Valproate and 4-methyloctanoic acid, an analogue of valproate, in animal models of epilepsy

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Thesis submitted to University College London for the degree of Doctor of Philosophy

September 2009

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Declaration

I, Pi-shan Chang, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. The wireless telemetric recording system presented in chapter 9 was developed in collaboration with opensource instruments, MA, USA. All experiments were conducted by me.

Abstract

Valproic acid (VPA) is a commonly used drug for the treatment of epilepsy, bipolar disorder and migraine, yet its mechanisms of action are unknown. The neuroprotective effect of VPA has been hypothesized to be secondary to inhibition of the cAMP/protein kinase A (PKA) pathway. Here, the result show that VPA (1mM) inhibited mossy fibre long-term potentiation induced (LTP) by application of high frequency stimulation in dentate gyrus. Furthermore, VPA (1mM) inhibited enhancement of mossy fibre responses induced by application of forskolin (50 μM), consistent with an effect on the PKA pathway. Using biochemical assays, it was further demonstrated that this was not due to a direct effect on PKA, but resulted from inhibition of adenylyl cyclase. The results further show using *in vitro* seizure models (Pentylenetetrazole model and low-Mg^{2+} model) that this mechanism cannot fully explain VPA's anti-seizure effect, but rather, by modifying synaptic plasticity, it may be more important for VPA's antiepileptogenic and neuroprotective action. VPA therefore has distinct mechanisms of action that contribute to its diverse biological activity. In hippocampi from epileptic rats (following pilocarpine-induced status epilepticus), but not in control tissue, VPA affects short-term plasticity, indicating that VPA may have specific effects in epileptic rather than control animals.

Using *in vitro* seizure models (Pentylenetetrazole model and low-Mg^{2+} model) and an *in vivo* status epilepticus model (the perforant pathway stimulation model), 4-methyloctanoic acid is further established that it is a more potent antiepileptic drug than VPA and provides neuroprotective effects which are similar to VPA. Furthermore, 4-methyloctanoic acid (1mM) inhibited enhancement of mossy fibre responses induced by application of forskolin (50 μM), indicating that 4-methyloctanoic acid shares the same effect as VPA on modulation of PKA.

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Abbreviations

AEDs	Anti-epilepsy drugs
aCSF	Artificial cerebrospinal fluid solution
AC	Adenylyl cyclase
AD	After discharges
AIF	Apoptosis-inducing factor
AMPA	Alpha-amino-3-hydroxy-5-methyl propionate
AMPAR	AMPA receptor
ATP	Adension triphosphate
BDNF	Brain-derived neurotrophic factor
B-gal	β-galactosidase
CA	Cornu ammonis
CA1	Cornu ammonis region 1
CA2	Cornu ammonis region 1
CA3	Cornu ammonis region 3
CA4	Cornu ammonis region 4
CaMKII	Calcium-calmodulin-dependent kinase II
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CMF-HBSS	Calcium and magnesium free Hank's balanced salt solution
CREB	cAMP response element binding protein
DAG	Diacylglycerol
DHPG	(S)-3,5-dihydroxyphenylglycine
DG	Dentate gyrus
DMSO	Dimethyl sulfoxide
EA	Enzyme acceptor
ED	Enzyme donor
ED-cAMP	Enzyme donor –labelled cAMP
EEG	Electroencephalography
EFC	Enzyme fragment complementation
EDTA	Ethylene glycol tetraacetic acid
eNOS	Endothelial NO synthase
ER	Endoplasmic reticulum
ERK	Extracellular signal regulated kinase
ERK1/2	Extracellular signal regulated kinase 1 and 2
ERK5	Extracellular signal regulated kinase 5
fEPSP	Field excitatory postsynaptic potentials
GABA	Gamma-aminobutyric acid
GSK	Glycogen synthase kinase
HAT	Histone acetyltransferases
HDAC	Histone deacetylase enzymes
HFS	High-frequency stimulation
iNOS	Inducible NO synthase
IBMX	Isobutylmethyl xanthine
IPSP	Inhibitory postsynaptic potentials
ip	Intraperitoneal administration
IP3	Inositol 1,4,5-triphosphate
IP3R	IP3 Receptor
LDH	Lactate dehydrogenase

LTP	Long-term potentiation
LWDAQ	Long-Wire Data Acquisition
MAPK	Mitogen-activated protein kinase
MARCKS	Myristoylated alanine-rich C kinase substrate
MEK1	Extracellular signal-regulated kinase activator kinases
MF	Mossy fibre
MOPS	3-(N-morpholino) propanesulfonic acid
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NTDs	Neural tube defects
NO	Nitric oxide
nNOS	Constitutive neuronal NO synthase
PEBP	Polyomavirus enhancer-binding protein
PED	Phosphodiesterase
pEPSP	Population excitatory post-synaptic potential
PLC	Phospholipase-C
PI3K	Phosphatidyloinositol-3-kinase
PIP3	Phosphatidylinositol 3,4,5-Trisphosphate
PKA	Protein kinase A
PKB	Protein kinase B
PKC	Protein kinase C
PS	Population spikes
PTP	Post-tetanic potentiation
PTZ	Pentylenetetrazole
RSK1	Phospho-ribosomal protein S6 kinase-1
SC	Schaffer/collaterals pathway
SE	Status epilepticus
SD	Sprague Dawley
SRS	Spontaneous recurrent seizures
SSSE	Self-sustained-status epilepticus
STP	Short term plasticity
TLE	Temporal lobe epilepsy
TrkB	Tyrosine kinase B
VPA	Valproate

Publications arising from this thesis

Chang, P, Chandler, KE, Williams, RSB, Walker, MC (2009) Inhibition of long-term potentiation by valproic acid through modulation of cyclic AMP. 2009. Epilepsia (accepted)

Orabi B*, Chang P*, Deranieh R, Dhaml M, Yagen B, Bialer M, Greenberg M, Walker MC, Williams RSB (2010) Seizure control predicted by acute phosphoinositide signalling attenuation in Dictyostelium. Nature (submitted) * Joint first authors

Published Abstracts

Chang, P, Williams, RS, Walker, MC (2009) 4-methyloctanoic acid is an effective compound in the treatment of an animal model of status epilepticus, Innsbruck Colloquium on Status Epilepticus. Innsbruck, Austria

Chang, P, Williams, RS, Walker, MC (2008) Effect of Valproates, its analogues and the action of PKA on epileptiform discharges induced by pentylenetetrazole, ILAE (UK chapter) Annual Scientific meeting. Dundee, UK

Chang, P, Williams, RS, Walker, MC (2008) Valproate's action on protein kinase A signalling, 8th European Congress on Epileptology. Berlin, Germany

Chang, P, Walker, MC (2007) Valproate's action on protein kinase A dependent LTP, ILAE (UK chapter) Annual Scientific meeting. Southampton, UK

Acknowledgements

I would like to thank all those who have contributed in various ways to help me finishing this thesis.

First I would like to take this opportunity to thank my principle supervisor, Professor Matthew Walker, who has been very supportive, generous and has helped me in every possible way. His warm encouragement and incredible enthusiasm in research have been a great inspiration to me. Without his encouragement, guidance and support, this thesis would not have been possible. I would like to thank my secondary supervisor Dr. Kate Chandler, who has been a great help in assisting me learning the *in vivo* experiment. Many thanks to Dr Robin Williams, Head of the Section Centre for Biomedical Sciences in Royal Holloway, University of London. He assisted me in completing the biomedical experiments. A very special thank you goes to Mr. Kevan Hashemi for development of wireless transmitter and resolving the problem as the problems I encountered when I conducted the experiment with the wireless transmitter.

A thank goes to Iris Oren who taught me how to prepare hippocampal slices; thanks also to Felicity Alder and Rainer Surges who helped me to prepare hippocampal cell cultures. I would like to thank Miss Eleanor Harris for assisting me in paper work, ordering and delivering. Many thanks go to everybody in the Department of Clinical and Experimental Epilepsy. I will always look back with fondness and gratitude for their kindness, assistance, and advice. I would also like to thank my friends who encouraged me through out my PhD. Finally, a very big thank you goes to my family, for being so understanding and for supporting me to fulfil my dream.

Chapter 1: Introduction

1.1 Epilepsy

Epilepsy is the commonest serious neurological condition, affecting 0.5-1 % of the population, of which 30% have epilepsy that is not adequately treated with our present antiepileptic drugs (AED). These patients have a high mortality and morbidity. The term epilepsy refers to a diverse family of common chronic disorders of the central nervous system (CNS). An epileptic syndrome is defined as recurrent provoked seizures that can arise either in a generalized fashion or from specific brain regions; moreover, the epilepsies are associated with psychosocial disability such as depression and decreased cognitive abilities.

Epilepsy syndromes can be broadly divided into two categories: generalized epilepsy and partial epilepsy. Each can be further subdivided by aetiology: (1) idiopathic, which is mainly genetically determined, (2) symptomatic, and (3) cryptogenic (or probable symptomatic) in which there is an assumed symptomatic cause. Most generalized epilepsies are idiopathic (e.g. Absence epilepsy, and Juvenile Myoclonic Epilepsy). Most partial epilepsies are symptomatic or cryptogenic, and often result from a brain insult such as head trauma or prolonged seizures (Johnson and Sander, 2001;Chang, 2003).

The mechanisms by which seizures occur have not been fully elucidated, but there appear to be certain key factors associated with epilepsy: changes in intrinsic properties of neurons, such as decreased potassium conductances or increased calcium conductances; changes in network inhibition in particular GABA(A) receptor-mediated inhibition, and enhancement of excitatory transmission, through “sprouting” of excitatory fibres and changes in glutamate receptors.

