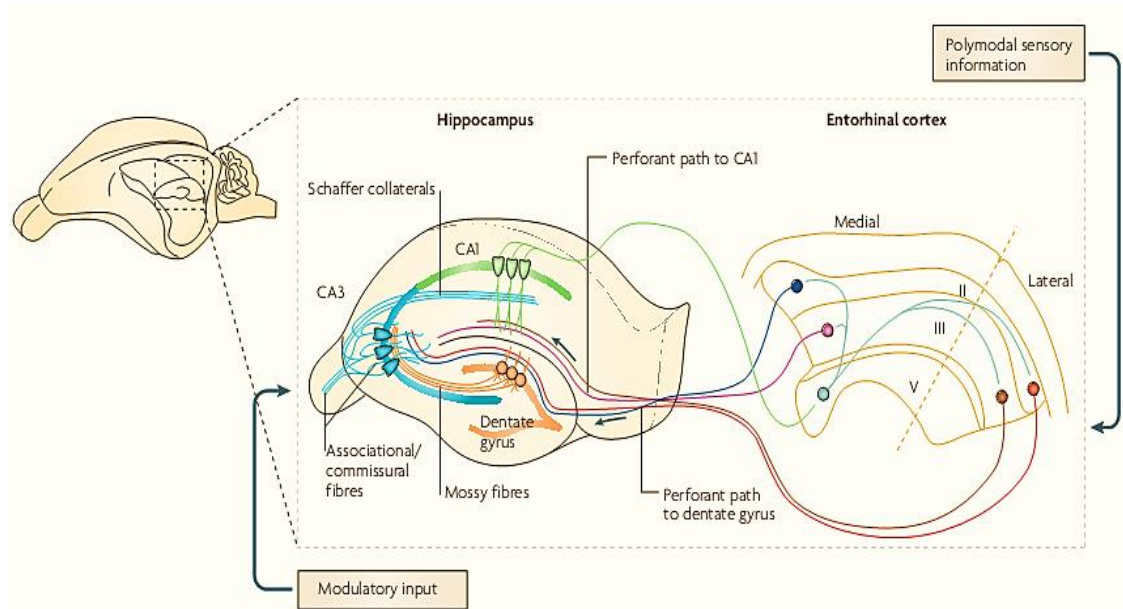


Figure 1.2 Three different pathways involved in the hippocampus.

The major input is carried by axons of the perforant path, from neurones in layer II of the entorhinal cortex to the dentate gyrus. Perforant path axons make excitatory synaptic contact with the dendrites of granule cells: axons from the lateral and medial entorhinal cortices innervate the outer and middle third of the dendritic tree, respectively. Granule cells project, through their axons (the mossy fibres), to the proximal apical dendrites of CA3 pyramidal cells which, in turn, project to ipsilateral CA1 pyramidal cells through Schaffer collaterals and to contralateral CA3 and CA1 pyramidal cells through commissural connections. (Neves et al., 2008).

Up to 75% of patients with epilepsy have been found to have had HS and medically refractory temporal lobe epilepsy (Thom, 2014). The underlying mechanism of HS in epilepsy is associated with the development of uncontrolled local hippocampal inflammation and blood-brain barrier damage (Yang et al., 2010; Wu et al., 2018). Other related factors are cytoskeletal abnormalities, neurotransmitter alterations, and hypoxia (Dhikav and Anand, 2007; Anand and Dhikav, 2012). A study by Eriksson et al. (2009) suggests that HS in epilepsy is a medial temporal sclerosis and may be associated with accompanying re-organisational dysplasia. It was observed that once hippocampus is damaged, the seizures become more intractable, indicating its inhibitory effect on seizure threshold (i.e., it keeps it elevated). The most successful treatment for medication-refractory medial temporal lobe epilepsy due to HS is surgical resection of the hippocampus (Bonilha et al., 2012; Huberfield et al., 2015). Another study by Dhikav and Anand (2007) also found that patients with Alzheimer's disease with seizures had hippocampal atrophy, and proposed autoimmunity as the mechanism.

Hippocampus has an important role as a map of space in humans and animals (O'Keefe and Nadel 1978; Moser et al., 2008). Neurones in the rat hippocampus seem to show activity that encodes the spatial position of the rat in the environment (Terry, 2006). Bilateral lesions of the hippocampus in animals affect specific behaviour (e.g., attention, arousal, and habituation) (Aly and Turke-Browne, 2017), cognitive operation (e.g., learning and memory) (Savage et al., 2004) and physiological reaction (e.g., skin conductance) (Wu et al., 2018). The behavioural deficits following hippocampal lesioning can be reversed by administration of clinically efficacious antipsychotics (Schatzberg and Nemeroff, 1998).

Moreover, the human brain does produce new nerve cells in adulthood, and newly-divided cells have been found in the hippocampus (Izquierdo and Medina, 1997; Morris et al., 2003; Anand and Dhikav, 2012; Wu et al., 2018). However, mice that live in stimulating environments and use exercise wheels have more new brain cells in their hippocampus and perform better in learning tasks rather than genetically-identical mice that live in standard cages (Campbell, 2002). The hippocampus plays an important role in spatial memory, as demonstrated through the Morris water maze,

where it determines the animal's ability to memorise spatial cues to locate a hidden underwater platform (Morris et al., 2003; Wu et al., 2018).

In addition, working memory is also dependent on both the hippocampus and the prefrontal cortex. The hippocampal-prefrontal connections are routed through the subiculum, which also receives inputs from the post-subiculum and the entorhinal cortex (Squire, 2008). This connection is important in the acquisition of motor skills and habits, and the memories associated with procedural memory as well as processing positional, directional, sensory and contextual information (Sendrowski and Sobaniec, 2013). As a result, when lesions appear in this area, learning deficiency may also develop.

The hippocampal pathway involving CA1 neurones projection to the perirhinal, postrhinal and entorhinal cortices also plays a vital role in learning and memory (Squire, 2008; Wu et al., 2018). The impact of lesions on the degree of impairment in recall and retrieval of remote memory depends on which brain part they affect, from only the CA1, CA3 and dentate gyrus to the entire hippocampal complex (refer to Figure 1.2). For instance, a partial lesion of the hippocampus will only affect recent memory, without depleting remote memory, while complete hippocampal lesions will result in comparable impairment of both recent and remote memory (Squire, 2008).

The hippocampus has a high level of plasticity, which can vary depending on how active or inactive the synapses are in the brain. As mentioned earlier, neurogenesis may also occur in the hippocampus; which is a lifespan change (Anand and Dhikav, 2012). Both factors are important for learning and memory, which may occur throughout our life. However, the downside of plasticity is an inclination for excitotoxic damage; the vulnerability of the hippocampus to various insults can result in damage that is limited to the hippocampal region (Wu et al., 2018).

Selective abnormalities of the CA1/subiculum have been shown to be present in schizophrenic individuals and to be differentially related to the subsequent conversion to psychosis (Wible, 2013). The hippocampal system (along with the adjacent amygdala) is also the most frequent cite of epileptic foci (Wiebe, 2000; Wu et al., 2018). The sensitivity of the hippocampus to insult may play a role in the

development of epilepsy following traumatic brain injury (Griesemer and Mautes, 2007). Seizures alter both the amount and the pattern of neurogenesis (Reddy and Kuruba, 2013). The hippocampus also contains the highest concentration of glucocorticoid (stress hormone) receptors in the brain, which can regulate LTP and may increase the likelihood of excitotoxic cell death with prolonged exposure (Sandi, 2011).

1.5.1 Physiological properties of the hippocampus and its function in memory and navigation.

Principal neurones and their networks underlie local information processing or storage and represent the major sources of output from any brain region. Principal cells are mostly excitatory, using glutamate as a neurotransmitter (Freund and Kali, 2008). The pyramidal cells are the principal neurones in CA1 and CA3, and are observed as complex spiking cells in extracellular recordings. Complex spiking happens when these neurones sometimes fire a burst of action potentials that are separated by a short interspike interval (Figure 1.3).

Interneurones do not fire complex spikes. Complex-spike bursting is a mechanism that allows neurones to fire together causing excitatory postsynaptic potentials (Deshmukh and Nierim, 2012). Interneurones are types of nerve cells, typically found in integrative areas of the central nervous system, whose axons (and dendrites) are limited to a single brain area. The functional roles of interneurones are to control activity levels within a brain area, coordination or generation of rhythmic and intermittent discharge patterns of neuronal ensembles, as well as the gating and gain control of excitatory inputs to principal cells. When a distant neurone excites the interneurones, they participate in feed-forward inhibition by inhibiting several other neurones (Buzsáki et al., 2007). In case of feed-back inhibition, the interneurones receive excitation from the same excitatory neuronal population that it is going to inhibit in return (Freund and Kali, 2008). The outcome of interneurones activity is often synchronisation of firing in large principal cell populations, which facilitates synaptic potentiation (enhancement of synaptic efficacy) and the formation of potentiated cell ensembles or networks as correlates of memory traces (Freund and Buzsaki 1996).

Hippocampal place cells were observed by monitoring extracellularly recorded action potentials from principal cells in CA1 and CA3 of freely behaving rats (Eichenbaum et al., 1999). Neurones in the hippocampus selectively respond when a rat is in a particular location in its environment, suggesting that these neurones are specialised for location memory (Bear et al., 2007). Place cells encode the conjunctions of ‘what’ and ‘where’ information, whereas timing information represents spatiotemporal sequences in some forms by the hippocampus (Moita et al., 2003; MacDonald et al., 2013; Wu et al., 2018). The CA3 region, in particular, is crucial for one-trial learning, which is a key component of episodic memory. CA3 place fields rapidly store information about the spatiotemporal sequence of other place fields, starting with the first exposure to an altered environment (Knierim et al., 2006). Mice with selectively deleted NMDA receptors from CA3 pyramidal neurones are also deficient in LTP at the CA3 recurrent synapses and show deficits in the rapid learning of new locations in the water maze task (Nakazawa et al., 2003). In humans, hippocampal neurones that respond during an experience are selectively reactivated when the person consciously recalls the experience (Deshmukh and Nierim, 2012).

Hippocampal lesions lead to impairment of spatial navigation, but not cue-based learning (Wu et al., 2018). Humans show a space-related signal in medial temporal lobe structures, which was also observed from animal studies (Maguire et al., 1997). Episodic memory requires a conscious memory of events in one’s past, which often involves a reconstruction of a spatially organized complex scene even when the specific detail recalled is not overtly spatial. The encoding of nonspatial information in the context of spatial information may indicate why episodic memory recall often involves reconstruction of space while recalling who said what after which event (Deshmukh and Nierim, 2012).



Figure 1.3 Complex spikings in the hippocampus.

The spikes were recorded by extracellular recordings of action potentials from an *in vivo* cell. The amplitude of the action potential drops during a burst (retrieved from Deshmukh and Nierim 2012).

1.6 Techniques for the study of neuronal function in epilepsy

The function and connectivity of neurones in the brain are studied to identify neuronal types, explain their molecular machinery, untangle their wiring, decipher principles of neural coding, and to attribute functional roles to specific regions of the brain (Obien et al., 2015; Wu et al., 2018). EEG is one of the common routine analyses in epilepsy diagnosis, and is still relevant until today. An EEG can detect epileptic seizures associated with abnormally synchronous brain activity or evoked electrical activity through electrodes on the scalp, but only with low spatial resolution (Jefferys, 2010; Obien et al., 2015). Other techniques available to indirectly measure the activity of large areas of the brain at a mesoscale are magnetic resonance imaging (MRI) and functional magnetic resonance imaging (fMRI). Electrophysiology at the mesoscale is also measured using microwire electrode arrays (MEA) which enables simultaneous long-term recordings of local field potentials (LFPs) and extra-cellular action potentials from a population of neurones at a time scale measured in milliseconds (Obien et al., 2015).

1.6.1 Electroencephalogram (EEG)

In clinical practice, the key feature of epilepsy is interictal epileptic activity described by its spikes, sharp waves, and spike-wave discharges (Stafstrom and Carmant, 2015; Zhou et al., 2018). Diagnosis is through the integration of the clinical description of the seizures, the age and comorbidities of the patient, the EEG patterns, and brain imaging. Prolonged EEG is beneficial to record habitual seizures in the case of frequent seizures and provides greater temporal interictal epileptic activity than a standard 30 min EEG. This information is crucial in the event of diagnostic uncertainty or if surgical treatment is being considered. For infrequent events with a possible cardiac cause, an implanted electrocardiography loop recorder is an essential diagnostic aid, often leading to specific and effective therapy (Duncan et al., 2006).

1.6.2 Magnetic Resonance Imaging (MRI)

MRI is the most useful and important non-invasive test with a high yield in identifying structural abnormalities in patients with partial epilepsy (Fitsiori et al., 2019). The most common underlying pathology of partial epilepsies involves the temporal lobe and HS (Wehner and Lüders, 2008). MRI is crucial in individuals with refractory partial seizures and in those with progressive neurological symptoms or psychological deficits (Stafstrom and Carmant, 2015; Ramli et al., 2015). Improvement of the sensitivity of MRI has led to the detection of subtle changes that could underlie refractory focal epilepsies, such as focal cortical dysplasia (Duncan et al., 2006).

1.6.3 Functional Magnetic Resonance Imaging (fMRI)

Through the so-called blood oxygenation-level-dependent (BOLD) response, fMRI can detect changes in brain bloodflow through the accompanying changes in blood oxygenation (Richardson, 2010; Dewiputri and Aeur, 2013; Stafstrom and Carmant, 2015). fMRI of BOLD is increasingly used in patients with TLE to lateralise the brain's activity before surgery, to determine the hemispheric dominance for language and to predict deficits after temporal lobe resection. fMRI can visualise the functional anatomy of memory tasks and the probable effects of surgery on individuals, hence aiding decision-making and planning of surgery. The simultaneous recording of EEG and fMRI can visualise the BOLD response to interictal epileptic activity. This emerging technique could assist in identifying targets for surgical treatment (Duncan et al., 2006). The advantages of using fMRI are that it is a non-invasive, risk-free technique, at lower cost, which is repeatable and generates a continuous measure of language lateralisation (Ladino et al., 2014).

1.6.4 Electrophysiology: microwire electrode array (MEA)

The function and connectivity of neurones within the brain can be studied through electrophysiology by understanding the temporal and spatial scales of the complex brain activity (Kini et al., 2016). Electrophysiology can capture a wide range of neural phenomena, from the spiking activity of individual neurones to the slower network oscillations of small populations (Obien et al. 2015). Because of this,

electrophysiology has become a tool for understanding epilepsy to assist the neurologist in identifying common lesions in patients with epilepsy and in localising the seizure onset zone and isolating the epileptic networks in preparation for surgery (Bertram, 2014; Kini et al., 2016; Clossen and Reddy, 2017). Oscillatory mechanisms have focused on the CA3 region of the hippocampus, which is an important pacemaker for generating network-wide activity. This feature makes the region susceptible to producing the pathological paroxysmal discharges associated with seizures (Ben-Ari and Cossart, 2000).

On-going research work in finding the mechanisms controlling seizure onset and propagation and the action of classic and new AEDs for treating epilepsy requires different types of analysis: at the neuronal network but also at the cellular level. MEA is one of the existing electrophysiological techniques which is still in its infancy and it helps in these analyses (Dossi et al., 2014; Kim et al., 2018).

MEA are customised devices embedding an arrangement of spatially distributed microelectrodes which allows simultaneous recording of neuronal activity and the action potentials (*in vivo*) of multiple neurones from different areas of the brain (Kim et al., 2018). This technique provides a macroscopic view of a complete brain structure, enabling spatio-temporal patterns of the spontaneous and evoked electrical activity of the neurones (Dossi et al., 2014; Obien et al., 2015). This technique also enables long-term recordings of local field potentials from a population of neurones at a time scale of milliseconds (Obien et al., 2015). This technique was first applied to monitor the developmental changes of neuronal cell culture activity and then adapted for acute and organotypic brain and spinal cord slices (Dossi et al., 2014). Early electrophysiological discoveries by Andersen (1964; 1966) and Bliss (1973) were made in the physical structure of the hippocampus, such as the identification and characterisation of specific inhibitory, excitatory synapses and the demonstration of long-term plasticity at these synapses. In a rodent hippocampus, as an example, several electrodes can record different regions (CA1, CA3, dentate gyrus) simultaneously. Such a recording configuration provides a plausible option for better understanding of a compound's effect on neuronal networks (Obien et al., 2015).

The MEA technique has been applied in neuroscience to understand neuronal communication, information encoding, propagation, and processing in neuronal cultures as well as in brain slices (Obien et al., 2015; Kim et al., 2018). MEA was used to detect burst firing or synchronisation from single neurones and repeated activation patterns (population wide synchronous neurones) when action potentials fire at a high frequency for a certain period, followed by a quiet period. Neuronal activity was also detected in brain slice perfused with artificial cerebrospinal fluid to keep the neurones viable, using MEA to detect electrical activity (Obien et al., 2015).

The microelectrodes of MEA sample spike and LFP signals. The LFP is assessed by the signal content in the low-frequency band of the recorded signal (<300 Hz). Synchrony of action potentials from many neurones can participate in the generation of LFPs. Synchronised synaptic currents in cortical neurones produce LFPs through the formation of dipoles (Obien et al., 2015)

1.6.4.1 Synchronisation, desynchronisation and seizure onset

Synchrony is traditionally inferred from linear correlation (in the time domain) or coherence (Klimes et al., 2016). Synchronisation at the cellular level is typically inferred from a linear measure of correlated firing between neurones (Jiruska et al., 2013; Asllani et al., 2018). Neuronal synchronisation refers to the correlated appearance of neuronal activity in the time of two or more events from a single cell to the whole brain (Timofeev et al., 2012; Jefferys, 2015; Nazemi and Jamali, 2019). Synchronisation (or desynchronisation) can be determined by a measurement exceeding (or falling below) a defined threshold, or determined through changes over time: an increasing trend in coupling can be interpreted as synchronisation, while a decreasing trend can be described as desynchronisation (Jiruska et al., 2013).

Synchronous firing clustered in tiny (<1 mm diameter) micro-domains were discovered in microelectrode studies of *in vivo* and *in vitro* models of epilepsy and seizures (Jiruska et al., 2013). During epileptogenesis, activity in these micro-domains was observed as high-frequency signals in extracellular recordings (Bragin et al., 2000; Jiruska et al., 2010). Seizures may arise from multiple, distributed cortical micro-domains, and affect cortical regions outside the seizure focus. This finding can

be explained by desynchronisation at the initiation of seizures, followed by increasing large-scale synchronisation as the seizure progresses. This desynchronisation has been observed in both neural activity and LFP *in vitro* and *in vivo* (Netoff and Schiff, 2002; Cymerblit-Sabba and Schiller, 2012) and has also been well documented in human intracranial and extracranial recordings (Figure 1.4; Wendling et al., 2003; Schiff et al., 2005).

There are two types of neuronal synchronisation; the first is local synchronisation, which generates LFPs; the second is long-range synchronisation, which can be detected with distantly located electrodes and mediated primarily via chemical synaptic interactions (Timofeev et al., 2012). Normal oscillations (sleep or wake oscillations) are generated as a result of both local and long-range synchronisation. Local or short-range synchrony can be detected by using one large field potential electrode or with two or more small, intracellular or extracellular unit (action potential) recording electrodes located at less than 1 mm from each other. On the other hand, the long-range synchrony can be observed using both LFP and EEG recordings detected with two or more electrodes placed some distance apart. This type of synchronisation leads to brain activity in widely separated areas that is correlated. LFP records a microscopic measure of brain activity involving the electrical activities of thousands of neurones (Traub, 1982). The synchronous activity of a few neurones does not necessarily lead to measurable EEG signals, but can be seen using LFP recordings (Timofeev et al., 2012).

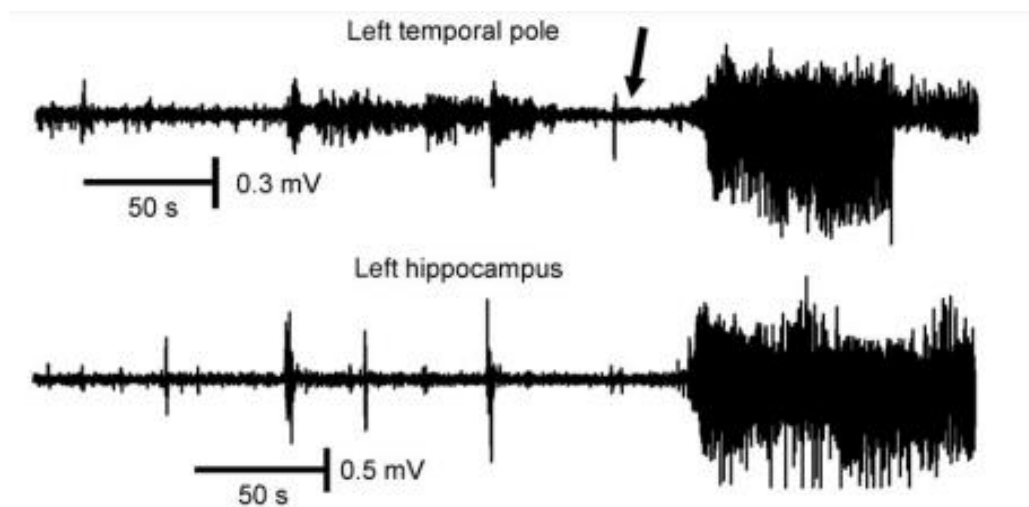


Figure 1.4 Desynchronisation at the onset of a seizure from mTLE patient. The seizure recorded with intracranial electrodes in a patient with temporal lobe epilepsy recorded from the left temporal pole contact located at the seizure onset zone. Ictal onset is characterised by the presence of gamma and fast gamma activity (60–120 Hz; arrow) and simultaneous recording from the left hippocampus (retrieved from Jiruska et al., 2013)

Synchronisation during sleep and wakefulness is related to rhythmic oscillations of neuronal activity, which are: slow oscillation, delta, spindle, beta, gamma and ripples (Watson and Buzsáki, 2015). The reduction in chemical synaptic interactions during seizure activity occurs due to changes in ionic composition (Timofeev et al., 2012). Synchronised activities may occur as almost simultaneous patterns across an entire population or as propagating waves in a large population of neurones (Timofeev et al., 2012).

A proposed mechanism of synchronisation is the synaptic mechanism (Diba et al., 2014), which involves a chain reaction process. Synaptic transmission occurs when an action potential fired by the presynaptic neurone invades the nerve terminals. This process allows Ca^{2+} entry, triggering neurotransmitter release, and leads to depolarisation or hyperpolarisation of the postsynaptic neurone (Timofeev et al., 2012). The chain reaction of excitation is produced by most acute convulsant through the interictal discharges related to abrupt paroxysmal depolarisation shifts in pyramidal cells (Traub and Wong, 1982; Traub et al., 1993). This interictal discharge is mainly generated in the CA3 region of the hippocampus (Prince, 1978; Johnston and Brown, 1981) through the axon collateral network of CA3 pyramidal cells which connect with other CA3 pyramidal cells and further connect to the CA1, septum and contralateral CA3.

The chain reaction requires different connections of CA3 pyramidal cells to other local pyramidal cells, which allows for the expansion of the synchronous population discharge, as illustrated in Figure 1.5. These synaptic networks, along with sprouting of new connections and other synaptic changes in chronic epileptic foci, are proposed to be the important mechanism involved in synchronisation during both seizures and interictal discharges (Jefferys et al., 2012; Jefferys, 2015).

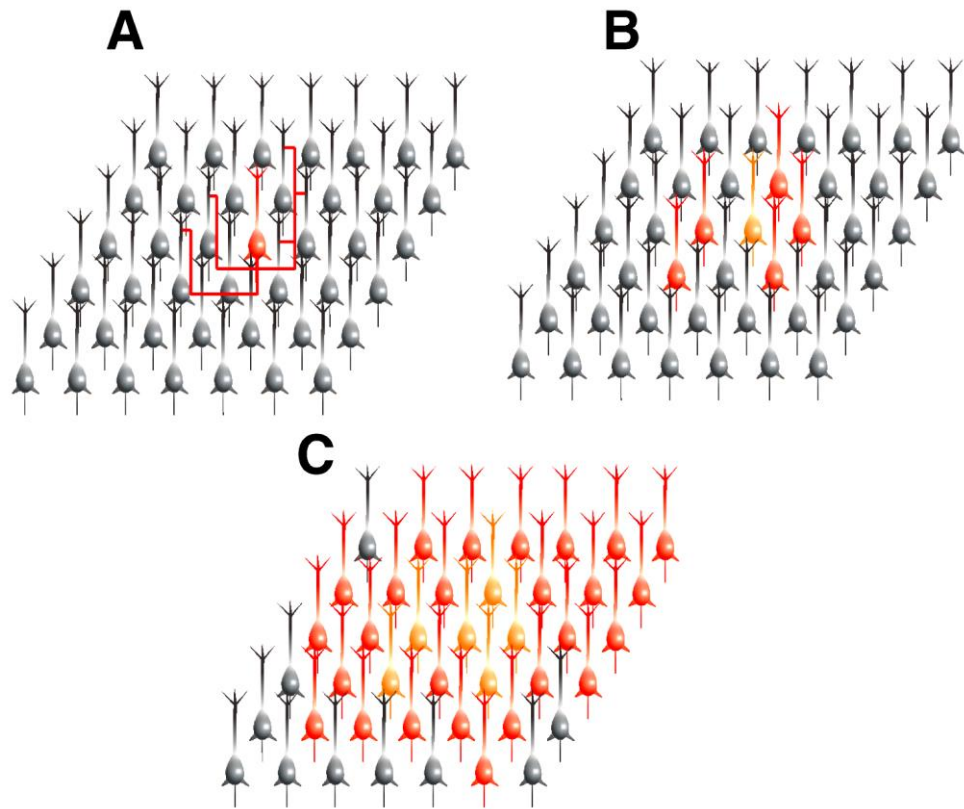


Figure 1.5 Chain reaction.

A: CA3 pyramidal neurone has divergent connections with (in this case) five other pyramidal cells.

B: Firing of the CA3 neurone will result in postsynaptic activation of the five pyramidal cells.

C: Each of the five then recruits its divergent postsynaptic targets, resulting in the recruitment of a large population of CA3 pyramidal neurones into synchronous firing within a small number of synaptic steps (retrieved from Jefferys et al., 2012).

Interaction among sets of neurones triggers the synchronisation of electrical activity in the brain (Diba et al., 2014). Apart from the chain reaction, interactions between brain cells such as neurones and glia occur through three other possible mechanisms. Firstly, gap junctions and synchronisation involve low-pass filters which prevent fast processes such as action potentials and absence of after-hyperpolarisation potential prevents slower synchronisation (Timofeev et al., 2012; Pernell et al., 2018). If a few cells are connected through electrical synapses, any change of membrane voltage in one neurone will generate current flow between coupled neurones, causing changes of membrane voltage in the latter cells, hence, producing a synchronising effect (Diba et al., 2014; Li et al., 2019).

Secondly, ephaptic interactions are the manifestation of neuronal excitability modulated by electric fields and field potentials generated by a population of neurones. Synchrony of electrical activity between neurones is attained through chemical or electrical interactions in epileptic activity (Klimes et al., 2016; Li et al., 2019). The weak effect of ephaptic interaction further enhances the synchrony (Jefferys et al., 2012).

Thirdly, there are non-synaptic mechanisms, which refer to the ability of hippocampal slices to generate seizure-like events where there is little or no extracellular Ca^{2+} and with the influence of drugs blocking synaptic transmission (Timofeev et al., 2012). This non-synaptic mechanism can also generate epileptiform synchronisation (Jefferys et al., 2012). Figure 1.6 demonstrates the different factors which increase or decrease epileptiform synchronisation.

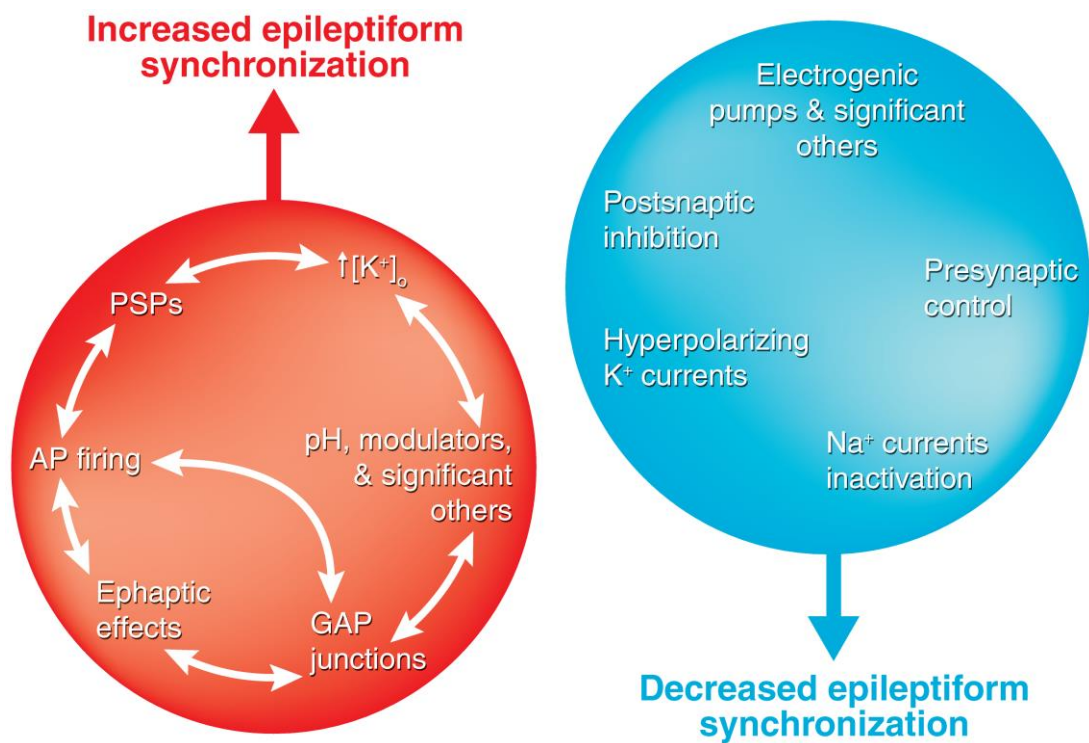


Figure 1.6 Schematic diagrams to illustrate synchronisation and desynchronisation. Mechanisms that promote epileptiform synchronisation and their broad relationships (left panel in red), and those that reduce epileptiform synchronisation (right panel in blue) (retrieved from Jefferys et al., 2012).

1.6.4.2 Neuronal cross-correlation

Cross correlation analysis is a standard method of estimating the degree to which two cells are correlated in the time domain (Perkel et al., 1967; Gerstein and Perkel, 1972). The cross-correlation technique has been used to characterise the neuronal interactions within the brain (Nowak and Bullier, 2000; Nazemi and Jamili, 2018). Essentially, a pair of cells (units) was selected, with cell 1 representing the ‘reference cell’. Firing in the second ‘target’ cell is compared with the temporal firing of the ‘reference cell’. The cross-correlation technique has been used to characterise the neuronal interactions within the central nervous system (Nowak and Bullier, 2000; Nazemi and Jamili, 2018). Understanding how populations of neurones encode information and correlate across brain regions is important in making inferences about the mechanisms that generate them, their functional interactions and their functional anatomy (Narayanan and Laubach, 2009; Cohen and Kohn, 2011; Nazemi and Jamili, 2018).

Cross-correlation is useful in providing information about the functional architecture of neuronal networks. The use of MEA allows large amounts of data to be obtained from simultaneously recorded neurones and correlation to be measured to make inferences about connectivity in multi-electrode data (Narayanan and Laubach, 2009; Cohen and Kohn, 2011; Nazemi and Jamili, 2018). Correlation analysis has been reported to infer connectivity between neurones in the cortex (Aertsen et al., 1989; Nazemi and Jamali, 2019).

A study by Poulet and Peterson (2008) reported that responses in the sensory cortex are desynchronised, although without any changes in neuronal firing rates or disruption of sensory input when an animal actively explores its environment. Narayanan and Laubach (2009) have explained cross-correlation techniques for investigating functional interactions between neurones to generate insights into how neurones interact. Figure 1.7 shows the application of linear correlation to measure cross-correlation.

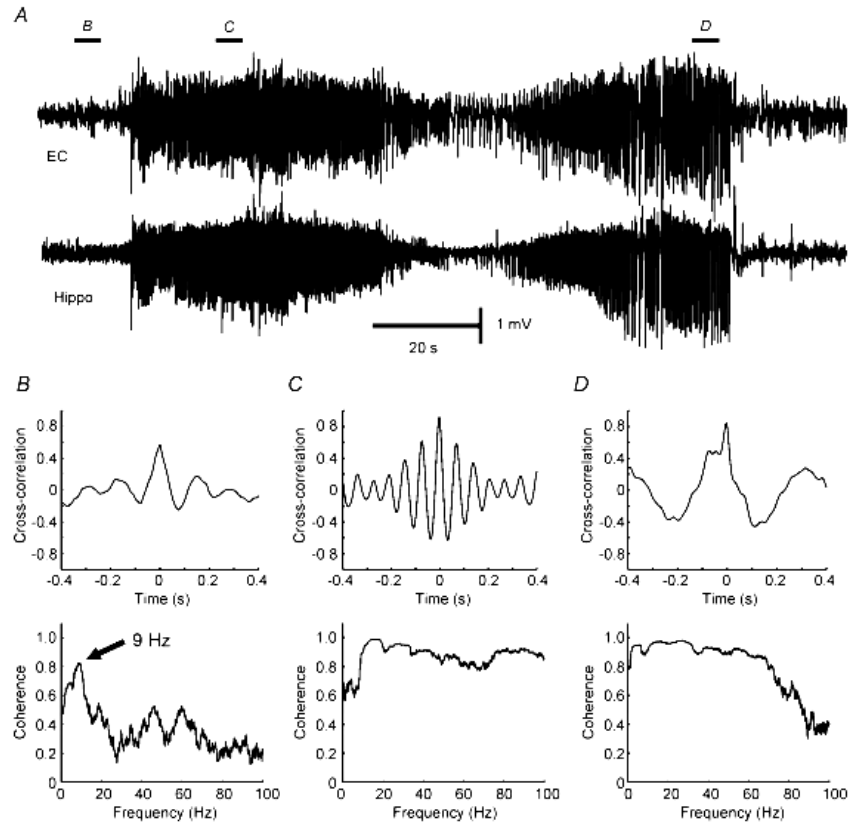


Figure 1.7 Application of linear correlation and coherence to measure synchronisation.

A: Synchronisation between the hippocampus and entorhinal cortex was examined before and during a seizure in a tetanus toxin model of temporal lobe epilepsy (Jiruska et al. unpublished results).

B: Correlation of 0.5 was observed between signals from the hippocampus and entorhinal cortex recorded before the onset of the seizure. Coherence, often interpreted as a correlation at each frequency, shows an obvious peak in coherence at frequency 9Hz.

C: Cross-correlation and coherence during early and final parts of seizure

D: (Jiruska P. and Jefferys J.G.R; unpublished results).

(retrieved from Jiruska et al., 2013).

Cross-correlation can be used to make inferences about synaptic connectivity between neurones by observing narrow peaks (a few milliseconds) in the latency of spikes between one neurone and another. Other than that, cross-correlation measures the distance in time between the spikes of one neurone and the spikes of another neurone through the compiled function in MATLAB (Narayanan and Laubach, 2009).

Furthermore, cross-correlation can also be used to look at long timescale interactions, i.e., in the order of several milliseconds or tens of seconds, which shows an increased correlation between the firing rates of two neurones (Narayanan and Laubach, 2009). Absolute cross-correlation values are contributed by detection thresholds (which affect observed firing rates), behavioural state, and the presence of multiunit activity (Cohen and Kohn, 2011). Figure 1.8 shows the correlation structure of the LFP.

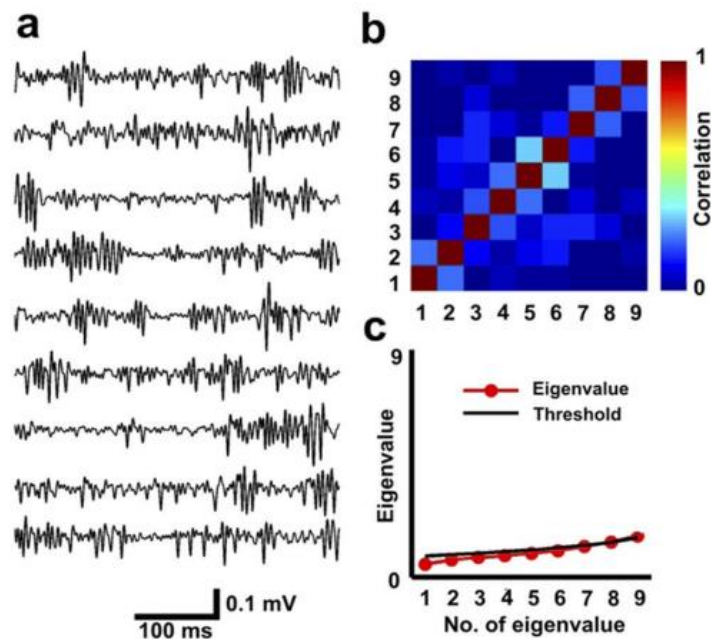


Figure 1.8 Correlation structure of local field potential (LFP).

A: LFP recordings of 9 channels

B: Correlation matrix, cold colours indicate a low correlation while hot colours mean a high correlation,

C: Eigenvalues and threshold of the correlation matrix (retrieved from Li et al., 2007).

.6.4.3 LFP oscillations and frequency bands

Brain generates oscillatory activities because of combination of neuronal populations (Nazemi and Jamili, 2018). Neuronal oscillations originated from the activation of currents intrinsic to the membrane, or from the activity of a group of interconnected hippocampal neurones, from the activity of their synapses (Hammond, 2003). LFP oscillations refer to the LFP rhythm in the brain (Buzsáki, 2006), in which the dominant frequencies depend on the specific mechanisms involved and the functional state of the brain (Salelkar et al., 2018). Slow (0.1–15 Hz) and fast (20–600 Hz) frequency bands are normal oscillatory activities which are mainly present during slow-wave sleep or anaesthesia (Timofeev et al., 2012).

Slow frequency bands

In slow frequency bands (0.3 – 1 Hz), the entire cortical network alternates between hyperpolarising and depolarising states. Hyperpolarisation refers to the periods of absence of synaptic activity, while depolarisation is the periods of intensive synaptic activity, which lead to fast oscillations within the brain (Mukoversuski et al., 2007). It has been proposed that the slow frequency bands originate from the action potential-independent release of transmitter vesicles and are regulated at the level of single synapses (Timofeev et al., 2012). During the hyperpolarised state of slow-sleep oscillations, the miniature excitatory post synaptic potential activates the persistent Na^+ current and depolarises the membrane of cortical pyramidal cells, which is enough for spike generation (Timofeev et al., 2000). This process generates the active phase of slow oscillation, which is sustained by synaptic activities and the Na^+ current. Another possible mechanism is selective synchronisation, which refers to the transitions from hyperpolarisation to depolarisation states; it is hypothesised that some neurones of the network still generate irregular spontaneous firing even during the hyperpolarising states (Timofeev et al., 2012). These mechanisms are likely to be responsible for producing synchronisation between depolarising and hyperpolarising states of slow oscillation, as observed in animals and humans during deep sleep, and leading to periodic LFP oscillations (Timofeev et al., 2012).

Delta frequency bands

Delta frequency bands (1-4 Hz) are present during sleep in the field potential recordings from the neocortex in human and animal models (Buzsáki, 2006; Timofeev et al., 2012; Roohi-Azizi et al., 2017). Cortical delta activity is hypothesised to be driven by the discharge of intrinsically bursting neurones (Amzica and Steriade, 1998). Another study showed that the delta oscillation during deep sleep when principal neurones in the thalamus are hyperpolarised sufficiently to de-inactivate Ca^{2+} . Slow wave sleep (SWS) is important for memory consolidation and memory formation (Maquet, 2000). Timofeev et al. (2012) have suggested that memory traces during wakefulness are due to the synaptic plasticity associated with slow and delta oscillations.

Alpha frequency bands

Alpha frequency bands are present in the resting-sleeping state in both the thalamus and cortex (Buzsáki, 2006). Alpha frequency bands change with age, being quite low in human infants but reaching their maximum in young adults. Alpha frequency bands reflect the first-order thalamic nuclei and their primary sensory and motor cortical partners. This band is hypothesised to originate from the endogenous rhythmicity of cortical or thalamic neuronal populations. Another hypothesis suggests that alpha frequency bands originate from the synaptic coupling of distributed populations of neurones, based on computational models (Buzsáki, 2006). This band is also associated with the waking immobility-related rhythm in the neocortex of rodents, with the largest amplitude being in the somatosensory cortex (Buzsáki, 2006).

Beta-gamma frequency bands

The beta (15–30 Hz) and gamma (30–80 Hz) ranges, categorised as fast frequency bands, are the major frequencies in the waking and sleep state of the brain (Bressler, 1990; Bazhenov et al., 2008; Buzsáki and Wang, 2012). During anaesthesia, natural SWS, and REM sleep, these fast frequency bands are synchronised between neighbouring cortical sites in delayed consciousness and the absence of thoughts (Timofeev et al., 2012). Gamma activity is linked with attentiveness, sensory perception and movement during waking (Buzsáki, 2006; Garcia-Rill, 2017).

Synchronisation in the gamma frequency range is important for temporal binding of sensory stimuli and is proposed to be related to cognitive processing (McDermott et al., 2018).

During REM sleep, gamma activity may also lead to the generation of dreams (Timofeev et al., 2012). Gamma oscillations develop from the coordinated interaction of excitation and inhibition (Garcia-Rill, 2017), which can be detected in LFPs (Buzsáki and Wang, 2012). Network oscillation in the gamma frequency range is found to be triggered by GABAergic interaction in isolated interneurons networks (Wang and Buzsáki, 1996; Khazipov, 2016), the conductance and decay time of GABA_A currents (Traub et al., 1996). Buzsáki and Wang, (2012) and Khazipov (2016) also reported that gamma activity depends on the activity of inhibitory interneurons specifically through GABA_A receptor-mediated inhibition. Gamma oscillations can be induced by tetanic stimulation of the hippocampus (Traub et al., 1997) and also by bath-application of carbachol or kainate, which can last for minutes to hours in CA3 and neocortex (Fisahn et al., 1998). Kainate and carbachol evoke network oscillations at gamma frequency in hippocampal slices *in vitro*; which are mechanistically similar to gamma rhythmic epileptic activity observed *in vivo* (Khazipov, 2016). Application of kainic acid (KA) to the hippocampus in rats *in vivo* evokes large population spikes in CA3 regions, regularly at gamma frequency bands (Khazipov and Holmes, 2003). A gamma range oscillation was also observed through infrequent increases in spiking synchrony within local groups of cortical neurons (Timofeev et al., 2012). Long-range interneurons may be important for gamma-phase synchrony in different brain regions (Buzsáki and Wang, 2012).

Under normal condition, gamma activity is not widely distributed, and is of low amplitude. It is thought to play an important role in the processing of episodic memory. During normal brain function, slower oscillations tend to be of higher amplitude, while higher frequency oscillations tend to be of lower amplitude. During a seizure, higher frequency oscillations occur at higher amplitudes than those which are present during normal brain function. The high amplitude of these oscillations represents the pathological hyper-synchrony of neuronal networks (Destexhe and Sejnowski, 2003). Neuronal synchronisation in hippocampal networks is of particular interest in the gamma band (30-100 Hz) (Kreuz, 2013; Obien et al., 2015).

Theta-Gamma frequency bands

Theta oscillations are large-amplitude neuronal oscillations observed in electrical recordings from the hippocampus when the oscillations are active in supporting the current behaviour (Buzsáki, 2002; Watson and Buzsáki, 2015). Theta oscillation is generated during voluntary movement in rodents at approximately 4–8 Hz, and is important in spatial processing in rodents (O’Keefe and Nadel, 1978; Aghajan et al., 2017). Several studies have reported the coupling of theta-gamma frequency bands and cognitive processes. In humans, the variation in the theta-gamma coupling in the hippocampal region is associated with working memory load in patients implanted with depth electrodes (Axmacher et al., 2010). Meanwhile, different task demands are influenced by the strength of theta-gamma coupling in the hippocampus and striatum of the rat (Tort et al., 2009). Gamma power in the frontal and temporal sites was phase-locked mainly to theta oscillations in an auditory task, while, in a visual task, the alpha rhythm showed the strongest phase modulation over occipital areas (Voytek et al., 2010).

1.7 Treatment of epilepsies - Anti-Epileptic Drugs (AED)

The aim of antiepileptic treatment is to control seizures as quickly as possible without adverse effects. Improved seizure control is likely to reduce morbidity and premature mortality associated with continuing seizures, especially convulsive attacks (Löscher, 2011; Löscher, 2017). Seizure remission is the major determinant of good quality of life. Drugs for epilepsy increase inhibition, decrease excitation or prevent aberrant burst firing of neurones. Some were discovered empirically through studies of induced seizures in animals without epilepsy (Löscher, 2011; Löscher, 2017).

The mechanisms of action are not fully understood, and many AEDs have multiple actions. The principal mechanisms of present drugs are thought to be the enhancement of the inhibitory GABAergic system (e.g., benzodiazepines, barbiturates, tiagabine, vigabatrin) or the blocking of sodium channels (e.g., carbamazepine, oxcarbazepine, lamotrigine, and phenytoin). Even within these groups, drugs can have very different modes of action (O’Dell et al., 2012). Benzodiazepines bind to GABA_A receptors, potentiate the response to GABA, and are used in generalised and partial seizures. Tiagabine, on the other hand, exerts antiepileptic effects by blocking GABA uptake

into the neurones and glia. GABA uptake inhibition sustains the level of endogenously released GABA at the synapse, thus preventing synchronous neuronal firing (hyperpolarise) and decreasing the excitability of neurones or seizures (Henry and Conway, 2012). Drugs that specifically target glutamate receptors have had little success because of unacceptable side effects, but some useful drugs (e.g., topiramate) might affect glutamatergic transmission. Some AEDs act on ion channels that affect neuronal excitability (Löscher, 2017). Ca^{2+} channels are crucial for cell excitability and also for neurotransmitter release (O'Dell et al., 2012). Ethosuximide might target T-type Ca^{2+} channels, which would explain its specificity for absence seizures. Other drugs (e.g., gabapentin, pregabalin) target presynaptic calcium channels, thus inhibiting neurotransmitter release (Henry and Conway, 2012). Modulation of neurotransmitter release might be an effective way of modifying network excitability.

More than 20 AEDs are licensed worldwide (Henry and Conway, 2012). Some of these drugs are used as first-line treatment and are selected mainly according to their clinical effectiveness for the epileptic syndrome or seizure type, and for tolerability and individual patients' circumstances (Henry and Conway 2012; Löscher, 2017). These drugs suppress the seizure rather than modify the disease process (epileptogenesis). AEDs are categorised as old drugs or new drugs, according to whether or not they were available before the 1990s (O'Dell et al., 2012). Since 1978, 11 antiepileptic drugs (AEDs) have been introduced: valproate, vigabatrin, tiagabine, lamotrigine, oxcarbazepine, felbamate, topiramate, gabapentin, levetiracetam, zonisamide, and pregabalin (Meldrum and Rogawski, 2007). Some AEDs promote neuronal inhibition by enhancing Cl^- current at GABA_A receptors such as benzodiazepines, barbiturates and topiramate, while other AEDs reduce GABA degradation or reuptake, e.g., vigabatrin and tiagabine (Asif, 2016). Recently, perampanel has been discovered as the first approved AED to provide an allosteric negative modulation of postsynaptic excitatory neurotransmission through the selective antagonism of glutamate AMPA receptors (Di Bonaventura et al., 2017; Lattanziani and Striano, 2019).

In view of the rapidly diminishing chances of becoming seizure-free after trying three AEDs, surgical treatment should be considered early on for individuals continuing to have focal seizures. The most common operations are temporal lobe resections, which

are cost-effective procedures carrying a 60–70% chance of making the individual seizure free with improved quality of life (Duncan, 2006). In our study, we investigated the effect of 1 commercial AED (valproate) and 2 potential AEDs, (WIN 55,212-2 and flurazepam) on kainic acid (KA) induced epileptiform activity in rats. Briefly, we explained the general characteristic and the use of the three drugs. A more detailed explanation of their mechanisms of action and previous studies existing in the literature are given in the introduction to Chapter 3 (Section 3.1), Chapter 4 (Section 4.1) and Chapter 5 (Section 5.1 and 5.2).

1.7.1 Valproate (VPA)

VPA is the most commonly used AED in the treatment of generalised and focal epilepsy and is also used against partial epilepsy absence seizures and myoclonic seizures (Johannessen and Johannessen, 2003). It was first discovered as an anticonvulsant in February 1962, and since then it has been commercialised in a large number of countries, including in the United States in 1978 (Chapman et al., 1982). The clear, colourless, eight-carbon branched chain fatty acid (Asif, 2016) derived the name ‘valproic acid’ from the chemical name of 2-propylvaleric acid (Lagace et al., 2004). VPA has a molecular weight of 144.21 and occurs as a colourless liquid. It is highly water soluble and very soluble in organic solvents (Bruni, 1979); valproate (VPA) (Löscher, 1999) differs from other anti-epileptic drugs in that it lacks nitrogen (Bruni, 1979).

VPA is widely used as an antiepileptic and is of major importance due to its therapeutic effect in treating other neurological and psychiatric disorders such as bipolar and schizoaffective disorders, for neuropathic pain and for prophylactic treatment of migraine. Its therapeutic effects have been studied in preclinical models, including a variety of animal models of seizures or epilepsy (Chapman et al., 1982; Löscher, 2017). The animal pharmacokinetics of VPA have been studied in the mouse, rat, dog, and monkey (Koshal et al., 2017; Löscher, 2017). VPA appears to accumulate preferentially in brain areas with high GABA-transaminase activity, such as the olfactory bulbs, caudate nucleus, putamen, nucleus accumbens, the midbrain reticular formation, substantia nigra, and the red nucleus (Ciesielsky et al., 1975).

VPA mediates GABA release (Olkola and Ahonen, 2008) by inhibiting α -ketoglutarate dehydrogenase, GABA transaminase, and succinate semialdehyde dehydrogenase, and enhances glutamate decarboxylase to elevate GABA levels in plasma and in several brain regions (Fan et al., 2016). VPA can also reduce aspartate concentration and decrease the potentiation of excitatory transmission at the NMDA receptor (Martin and Pozo, 2006).

1.7.2 WIN 55,212-2 (WIN)

Cannabis has been widely used in the medical field for treating menstrual fatigue, gout, rheumatism, malaria, constipation, pain, and absentmindedness and seizure (Suraev et al., 2017). Cannabis has also been used in the treatment of children and adults with epilepsy (Huntsman et al., 2019). Historically, the use of cannabis to treat seizure was documented in a Sumerian text from 2900 BCE and an Arabian document from the twelfth century (Rosenberg et al., 2015). One of the major psychoactive ingredients or phyto-cannabinoids derived from cannabis is Δ^9 -tetrahydrocannabinol (Δ^9 -THC) (Dixit et al., 2017; Perucca, 2017; Huntsman et al., 2019). Δ^9 -THC was isolated in 1964 and synthesised in 1971 (Gaoni and Mechoulam, 1964; 1971). Δ^9 -THC and other natural and synthetic cannabinoids produce characteristic motor, cognitive, and analgesic effects (Herkenham et al., 1990; Huntsman et al., 2019). Synthetic cannabinoids were found to have anti-seizure effects, and can provide an alternative to the use of marijuana (*Cannabis sativa*) with psychogenic effects (Huizenga et al., 2017).

WIN 55,212-2 is a synthetic cannabinoid receptor agonist (Vallée et al., 2014) which acts as a potent and efficacious agonist at the cannabinoid type 1 receptor (CB1R) (Auwärter et al., 2013). Cannabinoid compounds have been reported to have antiepileptic (Blair et al., 2006) and neuroprotective properties (Marsicano et al., 2003) via cannabinoid receptors (Perucca, 2017; Huntsman et al., 2019). The endogenous cannabinoid system is important in controlling neuronal excitability and synaptic plasticity, and consists of two G-protein coupled receptors (Freund et al., 2003; Rosenberg et al., 2015; Huntsman et al., 2019). The CB1R can be found predominantly in the brain and can be detected in the cerebral cortex, hippocampus, basal ganglia and cerebellum (Herkenham et al., 1990; Rosenberg et al., 2015).

CB1Rs are most densely expressed at cortical and hippocampal presynaptic boutons. Glutamatergic axon terminals in cortical and subcortical neurones contain fewer presynaptic CB1Rs than GABAergic terminals (Rosenberg et al., 2015). Cannabinoid type 2 receptor (CB2R) is mainly associated with immune tissues, but also expressed at a lower density in the brain (Bosier et al., 2010). The CB1R and CB2R are recognised by a variety of endogenous ligands and activate multiple signalling pathways (Bosier et al., 2010).

The aminoalkylindole WIN 55,212-2 as a cannabis substitute exerts similar pharmacological activity to Δ^9 -THC but has an entirely different chemical structure (Ferraro et al., 2001; Goonawardena et al., 2011). This finding drove the research in the 1970s and 1980s that resulted in the discovery of CB1R and CB2R (Pertwee et al., 2008). WIN 55,212-2 is a highly selective and potent activator of cannabinoid receptors *in vitro* and *in vivo* (Shen et al., 1996). In the study by Shen et al., (1996), cannabinoid receptor agonists acted presynaptically to inhibit glutamatergic synaptic transmission between cultured rat hippocampal neurones. Wallace et al. (2003) have reported that WIN 55,212-2 eliminated seizures in a pilocarpine model of epilepsy. In the KA-induced seizure model, removal of CB1R from glutamatergic neurones from the forebrain increased the susceptibility of mice to KA-induced seizures (Marsicano et al., 2003; Hofmann and Frazier, 2011). This result suggested that the activating effect of CB1R on excitatory neurones was required to protect from KA induced seizures.

1.7.3 Flurazepam (FLU)

Benzodiazepines were developed in the 1960s as they are efficient in reducing anxiety, have anticonvulsant properties, and promote myorelaxation, combined with rather low toxicity (Tyma et al., 1988; Riss et al., 2008; Vinkers and Olivier, 2012; Griffin et al., 2013). One of the main effects of benzodiazepines is the enhancement of the neurotransmitter gamma-aminobutyric acid (GABA) (Khazipov, 2016) and GABA receptor-mediated Cl^- conductance, which contribute to the effects of sedation, hypnosis, anxiolysis, anti-seizure medication, and muscle relaxation (Riss et al., 2008; Griffin et al., 2013; Fan et al., 2016). The anticonvulsant properties of benzodiazepines are due to their slowing down the central nervous system (Mahdavi

et al., 2010). Benzodiazepines are also used as a sedative, and may cause decreased motor activity during waking (Tyma et al., 1988). Benzodiazepines are the first line of treatment for SE, an emergency condition associated with continuous seizures lasting more than 30 min (Reddy and Kuruba, 2013).

In the earlier studies prior to 1970s, benzodiazepines in general, and nitrazepam and clonazepam in particular, were the most effective of the anticonvulsants in preventing pentylenetetrazol seizures in mice. Benzodiazepines alleviate seizures by enhancing GABAergic inhibition (Riss et al., 2008; Khazipov, 2016). Flurazepam (FLU) was mainly used to treat insomnia (Thangadurai, 2016) and as an anxiolytic to alleviate anxiety (Daga et al., 2019). FLU was not studied extensively as an anticonvulsant (Browne and Penry, 1973; Asnis et al., 2015; Lie et al., 2015). Rosenberg (1995) has studied the difference in anticonvulsant tolerance according to time after FLU treatment. His study showed that twelve hours after 1-week FLU treatment, all benzodiazepines were significantly less effective, showing tolerance.

1.8 Animal Model

To understand the mechanisms of epilepsy, much work is currently being conducted to elucidate the mechanisms behind the genesis of mTLE and other epilepsies and to use new information to design more effective treatment options for patients suffering from this difficult disease. Several animal models have been developed to study epileptogenesis. Our understanding of epileptic disorders has improved due to appropriate animal models (Pitkänen et al., 2006; Koshal et al., 2017; Löscher, 2017). Laboratory investigations have shown that various experimental manipulations can lead to epilepsy. Animal models of epilepsy are important tools for testing the hypotheses about the pathogenesis of epileptic disorder and for studying the efficacy of potential therapies and their mechanism of action (Sarkisian, 2001; Löscher, 2017). Many factors must be considered when selecting the animal model to be used, depending on the type of epilepsy to be modelled and the research aims. In this section, we explained some of the different animal models used in the literature, highlighting their uses and limitations. We have also included our motivation for selecting the KA model for this study.

1.8.1 Pilocarpine

Pilocarpine can be used to produce chronic animal models of TLE, particularly kindling or SE animal models (Lévesque et al., 2016; Clossen and Reddy, 2017). It has been demonstrated that intra-hippocampal pilocarpine administration is as efficient as the intraperitoneal (i.p.) route in inducing SE and then chronic epilepsy. In fact, animals receiving pilocarpine directly into the hippocampus show not only the same behavioural, electrographic and neuropathological alterations exhibited by those treated systemically, but have the advantage of a drastically reduced mortality (Furtado et al., 2002; Löscher, 2017). Indeed, the ratio of animals developing SE and surviving after the intra-hippocampal injection (71%) has been found to be higher than with systemic administration. Epileptiform discharges observed during SE were characterised by clonic movements of the head and forelimb that were mirrored by a larger amplitude signal in the amygdala than in hippocampus. Pilocarpine is an antagonist of muscarinic acetylcholine receptors, especially those expressed in the hippocampus, striatum, and cortex (Brady et al., 2012). Therefore, its seizure-producing effect arises from the increased activation of these receptors. The pilocarpine model may be useful for testing drugs which are potentially effective against complex partial seizure. Seizures induced by pilocarpine, however, are very persistent and long-lasting, and they result in severe neuropathological damage that far exceeds the damage seen in human mTLE (Velíšek, 2006).

1.8.2 Electrical stimulation

There are two electrical stimulation-induced models commonly used to study epilepsy; stimulation with an alternating current of 50 or 60 Hz (according to the local supply) frequency and low-frequency stimulation (usually 6 Hz) (Löscher, 2017). These stimulations may induce minimal clonic seizures involving the muscles of the head and forelimbs (righting ability is preserved) or maximal seizures with a loss of righting reflexes (Mazarati et al., 2006). Minimal seizure probably represents a model of myoclonic seizure. Minimal clonic seizures are generated in the forebrain: they are induced by threshold and slightly supra-threshold stimulation through corneal electrodes. The eyes must be moistened with a drop of physiological saline after previous local anaesthesia with 0.5% tetracaine. Less common is a transcranial electrode or ear clips. Maximal electroshock seizure (MES) is a model of generalised

tonic-clonic seizures. Inducing and evaluating minimal seizure and MESs are easy tasks that are not time consuming and are an ideal tool to screen potential AEDs. However, there are limitations of using the MES model; low predictive validity for some AEDs and animals can be used only once (Kandratavicius et al., 2014).

1.8.3 Kindling

The kindling model has been employed extensively as a chronic model of TLE (Koshal et al., 2017). Induction of epilepsy by kindling is initiated by periodic supply of a brief stimulus that evoked repetitive epilepsy spikes or an after-discharge (AD) and is accompanied by brief behavioural seizures (McIntyre, 2006). It is a progressive, permanent increase in susceptibility to evoked and spontaneous seizures induced by repeated episodes of network synchronisation, and it is robust (Löscher, 2017). The repetitive evoked seizures induced are subtle, but the accumulative neuronal loss and a variety of cellular alterations in neuronal circuits result in spontaneous seizure. There are two methods of kindling; electrical and chemical. Electrical kindling is most commonly induced by repeated electrical stimulation to create a simple phenomenon of provocation of focal seizure. There are limitations of kindling as an experimental model: 1) The time course for induction of spontaneous seizure is not relevant to human epilepsy. 2) Practical disadvantages include laborious handling and stimulation procedures, and the potential for loss of chronic head plugs and electrodes (Pitkänen et al., 2006; Pitkänen et al., 2015), 3) This model is only suitable for studying adult animal because of younger animals' skull fragility and growth (Kandratavicius et al., 2014).

1.8.4 Tetanus toxin

Tetanus toxin is one of the oldest methods of inducing chronic experiment epilepsies. The toxin is secreted by the bacteria *Clostridium tetani*. When injected into the brain, the toxin is taken up by peripheral nerve, and it enters the central nervous system via retrograde axonal transport along the motor nerve to the spinal cord (Koshal et al., 2017). Tetanus toxin produces epileptic foci centred on the injection site. It therefore models partial/focal epilepsies with the specific deficit subtype depending on the location at which the toxin is injected (Jefferys and Walker, 2006). There are two

main models based on the injected location: 1) injected into the hippocampus to create a model of complex partial seizure - temporal lobe or limbic epilepsy, 2) injected into the neocortex to create a model of focal neocortical epilepsy, secondary generalised seizure and epilepsy partialis continua.

The tetanus toxin model has a few limitations; 1) the toxin is relatively fragile – the chain can separate if the solution is agitated too vigorously. 2) Aseptic technique is required as the toxin can be destroyed by bacterial contamination. 3) It is heat labile, so refrigeration is essential. 4) Variation may occur between batches of toxin (Pitkänen et al., 2006).

1.8.5 Fluid percussion injury

Fluid percussion injury (FPI) produces several focal and diffuse characteristics of moderate to severe closed head injury in humans, including focal contusion, blood-brain barrier disruption, altered cerebral metabolism, altered blood cerebral flow, subdural haematoma, interaparenchymal and subarachnoid haemorrhage, local and remote axonal injury, progressive neuronal loss, altered electrical activity (acute seizures, alterations in evoked potentials) induced posttraumatic epilepsy (PTE), and acute and chronic behavioural abnormalities (Thompson et al., 2005). Rats with PTE were found to have neuronal damage in several hippocampus subfields, including the hilus, CA3 and CA1 (Pitkänen et al., 2006). Previous histological studies have demonstrated neuronal loss in CA4 (includes part of CA3 and the hilus), CA3 and CA1, whereas CA2 was preserved in the hippocampus of traumatic brain injury patients with or without epilepsy (Diaz-Arrastia et al., 2000; Maxwell et al., 2003). Spontaneous seizure has been described only after the induction of FPI (McIntosh et al., 1989; Thompson et al., 2005).

There are a few limitations of this model: 1) small differences in the location of the FPI in the cortex result in substantial variability in the distribution of structural damage and associated function impairment (Vink et al., 2001; Floyd et al., 2002). 2) Severe injury used to induce epileptogenesis results in 30% to 40% mortality after FPI. This increases the number of animals required to achieve statistical power in the final analysis. 3) Long follow-up (6-12 months) of animals is required to detect

spontaneous seizures. Low seizure frequency makes it difficult to detect seizures without long term video-EEG monitoring, which is costly and labour-intensive (Pitkänen et al., 2006).

1.8.6 Kainic acid (KA)

KA was among the first compounds used to induce TLE in rats (Sharma et al., 2007). The KA model can replicate several phenomenological features of human TLE (Leite et al., 1990; Lévesque et al., 2016). However, the exact mechanism of epileptogenesis in TLE is hard to achieve in clinical studies with humans. Therefore, KA models are used in animal studies to understand the basic mechanisms of epileptogenesis (Kandratavicius et al., 2014) by mimicking TLE. KA administration in rats has frequently been used as an animal model since it portrays three similar characteristics of TLE which are initial injury affecting the hippocampus and/or the temporal lobe (eg, SE) (Boullereit et al., 1999), a latent period between the injury, and the occurrence of spontaneous seizures (Sharma et al., 2009; Kandratavicius et al 2014). For instance, rodents were induced by KA to generate spontaneous continuous seizure activity, or a series of seizures without retaining full consciousness in between (Bertoglio et al., 2017). Previous studies have shown that local or systemic administration of KA in rodents triggers repetitive limbic seizures and SE (Leite et al., 1990; Furtado et al., 2002; Lévesque and Avoli, 2013). KA-induced experimental studies in rodents have caused seizures, behavioural changes, oxidative stress (Li et al., 2018), and neuronal degeneration of the selected population of neurones in the brain (Sairazi et al., 2017).

KA causes widespread neuronal loss in the hippocampus due to over-excitation and an excessive influx of Ca^{2+} , a phenomenon referred to as excitotoxicity (Fritschy, 2004). It is an agonist of ionotropic, non-NMDA glutamate AMPA and KA receptors. It generates acute continuous seizures at the hippocampus and, with time, recurrent spontaneous seizures with clear behavioural manifestations (Sharma et al., 2007; Kandratavicius et al., 2014; Grone and Baraban, 2015).