Our present pharmacotherapy of epilepsy prevents the symptom (seizures), but has not been shown to alter the course of the disease. There are several strategies by which AEDs suppress seizures: limiting sustained repetitive firing; increasing GABA-mediated inhibition; attenuating activity of voltage-sensitive Ca^{2+} channels; decreasing glutamate-mediated excitation (Perucca, 2001)(Table 1).

Table 1.1: Mechanism of AEDs and neuroprotective compounds

	Effect GABA system	Effect Calcium channels	Effect glutamate-mediated excitation.	Effect voltage-dependent Sodium channels
Old AEDs				
Benzodiazepines	+			
Carbamazepine	+	+		+
Ethosuximide		+		
Phenobarbital	+	+		
Phenytoin				+
Valproic acid	+	+		
New AEDs				
Felbamate	+	+	+	
Gabapentin	+	+		
Lacosamide				+
Lamotrigine	+	+		+
Levetiracetam	+	+		
Oxcarbazepine		+		
Pregabalin	+	+		
Tiagabine	+			
Topiramate	+	+	+	+
Vigabatrin	+			
Zonisamide		+		+

1.1.1 Temporal lobe epilepsy

The seizures associated with temporal lobe epilepsy (TLE) consist of simple partial seizures without loss of awareness and complex partial seizures. Further spread can result in secondary generalized seizures. Patients with temporal lobe epilepsy are predisposed to behavioural changes and deep emotionality. Furthermore, patients with TLE often respond poorly to AEDs (Sutula, 1990).

TLE can be subdivided into those in which the seizure onset zone involves the mesial temporal lobe and those involving the lateral temporal lobe. Mesial temporal lobe seizures can be localised to the limbic system, particularly the hippocampus, entorhinal cortex and amygdala (Bartolomei et al., 2005).

Mesial temporal lobe epilepsy is associated with hippocampal sclerosis and comprises the majority of the cases of epilepsy refractory to pharmacotherapy. Mesial temporal lobe seizures usually begin in late childhood or early adolescence (French et al., 1993). Generally, TLE is thought to be initiated by an "initial precipitating injury" such as status epilepticus, infection, febrile convulsions, traumatic brain injury etc. There is a seizure-free time interval which is known as the "latent period" followed by the appearance of spontaneous seizures. Hippocampal sclerosis is characterised by cell loss and gliosis in specific subfields of the Cornu Ammonis - CA1 ,CA3 and the dentate hilus, while the CA2 region is relatively spared (Sutula, 1990). Other, changes in the hippocampal formation changes are also observed, including mossy fibre sprouting, interneuron loss and granule cell dispersion in the dentate gyrus (Curia et al., 2008).

Lateral temporal lobe epilepsy (also known as neocortical temporal lobe seizures) is less common. Less than 10% of patients with TLE have seizures originating in the lateral

temporal neocortex (Walczak, 1995). There are various causes including genetic causes, such as Autosomal Dominant Lateral Temporal Lobe Epilepsy which is rare family epilepsy characterized by partial seizures with auditory disturbances.

1.2 Hippocampus formation

Human TLE is often accompanied by sclerosis in the hippocampus, also known as Ammon's horn sclerosis, with neuronal degeneration and dentate gyrus granule cell dispersion. The sclerotic hippocampus removed by surgery often improves this epileptic condition. Seizure-induced remodelling of hippocampus during the process of epilepsy contributes to its low seizure threshold leads spontaneous recurrent seizure. It has been suggested that the alteration in epileptic hippocampus includes mossy fibre sprouting and recurrent innervations of granule cells forming a feedback loop promoting synchronization of spike activity and deteriorating inhibitory network in strict correlation to epileptogenesis (Pitkänen and Sutula, 2002).

The hippocampus is a part of the brain located in the medial temporal lobe and adjacent to amygdala. The shape of hippocampus is like horseshoe. It forms a part of the limbic system and plays a part in memory, navigation, emotions and spatial orientation.

The hippocampus can be divided into two major U-shaped areas. One sector is called the fascia dentate, including area dentata, dentate gyrus. Another sector is called the hippocampus proper, cornu ammoni. The hippocampus proper can be further divided into four fields, CA1, CA2, CA3, and CA4. CA4, now, is frequently called the hilus and considered part of the dentate gyrus.

The fascia dentata comprises three layers: the granule cell layer which is the location of

the densely packed bodies of cell granule cells; the molecular layer which is formed by the apical dendrites of the granule cells and their afferents; and the polymorph layer in the hilus of the fascia dentata which merges with the CA4 field and contains the initial segments and axons of the granule-cell, forming the mossy fibre bundle. Also, it contains non-granule cells, such as basket cell.

The hippocampus proper is divided into several layers, defined by a particular feature of the large pyramidal cells or their afferents: The alveus containing the axons of the pyramidal cells towards the fimbria or subiculum; the stratum oriens which is the layer between alveus and the pyramidal cell bodies contains the basal dendrites of the pyramidal cells ; stratum pyramidale, or pyramidal cell layer is the location of the pyramidal cell bodies; the stratum radiatum and the stratum lacunosum/moleculare are formed by the proximal and distal segments of the apical dendritic tree; stratum lucidum which is between the CA3 pyramidal cell bodies and the stratum radiatum receives the mossy-fibre input from the dentate granule cells.

There are several sources of input to the cells in the hippocampus: intrinsic input from cells in same sector; intrinsic input from other sectors; commissural afferents from the opposite hippocampus; extrinsic afferents from outside the hippocampus (Summary in Figure 1.1).

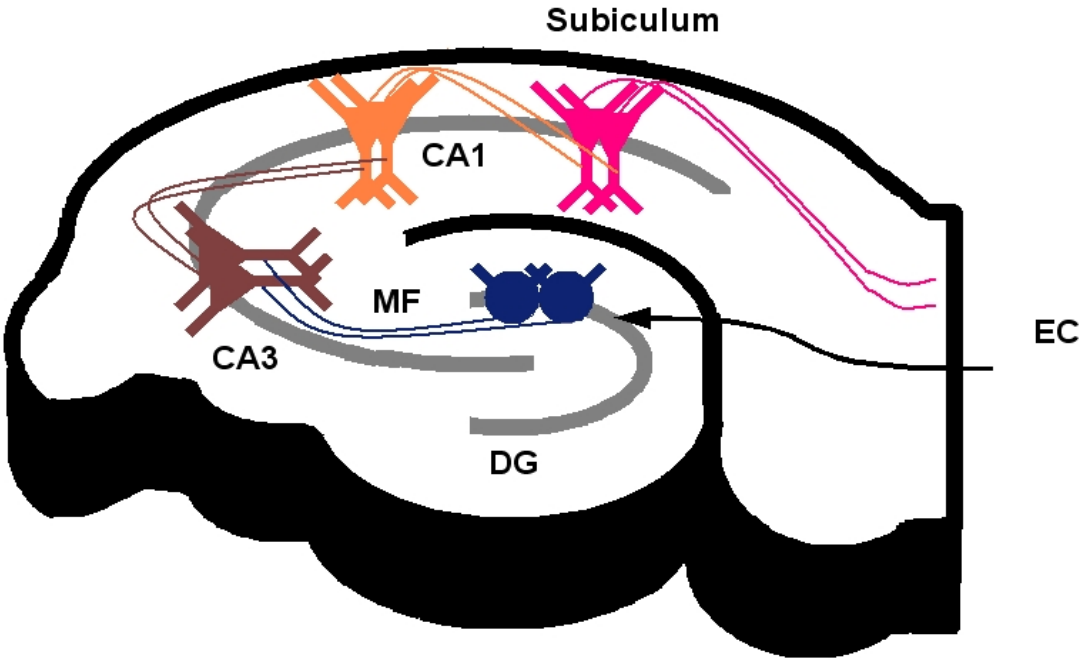


Figure 1.1: Summary of hippocampal formation. The entorhinal cortex (EC) projects to dentate gyrus (DG) via perforant pathway fibers. DG granule cells output mossy fibers (MF) to CA3. CA3 pyramidal cells send axons recurrently to CA1 through the Schaffer/collaterals (SC) pathway. CA1 pyramidal cells project back to EC.

1.2.1 Intrinsic input from cells in same sector

In the same CA field, the interactions between pyramidal cells fall into two types: direct excitatory and indirect inhibitory. The indirect inhibitory effect is mediated via interneurons, such as the basket cells. The axons of the basket cells synapse in the pyramidal region and make contact with numerous adjacent pyramidal cells. However the basket cell receives input from these same pyramidal cells. This feedback loop could form the basis for a Renshaw-type inhibitory circuit. There are also direct monosynaptic excitatory connections between pyramidal cells of the CA3 field (Lopes da Silva et al., 1990;Skrede and Westgaard, 1971).

1.2.2 Intrinsic input from other sectors

As described by Cajal, unmyelinated axons of the dentate granule cells gather together in the polymorph layer in the hilus and afferent out of the hilus. There are two different pathways, one is run above the pyramids of CA3 in the stratum lucidum as the mossy fibres, and one is run below the pyramids in the stratum oriens. The lower bundle stops in CA3 region (between CA3b and CA3c). Mossy fibre synapses on CA neurons are large aggregations of termini, with multiple transmitter release sites and post-synaptic densities.

Three pathways have been described as collaterals for the axons of both the giant CA3 pyramidal neurons and the pyramids of CA4, with one branch entering the fimbria and going to the septum: the Schaffer collateral pathway arises from the CA4, CA3 pyramidal cells and projects to the stratum radiatum of CA1 with excitatory synapses en passage; a pathway of collaterals of CA4 and CA3 around the fascia dentata runs parallel to the long axis of the hippocampus making contact with the proximal dendrites of the dentate granule cells; longitudinal and associational pathway arising in the CA2 and CA3 fields which is a collateral of the Schaffer collateral pathway or a collateral of the pathway project to the fimbria, connecting cells within the same sector (O'Keefe and Nadel, 1978).

There are input pathways from outside the hippocampus, including (a) entorhinal cortex; (b) brain-stem; (c) medial septal area.

The entorhinal afferents provide one of the main extrinsic inputs and consist of the perforant path, alvear path and crossed temporo-ammonic tract.
The perforant pathway is the major avenue of entorhinal afferents to hippocampus.

The axons of the perforant path arise from layer II and III of entorhinal cortex and project to the dentate gyrus and to the pyramidal cells of the CA1 and the subiculum as the lateral perforant path. Axons of perforant path also arise from layer IV and V of entorhinal cortex and project to the granule cells of the dentate gyrus (DG) and pyramidal cells of the CA3 region as the medial pathway. Glutamate is the main neurotransmitter in this pathway.

1.2.3 Brain-stem afferent

There are several brain-stem projections to the hippocampus, such as median raphe nucleus and particularly the nucleus centralis superior. This projection courses primarily through the fimbria, fornix, and cingulum, terminating mostly in stratum lacunosum or moleculare of CA1 and CA3, and in some specific hilar areas. The locus coeruleus also projects to the hippocampus. The pathway arises from locus coeruleus through the medial forebrain bundle and septum, entering hippocampus via restroplenial cortex. Fibres terminate in the stratum lacunosum/moleculare of CA1 and CA3, the hilus of the fascia dentata, and the stratum lucidum of CA3.

Septal afferents arise from septal area and project sparsely to all ipsilateral fields of the hippocampus, stratum oriens and stratum radiatum of the hippocampus and to the polymorph zone of the fascia dentata. There is a small projection to the molecular layer of CA4 and fascia dentata. These fibres travel in the fimbria to CA3-CA4, or travel in the dorsal fornix and alveus to CA1. The major termination zones are just above and below the main cell bodies, indicating the target cell might be interneurones.

Other afferents include an input from the fastigial nucleus of the cerebellum to CA2, CA3, and CA4 and to the fascia dentata, terminating in stratum oriens, stratum radiatum and stratum lacunosum in the hippocampus proper, and the polymorph layer in the fascia dentata. This pathway approaches the hippocampus through cingulate and uncinate, entering either through the alvear or perforant paths (Lopes da Silva et al., 1990).

1.2.4 Efferents from the hippocampal

The hippocampal efferents project to: (1) the lateral preoptic (2)medial and lateral hypothalamic areas, (3) the septal region, (4) the thalamus, (5) the mammillary bodies, (6) the rostral mid-brain, and (7) caudally to the subiculum and the entorhinal cortex.

1.3 Long term potentiation (LTP)

The mechanisms of memory which depends on formation of long-term potentiation (LTP) are similar to those mechanism underlying epileptogenesis by kindling. The stimulus parameters which best induce kindling are similar to LTP, such as high frequencies stimulation, resulting in structural synaptic changes through modulation of synaptic facilitation and protein synthesis (Meador, 2007). Additionally, *in vitro* experiment, LTP can be induced by spontaneous epileptiform activity, suggesting that LTP could be involved in the generation of seizure foci (Morgan and Teyler, 2001). Seizures can also reduce the ability to induce LTP, resulting in the impaired spatial learning in animals in repeated electroconvulsive seizures and kindling model (Reid and Stewart, 1997;Leung and Wu, 2003). LTP is markedly reduced in the hippocampal seizure focus in humans with temporal lobe epilepsy, resulting in deficient declarative memory (Beck et al., 2000).

The definition of LTP is an increase in strength of a chemical synapse in specific brain pathways that lasts from minutes to several days. In *in vitro* and *in vivo* experiments, applying high-frequency electric stimuli or excitatory chemical agents to a synapse can induce the LTP on the synapse for minutes to hours.

LTP contributes to plasticity in synaptic connections and formation of learning and memory. LTP can be divided into in three phases: Short-term potentiation, early phase and late phase (Andersen et al., 2007).

- Short-term potentiation is NMDA receptor independent without involving protein synthesis and protein kinase activity.
- Early phase which usually happened in the first hour, involves increased sensitivity of the synapse without any new gene transcription or mRNA translation occurring. There are several protein involve in early phase, including calcium/calmodulin-dependent protein kinase II (CaMKII) (Lledo et al., 1995), protein kinase C (PKC) (Otmakhova et al., 2000), protein kinase A (PKA), mitogen-activated protein kinase (MAPK) (English and Sweatt, 1997;Sweatt, 2001) and tyrosine Kinases (Huang and Hsu, 1999).
- Late phase usually persist for many hours and even many days, requiring new gene transcription, mRNA translation (Frey et al., 1996) and protein synthesis in the postsynaptic cell. AMPA receptors expression increases and formation of entirely synapses changes (Lynch, 2004).

Hippocampal LTP plays a crucial role in memory formation and learning, such as spatial

learning, recognition memory, working memory (Lynch, 2004).

In the hippocampus, LTP can be classified by different mechanisms into NMDA receptor mediated LTP and non-NMDA receptor mediated LTP. LTP in CA1 and dentate regions in hippocampus requires NMDA receptor activation and postsynaptic depolarization. In CA3 region of hippocampus, pyramidal cells receive two different synaptic inputs, an associational-commissural input and mossy fibre inputs. NMDA receptor antagonists are able to block the LTP induced in associational-commissural pathway, but not mossy fibre LTP. Paired-pulse facilitation, an index of increase transmitter release was reduced during mossy fibre LTP, indicating that the early and later phases of LTP in mossy fibres is expressed presynaptically (Zalutsky and Nicoll, 1990).

1.3.1 NMDA receptor mediated LTP

The influx of calcium through NMDA receptors contributes to a cascade of events which ultimately leads to an increase in synaptic efficacy. Such LTP is associational as it requires glutamate release and coincidental post-synaptic depolarisation to relieve the magnesium block of the NMDA receptors (Bliss and Collingridge, 1993).

The influx of Ca^{2+} into the neuron through NMDA receptors activates calcium-calmodulin-dependent kinase II (CaMKII) which phosphorylates critical proteins. For example, CaMKII phosphorylates AMPA receptors, increasing their permeability to sodium ions (Na^{+}). The activation of NMDA receptors varies with stimulation frequency. Activation dependent synaptic modifications depend on stimulation frequency. With high-frequency stimulation, the cellular responses result in LTP. With

low-frequency stimulation, the cell responses initiate long term depression. Moreover, sustained stimulation of NMDA receptors trigger the process of apoptosis (Popescu, 2005)

NMDA receptors have been shown to be a key factor for spatial memory function. NR1 knockout mice had poor performance in water maze task compared to the normal mice, indicating that their spatial memory was disrupted. Furthermore, these mice had impaired LTP in CA1 region (Tsien et al., 1996).

1.3.2 Non-NMDA receptor mediated LTP

The LTP at mossy fibre to CA3 pyramidal neuron synapses demonstrate NMDA receptor independent LTP (Zalutsky and Nicoll, 1990;Harris and Cotman, 1986). This LTP is similarly induced by tetanic stimulation and is expressed pre-synaptically (Summary in Figure 1.2).

Mossy fibre LTP has been proposed to be independent of postsynaptic calcium (Mellor and Nicoll, 2001;Castillo et al., 1994) and postsynaptic membrane potential (Zalutsky and Nicoll, 1990). However, there are some conflicting reports that claim that induction of mossy fibre LTP requires postsynaptic calcium entry and depolarization.