The KA-induced rat seizure model has been used by several studies in the literature (Liang and Patel, 2016; Bertoglio et al., 2017; Si et al., 2017; Li et al., 2018). Systemic or intracerebral injections of KA cause epileptiform seizures (Ambrogini et al., 2018; Pirttimäki et al., 2018) in the CA3 region of the hippocampus. These seizures propagate to other limbic structures and are followed by a pattern of cell loss that is similar to that seen in patients suffering from TLE (Nadler, 1981; Ben-Ari, 1985). Studies using the KA model of epilepsy have considerably improved our understanding of important issues in the field of the epilepsies such as the causes or the consequences of brain damage, hyperactivity leading to cell loss, and the places where seizures are generated. The combined approaches of molecular biology, patch clamp and imaging techniques, as well as novel *in vitro* preparations, have improved our knowledge of the mechanisms of action of KA (reviewed by Ben-Ari and Cossart, 2000).

However, there are a few limitations imposed by this model; namely, the dose of KA is specific to the rat strain, KA seizure-induced location also differs based on the rat strain (Bertoglio et al., 2017), the damage may be sex specific, and the resistance depend on the rat's age (Velíšek, 2006; Lidster et al., 2016). Furthermore, systemic KA administration is often associated with high mortality, experimental variability rates, and severe animal welfare issues (Lidster et al., 2016; Bielefeld et al., 2018). On the other hand, intrahippocampal KA injection is an excellent model of mTLE, as it reproduces the pathological signs of mTLE such as changes in gene expression, induction of reactive gliosis, granule cell dispersion, apoptosis, inflammation, the occurrence of chronic recurrent seizures, and deregulation of the neurogenic process (Schouten et al., 2015; Sierra et al., 2015). KA-induced seizures may be a valuable tool in the assessment of an AED's efficacy in a complex partial seizure. A more detailed explanation about KA will be given in Section 3.1 of Chapter 3. Table 1.2 lists some of the animal models used in the study of epilepsy.

Table 1.2 Summary of some of the existing animal models in the literature (modified from Löscher 2011; O'Dell et al. 2012; Kandravaticus et al. 2014)

Model	Induction type	Benefits	Limitations
Kainic acid/kainate	Chemical induction	Changes mimic human MTLE:CA1-CA3 neurone loss, aberrant mossy fiber sprouting, astrogliosis and microgliosis (Zheng et al., 2011)	The high mortality rate with high doses; source of damage: drug toxicity or seizure activity (Zheng et al., 2011; Sharma et al., 2007)
Pilocarpine	Chemical induction	Changes mimic human MTLE	Lesions are more prominent in the neocortex (Sharma et al., 2007)
Electroshock-induced seizures	Electrical induction	Can be either unilateral or bilateral; changes mimic human MTLE (Gorter et al., 2003)	Special equipment and time requirements for electrode implantation (Gorter et al., 2003)
Kindling	Electrical	Seizure onset can be controlled; specific sites can be individually stimulated (Sharma et al., 2007)	No spontaneous seizures are generated; lesion pattern is not as predictable (Sharma et al., 2007)
Tetanus toxin	Stereotaxic administration into the brain		Weak or temporary seizures; lesions differ from human MTLE;limited information on the model (Sharma et al., 2007)
Fluid percussion injury	By exposing the dura and using a fluid percussion instrument to deliver a traumatic force	Produces spontaneous seizures; useful for studying post-TBI epilepsy (Kharatishvili et al., 2006)	Seizures originate from frontal-parietal cortex and progress to medial temporal cortex; damage and seizure generation are highly heterogenic (Pitkänen et al., 2009)

1.8.6.1 Justification for the use of KA-induced model in our study

The KA model allows us to study the different stages between SE and the development of TLE, including SE, the acute and latent stages of epileptogenesis and chronic epilepsy. Our study focused on acute mTLE and systemic model system. The experiments were also continuity from previous study by Coomber et al. (2008) in our lab.

Based on the existing literatures described previously, other animal models were mainly focused on chronic model. These animal models also have more limitations compared to KA model. The KA model displays three similar main features of human TLE: 1) an initial epileptogenic injury, 2) latency period and 3) spontaneous seizure. These neuropathological characteristics of TLE patients showed that 65% had HS based on the histological patterns of neuronal loss and gliosis (Thom, 2014). About 60–80% of patients with mTLE are affected by type 1 HS, which is characterised by neuronal loss, predominantly in CA1 but also in CA3 and CA4. Consistent with this, the KA rat model of TLE also shows a high proportion of animals manifesting a histological pattern similar to type 1 HS in patients (Bertoglio et al., 2017).

Furthermore, the KA model also allows rapid investigation of epileptogenic mechanisms and screening of AEDs, which is vital for the progression of epilepsy and neurophysiological research. Hence, it was chosen as the focus of our study seeking to understand the mechanisms of epileptogenesis. The fact that KA-induced model fulfils the criterion of closely mimicking TLE in human temporal epilepsy justifies our selection of this model.

1.9 Contribution and significance of the study

Administration of KA is known to induce a sequence of well-characterised seizure syndromes and has resulted in behavioural changes in rodents, including motor and cognitive performance (Ben-Ari, 1985; Gayoso et al., 1994; Sairazi et al., 2017). KA-induced SE causes irreversible neuronal degeneration in the affected brain areas, particularly in limbic structures (in the CA1 and CA3 regions of the hippocampus) and the hilus of the dentate gyrus (Ben-Ari, 1985).

Local or systemic injection of KA was reported to activate CA3 pyramidal neurones, which subsequently propagate to other hippocampal regions through the activation of CA3 recurrent collateral synapses. This propagation generates synchronised network-driven glutamatergic currents (Ben-Ari and Gho, 1988). Zeng et al., (2009) found out that mammalian target of rapamycin signalling mediates mechanisms of epileptogenesis in the KA rat model, and its inhibitors have potential antiepileptogenic effects in this model. Karunakaran et al., (2012) measured changes in neuronal activity from CA3 hippocampus, ipsilateral and contralateral to a focal intrahippocampal_injection of KA during the full course of SE, 24 h post- SE, and one week post-SE. Sairazi et al. (2015) reviewed the potential of natural products and plants extracts to have a possible neuroprotective effect against KA-induced excitotoxicity through their antioxidant, anti-inflammatory, and anticonvulsive actions.

Bertoglio et al. (2017) investigated the effect of using two different rat strains in the KA model and found out that neuropathological differences may be related more to the distinct neurotoxic effects of KA in the two rat strains than being the outcome of seizure burden itself. These existing studies (Ben-Ari,1985; Ben-Ari and Gho, 1988; Gayoso et al., 1994; Zeng et al., 2009; Karunakaran et al., 2012; Sairazi et al., 2015; Bertoglio et al., 2017) have shown the effect of KA-induced epileptiform activity, but none of these studies observe the connectivity of CA1 and CA3 brain areas and also between left and right hippocampus. So, our motivation was triggered to answer the question: what is the characteristic of connectivity in KA-induced epileptiform activity within the hippocampus and between the left and right hippocampus?

Epileptic seizures are commonly associated with aberrant hyper-synchronisation of large neuronal aggregates in the hippocampus. Cannabinoid receptors have been shown to have antiepileptic and neuroprotective properties. Available evidence from human and animal seizure models suggests that various cannabinoid compounds (derived from the marijuana plant, *Cannabis sativa*) have antiepileptic properties. Coomber et al., (2008) investigated the role of the endocannabinoid system in regulating *in vivo* hippocampal neuronal activity under an acute KA-induced hyperexcitable condition. Their work found that administration of a CB1 antagonist during febrile seizures blocked seizure-induced potentiation of depolarisation-induced

suppression of inhibition, eliminated seizure-induced CB1R upregulation, and prevented the development of long-term limbic hyper-excitability.

A study by Mason and Cheer (2009) investigated the effects of cannabinoid receptor activation on KA-induced bursting activity in the CA1 hippocampal layer of anaesthetised rats. Using multiple simultaneous extracellular single-neurone recordings, the authors discovered that the cannabinoid agonist, HU210 100 µg/kg i.p following KA, markedly reduced the burst firing rate and reversed synchronised firing patterns back to basal levels. Previous *in vitro* and *in vivo* studies have shown that exogenously applied CB receptor agonists, such as WIN 55,212-2 are antiepileptic in a number of animal seizure models (Blair et al., 2006). Existing studies have studied the physiology and pharmacology of cannabinoid (Howlett et al., 2004; Ligresti et al., 2016), the therapeutic potential of cannabinoid receptors (Kreitzer and Stella 2009; Rosenberg et al., 2017) and the mechanisms of action and pharmacokinetics of currently available AEDs (Löscher, 1999; Conway and Henry, 2012).

Studies on benzodiazepines, in general, have focused on the treatment of epilepsy, particularly studies on chlordiazepoxide, diazepam, oxazepam and nitrazepam (Browne and Penry, 1973; Riss et al., 2008). FLU as a benzodiazepine derivative was used more as a hypnotic (Browne and Penry, 1973) and a sedative to treat insomnia (Mendelson and Martin, 1990; Asnis et al., 2015; Lie et al., 2015).

Even though this field of research has developed incredibly fast during the last decade and the different approaches of the experiments clarified some of the consolidated knowledge in animal models of epilepsy, the question of the connectivity between the left and right hippocampus during epilepsy still remains open. Therefore, our study intends to explore the connectivity between CA1 and CA3 brain regions and between both the left and right hippocampus, which may be the key to understanding the fundamental mechanism underlying epilepsy.

1.10 Aims and hypothesis of the study

This study was carried out to investigate connectivity between neurones in the hippocampus (intra-hippocampus) and also connectivity between hippocampal (inter-hemispheric) during epileptiform activity. The epileptiform states were induced by KA (injected intra-hippocampus) mimic the insult that leads to mTLE, and the connectivities were assessed via a few parameters such as normalised mean firing rate, normalised mean synchrony index, cross-correlation and LFP frequency band distribution, by employing multiple simultaneous extracellular single-neurone recordings. The changes in these parameters have also been evaluated when VPA, WIN and FLU were used.

We hypothesised that epileptiform activity induced by KA can not only occur ipsilaterally (injection site) but can also be propagated to the contralateral hippocampus. We believed that the neurones would transfer the information to the other side of the brain during a seizure. We also believed that the VPA, WIN and FLU could attenuate and/or prevent the KA-induced epileptiform activity.

Chapter 2

METHODS

2.1 Animals

All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986, UK. Experiments were performed on male Lister Hooded rats (Biomedical Sciences Services Unit, University of Nottingham) weighing 250-400g (n=53 rats) based on the study by Coomber et al., (2008). All experimental procedures were approved by the UK Home Office (project licence 40/3283 and personal licence 40/10438). These licences also included the application of 3Rs (replacement, reduction and refinement of animal use), which was considered when designing the experiments. The rats subjected to AC guideline were animals which have received general anaesthetic with no recovery.

2.2 Surgical procedure

The procedures were adapted from Coomber et al. (2008), which was carried out in our lab in the School of Life Sciences, University of Nottingham. A full level of anaesthetic (isoflurane) was introduced to the Fluotec vaporiser (O'Neill Medicalia Ltd, Liverpool, UK). The medical air cylinder valve was opened, and the flow regulator was set to 600 ml/min. The scavenging wall unit was turned on, and the induction box was connected to the vaporiser. The induction isoflurane level was set to 4% on the vaporiser unit. The animal was placed in the induction box until a surgical level of anaesthesia was obtained. This is indicated by a loss of balance, paw pinch, corneal reflexes, and a transition from thoracic to abdominal breathing. This system was already setup in our lab, and the anaesthetic agent (isoflurane) was already proven to be safe to be used in the electrophysiological experiment by Coomber et al. (2008), therefore we have carried out our experiment with the same setup. Our experiment was a continuity from previous study by Coomber et al. (2008).

2.2.1 Left femoral vein catheterisation

The animal was removed from the induction box and placed on the heated thermos blanket on the surgical platform in the dorsal recumbency position with the nose inserted into the anaesthetic mask (Kopf model 906, California, USA) to continue the procedure with femoral vein cannulation (to administer AED via intravenous). The anaesthetic mask was connected to the isoflurane vaporiser, and the isoflurane concentration was reduced to 3% to ensure the anaesthetic dose was not too high at this point. The lubricated rectal probe (Harvard Instruments, UK) was inserted into the rectum, and the animal's tail was secured with tape. The oxygen level (SPO₂) and pulse rate were monitored using an oximeter PM-60-1 (Burtons, UK) with the sensor clipped on the hind paw.

A small skin incision was made with a No. 10 A scalpel blade (Swann Morton, Sheffield, UK) on the proximal area of the thigh above the femoral vein. The size of the opening was increased to 1.5 cm by using blunt ended scissors. Blunt dissection was performed under the skin where the leg meets the body wall, between femoral vein and dorsal cervical incisions. The femoral vein (the largest vessel with a dark red colouring) was carefully dissected away from the femoral artery and nerve. Two ligatures (cotton thread) were placed loosely under the femoral vein. The distal ligature was tied, and the proximal ligature was left untied.

A small incision was made using a syringe needle from the distal ligature. The cannula was fed into the vein, and once it has entered the vessel, it is then checked to ensure the femoral vein is aspirating the vessel. The proximal ligature was tied around the cannula, including the vessel wall. The cannula patency was checked by flushing the catheter with saline. The free ends of the proximal and distal ligatures are tied loosely together, and the remaining ends cut to a 2 mm length. A loop of catheter material was left in the leg to allow movement of the leg without putting tension on the catheter. The incision area was covered with cotton wool and secured with autoclave tape. The rat was then turned to the sternal recumbency position.

2.2.2 Craniotomies procedures

The rat on the heated thermos blanket was briefly removed onto the modified stereotaxic frame (Kopf model 1730, California, USA) and the incisor teeth hooked over the incisor bar. The anaesthetics mask (Kopf model 906, California, USA) was slid over the snout and secured throughout the surgical procedure. The ear bars are positioned so the top of the head is flat and the eyes and ears symmetrical with no head movement.

A scalp incision was made using size 10 surgical scalpel blade no.10A (Swann Morton, Sheffield, UK) through the skin to expose the skull surface. The skin was clipped back at the top and bottom using the vessel clips. The final connective tissue layer was pushed away with a cotton bud and allowed to dry and revealed the bregma. The skull reference point of the bregma was located, and micro-wire electrode arrays (NB labs, Texas, USA) was positioned over the left and right of the dorsal hippocampus (AP: ± 4.5 mm; ML: ± 4.0 mm from the bregma). This step was conducted according to the coordinates of the rat atlas (Paxinos and Watson, 1998) using the dorsal-ventral (VD), SMM-100, three-axis stereotaxic micromanipulator micromanipulators (Narishige International, USA) on both left and right stereotaxic arms and frame. The electrode was lifted up and away from the areas (left and right hippocampus) and the spot marked with a fine permanent marker pen. The marked position was then checked with the electrode tip, and then the arm of the electrode was swung completely out of the way without catching the electrode tips or anything else. The surface of the skull was saturated with saline, and the microscope positioned over the hole and focused. The target hole was carefully drilled to a 3 mm diameter size circle using the micro trephine (hand-trephine) drill (Harvard Apparatus, Massachusetts, USA). The dura was retracted using a dura retractor (modified syringe needle) and microscope from the brain surface within the hole, and the exposed cortex was kept moist with saline. The anaesthetic concentration was adjusted to provide a constant level of surgical anaesthesia (1.75-2.0 %). The level of anaesthesia should be continually assessed by monitoring the reflexes and breathing rate of the rat for every 15-20 minutes.

2.3 Electrodes and recording procedure

Micro-wire electrode arrays (MEAs) comprised of two rows; each row has 4 electrodes; electrode 1-4 was the shorter electrode and electrode 5-8 was the longer electrode. Electrode 1-4 was separated from electrode 5-8 by 1 mm (Figure 2.1B). Each microwire is constructed from 0.50 mm Teflon insulated stainless-steel (NB Labs, Texas, USA). MEAs were used to record multiple single-unit (spike) activity and LFPs simultaneously in both right and left hippocampus. In this study, we used two MEAs; left hippocampus (CA1-ipsilateral, channels 1-4 and CA3-ipsilateral, channels 5-8) and right hippocampus (CA1-contralateral, channels 9-12 and CA3-contralateral, channels 13-16).

The MEA was customised ('MEA-injectrode') to record neural signals from the left hippocampus (ipsilateral) and to deliver KA or saline locally. KA or saline was administered via the cannula, which was attached to the electrode array at the position of 0.5 mm above the shorter electrode (Figure 2.1B). The cannula was constructed from a 4 cm length of 33G stainless steel syringe needle connected to 50 cm of fine bore polythene tubing (Portex tubing; PE 0.28x0.61 mm BxWall; Scientific Laboratory Supplies LTD, UK) and administered by a Hamilton syringe (1 µl 7101 Flat; Scientific Laboratory Supplies LTD, UK) as in Figure 2.1A. The KA injection was administered slowly with an air bubble in the tubing used as a marker over a period of about 2 min. The recording setup for the local injection of the cannula at the left and right MEA injectrode is shown in Figure 2.2.

The electrode was positioned over the targeted holes and slowly lowered to the brain surface using the SMM-100, three-axis stereotaxic micromanipulator (Narishige International, USA). Whilst looking down the microscope, the electrode was slowly and carefully lowered to puncture the brain tissue (cortex) then stopped to let the tissue settle. The electrode was slowly lowered until the correct depth (VD: -4.0 mm) was achieved and picked up the spike and LFP from both left and right of the dorsal hippocampus. The electrode arrays were allowed to settle for approximately 30-60 minutes after being lowered to get a stable signal and response from the neurones. The spike and LFP signal were recorded via the preamplifier which was connected to a *Plexon* Multichannel Acquisition Processor (MAP); Plexon pre-amplifier settings for spikes gain x1000; band pass filtered 150 Hz – 9 kHz and LFPs gain = x1000;

filtered at 0.07–170 Hz. Spikes and LFPs were recorded on-line with RASPUTIN (v 2.71, Plexon Inc.) and analysed off-line using NeuroExplorer (v4; NeuroExplorer Inc. USA).

At the end of the experiment, the electrode-cannula was taken out from the brain and cleaned using saline solution by brushing the electrode-cannula gently to remove any adhered tissue, blood and protein in order to maintain cannula patency in subsequent experiments.

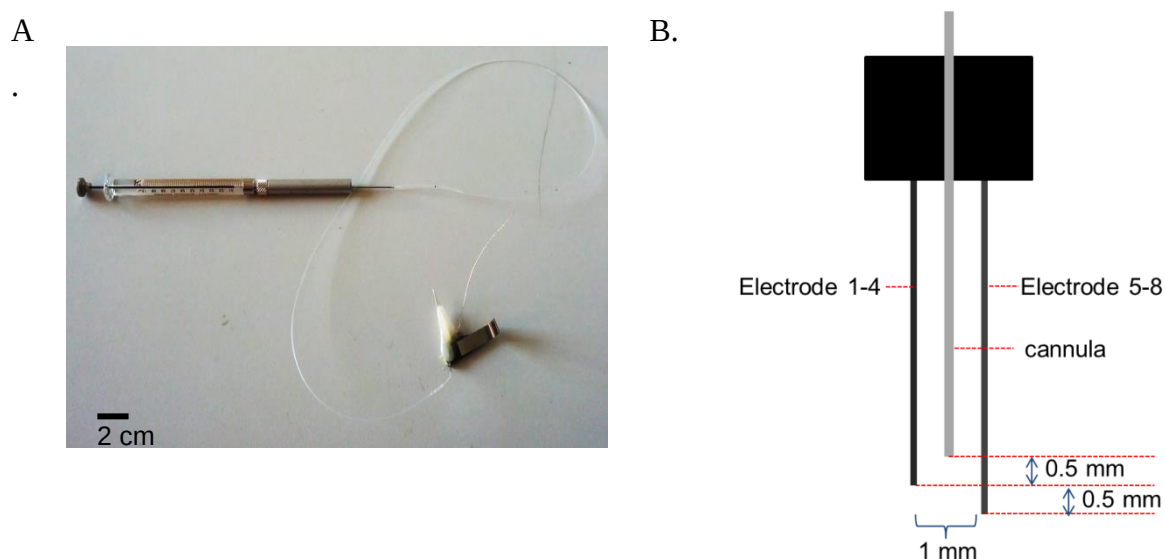


Figure 2.1 Diagram of cannula and electrode.

A: Cannula (stainless steel syringe needle tubing) connected to 50 cm of fine bore polythene tubing (0.65 mm x 0.12 mm) and Hamilton syringe (1 μ L).

B: Schematic Illustration of 'MEA-injectrode' (side view) used for intracerebral recording and local drug injection.

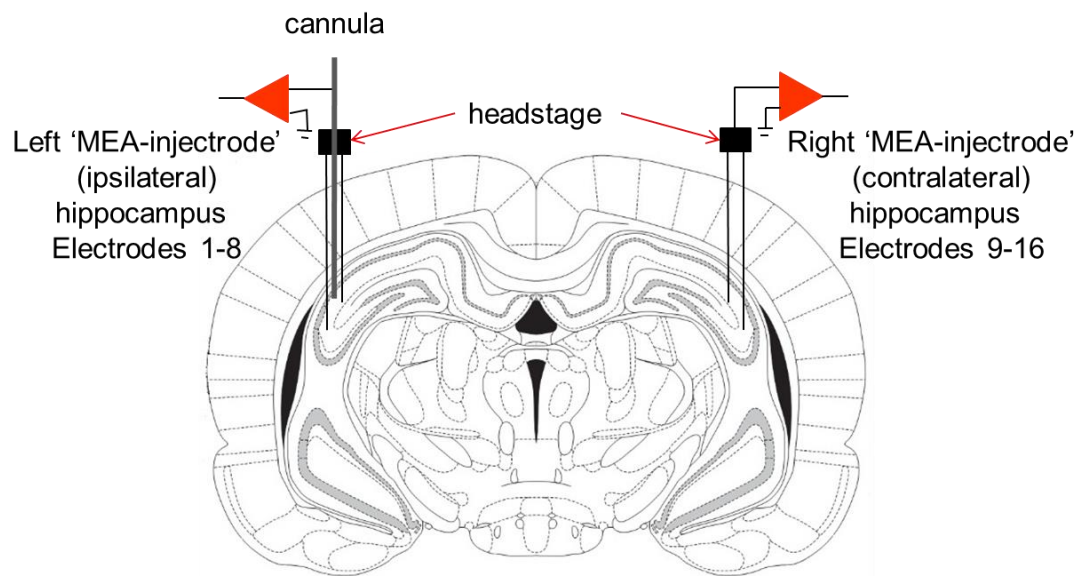


Figure 2.2 Schematic of the recording sites and setup.
Electrode placement in both the left and right region of the dorsal hippocampus. The left (ipsilateral) hippocampus shows the position of local injection cannula.

2.4 MAP system and electrodes

2.4.1 MAP system

The Multichannel Acquisition Processor (MAP) system allows programmable amplification, filtering and real-time spike sorting of multi-channel signals, with neuronal spike trains and LFPs recorded simultaneously.

2.4.2 Single unit activity (spikes)

The analogue output from the *Plexon PBX* preamplifier was fed to a 64 channel MAP system which provided additional computer-controlled amplification (x1000 to x32000; typically the gain was set at x1000 or x2000 for the current experiments), band-pass filtering (500 Hz – 5 Hz; plus a 50 Hz notch). Signals were digitised at 40 kHz (providing 25 μ s precision) on each channel at 12-bit resolution, then routed to digital signal processor (DSPs, Motorola 56002) boards running at 40 MHz. The MAP box was linked to a host PC via an MXI Bus board (National Instrument, UK) within the host PC (Dell OptiPlex 2.2 GHZ with 4 GB RAM and 240 GB hard drive) running C++ host software under Window XP Professional. Recorded files were archived to a 2 TB external hard drive (LaCie, USA).

Plexon MAP software referred to as the Real-Time Acquisition System Programs for Unit Timing in Neuroscience also known as RASPUTIN (*Plexon Inc*, Texas, USA) allowed on-line isolation and discrimination (using dual voltage-time windows or principal component analysis) of neuronal spikes from background noise (Mason and Cheer, 2009). A voltage threshold for capturing spikes was implemented based on visual inspection of the analogue signal on an oscilloscope and digitised signal (computer monitor) using the *Plexon* RASPUTIN software default setting (Nicolelis et al., 1995). Typically, one to four neurones were observed per micro-wire on-line (Sort Client V2.7.1; *Plexon Inc*), and discrimination was conducted off-line (Off Line Sorter v3.23; *Plexon Inc*, see Nicolelis et al., 1995). Unit activity was additionally displayed on a D11 5000 series dual-beam oscilloscope (Tektronix, OR) and a 507 analogue-digital oscilloscope (Hameg, Germany) and neural activity monitored aurally with the aid of a loudspeaker. The MAP system and the recording setup are shown in Figure 2.3.

2.4.3 Local field potentials (LFPs)

LFPs were recorded in parallel with spikes from the same electrode micro-arrays per region. The neural signal was split at the *Plexon* PBX preamplifier x1000 and filtered at 0.7-170 Hz PBX and was upgraded later in the study to 0.07-300 Hz. LFP signals were then fed via a National Instrument data acquisition (NIDAQ) card (PCI-6071E 125 MS/sec, 64 channels 12-bit resolution) in the host PC and LFP signals digitised at 1 kHz.

2.5 Drug administration

After 30-60 minutes, electrodes were lowered to get stable signals from the neurones, spikes and LFP activity was then recorded for a full 80 or 210 minutes (including 10 min basal time); waveforms were captured based on a spike amplitude threshold (>12 mV) set prior to initiating recording.

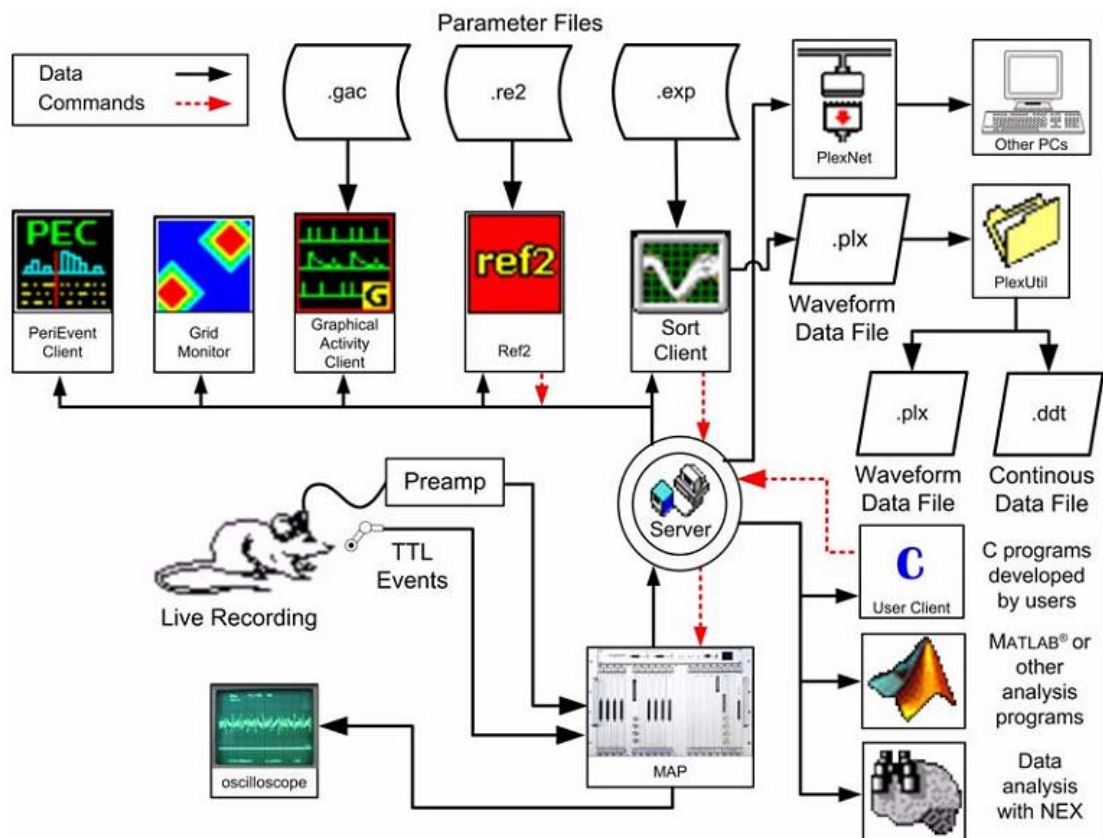


Figure 2.3 The following illustration shows the relationship between the various components of the MAP system and the RASPUTIN Software.

2.5.1 Systemic KA administration (to mimic generalised epilepsy model)

Basal recordings were obtained for 10 minutes prior to drug administration. The rats were administered 10 mg/kg of kainic acid (KA, Tocris, UK) which was dissolved in saline intraperitoneally (i.p). The neuronal activity was observed 20-30 min following the time of injection in agreement with other authors (Covolan and Mello, 2000; Coomber et al., 2008). Recordings were continued for 50 min after KA injection. The time period for systemic KA administration was chosen because the effect started (after 20 mins of KA administration) within the time frame (123 min) from previous study by Coomber et al. (2008).

2.5.2 Local KA administration (to mimic medial temporal epilepsy (mTLE) model)

KA (10 mM or 1 mM; 1 μ l) was locally injected in the left hippocampus (AP: -4.5 mm; ML: $\pm$ 4.0 mm; VD: -4.0mm; ipsilateral) (Pitkänen and Sutula, 2002; Karunakaran et al., 2012) via cannula attached to the MEAs (Figure 2.1). The experiment was further recorded for another 50 min. Overall this experiment totals up to 80 min (including 10 min basal time).

2.5.3 Dose selection of anti-epileptic drug (AED) administered intravenously

Basal recordings were obtained for 10 minutes. For drugs administered before KA experiments, the rats were administered with either 20 mg/kg of valproate (VPA, Tocris, UK) or 1.2 mg/kg of WIN 55,212-2 (WIN, Tocris, UK) followed by KA after 20 min.

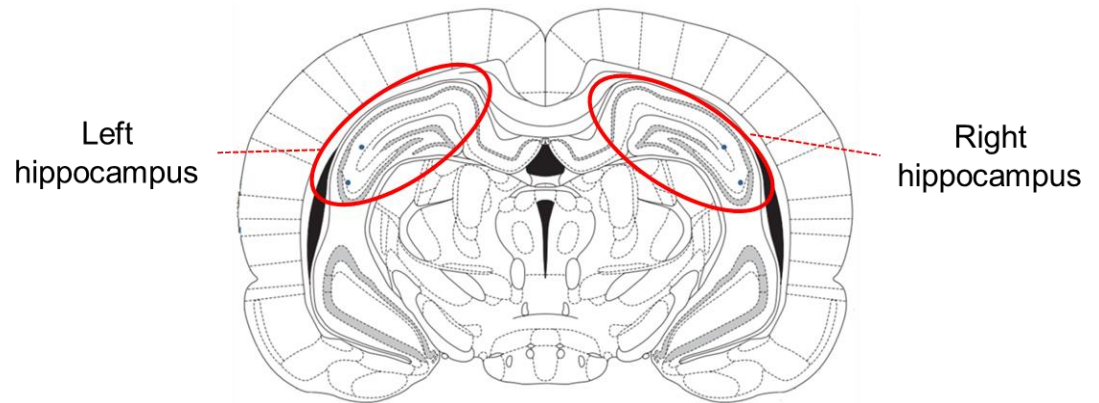
For drugs administered after KA experiments, the rats were administered with KA followed by either 20 mg/kg of valproate (VPA, Tocris, UK) or 1.2 mg/kg of WIN 55,212 (WIN, Tocris, UK) or 2 mg/kg of flurazepam (FLU, Sigma, USA) intravenously (i.v) after 20 min of KA administration.

2.6 Histological verification of electrode (MEAs) placement

At the end of the experiment, the animals were deeply anaesthetised with 5% isoflurane. The head-stage from the MEAs was carefully removed, leaving the MEAs in place. The positive (white wire) and negative (blue wire) gold pins were attached to the two pins on the head-stage with a minimum of one pin in-between to prevent the wires from touching. Once the animal had stopped breathing, the Grass D.C. constant current lesion maker (Grass Instruments, Quincy, MA) was used to allow a 0.1 mA current to pass for up to 10 seconds ensuring that the MEAs stayed in the correct position (both CA1 and CA3 of ipsilateral (left) and contralateral (right) hippocampus).

The MEAs was removed from the brain, and the pin head-stage was also removed. The tips of the electrode were cleaned using distilled water or saline with a paint brush. The MEAs was left to dry in a safe place. The animal was then removed from the stereotaxic frame. The neck was dislocated, and the head severed. The brain was removed and stored in 4% paraformaldehyde containing 4% potassium ferrous cyanate solution for at least 48 hours at room temperature. Histological verification confirmed the electrodes were at the targeted coordinate using a Prussian-Blue reaction (Coomber et al., 2008). Brains were sectioned transversely at 200 μ m using a Vibratome (Model 752 Campden Instruments; UK) and were further examined under a stereomicroscope (Olympus; MA, USA). Digital images were captured with a digital camera (Nikon CoolPix 4500; Nikon U.K Ltd, UK) attached to a microscope eye piece; files were saved as *.jpeg format, and the images were visualised using Windows Photo Viewer (Figure 2.4B). Recording sites were identified with reference to the coordinates (Figure 2.4A) of the Paxinos and Watson rat brain atlas (1998).

A.



B.

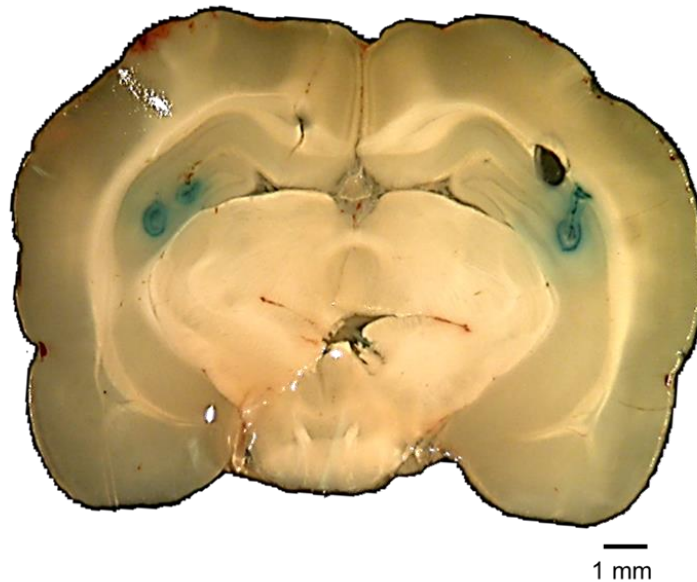


Figure 2.4 Representative histological verification of hippocampal recording sites.

A: Schematic representation of histological verification of hippocampal recording sites (blue dot) according to Paxinos and Watson rat brain atlas (1998).

B: An example of histological verification of hippocampal recording sites with electrode placement was confirmed by the visible Prussian blue dye mark (shown by the arrow). The recording was made using a 16-electrodes array at 4.0 mm posterior to bregma.

2.7 Data analysis

All of the recorded data were saved in *.plx file format and were archived on the host PC hard drive, with additional backup on an external hard drive (LaCie 2TB Porsche Design, UK). They were further analysed using Off-Line Sorter (v3.23; Plexon Inc., USA), NeuroExplorer (v4), Prism (v6; GraphPad, USA), and custom-written Matlab scripts (*Cross-Correlation Grid Plot_v9*; *spike train clustering*) (Fenton et al., 2015; www.nottingham.ac.uk/neuronal-network). Figure 2.5 shows the flow diagram of data processing.

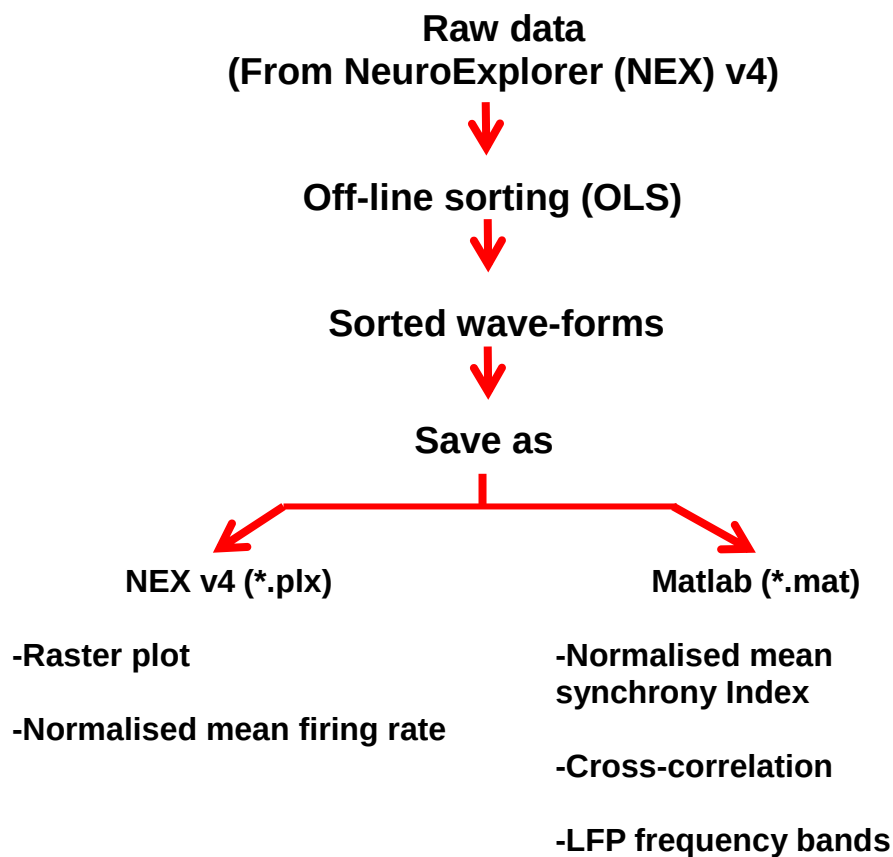


Figure 2.5 Flow diagram of data process.

The raw data were manually discriminated, and noise artefacts were removed based on the similar neuronal wave form. The cleaned and sorted wave forms were saved in two formats; NEX and Matlab for further analysis.

2.7.1 Off-Line Sorter

All the wave forms were manually discriminated, and noise artefacts (non-neuronal signals) were removed based on the similar neuronal wave form. The clustering of wave-forms was done automatically using t-distribution expectation maximisation (TDEM).

TDEM works on the assumption that the clusters in feature space (each cluster representing the spikes from one neurone) can be modelled as t-distributions so that the entire set of clusters on each channel is a mixture of t-distributions. The TDEM algorithm solves a global optimization problem to find the set of t-distributions which best fit the observed clusters on each spike channel. This algorithm is also capable of adjusting the number of clusters as it runs (by removing unfavourable clusters) to arrive at the optimum number of clusters (Offline Sorter v4.0 User Guide, Plexon Inc, Dallas, Texas, USA) as in Figure 2.6.

The TDEM algorithm is an iterative procedure that operates on the points in feature space to assign the wave-forms to an optimal number of clusters. The clustering also discriminated neuronal wave-forms from non-neuronal wave forms. There was usually more than one wave form clustered from single units recorded by one electrode. The discriminated and “sorted wave-forms” recorded from all 16 electrodes were saved in *.plx file format for further analysis using NeuroExplorer and/ or Matlab scripts.

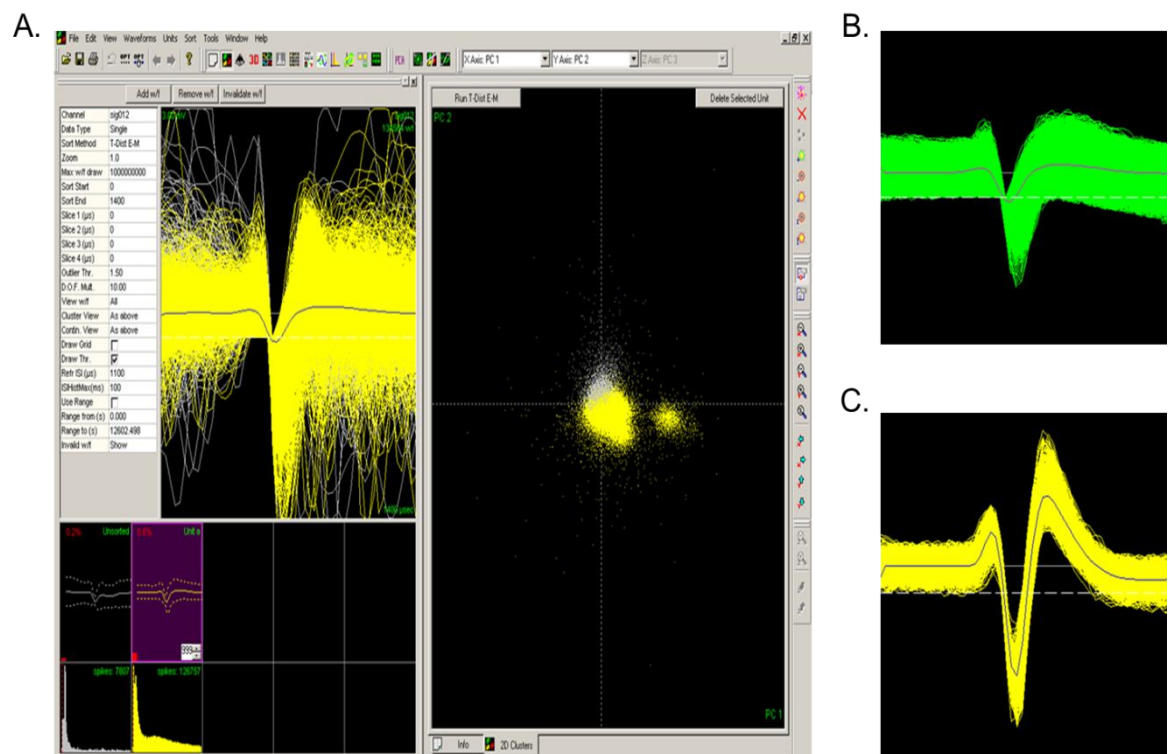


Figure 2.6 Representative screen shots of online and offline spike sorting. Right panel depicts a 2-D PCA plot of two sorted spike waveforms. **A:** waveform during online sorting recorded from one microwire of an electrode array. **B and C:** two resultant waveforms manually discriminated during offline sorting. (Offline Sorter v4.0 User Guide, Plexon Inc, Dallas, Texas, USA).

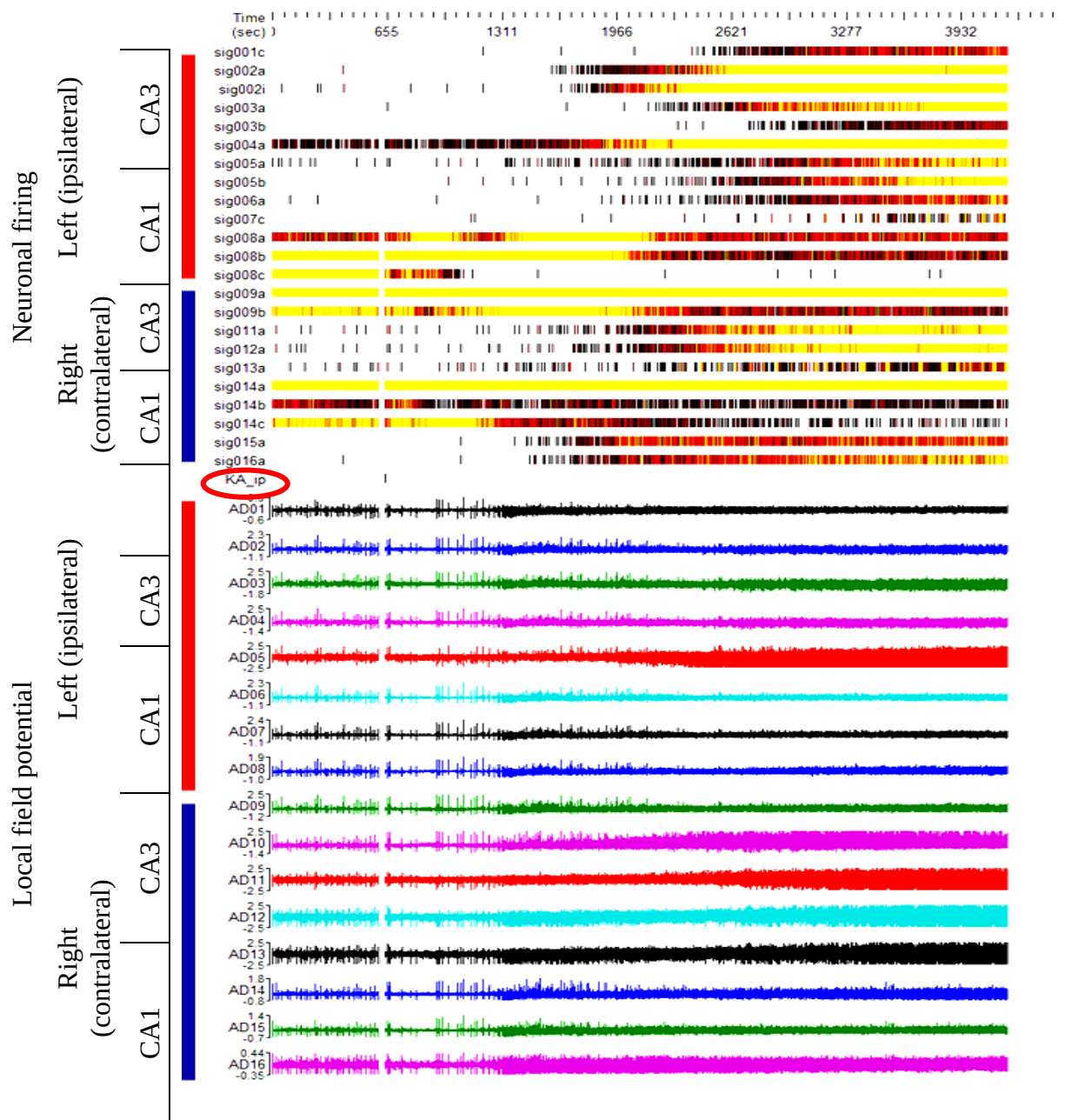


Figure 2.7 Example of a raster plot of systemic KA-induced epileptiform activity.

Simultaneous recordings of multiple hippocampal neuronal activities. Representative unit spike rasters and LFP traces showing basal activity in 16 discriminated individual hippocampal neurones, sig001-sig008 (red bar) are from left hippocampus and sig009-sig016 (blue bar) are from the right hippocampus. This trace illustrates a sample representation of spontaneous hippocampal unit firing observed over a ~600s period of basal control. Firing activity of the same neurones increase after ~15 min (~900s in the left hippocampus) and ~10 min (~600s in the right hippocampus), respectively after KA (10 mg.kg⁻¹, i.p) administration. The spike density of firing rate was qualitatively visualised by a colour code; **black** for individual spikes running through **red** to **yellow** as the firing rate increases. The recordings were ordered vertically from CA3 to CA1 to aid visualisation of the anatomical/functional direction of information flow through the hippocampus.

2.7.2 NeuroExplorer - based analysis scripts

2.7.2.1 Spike train raster plot

The sorted data were then initially analysed using NeuroExplorer (v4). Spike neuronal-trains were visualised as unit spike raster plots and LFPs (1D viewer). Each vertical tick in the raster display represents the ‘time-stamp’ of a single neuronal action potential. A representative recording of unit spike raster and LFP trace is shown in Figure 2.7 from 16 electrodes within the left (ipsilateral) and right (contralateral) hippocampus. The basal refers to the baseline activity which was recorded 10 min before saline/drug administration.

2.7.2.2 Normalised mean firing rate analysis

Unit firing rates were analysed based on the spike count per time bin (1 second bin) using *NeuroExplorer* software (firing rate histogram analysis). The total numbers mean of spikes from firing rate analysis was averaged every 1 second throughout 80 or 210 min total time of the experiment (including 10 min basal time) and were exported to Microsoft Excel to pool the unit spike data (Figure 2.8). Normalised mean firing rate graph line was generated using Prism (v6; GraphPad, USA).

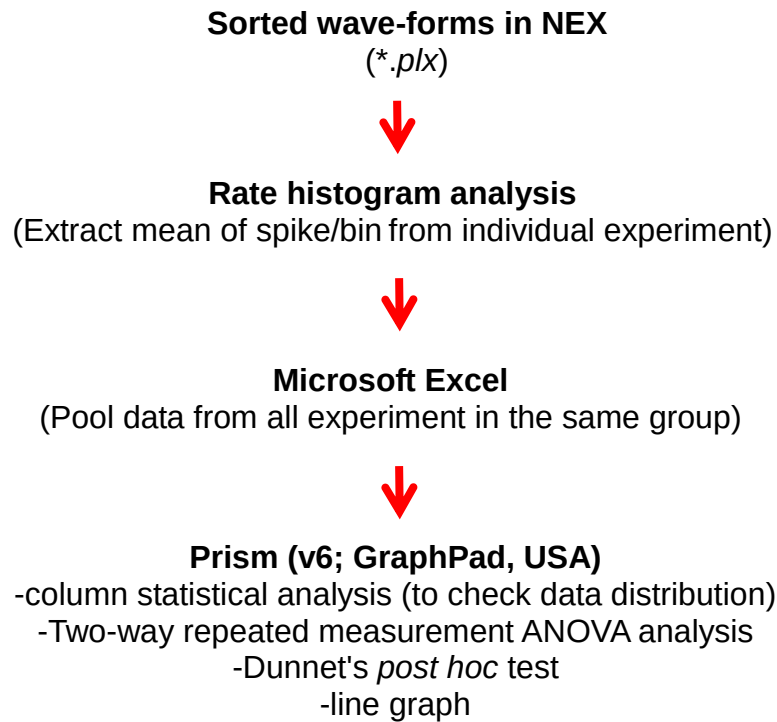


Figure 2.8 Flow of normalised mean firing rate analysis procedure.

Individual mean firing rate was extracted from sorted wave-form data. All mean firing rate data from all experiments from the same group of the study were transferred to Microsoft Excel to normalise the data set to the mean basal (0-600s) firing rate. The data were further analysed in Prism.

2.7.3 MATLAB-based analysis scripts

2.7.3.1 Normalised mean synchrony index (SI)

Neural Population Synchrony Index is a Matlab script developed by M Zachariou and R Mason to measure the synchrony in firing activity between neuronal populations. This analysis allows visualisation of synchrony between neuronal spike trains during an epileptic seizure (Bressloff and Coombes, 2000; Kreuz et al., 2012). Synchronisation and desynchronisation (Jiruska et al., 2013) were analysed based on a scale of 0 (not synchronised) to 1 (fully synchronised)(Bressloff and Coombes, 2000).

In this study, synchronisation was evaluated between ipsilateral-CA1 (**green**), ipsilateral-CA3 (**dark blue**), contralateral-CA1 (**pink**) and contralateral-CA3 (**light blue**) hippocampus, as shown in Figure 2.9. Normalised mean SI of individual experiment was measured in 10 min epoch (red boxes, Figure 2.9); epochs of -10-0 min (basal), 0-10 min, 20-30 min, and 50-60 min for experiment in Chapter 3 and epochs of -10-0 min (basal), 0-10 min, 20-30 min, 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min for experiments in Chapter 4 and 5. The data of each epoch were then normalised by dividing with basal times 1 ($x/\text{basal} \times 1$).

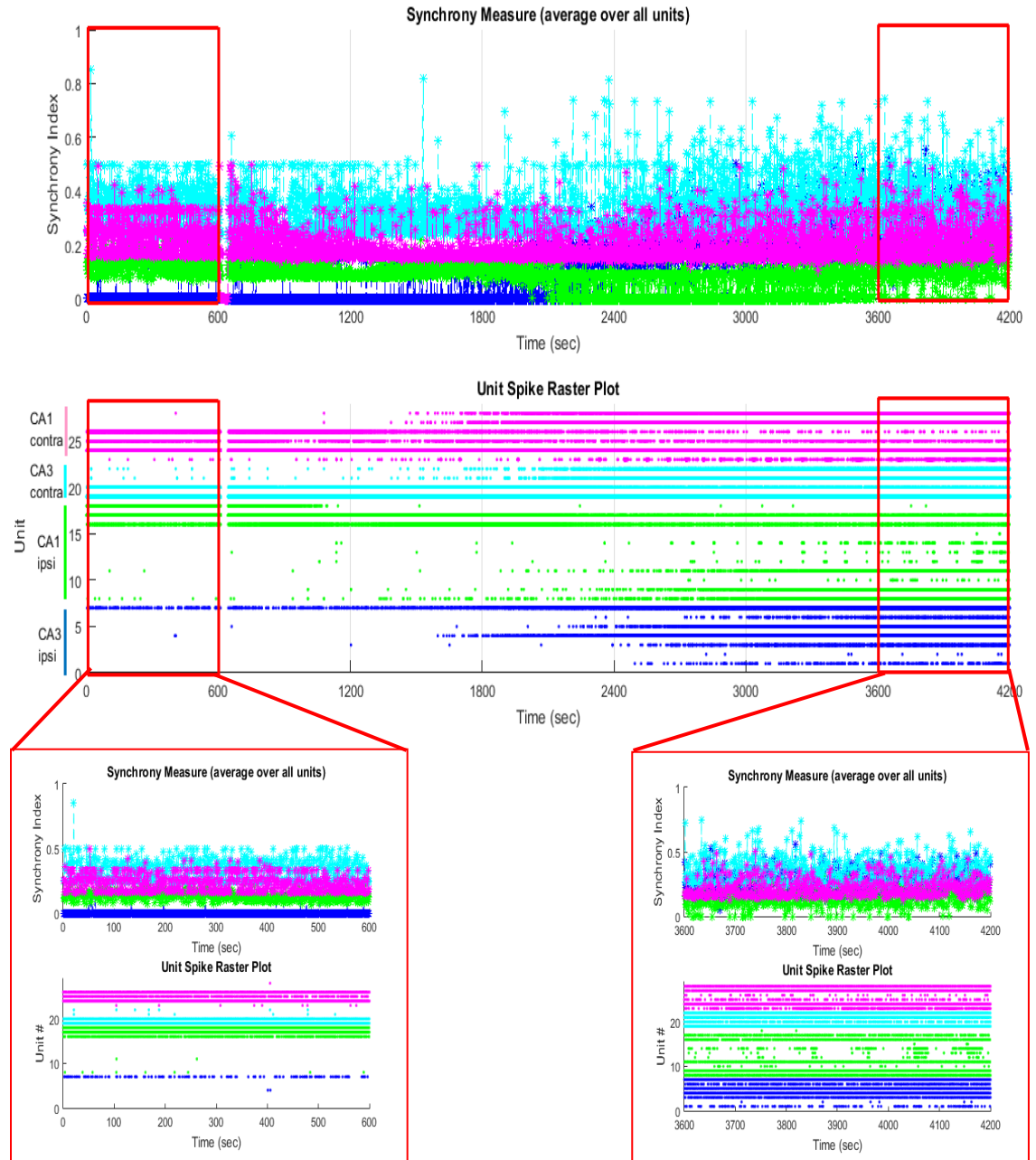


Figure 2.9 Computed SI (upper panel) and unit spike raster plot (lower panel). Computed SI measure over 4200 s, bin size=1 s. 10 min epochs was selected (red boxes). Dual-site recording in left (ipsilateral) hippocampus (units1-8; blue) and right (contralateral) hippocampus (units 9-16; green). Synchrony Index values; 0=no synchrony and 1=100% synchrony.

2.7.3.2 Cross-correlation analysis

Cross correlation analysis is a standard method of estimating the degree to which two cells are correlated in the time domain (Perkel et al., 1967; Gerstein and Perkel, 1972). Essentially a pair of cells (units) was selected, with cell 1 representing the ‘reference cell’. Firing in the second ‘target’ cell is compared with the temporal firing of the ‘reference cell’ (Figure 2.10).

Cross-correlation is generated by lining up the spike trains of ‘reference cell’ and ‘target cell’ (Fenton et al., 2015). The cross-correlation of spikes trains from cell 1 and cell 2 is defined by the number of spikes from cell 2 at time lag relative to spikes (bin width) from cell 1 (Figure 2.10). Peaks in the cross-correlation were defined according to the following criteria (Hilaire et al., 1984):

1. Uncorrelated or independence between the firing of the two cells (i.e., a flat cross-correlogram).
2. Direct dependence (either excitation - a delayed narrow peak count or if inhibited, a trough in the cross-correlogram).
3. Indirect dependence (the receiving common inputs shown by a broad peak overlapping zero time).

Conventional studies consider paired unit cross-correlograms, with a single pair of units. One of the units is taken as a reference and is compared with another unit (Figure 2.10). However, when considering multiple single-unit recording, there are large populations of potential neuronal pairs available for analysis.

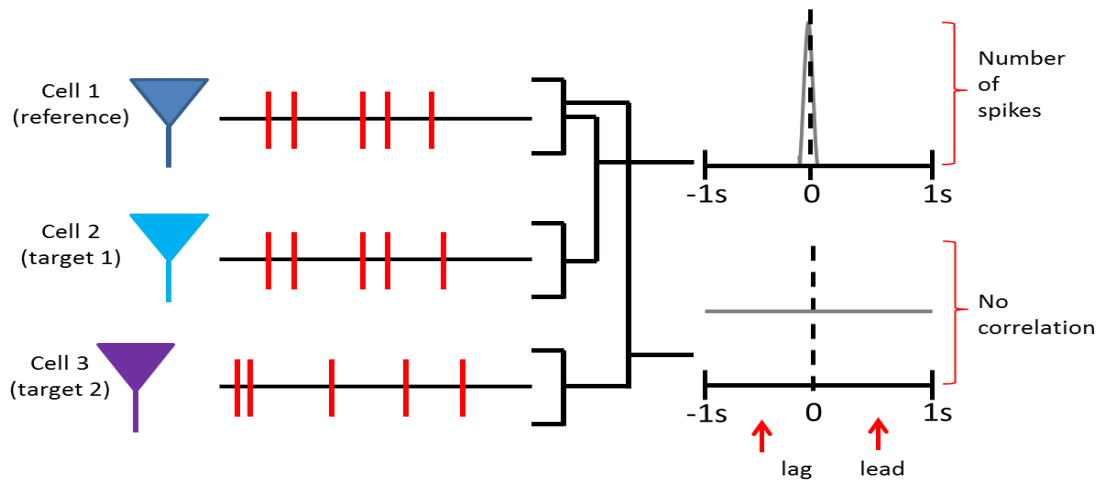


Figure 2.10 The pattern of cross-correlation and interaction between cells. Vertical ticks (in red) represent unit spike raster occurrence of unit action potentials. Cell 1 and cell 2 revealed a narrow peak at 0 seconds, an example of cell excitation and received common input. Cell 1 and cell 3 is not correlated as displayed by a straight grey line. If the peak shift to the right (+ s) of more 0 s indicating that the reference cell leads the target cell whereas if the peak located at left (- s) or less than 0 s, the reference cell lags the target cell.

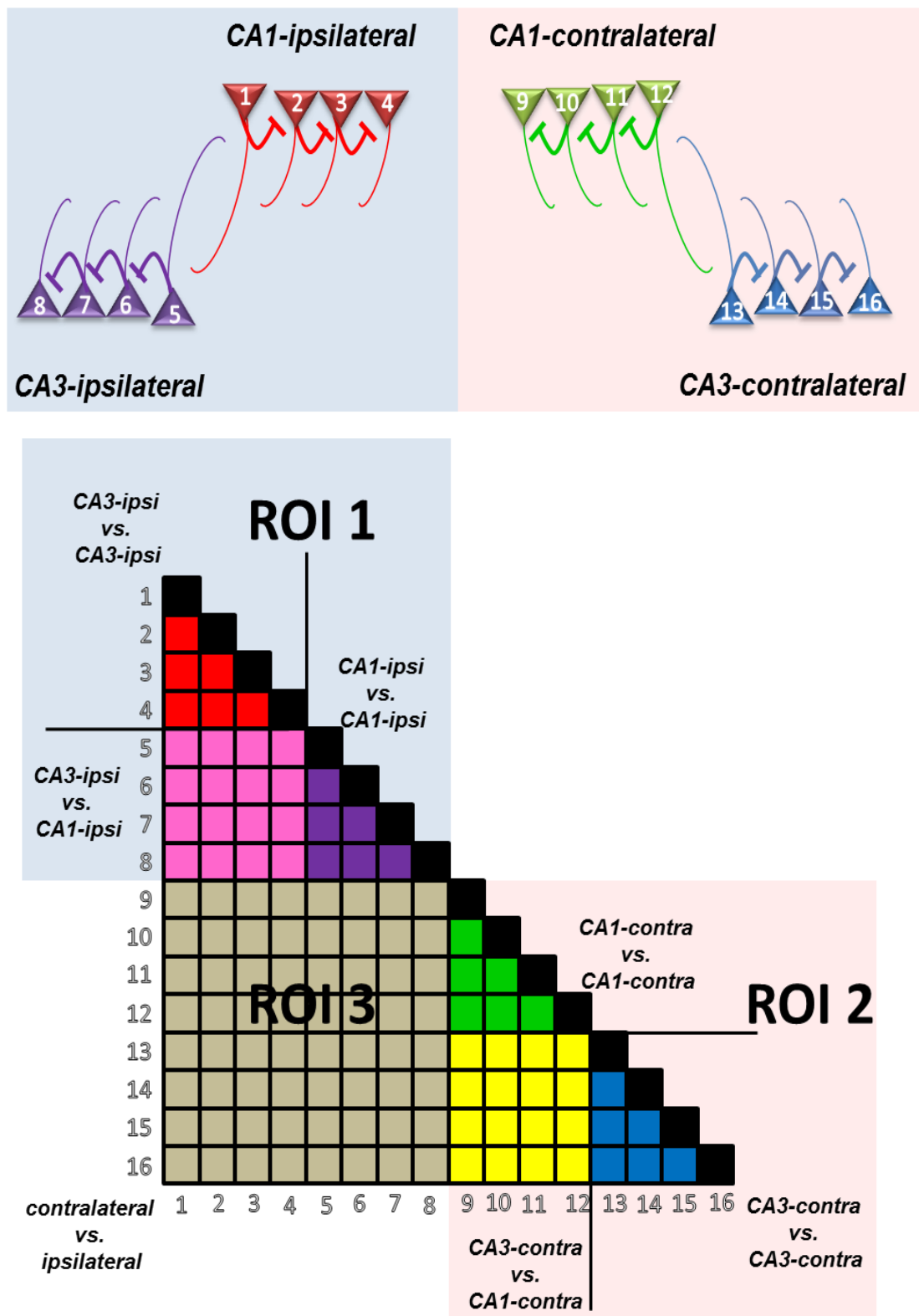


Figure 2.11 Schematic of the cross-correlation Grid/Matrix plot. Y axis shows the reference cell identity and x axis target cell identity. Colour of the grid reveals the intensity of correlation – outlining three user-defined Regions of Interest (ROI 1, 2, and 3) illustrated for dual-site MEA recording in the ipsilateral and contralateral hippocampus.

The grid (Figure 2.11) displays the reference units (y-axis) versus target units (x-axis), and the z-axis colour is proportional to the degree of correlation. Each pixel represents a correlation between pairs of units, with the grid displaying all the neural pair combinations available from the recording electrode array(s). Each individual box indicates the correlation between two neurones and the colour indicates the strength of the correlation; scaled as 0-100% normalised peak (0% refers to little or no correlation activity between any given units pair by a ‘cold’ (blue) colour, whereas higher correlation is shown by a ‘hotter’ (yellow to red) colour in Figure 2.12).

The cross-correlation enables an evaluation of the interaction of the neurones within and between the regions of the brain (ipsilateral and contralateral hippocampus). In addition, by placing the mouse cursor on an individual pixel, a conventional cross-correlation histogram is produced with descriptive statistics for that given unit-pair. The correlated activity was routinely computed in 1 ms bins for a user-defined lag time of ± 1000 ms. The mean $\pm$ SD of uncorrelated activity was computed from the “outer (± 200 ms) uncorrelated shoulders” of the correlogram. The peaks were normalised by count per bin of 10 min (-10-0 min (basal), 0-10 min, 20-30 min and 50-60 min).

The results of cross-correlation grid analysis were only applicable to unit pairs recorded in an individual rat. The correlation strength (mean K-index, Figure 2.13), i.e., peak (or trough) count/arithmetic mean (shoulder) count and the half-peak width (PW_{50})-index were measured as computed correlogram (Fenton et al., 2015). The k-index was extracted from the cross-correlation grid of ROI 1, 2 and 3 from all individual experiments at different epochs (in our case; -10-0 min (basal), 0-10 min, 20-30 min and 50-60 min). All k-indexes were pooled and averaged to get the mean k-index in each experimental group.

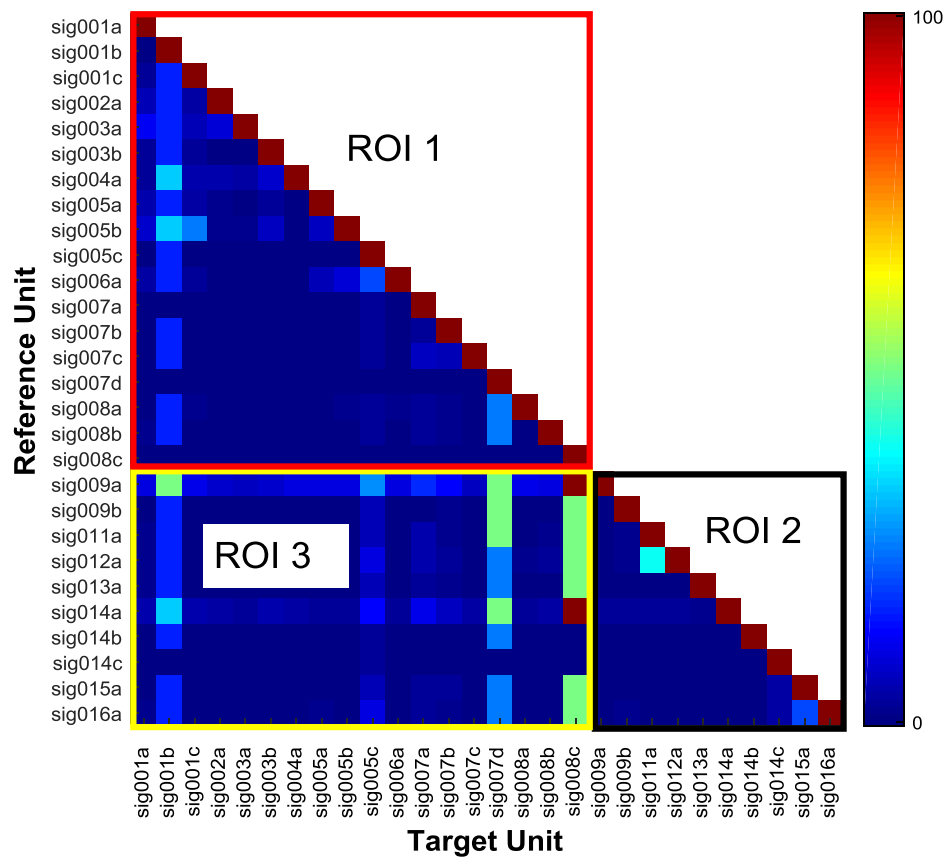


Figure 2.12 An example of cross-correlation grid.

18 neurones signal from the left (ipsilateral; sig001a-sig008c) and 10 neurones were detected from the right (contralateral; sig009a-sig016a) hippocampus, respectively with count per bin in 1 s for 600s period of recording. The colour-coded pixels represent the degree (%) of cross-correlation, ranging from **dark blue** (no correlation - 0%) to **dark red** (100% correlation). The colour grid shows examples of the various patterns of single unit cross-correlation in the population. +/- 1s; bin width 1ms (=1000 bins) and +/- 200 ms “shoulder” specified in computation.

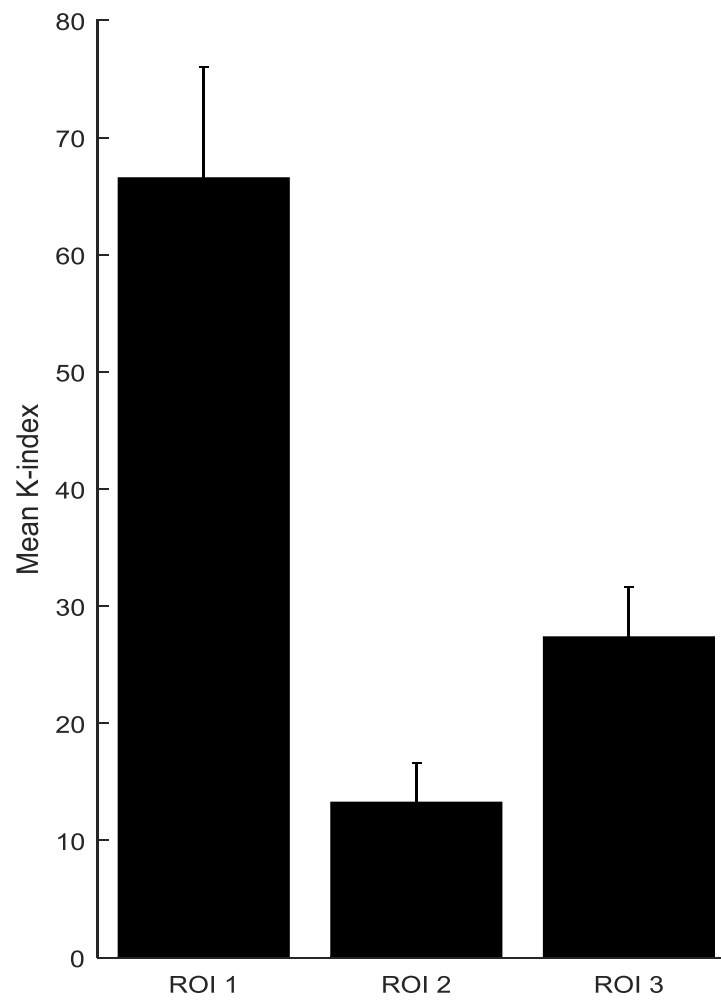


Figure 2.13 Computed indices/parameters for cross-correlation.
Mean K-index plot (mean $\pm$ SEM) for each ROI (represent the strength of correlation) based on the size of the amplitude of paring.

2.7.3.3 LFP frequency bands distribution

The MATLAB software designed for the lab by G. Aldam and R. Peter (LFP analyser 2007), allowed extraction of LFP signals into constituent frequency bands and the proportion of signal power contained within each frequency band to be quantified. LFP (usually signal examined between 1-170/300Hz with A/D sample rate of 1 kHz) reflects the average transmembrane currents of neurones in a volume of few hundred μm radius around electrode tip - negative values correspond to neuronal activation. The frequency band distribution extracted the power (mV^2) and the percentage of LFP signal distribution from the recording area into constituent frequency bands via:

Slow oscillation	<1Hz	"slow"
Delta δ	1-4Hz	"slow-wave complexes"
Theta θ	4-8Hz	"intermediate spindle-waves"
Alpha α	8-14Hz	"intermediate spindle-waves"
Beta β	15-30Hz	"fast"
Gamma γ	30-170Hz	"fast"

The frequency bands were calculated for time epochs of 10 min; epochs of -10-0 min (basal), 0-10 min, 20-30 min, 50-60 min for experiment in Chapter 3 and epochs of -10-0 min (basal), 0-10 min, 20-30 min, 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min for experiments in Chapter 4 and 5 to identify any changes before and after treatment. Values were then pooled, normalised and displayed in histograms.

The analysis of LFP frequency bands distribution is important to provide additional information about the physiology of functional connectivity in the hippocampus.

2.7.4 Statistical analysis

Normalised mean firing rate

Normalised individual data were pooled in Microsoft Excel, and then transferred to Graph- Pad Prism v6. Comparisons were made using two-way ANOVA repeated measures analysis to determine the significant differences in a group of different treatments, time course of the experiment and the interaction between groups of treatment versus time before and after-KA induced neuronal hyperactivity (epileptiform) at a particular brain region (ipsilateral-CA1, ipsilateral-CA3, contralateral-CA1 and contralateral-CA3 hippocampus). Two-way repeated measures were used because the group (brain regions) was subjected to repeated time.

Tukey's multiple comparisons *post hoc* test was done to see which groups of treatments differ from one another. Only the significant group was selected for one-way ANOVA with Dunnett *post hoc* test to determine at what time point the firing rate changes compared to basal. All data points represented the mean and standard error means (S.E.M).

Normalised mean SI

Normalised individual data were pooled in Microsoft Excel, and then transferred to GraphPad Prism v6 for two-way repeated measures to find any significant differences between brain regions versus time. Two-way repeated measures were used because the group (brain regions) was subjected to repeated time. Dunnett's multiple comparisons *post hoc* test was done to determine at what epoch the SI showed any changes before and after treatment compared to basal. All data points represented the mean and standard error means (S.E.M).

Cross-correlation (mean K-index)

Normalised individual data were pooled in Microsoft Excel, and then transferred to GraphPad Prism v6 for ordinary two-way ANOVA analysis to find any significant differences between neurones in region of interest (ROI). An ordinary two-way ANOVA analysis was used because two factors were involved, epoch (min) and ROI which were not repeated. Dunnett's multiple comparisons *post hoc* test was done to

determine at what epoch the K-index showed any changes compared to basal. All data points represented the mean and standard error means (S.E.M).

LFP frequency band

Normalised individual data were pooled in Microsoft excel, and then transferred to Graph- Pad Prism v6 for two-way repeated measures to find any significant interaction between group versus time. Two-way repeated measures were used because the group (brain regions) was subjected to repeated time. Dunnett's *post hoc* multiple comparisons test was conducted to determine at what epoch the normalised mean power changes compared to basal. All data points represented the mean and standard error means (S.E.M).

Chapter 3

KA-INDUCED EPILEPTIFORM-LIKE ACTIVITY IN A RAT MODEL

3.1 Introduction

Kainic acid (KA) is a natural marine acid present in a seaweed, *Digenea simplex* (Ben-Ari, 2004; Sharma et al., 2007; Contractor et al., 2011; Lévesque et al., 2016). It was used originally in the 1960s to eliminate parasitic nematode worms from the gut (Ben-Ari, 2004; Zheng et al., 2011). In 1974, a study by Olney and colleagues discovered that KA is an analogue of excitotoxic glutamate, which can trigger selective neuronal death in the brains of rodents (Ben-Ari, 2004; Zheng et al., 2011). After the study by Olney et al. (1974), KA was used for many years as a tool to destroy neurones. The ion channel formed by KARs is permeable to Na⁺ and K⁺ ions (Dingledine et al., 1999).

Administration of KA has resulted in behavioural changes in rodents, including wet dog shakes and loss of posture control (Ben-Ari, 2012; Kandratavicius et al., 2014; Koshal et al., 2017). Furthermore, rodents also exhibited behavioural changes in the water maze, object exploration tasks, and the open field test, which are related to selective hippocampus damage (Zheng et al., 2011). Other effects observed after KA administration include insufficiency in short term spatial memory and long term spatial learning, anxiety, and depression-like behaviours (Gröticke et al., 2008).

Neuropathological changes in KA-induced seizures in rats include irreversible neuronal degeneration in the CA1 and CA3 regions of the hippocampus and the hilus of the dentate gyrus (Ben-Ari, 1985; Represa et al., 1987; Ben-Ari and Cossart, 2000). Studies in KA-induced epileptiform-like activity in a rat model have shown that administration of KA resulted in seizures (Ahmad et al., 2013; Lévesque and Avoli, 2013; Sairazi et al., 2017). Jarrard (2002) has stated that specifically, focal injection

of KA caused hippocampal damage in the CA3 region, which is associated with the distribution of KARs. KA-induced seizures may be a valuable tool in the assessment of anti-epileptic drug efficacy in complex partial seizures (Nadler, 1981; Pitkänen et al., 2006; Pitkänen et al., 2015).

Systemic KA administration is a well-established animal model used to induce epileptiform activity and SE to mimic systemic or generalised epilepsy (Coomber et al., 2008; Mason and Cheer 2009; Zeng et al., 2009; Sairazi et al., 2015; Bertoglio et al., 2017; Si et al., 2017). Early studies (Nadler, 1981; Ben-Ari 1985) approximately 3 decades ago used KA at a single dose from 12 to 18 mg/kg via the intraperitoneal (i.p) or intravenous route. A study by Hellier et al. (1998) proposed a modified technique using multiple low doses (5 mg/kg i.p of KA) instead of using a single dose which showed about 15% lower mortality rates. 97% of the rats also developed epilepsy from this modified technique. In the KA model, the mortality ranges from 5 to 30%, but it can be decreased with the administration of multiple doses of 5 mg/kg until SE occurs (Lévesque and Avoli, 2013). Meanwhile, Leite et al. (2002) reported that KA doses of 10 to 15 mg/kg (i.p.) were used to produce neuronal hyper-excitability and SE in adult rats. Additionally, a study by Sharma et al. (2007) used a single dose of KA at 9 mg/kg in 7- to 8-week-old Fischer-344 rats. In their study, 95% of the rats survived; approximately 93% of them exhibited SE, and 80% developed spontaneous motor seizures. In another study by Ahmad et al. (2013), KA was usually applied to adult rodents either via i.p or intracerebral with the systemic doses, varying from 8 to 12 mg/kg for rats. Other than that, Lévesque et al. (2016) also agreed that the administration of a single intraperitoneal dose of kainic acid (6–15 mg/kg) in adult animals could trigger SE. Bertoglio et al., (2017) found that Wistar Han rats were more sensitive to KA when compared to the Sprague Dawley rats using the Hellier protocol (initial dose of 5 mg/kg KA subcutaneously). A modified protocol of repeated doses of 2.5 mg/kg KA in Wistar Han rats were subsequently used to improve survival and welfare requirements from local ethical committees.

KA has been widely used to model mTLE (Babb et al., 1995; Raedt et al., 2009; Bragin et al., 1999; Bragin et al., 2009; Karunakaran et al., 2012; Klee et al., 2017; Falcón-Moya et al., 2018). Early studies by Nadler (1981) and Ben-Ari (1985) showed that the CA3 region of the hippocampus is the most susceptible to epileptiform seizures after local injections of KA. Another early study by Cavalheiro et al. (1982) reported that intrahippocampal KA injection induced recurrent limbic seizures, observed through behaviour and EEG in awake rats. Babb et al. (1995) demonstrated that intrahippocampal KA injection produced hippocampus EEG spikes, EEG seizures and behavioural seizures in awake rats. A study by Raedt et al. (2009) stated that intrahippocampal KA injection in awake rats was able to induce SE, HS and spontaneous seizures. Moreover, Rattka et al., (2013) showed that intrahippocampal KA injection into the CA3 of the posterior hippocampus in awake rats has induced limbic SE (ranging from 4 to 20 hours). In addition, Klee et al., (2017) found out that only a few rats developed epilepsy when anaesthesia was used during intrahippocampal KA injection, while frequent electrographic seizures as observed in mice did not occur in rats, irrespective of the location of the kainate injection (CA1 or CA3) or EEG recording electrode (CA1, CA3 or dentate gyrus) or dose of kainate injected. Although these existing studies reported the use of KA injection directly to the hippocampus, a limited number of studies emphasised the use of anaesthetised rats. Thus, our study is another effort to expand the understanding of epilepsy under anaesthesia and to evaluate the characteristics of connectivity during epileptiform-like activity.

Generally, one of the disadvantages of systemic KA (i.p, subcutaneous and intravenous) is the widespread damage to other regions such as the pyriform, entorhinal and other neocortical regions (Schwob et al., 1980). The reason is that these regions have among the lowest thresholds for seizure generation in the rat brain (Ben-Ari, 2012). Morimoto et al. (2004) also mentioned that systemic injection caused a high mortality rate, that the expression of spontaneous seizures can be unpredictable, and that the neural damage can be extensive. On the other hand, KA administration to the specific site of the hippocampus is reported to result in a lower mortality rate (Löscher, 2002), and more discrete morphology alterations, and is largely restricted to the ipsilateral hippocampus (Gröticke et al., 2008). Moreover, a spontaneous recurrent seizure was normally only observed in EEG, without any

noticeable clinical manifestation (Gröticke et al., 2008; Ahmad et al., 2013). Therefore, our study was intended to evaluate the effect of systemic and local intrahippocampal KA injection to induce neuronal epileptiform excitability in anaesthetised rats.

3.1.1 Aims of this chapter

The first aim of this chapter is to compare the changes in normalised mean firing rate from both left (ipsilateral) and right (contralateral) hippocampus in anaesthetised rats between the sham and vehicle groups. The comparisons between the sham and vehicle groups are essential to ensure that the vehicle (saline) does not interfere with the neuronal signal.

The second aim of this chapter is primarily to compare the concentrations of KA (10 mM and 1 mM), to determine the best concentration for inducing hyperexcitable neurones (epileptiform activity) in the ipsilateral hippocampus, and propagating the excitability to the contralateral hippocampus, and to establish the best animal model of mTLE in anaesthetised rats. The comparison between these two concentrations is important to optimise this acute mTLE as a preliminary step prior to further investigation of the AEDs (VPA), cannabinoid compounds (WIN) and benzodiazepines derivatives (FLU), described in Chapter 4 and 5.

The third aim of this chapter is to evaluate the similarities and differences between the systemic epileptic model (KA administered intraperitoneally) and the chosen mTLE model (local intra-hippocampal KA injection). This evaluation will enhance our understanding of acute mTLE in anaesthetised rats.

3.1.2 Hypothesis of this chapter

We hypothesised that; saline as a drug vehicle would not alter the neuronal activity in normal conditions (in this case in anaesthetised rats) and can be used as a drug carrier in later studies. We also hypothesised that 10 mM of KA will cause the death of the rats, whilst a concentration of 1 mM of KA would be effective to generate mTLE and cause the neuronal hyper-excitability to propagate to the contralateral hippocampus. Our third hypothesis was that we would be able to find the similarities and differences between acute mTLE and the systemic epileptic rat model.

3.2 Methods

3.2.1 Experimental procedure

Electrophysiological procedures were performed, as described in Chapter 2. Briefly, male Lister Hooded rats (300-400g; n=25) were anaesthetised with 3.5% isoflurane in medical air (O₂) mixture (reduced to 1.5-2% before recording was initiated). Separate craniotomies were performed, and each of an eight-electrode stainless-steel micro-wire array (NB Labs, Texas, USA) connected to Plexon Multichannel Acquisition Processor system (Plexon Inc. Texas, USA) were placed stereotaxically in the left (ipsilateral) and right (contralateral) dorsal hippocampus (Hpc AP-4.5mm; ML-4.0mm; DV-4.0mm) respectively, according to the coordinates of the rat atlas (Paxinos and Watson, 1998). The dura was retracted, and the exposed cortex was kept moist with saline. Detailed procedures are explained in Chapter 2 (2.2 Surgical Procedure).

3.2.2 Control Studies

The electrodes were allowed to settle for ~60 min (before recording) after being lowered into the dorsal hippocampus (to make sure only stable signals of spikes and LFPs were recorded). Continuous recording was conducted without any vehicle or drug administration for a total period of 80 min in the sham group (Figure 3.1A). Rats in the vehicle group were administered with 0.5 ml saline intravenously (i.v) after 10 min of basal recording (Figure 3.1B). This was followed by intra-hippocampus (local) administration of 1 µl saline after 20 min. The experiment continued with a further 50 min of recording (Figure 3.1B).

3.2.3 Systemic KA administration

Prior to drug administration, basal recordings were obtained for 10 min after the 60 min equilibration period. The rats were administered KA (10 mg/kg, i.p.), and the recording was continued for 50 min after KA injection (Figure 3.2A).

3.2.4 Local KA administration

Saline (0.5 mL) was administered intravenously after 10 min of basal recording followed by 10 mM (Figure 3.2B) or 1 mM of KA (Figure 3.2C) after 20 min. KA (10 mM or 1mM; 1µl respectively) was locally injected in the left hippocampus. The experiment was recorded for a further 50 min. Overall the experiment duration totalled 80 min (including the basal time of 10 min; Figures 3.2B and 3.2C).

3.2.5 Data Analysis

Extracellular unit activity data from animals with confirmed hippocampal electrode placements were sorted into individual neuronal units using *Offline Sorter*, analysed with *NeuroExplorer* and also exported to MATLAB format for further analysis, described in detail in Chapter 2.

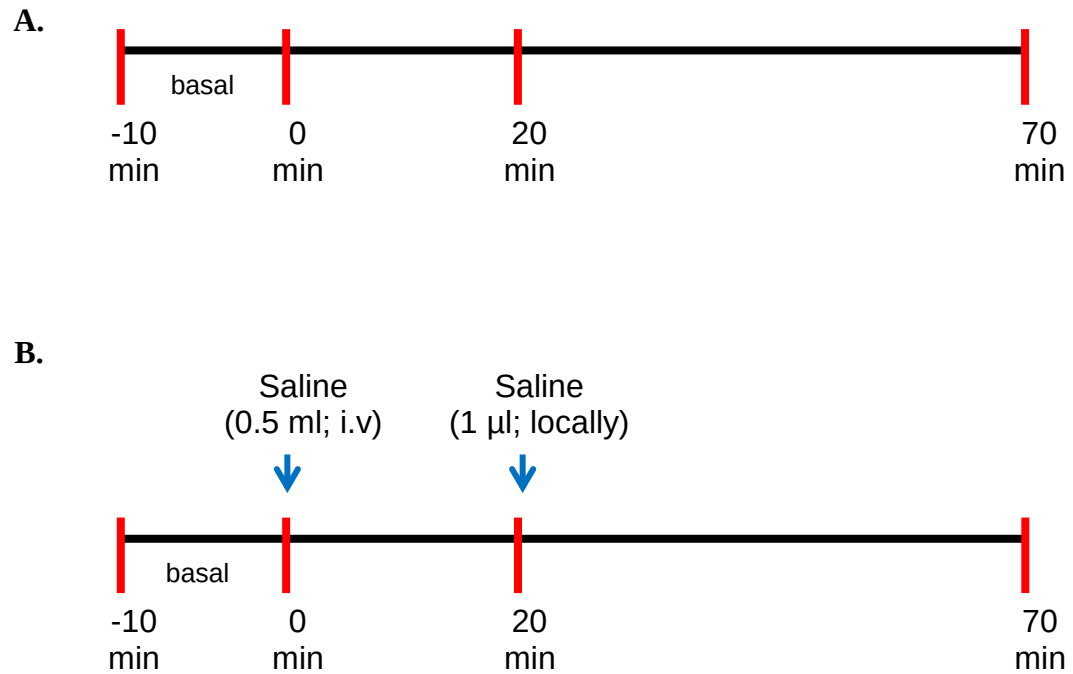


Figure 3.1 Time-line experimental protocols for *in vivo* no-drug control group.

A: Continuous recording (sham group) for a total of 80 min (n=3 rats).

B: Vehicle (saline; 0.5 ml) administered intravenously (i.v) after 10 min basal recording followed by saline (1 µl) administered intra-hippocampus (locally) after 20 min (n=7 rats).

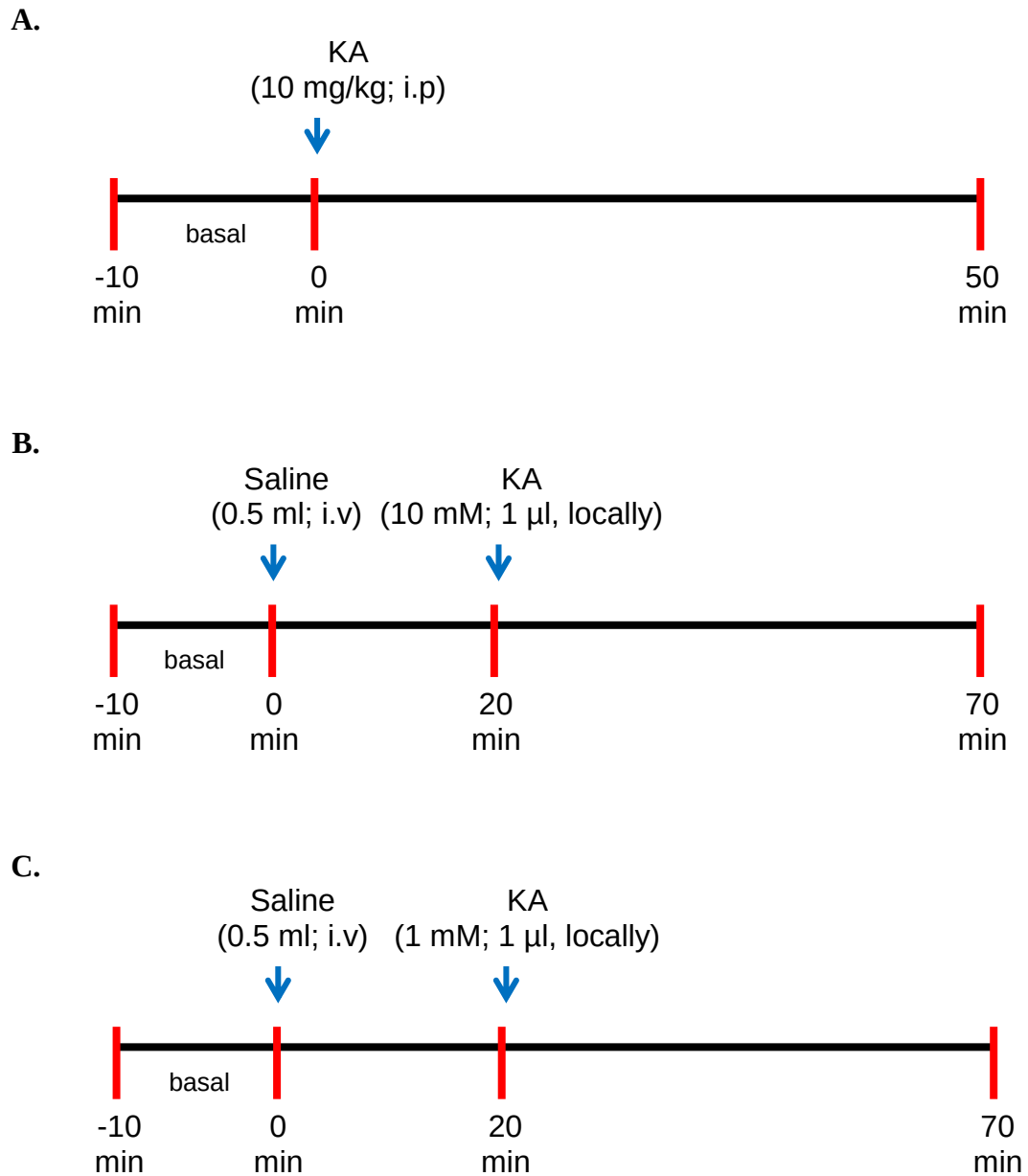


Figure 3.2 Time-line experimental protocols for kainic acid administration.

A: KA administered intraperitoneally after 10 min basal recording to mimic systemic or generalised epilepsy model, n=3 rats.

B: Saline was injected intravenously after 10 min basal recording followed by 10 mM of KA locally after 20 min and recorded for a further 50 min, n=6 rats.

C: Same procedure as **B** but with different concentration of KA (1 mM), n=6 rats.

3.3 Results

3.3.1 Histology

Control studies (continuous recording)

In this study, three rats (rat ID: 20140120, 20140121 and 20140122) were used to record hippocampus activity in both hemispheres without any influence of vehicle or drug. The histology of the rat's brains showed the area of the electrodes recording sites (based on Prussian-blue reaction, as explained under section 2.6 in Chapter 2). Each site was marked as coloured dots (Figure 3.3) where each colour represents one rat used in this experiment.

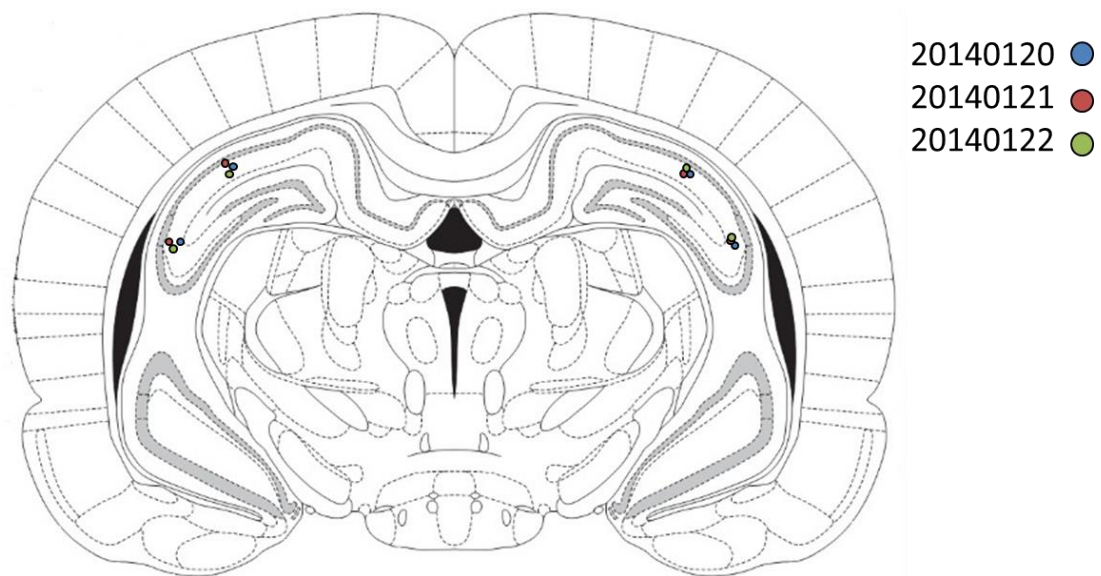


Figure 3.3 Schematic histological verification of hippocampal recording sites for control or sham group (n=3 rats). Each colour represents one rat used in this experiment..

Saline+saline (vehicle group) did not alter the neuronal signal

Seven rats (rat ID: 20130304, 20130305, 20130308, 20130311, 20130313, 20130513 and 20130514) were used to record hippocampus activity in both hemispheres during saline administration in the vehicle and drug groups. This experiment was carried out to show that the vehicle (saline) did not alter the neuronal signal. This information is important to validate that the effect comes from the drug itself and not from the vehicle. Figure 3.4 shows the position of electrode recording sites in each of the 7 rats used, marked as 7 different coloured dots.

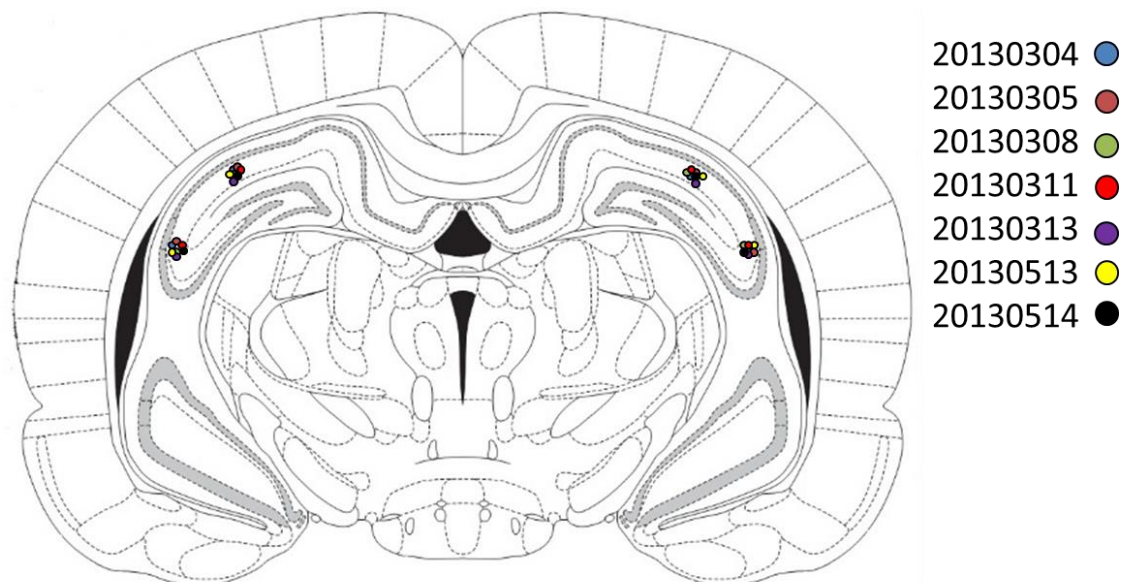


Figure 3.4 Schematic diagram of histological verification of hippocampal recording sites for vehicle group (n=7 rats). Each colour represents one rat used in this experiment.

Systemic (KA i.p) KA-induced epileptiform activity

Three rats were used in 10 mg/kg KA administered intraperitoneally, as shown in Figure 3.5. The position of the recording sites for the electrodes is labelled and marked with different colours representing the three different rats used (20120523, 20120524 and 20120525).

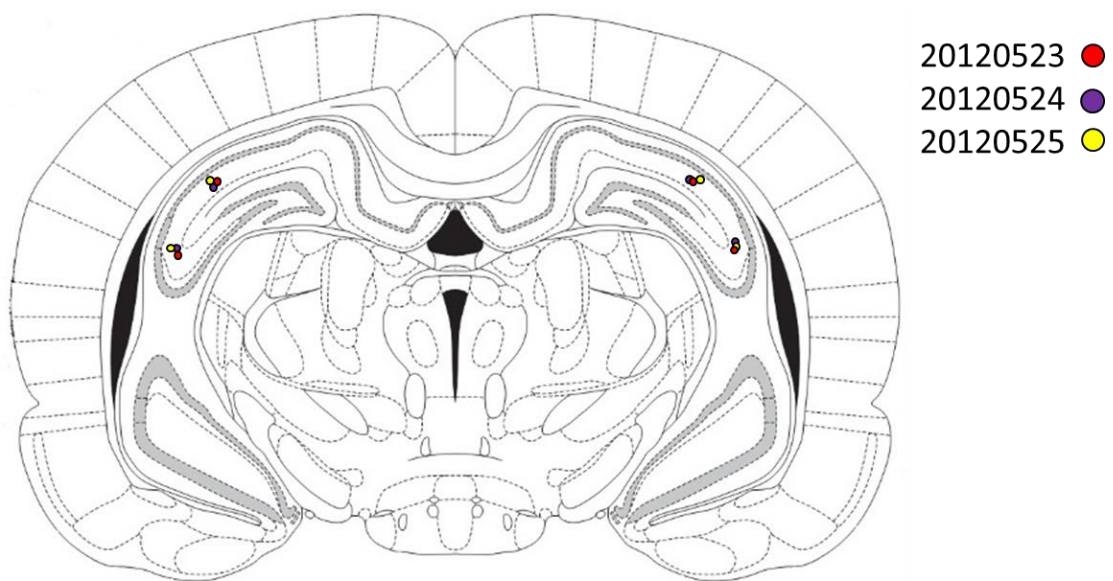


Figure 3.5 Schematic diagram of histological verification of hippocampal recording sites in KA systemic group (n=3 rats). Each colour represent one rat used in this experiment.

KA administered locally (10mM) induced epileptiform-like activity in the hippocampal

Figure 3.6 shows the recording sites for the six rats used in the 10 mM KA locally administered group, marked as 20130107, 20130110, 201330114, 20130117, 20130118 and 20130121 and plotted with 6 different colours.

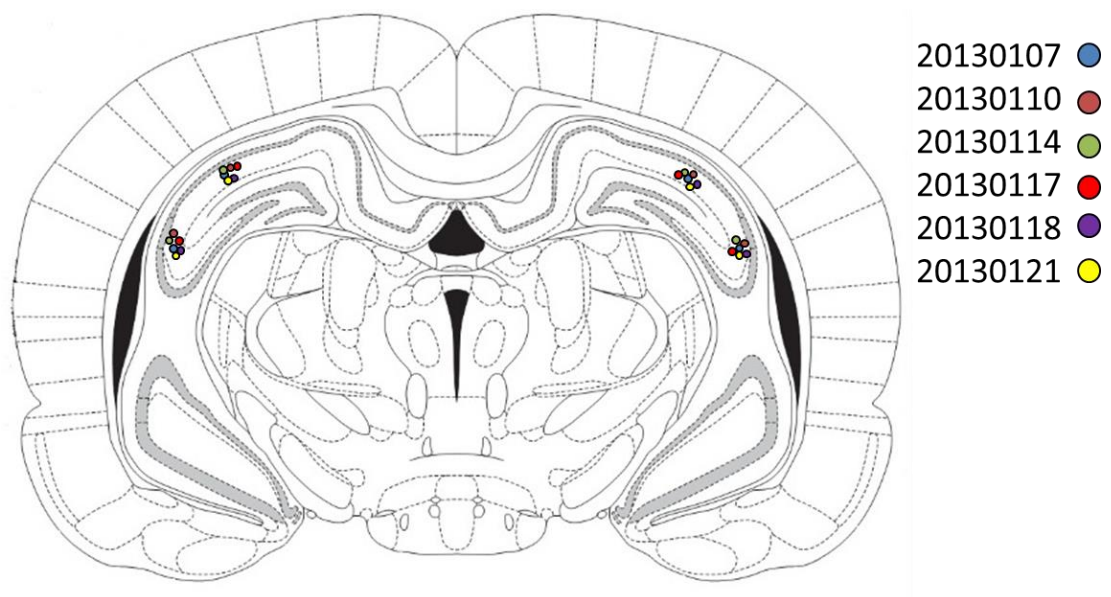


Figure 3.6 Schematic diagram of histological verification of hippocampal recording sites in saline+KA 10 mM group (n=6 rats). Each colour represents one of the six different rats used in this experiment.

KA administered locally (1 mM) induced epileptiform-like activity in the hippocampal

The recording sites for the six rats used in the 1 mM KA administered locally group were labelled as 20130321, 20130325, 20130326, 20130327, 20130516 and 20130517 and are plotted with six different colours in Figure 3.7.

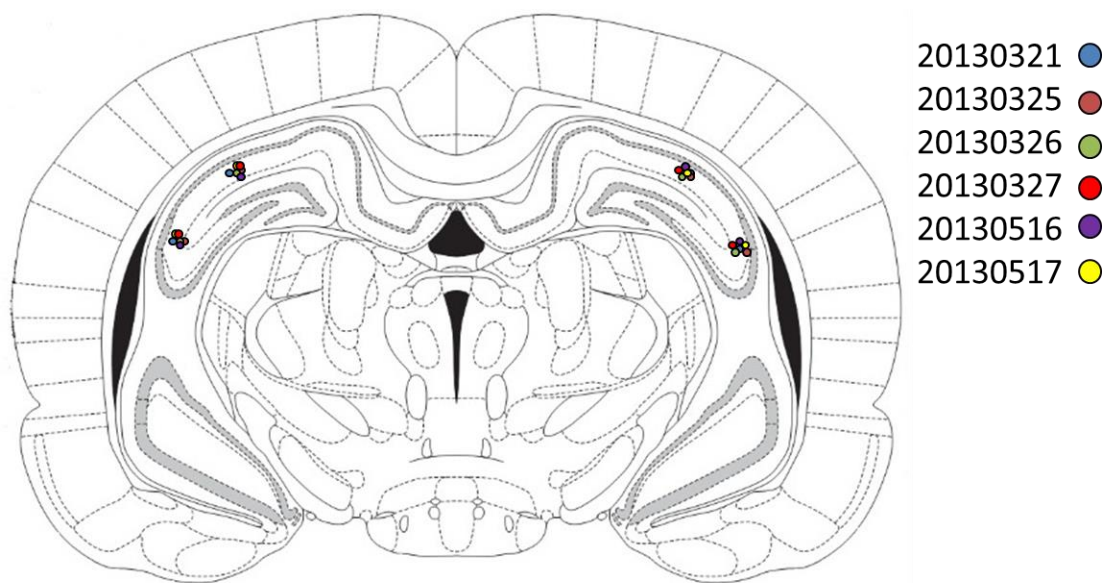


Figure 3.7 Schematic diagram of histological verification of hippocampal recording sites in the saline+KA 1mM group (n=6 rats). Each colour represents one of the six different rats used in this experiment

3.3.2 Normalised Mean Firing rate

The total numbers of spikes per second were applied to all data was segmented into bins of 10 min' duration. The 10 min bins in the 80 min experiment were normalised by dividing the number of spikes in each bin by the number in the first bin (basal).

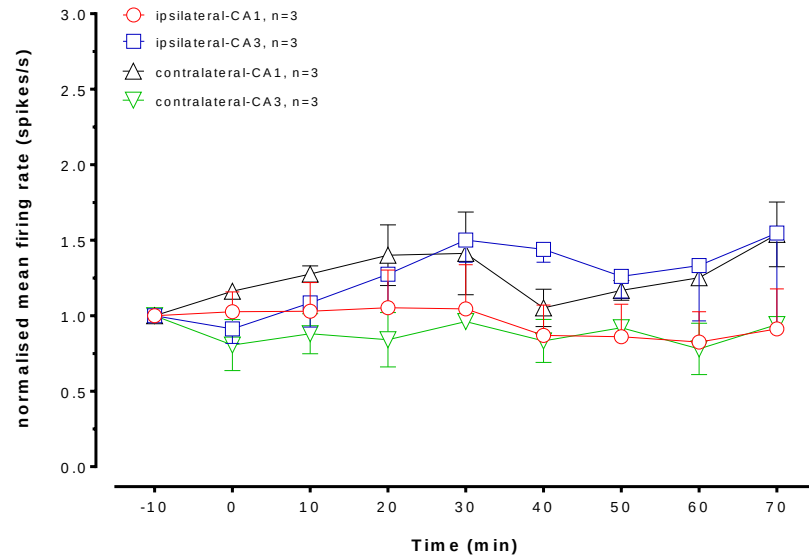
Control studies (continuous recording)

The normalised mean firing rate from all neurones in both left and right hippocampus in the sham group (continuous recording group) showed no significant changes when analysed using two-way-ANOVA repeated measures analysis. There were no significant changes in firing rate to the brain region (Figure 3.8A; $F_{(3, 6)} = 2.053$, $p > 0.05$), time course of the experiment ($F_{(8, 48)} = 0.8716$, $p > 0.05$) or interaction of brain region x time ($F_{(24, 48)} = 0.5488$, $p > 0.05$) between the two hemispheres or within the hippocampus (CA1 and CA3).

Saline+saline group

There were also no significant changes in the vehicle group in the normalised mean firing rate to the brain region ($F_{(3, 20)} = 0.765$, $p > 0.05$), and no interaction of brain region x time ($F_{(24, 160)} = 0.7728$, $p > 0.05$) between hippocampus regions (CA1 and CA3) in both hemispheres after saline was administered intravenously or locally. The only significant changes were found in the normalised mean firing rate according to time ($F_{(8, 160)} = 2.819$, $p < 0.01$). These observations indicate that saline, as a drug vehicle in this study, did not alter the neuronal signal (Figure 3.8B).

A. Continuous recording (no saline/drug)



B. Saline+saline

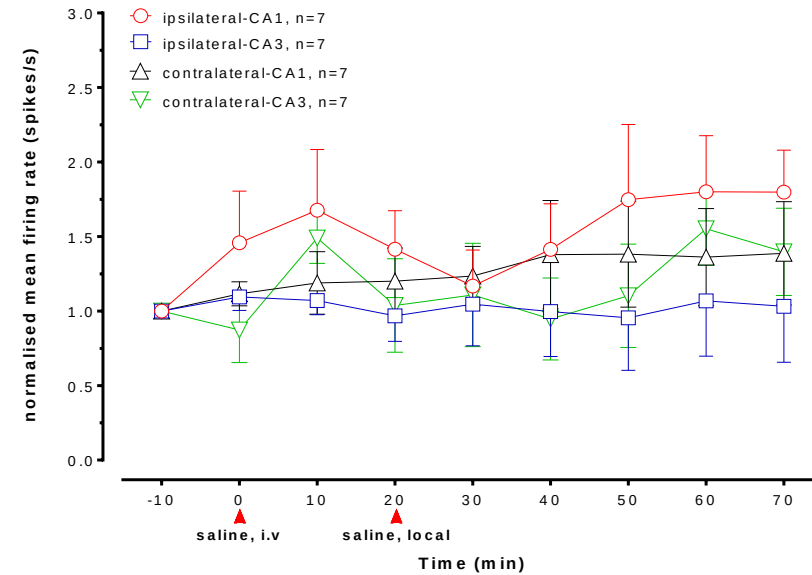


Figure 3.8 Normalised mean firing rate of the sham group and the saline+saline group.

Firing rate in populations of neurones from the left (CA3 and CA1) and right (CA3 and CA1) hippocampus, expressed as normalised mean firing rate (spikes/s $\pm$ SEM).

A: Continuous sham recording (80 min) from both left and right hippocampus with no administration of saline or drug, n=3 rats.

B: Saline vehicle (0.5 ml) was administered intravenously (i.v) after a 10 min basal recording. This was followed by saline (1 μ l) administered intra-hippocampus (locally) after 20 min, and further recording was continued for another 50 min, n=7 rats.

KA (i.p) group

There was a significant effect on the mean firing rate of neurones in brain region x time interaction (Figure 3.9; $F_{(18, 48)} = 2.93, p < 0.005$), time course of the experiment ($F_{(6, 48)} = 30.67, p < 0.0001$) and brain region ($F_{(3, 8)} = 11.04, p < 0.005$). This effect was expected since the rats were injected intraperitoneally with KA. Thus, we can see an increase in the normalised mean firing rate after KA (i.p) administration, indicating seizure in both hippocampi.

Since the main effects were significant, we further analysed the data using *post-hoc* Tukey's multiple comparisons test to determine how the firing rate patterns were different in each brain region. There were significant differences in the mean firing rate in ipsilateral-CA1 compared to ipsilateral-CA3 (^a $p < 0.05$) and contralateral-CA1 hippocampus (^b $p < 0.005$). A significant difference was also found between the firing rate in ipsilateral-CA3 and contralateral-CA1 (^c $p < 0.05$).

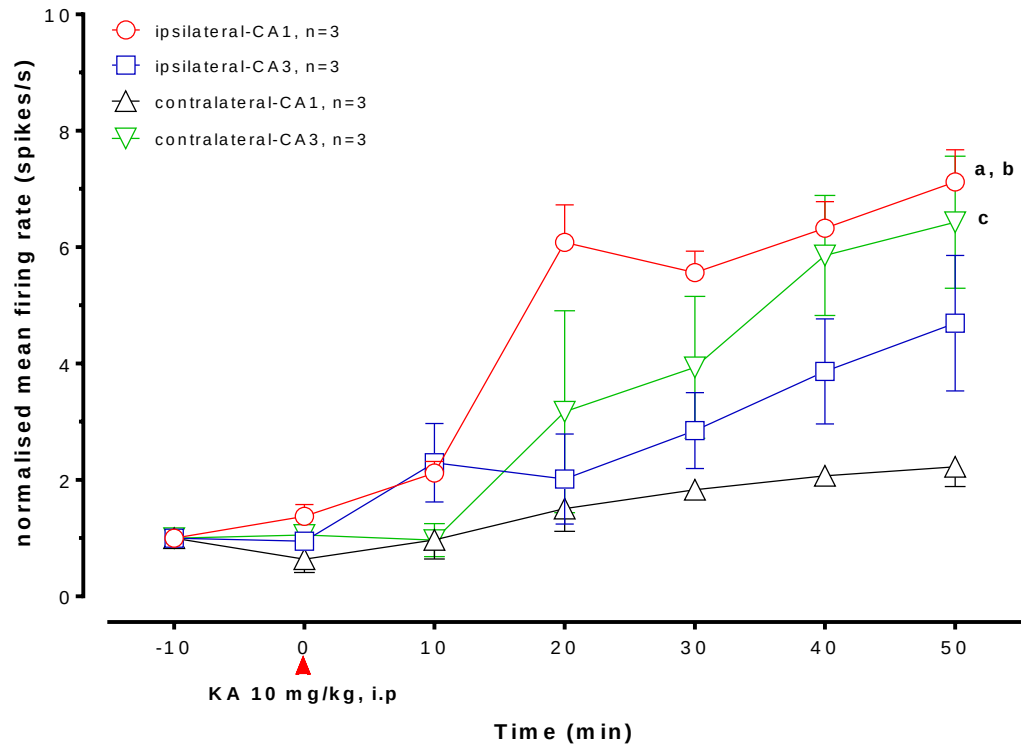


Figure 3.9 Normalised mean firing rate of the KA i.p group. Normalised mean firing rate of neurones from the ipsilateral (CA3 and CA1) and contralateral (CA3 and CA1) hippocampus from KA (10 mg/kg, i.p) experiments (n=3 rats). $F_{(18, 48)} = 2.93$, ^a $p < 0.05$ when compared to ipsilateral-CA3, ^b $p < 0.005$ when compared to contralateral-CA1 and ^c $p < 0.05$ when compared to contralateral-CA1. Two-way ANOVA repeated measures and *post hoc* Tukey's multiple comparisons tests.

We analysed the data using one-way ANOVA and Dunnett *post hoc* tests to determine at which time point the firing rate differs from basal in each brain region in systemic KA experiments (n=3 rats).

In the ipsilateral-CA1 hippocampus, there was a significant effect on firing rate ($F_{(6, 14)} = 41.71, p < 0.0001$). The firing rate was significantly increased after 20 min of KA i.p administration (6.08 spikes/s) until the end of the experiment compared to basal (1.0 spike/s) (Figure 3.10A).

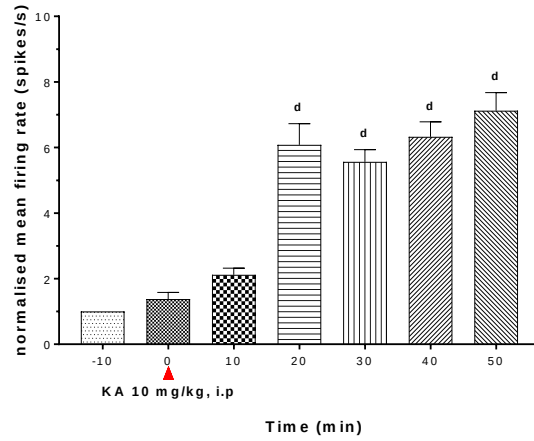
There was also significant effect in firing rate in ipsilateral-CA3 ($F_{(6, 14)} = 3.724, p < 0.05$). The firing rate was only significantly increased after 50 min of systemic KA administration (4.70 spikes/s) compared to basal in ipsilateral-CA3 (Figure 3.10B).

A significant difference was also found in the firing rate in contralateral-CA1 ($F_{(6, 14)} = 5.271, p < 0.005$). The firing rate was only significantly increased after 50 min of systemic KA injection (2.23 spikes/s) compared to basal (Figure 3.10C).

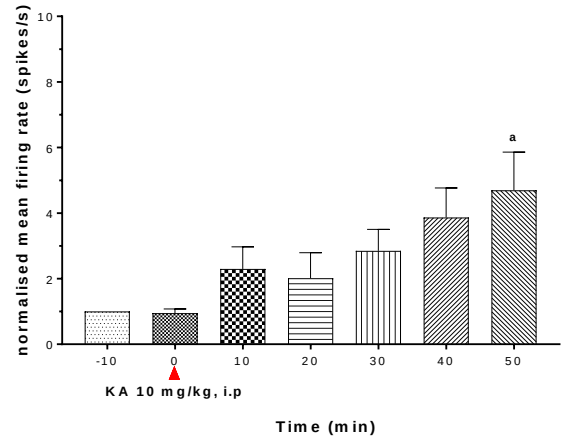
A significant difference was also found in the firing rate in contralateral-CA3 ($F_{(6, 14)} = 5.436, p < 0.005$). The firing rate was also significantly increased after 40 and 50 min of KA (i.p) administration (Figure 3.10D).

This experiment indicates that KA has induced neuronal hyper-excitability in both CA1 and CA3 in left and right hippocampus.

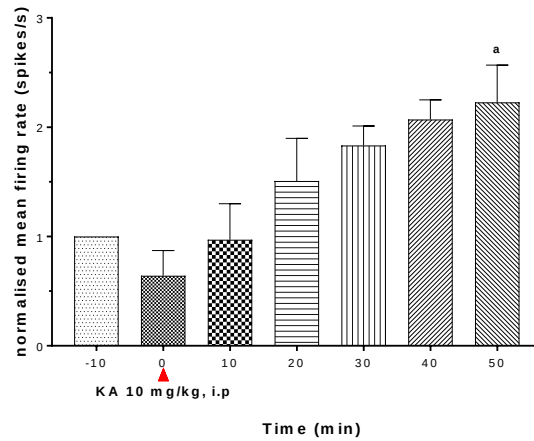
A. Ipsilateral-CA1



B. Ipsilateral-CA3



C. Contralateral-CA1



D. Contralateral-CA3

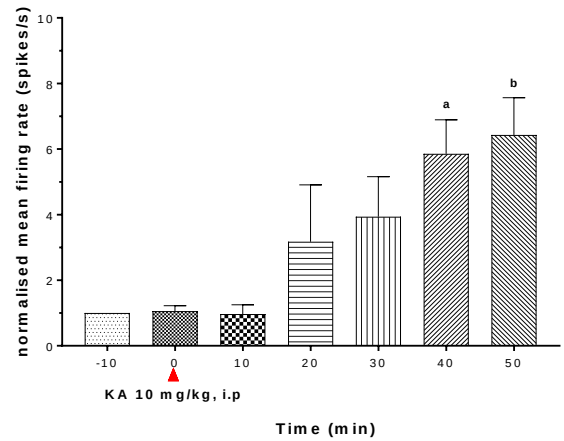


Figure 3.10 Normalised mean firing rate in KA (10 mg/kg, i.p) experiments (n=3 rats).

A: ipsilateral-CA1,

B: ipsilateral-CA3

C: contralateral-CA1

D: contralateral-CA3

^a $p < 0.05$, ^b $p < 0.005$ and ^d $p < 0.0001$ when compared to basal, respectively. (One-way ANOVA and *post hoc* Dunnett's multiple comparisons tests).

Saline+KA 10 mM group

The normalised mean firing rate of saline+10 mM KA experiments (n=6 rats) were significantly different between brain regions ($F_{(3, 19)} = 77.40$, $p < 0.0001$), time courses of the experiment ($F_{(8, 152)} = 123.1$, $p < 0.0001$) and brain regions x time interaction (Figure 3.11; $F_{(24, 152)} = 34.72$, $p < 0.0001$) (two-way ANOVA repeated measures).

Comparison using Tukey's *post hoc* test between the firing rate in the brain regions revealed that the mean firing rate in contralateral-CA1 was significantly different from that in ipsilateral-CA1 and contralateral-CA3 ($^a p < 0.0001$). The mean firing rate in contralateral-CA1 was also significantly different when compared to ipsilateral-CA3 ($^{aa} p < 0.005$).

The mean firing rate in ipsilateral-CA1 was significantly different when compared to ipsilateral-CA3 and contralateral-CA1 ($^b p < 0.0001$). In addition, significant differences were also found in the mean firing in ipsilateral-CA1 when compared to contralateral-CA1 ($^{bb} p < 0.0001$).

Apart from that, the mean firing rate in contralateral-CA3 was significantly different from that in ipsilateral-CA1 and contralateral-CA1 ($^c p < 0.0001$) and ipsilateral-CA3 ($^{cc} p < 0.05$).

The increased firing rate after 10 mM KA local administration, as shown in Figure 3.11, indicates that there was an epileptiform-like activity which propagated in all hippocampus regions except ipsilateral-CA3. Overall, there was an interaction between brain region x time during a seizure in both hippocampi.

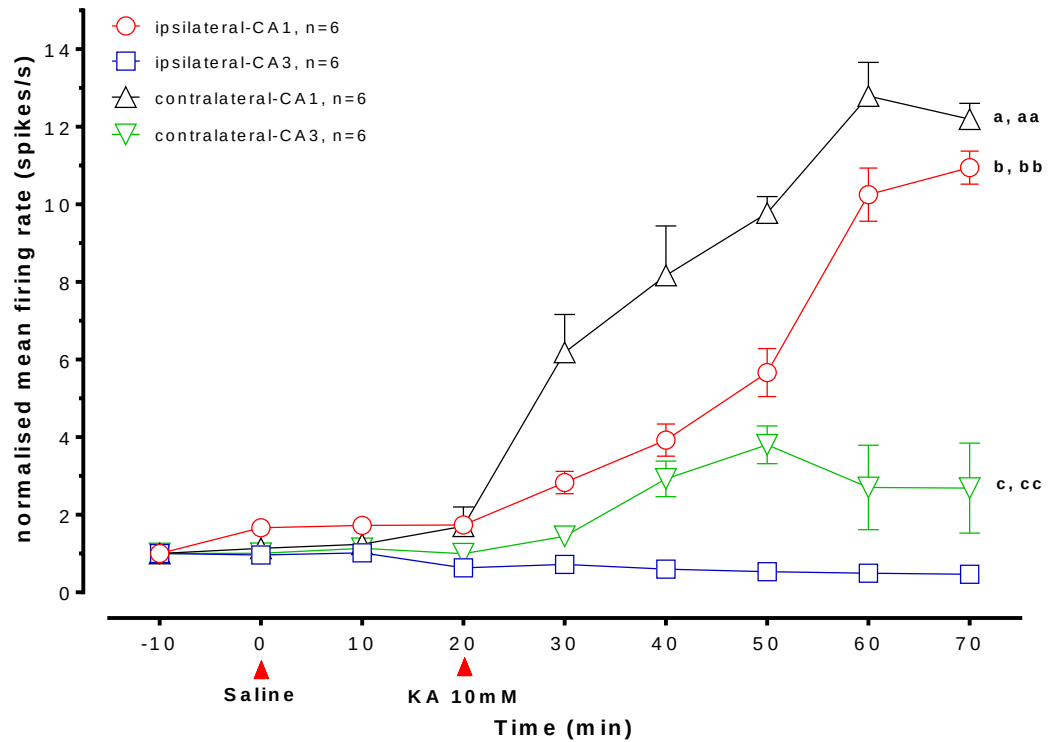


Figure 3.11 Normalised mean firing rate of saline+KA 10 mM group.

Normalised mean firing rate of neurones from the ipsilateral (CA3 and CA1) and contralateral (CA3 and CA1) hippocampus from KA (10 mM local) experiments (n=6 rats). $F_{(24, 152)} = 34.72$, $p < 0.0001$ (Two-way ANOVA repeated measures).

^a $p < 0.0001$ when compared to ipsilateral-CA1 and contralateral-CA3, ^{aa} $p < 0.005$ when compared to ipsilateral-CA3, ^b $p < 0.0001$ when compared to ipsilateral-CA3 and contralateral-CA1, ^{bb} $p < 0.0001$ when compared to contralateral-CA1, ^c $p < 0.0001$ when compared to ipsilateral-CA1 and contralateral-CA1 and ^{cc} $p < 0.05$ when compared to ipsilateral-CA3 (*post hoc* Tukey's multiple comparisons test).

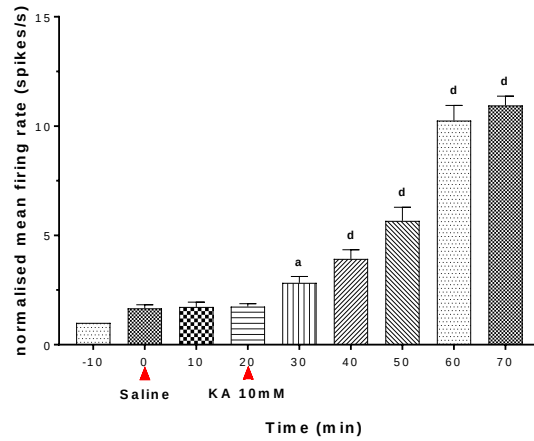
One-way ANOVA and Dunnett *post hoc* tests were carried out to examine at which time point the firing rate differs from basal in each brain region in KA 10 mM experiments (n=6 rats).

In ipsilateral-CA1, normalised mean firing rate was found to be significantly different ($F_{(8, 45)} = 92.30, p < 0.0001$). The mean firing rate was significantly increased (2.83 ± 0.29 spikes/s) after 10 min ($^a p < 0.05$) and 20-50 min ($^d p < 0.0001$) of KA administration compared to basal (-10 min, Figure 3.12A). No significant difference was found in ipsilateral-CA3 (data not shown).

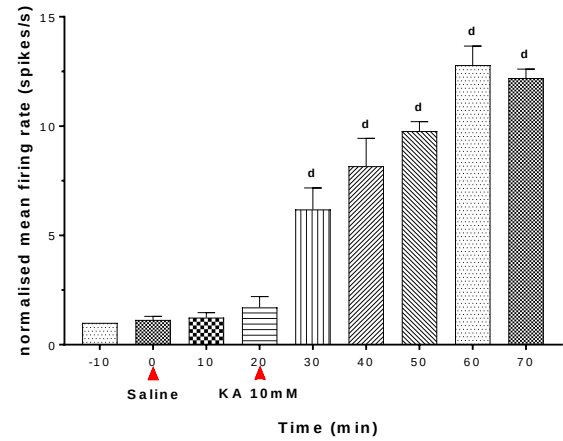
There was a significant difference in normalised mean firing rate in contralateral-CA1 ($F_{(8, 36)} = 54.11, p < 0.0001$). Normalised mean firing rate in contralateral-CA1 was significantly increased ($^d p < 0.0001$) after 10 min (6.19 ± 0.98 spikes/s) until the end of the experiment in local KA 10 mM administration when compared to basal (Figure 3.12B).

There was a significant difference in normalised mean firing rate in the contralateral-CA3 hippocampus ($F_{(8, 45)} = 3.347, p < 0.005$). As shown in Figure 3.12C, there was a significant difference ($^b p < 0.005$) after 30 min of KA administration (3.80 ± 0.5 spikes/s) when compared to basal (-10 min).

A. Ipsilateral-CA1



B. Contralateral-CA1



C. Contralateral-CA3

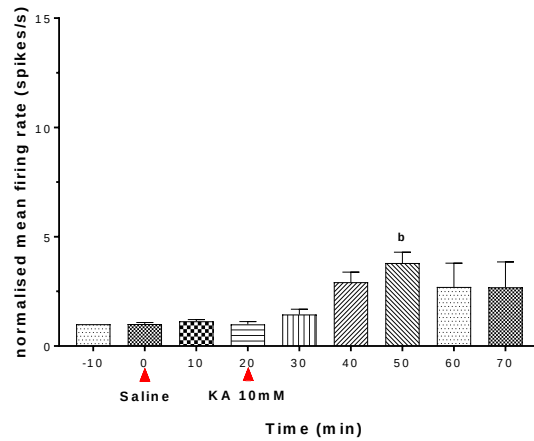


Figure 3.12 Normalised mean firing rate of A) ipsilateral-CA1, B) contralateral-CA1 and C) contralateral-CA3 from KA 10 mM local administration experiments (n=6 rats).
^a $p < 0.05$, ^b $p < 0.005$ and ^d $p < 0.0001$ when compared to basal, respectively.
 One-way ANOVA and *post hoc* Dunnett's multiple comparisons tests.

Saline+KA 1 mM group

There was a significant effect on the normalised mean firing rate to the brain region ($F_{(3, 18)} = 22.54$, $p < 0.0001$), time course of the experiment ($F_{(8, 144)} = 45.25$, $p < 0.0001$) and interaction between brain region and time ($F_{(24, 144)} = 29.06$, $p < 0.0001$) in saline+KA 1 mM experiment (n=6 rats; Figure 3.13).

Tukey's multiple comparisons test was conducted to determine which group was significantly different from the others. The normalised mean firing rate of neurones in ipsilateral-CA1 was significantly different ($^a p < 0.0001$) compared to all other groups. Meanwhile, the normalised mean firing rate in contralateral-CA3 was significantly different ($^b p < 0.001$) when compared to ipsilateral-CA3 and contralateral-CA1 (Figure 3.13).

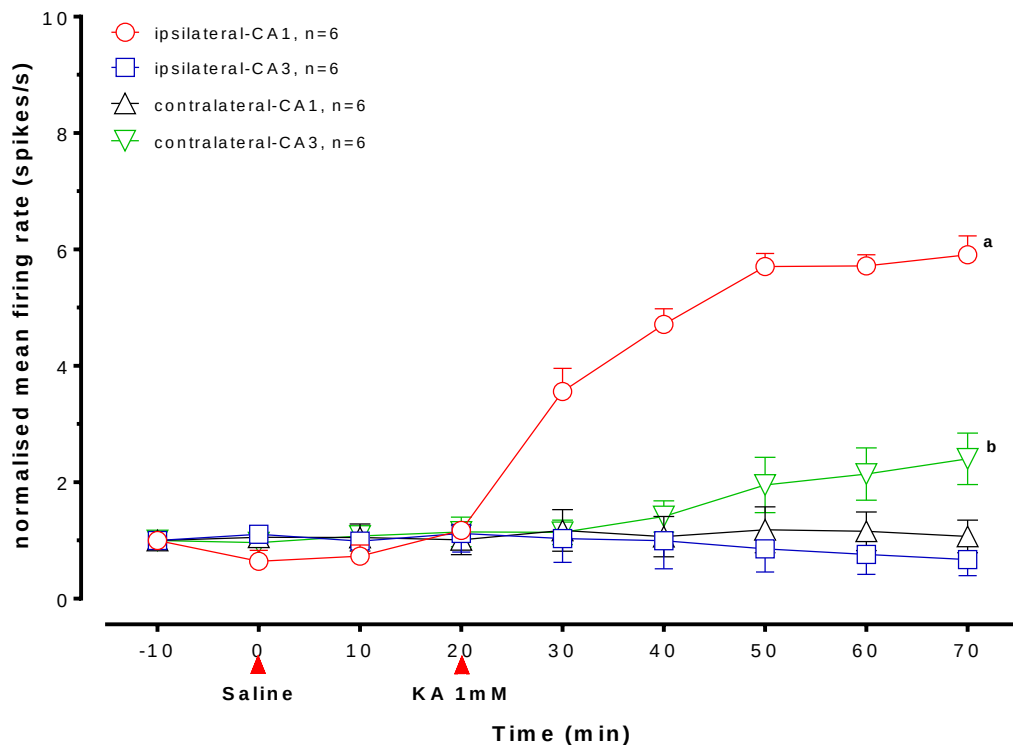


Figure 3.13 Normalised mean firing rate of saline+KA 1 mM group.