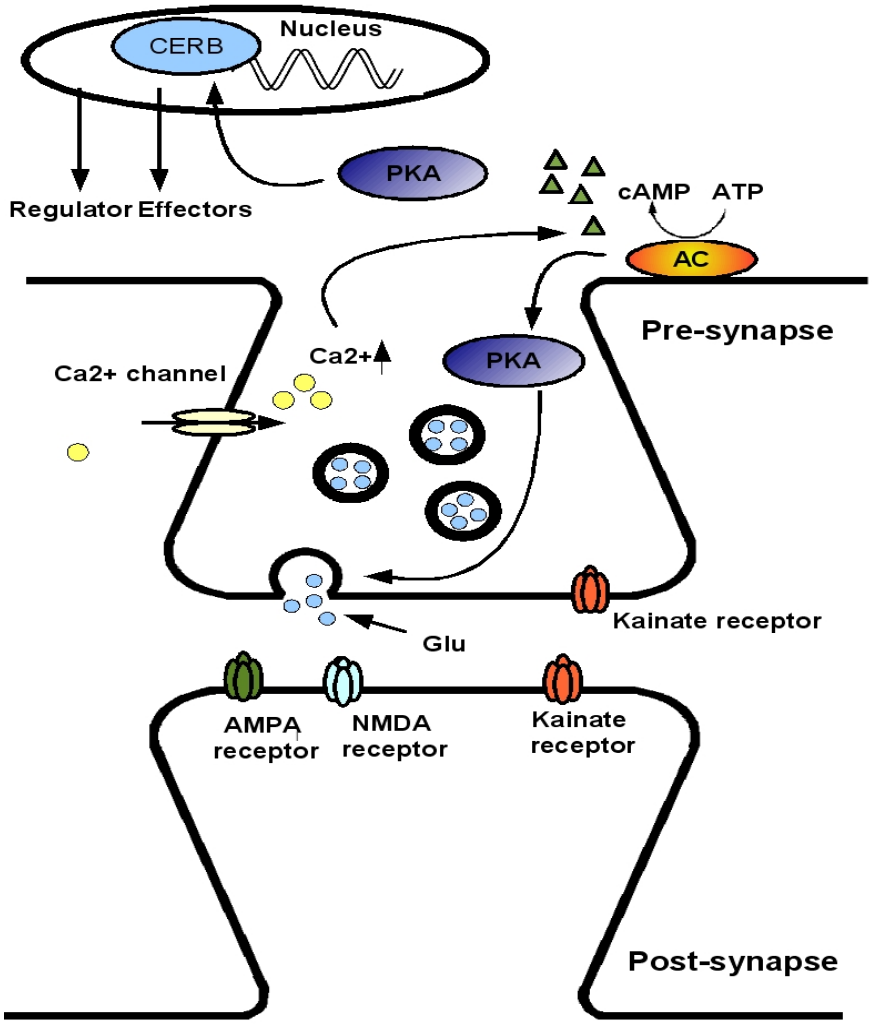


Figure 1.2: cAMP contributes to the long term potentiation in mossy fibre synapse to CA3 region. Stimulation synaptic activity results in the entry of presynaptic Ca^{2+}. The increase the level of Ca^{2+} activates adenylyl cyclase, a Ca^{2+}-sensitive kinase, leading to activate cAMP-dependent protein kinase A (PKA) via increase of cAMP level. This results in a long-lasting increase in glutamate release.

There is considerable evidence that the expression of mossy fibre LTP requires the involvement of cyclic adenosine monophosphate (cAMP). Application of adenylyl cyclase activator, forskolin, or membrane-permanent analogues of cAMP cause a long lasting presynaptic enhancement of mossy fibre responses which occlude mossy fibre LTP (Huang et al., 1994;Nicoll and Malenka, 1995;Tzounopoulos et al., 1998;Weisskopf et al., 1994). Giving the PKA antagonist, not only the antagonist of

catalytic but also regulatory sites, reverses mossy fibre enhancement after application of forskolin (Weisskopf et al., 1994). Also, applying high-frequency tetanic stimulation induces LTP at mossy fibre synapses. It has been suggested that high-frequency tetanic stimulation activates a calcium/calmodulin-dependent adenylyl cyclase via rising presynaptic calcium. This results in an increase in presynaptic cAMP levels and activation of the cAMP-dependent protein kinase. The activation of PKA increases neurotransmitter release (Nicoll and Malenka, 1995).

A recent study suggests that presynaptic hyperpolarization-activated cation channel (Ih) contributes to the LTP in mossy fibre and is modulated by cAMP (Mellor et al., 2002). However, the involvement of Ih in the expression of LTP in mossy fibre has been challenged (Chevaleyre and Castillo, 2002).

1.4 Epileptogenesis

Epileptogenesis is the process by which the brain develops the capability of generating spontaneous, recurrent seizures. It may take months to years to develop epilepsy. During this period an area of brain injury undergoes morphologic and biologic changes (Herman, 2002). The epileptogenic process can be divided into three phases. First, there is an initiating injury produced by events such as head trauma, stroke, infection or status epilepticus. Second, a latent phase follows which can last for weeks to years without seizure occurrence. In this phase, alterations in molecular, cellular and network properties occur. Finally, spontaneous epileptic seizures occur. Once seizures occur, the epileptic disease state probably continues to progress, as seizures may induce additional neuronal alterations, further lowering seizure threshold (Figure 1.3).

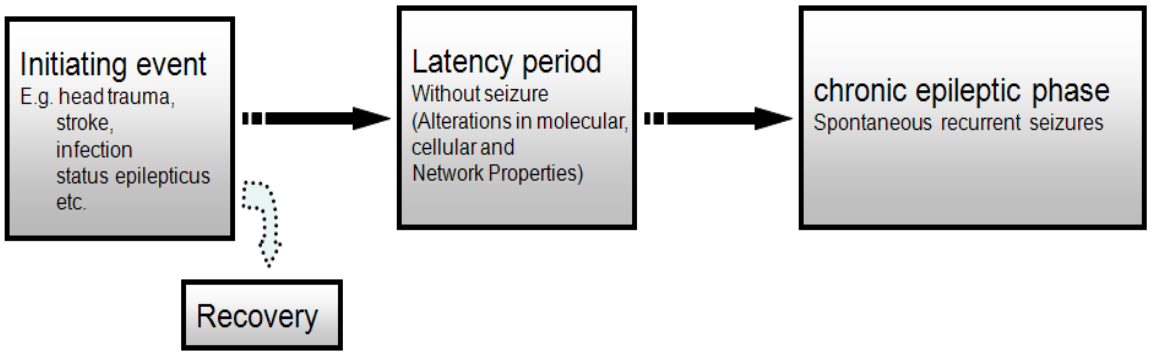


Figure 1.3: The process of development of epilepsy. The epileptogenic process can be divided into three phases: an initiating injury produced by events such as head trauma, stroke, infection or status epilepticus, a latent phase follows which can last for weeks to years without seizure occurrence, and chronic epileptic phase which spontaneous epileptic seizures occur.

1.5 Animal models of epileptogenesis

By development and characterization of insult- and age-specific animal models of epileptogenesis, there has been significant progress in our understanding of the factors that contribute to human epileptogenesis in recent years. To study the epilepsy in animal model allows us to elucidated the mechanism of epileptogenesis, and to develop the therapy to prevent the epileptogenic process (White, 2002).

1.5.1 Animal models of temporal lobe epilepsy

Several animal models have been developed to investigate epilepsy and epileptogenic process (Table 1.2). In addition, there are models based upon spontaneous genetic mutations in diverse animal species, or induced mutations in mice. The two most common models for studying epileptogenesis of TLE are the kindling and the post-status epilepticus models. These chronic animal models are used not only to investigate the mechanism of epilepsy, but also to evaluate the effect of AEDs to prevent epileptogenesis. Both types of model provide a reliable method to study the progression of epilepsy.

Table 1.2: A summary of animal model to investigate epilepsy and epileptogenesis

Acute seizure model	
Cell culture	
Low magnesium model	(Rose et al., 1990)
Acutely isolated hippocampus slices	
mGlu agonist model	(Thuault et al., 2002)
Combined hippocampal-parahippocampal slices	
Schaffer collateral model	(Rafiq et al., 1995)
Acutely combined entorhinal cortex-hippocampus slices	
Pentylenetetrazole model	(Armand et al., 1998)
Low magnesium model	(Armand et al., 1998)
Interconnected intact hippocampal formation	
Three-chamber model	(Khalilov et al., 2003)
Electroshock model	(Jones, 1991)
Chronic spontaneous seizure model	
Model of acquired epilepsy	
Kindling model	(Goddard, 1967)
Pilocarpine model	(Hamilton et al., 1997)
Kainic acid model	(Sperk, 1994)
Electrical stimulation model	(Walker et al.,, 1999)
Hypoxic seizure model	(Jensen, 1995)
Undercut cortex model	(Li and Prince, 2002)
Post-traumatic epilepsy model	(Kharatishvili et al., 2006)
Model of genetic epilepsy	
Spontaneous mutations in diverse animal species	(Frankel et al., 1994)

1.5.2 The kindling model

The kindling phenomenon was first described by Goddard (Goddard, 1967). It became one of the most common animal models for the study of temporal lobe epilepsy. In the kindling model, repeated exposure to an initial sub-convulsive stimulus eventually results in evoked seizures. Initially, electrical kindling stimuli only elicit short-duration after discharges which are produced by synchronous neuronal discharges from the stimulated region. Additional kindling stimulations induce longer afterdischarges and increase the area of brain involved. The seizures become more and more complex and longer with repeated stimuli. This increase in sensitivity to previously sub-convulsant stimuli may take a number of days or weeks. The kindling induced reduction in seizure threshold is permanent.

There are many advantages of the kindling model for research into the epileptogenic process, such as: the precise activation of defined brain regions; the development of epilepsy is easy to observe; the pattern of seizure propagation and generalisation can be readily monitored; inter-ictal, ictal and postictal periods are easily manipulated, and the pharmacology of kindled seizures are very similar to the clinical condition.

However, the kindling model rarely results in the development of spontaneous seizures unless a very large number of kindling stimulations are applied (the “over-kindling” model). Kindling can, however, cause some cell loss and sprouting of axonal collaterals (Morimoto et al., 2004).

1.5.3 The status epilepticus models

Status epilepticus (SE) is defined as prolonged seizure activity. Experimental SE can be induced by acute systemic exposure to convulsant agents, such as drugs which block

GABAergic inhibition or increase glutamatergic transmission. Kainic acid, a glutamate receptor agonist, and pilocarpine, a cholinergic agonist, are the most commonly used methods for chemical induction of SE. In addition, electrical stimulation of the hippocampus, amygdala or perforant pathway can induce SE. Usually, spontaneous seizures first occur after a latent period of about 3–4 weeks after the episode of SE in rats. Generally speaking, the development of epilepsy after SE can be classified into four stages: (1) the acute phase (2) the active phase (3) the latent phase and (4) the chronic phase (Morimoto, 2004)

The advantages of the SE model are that the neuronal alterations are similar to those seen in people with epilepsy. SE induces cell loss in specific neuronal populations in multiple brain regions, such as hippocampus, amygdala and entorhinal cortex. Other cells can undergo delayed cell death after seizure termination. Surviving brain cells transform their morphology, including axon sprouting and altered density of dendritic spines. Furthermore, SE also affects non-neuronal brain cells, changing the number and morphology of astrocytes and microglia. Therefore, SE can be used not only to help us understand the epileptogenic process, but also to test the antiepileptogenic properties of drugs by administering the drugs following SE and examining the effects on neuronal pathology and the expression of spontaneous seizures. However, the disadvantages of the SE model are that the SE can be unpredictable and difficult to control, and there may be considerable neuronal damage.