Normalised mean firing rate of neurones from the ipsilateral (CA3 and CA1) and contralateral (CA3 and CA1) hippocampus from KA (1 mM local) experiments (n=6 rats). $F_{(24, 144)} = 29.06$, $^a p < 0.0001$ when compared to all groups and $^b p < 0.001$ when compared to ipsilateral-CA3 and contralateral-CA1. Two-way ANOVA repeated measures and *post hoc* Tukey's multiple comparisons tests.

There was a significant effect of normalised mean firing rate in ipsilateral-CA1 when compared to basal (one-way ANOVA, $F_{(8, 36)} = 99.23$, $p < 0.0001$). The normalised mean firing rate in ipsilateral-CA1 was significantly increased ($^d p < 0.0001$) after 10 min (3.56 ± 0.40 spikes/s) of local KA 1 mM administration compared to basal (1 spike/s at -10 min, Figure 3.14A).

On the other hand, the normalised mean firing rate in contralateral-CA3 was significantly increased ($^a p < 0.005$) after 50 min of local KA 1 mM administration (2.40 ± 0.44 spikes/s, Figure 3.14B) when compared to basal. In contrast, no significant changes in firing rate were found in ipsilateral-CA3 and contralateral-CA1 after 1 mM KA administration compared to basal recordings (data not shown).

This pattern of neuronal firing in ipsilateral-CA1 shows that epileptiform-like activity occurred after local KA injection in the left intra-hippocampus. The epileptiform effect also can be observed in the contralateral-CA3 hippocampus, which indicates that the seizure spread to the other side of the brain region.

A. Ipsilateral-CA1

B. Contralateral-CA3

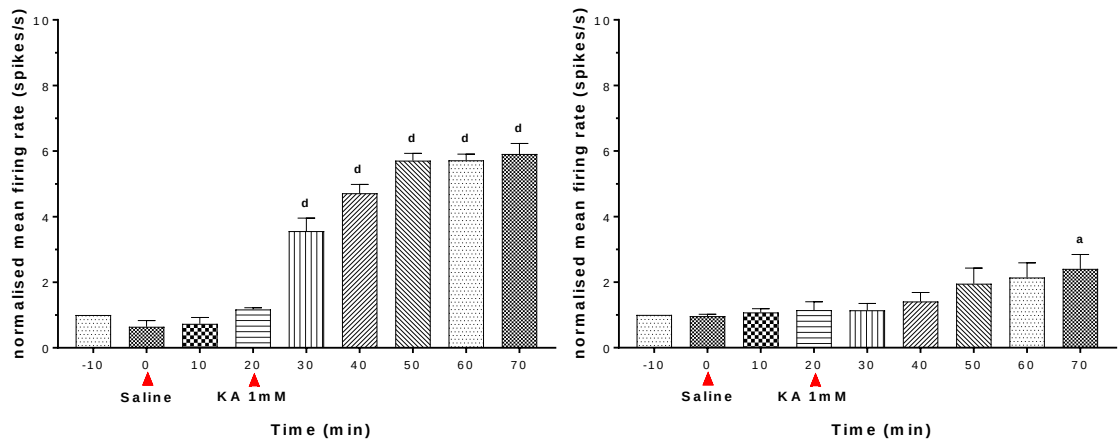


Figure 3.14 Normalised mean firing rate of A) ipsilateral-CA1 and B) contralateral-CA3 from local KA 1 mM administration experiments (n=6 rats).

$^a p < 0.05$ and $^d p < 0.0001$ when compared to basal, respectively.

One-way ANOVA and *post hoc* Dunnett's multiple comparisons tests.

KA (1 mM, local) administered after saline (0.5 mL, i.v) was chosen as a positive control group to be compared to AED treatment group.

In the previous section, experiments using KA 1 mM showed a significant increase in firing rate (Figure 3.13 and 3.14); therefore, only this group was considered for further analysis. The increases of normalised mean firing rate in KA 1mM (Figure 3.13 and 3.14) in ipsilateral-CA1 and contralateral-CA3 hippocampus was also in parallel with increases in normalised mean firing rate in the KA 10 mM group (Figure 3.11 and 3.12). Based on our experimental manipulations, the lower KA concentration of 1 mM effectively induced a seizure, eliminating the need to use a higher dose of KA, up to 10 mM concentration.

Since KA 1 mM administration was chosen as a model, we did not use rats administered with KA 10 mM for further analyses (synchrony index, unit pair cross-correlation or LFP frequency band distribution). Experiments using KA 10 mM were also not used in the subsequent experimental manipulations using VPA, WIN and FLU described later in this study (Chapter 4 and 5) because the rats died no longer than 2 hours after recording, preventing us from proceeding with a longer recording of 3.5 hours. Hence, we selected KA 1 mM based on the importance of avoiding the death of the rat throughout the experiment. The KA 1 mM local model was also selected on the basis of improving survival and welfare to comply with the requirements of local ethical committees. We wanted to optimise the efficiency of the epilepsy outcome and provide an excellent survival rate at the same time.

3.3.2 Normalised mean synchrony index

Normalised mean SI estimates the synchrony in firing rates for an individual population (e.g., CA1 and CA3) over a set epoch (e.g., 1 s) between ipsilateral (CA1 and CA3) and contralateral (CA1 and CA3) hippocampus, scaled as 0 (not synchronised) to 1 (fully synchronised). Normalised mean SI was measured in four 10 min epochs time (-10-0 min (basal), 0-10 min, 20-30 min and 50-60 min).

The normalised mean SI of the vehicle control group showed no significant changes before and after saline administration either intravenously or locally (two-way ANOVA repeated measures; Figure 3.15A).

KA i.p shows significant changes in SI in all brain regions after KA injection

The normalised mean SI was significantly different in the interaction between time and brain region ($F_{(9, 24)} = 3.53, p < 0.01$) for the KA systemic group. The normalised mean SI was significantly increased in ipsilateral-CA1 at the epoch of 20-30 min (2.68 ± 0.22 ; $^c p < 0.001$) and at the epoch of 50-60 min (3.88 ± 0.08 ; $^d p < 0.0001$), significantly increased in ipsilateral-CA3 at the epoch of 50-60 min (2.11 ± 0.15 ; $^b p < 0.005$), significantly increased in contralateral-CA1 at the epoch of 50-60 min (2.02 ± 0.49 ; $^a p < 0.05$) and significantly increased in contralateral-CA3 at the epoch of 50-60 min (1.99 ± 0.48 ; $^a p < 0.05$) compared to basal recordings (Figure 3.15B; Dunnett's *post hoc* test).

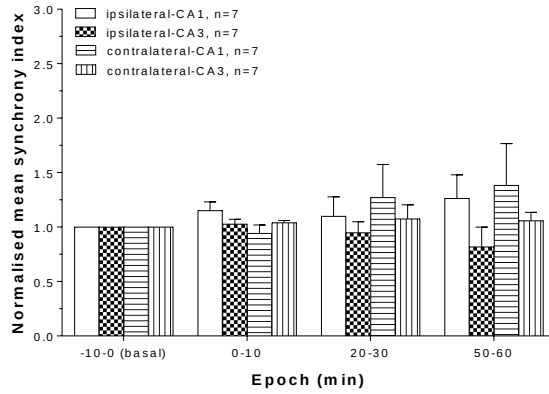
Significant changes of SI were found in ipsilateral-CA1 after KA (1 mM, local) administration

As a comparison, there was a significant difference in the interaction between time and brain region ($F_{(9, 51)} = 4.62, p < 0.005$) in the saline + KA 1mM group (Figure 3.15C). For Dunnett's *post hoc* test, mean SI was significantly increased in ipsilateral-CA1 at the epoch of 50-60 min (2.00 ± 0.20 ; $^c p < 0.001$).

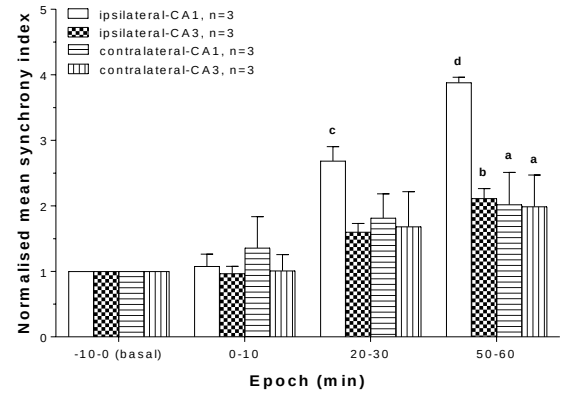
Overall, the systemic KA model shows a significant increase in SI in all brain regions after KA injection when compared to basal. This result is in parallel with the increase in normalised mean firing rate (Figure 3.9 and 3.10), indicating that the synaptic network of firing plays a role in synchronisation during seizures.

Significant changes in SI were found in the ipsilateral-CA1 after KA (1 mM, local) administration, which is also in parallel with the increased normalised mean firing rate (Figure 3.13 and 3.14). This finding indicates that repetitive firing and synchronisation are generated by a subpopulation of neurones (in this case, ipsilateral-CA1 hippocampus).

A. Saline+saline



B. KA i.p



C. Saline+KA 1 mM

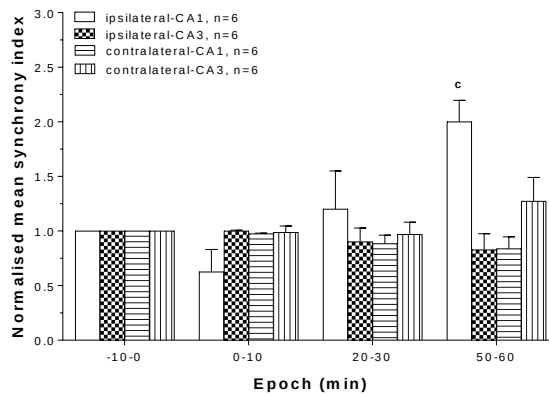


Figure 3.15 Normalised mean SI of saline+saline, KA i.p and saline+KA 1 mM groups.

The data were collected from neurones from the ipsilateral-CA1, ipsilateral-CA3, contralateral-CA1 and contralateral-CA3 hippocampus, (mean $\pm$ SEM).

A: Mean SI of vehicle group, n=7 rats.

B: Mean SI of KA administered intraperitoneally (10 mg/kg) group, n=3 rats.

C: Mean SI of saline (administered intravenously) followed by KA (1 mM, locally) administration, n=6 rats.

^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal (-10-0 min), respectively.

Two-way ANOVA repeated measures and *post hoc* Dunnett's multiple comparisons tests.

3.3.4 Unit-pair cross-correlation (K-index)

Neurones did not correlate in the vehicle group experiment

The mean k-index also showed that there was no significant change in the interaction between neurones in ROI 1, ROI 2 and ROI 3 compared to basal ($F_{(6, 2916)} = 0.5872$, $p > 0.05$; Figure 3.16).

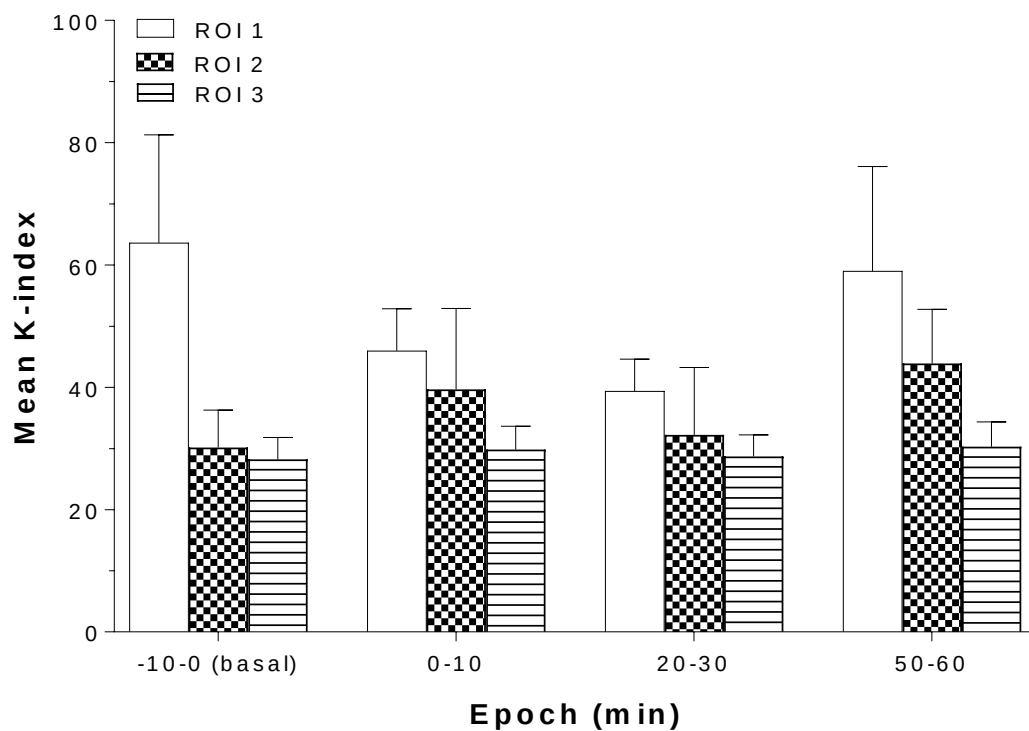


Figure 3.16 Normalised mean K-index of saline+saline group.

Mean k-index for saline administered intravenously after 10 min basal recording followed by saline locally administered after 20 min. Recording was continued for s further 40 min, mean $\pm$ SEM, n=7 rats.

No statistical significance.

(ROI 1= ipsilateral CA1 and CA3; ROI 2= contralateral CA1 and CA3; ROI 3=neurones in both ipsilateral and contralateral hippocampus)

KA i.p decreased correlation between neurones in contralateral (ROI 2) and between neurones in ipsilateral and contralateral (ROI 3)

Mean k-index shows significant differences in the interaction between neurones ($F_{(6, 3624)} = 3.261$, $p > 0.005$), particularly in ROI 2 and ROI 3 (Two-way ANOVA). Dunnett's multiple comparisons test shows that the mean k-index of ROI 2 was significantly reduced in the epochs of 20-30 min and 50-60 min ($^d p < 0.0001$; in Figure 3.17) compared to basal (90.53 ± 14.10). The mean K-index of ROI 3 was also significantly reduced in the epochs of 20-30 min ($^c p < 0.001$) and 50-60 min ($^d p < 0.0001$) compared to basal (65.77 ± 8.45).

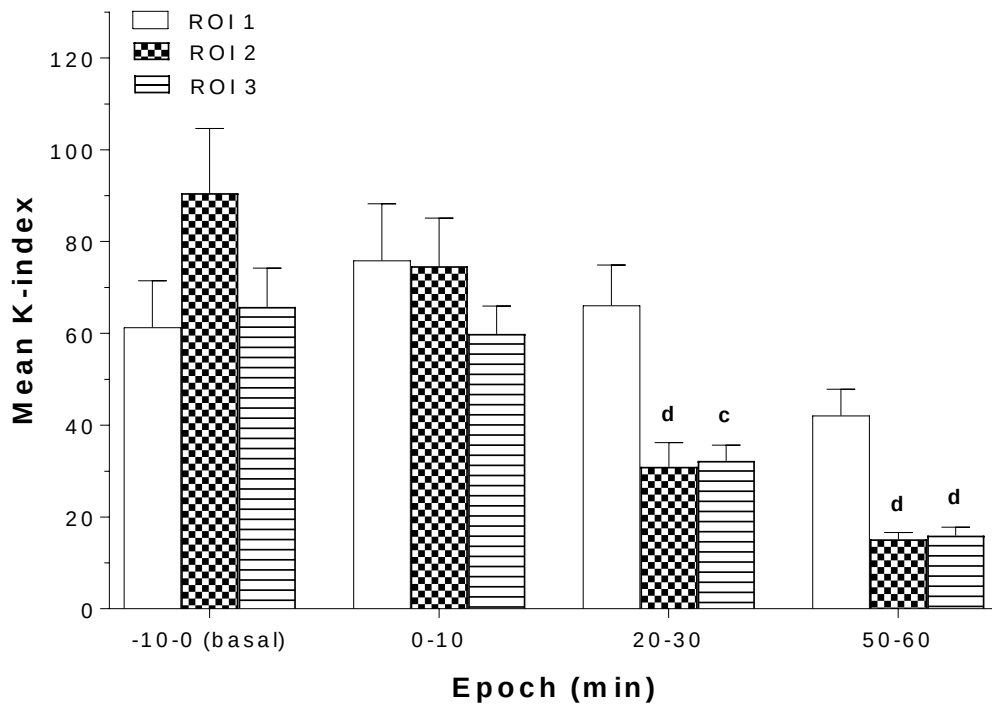


Figure 3.17 Mean K-index of systemic KA.

Mean k-index for KA administered intraperitoneally (10 mg/kg; i.p.) after 10 min basal recording (-10-0) and further recording for another 60 min, mean $\pm$ SEM, n=3 rats.

$^c p < 0.001$ and $^d p < 0.0001$ when compared to basal (-10-0 min), respectively. Two-way ANOVA and *post hoc* Dunnett's multiple comparisons tests.

(ROI 1 = ipsilateral CA1 and CA3; ROI 2 = contralateral CA1 and CA3; ROI 3 = neurones in both ipsilateral and contralateral hippocampus)

No changes in cross-correlation in the ipsilateral hippocampus after KA 1 mM

Based on two-way ANOVA of mean k-index, there were no significant differences compared to basal in any ROI ($F_{(6, 2280)} = 1.124, p > 0.05$ in Figure 3.18).

Overall, KA injected intraperitoneally to induce systemic epilepsy shows that the neurones lost their correlation after 10 min of KA administration, particularly in ROI 2 and ROI 3. However, the absence of significant differences in mean K-index compared to basal indicates that there were no changes in correlation among neurones in the vehicle group and local KA model.

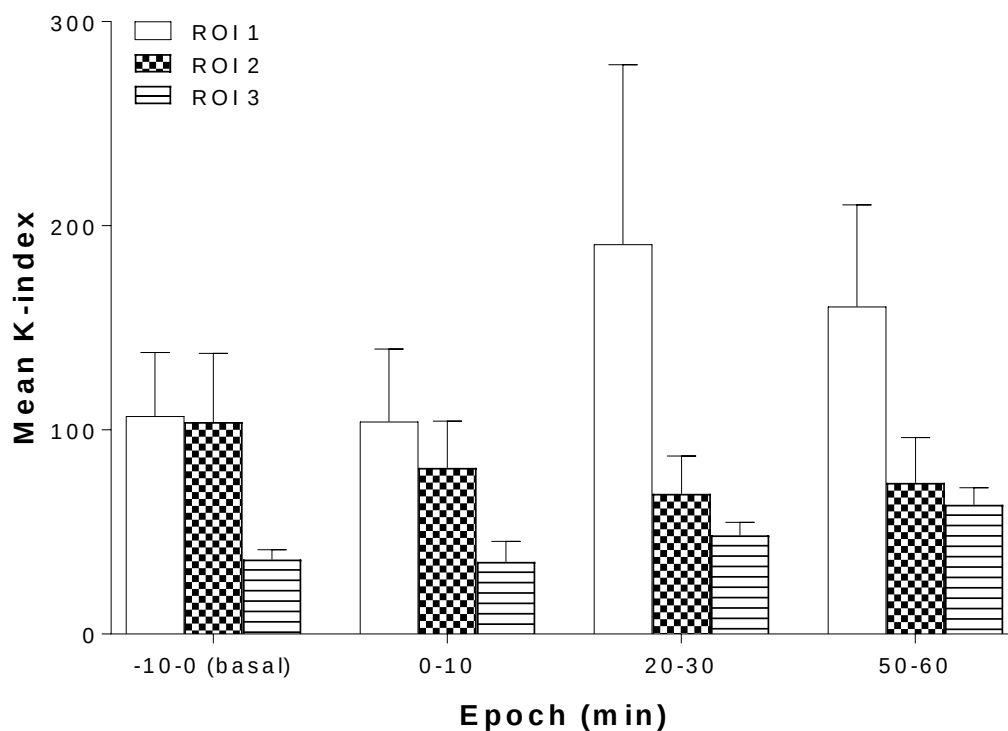


Figure 3.18 Mean k-index of saline+KA 1 mM group.

Saline administered intravenously after 10 min' basal recording (0 min) followed by local administration of 1 mM KA after 20 min and further recording for another 40 min, mean $\pm$ SEM, n=6 rats.

No significant changes.

(ROI 1 = ipsilateral CA1 and CA3; ROI 2 = contralateral CA1 and CA3; ROI 3 = neurones in both ipsilateral and contralateral hippocampus)

3.3.5 LFP frequency band distribution

Saline changed the frequency band (delta, theta, alpha, beta, gamma) in the ipsilateral and contralateral hippocampus

There were no significant changes in the interaction of the overall LFP power in any brain regions of rats in the vehicle group (Figure 3.19; $F_{(9, 60)} = 1.43$, $p > 0.05$; Two-way ANOVA repeated measures). However, the extracted frequency bands from the LFP power showed significant differences, as explained below and shown in Figure 3.20.

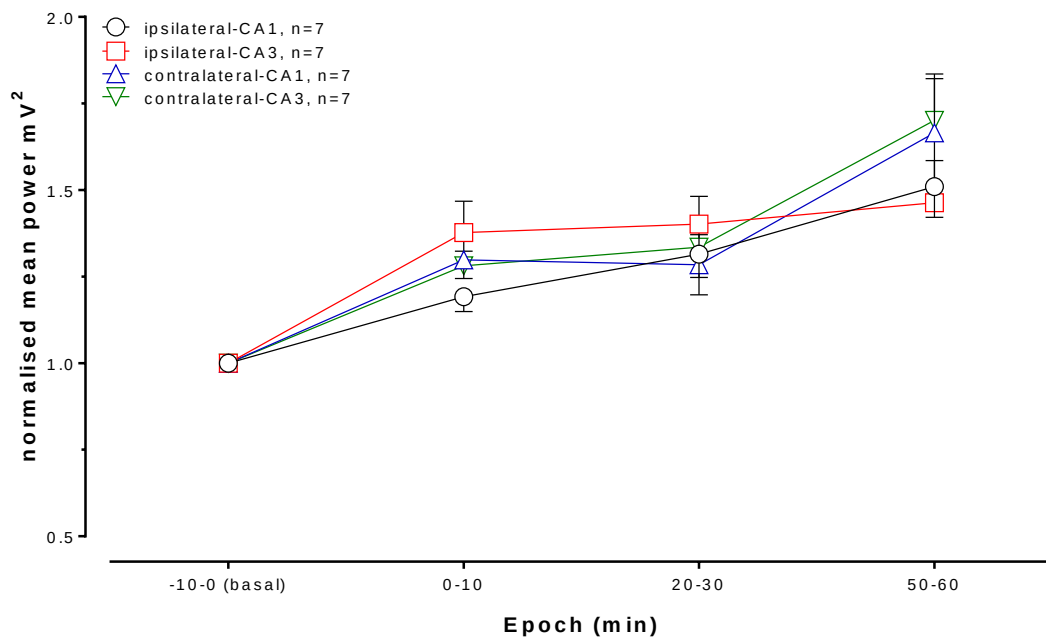
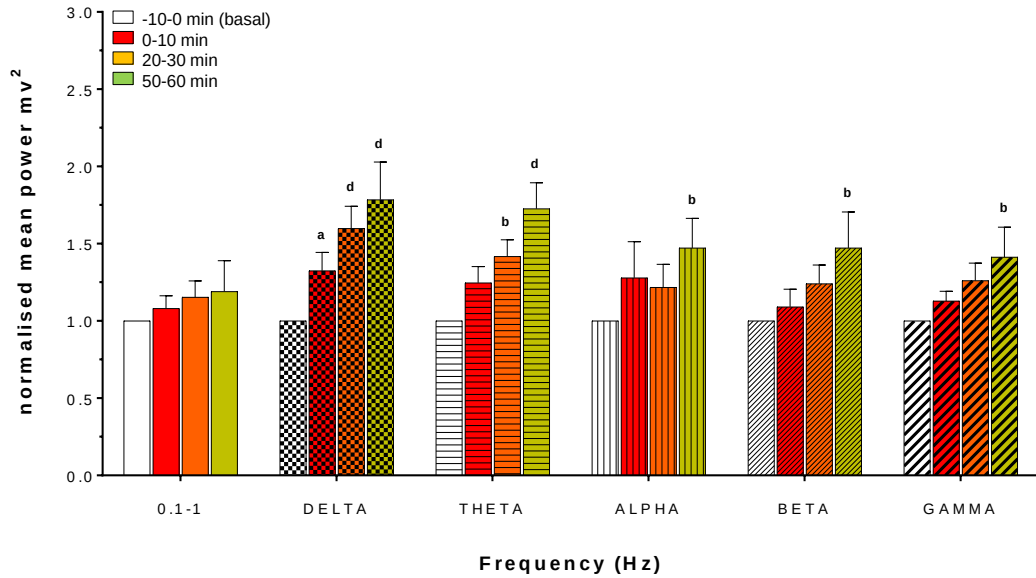


Figure 3.19 Normalised mean power of LFP in the vehicle group, mean $\pm$ SEM, $n=7$ rats. No significant difference, $F_{(9, 60)} = 1.43$, $p > 0.05$.

In **ipsilateral-CA1**, the normalised mean power of **delta** bands from vehicle group was significantly increased in the epochs of 0-10 min (1.32 ± 0.12 ; $^a p < 0.005$), 20-30 min and 50-60 min (1.60 ± 0.14 and 1.79 ± 0.24 ; $^d p < 0.0001$, respectively) compared to basal (-10-0 min; Dunnett's multiple comparisons test). In the **theta** band, normalised mean power was significantly increased in the epoch of 20-30 min (1.14 ± 0.11 ; $^b p < 0.005$) and at 50-60 min (1.73 ± 0.17 ; $^a p < 0.0001$) compared to basal. There were significant increases in normalised mean power in **alpha**, **beta** and **gamma** only at 50-60 min (1.47 ± 0.19 , 1.47 ± 0.23 and 1.41 ± 0.19 ; $^b p < 0.005$, respectively) compared to basal, as shown in Figure 3.20A.

Figure 3.20B shows that there are significant differences in normalised mean power in the **delta** band in **ipsilateral-CA3** at 20-30 min (1.59 ± 0.15 ; $^a p < 0.05$) and at 50-60 min (1.67 ± 0.18 ; $^b p < 0.005$) compared to basal. Significant increases in the **theta** band were also found at 20-30 min (1.53 ± 0.13 ; $^a p < 0.05$) and at 50-60 min (1.81 ± 0.16 ; $^c p < 0.001$) compared to basal. Normalised mean power in the **alpha** band was significantly increased at 50-60 min (1.71 ± 0.13 ; $^b p < 0.005$) whereas the **gamma** band also showed significantly increased normalised mean power at 0-10 min (1.79 ± 0.59 ; $^c p < 0.001$) and 20-30 min (1.51 ± 0.32 ; $^a p < 0.05$) compared to basal.

A. Ipsilateral-CA1



B. Ipsilateral-CA3

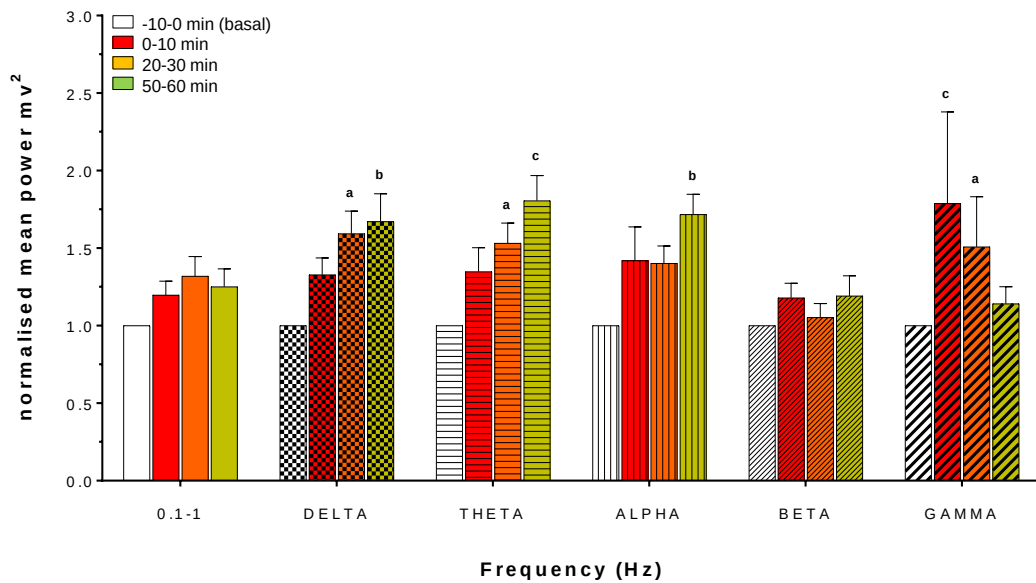


Figure 3.20 Normalised mean power of LFP frequency band distribution in the ipsilateral hippocampus from saline+saline group throughout the 80 min experiment (n=7 rats).

A: Frequency band distribution in ipsilateral-CA1, no significant interaction $F_{(15, 108)} = 0.8277$.

B: Frequency band distribution in ipsilateral-CA3, no significant interaction $F_{(15, 108)} = 1.543$

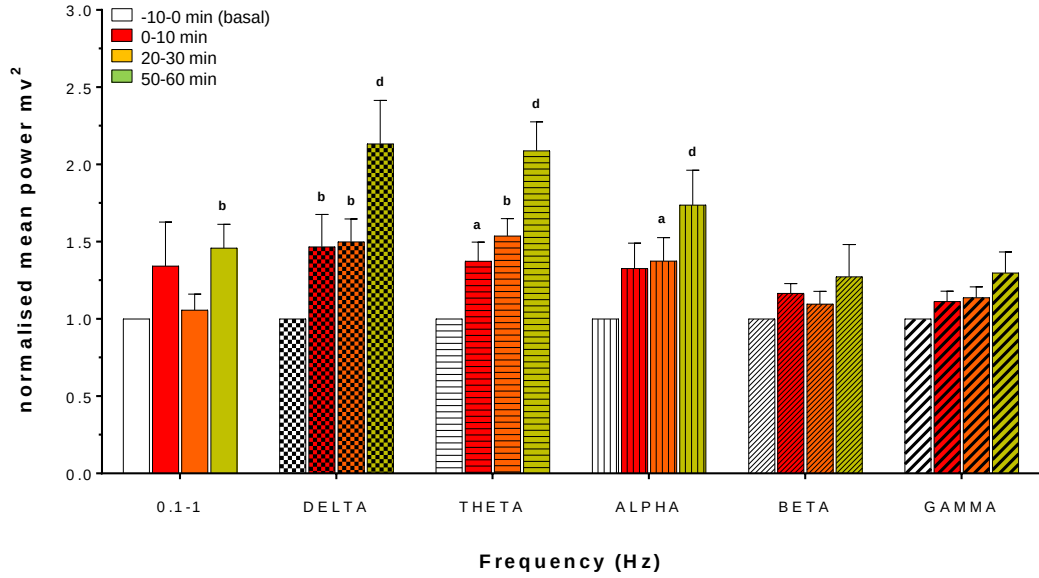
^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.

Two-way ANOVA and *post hoc* Dunnett's multiple comparisons tests.

Normalised mean power of **0.1-1 Hz** bands from the vehicle group of the **contralateral-CA1** (Figure 3.21A) were significantly different at 20-30 min (1.75 ± 0.31 ; $^b p < 0.05$) compared to basal. Significant increases in normalised mean power in the **delta** band were also found at 0-10 min (1.47 ± 0.21 ; $^b p < 0.05$), 20-30 min (1.50 ± 0.15 ; $^b p < 0.05$) and at 50-60 min (2.13 ± 0.28 ; $^d p < 0.0001$) compared to basal. Normalised mean power in the **theta** band was significantly increased at 0-10 min (1.37 ± 0.12 ; $^a p < 0.05$), 20-30 min (1.54 ± 0.11 ; $^b p < 0.005$) and 50-60 min (2.09 ± 0.19 ; $^d p < 0.0001$) compared to basal. Normalised mean power in the **alpha** band also showed significant increased at 20-30 min (1.38 ± 0.15 ; $^a p < 0.05$) and 50-60 min (1.74 ± 0.23 ; $^d p < 0.0001$) compared to basal.

As shown in Figure 3.21B, in the **contralateral-CA3**, normalised mean power of **0.1-1 Hz** from vehicle group was significantly increased at 50-60 min (1.75 ± 0.31 ; $^c p < 0.001$) compared to basal (-10-0 min). In the **delta** and **theta** bands, normalised mean power was significantly increased at 20-30 min (1.50 ± 0.20 and 1.50 ± 0.10 ; $^a p < 0.05$) and at 50-60 min (1.97 ± 0.34 and 2.03 ± 0.29 ; $^d p < 0.0001$). There was a significant increase in the **alpha** band only at 50-60 min (1.86 ± 0.30 ; $^c p < 0.001$, respectively) compared to basal.

A. Contralateral-CA1



B. Contralateral-CA3

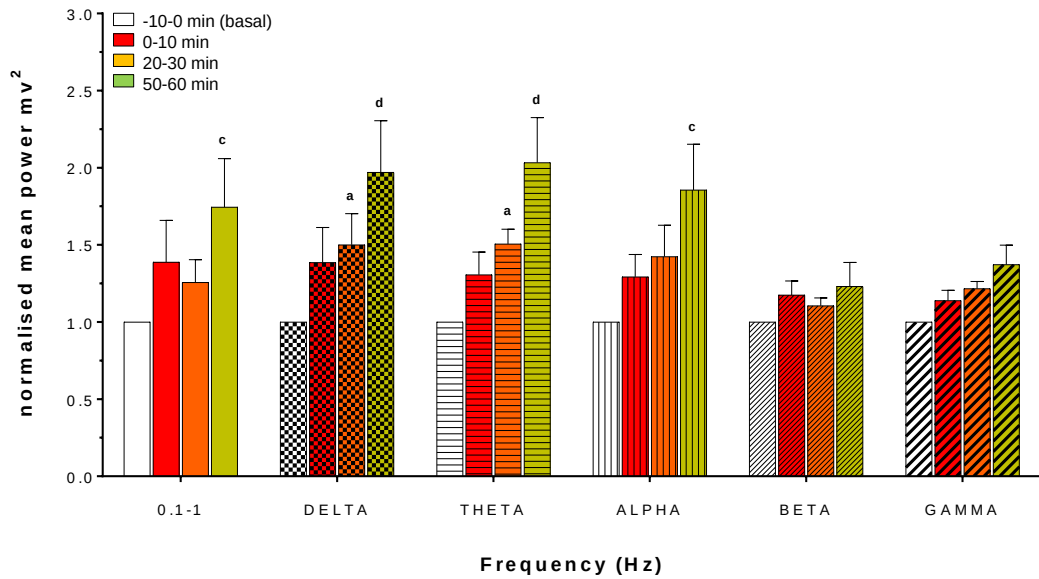


Figure 3.21 Normalised mean power of LFP frequency band distribution in the ipsilateral hippocampus from saline+saline group throughout the 80 min experiment (n=7 rats) .

A: Frequency band distribution in contralateral-CA1, no significant interaction $F_{(15, 108)} = 1.695$

B: Frequency band distribution in contralateral-CA3, no significant interaction $F_{(15, 108)} = 0.8936$

^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.

Two-way ANOVA and *post hoc* Dunnett's multiple comparisons tests.

Systemic KA administered intraperitoneally increased the frequency bands of beta and gamma in the ipsilateral and contralateral hippocampus

No significant changes were found in the interaction of the overall LFP power in any brain regions of the rats in the KA i.p group (Figure 3.22; $F_{(9, 45)} = 1.991$, $p > 0.05$; Two-way ANOVA repeated measures).

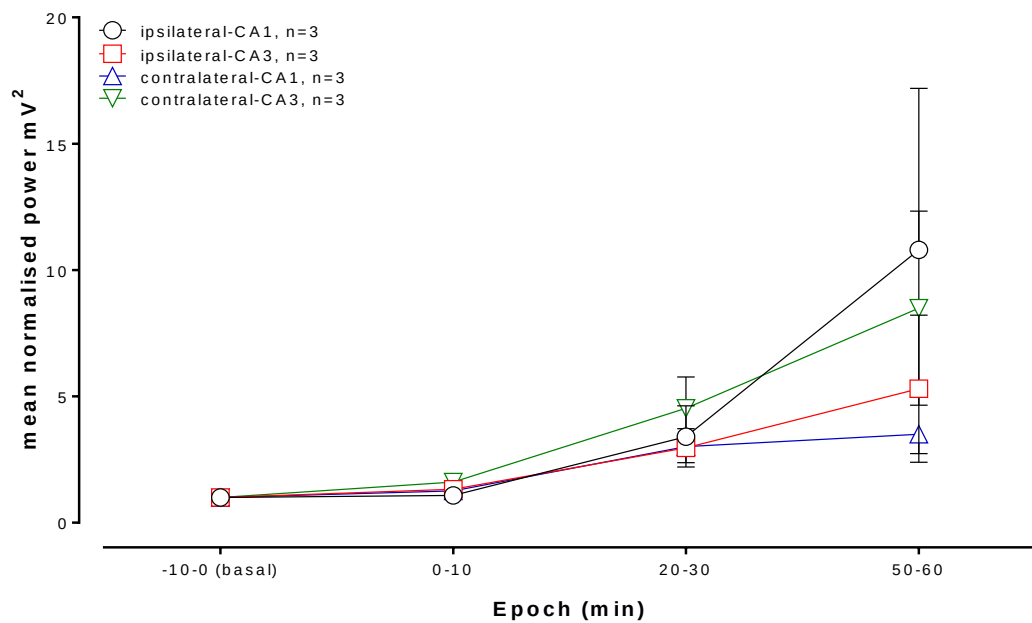
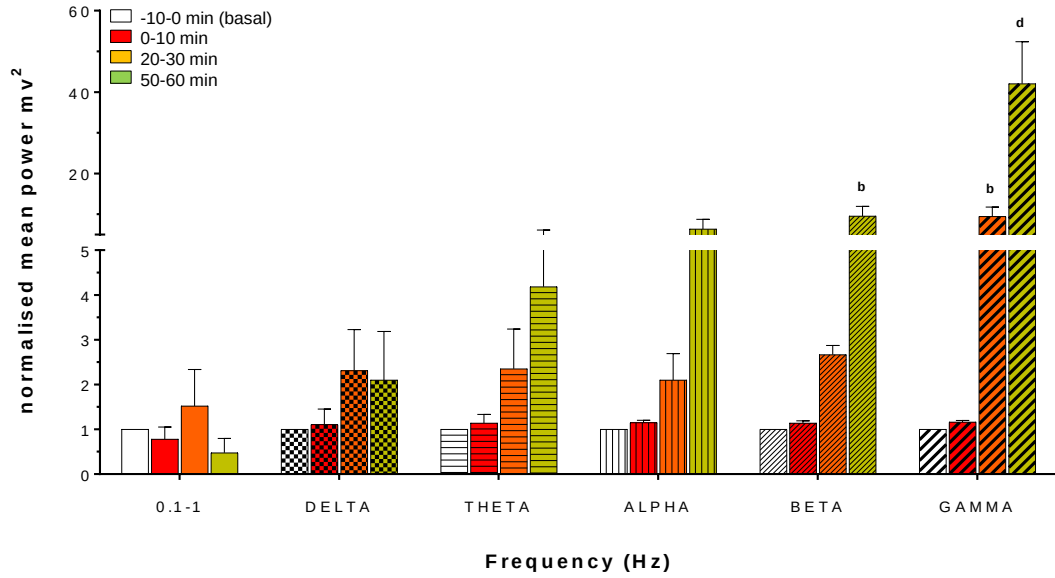


Figure 3.22 Normalised mean power of LFP in ipsilateral-CA1 and CA3 and contralateral-CA1 and CA3 in KA i.p group, mean $\pm$ SEM, n=3 rats.

In **ipsilateral-CA1**, the normalised mean power of the **beta** band in the KA i.p group was significantly increased at 50-60 min (9.58 ± 2.35 ; ^b $p < 0.005$) compared to basal (-10-0 min; Dunnett's multiple comparisons test). In the **gamma** band, normalised mean power was significantly increased at 20-30 min (9.48 ± 2.29 ; ^b $p < 0.005$) and at 50-60 min (42.08 ± 10.29 ; ^d $p < 0.0001$) as shown in Figure 3.23A.

Figure 3.23B shows that there was a significant increase in the normalised mean power in **beta** band in **ipsilateral-CA3** at 50-60 min (4.47 ± 0.91 ; ^a $p < 0.05$). A significant increase in the normalised mean power in the **gamma** band was also found at 20-30 min (6.64 ± 2.20 ; ^c $p < 0.001$) and at 50-60 min (19.65 ± 4.12 ; ^d $p < 0.0001$) compared to basal.

A. Ipsilateral-CA1



B. Ipsilateral-CA3

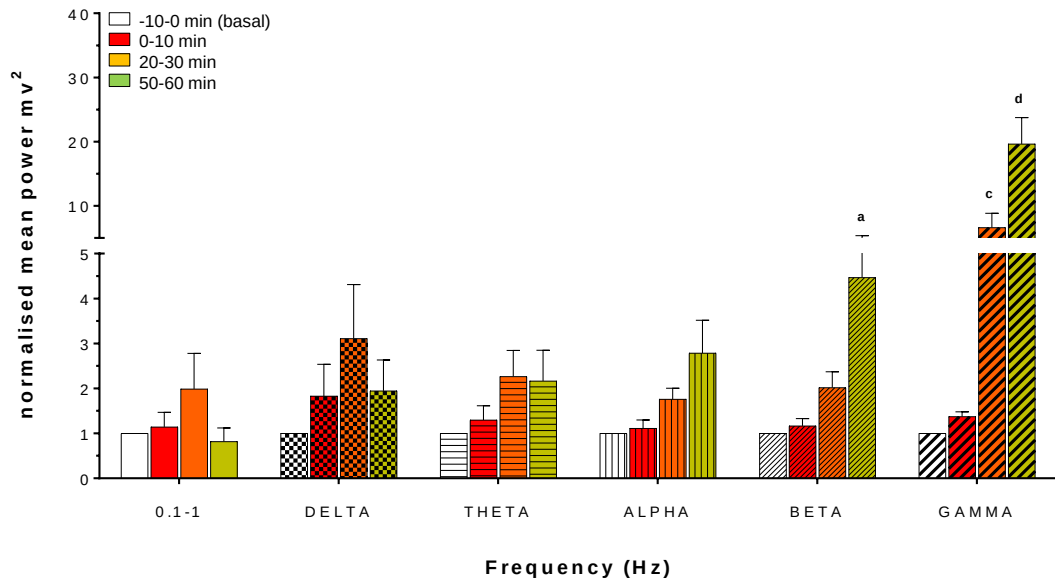


Figure 3.23 Normalised mean power of LFP frequency band in the ipsilateral hippocampus from KA i.p group throughout the 60 min experiment (n=3 rats).

A: Frequency band distribution in ipsilateral-CA1, $F_{(15, 30)} = 16.64$, $p < 0.0001$

B: Frequency band distribution in ipsilateral-CA3, $F_{(15, 30)} = 14.58$, $p < 0.0001$

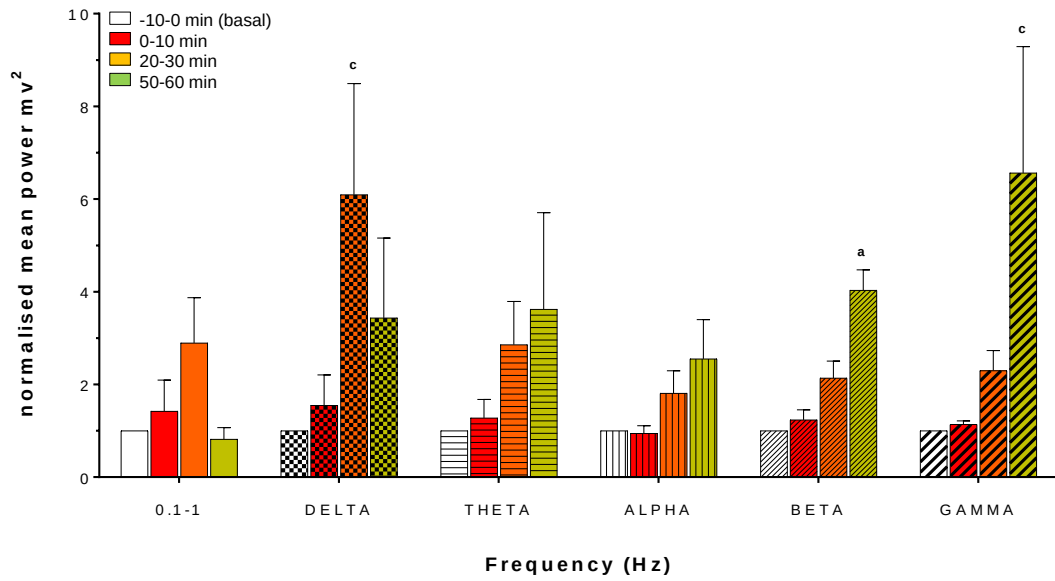
^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.

Two-way ANOVA and *post hoc* Dunnett's multiple comparisons tests.

The normalised mean power of the **delta** band in the KA i.p group of **contralateral-CA1** (Figure 3.24A) was significantly increased at 20-30 min (6.01 ± 2.40 ; $^c p < 0.001$) compared to basal. Significant increases in the **beta** and **gamma** bands were also found at 50-60 min (4.03 ± 0.44 ; $^a p < 0.05$ and 6.56 ± 2.73 ; $^d p < 0.0001$, respectively).

As shown in Figure 3.24B, in **contralateral-CA3**, the normalised mean power of **beta** from the KA i.p group was significantly increased at 50-60 min (8.79 ± 2.73 ; $^b p < 0.005$) compared to basal (-10-0 min). In the **gamma** band, normalised mean power was significantly increased at 20-30 min (10.21 ± 5.02 ; $^b p < 0.005$) and at 50-60 min (27.10 ± 6.21 ; $^d p < 0.0001$).

A. Contralateral-CA1



B. Contralateral-CA3

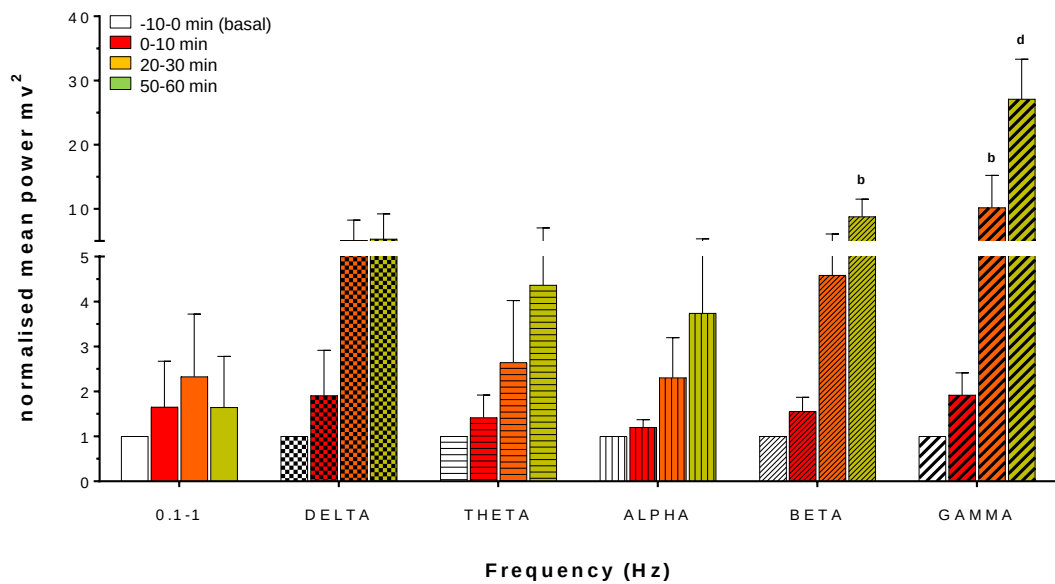


Figure 3.24 Normalised mean power of LFP frequency band in the ipsilateral hippocampus from KA i.p group throughout the 60 min experiment (n=3 rats).

A: Frequency band distribution in contralateral-CA1, $F_{(15, 30)} = 2.32$, $p < 0.05$

B: Frequency band distribution in contralateral-CA3, $F_{(15, 30)} = 6.56$, $p < 0.0001$

^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.

Two-way ANOVA and *post hoc* Dunnett's multiple comparisons tests.

KA 1 mM administered locally increased the frequency bands of 0.1-1 Hz, delta and theta in the contralateral hippocampus

Significant changes were found in the interaction of overall LFP power in all brain regions of rats in saline+KA 1 mM group (Figure 3.25; $F_{(9, 45)} = 9.88, p < 0.0001$; Two-way ANOVA repeated measures). The normalised mean power in contralateral-CA3 was significantly different when compared to ipsilateral-CA1 ($p < 0.05$), ipsilateral-CA3 ($p < 0.005$) and contralateral-CA1 ($p < 0.001$; Tukey's multiple comparisons test)

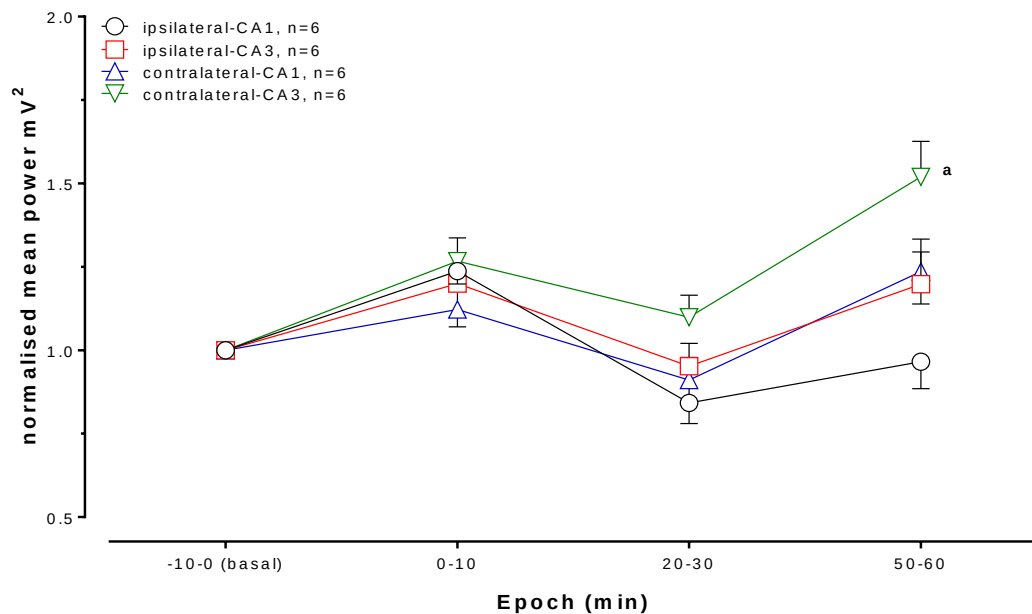


Figure 3.25 Normalised mean power of LFP in saline+KA group in 80 min experiment, mean $\pm$ SEM, n=6 rats.

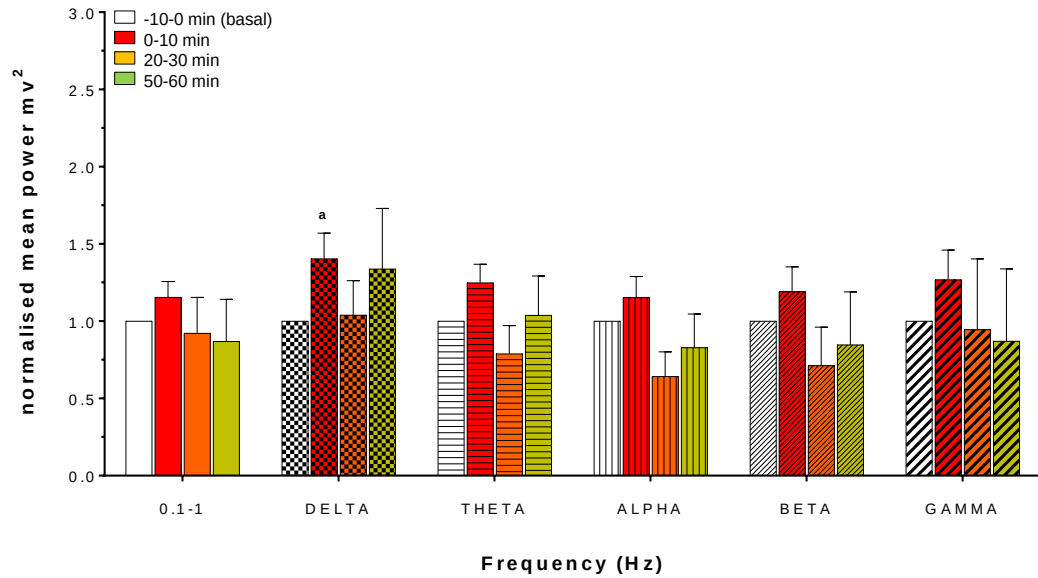
Significant interaction, $F_{(9, 45)} = 9.88, p < 0.0001$ (two-way ANOVA repeated measures).

Contralateral-CA3 (mark with **a**) is significantly different when compared to ipsilateral-CA1 ($p < 0.05$), ipsilateral-CA3 ($p < 0.005$) and contralateral-CA1 ($p < 0.001$) (*post hoc* Tukey's multiple comparisons test).

In **ipsilateral-CA1**, the normalised mean power of the **delta** band in the KA 1 mM group was significantly increased at 0-10 min (1.40 ± 0.17 ; $^a p < 0.05$) compared to basal (-10-0 min; Dunnett's multiple comparisons test) as shown in Figure 3.26A.

Figure 3.26B shows that there was a significant increase in the **delta** band in **ipsilateral-CA3** at 50-60 min (1.56 ± 0.13 ; $^a p < 0.05$) compared to basal.

A. Ipsilateral-CA1



B. Ipsilateral-CA3

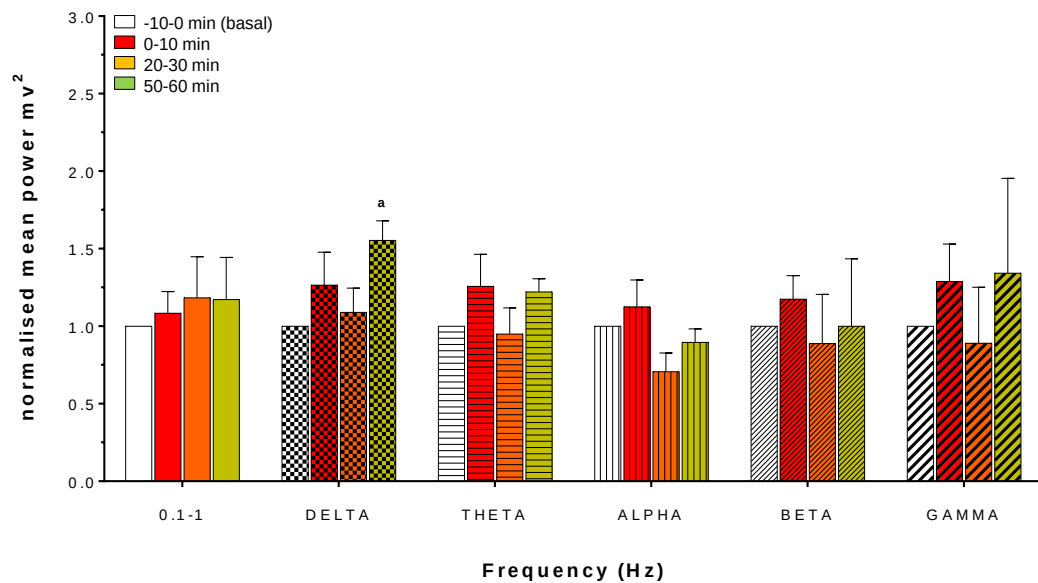


Figure 3.26 Normalised mean power of LFP frequency band distribution in the ipsilateral hippocampus from saline+KA 1 mM group throughout the 80 min experiment (n=6 rats).

A: Frequency band distribution in ipsilateral-CA1, no significant interaction $F_{(15, 75)} = 0.8644$, $p > 0.05$.

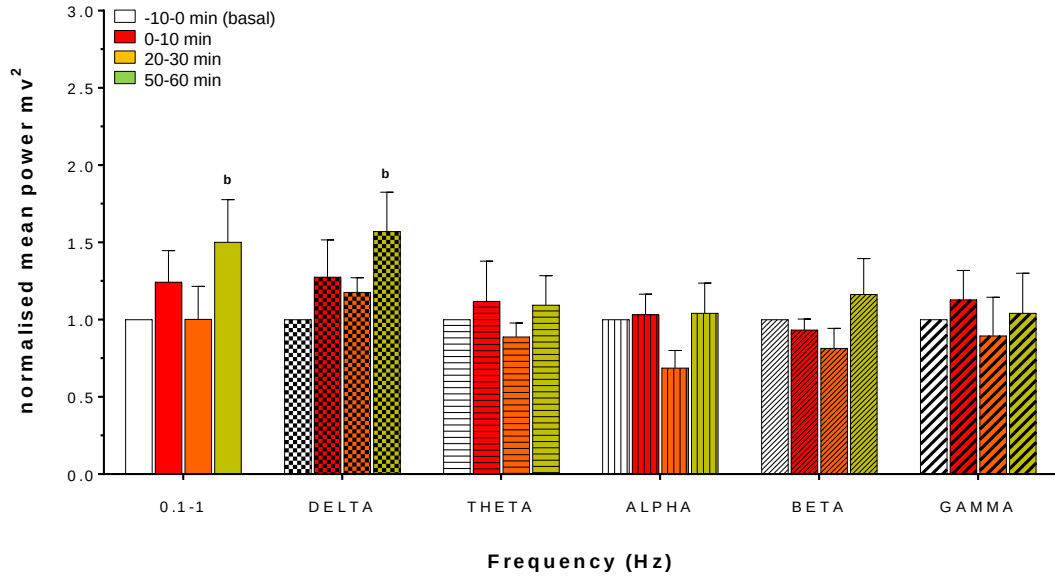
B: Frequency band distribution in ipsilateral-CA3, no significant interaction $F_{(15, 75)} = 0.7936$, $p < 0.05$
^a $p < 0.05$ when compared to basal, respectively (Dunnett's multiple comparisons test)

The normalised mean power of **0.1-1 Hz** from the KA 1 mM group of contralateral-CA1 (Figure 3.27A) was significantly increased at 50-60 min (1.50 ± 0.28 ; $^b p < 0.005$) compared to basal. A significant increase in the **delta** band was also found at 50-60 min (0.57 ± 0.26 ; $^b p < 0.005$) compared to basal.

As shown in Figure 3.27B, in **contralateral-CA3**, the normalised mean power of **0.1-1 Hz** in the KA 1 mM group was significantly increased at 0-10 min (1.44 ± 0.27 ; $^b p < 0.005$) and 50-60 min (1.66 ± 0.25 ; $^b p < 0.005$) compared to basal (-10-0 min). In the **delta** band, the normalised mean power was significantly increased at 0-10 min (1.39 ± 0.24 ; $^a p < 0.05$) and at 50-60 min (1.81 ± 0.19 ; $^d p < 0.0001$) compared to basal. Significant increases in the **theta** band were also found at 0-10 min (1.42 ± 0.20 ; $^a p < 0.05$) and at 50-60 min (1.74 ± 0.20 ; $^d p < 0.0001$). The normalised mean power of the **beta** band was significantly increased at 50-60 min (1.44 ± 0.30 ; $^a p < 0.05$) compared to basal.

In summary, for the LFP frequency bands, saline increased the normalised mean power of the frequency bands (delta, theta, alpha, beta, and gamma) in the ipsilateral and contralateral hippocampus. The **KA i.p group** increased the normalised mean power in frequency bands of beta and gamma in the ipsilateral and contralateral hippocampus. **Saline+KA 1 mM** administered locally increased the normalised mean power in frequency bands of 0.1-1 Hz, delta and theta in the contralateral hippocampus. Overall, in anaesthetised rats in a normal condition (without seizure), the frequency bands of delta, theta and alpha, beta and gamma were prominent in the saline group except 0.1-1 Hz. In systemic epileptic model (KA i.p group), only beta and gamma were prominent after KA-induced seizure. However, in our acute mTLE rat model (saline + KA 1 mM group) the frequency bands of 0.1-1 Hz, delta and theta were prominent, emphasising the different effect of the different animal model.

A. Contralateral-CA1



B. Contralateral-CA3

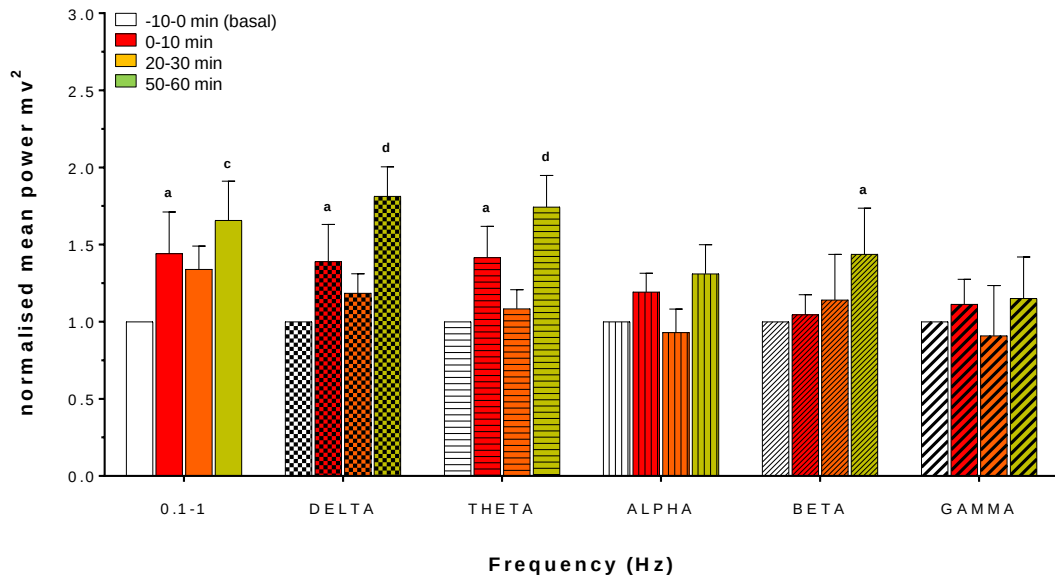


Figure 3.27 Normalised mean power of LFP frequency band distribution in the contralateral hippocampus from saline+KA 1 mM group throughout the 80 min experiment (n= 6 rats).

C: Frequency band distribution in contralateral-CA1, no significant interaction $F_{(15, 90)} = 0.5814$

D: Frequency band distribution in contralateral-CA3, no significant interaction $F_{(15, 90)} = 0.7122$

^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively (Dunnett's multiple comparisons test).

Table 3.1 summarises all the results presented in this chapter from Figure 3.8-3.27 to aid in interpretation and understanding of the whole chapter.

Table 3.1 A summary of all results reported in Chapter 3.

Group of study	Hippocampus area	Normalised mean firing rate	Normalised mean synchrony index	Unit-pair cross-correlation (mean k-index)	LFP frequency band distribution
Continuous recording, n=3 rats	Ipsilateral-CA1	No changes.	Not applicable	Not applicable	Not applicable
	Ipsilateral-CA3	No changes.	Not applicable		Not applicable
	Contralateral-CA1	No changes.	Not applicable		Not applicable
	Contralateral-CA3	No changes.	Not applicable		Not applicable
Saline (0.5 mL; i.v) followed by saline (1 µL; locally), n=7 rats.	Ipsilateral-CA1	No changes.	No changes.	No changes in ROI 1, ROI 2 and ROI 3.	Delta band increased at the epoch of 0-10 min, 20-30 min and 50-60 min. Theta band increased at 20-30 min and 50-60 min. Alpha, beta and gamma only increased at 50-60 min.
	Ipsilateral-CA3	No changes.	No changes.		Delta band increased at the epoch of 20-30 min and 50-60 min. Theta band increased at 20-30 min and 50-60 min. Alpha band increased at 50-60 min. Gamma

					band increased at 0-10 min and 20-30 min.
	Contralateral-CA1	No changes.	No changes.		0.1-1 Hz band increased at 50-60 min. Delta band increased at the epoch of 0-10 min, 20-30 min and 50-60 min. Theta band increased at 0-10 min, 20-30 min and 50-60 min. Alpha band increased at 20-30 min and 50-60 min.
	Contralateral-CA3	No changes.	No changes.		0.1-1 Hz band increased at 50-60 min. Delta band increased at the epoch of 20-30 min and 50-60 min. Theta band increased at 20-30 min and 50-60 min. Alpha band increased at 50-60 min.
KA (10 mg/kg; i.p), n=3 rats.	Ipsilateral-CA1	Increased after 20 min of KA injection.	No changes.	Decreased correlation in ROI 2 and ROI 3 and no changes in ROI 1.	Beta band increased at the epoch of 50-60 min. Gamma band increased at 20-30 min and 50-60 min.
	Ipsilateral-CA3	Increased after 50 min of KA injection.	Increased after 50 min of KA injection.		Beta band increased at the epoch of 50-60 min. Gamma

					band increased at 20-30 min and 50-60 min.
	Contralateral-CA1	Increased after 50 min of KA injection.	No changes.		Delta band increased at the epoch of 20-30 min. Beta band increased at the epoch of 50-60 min. Gamma band increased at 20-30 min and 50-60 min.
	Contralateral-CA3	Increased after 40 min of KA administration.	Increased after 50 min of KA injection.		Beta band increased at the epoch of 50-60 min. Gamma band increased at 20-30 min and 50-60 min.
Saline; i.v followed by KA (10 mM; locally), n=6 rats.	Ipsilateral-CA1	Increased after 10 min of KA administration	Not applicable	Not applicable	Not applicable
	Ipsilateral-CA3	No changes.	Not applicable		Not applicable
	Contralateral-CA1	Increased after 10 min of KA administration	Not applicable		Not applicable
	Contralateral-CA3	Increased only at 50 min of KA administration	Not applicable		Not applicable
Saline (0.5 i.v) followed by KA (1 mM; locally), n=6 rats.	Ipsilateral-CA1	Increased after 10 min of KA administration	No changes.	No changes in ROI 1, ROI 2 and ROI 3.	Delta band increased at the epoch of 0-10 min.
	Ipsilateral-CA3	No changes.	No changes.		Delta band increased at the epoch of 50-60 min.
	Contralateral-CA1	No changes.	No changes.		0.1-1 Hz band increased at 50-60 min. Delta band increased at

					the epoch of 50-60 min.
	Contralateral-CA3	Increased only at 50 min after KA administration	No changes.		0.1-1 Hz band increased at 0-10 min and 50-60 min. Delta band increased at the epoch of 0-10 min and 50-60 min. Theta band increased at 0-10 min and 50-60 min. Beta band increased at the epoch of 50-60 min.

3.4 Discussion

This *in vivo* multiple single-unit study has generated three main findings. First, the normalised mean firing rate was not altered in the left and right hippocampus in anaesthetised rats between sham and vehicle groups throughout the recording time. Saline was used to dissolve the drugs (VPA, WIN and FLU) for the experiments described in Chapter 4 and 5. The increased in normalised mean power in the delta, theta, alpha, beta and gamma bands in the ipsilateral and contralateral hippocampus in the saline+saline group indicated that these frequencies exist in anaesthetised rats in a normal condition.

Secondly, KA 1 mM was selected as the best animal model for further investigation into the effect of drugs (VPA, WIN and FLU) in the experiments detailed in Chapter 4 and 5. A low concentration of 1 mM KA has effectively induced a seizure in both left and right hippocampus, allowing consecutive experiments to be conducted.

Thirdly, the similarities and differences between the systemic epileptic model (KA administered intraperitoneally) and the chosen mTLE model (local intra-hippocampal KA injection) can be evaluated.

First and foremost, prior to any epilepsy treatment, validation of the methodology must be carried out to enhance the uniformity, reliability and reporting of early stage pre-clinical studies with animal epilepsy models (systemic and mTLE) that aim to develop and evaluate new therapies for seizures or epilepsies (Galanopoulou et al., 2013). Isoflurane was used as the anaesthetic agent in our study. Isoflurane is a volatile anaesthetic agent and is widely used in other reported studies in developing epilepsy (Bar-Klein et al., 2016; Bielefield et al., 2017; Newmann et al., 2017). Bielefield et al., (2017) recommend using isoflurane anesthesia as this allows for a tight control of the anesthesia plane and the fastest recovery after surgery. Isoflurane is an appropriate anaesthetic agent in pre-clinical experiments as long as its own pharmacological effects are borne in mind.

Our findings showed that saline did not alter the neuronal signal in anaesthetised rats in a normal condition. The use of saline created a placebo effect, similar with the sham group. In laboratory studies, drug formulation (ingredients) act as a carrier or

vehicle for the active drug (Galanopoulou et al. 2013). It is important to determine whether the vehicles exert their own effects, either directly or by altering the properties of the active drug. The use of saline to dissolve KA in this chapter and other drugs discussed in Chapter 4 and 5 did not change in colour due to drug-vehicle interaction (Galanopoulou et al. 2013), suggesting that saline did not change the neuronal signal in our experiment. Saline was also used as a vehicle in other reported studies of KA-induced epilepsy in rats (Gupta et al., 2009; Nam et al., 2017; Yow et al., 2017) which is in agreement with the use of saline as a vehicle for drug delivery in our experiment. **Saline+saline group** showed no changes in firing rate, compared to basal (Figure 3.8B). A study by Coomber et al. (2008) also found no changes in the firing rate in the no-drug control group using saline. The synchronisation of electrical activity in the brain occurs because of the interaction among sets of neurones between cells and within local networks (Timofeev et al., 2012; Jefferys et al., 2012). Our study showed that saline as the vehicle did not alter the normalised mean SI and did not interfere with the interaction among neurones in the ipsilateral and between neurones in the contralateral as shown in the result of our unit pair cross-correlation, where no changes were detected in mean K-index. Generally, the brain is dominated by beta (15-30 Hz) and gamma (30-80 Hz) ranges in the awake state (Timofeev et al., 2012). During slow-wave sleep or anaesthesia, normal oscillatory activities in the frequency of 0.1-15 Hz are usually present (Timofeev et al., 2012). This is in agreement with our findings on LFP frequency band distribution, which shows a significant increase in the normalised mean power in the delta (1-4 Hz), theta (4-8 Hz) and alpha bands (8-14 Hz). On the other hand, significant increases in the normalised mean power were also found in the beta and gamma bands in the ipsilateral hippocampus, implying that, these frequency bands were prominent in our experimental anaesthetised rats.

We also evaluated the potential acute mTLE rat model induced by two concentrations of KA; 1 mM and 10 mM administered locally. Neurones in ipsilateral-CA1 and contralateral-CA1 in the **saline+KA 10 mM group** showed high firing rates compared to the other groups (Figure 3.11); the firing rate was increased after 10 min of KA 10 mM administration (Figure 3.12A and B). This immediate firing after 10 min of KA administration is due to quicker development of epileptiform-like activity in the hippocampus. In contrast, the neuronal firing rate

was increased in contralateral-CA3 after 30 min of KA 10 mM administration (Figure 3.12C). During this hyperexcitable state, the inter-hemispheric hippocampal connectivity was initially disrupted and then increased due to the hippocampus contralateral to the epileptogenic focus, exerting more influence over the ipsilateral site (Morgan et al., 2011). In the **KA 1 mM group**, the firing rate of neurones in ipsilateral-CA1 was higher than in the other groups (Figure 3.13). The firing rate was increased after 10-50 min of KA administration in ipsilateral-CA1 (Figure 3.14A) and 50 min of KA administration in contralateral-CA3 (Figure 3.14B). Comparisons between both groups showed a similar increasing firing rate at the injection site (ipsilateral-CA1) after 10 min of KA administration, indicating that neuronal hyper-excitability or epileptiform-like activity were induced in both KA groups. On the other hand, the epileptiform-like activity was also induced in the contralateral hippocampus, specifically in contralateral-CA3 in both 10 mM and 1 mM KA groups. This result shows that although there was no direct administration of KA, the neuronal hyper-excitability effect spread to the contralateral hippocampus. The increase in firing rate that occurred bilaterally during epileptiform-like periods suggests that the hyper-excitability spreads to both hemispheres (Karunakaran et al., 2012). *In vitro* preparation by Finnerty and Jefferys (2002) suggested that individual bursts are propagated in either direction, both between the right and left dorsal hippocampus and between CA3 and the dentate gyrus within the same hippocampus. According to Ben-Ari and Cossart (2000), there are several factors that may contribute to KA-induced hyper-excitability cell activity, such as CA3 pyramidal neurones, which are highly vulnerable to network hyperactivity because of a sustained release of glutamate leading to activation of KARs. Repetitive high-frequency stimulation of CA3 pyramidal neurones also induced selective cell loss as a result of the sustained release of glutamate. Other than that, the mossy fibre synaptic region (stratum lucidum) is enriched with high-affinity KARs that can be activated by even small concentrations of KA crossing the blood–brain barrier (Ben-Ari and Cossart, 2000). The same authors asserted that disruption of the normal balance between excitation and inhibition in the brain results in seizure generation (Duncan et al., 2006).

One of the motivations to conduct this research is the lack of epilepsy studies using anaesthetised rats. In previous studies, the dose used for KA injection was 0.4 µg

(1.9 nM) in awake rats (Cavalheiro et al., 1982; Babb et al., 1995; Bragin et al., 1999; Bragin et al., 2005; Raedt et al., 2009, Rattka et al., 2013). In 2009, Bragin's research team modified the dose of KA to 9.3 mM in awake rats experiment (Bragin et al., 2009). Based on these previous studies, we initially modified the KA dose to 10 mM. A study by Li et al. (2018) also reported a modified dose of 8.6 mM of KA intrahippocampally administered in awake rats experiment. Their selected KA dose was adapted from the study by Bragin et al. (2009), which also supported our selection of a high KA dose of 10 mM. However, our laboratory experience demonstrated that 10 mM of KA caused mortality (drop out) to our experimental animals in an experiment of long duration (3.5 hours). Hence, we discontinued the experiment using 10 mM KA. To the best of our knowledge, there are no existing studies which have used up to 10 mM of KA in intrahippocampal injection (Cavalheiro et al., 1982; Babb et al., 1995; Bragin et al., 2005, Raedt et al., 2009, Rattka et al., 2013 and Klee et al., 2017). Also, the Home Office license for animal testing does not support dose response curve experiments. Thus, we chose the most effective dose based on the existing literature.

As mentioned previously, 10 mM KA has caused death to our anaesthetised rats. Hence, we selected a lower dose of 1 mM KA to reduce the likelihood of mortality in the rats. Based on this rationale and our data showing a hyper-excitable effect in **saline+KA 1 mM** experiments (Figure 3.13 and 3.14), KA 1 mM was selected to be the acute mTLE model in this study. From this point onwards, the saline+KA 1 mM group is referred to as the mTLE model. Klee et al. (2017) reported that the use of 0.4 µg (1.9 nM), 0.6 µg (2.8 nM) and 0.8 µg (3.8 nM) KA injected into anaesthetised rats for a study of 2.58 hours showed that only a few rats developed epilepsy (5/36 rats using five different protocols). Our study has shown that the use of 1 mM KA is effective to induce neuronal hyper-excitability relative to the low doses of KA used by Klee et al. (2017).

We have also evaluated the similarities and differences between the acute systemic epilepsy rat model (KA administered intraperitoneally) and the acute mTLE rat model (KA 1 mM) as shown in Table 3.2. This study compared the effect of KA administration, either systemically or locally in the hippocampus of anaesthetised rats. Our findings revealed that both models showed increased firing in ipsilateral-CA1 and contralateral-CA3. However, in the systemic model, increased firing also occurred in ipsilateral-CA3 and contralateral-CA1. As expected, intraperitoneal KA injection increased the firing rate in both CA1 and CA3 in left and right hippocampus, indicating seizure in both hippocampi.

Table 3.2 Similarities and differences between KA i.p and saline+KA 1 mM.

PARAMETERS		SIMILARITIES	DIFFERENCES
Normalised firing rate	mean	Increase firing in ipsilateral-CA1 and contralateral-CA3	Only KA i.p increase firing rate in ipsilateral-CA3 and contralateral-CA1
Mean index	synchrony		Increased in ipsilateral-CA3 and contralateral-CA3 in KA i.p group but no changes in saline + KA 1 mM group.
Unit pair cross-correlation (mean K-index)			Decreased in ROI 2 and ROI 3 in KA i.p group but no changes in saline + KA 1 mM group.
LFP band distribution	frequency		Beta and gamma were increased in ipsi- and contralateral hippocampus in KA i.p group. Increased normalised mean power in delta band in ipsilateral-CA1, ipsilateral-CA3 and contralateral-CA1. Increased normalised mean power in 0.1-1 Hz, delta, theta and beta in contralateral-CA3 in saline + KA 1 mM group

The neurones fire at a different time point in each brain region (Figure 3.10); after 20 min of KA administration in ipsilateral-CA1, 50 min in ipsilateral-CA3 and contralateral-CA1 and 40 min in contralateral-CA3. The basal firing characteristics may play a role in the neural firing pattern observed (Bernasconi et al., 2003). Other factors such as pharmacokinetic considerations, i.e., time for KA to cross the blood brain barrier, time to reach target structure(s), and time to reach an effective concentration to activate KARs also contributes to the delayed response in firing rate (Ben-Ari and Cossart, 2000; Pinheiro and Mulle, 2006). There is also the possibility that KA caused depolarisation block in firing and/or activated a population of inhibitory GABAergic cells to delay any KA-induced increase in firing (Ben-Ari and Cossart, 2000).

In our mTLE model, local administration of KA 1 mM induced neuronal epileptiform-like activity in the ipsilateral-CA1 hippocampus and also in the contralateral-CA3 hippocampus, implying that seizure spread from the site of injection on the left to the right side of the hippocampus (Figure 3.14B). Although the seizure is initiated in the hippocampus, it could spread to the amygdala, suggesting that other structures could be involved in the generation and maintenance of temporal lobe epilepsy (De A. Furtado et al., 2002). Functional connectivity between inter-hemispheric areas is dependent on GABAergic transmission, while intra-hemispheric functional connectivity is KA receptor-dependent (Karunakaran et al., 2012; Bragin et al., 2014). KARs are widely distributed in the hippocampus, presynaptically regulating glutamate release at mossy fibre-CA3 synapses and GABA release between interneurons and at the interneurons-pyramidal cell synapses (Ben-Ari and Cossart, 2000; Bernard et al., 2000; Falcón-Moya et al., 2018). Activation of high affinity KARs is proposed to underlie the epileptiform-like activity generated by the KA administration (Coomber et al., 2008; Falcón-Moya et al., 2018). Another mechanism may be via activation of the glutamatergic pyramidal cells located in CA3, which project via Schaffer collaterals to pyramidal cells in CA1 (Amaral and Witter 1989). Epileptiform-like activity subsequently propagates from CA3 to CA1, then via the subiculum, to other limbic structures (Ben-Ari and Cossart 2000).

Our mTLE model also demonstrated that the firing started after 10 min of KA administration in ipsilateral-CA1 (Figure 3.14A). The result indicates that the KARs (Karschin et al., 1997) were activated immediately after KA-induced seizures occurred (Lerma, 2006; Pinheiro and Mulle, 2006). We suggest that the neuronal aggregate necessary for hyper-excitability comprises multiple spatially distributed neuronal networks and that the increased synchrony of the output (population spike firing) of these networks during the early part of hyper-excitability may contribute to seizure generation (Finnerty and Jefferys, 2002). There were no changes in firing rate in ipsilateral-CA3 and contralateral-CA1 in the mTLE model. This event can be accounted for by neuronal depolarisation, which requires the neurones to rearrange their network dynamic (including neuronal plasticity) to act as normal prior to the interference (Karunakaran et al., 2012). Another contributing factor may include the KA-induced release of GABA from interneurons, which lead the neurones to an inhibitory state (Karunakaran et al., 2012; Khazipov, 2016). The initial suppression of neuronal firing at the injection site may reflect either prolonged KA-induced depolarisation and/or stimulation of GABA interneurons. There are at least three main components that control the activity of GABA (Bernard et al., 2000). The first component is the activation of post-synaptic GABA receptors, which counteract the membrane depolarisation induced by excitatory inputs via direct hyperpolarising or shunt effects. Secondly, GABA receptor-mediated synaptic responses can directly block action potential firing, and thirdly, interneurons can synchronise the firing of principal neurones during oscillations (Bernard et al., 2000; Khazipov, 2016).

There were differences between the systemic KA model and the mTLE model in our study, in terms of normalised mean SI. Epileptiform-like activity interpreted as the occurrence of abnormally enhanced neuronal excitability and synchronisation (Jiruska et al., 2013; Jefferys, 2015) is in agreement with our systemic model. Our result showed that normalised mean SI increased in ipsilateral-CA3 and contralateral-CA3 in the systemic model, but no change was observed in mTLE model (Table 3.2). Several neurological and psychiatric disorders, such as epilepsy, Alzheimer's and schizophrenia, cause alteration of synchronisation (Jiruska et al., 2013). Interactions between neuronal populations are determined using LFP, reflecting that the synaptic currents arise locally from these neuronal populations (Jiruska et al., 2013). Chemical synaptic interaction is a common mechanism of

communication between neurones (Diba et al., 2014; Jefferys, 2015). Both excitatory and inhibitory synaptic interactions may contribute to the synchronisation of neuronal activity (Timofeev et al., 2012). Neurotransmitter release by synaptic terminal leads to receptor activation on the post synaptic cell and generates inward or outward currents which depolarise or hyperpolarise the post-synaptic cells (Jiruska et al., 2013). The increasing firing rate in our systemic model indicates depolarisation at the post-synaptic cells. This depolarisation caused recruitment of a large population of neurones into increased synchronisation in ipsilateral-CA3 and contralateral-CA3 hippocampus in our study.

In our case, no changes in the normalised mean SI were found in the mTLE model (Figure 3.15C). We postulate that membrane impedance shunting could be one of the factors. Increased membrane conductance disrupts synaptic integration and results in decreased efficacy of excitatory transmission, which promotes termination of synchronisation. Another factor could be the inhibitory transmission mediated by interneurons. Their interconnectivity and connectivity with principal cells may contribute to synchronised excitation that causes strong synchronisation of inhibition (Pavlov et al., 2013). Thirdly, the absence of changes in membrane voltage in one neurone would not trigger current flow between coupled neurones, hence causing no changes in synchronising effect (Timofeev et al., 2012). The low-pass filter properties of gap junctions also prevent efficient transmission of a fast spike to coupled neurones (Salelkar et al., 2018).

Mean K-index decreased in ROI 2 and ROI 3 in the systemic model (Figure 3.17), but no changes were recorded in the mTLE model (Figure 3.18). Decreasing cross-correlation analysis indicates that neurones are uncorrelated, or that the two cells fire independently (Hilaire et al., 1984; Cohen and Kohn, 2011). The mTLE model indicates no changes between the degrees to which two cells are correlated in the time domain (Perkel et al., 1967; Gerstein and Perkel, 1972). Responses in the sensory cortex are desynchronised, although without any changes in neuronal firing rates (Poulet and Peterson (2008), but, this is not the case for the systemic model, where increasing neuronal firing rates were observed.

The hippocampal formation has well developed anatomical and physiological mechanisms that promote neuronal synchronisation, including the theta rhythm, beta and gamma oscillations and sharp-wave ripples. Gamma activity is found in CA3 (Timofeev et al., 2012). In our systemic model, beta and gamma were increased in both ipsi- and contralateral hippocampus. The present results suggest that the systemic model disrupts network oscillations in the hippocampus of rats under isoflurane-anaesthesia. This finding may have implications in understanding the functional development of epileptic activity and seizure propagation. Lehmkuhle et al. (2009) demonstrated that the gamma band could be used as an indicator of seizure severity during KA-induced SE. KA-induced gamma oscillations have been attributed to GABA_A receptor mediated inhibitory post synaptic potentials produced by inhibitory interneurons (Traub et al., 1996; Khazipov and Holmes, 2003; Khazipov, 2016). GABAergic interneurons respond with the highest temporal precision to frequencies in the gamma band, whereas pyramidal cells respond more reliably to lower-frequency rhythmic inputs (Buzsáki 2006). In the hippocampus, the CA3 subfield can essentially generate population oscillations at the theta and gamma frequencies, and such activity can propagate to CA1. GABAergic interneurons regulate when and where information flows in the structure of neurones and neuronal networks (Jacob et al., 2008; Khazipov, 2016). During anaesthesia, natural slow-wave sleep and REM, beta and gamma were also found to be synchronised between neighbouring cortical sites (Timofeev et al., 2012). During natural waking and sleep behaviour, the occurrence of spontaneous rhythmic field potential, for example, theta and gamma, correspond with an increase in the rate and synchrony of neuronal firing (Staba, 2012). The waking state of the brain is also characterised by the predominance of frequencies in the beta and gamma bands (Timofeev et al., 2012).

In our mTLE model, increased in the normalised mean power in the delta band was found in CA1 and CA3 in both ipsilateral and contralateral hippocampus (Figure 3.26 and 3.27). The interpretation of the delta wave is in agreement with the delta sleep pattern (Roohi-Azizi et al., 2017). During delta oscillation, the delta wave is associated with an increase in K⁺ conductance, which can be eliminated by the activating neurotransmitters: acetylcholine or norepinephrine (Buzsáki, 1988). Various factors such as decreasing input resistance of neurones, activity-dependent K⁺ current and gain of inhibition over excitation are considered opposing forces to

excitation that may return the network to a silent state (Metherate and Ashe, 1993). An anaesthetic that increases K^+ conductance or potentiates the action of GABA can prolong the down state in sleep, which could be one possible mechanism (Buzsáki, 2006) explaining the increased in delta band in our isoflurane-anaesthetised mTLE model. Neurones can sustain oscillation in the delta frequency band in the deepest stage of sleep (Roohi-Azizi et al., 2017). In contrast, cortical neurones in the waking brain stay virtually consistently in the up state. A major reason for this is that the main action of subcortical neurotransmitters is to decrease the K^+ conductance of cortical neurones (Buzsáki, 2006).

The present study also recorded that normalised mean power in the 0.1-1 Hz, delta, theta and beta bands were increased in the contralateral-CA3 hippocampus in our mTLE model. Normal oscillatory activities in the frequency of 0.1-15 Hz (0.1-1 Hz, delta, and theta) are present during SWS or anaesthesia (Timofeev et al., 2012) which is shown in our isoflurane-anaesthetised mTLE model. Theta frequency in rodents is considered to represent the ‘online state’ of hippocampal processing and is associated with information acquisition during exploration, spatial navigation, and various memory tasks, as well as memory consolidation and during rapid-eye-movement (REM) sleep in both rodents and humans (Buzsáki, 2006; Cobb and Vida, 2010). Pyramidal cells and some subpopulations of interneurones show intrinsic oscillations at theta frequencies, and other types of interneurones are known to play an important role in gamma oscillations (Khazipov and Holmes, 2003). However, the way in which network oscillations affect the activity of individual neurones in the network is still unknown.

Our results from the isoflurane-anaesthetised mTLE model contradict the findings of some other animal models: the awake pilocarpine-induced epilepsy animal model (Karunakaran et al., 2012) and the *in vivo* patch clamp recording (Khazipov and Holmes, 2003). These authors reported that the oscillations at low frequencies (up to 25 Hz) were significantly decreased after KA injection in an intact awake animal, but with no increase in gamma frequency (Karunakaran et al., 2012), while Khazipov and Holmes (2003) reported an increase in LFP gamma oscillations 5-10 mins post-KA injection. The difference in our model with the previous awake models could also in part be due to effects of isoflurane rather than just different KA doses.

Overall, the normalised mean power in the frequency bands indicated for seizure severity (beta and gamma frequency bands) did not appear in our mTLE model. Our mTLE model only showed increases in low frequency bands (delta) in ipsilateral-CA1, ipsilateral-CA3 and contralateral-CA1 hippocampus and 0.1-1 Hz, delta, theta and beta frequency bands in contralateral-CA3 (Fig. 3.26 and 3.27), which showed more or less similar results to the control group (saline+saline group). Based on these results, we postulate that the concentration of KA 1 mM used to induce epileptiform-like activity did not reach the threshold of severity which would alter the neuronal synchrony and oscillation, and subsequently alter the distribution of frequency bands. The function of the brain depends on cooperation between different networks that are mediated through oscillations within these networks. The transition from normal to epileptiform-like activity is caused by greater spread and neuronal recruitment secondary to a combination of enhanced connectivity, enhanced excitatory transmission, failure of inhibitory mechanisms (McCormick and Contreras, 2001), and changes in intrinsic neuronal properties (Duncan et al., 2006).

Considering all the results in this chapter, we have constructed a conceptualised diagram explaining the connectivity within the hippocampus and between hippocampi (Figure 3.28). The increased in firing rate and normalised mean power in the delta bands was shown in our mTLE model of KA-induced epileptiform-like activity in the ipsilateral-CA1.

We postulate that the signal travelled to subiculum (Sb) and further projected to the entorhinal cortex (EC) via the medial entorhinal cortex (MEC) and the lateral entorhinal cortex (LEC). Following the uni-directional network, the signal inputs from the EC were transferred to the dentate gyrus (DG) and ipsilateral-CA3 via the perforant path (PP) which splits into the lateral perforant path (LPP) and medial perforant path (MPP). However, there was no neuronal firing recorded in the ipsilateral-CA3 hippocampus. We conceptualised that the input was further transferred to contralateral-CA3 (as observed from the increase of firing rate and increases of 0.1-1 Hz, delta, theta and beta frequency bands) and contralateral-CA1 hippocampus (no neuronal firing) through the associational commissural (AC).

In conclusion, we were able to optimise and validate a rat model of KA-induced hyper-excitabile neuronal (epileptiform-like) activity for further investigation. Establishment of these hyper-excitabile models is crucial for the present study to investigate the effect of AEDs in the interaction of intra-hemispheric interaction in epileptogenesis and progression of epileptic seizure activity in human subjects.

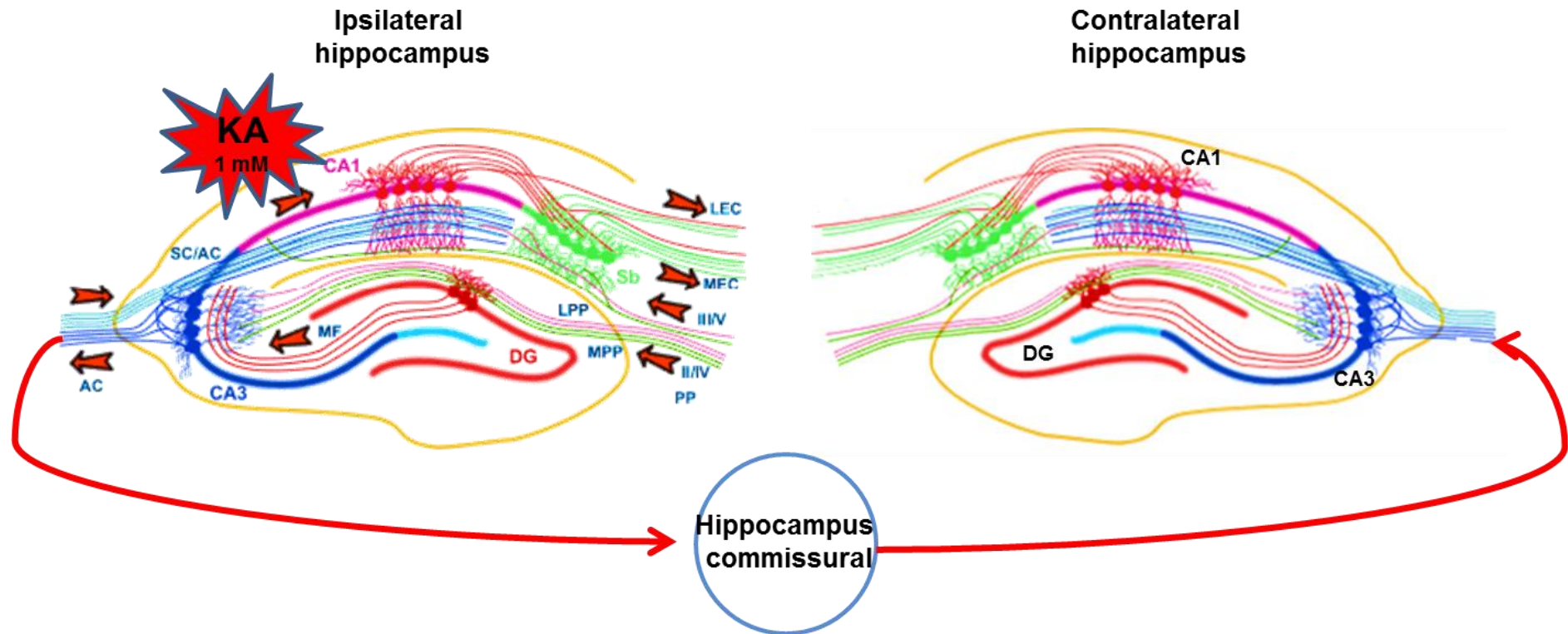


Figure 3. 28 Diagram of the mTLE model used in this study.

Kainic acid (KA 1 mM) administered locally following saline (0.5 mL) administered intravenously as an acute model of epileptiform-like activity in this thesis. This diagram of the hippocampus was adapted from <http://www.bristol.ac.uk/synaptic/pathways/>. The hippocampal functional connectivities were adapted from Amaral et al. (1999) and Neves et al. (2008).

DC=Dentate gyrus, **MF**=mossy fibres, **AC**=associational commissural, **SC**=Shaffer collateral pathway, **Sb**=subiculum, **LEC**=lateral entorhinal cortex, **MEC**=medial entorhinal cortex, **PP**=perforant path, **MPP**=medial perforant path, **LPP**=lateral perforant path.

Chapter 4

EFFECT OF THE ANTI-EPILEPTIC DRUG VALPROATE ON LOCAL KA-INDUCED EPILEPTIFORM-LIKE ACTIVITY

4.1 Introduction

Valproate (VPA) is categorised as a second-generation AED along with carbamazepine and the benzodiazepines, which differ chemically from the barbiturates (Shorvon, 2009; Löscher and Schmidt, 2011; Löscher, 2017; Musumeci et al., 2019). The first clinical trial for VPA in the treatment of epilepsy was reported in 1968, and subsequently in the United States in 1978. VPA was shown to be efficient in animal models of epilepsy and in humans for the treatment of partial and generalised seizures (Löscher, 1999; Lagace et al., 2004; Stafstrom and Carmant, 2015).

Several factors determine the anticonvulsant potency of VPA: the species, the route of administration, the type of seizure induction, and the time interval between drug administration and seizure induction. Different doses of VPA have been used over the years in various experiments (Chapman et al., 1982; Löscher, 1999; Nandhagopal, 2006). In general, the anticonvulsant potency of VPA increases in parallel with the size of the animal (Löscher, 1999). VPA administered via i.v was used in cats and baboons with doses of 100-200 mg/kg (Chapman et al., 1982). In humans, active doses of 20-30 mg/kg VPA i.v were used for the anticonvulsant activity of VPA (Chapman et al., 1982) and a loading dose of 20-25 mg/kg i.v of VPA for 40-65 min in refractory SE (Nandhagopal, 2006). These studies point out that humans require lower doses of VPA than animals because humans cannot tolerate high doses, which can be toxic (Chateauviac et al., 2010). Therefore, in our study, we administered a lower dose of 20 mg/kg of VPA to anaesthetised rats (which is within the dose concentration (20-30 mg/kg) used in humans), to determine

whether this dose is effective in a preclinical animal model of neuronal epileptiform-like activity.

The parenteral route of VPA administration into the brain (via i.p. or i.v. injection) is rapid, within 2-15 min (Löscher, 1999; Löscher, 2002). VPA also has a short half-life in most species; for example, 2-5 hours in rats and 9-18 hours in humans (Löscher, 1985; Löscher, 1999; Löscher, 2002). In our study, we selected i.v. as the route of administration for the drugs based on its instant effect on the brain (Löscher, 1999), its non-sedating effects and safe use (Martin and Pozo, 2006). The timing of drug administration up to 20 minutes before and after KA administration was chosen on the basis that i.v. injections are effective within 2-15 min (Löscher, 1999). A time window of 20 min up to 50 min of recording time after i.v. injection of 100 mg/kg VPA was used in the study by Löscher et al. (1995) and Rohlfs et al. (1996). The recording time of our experiment was 210 min (3.5 hours), which is in the range of the half-life of VPA, 2-5 hours in rats (Löscher, 1999). Therefore, in our study, the timing of drug administration and the recording time were sufficient to test the effect of VPA on epileptiform-like activity in anaesthetised rats.

The various mechanisms of VPA action are due to the numerous effects of the drug on neuronal tissue and its broad clinical activity in epilepsy and other brain diseases. The broad antiepileptic efficacy of VPA could be explained through the combination of several neurochemical and neurophysiological mechanisms in a single drug molecule (Löscher, 2002). The anticonvulsant identified in experimental and clinical studies of VPA suggest it has both direct and indirect action. The first is a direct pharmacological effect related to acute plasma and brain concentrations from high doses of VPA in animals. The other type of action is indirect and presumably relates to metabolites of VPA persisting in the brain, or to adaptive or other changes in membranes, receptors, or enzymes (Löscher, 1999; Löscher, 2002). One of the most extensively studied metabolites of VPA is trans-2-en-valproate. Previous studies by Semmes and Shan (1991) and Löscher (1992) have reported that this metabolite is more potent than VPA.

In a study by Lagace et al., (2004), the pharmacokinetics, mechanism of action and the clinical efficacy of VPA were reviewed. Löscher (1999) also reviewed the

pharmacodynamic properties and mechanism of action of VPA. The actions of VPA were reported to increase the level of GABA (Bruni, 1979; Ghodke-Puranik et al., 2013; Georgoff et al., 2019) and may result from inhibition of excitatory synaptic transmission following repetitive cell firing (Griffith and Taylor, 1988). VPA increases GABA synthesis and release, and thereby potentiates GABAergic functions in the substantia nigra, a brain region thought to be involved in the control of seizure generation and propagation (Löscher, 1999). The elevation of cerebral GABA levels and protection against different types of seizures after VPA administration depend on different dose responses used (Goldenberg, 2010; Tolou-Gamari and Palizban, 2015). For instance, GABA was induced during the first hour following acute VPA administration (200-600 mg/kg) in rodents by measuring the cortical or whole-brain GABA level (Chapman et al., 1982). Gent and Phillips (1980) reported that 200-400 mg/kg i.v VPA administration potentiated GABA responses in the medullary reticular formation. Meanwhile, a dose of 3-10 mM VPA potentiated GABA responses in cuneate afferent fibres in the rat (Harrison and Simmonds, 1982). The anticonvulsant effect of VPA depends on the attenuation of NMDA receptor-mediated excitation (Löscher, 1999 ;Löscher, 2002; Asif, 2016).

The mode of action of VPA also depends on the Na⁺ channel blockade (Cunningham et al., 2003; Ghodke-Puranik et al., 2013; Georgoff et al., 2019). In epilepsy therapy, VPA and other Na⁺ channel blockers including phenytoin, carbamazepine, lamotrigine, topiramate and lacosamide are thought to act selectively on Na⁺ channels (Mantegazza et al., 2010). Na⁺ channels are the molecular targets for drugs used in the treatment of epilepsy, cardiac arrhythmias, bipolar disorder and migraine, and in the prevention of acute pain (Mantegazza et al., 2010). Their therapeutic effect depends on the use-dependent action of the Na⁺ channel blocking drugs (Mantegazza and Catterall, 2012). A dose of 0.2–2 mM extracellularly applied VPA in the rat neocortical neurones in cell culture has reduced Na⁺ currents by ~25% (Zona and Avoli, 1990).

Previous studies have used VPA in treating different types of epilepsies. VPA enhances GABA release in neuronal cultures in mice and cortical slice in rats (Löscher, 1999). A study by Albus et al. (2008) has used VPA to block epileptiform activity in rat organotypic hippocampal slice cultures with other standard AEDs such

as carbamazepine, phenytoin, clonazepam and diazepam. VPA did not increase brain GABA significantly in patients with epilepsy, whereas increased glutamine suggested mild hyperammonemia associated with VPA therapy in patients with refractory complex partial seizures (Petroff et al., 1999). A study by Löscher and Hönek (1996) has shown that VPA administration of 200 mg/kg i.p also alters dopaminergic and serotonergic functions in rats. Acute VPA treatment can stimulate glutamate release in the cerebral cortex in rats (Löscher, 1999; Lagace et al., 2004). Based on the pharmacology, pharmacodynamic properties and mechanism of action of VPA, the diverse effects of VPA indicate that the drug has not only anticonvulsant but also have other pharmacodynamic and pharmacotherapeutic actions, such as antipsychotic and antidystonic efficacy. GABAergic potentiation and glutamate, or NMDA inhibition could be likely explanations for its anticonvulsant action on focal and generalised convulsive seizures, whereas the reduction of γ -hydroxybutyrate (GHB) release could plausibly be the effect of VPA on non-convulsive seizures, such as absences. VPA has reduced the release of the epileptogenic amino acid GHB in rats with a dose of 250-500 μ M (Vayer et al., 1988). Apart from that, VPA apparently exerts a direct action on ion channels, thereby limiting sustained repetitive neuronal firing (Löscher, 1999).

Furthermore, VPA works efficiently for the treatment of adults with generalised epilepsy (Löscher 1999; Kälviäinen and Keränen 2006; Tang et al., 2017). VPA has a wide spectrum of antiepileptic efficacy, and it is used in both localisation-related epilepsy and idiopathic generalised epilepsy, and also in childhood epilepsies (Shorvon, 2000; Chateauvieux et al., 2010; Mehndiratta et al., 2016). VPA was the drug least likely to be associated with treatment failure through inadequate seizure control and was the preferred drug to achieve a 12-month remission.

4.1.1 Aim and Hypothesis of this chapter

Considering the numerous mechanisms of action of VPA reported in the literature and its continuous use as an anticonvulsant against different types of seizures, in this chapter, we investigate the effect of AED, VPA on the rat model of KA-induced hyper-excitable neuronal activity before and after KA to evaluate the changes in the pattern of hippocampal neuronal unit firing, normalised mean SI, cross-correlations, and LFP frequency band distribution using an electrophysiological technique. We hypothesised that VPA would attenuate the epileptiform activity induced by KA due to its anticonvulsant effect.

4.2 Methods

4.2.1 Experimental procedure

Electrophysiological procedures were performed, as described in Chapter 2, Section 2.2 and Section 2.2.2. Briefly, male Lister Hooded rats (300-400g; n=12) were anaesthetised with 3.5% isoflurane in medical air (O₂) mixture (reduced to 1.5-2% before recording was initiated). Separate craniotomies were performed, and an eight-electrode stainless-steel microwire array (NB Labs, Texas, USA) connected to a Plexon Multichannel Acquisition Processor system (Plexon Inc. Texas, USA) were placed stereotaxically in the left (ipsilateral) and right (contralateral) dorsal hippocampus (Hpc AP-4.5mm; ML-4.0mm; DV-4.0mm) respectively, according to the coordinates in a rat atlas (Paxinos and Watson, 1998). The dura was retracted, and the exposed cortex was kept moist with saline.

4.2.2 Recording procedure

As explained in Section 2.5, the electrodes were allowed to settle for ~ 60 min after they were lowered into the dorsal hippocampus, before recordings were made over a total period of 210 min. In this chapter, three groups were examined, following a 10 min basal period. Rats were administered with either:

1. KA-induced epileptiform-activity group (positive control; **saline+KA 1 mM**), n= 6 rats: 0.5 mL saline (i.v) followed by local KA administration (1 mM, 1 μ L) after 20 minutes Figure 4.1A).

2. Valproate before KA group administration (**VPA+KA**), n= 6 rats: Valproate (VPA; 20 mg/kg, i.v) followed by local KA administration (1 mM, 1 μ L) after 20 minutes (Figure 4.1B).

3. Valproate after KA group administration (**KA+VPA**); n= 6 rats: local KA (1 mM, 1 μ L) followed by Valproate (VPA; 20 mg/kg, i.v) after 20 minutes (Figure 4.1C).

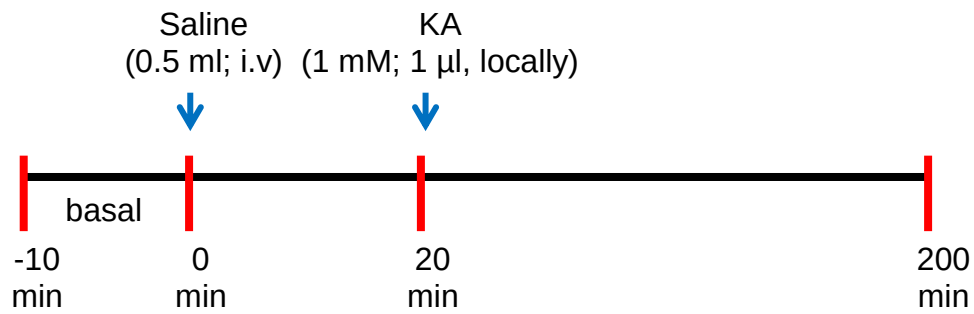
KA (1 mM; 1 μ L) was locally injected into the left (AP: -4.5 mm; ML: $\pm$ 4.0 mm; VD: -4.0mm; ipsilateral) hippocampus (Karunakaran et al., 2012; Pitkänen et al., 2006). This was administered via a cannula attached to the electrode array at a position 0.5 mm above the shorter electrode using a Hamilton syringe (1 μ L 7101 Flat; Scientific Laboratory Supplies LTD, UK). The KA injection was administered slowly with an air bubble as a marker over a period of about 2 min.

4.2.3 Data analysis

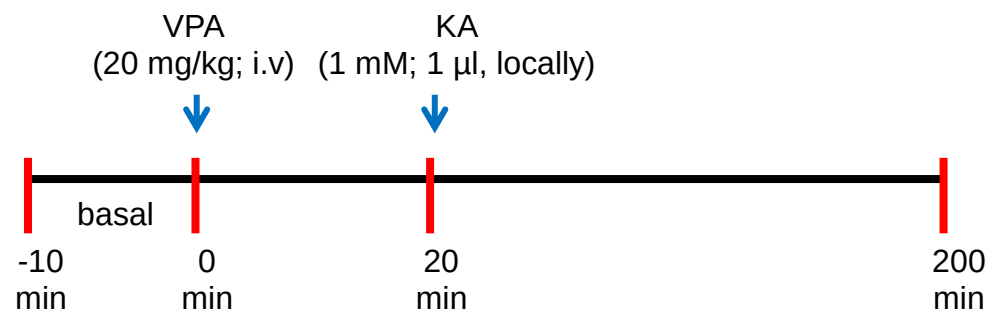
Extracellular unit activity data from the animals with confirmed hippocampal electrode placements were sorted into individual neuronal units using *Offline Sorter*, analysed with *NeuroExplorer* and also exported in MATLAB format for further analysis, described in detail in Chapter 2, Section 2.7, 2.7.1, 2.7.2, 2.7.3 and 2.7.4.

Due to the longer duration of the experiment, the frequency band distribution extracted the power (mV^2) from the recording area into constituent frequency bands [0.1-1, delta (1-4 Hz), theta (4-8 Hz), alpha (8-14 Hz), beta (14-30 Hz) and gamma (30-170 Hz)]. The frequency bands were calculated for time epochs of 10 min (epochs at -0-10 min, 0-10 min, 20-30 min, 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min) to identify any changes before, during or after treatment. Values were then pooled, normalised and displayed in histograms. Overall normalised mean power from each brain region was statistically assessed using two-way ANOVA repeated measures (main effect) followed by Tukey for *post-hoc* comparison. The data were further analysed with two-way ANOVA repeated measures to see the interaction between frequency bands with time followed by a Dunnett's multiple comparisons *post hoc* test to see at what time point the changes started.

A. Saline+KA



B. VPA+KA



C. KA+VPA

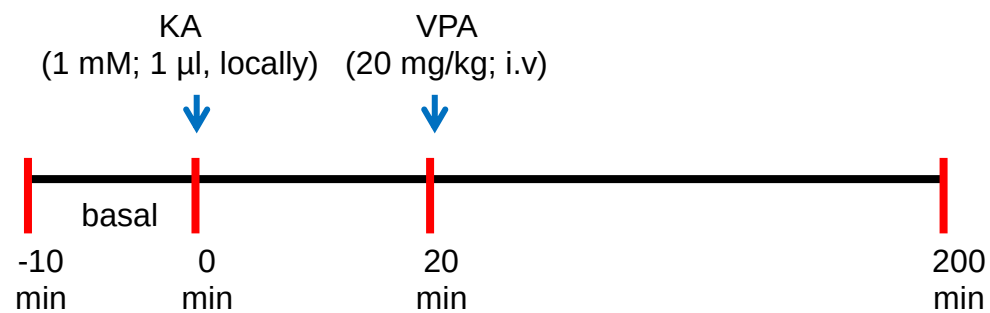


Figure 4.1 Time-line experimental protocols for KA and sodium VPA administration.

A: Saline was injected intravenously after 10 min basal recording followed by 1 mM of local KA administration after 20 min (**saline+KA 1 mM**). The recording was continued until 200 min, n=6 rats.

B: Valproate (VPA, 20 mg/kg) was injected intravenously after 10 min basal recording followed by 1 mM of local KA administration after 20 min (**VPA+KA**). The recording was continued until 200 min, n=6 rats.

C: 1 mM of KA was administered locally after 10 min basal recording followed by valproate (VPA, 20 mg/kg) intravenous injection after 20 min and further recording until 200 min (**KA+VPA**), n=6 rats.

4.3 Results

4.3.1 Histology of the location of electrodes recording sites

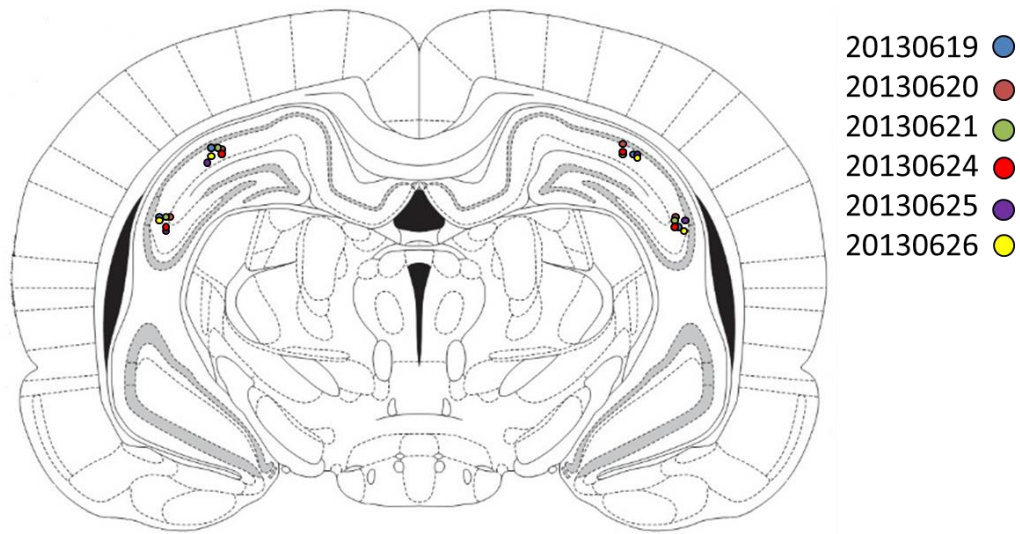
In this chapter, we are comparing three groups of rats and treatments (saline+KA, VPA+KA and KA+VPA). The rats in the saline+KA 1 mM are the same group of rats used in Chapter 3; however, the experiment time was extended to 210 min (3.5 hours). The histology of the saline+KA group has been given in Section 3.3.1.

In the VPA+KA group, six rats (rat ID: 20130619, 20130620, 20130621, 20130624, 20130625 and 20130626) were used to record hippocampus activity in both hemispheres (Figure 4.2A).

Another set of six rats (rat ID: 20130520, 20130523, 20130617, 20130618, 20130715, 20140124) were used in the KA+VPA group, as shown in Figure 4.2B. Two of the rats died during the recording; therefore these rats were excluded from the analysis (labelled as withdraw in Figure 4.2B).

The histology of the rat's brains showed the area of electrodes recording sites and each site was marked as a coloured dot (each dot representing one rat used in this experiment).

A. VPA+KA



B. KA+VPA

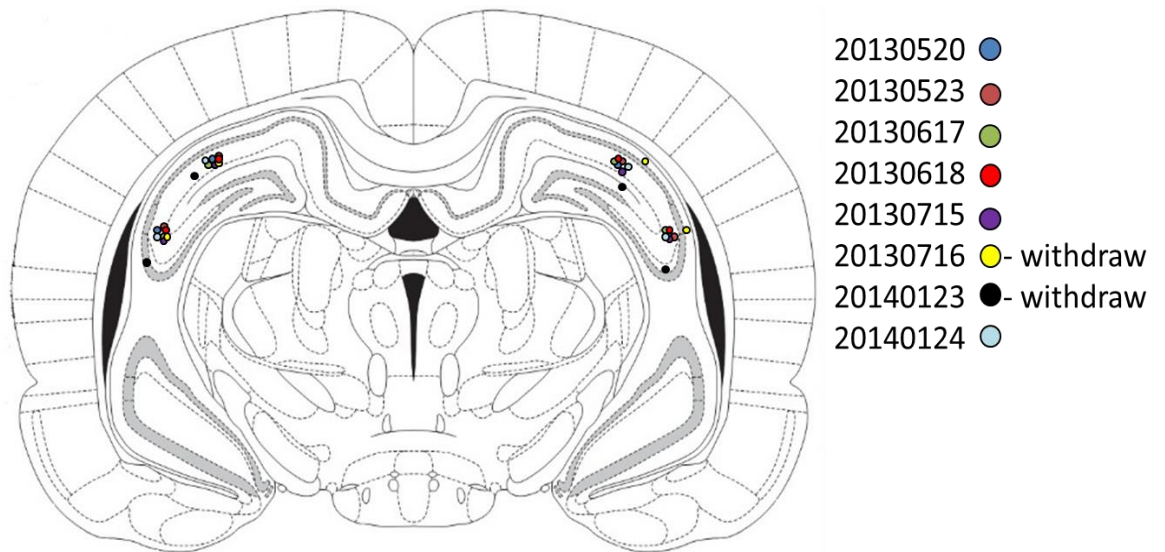


Figure 4.2 Schematic histological verification of hippocampal recording sites.

A: VPA+KA group of n=6 rats

B: KA+VPA group of n=6 rats

Each colour represents one rat used in this experiment.

4.3.2 Normalised mean firing rate

Comparisons of normalised mean firing rate were made between 3 groups of rats to see the effect of the drug before and after seizure. From the validation of the previous experiment in Chapter 3, we selected the epileptic rat model of saline+KA 1 mM administered locally.

The first group of rats was administered with VPA before KA-induced neuronal epileptiform-like activity (VPA+KA) to determine the effect of the drug in preventing the seizure. The second group of rats was injected with VPA after KA-induced neuronal epileptiform-like activity (KA+VPA) to see the effect of the drug in attenuating the seizure induced.

Comparisons were made using two-way ANOVA repeated measures analysis to determine the significant differences between groups of different treatments (saline+KA, VPA+KA and KA+VPA), the time course of the experiment, the interaction between groups of treatments, and the time before and after-KA induced neuronal hyper-excitability (epileptiform-like) at a particular brain region (ipsilateral-CA1, ipsilateral-CA3, contralateral-CA1 and contralateral-CA3 hippocampus).

Tukey's multiple comparisons *post hoc* test was done to see which groups of treatments differed from one another. Only the significantly different group was selected for one-way ANOVA with Dunnett's *post hoc* test to see at what time point the effect started, compared to basal.

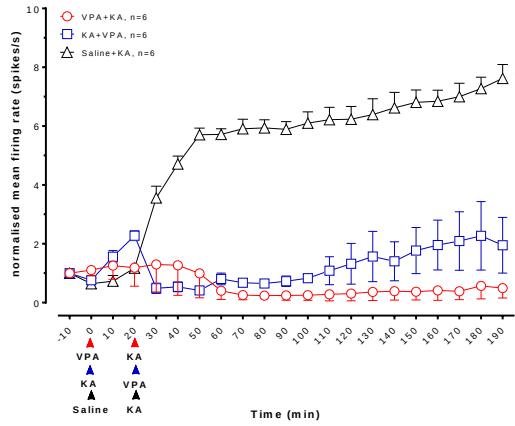
Based on two-way ANOVA repeated measures, KA had significant effects on the hippocampal mean firing rate of neurones in **ipsilateral-CA1** hippocampus in group of treatments x time interaction (Figure 4.3A; $F_{(40, 200)} = 20.3, p < 0.0001$), time course of the experiment ($F_{(40, 200)} = 14.48, p < 0.0001$) and group of treatments ($F_{(2, 10)} = 64.90, p < 0.0001$). No significant differences in normalised mean firing rate of neurones were found in **ipsilateral-CA3** hippocampus (Figure 4.3B) between group of treatments x time interaction ($F_{(4, 200)} = 1.011, p > 0.05$) or group of treatments ($F_{(2, 10)} = 0.7644, p > 0.05$). There were significant differences in the time course of the experiments ($F_{(20, 200)} = 4.602, p < 0.0001$). No significant differences in normalised

mean firing rate of neurones were found in **contralateral-CA1** hippocampus (Figure 4.3C) between group of treatments x time interaction ($F_{(40, 300)} = 1.229, p>0.05$), group of treatments ($F_{(2, 15)} = 0.4729, p>0.05$) or time course of the experiment ($F_{(20, 300)} = 0.7631, p>0.05$). There were significant differences in normalised mean firing rate of neurones in the **contralateral-CA3** hippocampus (Figure 4.3D) between group of treatments x time interaction ($F_{(40, 280)} = 9.48, p<0.0001$), time course of the experiment ($F_{(20, 280)} = 18.41, p<0.0001$) and group of treatments ($F_{(2, 14)} = 11.18, p<0.005$).

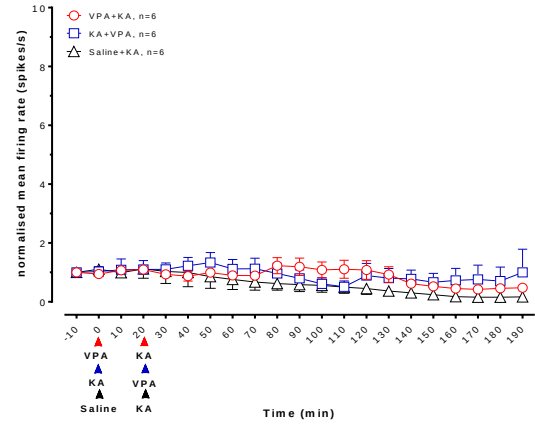
We would like to highlight that the recording was longer in the **saline+KA** group (n=6 rats) in this experiment (210 min) than in the ones described in Chapter 3 (80 min). Using Tukey's multiple comparison test, the firing rate in the mTLE group was significantly different in **ipsilateral-CA1** ($^d p<0.0001$) when compared to other groups (Figure 4.3A). No significant changes were found in **ipsilateral-CA3** or **contralateral-CA1** (Figure 4.3B and C). However, significant changes were found in the normalised mean firing rate in **contralateral-CA3** ($^a p<0.005$) when compared to KA+VPA (Figure 4.3D). Apart from that, a significant difference was also found between VPA+KA and KA+VPA in contralateral-CA3 ($^b p<0.05$; Figure 4.3D).

Only groups of treatments which showed significant changes in Tukey's multiple comparisons test were selected for further analysis using one-way ANOVA to determine at what time point the changes started.

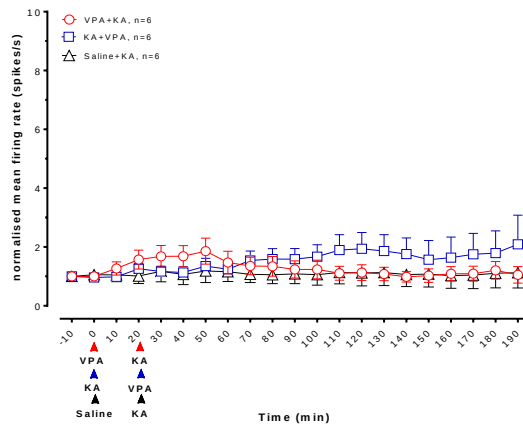
A. Ipsilateral-CA1



B. Ipsilateral-CA3



C. Contralateral-CA1



D. Contralateral-CA3

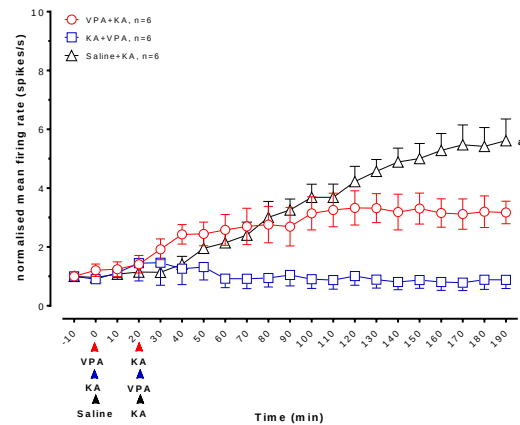


Figure 4.3 Normalised mean firing rate of neurones from the ipsilateral (CA3 and CA1) and contralateral (CA3 and CA1) hippocampus from **saline+KA** experiments (n=6 rats), **VPA+KA** experiments (n=6 rats) and **KA+VPA** experiments, (n=6 rats).

A: Firing rate of neurones from the ipsilateral-CA1 hippocampus, $F_{(40, 200)} = 20.3$ (interaction), $p < 0.0001$

B: Firing rate of neurones from the ipsilateral-CA3 hippocampus, $F_{(40, 200)} = 1.011$ (interaction), $p > 0.05$

C: Firing rate of neurones from the contralateral-CA1 hippocampus, $F_{(40, 200)} = 1.229$ (interaction), $p > 0.05$

D: Firing rate of neurones from the contralateral-CA3 hippocampus, $F_{(40, 280)} = 9.480$ (interaction), $p < 0.0001$

Expressed as normalised mean firing rate $\pm$ SEM.

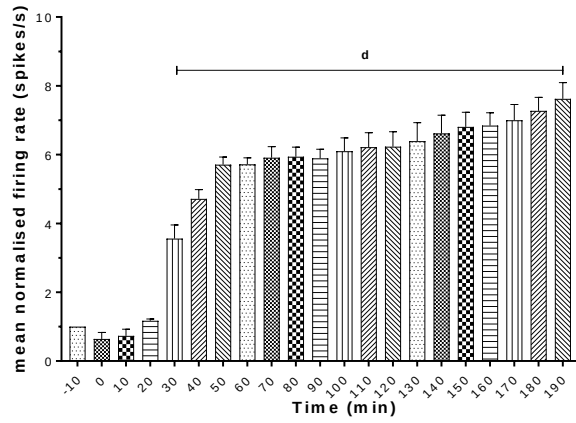
Two-way ANOVA repeated measures.

For *post hoc* Tukey's multiple comparisons test; ^a $p < 0.0001$ when compared to other groups, ^b $p < 0.05$ when compared to KA+VPA, ^b $p < 0.005$ when compared to KA+VPA

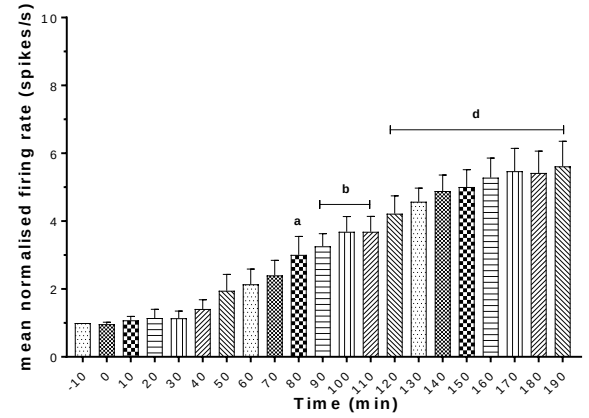
The normalised mean firing rate of the **saline+KA** group in ipsilateral-CA1 was significantly different 10 min after 1 mM of KA was administered locally (one-way ANOVA, Figure 4.4A, $F_{(20, 84)} = 41.71, p < 0.0001$) when compared to basal. KA also significantly increased normalised mean firing rate in contralateral-CA3 (Figure 4.4B, $F_{(20, 105)} = 14.39, p < 0.0001$). These significant differences show that KA induced seizure after 60 min (^a $p < 0.05$), 70-90 min (^b $p < 0.005$) and 100-170 min (^d $p < 0.0001$) of KA administration compared to basal (Figure 4.4B).

In the **VPA+KA** group, the normalised mean firing rate of neurones was significantly different in contralateral-CA3 (Figure 4.4C, $F_{(20, 105)} = 2.614, p < 0.001$). The firing rate increased at 100-190 min (^a $p < 0.05$) after 70 min of KA administration compared to basal in contralateral-CA3.

A. ipsilateral-CA1 of Saline+KA



B. Contralateral-CA3 of Saline+KA



C. Contralateral-CA3 of VPA+KA

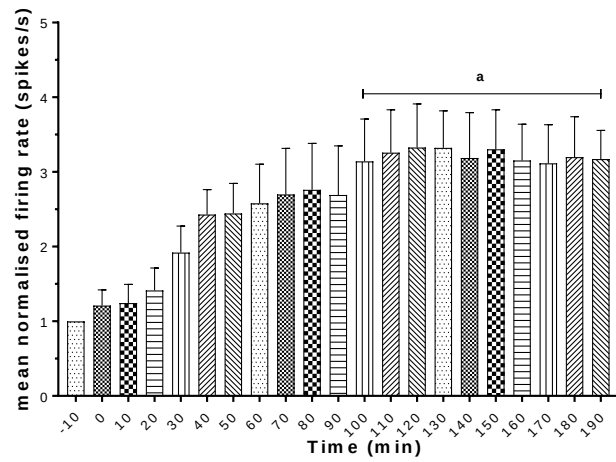


Figure 4.4 Normalised mean firing rate of A) ipsilateral-CA1 of saline+KA and B) contralateral-CA3 of saline+KA and C) contralateral CA3 of VPA+KA experiments (n=6 rats).
^a $p < 0.05$, ^b $p < 0.005$ and ^d $p < 0.0001$ when compared to basal, respectively.
 One-way ANOVA and *post hoc* Dunnett's multiple comparisons test.

In summary, these significant differences showed that firing increased in ipsilateral-CA1 and contralateral-CA3 in the saline+KA experiment, indicating that seizure was induced by KA in these two brain regions. The increasing normalised mean firing rate in ipsilateral-CA1 is expected, as KA was directly delivered in between CA1 and CA3 ipsilateral hippocampus. The increasing normalised mean firing rate in contralateral-CA3 shows that a seizure induced at the ipsilateral can spread to the contralateral, specifically in CA3 hippocampus, without direct local injection of KA (Figure 4.3A and D). This pattern shows connectivity between ipsilateral and contralateral hippocampus. KA has increased the firing rate in ipsilateral-CA1 and contralateral-CA3 but suppressed the firing rate in ipsilateral-CA3 and contralateral-CA1. With a longer experiment extending from 80 min (refer to Chapter 3), increasing the effect of the firing rate can be observed particularly in contralateral-CA3 where the firing increased further from 70 min onwards. In contrast, if we refer to Chapter 3, we can only observe the effect up to 70 min without knowing what will happen when longer experiments are conducted.

In the VPA+KA group, VPA was observed to prevent the increase in firing rate induced by KA in ipsilateral-CA1, ipsilateral-CA3 and contralateral-CA1 (Figure 4.3A-C). However, in contralateral-CA3, the significant difference indicates that VPA did not prevent neuronal firing induced by KA but reduced the KA effect to about 55% compared to saline+KA (Figure 4.3D).

On the other hand, in the KA+VPA group, VPA administered after KA attenuated KA-induced epileptiform activity in ipsilateral-CA1, ipsilateral-CA3, contralateral-CA1 and contralateral-CA3 hippocampus (Figure 4.3A-D).

4.3.3 Normalised mean synchrony index (SI)

Two-way repeated measures ANOVA with Dunnett *post hoc* multiple comparison tests was conducted to see the significant changes in normalised mean SI.

Saline+KA: SI increases in ipsilateral-CA1 and contralateral-CA3 hippocampus

The pooled data (n= 6 rats) of eight 10 min epochs of synchrony index in the saline+KA group were analysed by normalised mean SI, as shown in Figure 4.5B. Significant differences were detected in interaction of brain region x time ($F_{(21, 119)} = 5.768$, $p > 0.0001$), brain region ($F_{(3, 17)} = 13.970$, $p > 0.0001$) and time ($F_{(7, 119)} = 7.066$, $p > 0.0001$) (Two-way repeated measures ANOVA: Figure 4.5A). Normalised mean SI was significantly increased in ipsilateral-CA1 during epochs from 50-60 min onwards ($^d p < 0.0001$, respectively) and significantly increased in contralateral-CA3 during the epochs at 80-90 min (1.525 ± 0.218 ; $^a p < 0.05$) and during the epochs from 110-120 min onwards ($^b p < 0.005$) compared to basal recordings, respectively (Figure 4.5A; Dunnett's *post hoc* test).