1.5.3.1 Pilocarpine model

Pilocarpine is a non-selective muscarinic receptor agonist. M1 receptor knockout mice are resistant to the development of seizure activity in the pilocarpine model (Hamilton

et al., 1997), indicating that the M1 receptor is required for the initiation of epileptic seizures. Furthermore, pretreatment with atropine, the muscarinic antagonist, blocks pilocarpine-induced seizure activity (Clifford et al., 1987;Priel and Albuquerque, 2002). In cultured hippocampal neurons, application of pilocarpine caused an increase of excitation through modulation of muscarinic receptors, resulting in generation of seizure activity (Priel and Albuquerque, 2002).

Injection of pilocarpine induces limbic and tonic–clonic seizures. The animal has oro-facial movements, salivation, eye-blinking, twitching of vibrissae, and yawning 5-15 minutes after administration of pilocarpine. This activity persists for up to 45 minutes. Discontinuous seizures are observed 30 minutes after injection and last up to 90–150 min. Finally, the seizure activity develops into limbic motor seizures with intense salivation, rearing, forearm clonus, and falling. After pilocarpine injection, around 60% of the rats have been described to develop SE (Curia et al., 2008) Several hours after SE, the seizure activity in pilocarpine-treated animals ceases spontaneously and the animals enter a seizure-free period. 14-15 days after SE, pilocarpine-treated animals have spontaneous recurrent seizures and enter a chronic epilepsy stage.

Animals with several hours of SE have lesions and histopathological alterations in many brain areas, such as olfactory cortex, amygdala, thalamus, hippocampal formation, and neocortex. The hilus, area CA1 and area CA3 of the hippocampus are most affected (Lemos and Cavalheiro, 1995). There is also extensive network reorganization, which may result from neuronal loss. There is not only mossy fibre sprouting, but also sprouting of CA1 axons into area CA3 increased connectivity between CA1 neurons and sprouting of subicular neurons into area CA1 (Lehmann et al., 2001). These

structural alterations result in enhanced excitatory feedback loops within and between hippocampal regions, resulting in increased synchronization of pathological discharges in the hippocampus.

1.5.3.2 Kainic acid model

Kainic acid is a natural marine acid which is a specific agonist for the kainate receptor, an ionotropic glutamate receptor subtype. Kainic acid given locally into the brain or systemically has been used as experimental models of temporal lobe epilepsy, because the clinical signs and pathology are similar to those seen in patients with temporal lobe epilepsy.

Rat behaviour during kainic-acid induced status epilepsy can be divided into several stages: Stage 1, staring and movement arrest is observed 5-6 min after administration; Stage 2, masticatory movements, head nodding, myoclonic twitches of the head, face and fore limbs, and ‘wet dog’ shakes are observed after 5 minutes of administration and last for 30 minutes; stage 3, about 1 hours after injection kainic acid, the rats have recurrent generalised tonic-clonic seizures, rearing, loss of postural control and increased salivation, production of foam at the mouth often mixed with blood. Once the recurrent generalised tonic-clonic seizures develop, they continue for 4-5 hours and remit spontaneously. Approximately 60-80 % of animal develop the full seizure syndrome after systemic kainic acid administration, via the subcutaneous or intraperitoneal route. In addition to the involvement of entorhinal cortex and hippocampus, the epileptic activity recruits other limbic structures, including the median thalamic complex, and medial frontal cortex (Sperk, 1994). Kainic acid preferentially induces seizures in limbic structures, perhaps secondary to the high

density of kainate receptors in the CA3 region of the hippocampus, increasing the release of the endogenous excitatory amino acids, glutamate and aspartate and further activating all types of glutamate receptors (Oprica et al., 2003;Ferkany et al., 1982).

Administration of kainic acid into rats not only induces ongoing convulsions, but also causes degeneration of neurons and hyperexcitability of surviving neurons. Kainic acid can directly induce cell death because of its excitotoxic properties, increasing intraneuronal calcium levels and the activation of calcium-dependent proteases. In addition, kainic acid also results in neuronal death through the induction of seizure activity (Ben-Ari et al., 1979).

1.5.3.3 Electrical stimulation model

Sustained electrical stimulation of the perforant pathway (the angular bundle i.e. the pathway from entorhinal cortex to hippocampus) evokes hippocampal granule cell population spikes and epileptiform discharges (Sloviter, 1983). By using biphasic electrical stimulation with continuous low frequency, 20Hz to 50 Hz, of the perforant path in awaken animals; it is possible to evoke self-sustaining SE (Walker et al., 1999; Martín and Pozo,, 2006;Prasad et al., 2002).

During stimulation, the rats first have ‘wet dog shakes’ and progress to limbic seizure consisting of chewing, head nodding, forelimb clonus and few tonic seizures. The seizure activity remits spontaneously over a period of hours. The seizure may reappear in few weeks after stimulation procedure.

Even though perforant path stimulation is unilateral, it evokes bilateral granule cell

discharges and causes bilateral hippocampal damage. In this model, the hilar somatostatin-containing cells of the dentate gyrus are the most susceptible, followed by the pyramidal cells of CA1 and CA3. The CA2 pyramidal cells and the dentate gyrus granule cells appear to be most resistant (Sloviter, 1983;Kelsey et al., 2000).

The effects of perforant path induced SE extend beyond hippocampal neuronal loss, and include impairment of learning and memory (Rogers et al., 1989;Kelsey et al., 2000). The learning and memory deficits are associated with hippocampal cell loss, the frequency of inter-ictal spikes and the age of seizure onset (Ylinen et al., 1991;Thurber et al., 1992).

1.6 Mechanisms of epileptogenesis

The mechanisms of epileptogenesis are still unclear. However, different initiating events may lead to a similar sequence, including acute and delayed excitotoxicity, resulting in the death of specific, susceptible neuronal populations. Long-term alterations which are evoked by inducing gene activity expression or compensatory responses to cell damage and death appear to produce effects on neuronal circuitry, dendritic plasticity, neurogenesis and molecular reorganization of membranes and extracellular space (Jutila et al., 2002).

1.6.1 Excitatory systems in epileptogenesis

A number of studies have pointed out that increased activity in the excitatory system may play an important role in epileptogenesis. Enhancement of excitatory activity can be due to increased presynaptic neurotransmitter release, increased postsynaptic receptor sensitivity, and changes in glutamate uptake and metabolism.

1.6.1.1 Glutamate receptors in epileptogenesis

Glutamate receptors can be divided into two classes: ionotropic glutamate receptors and metabotropic glutamate receptors. Ionotropic glutamate receptors consist of N-methyl-D-aspartate (NMDA) receptors, alpha-amino-3-hydroxy-5-methyl propionate (AMPA) receptors and kainate receptors. Metabotropic glutamate receptors are divided into three groups: Group one receptors, which include mGluR1 mGluR5, are coupled via Gq/11 protein and phospholipase Cβ (PLCβ) to inositol phospholipids metabolism; Group two receptors, which include mGluR2 and mGluR3, and are negatively coupled by Gi to adenylyl cyclase; Group three receptors, which include mGluR4, mGluR6, mGluR7, and mGluR8 are negatively coupled by Gi to adenylyl cyclase. Both ionotropic receptors (especially NMDA receptors) and metabotropic glutamate receptors have been linked to excitotoxicity.

1.6.1.2 NMDA receptor in epileptogenesis

The NMDA receptor (NMDAR) is an ionotropic receptor, allowing flow of Na^+, K^+, and Ca^{2+} ions. Calcium influx through NMDARs plays an important role in Hebbian plasticity. NMDA receptors probably consist of 5 subunits per channel. The NMDA receptor subunits include: NR1, NR2A, NR2B, NR2C, NR2D, and NR3. The functional receptor requires two NR1 and a subtype of the NR2 subunits. The NR1 subunits bind the co-agonist glycine and NR2 subunits bind the neurotransmitter, glutamate (Yamakura and Shimoji, 1999).

NMDA receptors are blocked by magnesium at hyperpolarized potentials and this block is removed by depolarization. NMDA receptor currents lead to a prolonged depolarization. Over-activation of glutamate receptor causes the influx of chloride and water, resulting in neuronal swelling and cellular necrosis. Additionally, over-activation

of NMDA receptor triggers intracellular signalling and regulatory pathways, resulting in delayed cell degeneration (apoptosis or "programmed cell death"). This depends upon calcium entry through NMDA receptors. Other routes of calcium entry, such as through L-type voltage-sensitive Ca^{2+} channels do not usually initiate apoptosis. This may be because the enzymes or substrates responsible for excitotoxicity co-localize with NMDA receptors (Arundine and Tymianski, 2003).

Calcium entering through NMDA receptors during SE may initiate a cascade of cellular events, including activation of catabolic enzymes, impairment of energy metabolism and generation of reactive oxygen species and ultimately leading to cell death. NMDA receptor activation also leads to permanent alterations in neuronal circuits and excitability.

Recent evidence suggests that NMDA receptor currents are increased following SE (Scimemi et al., 2006). This may also occur in other models of epileptogenesis. NMDAR1 expression is increased after Fe^{3+}-induced epileptogenesis (Doi et al., 2001). In humans, changes in NMDA receptors in TLE patients are region specific. For example, the expression of NR1 increases in CA3-1 stratum radiatum, while the expression of NR2 increases through all hippocampal subfields (Mathern et al., 1998).