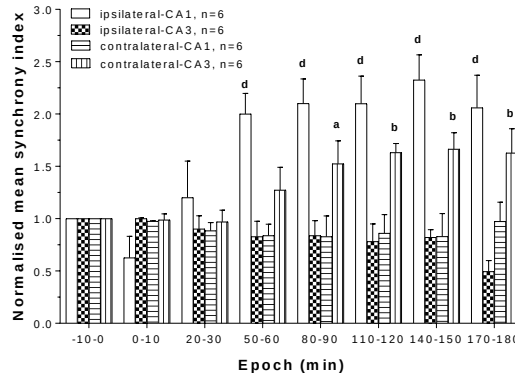
VPA+KA: SI increases in contralateral-CA3

There was no significant interaction in the VPA+KA group ($F_{(21, 105)} = 1.289$, $p > 0.05$), brain region ($F_{(3, 15)} = 1.987$, $p > 0.05$) or time ($F_{(7, 105)} = 0.9061$, $p > 0.05$). However, Dunnett's *post hoc* test showed that SI in contralateral-CA3 was significantly increased at the epochs of 110-120 min ($^b p < 0.005$), 140-150 min and 170-180 min ($^a p < 0.05$) when compared to basal (-10-0 min; Figure 4.5B).

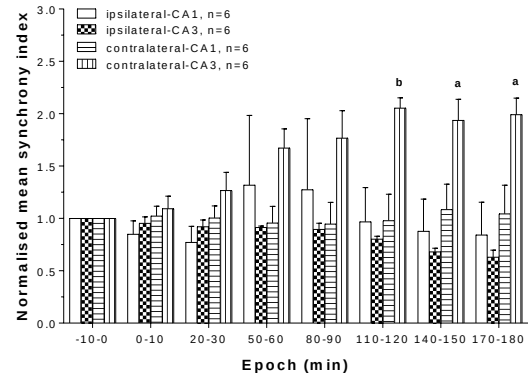
KA+VPA: No significant changes in SI

No significant differences in interaction of brain region x time ($F_{(21, 112)} = 1.256$, $p > 0.05$), brain region ($F_{(3, 16)} = 0.9305$, $p > 0.05$) and time $F_{(7, 112)} = 1.933$, $p > 0.05$) were shown in any brain regions (Figure 4.5C).

A. Saline+KA 1 mM



B. VPA+KA



C. KA+VPA

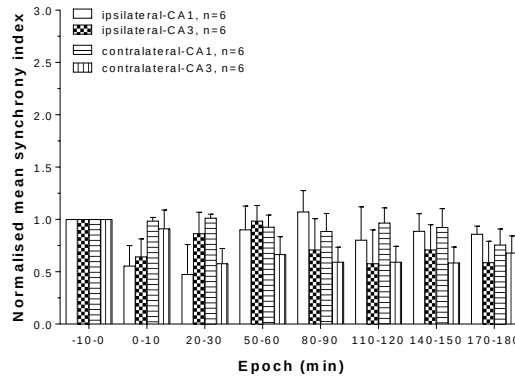


Figure 4.5 Histograms of normalised mean SI.

SI of neurones from the ipsilateral-CA1, ipsilateral-CA3, contralateral-CA1 and contralateral-CA3 hippocampus, (mean $\pm$ SEM).

A: Normalised mean synchrony index of saline+KA, n=6 rats.

B: Normalised mean synchrony index of VPA+KA, n=6 rats.

C: Normalised mean synchrony index of KA+VPA, n=6 rats.

^a $p < 0.05$, ^b $p < 0.005$ and ^d $p < 0.0001$ when compared to basal (-10-0 min), respectively.

Two-way ANOVA repeated measures and *post hoc* Dunnett's multiple comparisons test.

Overall, the saline+KA group shows a significant increase in normalised mean SI in ipsilateral-CA1 and contralateral-CA3 hippocampus after KA administration when compared to basal, whereas, in VPA+KA group, normalised mean SI was significantly increased in contralateral-CA3. Increasing normalised mean SI in the saline+KA group is in parallel with the increasing of normalised mean firing rate (Figures 4.3 and 4.4), indicating that a synaptic network of repetitive firing generates synchronisation during seizures.

4.3.4 Unit-pair cross-correlation

Mean k-index (pooled data of n= 6 rats in each group: saline+KA, VPA+KA and KA+VPA) was calculated to determine the strength of the correlation.

Saline+KA: High correlation among neurones in ROI 1

Ordinary two-way ANOVA analysed with Dunnett's *post hoc* multiple comparisons tests were conducted to see the significant differences in neurones in region of interest (ROI). Significant differences were found between region of interest ($F_{(2, 4560)} = 32.31, p < 0.0001$), time course of the experiment ($F_{(7, 4560)} = 4.018, p < 0.001$) and interaction of ROI x time ($F_{(14, 4560)} = 3.83, p < 0.0001$).

The mean k-index of ROI 1 from the saline+KA group was significantly increased at epochs of 110-120 min (333.70 ± 122.2 ; $^d p < 0.0001$) and 170-180 min (276.79 ± 86.99 ; $^b p < 0.005$) compared to basal (Figure 4.6A, -10-0 min, 160.42 ± 49.7). The pattern indicates a high correlation among neurones in ROI 1.

VPA+KA: Neurones in VPA administered before KA were highly correlated in ROI 1

The mean k-index of neurones from the VPA+KA group showed only neurones in the ipsilateral hippocampus (ROI 1) were significantly different at the epochs of 110-120 min (Figure 4.6B; 188.74 ± 127.44 , $^b p < 0.005$) and 140-150 min (249.69 ± 106.24 , $^d p < 0.0001$) compared to basal (61.57 ± 33.23). This pattern shows that

neurones in ROI 1 were highly correlated after 90 min of KA administration compared to basal.

KA+VPA: Mean K-index increased in ROI 2

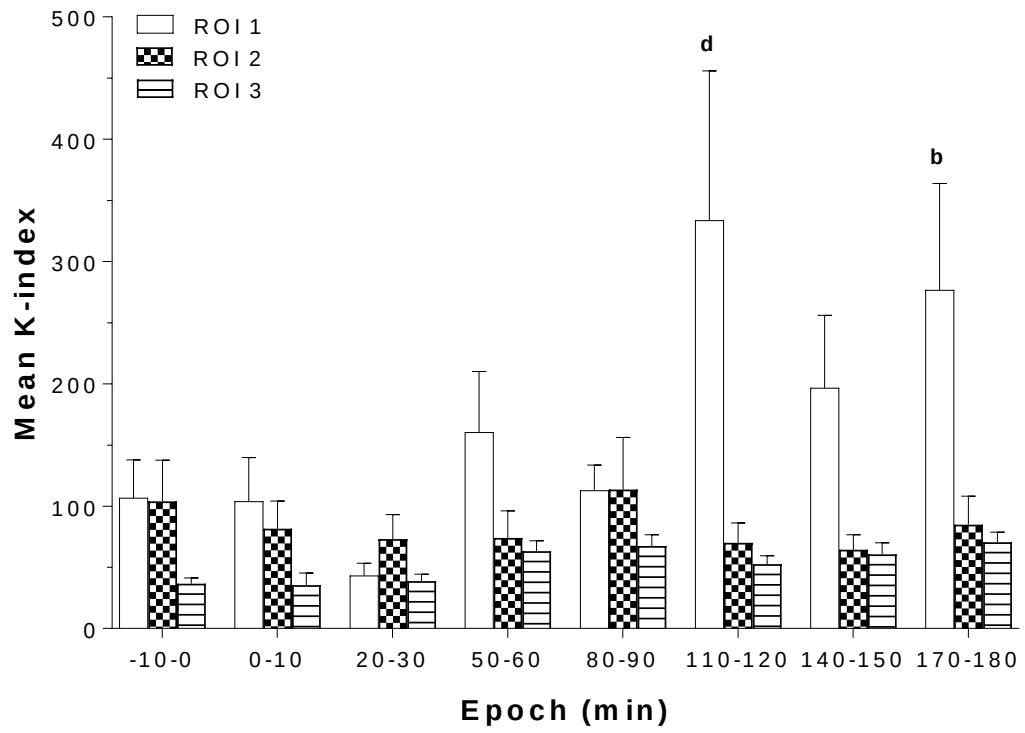
Significant increase in the mean k-index of neurones at the epochs of 140-150 min (92.85 ± 60.11 , ^a $p < 0.05$) were found in ROI 2 compared to basal (Figure 4.6C).

In summary, the mean K-index result shows that the neurones in ipsilateral (ROI 1) were highly correlated after KA administration in the **saline+KA** group, indicating that the neurones were highly connected with one another during the seizure. However, there were no changes of correlation among neurones in contralateral (ROI 2) or between neurones of ipsilateral and contralateral (ROI 3).

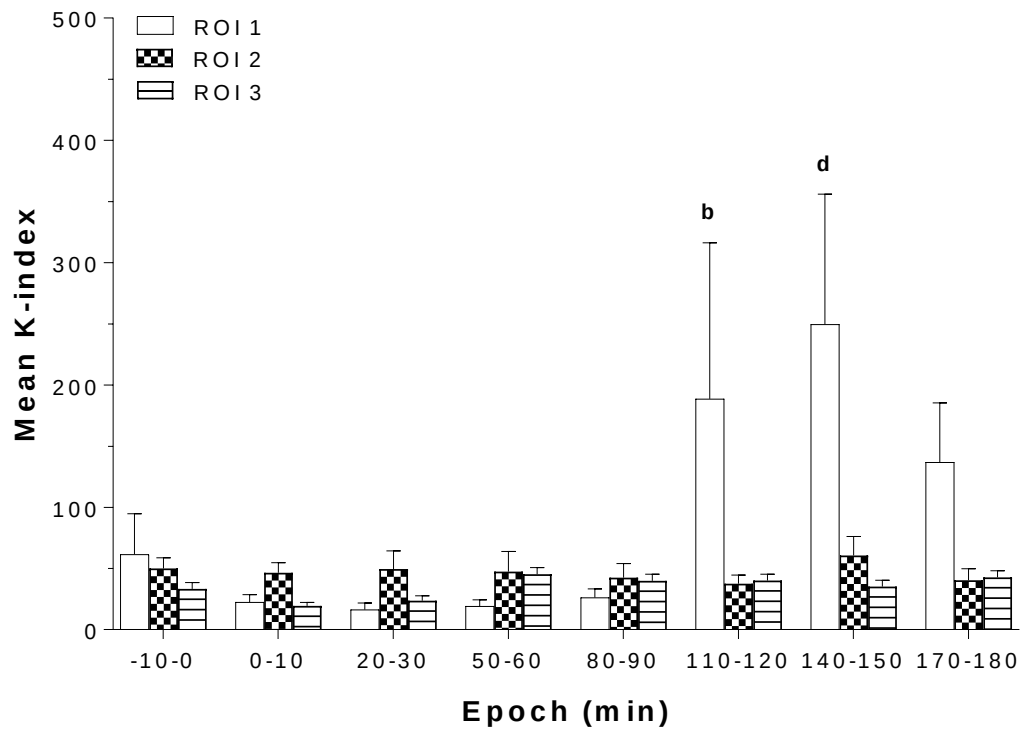
In the **VPA+KA** group, the mean K-index also showed a significant increase in ROI 1, indicating that the neurones were highly connected ipsilaterally after 90 min of KA administration and there were no changes in correlation in ROI 2 or ROI 3.

In contrast, there were no significant changes of mean k-index in ROI 1 or 3 in the **KA+VPA** group; however, the neurones were highly correlated with one another contralaterally (ROI 2).

A. Saline+KA



B. VPA+KA



C. KA+VPA

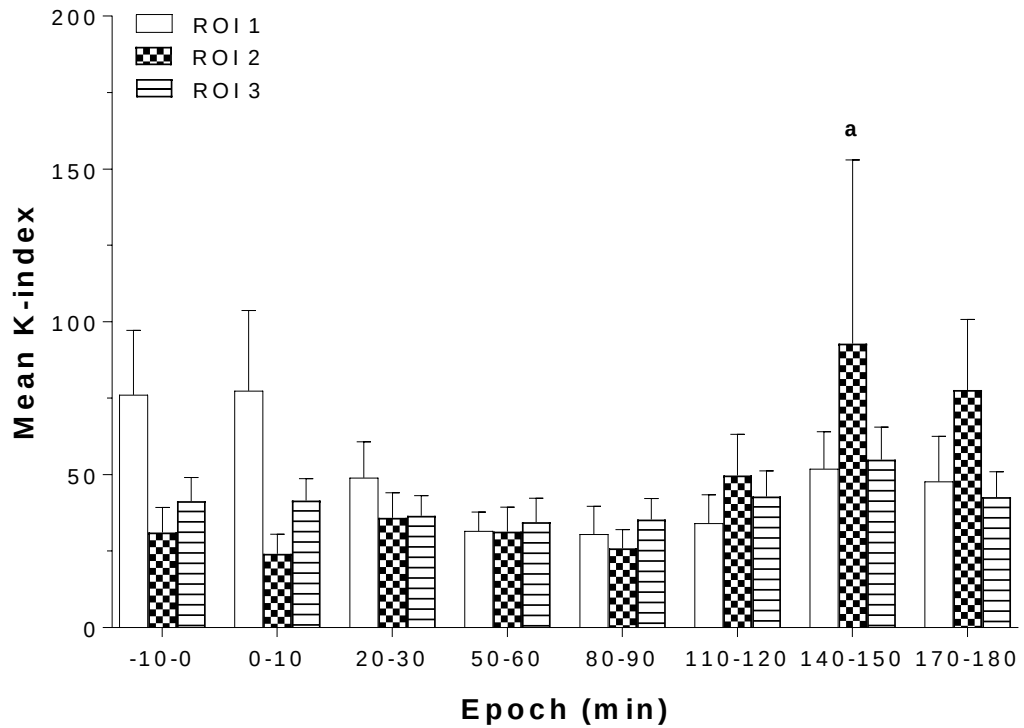


Figure 4.6 Graphs of mean k-index of all neurones in the experimental group.

A: Saline+KA group, n=6 rats.

B: VPA+KA group, n=6 rats.

C: KA+VPA group, n=6 rats.

^a $p < 0.05$, ^b $p < 0.005$ and ^d $p < 0.0001$ when compared to basal, respectively.

Ordinary two-way ANOVA with Dunnett's multiple comparisons test.

ROI 1=ipsilateral CA1 and CA3; ROI 2=contralateral CA1 and CA3; ROI 3=neurones in both ipsilateral and contralateral hippocampus.

4.3.5 LFP Frequency Band Distribution

Changes in LFP frequency band distribution provide information about which frequency bands were prominent through the time course of the experiment and how KA and VPA might affect the pooled frequency band distribution from six rats. The prominent frequency bands provide additional information about the physiology of functional connectivity in the hippocampus in acute mTLE model and the changes associated with VPA administration (before or after KA-induced epileptiform-like activity).

Saline+KA increased the delta and theta frequency bands in the ipsilateral and contralateral hippocampus

Significant changes in the interaction of overall LFP power were found in the interaction between brain region x time ($F_{(21, 105)} = 5.634, p < 0.0001$), time course of the experiment ($F_{(7, 35)} = 8.954, p < 0.0001$) and all brain regions of rats (Figure 4.7; $F_{(3, 15)} = 10.84, p < 0.001$; two-way ANOVA repeated measures).

Using Tukey's multiple comparisons test, normalised mean power in ipsilateral-CA3 was significantly different compared to ipsilateral-CA1 (^a $p < 0.05$). A significant difference was also found in contralateral-CA1 compared to ipsilateral-CA1 (^b $p < 0.005$). There was a significant difference in normalised mean power between contralateral-CA3 and ipsilateral-CA1 (Figure 4.7; ^c $p < 0.001$).

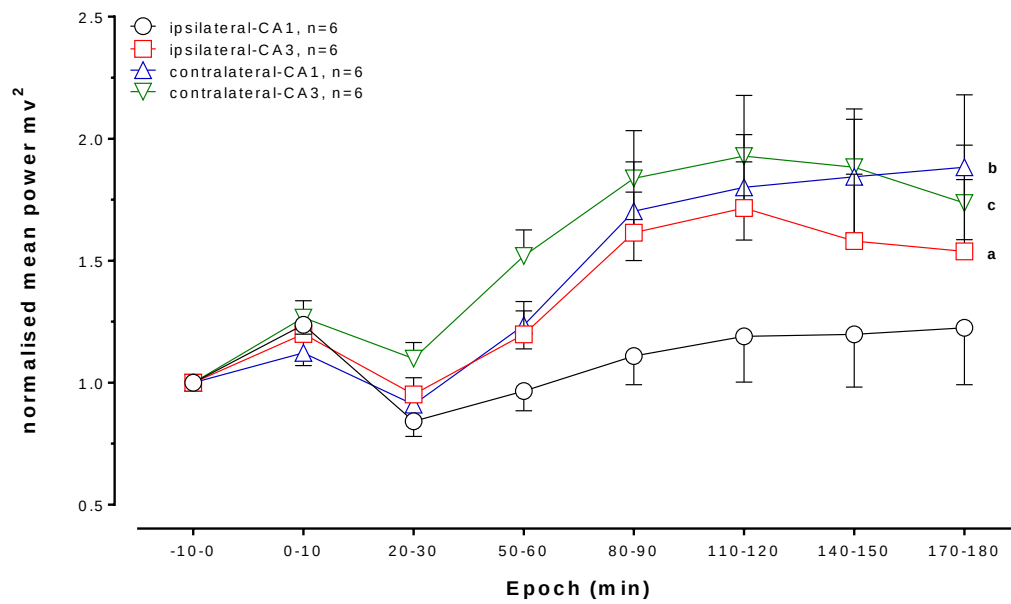


Figure 4.7 Normalised mean power of LFP in **saline+KA** group in 210 min experiment, mean $\pm$ SEM, n=6 rats.

Significant interaction, $F_{(21, 105)} = 5.634$, $p < 0.0001$.

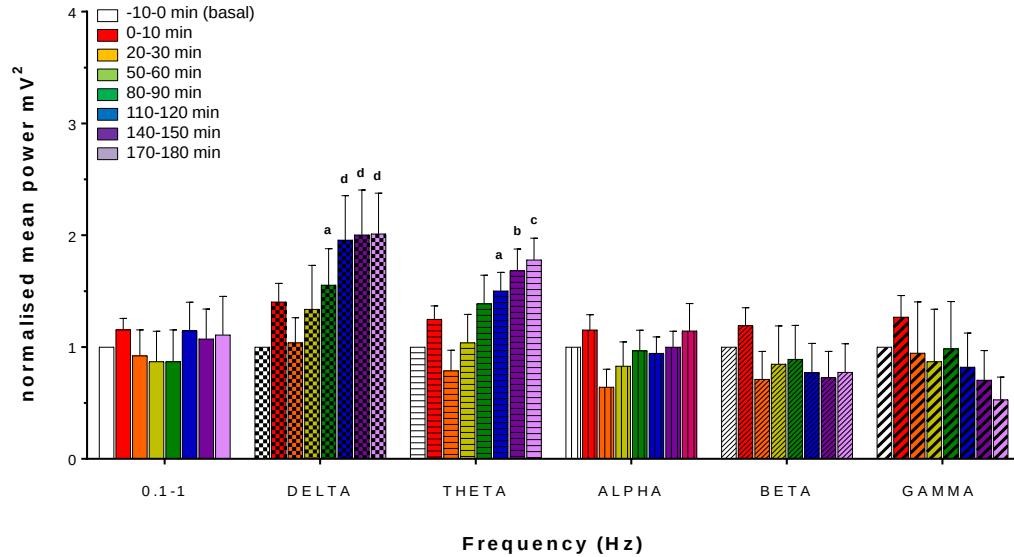
^a $p < 0.05$, ^b $p < 0.005$ and ^c $p < 0.001$ when compared to ipsilateral-CA1, respectively.

Two-way ANOVA repeated measures and *post hoc* Tukey's multiple comparisons tests.

In **ipsilateral-CA1**, there is significant interaction between time x frequency band ($F_{(35, 175)} = 3.68$, $p < 0.0001$; two-way ANOVA repeated measures). Using Dunnett's multiple comparisons test, the normalised mean power of the **delta** band in the saline+KA group was significantly different at epoch of 80-90 min (1.56 ± 0.33 ; $^a p < 0.05$), epochs of 110-120 min, 140-150 min and 170-180 min ($^d p < 0.0001$) compared to basal (-10-0 min;) as shown in Figure 4.8A. There was also significant difference in the normalised mean power of the **theta** band at 110-120 min (1.50 ± 0.17 ; $^a p < 0.05$), 140-150 min (1.68 ± 0.19 ; $^b p < 0.005$) and 170-180 min (1.78 ± 0.19 ; $^c p < 0.001$) compared to basal.

Significant interaction was found in the **ipsilateral-CA3** ($F_{(35, 175)} = 2.593$, $p < 0.0001$; two-way ANOVA repeated measures). Figure 4.8B shows that there are significant differences in the **delta** band in ipsilateral-CA3 at the epochs of 80-90 min (2.31 ± 0.51 ; $^a p < 0.05$), 110-120 min (2.10 ± 0.21 ; $^b p < 0.005$), 140-150 min and 170-180 min ($^d p < 0.0001$). A significant difference was also found in the **theta** band in ipsilateral-CA3 at the epochs of 110-120 min (2.06 ± 0.34 ; $^c p < 0.001$), 140-150 min and 170-180 min ($^d p < 0.0001$) compared to basal.

A. Ipsilateral-CA1



B. Ipsilateral-CA3

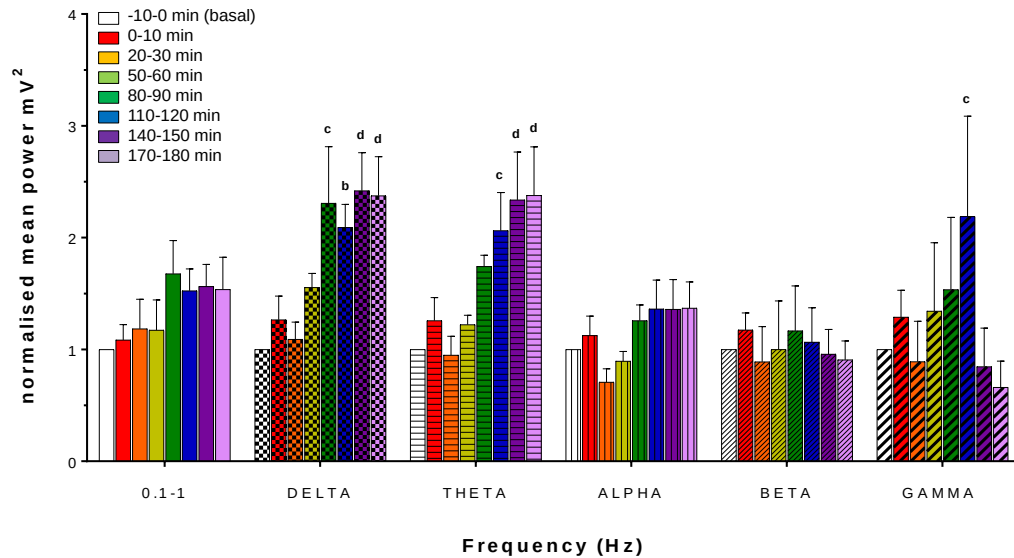
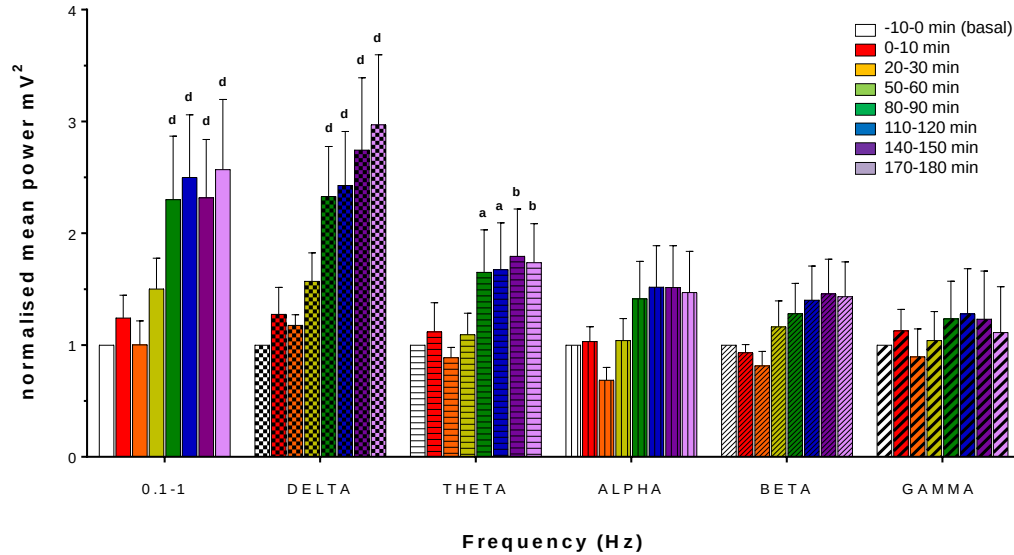


Figure 4.8 Normalised mean power of LFP frequency band distribution in the ipsilateral hippocampus from **saline+KA** group throughout the 210 min experiment (n=6 rats).
A: Frequency band distribution in ipsilateral-CA1, significant interaction ($F_{(35, 175)} = 3.68, p < 0.0001$).
B: Frequency band distribution in ipsilateral-CA3, significant interaction ($F_{(35, 75)} = 2.593, p < 0.0001$).
^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.
Two-way ANOVA repeated measures and *post hoc* Dunnett's multiple comparisons tests.

There was significant interaction between time x frequency band in **contralateral-CA1** ($F_{(35, 175)} = 2.87, p < 0.0001$; two-way ANOVA repeated measures). The normalised mean power of **0.1-1 Hz** from the saline+KA group of contralateral-CA1 (Figure 4.9A) was significantly different at the epochs of 80-90 min, 110-120 min, 140-150 min and 170-180 min ($^d p < 0.0001$) compared to basal. Significant differences in the **delta** band were also found in contralateral-CA1 at the epochs of 80-90 min, 110-120 min, 140-150 min and 170-180 min ($^d p < 0.0001$) compared to basal. The normalised mean power of the **theta** band was also significantly different at the epochs of 80-90 min and 110-120 min ($^a p < 0.05$), 140-150 min and 170-180 min ($^b p < 0.005$) compared to basal.

A significant interaction was found between time x frequency band in **contralateral-CA3** ($F_{(35, 175)} = 2.275, p < 0.001$; two-way ANOVA repeated measures). As shown in Figure 4.9B, in contralateral-CA3, the normalised mean power of **0.1-1 Hz** from saline+KA group was significantly different at the epochs of 50-60 min (1.66 ± 0.25 ; $^a p < 0.05$), 80-90 min, 110-120 min, 140-150 min, and 170-180 min ($^d p < 0.0001$, respectively) compared to basal (-10-0 min). In the **delta** band, normalised mean power was significantly different at the epochs of 50-60 min (1.81 ± 0.19 ; $^b p < 0.005$), 80-90 min, 110-120 min, 140-150 min and 170-180 min ($^d p < 0.0001$, respectively) compared to basal. Significant differences in the **theta** band were also found at the epochs of 50-60 min (1.74 ± 0.20 ; $^a p < 0.05$), 80-90 min, 110-120 min, 140-150 min and 170-180 min ($^d p < 0.0001$, respectively) compared to basal.

A. Contralateral-CA1



B. Contralateral-CA3

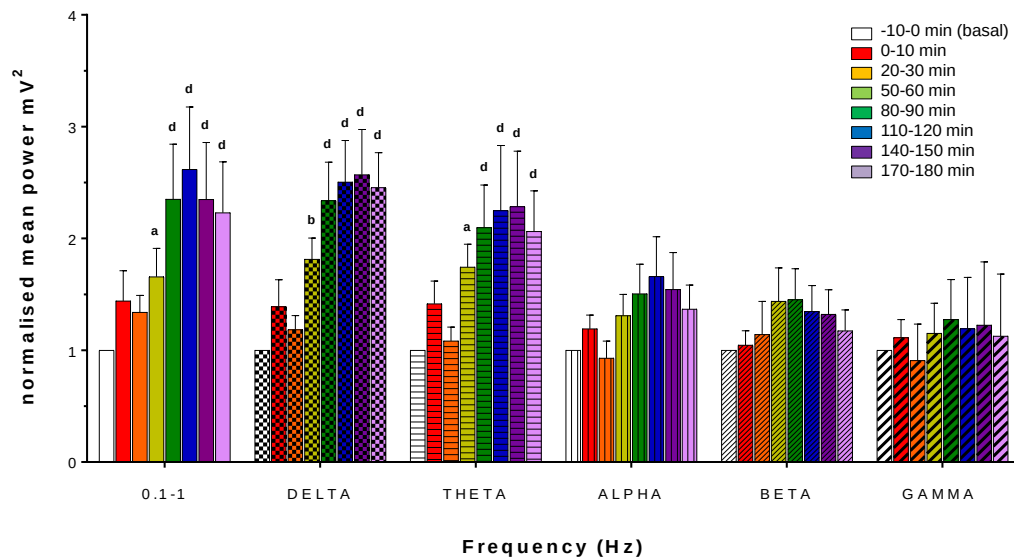


Figure 4.9 Normalised mean power of LFP frequency band distribution in the contralateral hippocampus from **saline+KA** group throughout the 210 min experiment (n=6 rats).

A: Frequency band distribution in contralateral-CA1, significant interaction ($F_{(35, 175)} = 2.87$, $p < 0.0001$).

B: Frequency band distribution in contralateral-CA3, significant interaction ($F_{(35, 175)} = 2.275$, $p < 0.001$).

^a $p < 0.05$, ^b $p < 0.005$ and ^d $p < 0.0001$ when compared to basal, respectively.

Two-way ANOVA repeated measures and *post hoc* Dunnett's multiple comparisons tests.

VPA administered before KA (VPA+KA group) increased the low frequency band (0.1-1 Hz, delta, theta and alpha) in the ipsilateral and contralateral hippocampus

Significant changes in the interaction of the overall LFP power was found in the interaction between brain region x time ($F_{(21, 105)} = 15.71, p < 0.0001$), time course of the experiment ($F_{(7, 35)} = 15.5, p < 0.0001$) and all brain regions of rats (Figure 4.10; $F_{(3, 15)} = 20.78, p < 0.001$; two-way ANOVA repeated measures).

Using Tukey's multiple comparison test, normalised mean power in contralateral-CA3 was significantly different compared to contralateral-CA1 ($^b p < 0.005$), ipsilateral-CA3 ($^c p < 0.001$) and ipsilateral-CA1 ($^d p < 0.0001$). A significant difference was also found in contralateral-CA1 compared to ipsilateral-CA1 (Figure 4.11; $^a p < 0.05$).

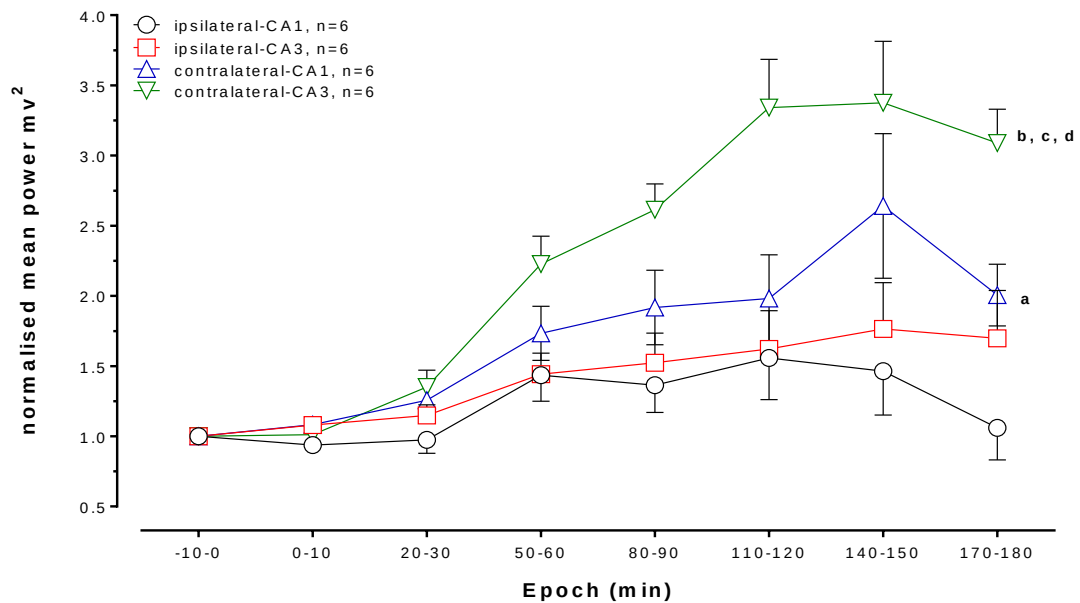


Figure 4.10 Normalised mean power of LFP in **VPA+KA** group in 210 min experiment, mean $\pm$ SEM, n=6 rats.

Significant interaction, ($F_{(21, 105)} = 15.71, p < 0.0001$).

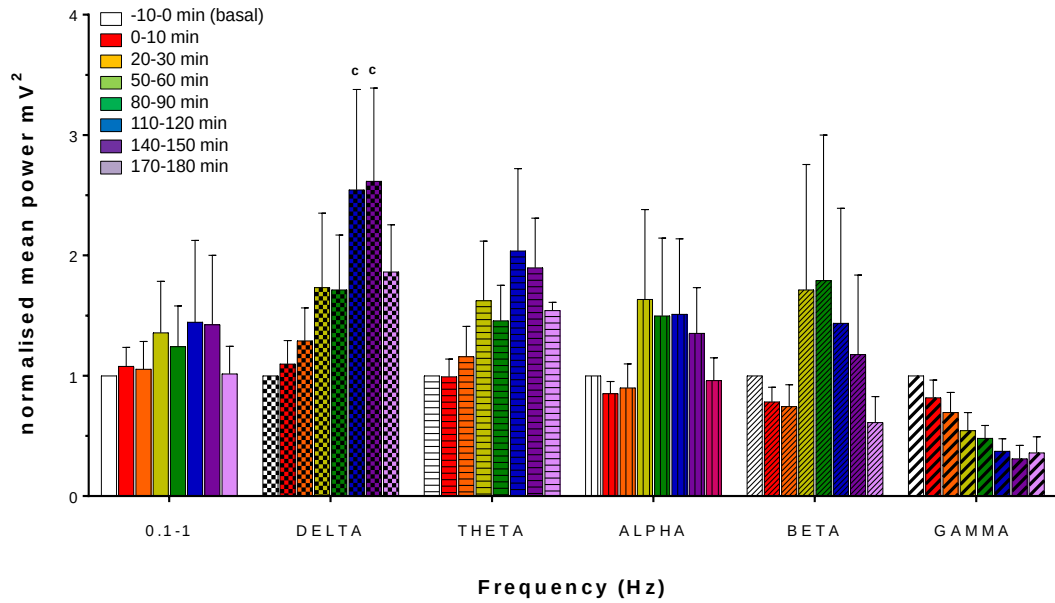
$^a p < 0.05$ when compared to ipsilateral-CA1, $^b p < 0.005$ when compared to contralateral-CA1, $^c p < 0.001$ when compared to ipsilateral-CA3 and $^d p < 0.0001$ when compared to ipsilateral-CA1.

Two-way ANOVA repeated measures and *post hoc* Tukey's multiple comparisons tests.

There was significant interaction between time x frequency band in the **ipsilateral-CA1** ($F_{(35, 140)} = 1.592, p < 0.05$; two-way ANOVA repeated measures). Figure 4.11A shows that normalised mean power of the **delta** band in ipsilateral-CA1 from VPA+KA group were significantly different at 110-120 min and 140-150 min ($p < 0.001$, respectively) compared to basal (-10-0 min; Dunnett's multiple comparisons test).

A significant interaction was found between time x frequency band in the **ipsilateral-CA3** ($F_{(35, 140)} = 4.214, p < 0.0001$; two-way ANOVA repeated measures). There are significant differences in the **delta** band in ipsilateral-CA3 (Figure 4.11B) at the epochs of 50-60 min (1.95 ± 0.60 ; $^c p < 0.001$), 80-90 min, at 110-120 min, 140-150 min and 170-180 min ($^d p < 0.0001$). Significant difference was also found in the **theta** band in the ipsilateral-CA3 at the epochs of 50-60 min (1.69 ± 0.25 ; $^a p < 0.05$), 80-90 min (2.00 ± 0.43 ; $^c p < 0.001$), 110-120 min (2.16 ± 0.46 ; $^d p < 0.0001$), 140-150 min and 170-180 min ($^d p < 0.0001$) compared to basal. There were also significant differences in the **alpha** band at the epochs of 80-90 min and 110-120 min ($^a p < 0.05$), 140-150 min and 170-180 min ($^c p < 0.001$) compared to basal.

A. Ipsilateral-CA1



B. Ipsilateral-CA3

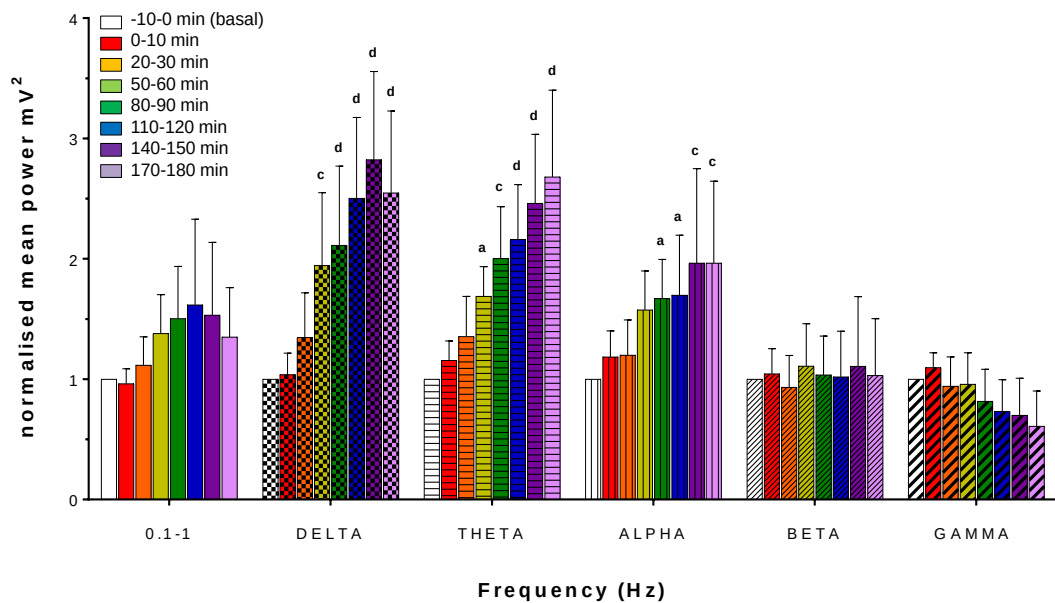


Figure 4.11 Normalised mean power of LFP frequency band distribution in the ipsilateral hippocampus from VPA+KA group throughout the 210 min experiment (n=6 rats).

A: Frequency band distribution in ipsilateral-CA1, significant interaction ($F_{(35, 140)} = 1.592, p < 0.05$).

B: Frequency band distribution in ipsilateral-CA3, significant interaction ($F_{(35, 140)} = 4.214, p < 0.0001$).

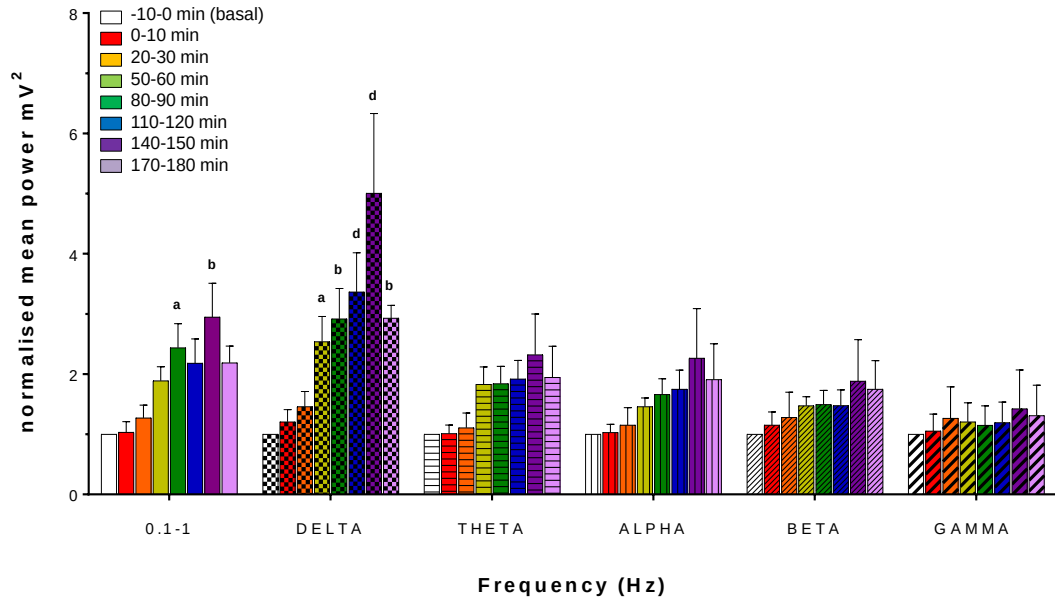
^a $p < 0.05$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.

Two-way ANOVA repeated measures and *post hoc* Dunnett's multiple comparisons tests.

Significant interaction was found between time x frequency band in the **contralateral-CA1** ($F_{(35, 140)} = 3.604$, $p < 0.0001$; two-way ANOVA repeated measures). There was significant difference in the normalised mean power of **0.1-1 Hz** from the VPA+KA group of contralateral-CA1 (Figure 4.12A) at the epochs of 80-90 min (2.44 ± 0.40 ; $^a p < 0.05$) and 140-150 min (2.95 ± 0.56 ; $^b p < 0.005$) compared to basal. Significant differences in the **delta** band were also found in contralateral-CA1 at 50-60 min (2.54 ± 0.42 ; $^a p < 0.05$), 80-90 min (2.92 ± 0.50 ; $^b p < 0.005$), 110-120 min and 140-150 min ($^d p < 0.0001$) and 170-180 min (2.93 ± 0.21 ; $^b p < 0.005$) compared to basal.

No significant interaction was found between time x frequency band in the **contralateral-CA3** ($F_{(35, 140)} = 1.353$, $p > 0.05$; two-way ANOVA repeated measures). As shown in Figure 4.12B, in contralateral-CA3, the normalised mean power of **0.1-1 Hz** from VPA+KA group was significantly different at the epochs of 50-60 min and 80-90 min ($^b p < 0.005$), 110-120 min, 140-150 min and 170-180 min ($^d p < 0.0001$) compared to basal (-10-0 min). In **delta** band, the normalised mean power was significantly different at the epochs of 50-60 min and 80-90 min ($^c p < 0.001$), 110-120 min and 140-150 min ($^d p < 0.0001$) and 170-180 min (3.80 ± 0.82 ; $^c p < 0.001$) compared to basal. Significant differences in **theta** band were also found at the epochs of 50-60 min (2.35 ± 0.68 ; $^a p < 0.05$), 80-90 min (2.57 ± 0.75 ; $^b p < 0.005$), 110-120 min and 140-150 min ($^d p < 0.0001$) and 170-180 min (3.14 ± 0.68 ; $^c p < 0.001$) compared to basal. We can also find significant differences in the normalised mean power of the **alpha** band at the epochs of 110-120 min (2.52 ± 0.66 ; $^a p < 0.05$), 140-150 min and 170-180 min ($^b p < 0.005$, respectively) compared to basal.

A. Contralateral-CA1



B. Contralateral-CA3

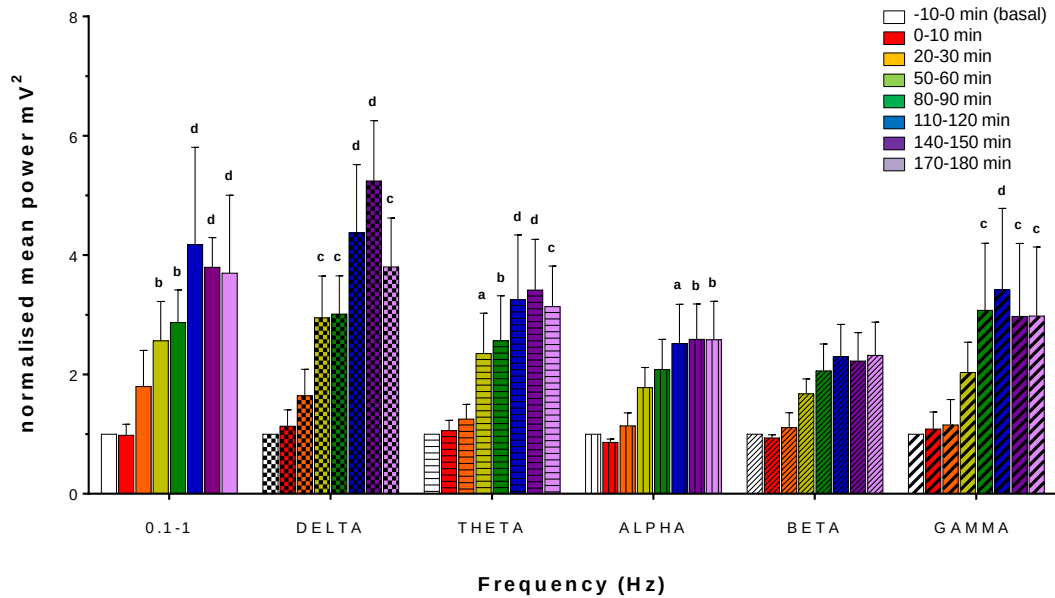


Figure 4.12 Normalised mean power of LFP frequency band distribution in the contralateral hippocampus from **VPA+KA** group throughout the 210 min experiment (n=6 rats).

A: Frequency band distribution in contralateral-CA1, significant interaction ($F_{(35, 140)} = 3.604, p < 0.0001$).

B: Frequency band distribution in contralateral-CA3, no significant interaction ($F_{(35, 140)} = 1.353, p > 0.05$).

^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.

Two-way ANOVA repeated measures and *post hoc* Dunnett's multiple comparisons tests.

VPA administered after KA (KA+VPA) increased the frequency bands of 0.1-1 Hz, delta, theta, alpha and beta in the contralateral hippocampus

Significant changes in overall LFP power were found in the interaction between brain region x time ($F_{(21, 105)} = 9.165, p < 0.0001$), time course of the experiment ($F_{(7, 35)} = 14.11, p < 0.0001$) and all brain regions of rats (Figure 4.13; $F_{(3, 15)} = 19.72, p < 0.0001$; two-way ANOVA repeated measures).

Tukey's multiple comparisons test showed that the normalised mean power in contralateral-CA3 was significantly different compared to ipsilateral-CA3 ($^d p < 0.0001$), and ipsilateral-CA1 ($^a p < 0.05$). A significant difference was also found in contralateral-CA1 ($^b p < 0.005$) compared to ipsilateral-CA3 (Figure 4.13). There was a significant difference in ipsilateral-CA1 compared to ipsilateral-CA3 ($^c p < 0.001$) and contralateral-CA3 ($^{cc} p < 0.001$).

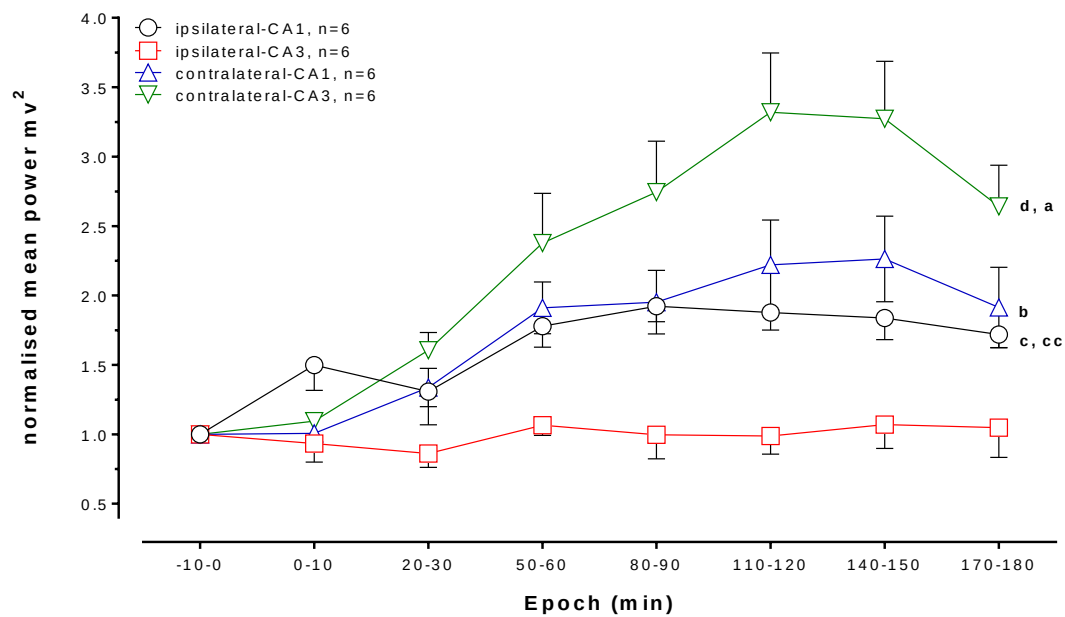


Figure 4.13 Normalised mean power of LFP in **KA+VPA** group in 210 min experiment, mean $\pm$ SEM, n=6 rats.

Significant interaction, ($F_{(21, 105)} = 9.165, p < 0.0001$).

$^a p < 0.05$ when compared to ipsilateral-CA1, $^b p < 0.005$ when compared to ipsilateral-CA3, $^c p < 0.001$ when compared to ipsilateral-CA3 and $^{cc} p < 0.0001$ when compared to contralateral-CA3.

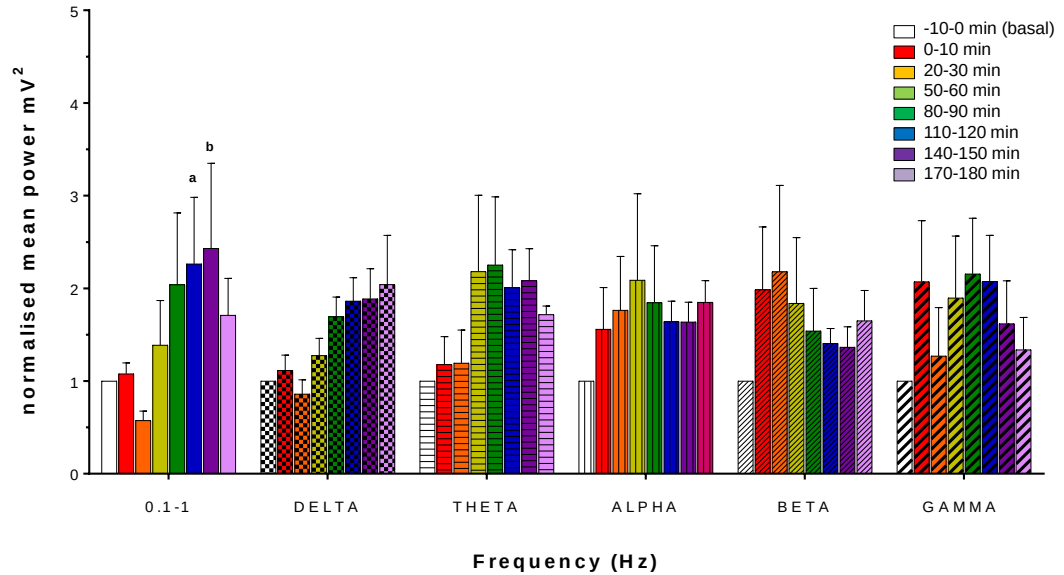
Two-way ANOVA repeated measures and *post hoc* Tukey's multiple comparisons tests.

In **ipsilateral-CA1**, no significant interaction was found between time x frequency band ($F_{(35, 175)} = 1.487, p > 0.05$; two-way ANOVA repeated measures). The normalised mean power of **0.1-1 Hz** from the KA+VPA group was significantly different at 110-120 min (Figure 4.14A; 2.26 ± 0.72 ; $^a p < 0.05$) and 140-150 min (2.43 ± 0.92 ; $^b p < 0.005$) compared to basal (-10-0 min; Dunnett's multiple comparisons test).

No significant interaction was found between time x frequency band in **ipsilateral-CA3** ($F_{(35, 175)} = 1.375, p > 0.05$; Two-way ANOVA repeated measures). Also, no significant difference was found in frequency bands in ipsilateral-CA3 from the KA+VPA group compared to basal (Figure 4.14B).

In **contralateral-CA1**, a significant interaction was found between time x frequency band ($F_{(35, 175)} = 1.676, p < 0.05$; two-way ANOVA repeated measures). There was significant difference in the normalised mean power of **0.1-1 Hz** from the KA+VPA group (Figure 4.15A) at the epochs of 50-60 min (2.17 ± 0.33 ; $^a p < 0.05$), 80-90 min (2.49 ± 0.60 ; $^c p < 0.001$), 110-120 min (2.85 ± 0.75 ; $^d p < 0.0001$), 140-150 min and 170-180 min ($^c p < 0.001$) compared to basal. There was also a significant difference in the **delta** band in contralateral-CA1 at the epochs of 50-60 min and 80-90 min ($^b p < 0.005$), 110-120 min, 140-150 min and 170-180 min ($^d p < 0.0001$) compared to basal. Significant differences in **theta** band were also found at the epochs of 50-60 min and 80-90 min ($^a p < 0.05$), 110-120 min (2.34 ± 0.81 ; $^b p < 0.005$) and 140-150 min (2.64 ± 1.02 ; $^c p < 0.001$) compared to basal. We can also find significant differences in the normalised mean power of **beta** band at the epochs of 50-60 min and 80-90 min ($^a p < 0.05$, respectively), 110-120 min (2.35 ± 0.81 ; $^b p < 0.005$) and 140-150 min (2.21 ± 0.58 ; $^a p < 0.05$) compared to basal.

A. Ipsilateral-CA1



B. Ipsilateral-CA3

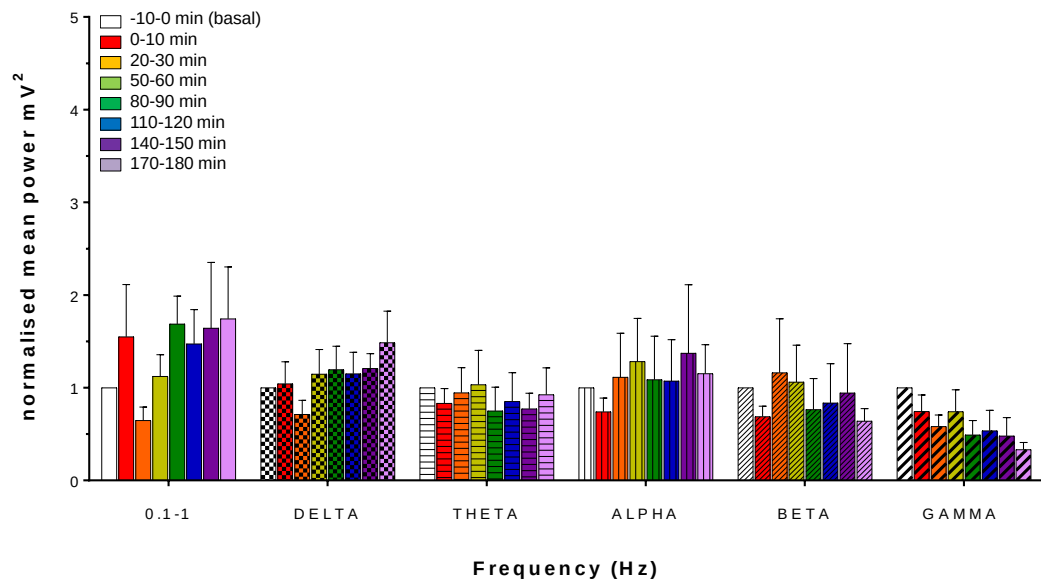


Figure 4.14 Normalised mean power of LFP frequency band distribution in the ipsilateral hippocampus from VPA+KA group throughout the 210 min experiment (n=6 rats).

A: Frequency band distribution in ipsilateral-CA1, no significant interaction ($F_{(35, 175)} = 1.487, p > 0.05$).

B: Frequency band distribution in ipsilateral-CA3, no significant interaction ($F_{(35, 175)} = 1.375, p > 0.05$).

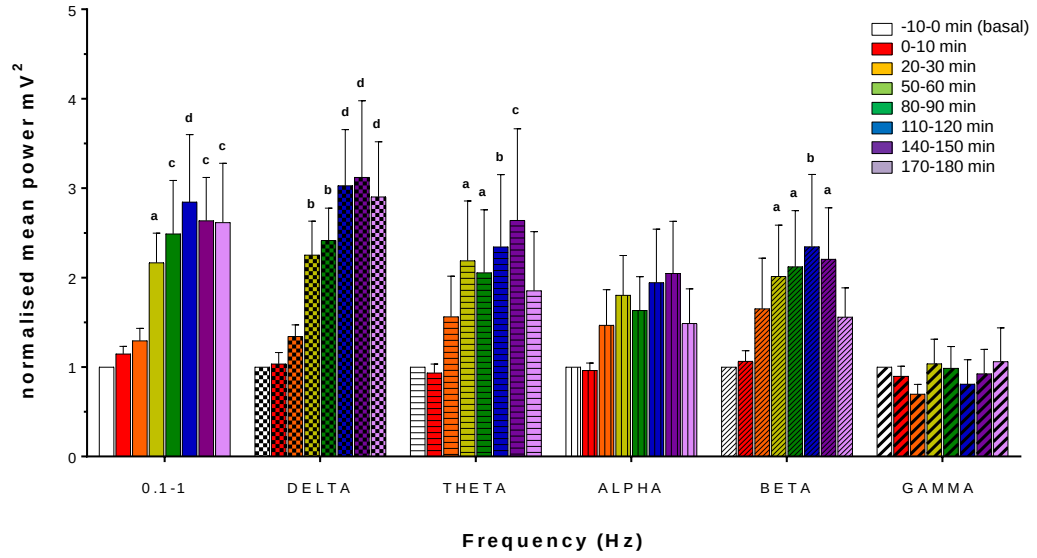
^a $p < 0.05$ and ^b $p < 0.005$ when compared to basal, respectively.

Two-way ANOVA repeated measures and *post hoc* Dunnet's multiple comparisons tests.

A significant interaction was found between time x frequency band in **contralateral-CA3** ($F_{(35, 175)} = 1.547, p < 0.05$; two-way ANOVA repeated measures). Figure 4.15B shows that in contralateral-CA3, the normalised mean power of **0.1-1 Hz** from the KA+VPA group was significantly different at the epochs of 80-90 min and 110-120 min ($^d p < 0.0001$, respectively), 140-150 min and 170-180 min ($^a p < 0.05$) compared to basal (-10-0 min). In the **delta** band, the normalised mean power was significantly different at 50-60 min and 80-90 min ($^b p < 0.005$, respectively), 110-120 min (4.09 ± 0.62 ; $^d p < 0.0001$), 140-150 min and 170-180 min ($^c p < 0.001$, respectively) compared to basal. Significant differences in the **theta** band were also found at 50-60 min and 80-90 min ($^b p < 0.005$, respectively), 110-120 min and 140-150 min ($^c p < 0.001$, respectively) and 170-180 min (2.86 ± 0.26 ; $^a p < 0.05$) compared to basal. We can also find significant differences in the normalised mean power of **alpha** band at 80-90 min (2.77 ± 0.94 ; $^a p < 0.05$), 110-120 min (3.69 ± 1.56 ; $^c p < 0.001$), 140-150 min (3.89 ± 1.48 ; $^d p < 0.0001$) and 170-180 min (2.75 ± 0.80 ; $^a p < 0.05$) compared to basal. There were significant differences in the normalised mean power of **beta** band at the epochs of 80-90 min (2.87 ± 1.30 ; $^a p < 0.05$), 110-120 min (3.74 ± 1.86 ; $^c p < 0.001$) and 140-150 min (4.14 ± 1.89 ; $^d p < 0.0001$) compared to basal.

In summary, **saline+KA** increased the frequency band of delta and theta in the ipsilateral and contralateral hippocampus (Fig. 4.8 A-D). VPA administered before KA (**VPA+KA** group) increased the low frequency band (0.1-1 Hz, delta, theta and alpha) in the ipsilateral and contralateral hippocampus (Fig. 4.11 and 4.12). VPA administered after KA (**KA+VPA**) increased the frequency bands of 0.1-1 Hz, delta, theta, alpha and beta in the contralateral hippocampus (Fig. 4.15A and B). Overall, only in groups of rats administered with VPA before epileptiform-like activity induced by KA (VPA+KA) increased the high frequency band of gamma in contralateral-CA3 (Fig. 4.12B). The other two groups, saline+KA and KA+VPA did not increase the LFP frequency bands of gamma in the contralateral-CA3 hippocampus (Fig. 4.9B and 4.15B).

A. Contralateral-CA1



B. Contralateral-CA3

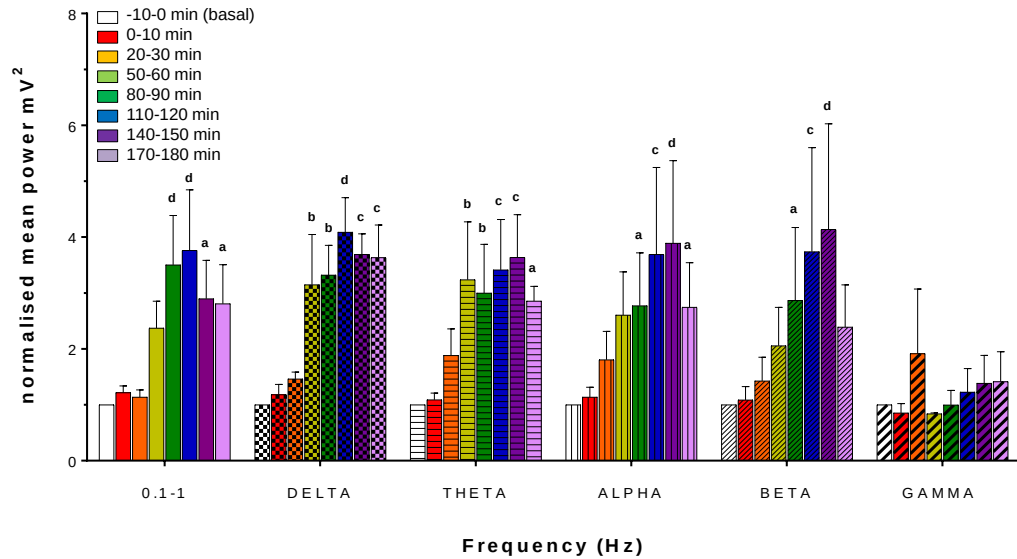


Figure 4.15 Normalised mean power of LFP frequency band distribution in the contralateral hippocampus from KA+VPA group throughout the 210 min experiment (n=6 rats).

A: Frequency band distribution in contralateral-CA1, significant interaction ($F_{(35, 175)} = 1.676, p < 0.05$).

B: Frequency band distribution in contralateral-CA3, significant interaction ($F_{(35, 175)} = 1.547, p < 0.05$).

^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.

Two-way ANOVA repeated measures and Dunnett's *post hoc* multiple comparisons tests.

Table 4.1 summarises the results presented in this chapter for ease of understanding.

Table 4.1 A summary of all results reported in Chapter 4.

Group of study	Hippocampus area	Normalised mean firing rate	Normalised mean synchrony index	Unit-pair cross-correlation (mean k-index)	LFP frequency band distribution
Saline; i.v followed by KA (1 mM; locally), n=6 rats.	Ipsilateral-CA1	Increased after 20 min of KA administration.	No changes.	ROI 1 increased at epochs of 110-120 and 170-180 min. No significant difference in ROI 2 and ROI 3.	Delta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Theta increased at epochs of 110-120 min, 140-150 min and 170-180 min.
	Ipsilateral-CA3	No changes.	No changes.		Delta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Theta increased at epochs of 110-120 min, 140-150 min and 170-180 min. Gamma only increased at the epoch of 110-120 min.
	Contralateral-CA1	No changes.	No changes.		0.1-1 Hz increased at epochs of 80-90 min, 110-120 min, 140-150 min and 170-180 min. Delta increased at epochs of 80-90 min, 110-120 min, 140-150 min and 170-180 min. Theta

					increased at epochs of 80-90 min, 110-120 min, 140-150 min and 170-180 min.
	Contralateral-CA3	Increased after 60 min of KA administration.	No changes.		0.1-1 Hz increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Delta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Theta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min.
VPA (20 mg/kg; i.v) followed by KA (1 mM; locally), n=6 rats.	Ipsilateral-CA1	No changes.	Decreased at the epoch of 140-150 min (2 hours after KA administration) and 170-180 min.	ROI 1 increased at epochs of 110-120 and 140-150 min. No significant difference in ROI 2 and ROI 3.	Delta increased at epochs of 110-120 min and 140-150 min.
	Ipsilateral-CA3	No changes.	No changes.		Delta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Theta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Alpha increased at epochs of 80-90 min, 110-120 min, 140-150 min and

					170-180 min.
	Contralateral-CA1	No changes.	No changes.		0.1-1 Hz increased at epochs of 80-90 min and 140-150 min. Delta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min.
	Contralateral-CA3	Increased after 80 min of KA administration	No changes.		0.1-1 Hz increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Delta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Theta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Alpha increased at epochs of 110-120 min, 140-150 min and 170-180 min. Gamma increased at epochs of 80-90 min, 110-120 min, 140-150 min and 170-180 min.
KA (1 mM; locally) followed by VPA	Ipsilateral-CA1	No changes.	No changes.	ROI 2 increased at epochs of 140-150 min. No significant	0.1-1 Hz increased at epochs of 110-120 min and 140-150 min.