1.6.1.3 AMPA receptor activation in epileptogenesis

AMPA receptors are a four subunit receptor composed of, GluR1, GluR2, GluR3, and/or GluR4. The AMPA receptor mediates fast glutamatergic synaptic transmission. The combination of GluR1 and GluR2 constitute the majority of AMPA receptor complexes in the neocortex and hippocampus (Wenthold et al., 1996)

Some researchers have suggested that AMPA receptors play a role in epileptogenesis and glutamate-induced neuronal death. In patients with chronic epilepsy, it has been reported that there was a significant increase of GluR2/3 on the dendrites of dentate granule cells in the hippocampus (de Lanerolle et al., 1998). In animals, a variety of changes in AMPA receptor subunits have been described. The expression of GluR1and GluR2 in the hippocampus change with epileptogenesis (Doi et al., 2001). The GluR2 subunit is reduced in limbic forebrain and amygdala 24 hours after amygdaloid kindling (Prince et al., 1995). After lithium–pilocarpine induced SE, the expression of different AMPA receptor subunits changes (e.g. GluR2 increases and GluR3 decreases) in dentate granule neurons (Porter, 2006). Perhaps the most direct evidence for a role of AMPA receptors in epileptogenesis is provided by the observation that application of the AMPA receptor antagonist, YM90K, markedly retarded the evolution of kindling (Kodama et al., 1999). Changes of AMPA receptor subunits during epileptogenesis alter excitatory neurotransmission and may contribute to seizure propagation.

1.6.1.4 Kainate receptor activation in epileptogenesis

The kainate receptor is a four subunit receptor composed from subunits: GluR5, GluR6, GluR7, KA1 and KA2 (Dingledine et al., 1999). Kainate receptors are permeable to sodium and potassium ions. The kainate receptor plays a role in: modulation of transmitter release, including regulation of excitatory synaptic transmission (Lauri et al., 2001b;Vignes and Collingridge, 1997); regulation of inhibitory synaptic transmission (Lerma, 2003); synaptic plasticity- long-term potentiation (Lauri et al., 2001a;Vissel et al., 2001). The CA3 area, which is considered as a pacemaker for the generation of synchronized activity that subsequently propagates to CA1 and other brain regions, is highly vulnerable to network hyperactivity and readily degenerates following recurrent

seizures probably because of a sustained release of glutamate leading to over-activation of kainate receptors (Ben-Ari and Cossart, 2000). In both humans and various animal species, high-affinity kainite receptors are greatly enriched at mossy fibre synapses onto CA3 pyramidal cells (stratum lucidum) (Monaghan and Cotman, 1982;Tremblay et al., 1985). Activation of kainate receptor generates seizures in CA3 pyramidal neurons that propagate to CA1 and to other limbic structures (Robinson and Deadwyler, 1981;Fisher and Alger, 1984;Ben-Ari and Gho, 1988). Furthermore, long-lasting changes of synaptic responses result from the spontaneous synchronized discharges present during and shortly after the application of kainic acid (Ben-Ari and Gho, 1988).

1.6.1.5 Metabotropic glutamate receptor in epileptogenesis

Metabotropic glutamate receptors, or mGluRs, are G-protein-coupled receptors, activated by glutamate binding. The mGluRs are located pre- and postsynaptically in the brain.

Metabotropic glutamate receptors play an important role in the central nervous system (CNS), regulating both neuronal excitability (Colwell and Levine, 1999) and neurotransmitter release (Cartmell and Schoepp, 2000). mGluRs may also play an important role in synaptic plasticity (long term potentiation and long-term depression) (Riedel and Reymann, 1996). Group I metabotropic glutamate receptors have been implicated in epileptogenesis. The group I mGluRs are comprised of two receptor subtypes, mGluR1 and mGluR5, both of which are present in the hippocampal CA3 region (Blümcke et al., 1996;Lujan et al., 1996). *In vitro* studies indicate that ictal-like discharges, which persist for hours, can be induced by transient application of the selective group I mGluR agonist (S)-3,5-dihydroxyphenylglycine (DHPG) (Merlin,

1999). Furthermore, application of group I mGluR antagonists block persistent ictal-length discharges induced by the transient activation of group I mGluRs (Merlin and Wong, 1997;Merlin, 1999). By using subtype-selective antagonists to evaluate the separate roles of mGluR1 and mGluR5 in the induction of epileptogenesis, both mGluR1 and mGluR5 participate in both the induction and maintenance of mGluR-mediated burst prolongation. However, mGluR1 activation plays a greater role in sustaining the expression of prolonged bursts, whereas mGluR5 activation may be a more critical contributor to the induction process underlying epileptogenesis (Merlin, 2002;Wong et al., 2005). In human, expression of mGluR5 increases have been observed in the hippocampus in TLE patients (Notenboom et al., 2006;Wong et al., 2005).

Even though activation of group I mGluRs are proconvulsant, activation of group II and group III mGluRs are anticonvulsant. Activated Group II and Group III mGluRs show anticonvulsant efficacy in a number of models of limbic and generalized motor seizures (Alexander and Godwin, 2006). Application of LY379268 and LY389795, selective group II mGlu agonists, showed anti-epileptic effect in a variety of *in vivo* seizure models (Moldrich et al., 2001). Additionally, in fully kindled rats following daily electrical stimulation of the basolateral amygdale, application with DCG-4, a group II mGlu agonist, increased the generalized seizure threshold in these seizure susceptible animals and inhibited depolarization-induced release of aspartate from rat cerebrocortical synaptosomes, suggesting that the anticonvulsant property of group II mGlu receptors may be due to their modulatory action on neuronal glutamate release (Attwell et al., 1998). Selective activation of group III mGluRs by (R,S)-4-phosphonophenylglycine, a select selective agonist for group III mGluRs results in

neuroprotection against NMDA and quinolinic acid-induced striatal lesions in rats and showed anticonvulsive effect in the maximal electroshock model in mice (Gasparini et al., 1999)

1.6.2 Inhibitory systems in epileptogenesis

1.6.2.1 GABA(A) receptors in epileptogenesis

The neutral amino acid GABA interacts with post-synaptic GABA(A) receptors, resulting in fast synaptic inhibition. GABA(A) receptors are heteromeric protein complexes composed of five subunits that form ligand-gated anion-selective channels. Once GABA binds to GABA(A) receptors, the pore opens to allow chloride ion influx which contributes to inhibitory postsynaptic potentials (IPSP).

There are numerous subunit isoforms for the GABA(A) receptor (GABAR). GABA(A) receptors are pentameric structures composed of 2 alphas and 2 betas and one other subunit. There are 6 alpha subunits, 3 beta subunits, 3 gamma subunits, and also delta, epsilon, pi and rho subunits. The composition of subunits determines the GABA(A) receptor's properties, such as agonist affinity, opening probability and conductance (Barnard et al., 1998).

Impaired GABA-ergic function is associated with epilepsy (De Deyn et al., 1990;Jeffrey and Dudek, 1991). It has been demonstrated that post-synaptic GABAR function undergoes long-lasting alterations in temporal lobe epilepsy (Gibbs et al., 1997;Buhl et al., 1996;Nusser et al., 1998;Schwarzer et al., 1997). Epilepsy-associated changes in GABAR function may result from altered subunit composition (Brooks-Kayal et al., 1998;Fritschy et al., 1999).

Alterations of GABAR subtypes may lead to changes of the pharmacological properties of synaptic GABAR. Molecular reorganization of GABAR subunits significantly increases the blockade of GABA evoked currents by zinc in epileptic patient (Shumate et al., 1998) and in animal models of TLE (Buhl et al., 1996). In rats with SE induced by lithium–pilocarpine, there were drastic changes in GABAR-mediated inhibition within dentate gyrus granule cells that were sensitive to modulators such as benzodiazepine and neurosteroids (Leroy et al., 2004). In addition, GABAR endocytosis during prolonged seizures may contribute to decreased expression of specific GABAR subtypes (Blair et al., 2004). The change of pharmacological properties of synaptic GABAR may be crucial to epileptogenesis and the occurrence of seizures.

1.6.2.2 GABA(B) receptors in epileptogenesis

GABA(B) receptors are composed of two subunits GABA-R1 and GABA–R2. GABA(B) receptors are G protein coupled receptors which are involved in a number of physiological and disease processes, including epilepsy. The activation of presynaptic GABA(B) receptors inhibits neurotransmitter release; the activation of post-synaptic receptors increases potassium channel activation, resulting in prolonged synaptic inhibition. In addition, GABA(B) receptors alter cAMP formation via adenylyl cyclase (Kerr and Ong, 1995;Couve et al., 2000).

A number of studies have indicated that GABA(B) receptor function is altered during epileptogenesis. GABAB1 subunit knockout mouse develop generalized epilepsy resulting in premature death (Prosser et al., 2001). In rats with kindling-induced epileptogenesis, the sensitivity of presynaptic GABA(B) receptors located on the nerve

terminals of glutamatergic afferents was reduced, leading to the enhancement of excitatory transmission. This alteration lasted 4-6 weeks after kindled seizure, indicating a long-lasting alteration (Asprodini et al., 1992). Additionally, there is evidence of decreased presynaptic GABA(B) receptor inhibition of excitatory transmission at mossy fibre during the development of epilepsy following perforant path stimulation or pilocarpine administration (Chandler et al., 2003).

1.6.3 Non-synaptic mechanisms in epileptogenesis

Non-synaptic mechanisms refer to those mechanisms that are independent of active chemical synaptic transmission, resulting in the synchronization of neuronal activity during seizures. These “non-synaptic” mechanisms include electronic coupling of gap junctions, electrical field effects and ionic interactions. All of these may be enhanced during epileptogenesis due to changes in the extracellular space, gliosis and the formation of gap junctions (Dudek et al., 1998). Blocked chemical synaptic transmission both pre and post-synaptically, and decreases in osmolality, which shrink the extracellular volume, cause or enhance epileptiform bursting, indicating the enhancement of synchronization among CA1 hippocampal neurons through non-synaptic mechanisms. Increases in osmolality, which expand the extracellular volume, block or greatly reduce epileptiform discharges (Dudek et al., 1990).

Gap junctions are intercellular channels composed of connexin proteins, which can be modulated by a number of intracellular and extracellular factors, allowing certain molecules and ions to move freely between neurons. This permits direct electrical transmission between cells, chemical transmission between cells via small second messengers, and the passage of small molecules (1000 Dalton). Gap junction channels

contribute to neuronal synchronization in the brain (Draguhn et al., 1998;Draguhn et al., 1998;Traub et al., 2003). In addition, they may also play a role in hypersynchrony in epilepsy models (Kohling et al., 2001;Perez Velazquez and Carlen, 2000). Recent studies indicate that gap junctions can significantly modify the expression, duration, and propagation of seizures in *in vitro* (Gajda et al., 2003) and in *in vivo* (Gajda et al., 2005) epilepsy models.

Electrical-field effects depend on neuronal orientation, polarity and the size of the extracellular space (Ghai et al., 2000). Tight packed neurons that are arranged in parallel are susceptible to the effect of activity-induced electrical fields. Electric fields can modulate neuronal activity in the central nervous systems. They influence a variety of cellular events, such as membrane differentiation, neurite growth during both development and structural regeneration, organization of local neuronal circuits, and even receptor localization (Faber and Korn, 1989). When synaptic activity is blocked in hippocampal slices by removing extracellular Ca^{2+}, recurrent spontaneous paroxysms, termed seizure-like events, occur in the CA1 region (Konnerth et al., 1986).

Ionic interactions involve activity dependent shifts in the intracellular and extracellular concentration of ions. Intense electrical activity during seizures is associated with transmembrane ionic currents, causing K^+ and Cl^- redistribution. The increase of K^+ concentration in the extracellular milieu increases membrane excitability, resulting in a slow synchronizing effect on neuronal networks (Yaari et al., 1986;Jensen and Yaari, 1997;Bikson et al., 1999). Additionally, extracellular chloride may also play a critical role in neuronal synchronization (Hochman et al., 1999;Hochman et al., 1995).

1.6.4 Cell loss and epileptogenesis

Damage to the central nervous system can be modulated by excessive excitatory and reduced inhibitory neurotransmission. Furthermore, increased Na^+ and Ca^{2+} loading, through impaired ion channels or alterations in the acid-base balance, contributes to both excitotoxic and apoptotic cell death. It has been suggested that cell loss in TLE, including principal neurons in CA1, CA3 and CA4 regions of the hippocampus (Margerison and Corsellis, 1966) may indirectly result in the later occurrence of recurrent limbic seizures (Meldrum, 1997).

1.6.4.1 Apoptosis

After brain injury, such as ischemic injury, stroke, or traumatic brain injury, apoptosis (programmed cell death) plays a critical role in neuronal death (Liou et al., 2003). Apoptosis is a physiological process for killing cells and is important for the normal development and function of multicellular organisms (Strasser et al., 2000). Apoptosis results from a highly ordered molecular cascade, including inflammatory/cytokine-processing, Caspases- 1, 5, and 11 and apoptosis-regulatory caspases- 2, 3, 6, 7, 8, 9, and 10. Apoptosis may also involve changes in gene transcription (Henshall and Simon, 2005).

Repeated tonic-clonic seizures result in mild cell loss in the hippocampal formation, initially in the dentate gyrus (Cavazos and Sutula, 1990;Cavazos et al., 1994). Furthermore, there are small numbers of cells that die after brief seizures which exhibit 'apoptotic' DNA fragmentation.

Apoptosis after seizures may involve: extrinsic pathway activation which involves caspases 2 and 8; intrinsic pathway activation which relates to mitochondrial calcium

loading, cytochrome c release and calpain-mediated release of apoptosis-inducing factor (AIF); executioner caspase activation which includes caspase 3, 6 and7 (Henshall and Simon, 2005).

More recent evidence suggests that executioner caspases-3 and -6 continue to be active during epileptogenesis. Increased caspase 3 expression was observed following SE and this was suggested to contribute to cell death occurring within the first week after SE (Narkilahti et al., 2003). Further, caspase 6 expression remains elevated up to 4 weeks after inducing SE (Narkilahti and Pitkänen, 2005).

Bcl-2 family proteins are crucial for regulating apoptosis. The Bcl-2 family comprises pro-apoptotic and anti-apoptotic proteins, involved in the process of stimuli targeted at the mitochondrion (Adams and Cory, 1998). BAD, Bcl-2-associated death protein, has been shown to dissociate from its sequestered site with the molecular chaperone protein 14-3-3. After calcineurin-induced dephosphorylation, BAD is released from 14-3-3 and dimerizes with the anti-apoptotic protein BCL-Xl and releases BAX (Bcl-2-associated X) from BCL-Xl (Yang et al., 1995). Thereafter, BAX translocates to the mitochondrion, promoting release of cytochrome c which contributes to the formation of the apoptosome, and activation of the caspase cascade (Li et al., 1997;Zou et al., 1997). A recent study suggests that seizures elicit cell death and survival responses within neuronal populations and that the BAD cell death pathway contributes to seizure-induced neuronal death (Henshall et al., 2002).

1.6.4.2 Mitochondrial dysfunction in epileptogenesis

Mitochondria are important for cellular ATP production, ROS formation, control of apoptotic/necrotic cell death and fatty acid metabolism (Patel, 2002). Mitochondrial dysfunction can alter neuronal excitability. The inhibition of the mitochondrial respiratory chain enzymes, such as cytochrome c oxidase and succinate dehydrogenase, evokes seizures. This may be due to an intracellular decrease in ATP levels and alterations in neuronal calcium homeostasis (Kunz, 2002). Free radicals may "attack" mitochondrial, inhibit the activity of the respiratory chain and induce transient mitcohondrial calcium permeability. This results in ATP production decline and excessive release of free radicals, consequently causing cell death (Arzimanoglou et al., 2002).

1.6.5 Nitric oxide

Nitric oxide (NO) is a free radical oxidant with relevance to pathological conditions in the brain, and reactive nitrogen intermediates could damage DNA. NO is an endothelium-derived relaxing factor (Palmer et al., 1987), a potent vasodilator that regulates cerebral blood flow during certain types of neuronal activation, including seizures (de Vasconcelos et al., 1995).

NO is synthesized from L-arginine in CNS by Ca^{2+} calmodulin-dependent NO synthase (NOS) which is activated by NMDA receptors. There are three isoforms of NOS- the constitutive neuronal (nNOS), endothelial (eNOS), and the inducible (iNOS). nNOS and eNOS are essential for certain forms of long-term potentiation. Nitrites increase markedly in the hippocampus of rats experiencing repetitive seizures (Murashima et al., 2000). nNOS and iNOS increase in parallel during epileptogenesis. However, eNOS decreases during epileptogenesis (Murashima et al., 2000;Murashima et al., 2002).

1.6.6 Neural reorganization in epileptogenesis

It is thought that functional and structural reorganization of neural networks is crucial for epileptogenesis. The alteration of neuronal circuits leading to recurrent seizure activity may result from reorganisation following neuronal death and changes in excitatory and inhibitory transmission.

1.6.6.1 Neurogenesis in epileptigenesis

Adult neurogenesis is a mechanism by which there is repair of tissues and replacement of dead cells. Neurogenesis in the hippocampus has been demonstrated in human with TLE and a number of animal models of TLE (Parent et al., 2002;Parent et al., 2006a;Bengzon et al., 1997).

In human specimens, granule cell somata extend into the molecular layer to varying extents, creating an irregular boundary between the lamina, which is related to the amount of cell loss in the polymorph layer of the dentate gyrus. The abnormal extension of granule cells could lead to changes in both the afferent and efferent connections of these neurons and, lead to altered circuitry (Houser, 1990;Scharfman et al., 2000).

In rats with SE induced by pilocarpine, the subventricular zone neuroblast population, which is known to express a variety of immature neuronal markers before they enter the migratory pathway, expands and alters neuronal precursor migration in the adult rat forebrain (Parent et al., 2002). Cauda subventricular zone progenitors, which migrate into the corpus callosum to form oligodendrocytes or oligodendrocyte progenitors in normal condition, migrate to the injured hippocampus and differentiate into glial cells after SE (Parent et al., 2006b).

Prolonged seizures induced by administration of pilocarpine or by kindling induce aberrant neurogenesis in the adult rat dentate gyrus (Parent et al., 2006a;Parent et al., 1998). After SE, newly generated neurons migrate from the dentate subgranular zone to hilus and molecular layer, and integrate abnormally, leading to abnormal network function (Parent et al., 2006a).

The robust alteration in neurogenesis following SE may be due to neurodegeneration. Such neurogenesis may result in aberrant circuits, leading to hyperexcitability and an increased propensity for the propagation of epileptic activity (Curtis et al., 2007).

1.6.6.2 Growth factors in epileptogenesis

Growth factors regulate a variety of cellular processes. They bind to receptors on the cell surface, activating cellular proliferation and differentiation, contributing to altering the function of the hippocampal network.