(20 mg/kg; i.v), n=6 rats.	Ipsilateral-CA3	No changes.	No changes.	difference in ROI 1 and ROI 3.	No changes.
	Contralateral-CA1	No changes.	No changes.		0.1-1 Hz increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Delta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Theta increased at epochs of 50-60 min, 80-90 min, 110-120 min and 140-150 min. Beta increased at epochs of 50-60 min, 80-90 min, 110-120 min and 140-150 min.
	Contralateral-CA3	No changes.	No changes.		0.1-1 Hz increased at epochs of 80-90 min, 110-120 min, 140-150 min and 170-180 min. Delta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Theta increased at epochs of 50-60 min, 80-90 min, 110-120 min and 140-150 min and 170-180 min. Alpha increased at epochs of 80-90 min, 110-120 min and 140-150 min

					and 170-180 min. Beta increased at epochs of 80-90 min, 110-120 min and 140-150 min.
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4.4 Discussion

There are two main findings in this chapter: VPA prevented and reduced the KA effect in **VPA+KA** group and attenuated the KA effect in **KA+VPA** group. As mentioned previously, we would like to highlight that the rats in the **saline+KA 1mM** group are the same group of rats used in Chapter 3; however, the experiment time was extended to 210 min (3.5 hours). Although it was discussed in Chapter 3 (for experiments up to 80 min), this chapter will discuss this group in comparison to the groups where VPA was administered before and after KA. From this point onwards, saline+KA 1 mM will be referred to as the mTLE model, as we selected this group as our mTLE model in Chapter 3.

In the **mTLE** group (Figures 4.3 and 4.4); the normalised mean firing rate was increased in ipsilateral-CA1 and contralateral-CA3 after 10 min and 80 min of KA administration, respectively. The normalised mean SI increased in ipsilateral-CA1 (at the epoch of 50-60 min onward) and contralateral-CA3 (at the epoch of 80-90 min onward; Fig. 4.5). The increasing normalised mean SI, in conjunction with the increasing normalised mean firing rate, indicates that the synaptic networks of firing play a role in synchronisation during seizures. An epileptic seizure is associated with abnormal synchronisation of neurones (Jiruska et al., 2013; Kreuz, 2013). The neurones firing and the parallel synchronisation also increased the unit pair cross-correlation (mean K-index) in ROI 1 after 90 min of KA administration (Fig. 4.6A) and increased the LFP frequency band in delta and theta bands in all brain regions ~40 min after KA administration (Fig. 4.8A and B). Our mTLE model showed that epileptiform-like activity not only occurs at the injection site (ipsilateral hippocampus) but also propagates the neuronal hyper-excitability to the contralateral hippocampus (in our case, contralateral-CA3) via functional connectivity.

In the **VPA+KA** group, VPA prevented the normalised mean firing rate in the ipsilateral-CA1 hippocampus when compared to basal (Figure 4.3A). VPA administered 20 min before KA seems to prevent the neuronal hyper-excitability induced by KA in ipsilateral-CA1. As we can see in the mTLE model, the neurones started to fire 10 min after KA administration; however, we did not see this effect when we injected the VPA beforehand. There were no changes detected in

ipsilateral-CA3 or contralateral-CA1 hippocampus when compared to basal (Fig. 4.3B and C). The pattern in these brain regions could not be compared to the mTLE model since there were no changes of firing found in these regions (Figure 4.3B and C). The effect of VPA on sustained repetitive firing depends on the reduction of incoming Na^+ current (McLean and Macdonald, 1986). VPA delayed the recovery from the inactivation of Na^+ channels, which is consistent with the reduction of Na^+ conductance (Van den Berg et al., 1993). However, the normalised mean firing rate increased in contralateral-CA3 hippocampus 80 min after KA administration compared to basal (Figure 4.4C). This result indicates that VPA decreased the neuronal hyper-excitability by 55%. We could still see the increment of neuronal firing in contralateral-CA3 even when we had injected VPA 20 min before KA administration. However, the firing rate in contralateral-CA3 reduced compared to the neuronal hyper-excitability induced by KA in the mTLE model (Figure 4.3D). The normalised mean SI was increased at the epoch of 110-120 min onwards in the contralateral-CA3 hippocampus. The result of SI is in parallel with our normalised mean firing rate result, which can be plausibly explained by the chain reaction mechanism in the contralateral-CA3 hippocampus. As explained by Jefferys et al. (2012), the CA3 pyramidal neurone has different connections with other pyramidal cells through the chain reaction. The firing of the CA3 neurone causes postsynaptic activation of the other pyramidal cells which then subsequently cause the synchronous firing of a large population of CA3 pyramidal neurones within a small number of synaptic steps (Jefferys et al., 2012). The neurones firing may also increase the oscillation of low frequency bands (0.1-1 Hz, delta, theta and alpha) in both ipsilateral and contralateral hippocampus (Figure 4.12 and 4.13). Our mean k-index result showed that neurones in the ipsilateral were highly correlated in ROI 1 at the epochs of 110-120 min and 140-150 min when compared to basal (Figure 4.7B).

In the **KA+VPA** group, VPA attenuated the normalised mean firing rate in the ipsilateral-CA1 hippocampus compared to basal as well as compared to the mTLE model (Figure 4.3A). Similar to the VPA+KA group, no effect was detected in any brain regions when compared to basal. VPA administered 20 min after KA seems to attenuate the neuronal hyper-excitability induced by KA. Our results showed that

there was no neuronal hyper-excitability in any brain regions (Figure 4.3A-D). These results indicated that VPA administered 20 min after KA does not allow KA to further induce neuronal hyper-excitability. No changes were found in the normalised mean SI of any brain regions in **KA+VPA** group (Figure 4.5C) which is in parallel with there being no significant changes in normalised mean firing rate. Mean K-index in the **KA+VPA** group showed that neurones in the contralateral hippocampus were highly correlated in ROI 2 at the epoch of 140-150 min compared to basal (Figure 4.6C), indicating that there is a strengthening of the correlation of neurones only in the contralateral hippocampus. The frequency bands 0.1-1 Hz, delta, theta, alpha and beta increased in the **KA+VPA** group in the contralateral hippocampus (Figures 4.15) indicating that these are the dominant frequency bands in anaesthetised rats (Timofeev et al., 2012).

Our electrophysiological results indicated that VPA prevented, reduced (based on the **VPA+KA** group) and attenuated (based on **KA+VPA** group) neuronal hyper-excitability induced by KA through a few possible actions. We propose that VPA may act to reduce the excitatory synaptic potentiation necessary for network synchronisation (Griffith and Taylor, 1988). Our findings are in agreement with those of Griffith and Taylor, (1988), who showed that VPA may inhibit the excitatory synaptic transmission. We postulate that VPA treatment may alter the expression or function of glutamate receptors, including NMDA and AMPA receptors. VPA suppresses NMDA-induced depolarisation and attenuates the NMDA receptor-mediated excitation (Walton and Treiman, 1992; Johannessen and Johannessen, 2003; Lagace et al., 2004). VPA has shown an anticonvulsant effect in almost all animal models of acute or chronic seizure (Löscher, 1999). VPA's mechanisms are also said to be driven by the action of membrane electrical properties that tend to reduce neuronal excitability (Martin and Pozo, 2006). VPA's mode of action is also suggested to be through decreasing repetitive cell firing (McLean and MacDonald, 1986) which was observed in our **VPA+KA** and **KA+VPA** groups.

As we know, the glutamate receptor will allow cations influx into the neurones for depolarisation and further firing to occur. VPA however, acts selectively on Na^+ channels, which are the molecular targets for drugs used in the treatment of epilepsy. VPA and glutamate receptor binding blocks ion movement through the pore and stabilises the inactivated state of Na^+ channels. Anti-arrhythmic and anti-epileptic drugs share similar, overlapping receptor sites. However, these drugs selectively block Na^+ channels in depolarised and/or rapidly firing cells, such as axons carrying high-intensity pain information and rapidly firing nerve and muscle cells that drive epileptic seizures or cardiac arrhythmias (VanDongen et al., 1986; Mantegazza and Catterall, 2012).

VPA has been shown to diminish high frequency repetitive firing of action potentials of the spinal cord and cortical neurones in mouse cell culture (Löscher, 1999). However, our result in the **VPA+KA** experiment showed that VPA prevented 55% of the firing induced by KA in the contralateral-CA3 hippocampus. This finding could possibly be explained by the effect of VPA on voltage-gated Na^+ channel activity. VPA regulates both Na^+ and K^+ channel gating (Fohlmeister et al., 1984). Chronic treatment with VPA has been postulated to up-regulate cell surface expression of Na^+ channels (Griffith and Taylor, 1988; Georg Margineanu and Klitgaard, 2000). It has been reported that the gating properties of Na^+ channels differ between excitatory and inhibitory neurones in the hippocampus, which was much faster in interneurones (Martina and Jonas, 1997). Nevertheless, VPA had no effects on the bursting behaviour of neurones when a rat hippocampal slice was used for studying the neurophysiological effects of VPA (Albus and Williamson, 1998). Given the contrasting outcome, therefore, it cannot be concluded that the mechanism of action of VPA is similar to that of the classical voltage-gated Na^+ channel inhibiting antiepileptic drugs (Yoshida et al., 2010). Potentially, other factors might be involved in giving this effect. Further investigations should incorporate the studying of ion channel to support our findings.

VPA reduces epileptiform activity by inhibiting the high-frequency firing of neurones (Johannessen, 2000). This effect may contribute to the efficacy of VPA in the treatment of epilepsy, where excessive high-frequency bursting of neuronal aggregates is commonly observed (Johannessen and Johannessen, 2003). This finding is also seen in our results where hyper-excitable neurones induced by KA were inhibited. The mechanisms of VPA which seem to be of clinical importance include increased GABAergic activity, reduction in excitatory neurotransmission, and modification of monoamines (Johannessen and Johannessen, 2003). Early studies reported that acute VPA administration elevated the cerebral GABA levels in rodents which coincided with the period of protection against audiogenic seizures (Simler et al., 1973; Schechter et al., 1978). Interconnected GABAergic interneurons may drive network synchrony by phase locking firing in principal neurones, which could plausibly be one of the mechanisms involved in promoting epileptogenesis (Cobb et al., 1995; Wang and Buzsáki, 1996; Tamas et al., 2000). Synchrony in neuronal networks is reduced by increasing the amplitude or the duration of GABAergic inhibitory post-synaptic currents (IPSCs) or decreasing their frequency (Whittington et al., 2000). The various anticonvulsant effects of VPA could involve its ability to compromise GABA release and to prolong IPSCs and the simultaneous reduction of glutamate release, thus reducing synchronous drive within the interneurons-principal cell network.

In summary, our mTLE model showed that neuronal hyper-excitability does not only occur at the injection site (ipsilateral hippocampus) but also propagates the neuronal hyper-excitability to the contralateral hippocampus (in our case, contralateral-CA3) via functional connectivity. Our findings, through the selected parameters of normalised mean firing rate, normalised mean SI, cross-correlations, and LFP frequency bands distribution using electrophysiological technique showed that VPA administered intravenously prevented, reduced (based on the **VPA+KA** group) and attenuated (based on the **KA+VPA** group) neuronal hyper-excitability induced by KA through a few possible actions. We believe that the wide spectrum of the mechanism of action of VPA cannot completely account for the many aspects of the drug's effect on neurones and its broad pre-clinical and clinical activity in epilepsy and other brain diseases. The action of VPA in our study may, therefore, be proposed

to be through several different mechanisms which contribute to increasing our understanding of its various activities. We propose that VPA administered after KA is a better treatment than VPA administered before KA, as it fully attenuated the neuronal hyper-excitability induced by KA in all brain regions in the hippocampus (in our case, CA1 and CA3 in both ipsilateral and contralateral hippocampus).

Chapter 5

EFFECT OF WIN 55,212-2 AND FLURAZEPAM ON KA-INDUCED EPILEPTIFORM-LIKE ACTIVITY

5.1 Introduction

5.1.1 WIN 55,212-2 (WIN)

Synthetic cannabinoids have been used in animal models of epilepsy as a potential alternative or supplement to existing antiepileptic drugs (Rosenberg et al., 2015 and 2017). WIN 55,212-2 (WIN), as one of the synthetic cannabinoids, has been reported to exert both anti- and pro-convulsant effects in the pentylenetetrazole model (Vilela et al., 2013). Goonawardena et al. (2011) reported that WIN reduced average firing rates in CA3 and CA1 principal cells, reduced burst characteristics and disrupted synchronous activity within and between CA1 and CA3 in anaesthetised Long-Evan rats. WIN was also found to produce an anticonvulsant effect in 68.1 % (47/69) of seizure models in rats and mice (Rosenberg et al., 2015). In another study by Rudenko et al. (2013), WIN reduced seizure severity, burst frequency, and amplitude at a lower dose (0.5 mg/kg, i.p.) in KA-induced seizures in juvenile (P20) rats but increased seizures and behavioural impairments at the higher dose (5 mg/kg, i.p.), when given 90 min before testing. Schneider and Koch (2002) reported that injections of 1.2 mg/kg WIN 55,212-2 impaired recognition memory and prepulse inhibition but had no effect on lever-pressing acquisition or expression, or on food preference. A dose of 1.2 mg/kg WIN i.p caused temporal impairment of short term memory in the water maze and the object recognition tasks, as well as long-term potentiation in the ventral subiculum-nucleus accumbens pathway, in late adolescent rats (40-65 days post-natal) (Abush and Ikirav, 2012). Another study using 1.2 mg/kg WIN i.p showed that acute co-administration of the illegal drug 3,4-methylenedioxymethamphetamine (MDMA) and WIN did not substantially affect the MDMA-induced behavioural effects. Locomotion, exploratory behaviour and food intake were also comparable in MDMA and MDMA+WIN-treated animals

(Schulz et al., 2014). To our knowledge, so far, no study has tested the effect of using 1.2 mg/kg via i.v administration on neuronal epileptiform-like activity in anaesthetised rats. Therefore, we wanted to investigate whether 1.2 mg/kg WIN i.v administration is effective in preventing the neuronal epileptiform-like activity in anaesthetised rats.

Animal models demonstrate that activation of CB1 receptors (CB1Rs) reduces seizure severity (Rosenberg et al., 2017; Huizenga et al., 2018). WIN 55,212-2 was able to block the production of postsynaptic neuronal spiking and excitatory postsynaptic potential production through the activation of CB1R (Huntsman et al., 2019). In the case of mice with conditional deletion of CB1Rs in their principal forebrain, the excitatory neurones exhibited more severe KA-induced seizures (30 mg/kg) than wild-type controls (Antonucci et al., 2012). Conditional deletion of the CB1Rs increased gliosis and apoptosis following KA-induced seizures and prevented activation of the protective immediate early genes (c-Fos, Zif268, brain-derived neurotrophic factor) (Rosenberg et al, 2015). WIN reduced the amplitudes of excitatory postsynaptic currents in wild type and CB1^{-/-} mice, and at the same time decreased the inhibitory postsynaptic currents in wild type mice (Hajos et al., 2001). The underlying mechanism responsible for the inhibition of glutamatergic neurotransmission is the cannabinoid-sensitive receptor. This finding was consistent with a CB1R-dependent modulation of GABAergic postsynaptic currents (Huntsman et al., 2019). Activation of the presynaptic CB1R receptors by the endocannabinoids temporarily suppress voltage gated Ca²⁺ channels and the activation of K⁺ channels subsequently suppress further neurotransmitter release from the presynaptic neurone (Sugaya et al., 2018).

It is essential to mention the important role of cannabinoids and their receptors in the development of KA-induced seizures (Rosenberg et al., 2015 and 2017). Marsicano et al. (2003) found out that CB1R^{-/-} mice were more susceptible to KA-induced seizures than wild type mice, due to the fact that CB1Rs were selectively removed from all principal forebrain neurones, but not from associated interneurones. This result suggests that to protect from KA-induced seizures, activation of CB1Rs on excitatory neurones is vital, although it does not confirm whether activation of

CB1Rs on GABAergic neurones was also required. Nevertheless, a study by Khaspekov et al. (2004) suggested that CB1Rs had a role in protecting the brain from KA-induced seizures. The antiepileptic action of endogenous cannabinoids requires selective activation of CB1Rs on glutamatergic neurotransmitters (Huntsman et al., 2019). Variations in the pro- vs anticonvulsant effects in each system may reflect specific effects of the species, seizure models (acute vs chronic, focal vs generalised), dose ranges, timing, or experimental design (Blair et al., 2009).

A study conducted by Bhaskaran and Smith (2010) has demonstrated a change in the sensitivity of glutamatergic afferents to cannabinoids in the pilocarpine model of epilepsy. This is supported with increased expression of CB1Rs in a Western blot of tissue including the molecular and the granule cell layers (Monory et al., 2006). The first observation of total suppression of SE by a selective pharmacological agent was observed based on the inhibitory effect of WIN on low Mg^{2+} -induced SE (Blair et al., 2009). In addition, WIN produces an additive effect when combined with several commonly prescribed AEDs (Blair et al., 2009). In 18 studies of mice, rats, and guinea pigs, CB1R antagonists were pro-convulsant in 38.9 %, anti-convulsant in 5.6 %, and showed no significant effect in 55.6 % of trials. Although CB1R agonists were anticonvulsant in 68.1% of the studies, only 38.9% of CB1R antagonists were pro-convulsive (Blair et al., 2009). Another study showed that WIN inhibits glutamate release even at low micromolar concentrations in the hippocampus in mice (Hajos et al., 2001; Hajos and Freund, 2002; Köfalvi et al., 2003). On the other hand, Ferraro et al. (2001) speculated that WIN might affect other neurotransmitters, subsequently trigger the reduction of cortical glutamate levels and in turn balance the WIN-induced excitatory effect.

5.1.2 Flurazepam (FLU)

Flurazepam (FLU) is one of the classic derivatives of benzodiazepine and acts as a GABA_A receptor modulator via binding to the benzodiazepine site on the GABA_A receptor (Bai et al., 2001; Dudek, 2007). Potentiation of inhibitory synaptic transmission mediated by GABA acting at the GABA_A receptor subtype is increasingly being regarded as a primary mechanism of action for drugs with

hypnotic, anaesthetic and anticonvulsant properties (Reynolds and Maitra, 1996; Riss et al., 2008; Griffin et al., 2013). GABA receptors play an active role in processing information and controlling the balance of excitability and inhibitory states in the cortex, hippocampus and the interneurons (Rezayof et al., 2007; Khazipov, 2016). GABA acts at the inhibitory synapses in the brain, through binding two specific transmembrane receptors in the plasma membrane of both pre- and postsynaptic cells (Huntsman et al., 2019). Upon activation, this binding process increases the influx of Cl^- ions into the GABA_A neurone (Riss et al., 2008). This increased Cl^- ion influx hyperpolarises the neurone's membrane potential (Khazipov, 2016). As a result, the difference between resting potential and threshold potential is increased and firing is less likely (Bai et al., 2001).

Mendelsen and Martin (1990) have reported that FLU cause shortening of sleep latency, decreasing REM sleep, and increasing total sleep and REM latency, as measured by EEG. In their study, FLU exhibited hypnotic properties, which refers to EEG measures of sleep and waking. Tyma et al. (1988) reported that chronic treatment of FLU suppressed the neuronal activity in the pars reticulata with substantia nigra. This suppression may be related to the anticonvulsant activity of systemically administered benzodiazepines. Fairngold et al. (1986) have found that FLU was more effective in reducing the firing in the inferior colliculus in normal Sprague-Dawley rats than in the genetically epilepsy-prone rat. Their result indicated that GABA is less effective as an inhibitory agent when applied to inferior colliculus neurones in the epileptic animal, which may have occurred as the function of the postsynaptic GABA_A receptor was altered (Riss et al., 2008). Marchionni et al. (2007) have shown that FLU, as a positive allosteric modulator of γ^2 -containing receptors and extra-synaptic GABA_A receptor subunits, mediates tonic currents in CA1 principal cells.

In clinical use, benzodiazepines are often given intravenously in the acute treatment of seizures, especially SE but also occasionally for febrile seizures (Rogawski and Porter, 1990; Griffin et al., 2013). Benzodiazepines potentiate the inhibitory effects of GABA throughout the central nervous system, resulting in anxiolytic, sedative, hypnotic, muscle relaxant and antiepileptic effects (Stallman and Kohler, 2017).

Diazepam is the most widely used benzodiazepine for acute management of seizures, because high levels in the brain are rapidly obtained during i.v administration (Rogawski and Porter, 1990). Anxiety disorders represent a common psychiatric comorbidity in patients with epilepsy (Mula, 2016). In clinical practice, benzodiazepines are the most widely used as anxiolytics (Mula, 2016). FLU is a commonly prescribed drug for anxiety and sleep disorder (Thangadurai, 2016; Daga et al., 2019), which could be hypothesised as a potential acute antiepileptic drug. In this context, we chose to test the effect of i.v administration of FLU in KA-induced epileptiform-like activity. FLU was reported to be both convulsant and anticonvulsant depending on the dose used. Kalichman (1985) reported a significant anticonvulsant effect against picrotoxin in the range of 30-60 mg/kg i.p. of FLU. However, when doses of FLU were increased from 60 to 200 mg/kg i.p., epileptogenicity was also increased, and at a dose of 400 mg/kg all subjects died. With this in mind, we selected a low dose of 2 mg/kg i.v to see whether FLU could exert anticonvulsant or anti epileptiform-like activity.

5.1.3 Aims and hypothesis of this chapter

The aim of this chapter was to investigate the effect of WIN and FLU in the selected mTLE model (saline+KA) mentioned in Chapter 4. The changes in the pattern of neuronal unit firing, synchronisation, hippocampal LFP oscillatory activity and mean K-index in the models in response to locally applied KA were monitored. Since we know that WIN is a full agonist of CB1Rs belonging to the G protein-coupled receptor family, and its activation is associated with a reduction of glutamate release, we hypothesised that WIN would prevent and attenuate KA-induced epileptiform activity. Well-known benzodiazepines used for the treatment of epilepsy are diazepam, clonazepam, clobazam and nitrazepam; however, to our knowledge, only a limited number of studies have considered the potential use of FLU as an alternative AED. Since all benzodiazepines share similar neuro-pharmacologic properties, including anxiety reduction, sedation, sleep induction, anticonvulsant effects and muscle relaxation, epileptiform-like activity induced by KA is also hypothesised to be attenuated by FLU. We were interested in whether FLU could act

as anti-epileptic in the case of epileptiform-like activity induced via electrophysiological techniques.

5.2 Methods

5.2.1 Experimental procedure

Electrophysiological procedures were performed, as described in Chapter 2. Briefly, male Lister Hooded rats (300-400g; n=12) were anaesthetised with 3.5% isoflurane in medical air mixture (reduced to 1.5-2% before recording was initiated). Separate craniotomies were performed, and an eight-electrode stainless-steel microwire array (NB Labs, Texas, USA) connected to a Plexon Multichannel Acquisition Processor system (Plexon Inc. Texas, USA) was placed stereotactically in the left (ipsilateral) and an 8-channel U-probe in the right (contralateral-CA3) dorsal hippocampus (Hpc AP-4.5mm; ML-4.0mm; DV-4.0mm) respectively, according to the coordinates of the rat atlas (Paxinos and Watson, 1998). The dura was retracted and the exposed cortex was kept moist with saline.

In these experiments, an eight Channel U-probe, 0.050" pitch headstage (HST/8o50-Gx-xR) Plexon (Texas, USA) was used to record neuronal signals in the CA3 region of the contralateral hippocampus (Figure 5.1). Previously, no firing of neurones was detected in contralateral CA1 in the mTLE model or VPA experiments using MEA in Chapter 3 (Figure 3.13) and 4 (Figure 4.3), therefore, we used an advanced U-probe electrode specifically for contralateral-CA3 hippocampus recording only. We also compared the firing of neurones between MEA and U-probe in WIN+KA experiment (Appendix). This experiment showed that there was no difference in firing rate detected between MEA and U-probe in contralateral-CA3. U-probes are robust due to their stainless-steel construction and are able to penetrate deep within the brain for long periods. The U-probe was directly inserted into contralateral-CA3, as no firing was detected from the contralateral-CA1 hippocampus when we used MEA.

After completion of the experiment, the U-probe was cleaned by soaking the probe in the diluted enzymatic detergent for at least 30 minutes. In these experiments, we used protein remover tablets (Boots Pharmaceuticals, UK). Each tablet contains a 2 mg multi-enzyme complex (trypsin, lipase and amylase) which was diluted in 10 ml sterile saline solution. The tablet is used to remove deposits such as proteins, and contains surfactants to remove carbohydrates, lipids and other proteins from the U-probe electrode.

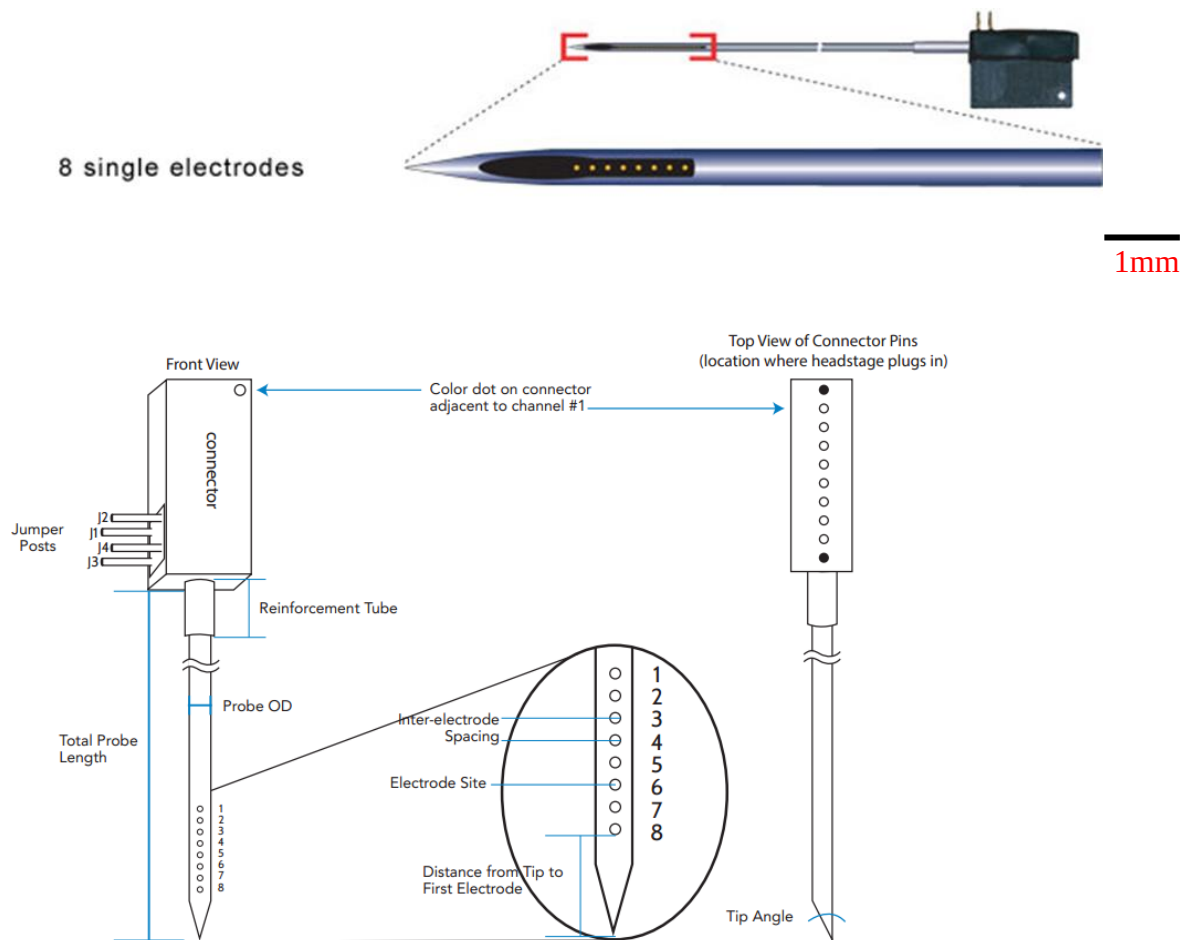


Figure 5.1 Diagram of an 8 channel U-probe. 0.050" pitch headstage (HST/8o50-Gx-xR) Plexon and the electrodes constructed. The total probe length is 30 mm and the small platinum/iridium electrode site diameter (15 μ m) enables effective single-unit recording, while a unique etching process of the electrode surface results in impedances of only 275 (+/- 50) k Ω at 1kHz. This etching drops the impedance of the electrode without significantly changing the overall surface area of the exposed recording site. (U-probe technical guide, Plexon (Texas, USA)).

5.2.2 Recording procedure

In order to standardise the route and timing of drug administration, we referred to the VPA experiment for all drug administrations. In this context, we wanted to investigate whether 1.2 mg/kg WIN and 2 mg/kg FLU would affect neuronal epileptiform-like activity in anaesthetised rats.

The electrodes were allowed to settle for ~60 min after they were lowered into the dorsal hippocampus, before recordings were made over a total period of 210 min (including basal time of 10 minutes, -10-0 min in Figure 5.2 A-C). This chapter discusses the three groups that were examined following a 10 min basal period. The rats were administered with one of the following:

1. WIN 55,212-2 administered before KA group (**WIN+KA**; n=8 rats): WIN 55,212-2 (1.2 mg/kg, i.v) followed by local KA (1 mM, 1 μ L) after 20 minutes (Figure 5.2A).
2. WIN 55,212-2 administered after KA group (**KA+WIN**; n=7 rats): local KA (1 mM, 1 μ L) followed by WIN 55,212-2 (1.2 mg/kg, i.v) after 20 minutes (Figure 5.2B).
3. Flurazepam administered after KA group (**KA+FLU**; n=4 rats): local KA (1 mM, 1 μ L) followed by FLU (2 mg/kg, i.v) after 20 minutes (Figure 5.2C).

FLU was only administered after KA group because based on our experience in the laboratory, FLU 2 mg/kg caused mortality (drop out) to our experimental animals in experiments of long duration of (3.5 hours). Therefore, we decided not to proceed with experiments for FLU administered before KA.

5.2.3 Data analysis

Extracellular unit activity data from animals with confirmed hippocampal electrode placements were sorted into individual neuronal units using *Offline Sorter*, and analysed with *NeuroExplorer*, and also exported to MATLAB format for further analysis, described in detail in Chapter 2.

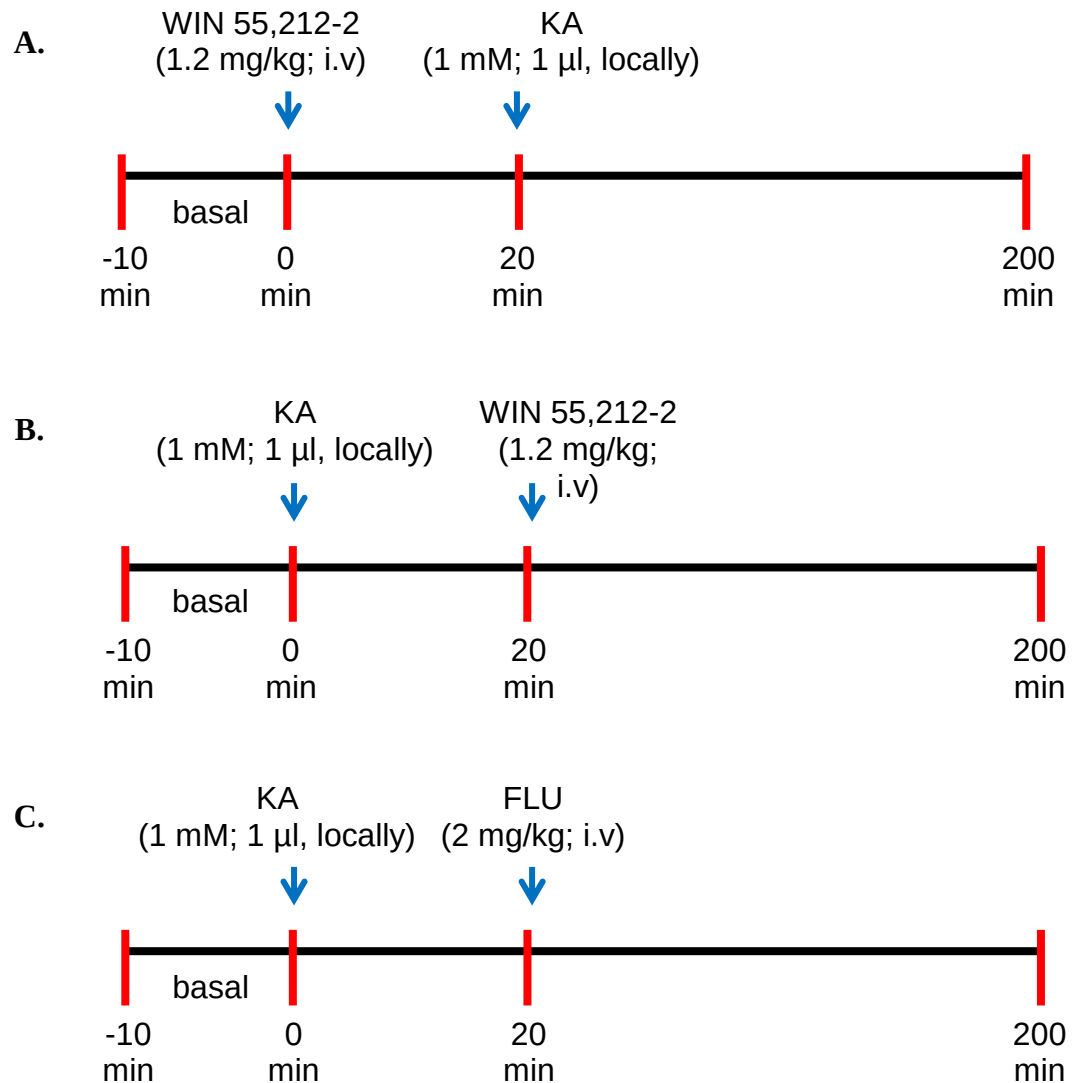


Figure 5.2 Time-line experimental protocols for KA, WIN and FLU administration.

A: WIN 55,212-2 (1.2 mg/kg) was injected intravenously after 10 min basal recording followed by 1 mM of KA locally after 20 min and recorded for a further 190 min (**WIN+KA**), n=8 rats.

B: 1 mM of KA was administered locally after 10 min basal recording followed by WIN 55,212-2 (1.2 mg/kg) intravenous injection after 20 min and recorded for a further 190 min (**KA+WIN**), n=7 rats.

C: 1 mM of KA was administered locally after 10 min basal recording followed by flurazepam (FLU, 2 mg/kg) intravenous injection after 20 min and recorded for a further 190 min (**KA+FLU**), n=4 rats.

5.3 Results

5.3.1 Histology of electrodes recording sites

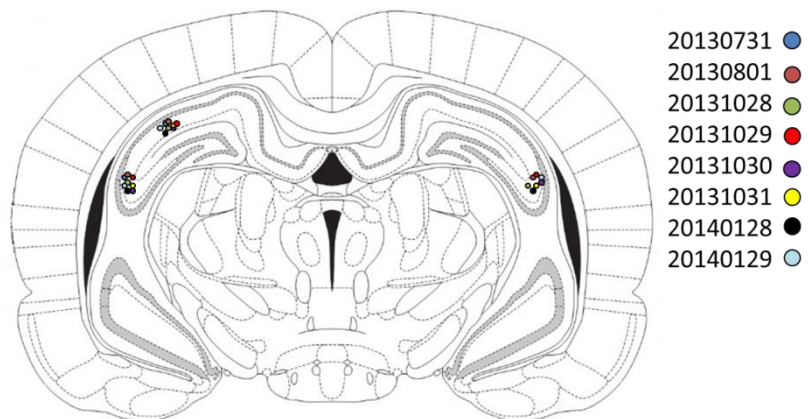
In this chapter, we are comparing three groups of rats and treatments (WIN+KA, KA+WIN and KA+FLU). In this experiment, we only focused on ipsilateral-CA1, ipsilateral-CA3 and contralateral-CA3. The contralateral-CA1 was excluded because of the use of the U-probe.

In the **WIN+KA** group, eight rats (rat ID: 20130731, 20130801, 20131028, 20131029, 20131030, 20140128 and 20140129) were used to record hippocampus activity in both hemispheres (Figure 5.3A).

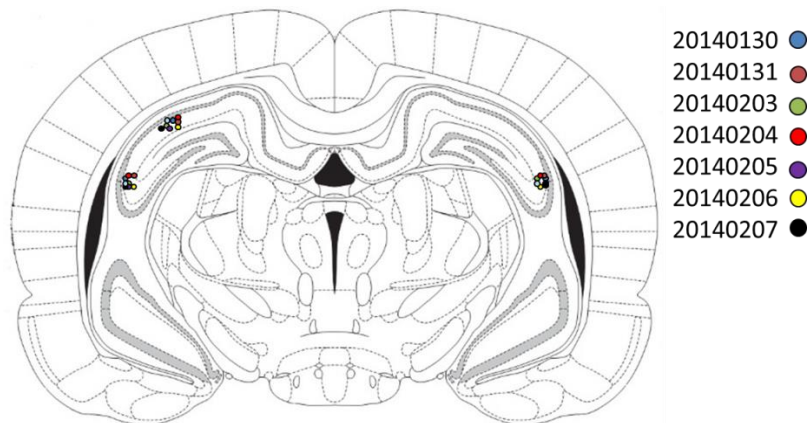
As shown in Figure 5.3B, seven rats (rat ID: 20140130, 20140131, 20140203, 20140204, 20140205, 20140206 and 20140207) were used in the **KA+WIN** group.

The third group used four rats in **KA+FLU** with the rat IDs of 20140211, 20140212, 20140214 and 20140218 (Figure 5.3C). Three rats died after ~20 min of FLU administration, reducing the number of rats used in this group. Despite this dropout, we decided not to use any additional rats for this experiment. The area of the electrodes' recording sites is shown in the histology of the rat's brains and each site is marked as a coloured dot (representing each rat used in this experiment).

A. 1.2 mg/kg WIN + 1 mM KA (WIN+KA)



B. 1 mM KA + 1.2 mg/kg WIN (KA+WIN)



C. 1 mM KA + 2 mg/kg Flurazepam (KA+FLU)

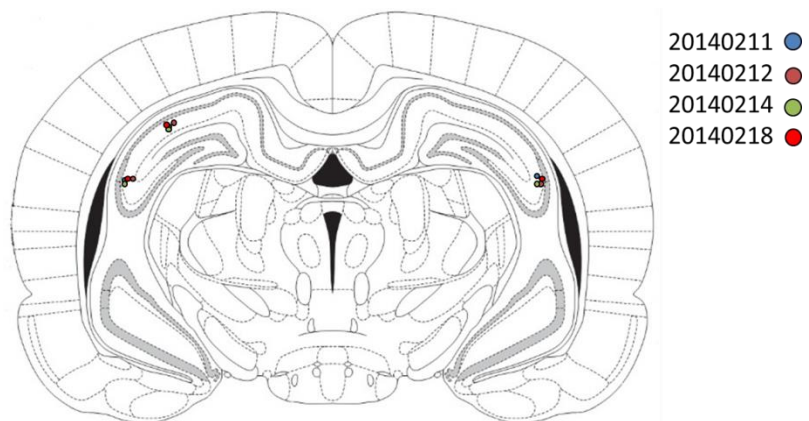


Figure 5.3 Schematic histological verification of hippocampal recording sites.

A: 1.2 mg/kg WIN + 1 mM KA (WIN+KA) group, n=8 rats

B: 1 mM KA + 1.2 mg/kg WIN (KA+WIN) group, n=7 rats

C: 1 mM KA + 2 mg/kg Flurazepam (KA+FLU) group, n=4 rats

Each colour represents one rat used in this experiment.

5.3.2 Normalised mean firing rate

Firing rate analyses were run to delineate the changes of firing pattern in ipsilateral-CA1 and CA3 and contralateral-CA3 hippocampus before and after KA administration.

Comparisons of normalised mean firing rate were made between 4 groups of rats to determine the effect of drug before and after neuronal epileptiform-like activity. These 3 groups of rats (WIN+KA, KA+WIN and KA+FLU) were compared with saline+KA group (the chosen mTLE rat model). The saline+KA group is not explained in detail in this chapter as it has been thoroughly explained in Chapters 3 and 4. As mentioned in Chapter 4, we also selected the epileptic rat model of saline+KA 1 mM administered locally (saline+KA) based on the validation experiment explained in Chapter 3.

In this chapter, we injected the drug, WIN, before KA in the first group of rats to assess whether WIN can prevent epileptiform-like activity induced by KA, whereas in the other two groups, WIN and FLU were injected after KA to determine whether WIN and FLU can attenuate or suppress the epileptiform-like activity induced by KA.

Comparisons were made using two-way ANOVA repeated measures analysis to determine the significant differences in groups of different treatments (WIN+KA, KA+WIN and KA+FLU), time course of the experiments and the interaction between group of treatments and time before and after KA-induced neuronal hyper-excitability in a particular brain region (ipsilateral-CA1, ipsilateral-CA3, contralateral-CA1 and contralateral-CA3 hippocampus).

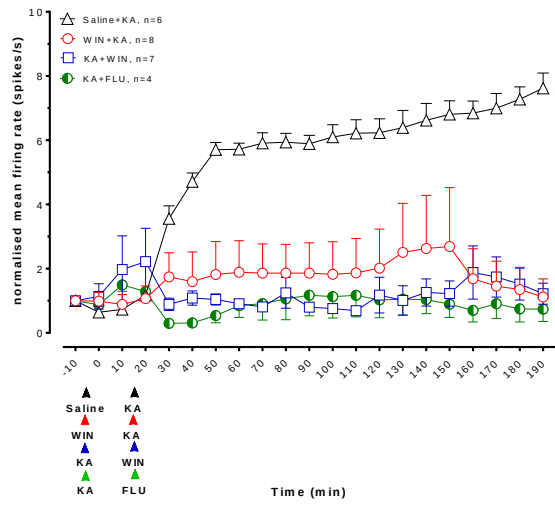
Tukey's multiple comparisons *post hoc* test was conducted to determine which groups of treatments differed from one another. Only the significant group was selected for one-way ANOVA with Dunnett's *post hoc* test to see at what time point the effect started compared to basal.

There were significant differences in normalised mean firing rate of neurones in **ipsilateral-CA1** hippocampus (Figure 5.4A) between group of treatments x time interaction ($F_{(60, 360)} = 6.537, p < 0.0001$), time course of the experiment ($F_{(20, 360)} = 7.449, p < 0.0001$) and group of treatments ($F_{(3, 18)} = 13.79, p < 0.0001$). No significant differences in normalised mean firing rate of neurones were found in **ipsilateral-CA3** hippocampus (Figure 5.4B) between group of treatments x time interaction ($F_{(60, 380)} = 0.9966, p > 0.05$) or group of treatments ($F_{(3, 19)} = 2.834, p > 0.05$). There were significant differences in normalised mean firing rate of neurones in **contralateral-CA3** hippocampus (Figure 5.4C) between group of treatments x time interaction ($F_{(60, 420)} = 7.298, p < 0.0001$), time course of the experiment ($F_{(20, 420)} = 15.42, p < 0.0001$) and group of treatments ($F_{(3, 21)} = 7.771, p < 0.0001$).

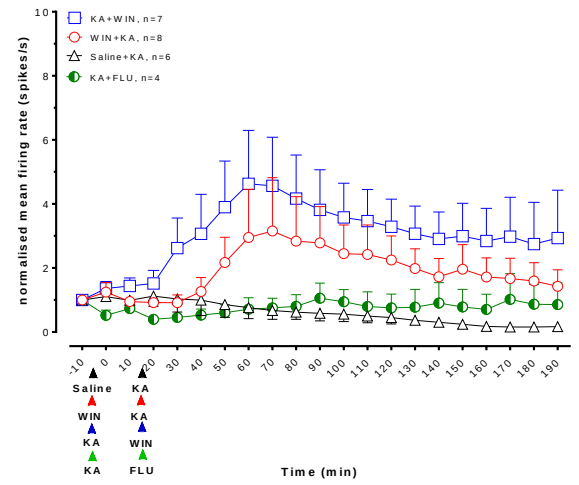
Using Tukey's multiple comparison test, only the firing rate in the saline+KA group was significantly different in ipsilateral-CA1 ($^c p < 0.001$) when compared to other groups (Figure 5.4A). No significant changes were found in ipsilateral-CA3 (Figure 5.4B); however, a significant difference was found in contralateral-CA3 when compared to WIN+KA and KA+FLU group ($^b p < 0.005$; Figure 5.4C). There was no significant difference in two-way ANOVA; therefore, one-way ANOVA with Dunnett's multiple comparisons *post hoc* test was not carried out for the other groups. We will not discuss the saline+KA group in this chapter as it has been explained in Chapter 4 (section 4.3.1).

In summary, there were no significant changes in normalised mean firing rate in the WIN+KA or KA+WIN experiments, indicating that WIN attenuates and prevents neuronal firing (epileptiform-like activity induced by KA) in ipsilateral and contralateral hippocampus, respectively. No significant changes in normalised mean firing rate were found in the KA+FLU experiment either, indicating that FLU attenuated epileptiform activity induced by KA in the ipsilateral and contralateral hippocampus.

A. Ipsilateral-CA1



B. Ipsilateral-CA3



C. Contralateral-CA3

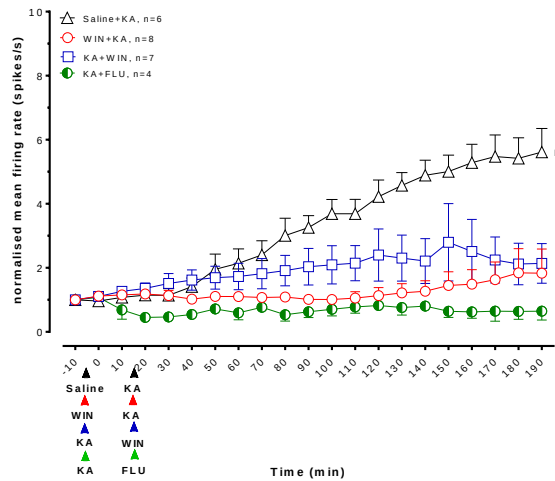


Figure 5.4 Normalised mean firing rate of neurones from in ipsilateral (CA1 and CA3) and contralateral (CA3) hippocampus from the **saline+KA** (n=6 rats), **WIN+KA** (n=8 rats), **KA+WIN** (n=7 rats) and **KA+FLU** experiments (n=4 rats).

Two-way ANOVA repeated measures.

A: Firing rate of neurones from the ipsilateral-CA1 hippocampus, $F_{(60, 360)} = 6.537$, $p < 0.0001$ (interaction).

B: Firing rate of neurones from the ipsilateral-CA3 hippocampus, $F_{(60, 380)} = 0.9966$, $p > 0.05$ (interaction).

C: Firing rate of neurones from the contralateral-CA3 hippocampus, $F_{(60, 420)} = 7.298$, $p < 0.0001$ (interaction).

Result of *post hoc* Tukey's multiple comparisons test: ^b $p < 0.005$ when compared to WIN+KA and KA+FLU groups, (^c $p < 0.001$) when compared to other groups, respectively.

5.3.3 Normalised mean synchrony index (SI)

Two-way repeated measures ANOVA with Dunnett's *post hoc* multiple comparisons test was conducted to see whether there were significant changes in synchrony index when compared to basal in each group.

WIN administered before KA (WIN+KA) and after KA (KA+WIN) did not alter SI in either hippocampus

There is no significant interaction in the WIN+KA group ($F_{(14, 140)} = 0.5492$, $p > 0.05$), brain region ($F_{(2, 20)} = 0.1697$, $p > 0.05$) or time ($F_{(7, 140)} = 1.073$, $p > 0.05$; Figure 5.5 A). SI of ipsilateral and contralateral hippocampus in WIN+KA group was not significant using Dunnett's multiple comparison test when compared to basal, with no changes in synchrony before and after KA administration (Figure 5.5A).

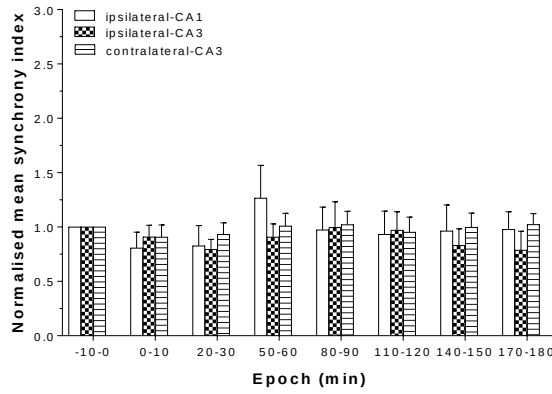
There is no significant interaction in the KA+WIN group ($F_{(14, 119)} = 0.2785$, $p > 0.05$) and no significant difference in brain region ($F_{(2, 17)} = 0.2502$, $p > 0.05$; Figure 5.5B).

Flurazepam administered after KA (KA+FLU) did not alter SI in either hippocampus

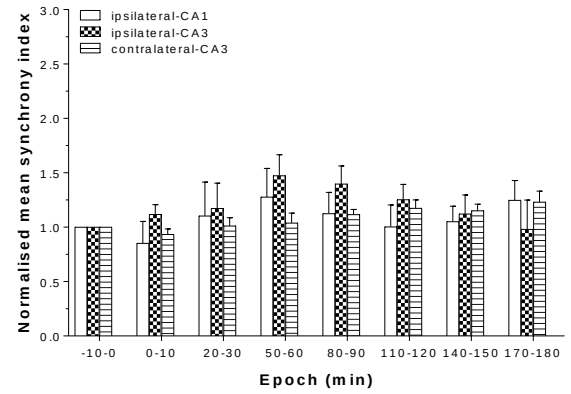
There is no significant interaction in the KA+FLU group ($F_{(14, 63)} = 0.7534$, $p > 0.05$) or brain region ($F_{(2, 9)} = 1.425$, $p > 0.05$; Figure 5.5C).

Overall, WIN 55,212-2 administered before KA (WIN+KA) and after KA (KA+WIN) did not alter SI in either hippocampus, reflecting no changes in synchronisation. Similarly, no changes in synchronisation were observed when FLU was administered after neuronal epileptiform-like activity by KA.

A. WIN+KA



B. KA+WIN



C. KA+FLU

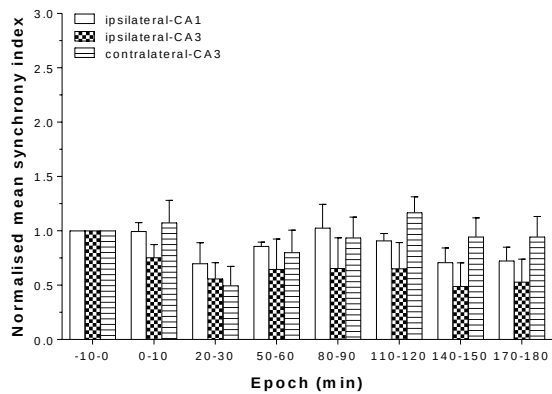


Figure 5.5 Histograms of normalised mean synchrony index of neurones from the ipsilateral-CA1, ipsilateral-CA3 and contralateral-CA3 hippocampus.

A: Normalised mean synchrony index of WIN+KA group, n=8 rats.

B: Normalised mean synchrony index of KA+WIN group, n=7 rats.

C: Normalised mean synchrony index of KA+FLU group, n=4 rats.

5.3.4 Unit-pair cross-correlation

The mean k-index (pooled data of n=8 rats in WIN+KA, n=7 rats in KA+WIN and n=4 rats in KA+FLU) was calculated to determine the strength of the correlation.

No correlation between neurones in any region of interest (ROI) when WIN was administered before KA (WIN+KA)

An ordinary two-way ANOVA analysis was conducted to see if there were significant differences in the neurones in the ROIs. No significant differences were found between region of interest ($F_{(2, 12048)} = 2.81, p > 0.05$), time course of the experiment ($F_{(7, 12048)} = 1.503, p > 0.05$) or interaction of region of interest x time ($F_{(14, 12048)} = 0.4645, p > 0.05$).

Dunnett's *post hoc* multiple comparisons test on the mean k-index from pooled data (n=8 rats) of WIN+KA showed that there were no significant changes. This result indicates that there is no correlation among neurones in ROI 1, ROI 2 or ROI 3 (k-index during basal, -10-0 min; ROI 1=39.58 $\pm$ 7.1, ROI 2=42.57 $\pm$ 7.6 (no contralateral-1), ROI 3=31.46 $\pm$ 3.9) (Figure 5.6A).

WIN administered after KA (KA+WIN) suppressed correlation between neurones in ROI 2 and ROI 3

Significant differences were found between region of interest ($F_{(2, 7624)} = 5.221, p < 0.005$), time course of the experiment ($F_{(7, 7624)} = 11.14, p < 0.0001$) and interaction of region of interest x time ($F_{(14, 7624)} = 3.744, p < 0.0001$).

Pooled data (n=7 rats) of mean k-index (basal -10-0 min =39.50 $\pm$ 12.0) showed the correlation strength of ROI 1 was significantly increased during the epoch at 20-30 min (80.74 $\pm$ 40.1; $^b p < 0.005$) after 10 min of WIN 55,212-2 administration. In contrast, the mean k-index of the contralateral-CA3 hippocampus (ROI 2) showed a significant reduction from 50.90 $\pm$ 5.5 during basal to 29.94 $\pm$ 3.38 at the epochs of 50-60 min ($^b p < 0.005$), 80-90 min and 110-120 min ($^c p < 0.001$), 140-150 min and 170-180 min ($^d p < 0.0001$). Meanwhile, significant reduction was also found in

ipsilateral-contralateral hippocampus (ROI 3), from 40.61 ± 4.4 during basal to 29.96 ± 3.33 at the epochs of 20-30 min ($^ap < 0.05$), 50-60 min (11.52 ± 1.64 ; $^dp < 0.0001$), 80-90 min (17.28 ± 2.38 ; $^bp < 0.005$), 110-120 min (21.39 ± 3.26 ; $^ap < 0.05$), 140-150 min (13.74 ± 1.92 ; $^dp < 0.0001$) and 170-180 min (18.72 ± 2.81 ; $^bp < 0.005$; Figure 5.6B)

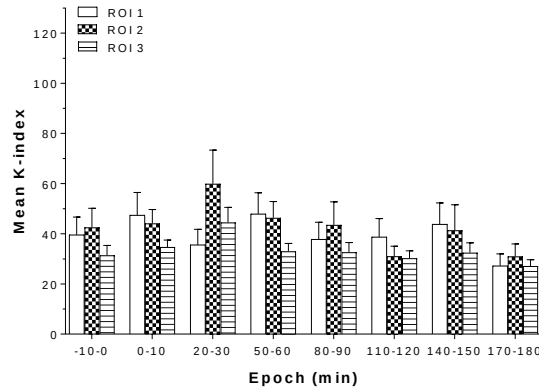
FLU suppressed the correlation between neurones in ROI 2 at epoch of 170-180 min

No significant differences were found for time course of the experiment ($F_{(7, 4480)} = 1.204$, $p > 0.05$) or the interaction of region of interest x time ($F_{(14, 4480)} = 1.001$, $p > 0.05$). There is a significant difference between region of interest ($F_{(2, 4480)} = 16.92$, $p < 0.0001$; two-way ANOVA).

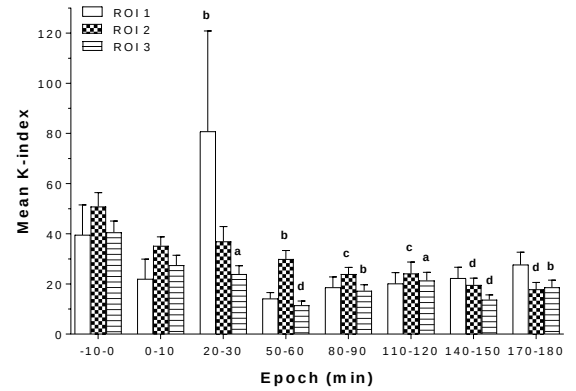
The pooled data (n=4 rats) of mean k-index showed that only correlation between neurones from contralateral hippocampus (ROI 2) was significantly decreased at epoch of 170-180 min (36.28 ± 7.39 ; $^ap < 0.05$) compared to basal (65.05 ± 13.85 ; Figure 5.6C).

Overall, no correlation was found between neurones in any region of interest (ROI) when WIN was administered before KA (WIN+KA). On the other hand, WIN administered after KA (KA+WIN) suppressed the correlation between neurones in ROI 2 and ROI 3. FLU did not alter the correlation between neurones when administered after KA (KA+FLU).

A. WIN+KA



B. KA+WIN



C. KA+FLU

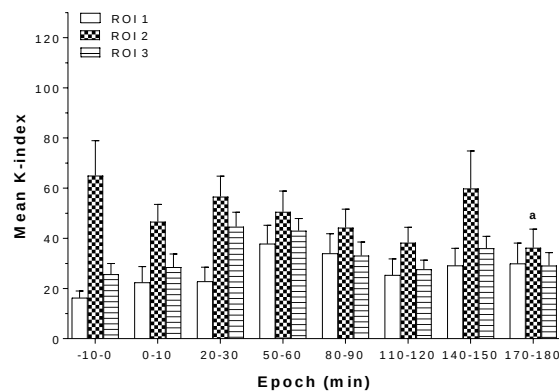


Figure 5.6 Histograms of mean k-index of all neurones in the experimental group.

A: WIN+KA group, n=8 rats.

B: KA+WIN group, n=7 rats.

C: KA+FLU group, n=4 rats.

^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.

Ordinary two-way ANOVA with Dunnett's multiple comparisons test.

ROI 1=ipsilateral CA1 and CA3; ROI 2=contralateral CA1 and CA3; ROI 3=neurones in both ipsilateral and contralateral hippocampus.

5.3.5 LFP Frequency band distribution

Changes in LFP frequency band distribution provide information about which frequency bands were prominent throughout the experiment and how KA, WIN and FLU might have affected the frequency band distribution. The prominent frequency bands provide additional information about the physiology of functional connectivity in the hippocampus in acute mTLE model and the changes associated with WIN administration (before or after KA-induced epileptiform-like activity) and FLU administration given after KA-induced epileptiform-like activity.

WIN administered before KA (WIN+KA) increased 0.1-1Hz, delta, theta, beta and gamma bands in ipsilateral-CA1 and gamma band in ipsilateral-CA3 and contralateral-CA3 hippocampus

Significant changes in the interaction of the overall LFP power were found between brain region x time ($F_{(14, 70)} = 2.06, p < 0.05$) and time course of the experiment ($F_{(7, 35)} = 8.495, p < 0.0001$). However, no significant changes were found in any brain regions of the rats (Figure 5.7; $F_{(2, 10)} = 1.767, p > 0.05$; two-way ANOVA repeated measures).

Using Tukey's multiple comparisons test, normalised mean power in ipsilateral-CA3 was not significantly different compared to all brain regions.

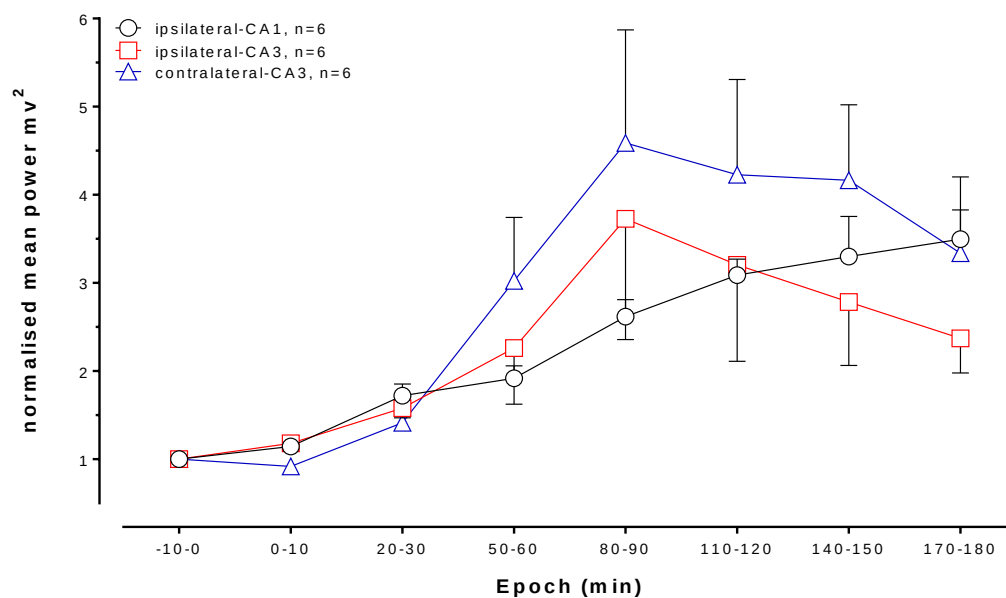


Figure 5.7 Normalised mean power of LFP in **WIN+KA** group in 210 min experiment, mean $\pm$ SEM, n=8 rats.

Significant interaction, $F_{(14, 70)} = 2.06, p < 0.05$.

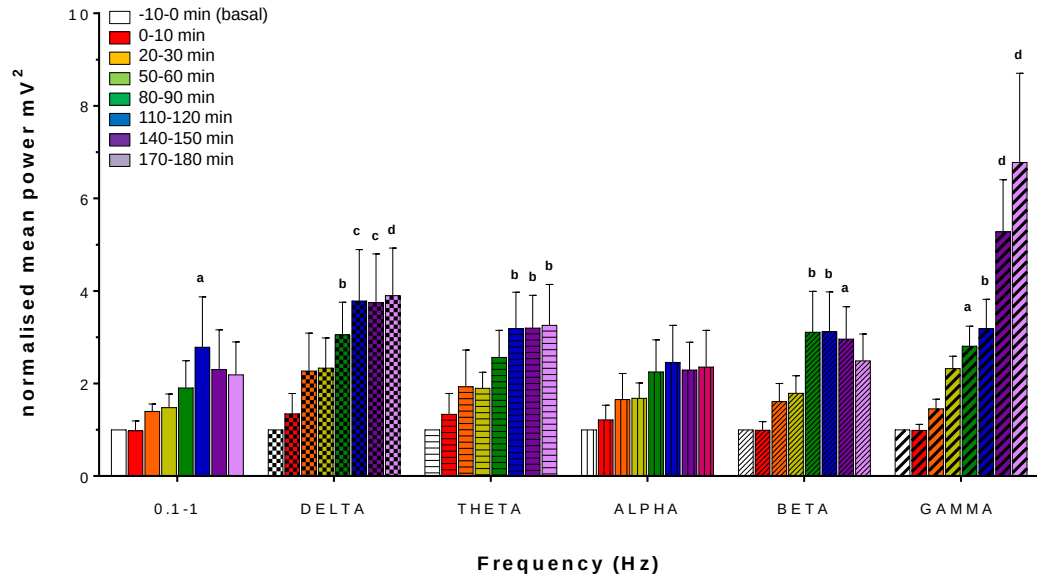
Two-way ANOVA repeated measures and *post hoc* Tukey's multiple comparisons tests

In **ipsilateral-CA1**, there is significant interaction between time x frequency band ($F_{(35, 245)} = 2.179$, $p < 0.001$; two-way ANOVA repeated measures). Using Dunnett's multiple comparisons test, normalised mean power in **0.1-1 Hz** from WIN+KA group was significantly increased at 110-120 min (2.78 ± 1.08 ; $^a p < 0.05$), normalised mean power in **delta** band was significantly increased at the epochs of 80-90 min (3.06 ± 0.70 ; $^b p < 0.005$), epochs of 110-120 min and 140-150 min ($^c p < 0.001$, respectively) and 170-180 min (3.90 ± 1.02 ; $^d p < 0.0001$) compared to basal (-10-0 min;) as shown in Figure 5.8A. There were also significant increases in the normalised mean power of **theta** band at 110-120 min, 140-150 min and 170-180 min ($^b p < 0.005$, respectively) compared to basal. Significant increases in normalised mean power were also found in **beta** band at 80-90 min, 110-120 min ($^b p < 0.005$, respectively) and 140-150 min (2.96 ± 0.70 ; $^a p < 0.05$) compared to basal. Significant increases in normalised mean power were also found in **gamma** band during the epochs at 80-90 min (2.81 ± 0.43 ; $^a p < 0.05$), 110-120 min (3.19 ± 0.63 ; $^b p < 0.005$) and 140-150 min and 170-180 min ($^d p < 0.0001$, respectively) compared to basal.

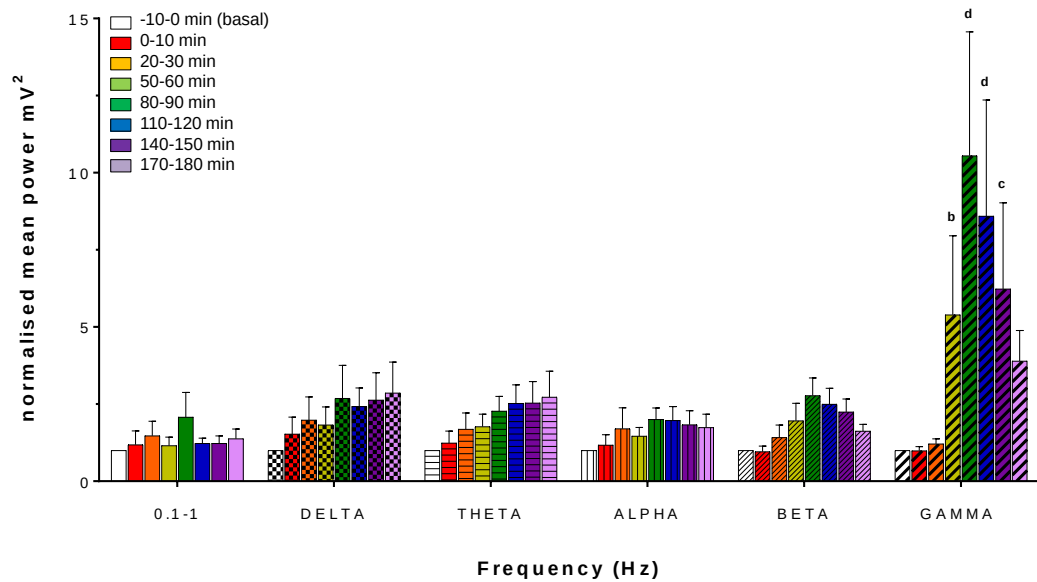
Significant interaction was found in **ipsilateral-CA3** ($F_{(35, 245)} = 2.609$, $p < 0.0001$; Two-way ANOVA repeated measures). Figure 5.8B shows that significant increases were only found in the normalised mean power in **gamma** band 30 min after KA administration during the epochs at 50-60 min (5.39 ± 2.56 ; $^b p < 0.005$), 80-90 min and 110-120 min ($^d p < 0.0001$, respectively), and 140-150 min (6.23 ± 2.79 ; $^c p < 0.001$) compared to basal.

No significant interaction was found between time x frequency band in **contralateral-CA3** ($F_{(35, 245)} = 1.138$, $p > 0.05$; two-way ANOVA repeated measures). As shown in Figure 5.8C, in contralateral-CA3, the normalised mean power of **gamma** band was significantly increased at 50-60 min (6.59 ± 3.98 ; $^a p < 0.05$), 80-90 min and 110-120 min ($^d p < 0.0001$, respectively) and at 140-150 min (8.01 ± 5.36 ; $^c p < 0.001$) compared to basal.

A. Ipsilateral-CA1



B. Ipsilateral-CA3



C. Contralateral-CA3

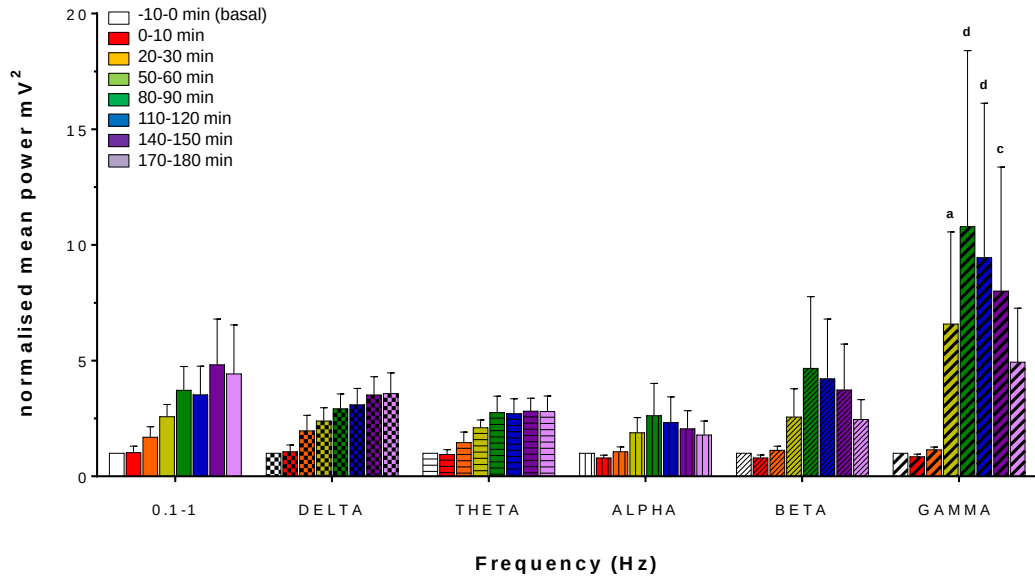


Figure 5.8 Normalised mean power of LFP frequency band distribution (n= 8 rats) of ipsilateral-CA1 and CA3 and contralateral-CA3 hippocampus from **WIN+KA** group throughout the 210 min experiment.

A: Frequency band distribution in ipsilateral-CA1, significant interaction, $F_{(14, 70)} = 2.06$, $p < 0.05$.

B: Frequency band distribution in ipsilateral-CA3, significant interaction, $F_{(35, 245)} = 2.609$, $p < 0.0001$.

C: Frequency band distribution in contralateral-CA3, no significant interaction, $F_{(35, 245)} = 1.138$, $p > 0.05$.

^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.

Two-way ANOVA repeated measures and *post hoc* Dunnett's multiple comparisons tests.

WIN administered after KA (KA+WIN) increased delta, theta, alpha, beta and gamma band in ipsilateral-CA1 and contralateral-CA3

Significant differences in overall LFP power were found in the interaction between brain region x time ($F_{(14, 70)} = 5.314, p < 0.0001$), time course of the experiment ($F_{(7, 35)} = 22.18, p < 0.0001$) and all brain regions of the rats (Figure 5.9; $F_{(2, 10)} = 24.77, p < 0.001$; two-way ANOVA repeated measures).

Tukey's multiple comparisons test showed that the normalised mean power in ipsilateral-CA1 was significantly different compared to ipsilateral-CA3 ($^c p < 0.001$). A significant difference was also found in contralateral-CA3 ($^c p < 0.001$) compared to ipsilateral-CA3 (Figure 5.9).

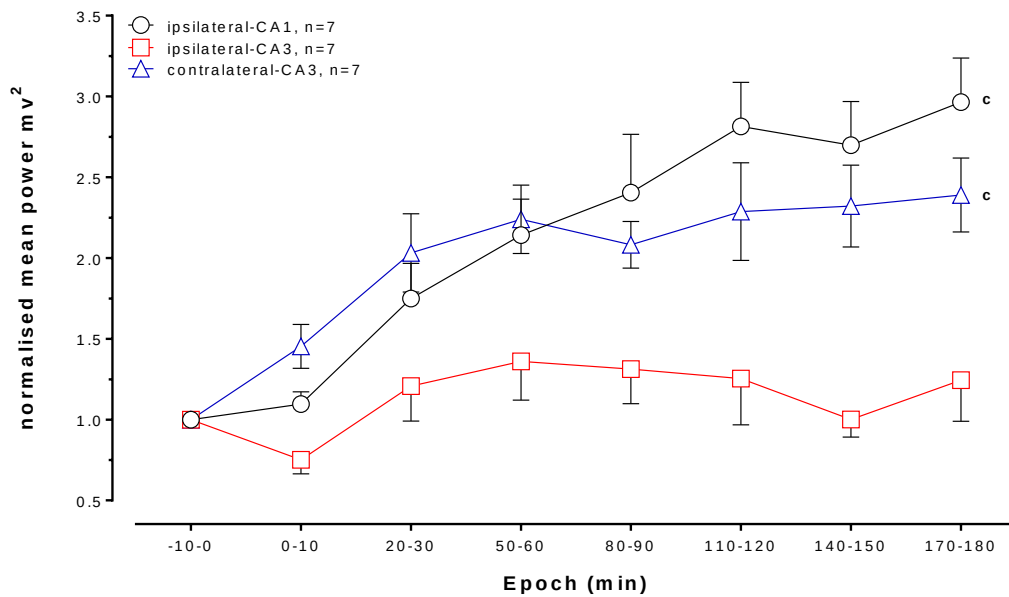


Figure 5.9 Normalised mean power of LFP in **KA+WIN** group in 210 min experiment, mean $\pm$ SEM, n= 6 rats.

Significant interaction, $F_{(14, 70)} = 5.314, p < 0.0001$.

$^c p < 0.001$ when compared to ipsilateral-CA3.

Two-way ANOVA repeated measures and *post hoc* Tukey's multiple comparisons tests.

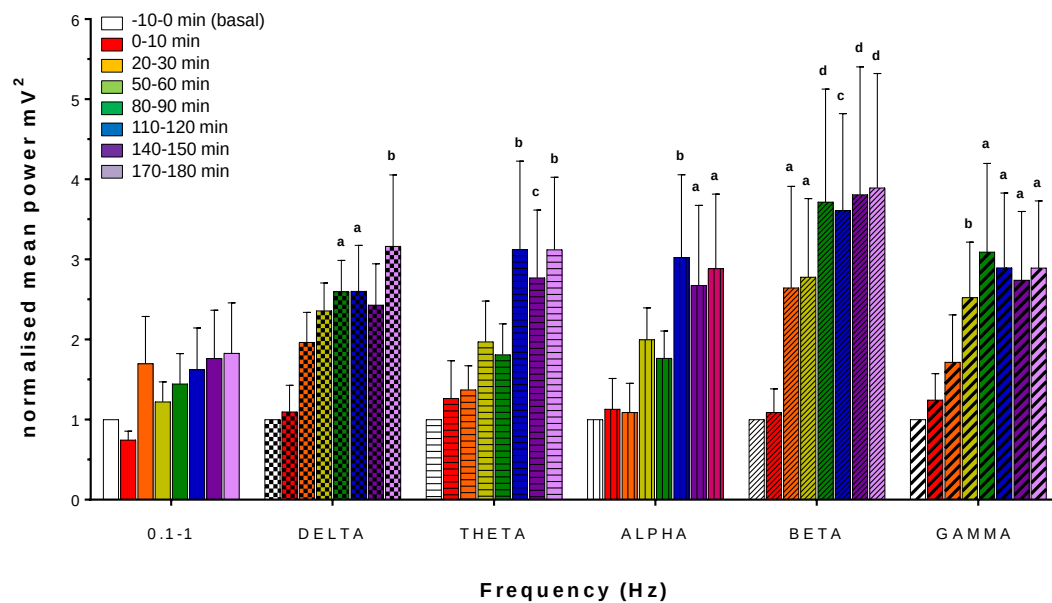
In **ipsilateral-CA1**, no significant interaction was found between time x frequency band ($F_{(35, 210)} = 0.8361$, $p > 0.05$; two-way ANOVA repeated measures). The normalised mean power in **delta** band from KA+WIN group was significantly increased during the epochs of 80-90 min, 110-120 min ($^a p < 0.05$) and 170-180 min (Figure 5.10A; 3.16 ± 0.90 ; $^b p < 0.005$) compared to basal (-10-0 min; Dunnett's multiple comparisons test). There was a significant increase in normalised mean power in **theta** band at 110-120 min (3.13 ± 1.10 ; $^b p < 0.005$), 140-150 min (2.77 ± 0.85 ; $^c p < 0.001$), and at 170-180 min (3.12 ± 0.90 ; $^b p < 0.005$) compared to basal. There were also significant increases in normalised mean power in **alpha** band at 110-120 min (3.03 ± 1.03 ; $^b p < 0.005$), 140-150 min and 170-180 min ($^a p < 0.05$, respectively) compared to basal. Significant increases in normalised mean power in **beta** band were also found at 20-30 min and 50-60 min ($^a p < 0.05$, respectively), 80-90 min (3.72 ± 1.41 ; $^c p < 0.0001$), 110-120 min (3.61 ± 1.21 ; $^c p < 0.001$), 140-150 min and 170-180 min ($^d p < 0.0001$, respectively) compared to basal. There was also a significant increase in the normalised mean power in **gamma** band at 50-60 min (2.52 ± 0.69 ; $^b p < 0.005$), 80-90 min, 110-120 min, 140-150 min and 170-180 min ($^a p < 0.05$, respectively) compared to basal.

No significant interaction was found between time x frequency band in **ipsilateral-CA3** ($F_{(35, 210)} = 1.111$, $p > 0.05$; two-way ANOVA repeated measures). Significant increases were only found the normalised mean power in **gamma** band at the epoch of 20-30 min (2.16 ± 0.78 ; $^b p < 0.005$), 50-60 min (2.44 ± 1.04 ; $^c p < 0.001$), 80-90 min (2.28 ± 0.74 ; $^b p < 0.005$), 110-120 min (2.64 ± 1.07 ; $^d p < 0.0001$) and 170-180 min (2.46 ± 1.11 ; $^c p < 0.001$) compared to basal (Figure 5.10B).

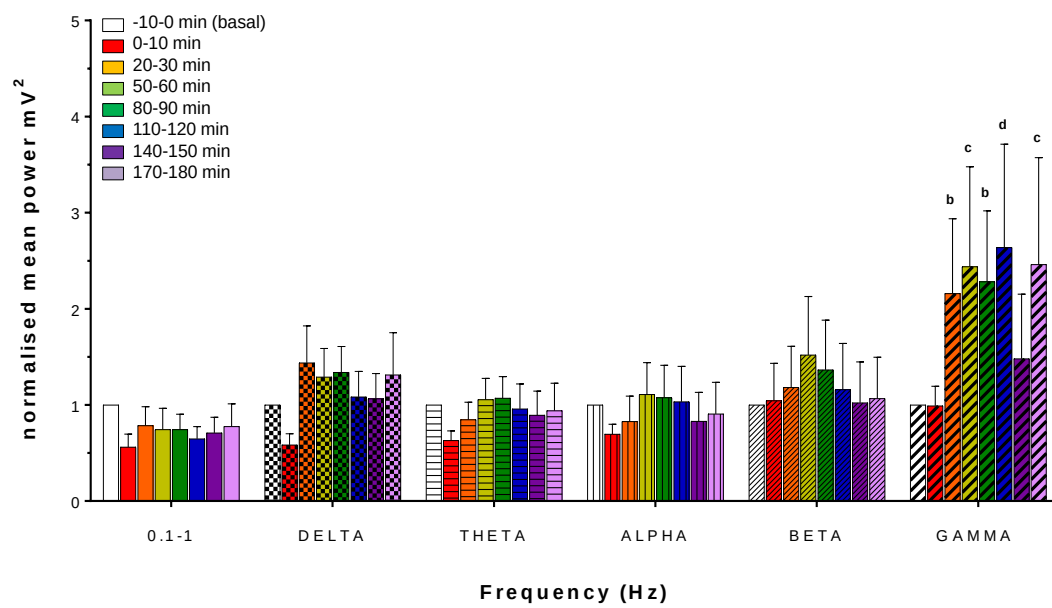
In **contralateral-CA3**, significant interaction was found between time x frequency band ($F_{(35, 210)} = 1.84$, $p < 0.005$; two-way ANOVA repeated measures). There were significant differences in normalised mean power in **0.1-1 Hz** from KA+WIN group (Figure 5.10C) at the epochs of 140-150 min and 170-180 min ($^b p < 0.005$, respectively) compared to basal. There were also significant increases the normalised mean power in **delta** band at 140-150 min (2.47 ± 0.58 ; $^a p < 0.05$) and 170-180 min (2.69 ± 0.68 ; $^b p < 0.005$) compared to basal. A significant increase in normalised mean power in **theta** band was also found at the epochs of 20-30 min (2.29 ± 0.77 ;

^a $p < 0.05$), compared to basal. We can also find a significant difference in the normalised mean power in **alpha** band during the epoch of 20-30 min (2.47 ± 0.93 ; ^a $p < 0.05$) compared to basal. Significant increases the normalised mean power in **beta** band were also found at 20-30 min (2.80 ± 0.94 ; ^b $p < 0.005$), 80-90 min and 110-120 min (^a $p < 0.05$, respectively). There were significant increases the normalised mean power in **gamma** band at the epochs of 50-60 min (3.28 ± 1.06 ; ^d $p < 0.0001$), 80-90 min (2.71 ± 0.85 ; ^b $p < 0.005$), 110-120 min, 140-150 min and 170-180 min (^d $p < 0.0001$, respectively) compared to basal (Figure 5.10C).

A. Ipsilateral-CA1



B. Ipsilateral-CA3



C. Contralateral-CA3

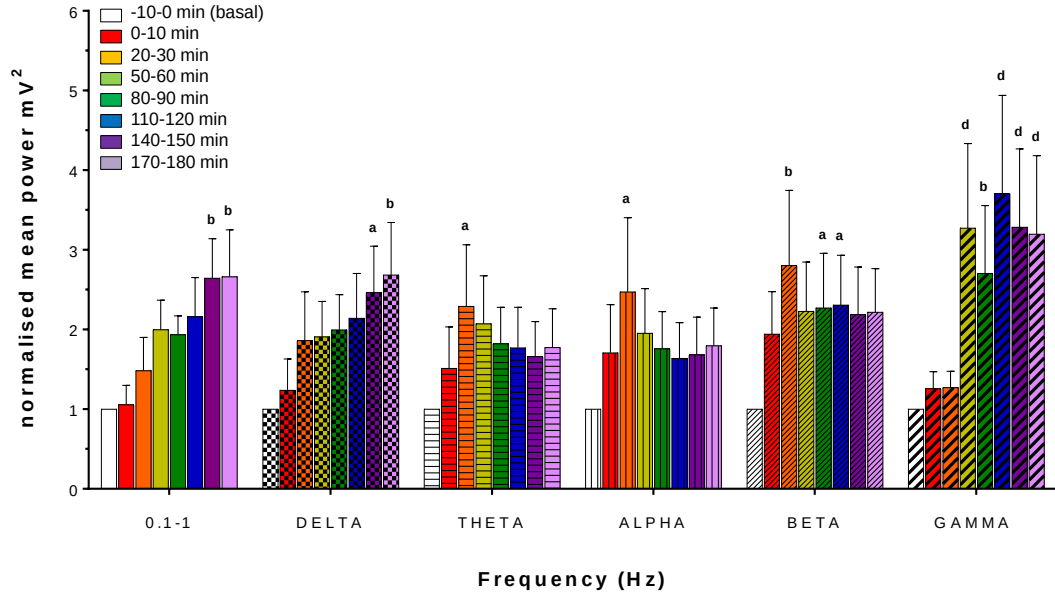


Figure 5.10 Normalised mean power of LFP frequency band distribution (n=7 rats) of ipsilateral hippocampus from **KA+WIN** group throughout the 210 min experiment.

A: Frequency band distribution in ipsilateral-CA1, no significant interaction, $F_{(35, 210)} = 0.8361$, $p > 0.05$.

B: Frequency band distribution in ipsilateral-CA3, no significant interaction, $F_{(35, 210)} = 1.111$, $p > 0.05$.

C: Frequency band distribution in contralateral-CA3, significant interaction, $F_{(35, 210)} = 1.84$, $p < 0.005$.

^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.

Two-way ANOVA repeated measures and *post hoc* Dunnett's multiple comparisons tests.

FLU administered increased LFP power in delta, theta, alpha and beta bands in ipsilateral hippocampus

Significant differences in overall LFP power were found in the interaction between brain region x time ($F_{(14, 70)} = 7.818, p < 0.0001$), time course of the experiment ($F_{(7, 35)} = 34.66, p < 0.0001$) and all brain regions of the rats (Figure 5.11; $F_{(2, 10)} = 15.91, p < 0.001$; two-way ANOVA repeated measures).

Tukey's multiple comparisons test showed that the normalised mean power in ipsilateral-CA1 was significantly different compared to ipsilateral-CA3 ($^a p < 0.05$) and ipsilateral-CA3 compared to contralateral-CA3 ($^a p < 0.05$). Significant differences were also found between ipsilateral-CA1 and contralateral-CA3 ($^c p < 0.001$; Figure 5.11).

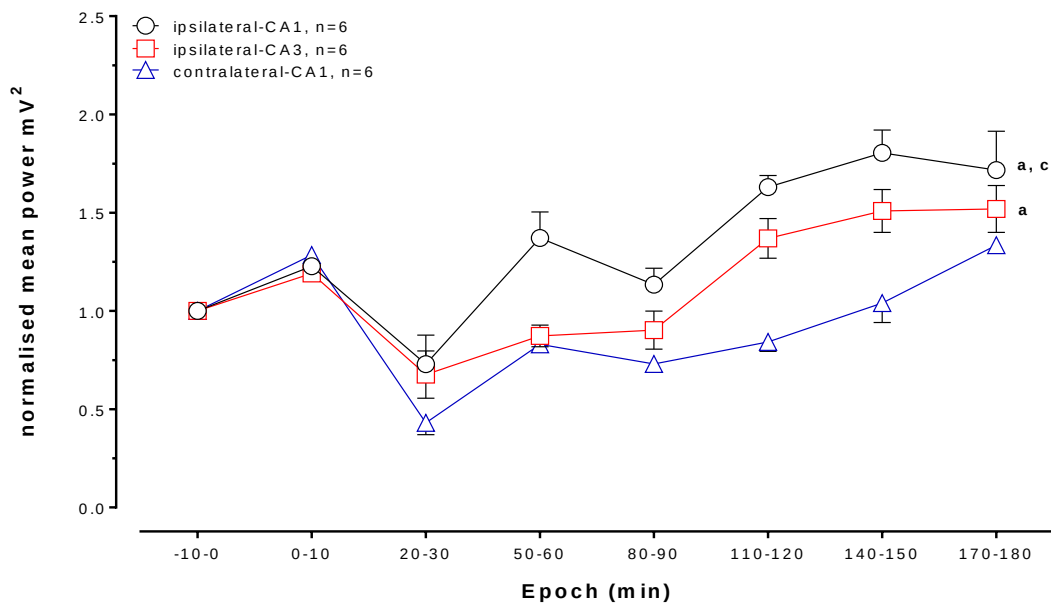


Figure 5.11 Normalised mean power of LFP in **KA+FLU** group in 210 min experiment, mean $\pm$ SEM, n= 4 rats.

Significant interaction, ($F_{(14, 70)} = 7.818, p < 0.0001$).

$^a p < 0.05$ when compared to contralateral-CA1 and $^c p < 0.001$ when compared to ipsilateral-CA3.

Two-way ANOVA repeated measures and *post hoc* Tukey's multiple comparisons tests.

In Figure 5.12A, FLU administered after KA showed no significant interaction between time x frequency band, $F_{(35, 105)} = 1.103$, $p > 0.05$ (two-way ANOVA repeated measures). Significant increases were found in ipsilateral-CA1 in the normalised mean power in **delta** band at the epochs of 140 -150 min (1.90 ± 0.30 ; $^a p < 0.05$) and 170-180 min (2.38 ± 1.03 ; $^c p < 0.001$). There were significant increases in the normalised mean power of **theta** band at 110-120 min (2.09 ± 0.27 ; $^b p < 0.005$), 140-150 min (2.63 ± 0.48 ; $^d p < 0.0001$), and at 170-180 min (2.33 ± 0.50 ; $^c p < 0.001$) compared to basal. There were also significant increases in the normalised mean power in **alpha** band at 140-150 min (2.06 ± 0.28 ; $^b p < 0.005$ and 170-180 min ($^a p < 0.05$, respectively) compared to basal. Significant increases in the normalised mean power in **beta** band were also found at the epochs of 140-150 min ($^a p < 0.05$) compared to basal.

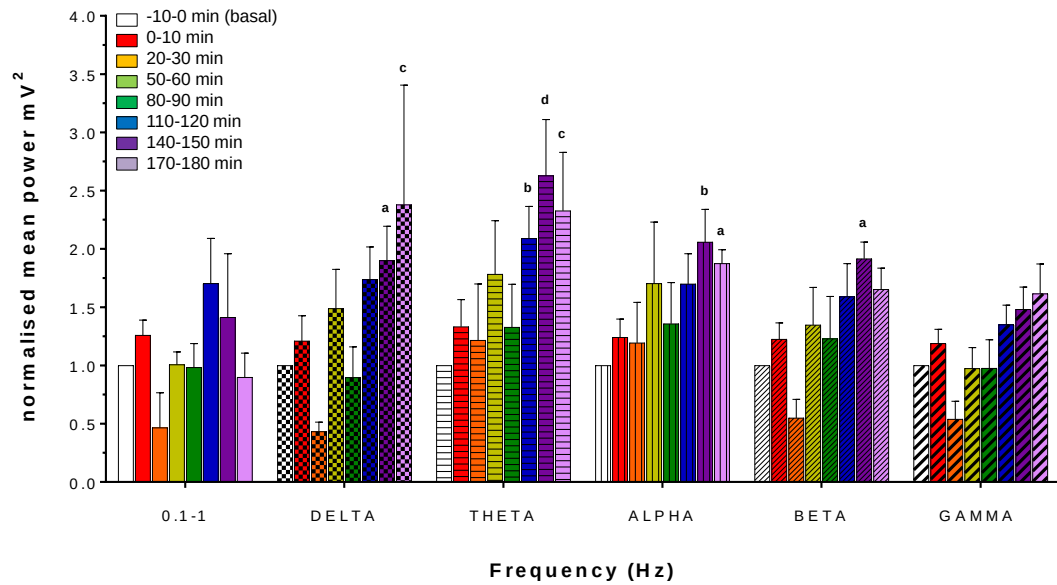
In **ipsilateral-CA3** (Figure 5.12B), no significant interaction was found between time x frequency band, $F_{(35, 105)} = 1.417$, $p > 0.05$ (two-way ANOVA repeated measures). There were significant increases in the normalised mean power in **delta** band at the epochs of 110-120 min (1.58 ± 0.49 ; $^a p < 0.05$), 140-150 min and 170-180 min ($^b p < 0.005$, respectively) compared to basal. Significant increases in the normalised mean power in **theta** band were also found at 110-120 min (1.74 ± 0.18 ; $^b p < 0.005$), 140-150 min and 170-180 min ($^d p < 0.0001$, respectively) compared to basal. We can also find significant increases in the normalised mean power of **alpha** band at 110-120 min (1.56 ± 0.08 ; $^a p < 0.05$), 140-150 min and 170-180 min ($^b p < 0.005$, respectively) compared to basal. A significant increase in in the normalised mean power **beta** band was also found at the epoch of 170-180 min (1.58 ± 0.22 ; $^a p < 0.05$).

No significant interaction was found between time x frequency band in **contralateral-CA3** (Figure 5.12C), $F_{(35, 105)} = 1.01$, $p > 0.05$ (Two-way ANOVA repeated measures). A significant decrease was found in the normalised mean power in **0.1-1 Hz** band at the epochs of 20-30 min (0.26 ± 0.22 ; $^c p < 0.001$), while significant increases were found at the epochs of 140-150 min and 170-180 min ($^a p < 0.05$) compared to basal. There were significant decreases in the normalised

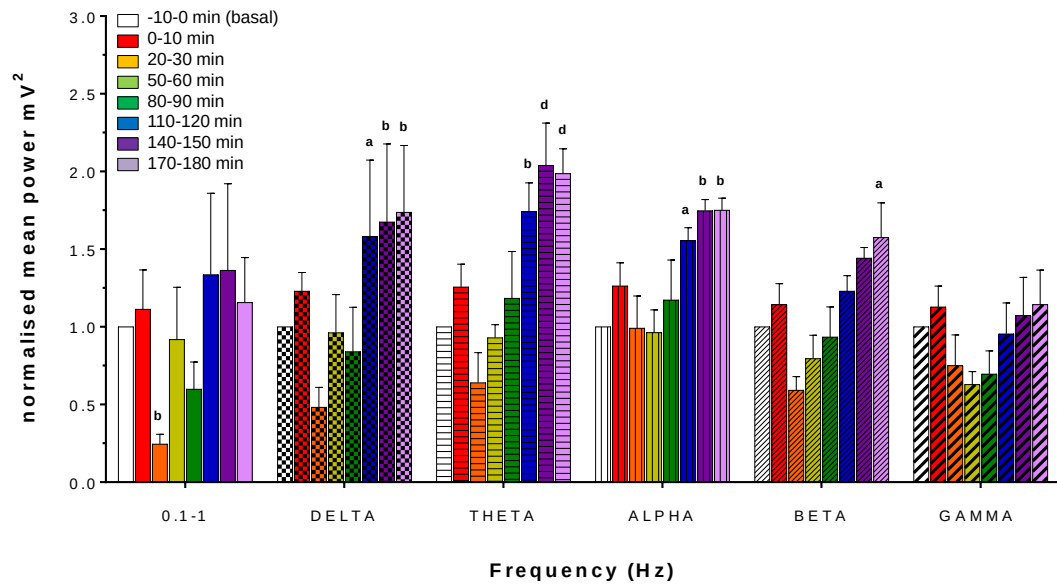
mean power in **delta**, **beta** and **gamma** at 20-30 min (0.25 ± 0.16 , 0.44 ± 0.17 , 0.48 ± 0.19 ; $^a p < 0.001$, respectively) when compared to basal.

In summary, WIN administered before KA (**WIN+KA**) increased the normalised mean power in **0.1-1 Hz**, **delta**, **theta**, **beta** and **gamma** bands in ipsilateral-CA1 and gamma band in ipsilateral-CA3 and contralateral-CA3 hippocampus. WIN administered after KA (**KA+WIN**) increased the normalised mean power in **delta**, **theta**, **alpha**, **beta** and **gamma** band in ipsilateral-CA1 and contralateral-CA3. FLU administered after KA (**KA+FLU**) increased the normalised mean power in **delta**, **theta**, **alpha** and **beta** bands in the ipsilateral hippocampus. Overall, the normalised mean power in **delta**, **theta**, **beta** and **gamma** bands in ipsilateral-CA1 were prominent in WIN experiment administered before and after KA-induced seizure. The normalised mean power in **delta**, **theta**, **alpha** and **beta** bands were also prominent in **KA+FLU** in the ipsilateral hippocampus.

A. Ipsilateral-CA1



B. Ipsilateral-CA3



C. Contralateral-CA3

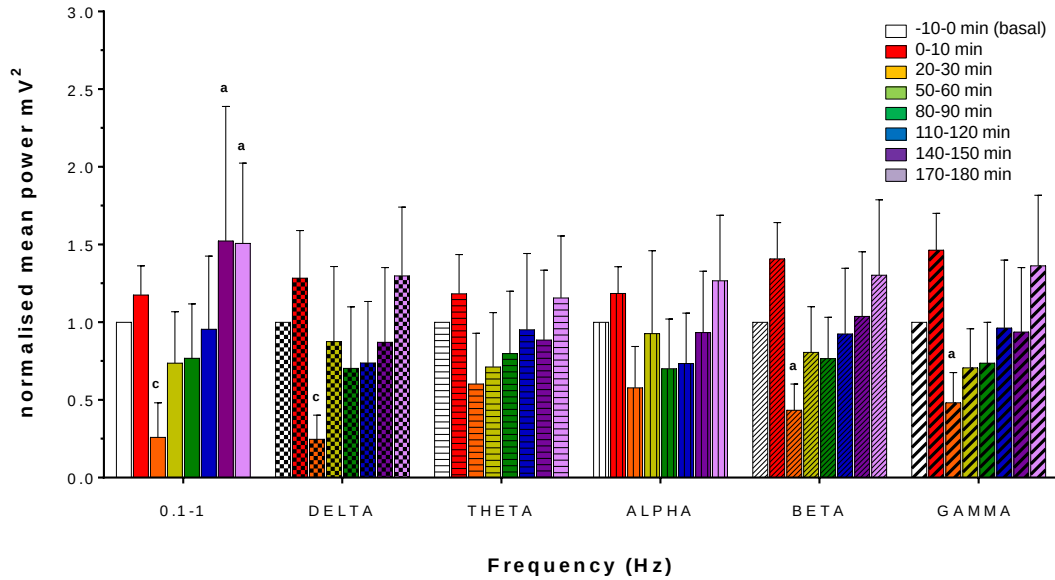


Figure 5.12 Normalised mean power of LFP frequency band distribution (n=4 rats) of ipsilateral hippocampus from KA+FLU group throughout the 210 min experiment.

A: Frequency band distribution in ipsilateral-CA1, no significant interaction, $F_{(35, 105)} = 1.103$, $p > 0.05$

B: Frequency band distribution in ipsilateral-CA3, no significant interaction, $F_{(35, 105)} = 1.417$, $p > 0.05$.

C: Frequency band distribution in contralateral-CA3, no significant interaction, $F_{(35, 105)} = 1.02$, $p > 0.05$.

^a $p < 0.05$, ^b $p < 0.005$, ^c $p < 0.001$ and ^d $p < 0.0001$ when compared to basal, respectively.

Two-way ANOVA repeated measures and *post hoc* Dunnett's multiple comparisons tests.

Table 5.1 summarises all the results presented in this chapter.

Table 5.1 A summary of all results reported in Chapter 5.

Group of study	Hippocampus area	Normalised mean firing rate	Mean Synchrony index	Unit-pair cross-correlation (mean k-index)	LFP frequency band distribution
Saline; i.v followed by KA (1 mM; locally), n=6 rats.	Ipsilateral-CA1	Increased after 20 min of KA administration.	No changes.	ROI 1 increased at epochs of 110-120 and 170-180 min. No significant difference in ROI 2 and ROI 3.	Delta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Theta increased at epochs of 110-120 min, 140-150 min and 170-180 min.
	Ipsilateral-CA3	No changes.	No changes.		Delta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Theta increased at epochs of 110-120 min, 140-150 min and 170-180 min. Gamma only increased at epoch of 110-120 min.
	Contralateral-CA3	Increased after 60 min of KA administration.	No changes.		0.1-1 Hz increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. Delta increased at epochs of 50-60 min, 80-90 min, 110-120 min.

					min, 140-150 min and 170-180 min. Theta increased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min.
WIN (1.2 mg/kg; i.v) followed by KA (1 mM; locally), n=8 rats.	Ipsilateral-CA1	No changes.	No changes.	No changes.	0.1-1 Hz increased at epoch of 110-120 min. Delta increased at epochs of 80-90 min, 110-120 min, 140-150 min and 170-180 min. Theta increased at epochs of 110-120 min, 140-150 min and 170-180 min. Beta increased at epochs of 80-90 min, 110-120 min and 140-150. Gamma increased at epochs of 80-90 min, 110-120 min 140-150 min and 170-180 min.

	Ipsilateral-CA3	No changes.	No changes.		Gamma increased at epochs of 50-60 min, 80-90 min, 110-120 min and 140-150 min.
	Contralateral-CA3	No changes.	No changes.		Gamma increased at epochs of 50-60 min, 80-90 min, 110-120 min and 140-150 min.
KA (1 mM; locally) followed by WIN (1.2 mg/kg; i.v), n=7 rats.	Ipsilateral-CA1	No changes.	No changes.	ROI 1 increased at epochs of 20-30 min. ROI 2 decreased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min. ROI 3 decreased at epochs of 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min.	Delta increased at epochs of 80-90 min, 110-120 min and 170-180 min. Theta increased at epochs of 110-120 min, 140-150 min and 170-180 min. Alpha increased at epochs of 110-120 min, 140-150 min and 170-180 min. Beta increased at epochs of

					20-30 min, 50-60 min, 80-90 min, 110-120 min 140-150 min and 170-180 min. Gamma increased at epochs of 50-60 min, 80-90 min, 110-120 min 140-150 min and 170-180 min.
	Ipsilateral-CA3	No changes.	Increased at epochs of 0-10 min, 50-60 min, 80-90 min and 110-120 min.		Gamma increased at epochs of 20-30 min, 50-60 min, 80-90 min, 110-120 min and 170-180 min.
	Contralateral-CA3	No changes.	No changes.		0.1-1 Hz increased at epoch of 140-150 min and 170-180 min. Delta increased at epochs of 140-150 min and 170-180 min. Theta increased at epoch of 20-30 min. Alpha increased at epoch of 20-30 min. Beta increased at epochs of 20-30 min, 80-90 min, and 110-120 min. Gamma increased at epochs of 50-60 min, 80-90 min, 110-120 min 140-150 min and 170-180 min.
KA (1 mM; locally) followed by flurazepam	Ipsilateral-CA1	No changes.	No changes.	ROI 2 decreased at epoch of 170-180 min. No changes	Delta increased at epochs of 140-150 min and 170-180 min.

(2 mg/kg i.v), n=4 rats.				in ROI 1 and ROI 3.	Theta increased at epoch of 110-120 min, 140-150 min and 170-180 min. Alpha increased at epochs of 140-150 min and 170-180 min. Beta increased at epoch of 140-150 min.
	Ipsilateral-CA3	No changes.	Decreased at epochs of 20-30 min, 140-150 min and 170-180 min.		Delta increased at epochs of 110-120 min, 140-150 min and 170-180 min. Theta increased at epochs of 110-120 min, 140-150 min and 170-180 min. Alpha increased at epochs of 110-120 min 140-150 min and 170-180 min. Beta increased at epoch of 170-180 min.
	Contralateral-CA3	No changes.	No changes.		0.1-1 Hz decreased at epoch of 20-30 min and increased at epoch of 140-150 min and 170-180 min. Delta decreased at epoch of 20-30 min. Beta decreased at epoch of 20-30 min. Gamma decreased at epoch of 20-30 min.

5.4 Discussion

This chapter has produced three main findings: WIN prevented neuronal epileptiform activity induced by KA in the **WIN+KA** group whereas WIN and FLU attenuated neuronal hyper-excitability induced by KA in the **KA+WIN** and the **KA+FLU** groups.

In the **WIN+KA** group, no significant changes were found in normalised mean firing rate in any brain regions (ipsilateral-CA1, ipsilateral-CA3 and contralateral-CA3) when compared to the other groups (Figure 5.4A, B and C). In our case, no significant change indicates that WIN prevented neuronal hyper-excitability induced by KA, as no firing was observed since the firing was alleviated by the administration of WIN. In the **mTLE** group (Figure 4.3 and 4.4), normalised mean firing rate was increased in ipsilateral-CA1 and contralateral-CA3 after 10 min and 80 min of KA administration, respectively. However, there were no changes in firing rate when we injected WIN 20 min before KA. Also, no changes were found in normalised mean synchrony index in either the ipsilateral or contralateral hippocampus (Figure 5.5A). This result is in parallel with our normalised mean firing rate. No significant changes of normalised mean firing rate imply that there were no abrupt depolarisation shifts in pyramidal cells, and therefore no synchronisation occurred. An abrupt depolarisation shift in pyramidal cells, which is produced by most acute convulsant, is likely to be caused by a chain reaction of excitation (Traub and Wong, 1982; Traub et al., 1993). There was no correlation between neurones in any region of interest based on the unit pair cross-correlation (mean K-index) (Figure 5.6A). The increase in the normalised mean power in 0.1-1 Hz, delta, theta, beta and gamma bands in ipsilateral-CA1 hippocampus, and gamma band in ipsilateral-CA3 and contralateral-CA3 hippocampus, in the **WIN+KA** group (Figure 5.8A, B and C) implies that these frequencies were present when there was no effect from KA (i.e. normal condition in anaesthetised rats). Timofeev et al. (2012) reported that 0.1-1 Hz, delta and theta bands are normally found in the anaesthetised condition.

In **KA+WIN** group, no significant changes were found in normalised mean firing rate in any brain regions when compared to basal and the other groups (Figure 5.4A,

B and C) which is similar with the finding in **WIN+KA** group. When KA was induced, KA caused neuronal hyper-excitability through an increased firing rate. But, in this group, with the administration of WIN 20 min after KA, the firing rate was not increased, indicating that WIN attenuated the firing induced by KA. No changes were also found in normalised mean synchrony index in both hippocampus (Figure 5.5B) while unit pair cross-correlation (mean K-index) showed that WIN reduced the correlation between neurones in ROI 2 and ROI 3 (Figure 5.6B). WIN administered after KA also increased the LFP frequency bands of delta, theta, alpha, beta and gamma in ipsilateral-CA1 (Figure 5.10A) and contralateral-CA3 hippocampus (Figure 5.10C). A significant increase was also found in the normalised mean power in gamma frequency in ipsilateral-CA3 (Figure 5.10B).

Our findings in **KA+WIN** and **WIN+KA** groups suggest that WIN acted as an anticonvulsant and an antiepileptic agent when administered before and after KA-induced neuronal epileptiform activity. Our finding is also consistent with the study by Blair et al. (2009) which reported that WIN 55, 212-2 produced dose-dependent anticonvulsant effects against spontaneous recurrent epileptiform discharges by suppressing seizure activity in a study conducted on primary hippocampal neuronal culture models of acquired epilepsy and SE. Another study by Wallace et al. (2002) stated that when treating rats with CB1Rs, WIN 55,212-2 essentially eliminated seizures in a pilocarpine model of epilepsy. The mechanism underlying our result in both groups could be said to be driven by the activation of CB1Rs which decreased the glutamate function in the hippocampus and reduced seizure severity (Antonucci et al., 2012; Huntsman et al., 2019). CB1R expression in hippocampal glutamatergic inputs protects against KA-induced seizures (Rosenberg et al., 2015; Huntsman et al., 2019). Endogenous cannabinoids are believed to be retrograde mediators of depolarisation-induced suppression of inhibition (Wilson and Nicoll, 2001). Furthermore, viral-induced overexpression of CB1Rs targeted at the hippocampus reduced KA-induced seizure severity, seizure-induced CA3 pyramidal cell death, and mortality (Blair et al., 2009). WIN as a full CB1R agonist also plays a major role in the psychotropic and behavioural effects of cannabis (Ferraro et al., 2001; Huntsman et al., 2019). The hippocampus cannabinoid receptors are present at high density on the presynaptic terminals of glutamatergic synapses (Twitchell et al.,

1997). Their activation is associated with a reduction of glutamate release (Piomelli et al., 2000; Ferraro et al., 2001). All of these characteristics demonstrate that CB1Rs could limit seizure activity and protect neurones from subsequent cell death and reactive gliosis.

Monory and Lutz (2008) proposed that circuits may be involved in the CB1R-mediated suppression of excitatory drive in the hippocampus. The CB1R-positive glutamatergic terminals synapse to granule cells of the dentate gyrus at the inner molecular layer of the dentate gyrus; these terminals project from ipsilateral and contralateral mossy cells, located in the hilus of the dentate gyrus, and from the CA3 of the hippocampus. CB1R-negative projections are from the dentate gyrus to the mossy cells and to the CA3 region. This proposed circuit shows that the CA3 region is involved in the control of excitatory drive in the hippocampus (Blair et al., 2009; Huntsman et al., 2019), as shown in our findings in the ipsilateral and contralateral hippocampus.

Apart from the mechanism of CB1Rs, the most likely explanation for the anticonvulsant action of cannabinoids is that it depends on the pathology of epilepsy which may cause a compensatory shift in the balance between CB1 receptor-mediated inhibition of presynaptic glutamate and GABA release (Barker-Haliski and White, 2015). Similarly, depolarisation-induced suppression of excitation can be induced in hippocampal tissue (Ohno-Shosaku et al., 2002). The induction depends on the sensitivity of the pre-synaptic neurone to cannabinoids and the duration of post-synaptic depolarisation. With extended depolarisation, the result of CB1R activation is a shift from depolarisation-induced suppression of inhibition to depolarisation-induced suppression of excitation. Therefore, the extended neuronal depolarisation of an epileptiform discharge may cause a switch from suppression of GABA release to suppression of glutamate release.

A study by Robbe et al. (2006) has reported that cannabinoids, Δ^9 -THC (5 mg/kg) and CP 55,940 (0.1-0.3 mg/kg) can decrease the power of theta and gamma oscillations in the hippocampus of head-restrained and freely moving rats, causing desynchronisation of neuronal assemblies in the hippocampus. In our case, WIN, as

one of the cannabinoids, increased the theta and gamma bands in anaesthetised rats in both the WIN+KA and KA+WIN groups, suggesting that the theta and gamma bands were prominent in anaesthetised rats. Hippocampal theta and gamma oscillations play a fundamental role in the coordination of synchronous firing between hippocampus and cortex and may be critical to memory formation (Siapas et al., 2005). The GABA as an inhibitory neurotransmitter is postulated not to be activated since no changes in synchronisation were found. The GABAergic system can become a depolarising force capable of synchronising abnormal bursting in human epileptic temporal lobe, and brain slice preparations (Cohen et al., 2002; Wallace et al., 2003; Khazipov, 2016). Our study showed that WIN (1.2 mg/kg i.v) did not change synchronisation in either the ipsilateral or contralateral hippocampus in anaesthetised rats, whether administered before or after KA-induced neuronal hyper-excitability. Goonawardena et al. (2011) reported that WIN (1 mg/kg i.p) disrupted synchronous activity of principal neurones within and between CA1 and CA3 hippocampus in anaesthetised rats. The difference between our study and the finding by Robbe et al. (2006) emphasises the different outcome shown in awake and anaesthetised rats, which plays an important role in enhancing our understanding in order to select the most appropriate animal model. Robbe et al. (2006) mentioned that these cannabinoid-induced decreases in theta power were brought about by a CB1R-mediated mechanism, as observed from the prevention of power spectra changes when pre-treated with a CB1R antagonist, SR141716A (SR). We speculate that the increasing of the normalised mean power of theta and gamma bands by WIN before and after KA in our study could be due to the CB1R mechanism in anaesthetised rats, but further study is required to confirm this.

In our **KA+FLU** experiment, FLU attenuated epileptiform-like activity induced by KA, as evidenced by no firing being observed in any brain regions when compared to the other groups (Figure 5.4A, B and C), and no change in normalised mean synchrony index (Figure 5.5C). Furthermore, it suppressed correlation between neurones in ROI 2 at the epoch of 170-180 min compared to basal (Figure 5.6C), and increased the normalised mean power in delta, theta, alpha and beta frequency bands in the ipsilateral hippocampus (Figure 5.12A and B), and significant decreases in the normalised mean power in delta, beta and gamma frequency bands were also found

in contralateral-CA3 hippocampus (Figure 5.12C). No change in firing in this case implies that FLU administered after neuronal epileptiform-like activity induced by KA diminishes the firing by KA, hence FLU has a positive effect as an antiepileptic agent. However, based on our experience in the laboratory, FLU 2 mg/kg caused mortality (drop out) to our experimental animals in experiments of long duration of (3.5 hours).

The attenuation effect observed in the **KA+FLU** group could be explained by relating it to the pharmacology and pharmacokinetics of the other reported benzodiazepines. One plausible mechanism would be GABA-mediated enhancement of the Cl⁻ channel function (Khazipov, 2016), which is involved in muscle relaxation (Mendelson and Martin, 1990) and anticonvulsant action (Rosenberg et al., 1983) in benzodiazepines. Binding of benzodiazepines to the γ and α subunit of the receptor enhances the inhibitory effects of GABA_A (Mihic et al., 1994; Sieghart et al., 2012; Griffin et al., 2013; Khazipov, 2016) by inducing a conformational change in the GABA_A receptor's Cl⁻ channel (Griffin et al., 2013). Benzodiazepines allosterically bind to the receptor at a different location than GABA does and enhances the Cl⁻ channel's conductance by increasing the frequency of gated channel opening (Campo-Soria et al., 2006; Riss et al., 2008). GABA regulates membrane hyperpolarisation and the “shunting” of excitatory input by changing the neurones electrical activity (Bai et al., 2001; Griffin et al., 2013). Hyperpolarisation occurs as the surrounding is more negative, due to an influx of Cl⁻ into the cell. As a result of this process, the GABA_A channel opens rapidly and contributes to the early part of the inhibitory post-synaptic potential (Cooper et al., 2003; Riss et al., 2008). GABA signalling is simply decreased as part of the epileptogenic mechanism, which allows the release of glutamate signalling to continue out of control.

Another factor which contributes to FLU's attenuation effect could be related to the general characteristic of benzodiazepines showing rapid distribution into the central nervous system and highly perfused tissues (Riss et al., 2008). We postulate that FLU's effect is distributed promptly in the ipsilateral and contralateral hippocampus, as observed from the attenuating effect after KA-induced epileptiform activity within the 3.5 hours of our experiment. The biological half-life of FLU was reported to vary

from 2-4 hours after the administration of a single oral dose (Mula, 2016). Our experiment on the KA+FLU group lasted for 3.5 hours, which is within the period that FLU persists in the body. This half-life characteristic indicates that FLU could exert its effect in our experimental manipulations. Based on our findings showing efficacy in acute neuro-epileptiform activity, we could propose FLU as an alternative AED for immediate, temporary and short-term effects. This recommendation is due to its rapid hypnotic properties, general anaesthetics use and low toxicity. It develops tolerance to anti-epileptic activity for long term use. For clinical use, short acting benzodiazepines such as FLU are preferable for elderly patients because such a drug is better tolerated than a benzodiazepine or its active metabolite with a long elimination half-life.

In conclusion, WIN could be proposed as an epileptic agent if taken before and after neuronal epileptiform-like activity. This recommendation is based on changes in the normalised mean firing rate, normalised mean synchrony index, cross correlation (mean k-index) and LFP frequency band in response to locally applied KA, and the effect of the cannabinoid agonist (WIN). Our findings provide additional information about WIN if we want to consider WIN as an epilepsy therapeutic in the future. In the case of FLU, based on the findings in this chapter, FLU attenuated the KA-induced epileptiform-like activity, therefore suggesting the potential use of FLU as an AED in the mTLE model in addition to its conventional use as a hypnotic drug.

Chapter 6

GENERAL DISCUSSION

This thesis aimed to examine the effect of pharmacologically induced epileptiform-like activity (KA effect) on inter-hemispheric hippocampal functional connectivity in the anaesthetised rat model. This thesis produced four main contributions: the mTLE model for anaesthetised rats is proposed; VPA administered after KA is the most effective treatment to attenuate KA's effects than WIN and FLU; WIN and FLU are proposed as potential AEDs in the mTLE model. Moreover, functional connectivity was detected in the mTLE model. Our study has highlighted the significance of using the electrophysiological approach of the mTLE model in anaesthetised rats, and of the functional connectivity in the hippocampi, which are lacking in the current literature.

The main findings in this thesis are divided into 3 results chapter as each chapter was carried out to achieve specific aims respectively. Nonetheless, the results from all three chapters are inter-correlated. As mentioned in Chapter 3, the ultimate aim of this chapter is to establish an mTLE model by administration of KA locally and also to investigate its electrophysiological characteristics by comparison with the sham and systemic epilepsy group. In Chapter 4, VPA, which is a commercial AED, was studied using an electrophysiological technique to discover whether VPA administration pre- or post- seizure is the best approach to alleviate KA-induced epileptiform-like activity. Concurrently, this finding was compared with the chosen mTLE model in Chapter 3 but the recording was lengthier than the experiment conducted as described in Chapter 3. The reason underlying the decision to conduct longer experiments is related to the fact that more effects of KA and VPA can be observed on KA-induced epileptiform-like activity. In Chapter 5, investigations of WIN (cannabinoid agonist) and FLU (benzodiazepine derivative) were carried out to

determine whether those drugs could be proposed as potential AEDs. We also carried out the experiment described in Chapter 5 using different electrodes from the ones used in Chapter 3 and 4. Since no firing of neurones was detected in contralateral CA1 in the mTLE model or VPA experiments, we used an advanced U-probe electrode specifically for contralateral CA3 hippocampus recording only.

The mTLE animal model is one of the established animal models and it has been studied for almost 40 years (Ben-Ari, 2010) to understand the mechanisms involved in seizure propagation. To date, the pathological mechanisms starting from the induction to the expression of spontaneous recurrent seizures are still not clear; the classical view indicates an imbalance between excitation and inhibition. In the search to identify factors causing such an imbalance, past studies have mainly focused on functional alteration in inhibition as well as on morphological changes such as neuronal loss (excitatory and inhibitory) and mossy fibre sprouting (Pitkänen and Sutula, 2002; Abegg et al., 2004). Thus, this study was carried out to investigate the connectivity between neurones in the hippocampus (intra-hippocampus) and also connectivity between the both ipsilateral and contralateral hippocampus (inter-hemispheric) during epileptiform activity. The connectivities were assessed via a few parameters such as normalised mean firing rate, normalised mean synchrony index, cross-correlation (K-index) and LFP frequency band distribution.

Our result showed that saline as the vehicle (no drug) did not alter the normalised mean SI and did not interfere with the interaction between neurones in the ipsilateral or contralateral hippocampus in isoflurane-anaesthetised rats. Neurones are generally in a resting state where there is no neuronal excitatory activity to trigger neuronal firing during anaesthesia (Timofeev et al., 2012). Both excitatory and inhibitory synaptic interaction may contribute to the synchronisation of neuronal activity. Our result from unit pair cross-correlation was also consistent with the findings of normalised mean SI, where no changes were detected in cross-correlation (mean K-index). Bower et al. (2015) mentioned that no change in firing rate was observed during either slow wave sleep (SWS) or awake conditions after seizures relative to before seizures. Cross-correlation values which depend on the underlying firing rate of neurones (de la Rocha et al., 2007) can trigger fake increases in observed

correlation coefficients during periods of increased firing, which was not the case in our study. Slow frequency waves (delta (1-4 Hz), theta (4-8 Hz) and alpha (8-14 Hz)) were detected in both the ipsilateral and contralateral hippocampus. This is in agreement with Timofeev et al. (2012) who stated that normal oscillatory activities within the frequencies of 0.1-15 Hz are detected during SWS or anaesthesia. Our result, however, showed that beta and gamma bands were also detected in the ipsilateral hippocampus in anaesthetised rats.

In our study, the isoflurane-anaesthetised rat without any drug treatment (Section 3.2.2) acted as a benchmark for comparing the electrophysiological characteristics with our selected mTLE model, as explained in Chapter 3. The brain connectivity of the mTLE model was addressed via the same four parameters as in our anaesthetised rat without the influence of any drug. In the **mTLE** rat model, increasing neuronal firing in ipsilateral-CA1 and contralateral-CA3, parallel with increasing synchronisation, increased the unit pair cross-correlation (mean K-index) in ROI 1 (among neurones in the ipsilateral hippocampus). The increasing normalised mean firing rate which generated the increasing normalised mean SI indicated that the synaptic networks of firing were involved in the synchronisation during seizures (Jefferys et al., 2012; Kreuz, 2013), which shows that epileptiform activity was generated in our mTLE model. During seizures, repetitive firing and synchronisation are generated by a subpopulation of neurones while most neurones remain silent (Babb et al., 1973; Bower and Buckmaster, 2008; Truccolo et al., 2011; Bower et al., 2012). However, in our study, most neurones in ipsilateral-CA3 and contralateral-CA1 remained silent, no KA effect was observed in these brain regions. Several mechanisms are proposed for seizures, including synchronous excitatory synaptic input, decreased volume of the extracellular space, increased extracellular potassium and loss of inhibitory restraint (Jiruska et al., 2010). Delta frequency band was detected in both ipsilateral and contralateral hippocampus. In addition, 0.1-1 Hz, theta and beta frequency bands were only detected in contralateral-CA3. Beta and gamma frequency bands, which indicate seizure severity (Timofeev et al., 2012), were absent in our mTLE model; hence, we postulate that the concentration of KA 1 mM used to induce neuronal hyper-excitability did not achieve the threshold of severity. Neuronal hyper-excitability will alter the neuronal synchrony and

oscillation and consequently alter the frequency band distribution (Jefferys et al., 2012). The action of KA in our study may therefore be proposed to be through several different mechanisms, such as activation of glutamate receptors (NMDA, AMPA and KA receptors) and suppression of inhibitory neurotransmitter (GABA), as has been explained in Chapter 3 (Section 3.4). Functional connectivity was detected from the findings that the neurones propagated the neuronal hyper-excitability from the ipsilateral-CA1 hippocampus (injected site) to the other side of the brain (contralateral-CA3 hippocampus), which was not directly injected with KA. This finding is supported by the classic axonal pathways that interconnect limbic structures and their projection target (Neves et al., 2008).

In Chapter 4 of this thesis, we evaluated the effect of the established AED, VPA, which was administered before and after KA. This experiment was designed to mimic how the drug is taken up by epilepsy patients. Our findings showed that VPA administered intravenously before KA prevented and reduced neuronal hyper-excitability induced by KA, while VPA administered after KA attenuated the KA effect. No firing was observed in these experiments; thus, no synchrony changes were generated. LFP oscillations can be observed if individual neurones fire action potentials periodically, and these events are synchronised among many cells (Timofeev et al., 2012), but this was not observed in our VPA experiments. The action of VPA may therefore be proposed to be through several different mechanisms, which are: alteration of the expression or function of glutamate receptors (NMDA and AMPA receptors), action of membrane electrical properties, voltage-gated Na^+ channel activity, increased GABAergic activity and reduction in excitatory neurotransmission. This information contributes to increase our understanding of its various activities, which have been explained in Chapter 4 (section 4.4). Based on our findings, we proposed that VPA administered after KA is a better treatment than VPA administered before KA, as it fully attenuated the neuronal hyper-excitability induced by KA in CA1 and CA3 in both the ipsilateral and contralateral hippocampus. The electrophysiological characteristics we found in these groups were different from the ones in our mTLE rat model; therefore, these VPA experiments may represent a benchmark or standard for potential AED references.

In Chapter 5, a cannabinoid agonist (WIN 55,212-2) administered before and after KA was evaluated, as well as flurazepam (FLU) administered after KA. Our findings showed that WIN administered before KA (WIN+KA) prevented KA inducing neuronal epileptiform-like activity, whereas WIN administered after KA (KA+WIN) attenuated the KA effect. No neuronal firing, no synchrony, no correlation (in WIN+KA) and reduced correlation (in KA+WIN) were detected. The plausible mechanism underlying WIN's effect is believed to be driven by CB1Rs. There is morphological and biochemical evidence that the hippocampus cannabinoid receptors are present at high density on the presynaptic terminals of glutamatergic synapses (Twitchell et al., 1997). Their activation is associated with a reduction of glutamate release (Piomelli et al., 2000; Ferraro et al., 2001). The induction of this process was dependent on the sensitivity of the presynaptic neurone to cannabinoids, as well as the duration of postsynaptic depolarisation. With extended depolarisation, the result of CB1R activation was a shift from depolarisation-induced suppression of inhibition to depolarisation-induced suppression of excitation. Therefore, the extended neuronal depolarisation of an epileptiform discharge may cause a switch from suppression of GABA release to suppression of glutamate release. Based on our study, WIN has potential as an AED since the electrophysiological characteristics examined in this study showed similarities with the effect of VPA in attenuating and preventing the effect of KA.

FLU fulfils our hypothesis as our findings showed that FLU can attenuate KA-induced epileptiform activity since there was no neuronal firing, no synchrony and no correlation in any brain regions. The mechanism could be proposed to be a result of activation of GABA, the major inhibitory neurotransmitter in the central nervous system (Khazipov, 2016). Apart from that, FLU attenuates KA's effects through rapid distribution into the central nervous system and highly perfused tissues, and is within its biological half-life. Nonetheless, 2 mg/kg of FLU caused mortality (drop out) to our experimental animals in experiments of long duration of (3.5 hours). From this experiment, we propose that FLU can act as a potential AED based on its electrophysiological characteristics evaluated in this study; however further experiment is required to confirm its pharmacokinetic and pharmacodynamic properties.

The mechanism of KA as a model of spontaneous seizure in the rat, however, is not fully understood. There are a few gaps in this study which should be addressed in future:

1. Investigation at the cellular level, to understand the mechanism and ions profile that underlie the epileptic event and how the anti-epileptic drugs react/work on the ion channel (patch clamp approach).
2. Comparison between awake and anaesthetised rats should be carried out in order to understand the electrophysiological characteristics (neuronal firing, synchronisation, cross-correlation and LFP frequency bands) without the influence of anaesthesia.
3. Supporting studies should incorporate the pharmacokinetic and pharmacodynamic properties of FLU to determine its underlying mechanism as a potential AED.
4. The connectivity between hippocampi should be evaluated by using non-parametric multivariate analysis to investigate the flow of information in the hippocampus.
5. The generalisation of this study pertaining to VPA, WIN and FLU use in humans is limited, because extrapolating conclusions from preclinical animal model to humans requires evaluation of the drug's safety and efficacy. Additionally, the animal dose used for VPA (20 mg/kg), WIN (1.2 mg/kg) and FLU (2 mg/kg) cannot be extrapolated to human-equivalent doses until further pharmacokinetic studies of VPA, WIN and FLU have been conducted.

In conclusion, the connectivity between the ipsilateral and contralateral hippocampus (inter-hemispheric) and connectivity between neurones in the hippocampus (intra-hippocampus) during epileptiform activity in the mTLE model was evaluated employing multielectrode array recordings, based on our four selected parameters (normalised mean firing rate, normalised mean synchrony index, cross-correlation (k-index) and LFP frequency band distribution). We were also able to come up with an mTLE model of anaesthetised rats. The data presented in this thesis have addressed the aims and hypothesis of this study by providing additional information using an electrophysiological approach supporting the use of VPA, WIN 55,212-2 and flurazepam in controlling hyper-excitable neurones in the hippocampi. This pre-clinical study can assist clinician in choosing the appropriate administration (pre or post seizure) of AED and to select the best AED in order to alleviate seizure in epileptic patients. A better understanding of the dynamics of how seizures spatially initiate, propagate, and terminate may be an important step towards the development of seizure intervention therapies.

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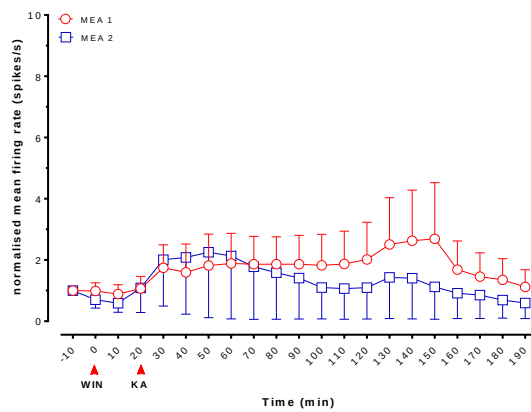
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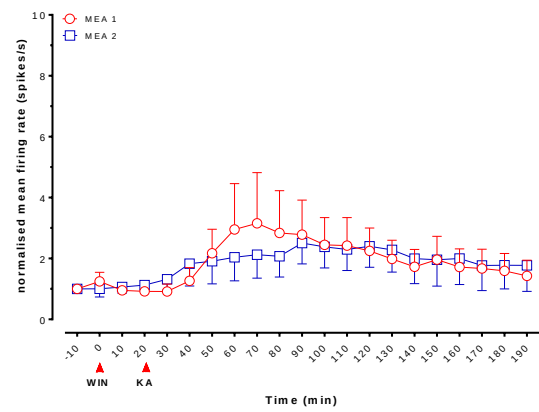
APPENDIX

Comparison between the microwire electrode array (MEA) and U-probe used in Chapter 5 is shown in A-D. The use of U-probe was directly injected to contralateral-CA3 (D), as no firing was detected from the contralateral-CA1 hippocampus when we used MEA (C). T-test analysis showed that there was no significant difference of normalised mean firing rate ($p>0.05$) ($F_{(20, 200)} = 2.225$) recorded between MEA and U-probe (in contralateral-CA3).

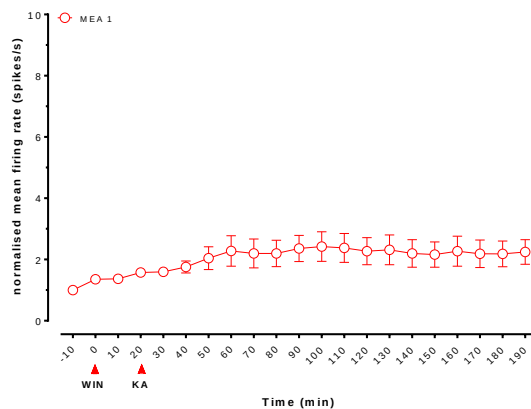
A. Ipsilateral- CA1



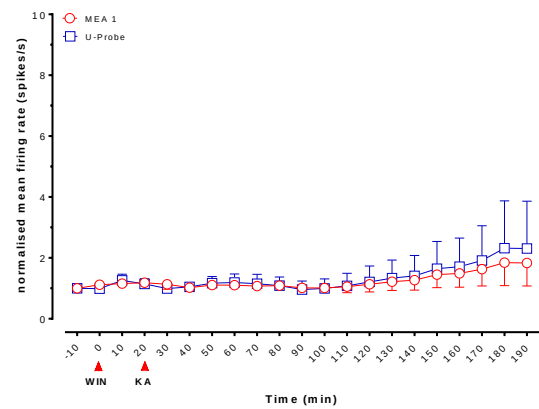
B. Ipsilateral-CA3



C. Contralateral-CA1



D. Contralateral-CA3



Comparison of the normalised mean firing rate of WIN+KA experiments using MEA in both ipsi- and contralateral hippocampus ($n=8$ rats) and MEA in ipsilateral and U-probe in the contralateral hippocampus ($n=4$ rats).

A: ipsilateral-CA1

B: ipsilateral-CA3

C: contralateral-CA1

D: contralateral-CA3