Growth factors are involved in the development of epilepsy. There is evidence that nerve growth factor (NGF) accelerates epileptogenesis in the kindling model and increases mossy fibre sprouting in the CA3 region and inner molecular layer (Adams et al., 1997). BDNF mRNA was markedly increased in hippocampus and neocortex in fully kindled rats (Simonato et al., 1998). In the kindling model, the expression of BNDF mRNA was increased in dentate granule cell layers, and was influenced by neurotrophin-3 (Elmer et al., 1997). There evidence also shows that synthetic peptides designed to prevent neurotrophin binding to their receptors significantly retard kindling induced epilepsy and inhibit mossy fibre sprouting (Rashid et al., 1995).

Tyrosine kinase B (TrkB), the primary target of BDNF, plays a vital role in epileptogenesis. Characteristic neuropathologic changes result from the interaction of BDNF with TrkB, such as neuronal loss and axonal sprouting, plastic changes in neuronal networks and synapses, neurogenesis, and dendritic outgrowth. Increased BDNF causes neuronal hyperexcitability and promotes long-term potentiation of excitatory synaptic transmission. Further studies suggest that BDNF is redistributed (e.g. to distal dendrites) during the development of epilepsy.

1.6.6.3 Mossy fibre sprouting in epileptogenesis

Hippocampal mossy fibres, which are the axons of dentate granule cells, converge in the dentate hilus and innervate hilar and CA3 neurones (Frotscher et al., 1994). In the rodent, the main mossy fibre axons leave the hilus and travel through CA3 in stratum lucidum, which corresponds to the apical dendrites of CA3 pyramidal cells (Henze et al., 2000). Mossy fibres transmit information from dentate gyrus into the hippocampus proper.

Mossy fibre axons form synapses with excitatory and inhibitory cells of the hilus and area CA3. There are inhibitory presynaptic G-protein coupled receptors at mossy fibre synapses for a number of transmitters, such as GABA, adenosine and dynorphin. The release of glutamate and GABA can therefore have a depressant effect on mossy fibre synaptic transmission. The mossy fibre pathway predominantly synapses onto inhibitory interneurons, rather than excitatory hilar mossy cells and CA3 pyramidal cells.

The presynaptic terminals of mossy fibbers are large, complex and completely surround branched dendritic spines with up to 20 independent release sites. The mossy fibre

synapses demonstrate strong activity-dependent plasticity, and a presynaptic form of LTP, which does not require NMDA receptor activation (Nicoll and Malenka, 1995). A CA3 pyramidal neuron receives only approximately fifty synapses from mossy fibres, whereas it receives about 12,000 synapses from other CA3 neurons located in the ipsilateral and contralateral hippocampus. Mossy fibre synapses are however very efficient and are able to bring CA3 pyramidal cells to firing threshold. Therefore, stimulating mossy fibres can lead to activation of recurrent excitatory synapses by firing of CA3 pyramidal neurons, resulting in epileptiform activity (Johnston et al., 1992).

Mossy fibre sprouting is secondary to synaptic reorganization in the dentate gyrus; mossy fibres branch out of the dentate hilus and abnormally innervate the dentate inner and outer molecular layer (Dalby and Mody, 2001). Sprouting is seen as a response to the loss of neuronal targets. These alterations in TLE have been found in different chronic epilepsy animal models, such as kindling models (Bengzon et al., 1997;Sutula et al., 1988) and post-status epilepticus models (Elmer et al., 1996;Molnar and Nadler, 1999). Mossy fibre sprouting is also found in hippocampal tissue TLE patients (Sutula et al., 1989;Proper et al., 2000).

What role does mossy fibre sprouting play? Mossy fibres form excitatory recurrent circuits through collaterals synapsing onto granule cells and interneuron dendrites in the supragranular layer in the dentate gyrus (Proper et al., 2000). Also, mossy fibre spouting is found in the infrapyramidal CA3 region and CA1 pyramidal neurons (Holmes and Ben-Ari, 2001). Mossy fibre sprouting forms new excitatory connections between granule cells and may therefore lead to increased seizure susceptibility in the dentate gyrus (Wuarin and Dudek, 1996).

Mossy fibre sprouting could also serve a compensatory role. The granule cell mossy fibre system produces a strong activation of GABA-ergic hilar neurons. Therefore, activating the entorhinal cortical input to the dentate causes the interneuronal population to burst and will depress intrahippocampal associational pathways. Indeed, mossy fibre sprouting is aimed predominantly at inhibitory basket cells, possibly re-establishing normal levels of inhibition to the granule cells (Sloviter, 1992). Thus, it has been suggested that mossy fibre sprouting may not be a primary epileptogenic mechanism (Harvey and Sloviter, 2005;Timofeeva and Peterson, 1999).

1.7 Interventions to prevent epileptogenesis

The multiple processes of epileptogenesis provide a number of sites for potential interventions to prevent epilepsy. Elucidation of the epileptogenic process should provide significantly more effective methods of treatment. The opportunities for intervention can be divided into four stages: Initial insult modification, which can be achieved by early pharmacological intervention and neuro-surgery, neuroprotection, antagonism of epileptogenesis, and disease modification (Stefan et al., 2006). Interventions aimed at the epileptogenic cascade may prevent neuronal injury or death, preserving or restoring neuronal function, and neuronal recovery or regeneration (Walker et al., 2002). Epileptogenesis depends on different processes from those involved in seizure generation. Drugs can be designed to inhibit the initial damage which is produced by brain insults, such as excitotoxic cell death, and to prevent or reverse alterations in neuronal circuits that contribute to lowered seizure thresholds.

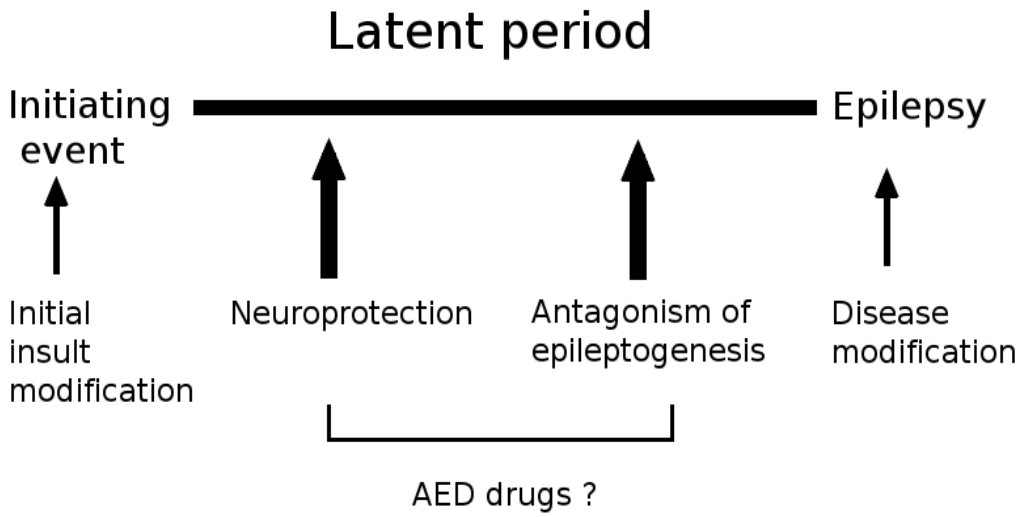


Figure 1.4: The interventions to modify the epileptogenic process. The multiple processes of epileptogenesis provide a number of sites for potential interventions to prevent epilepsy: initial insult modification, neuroprotection and disease modification.

1.7.1 Initial insult modification

Initial insult modifications may have an antiepileptogenic and disease-modifying effect. The effect of phenobarbital (80mg/kg), MK-801 (4mg/kg), or phenytoin (100mg/kg) at 1, 2, and 4 hours after initiation of "continuous" hippocampal stimulation on the development of epilepsy has been investigated. Both phenobarbital and MK-801 reduced the percentage of animals developing epilepsy (Prasad et al., 2002). The mechanism may have involved inhibition of the cascade of events that leads to the death of critical neuronal populations and cell death-induced reactive gliosis, sprouting, and

other types of damage-induced network reorganization underlying the development of spontaneous seizures (Pitkänen, 2002).

1.7.2 Neuroprotection to prevent epileptogenesis

Neuronal injury and death may play an important role in epileptogenesis. Pharmacological neuroprotection in epileptogenesis can be considered as primary and secondary. Primary neuroprotection lessens the initial insult by suppressing seizure activity or diminishing the associated ionic fluxes. Secondary neuroprotection refers to targeting later events in the chain linking ionic changes to altered brain morphology or function. AEDs and compounds designed to act on voltage- sensitive Na^+ and Ca^{2+} channels or on glutamate receptors are considered primary neuroprotection. Secondary neuroprotection refers to interventions that act on the cascade leading to necrosis or apoptosis (Artemowicz and Sobaniec, 2005).

1.7.2.1 Neuronal survival signalling pathways

There are several signalling pathways which are involved in neuronal survival, such as phosphatidyloinositol-3-kinase (PI3K), extracellular signal regulated kinase 1/2 (ERK1/2) and extracellular signal regulated kinase 5 (ERK5). The activation of neuronal survival signalling pathways to protect neurodegeneration after SE may provide novel methods of the prevention of neuronal death and the regulation of epileptogenesis.

ERK1/2 is a member of mitogen activated protein kinases. ERK 1/2 can be activated by various growth factors and cAMP-signalling pathway (Vossler et al., 1997;Hetman and Gozdz, 2004). ERK1/2 contributes to the regulation of proliferation, differentiation,

actin cytoskeleton reorganization and cell migration (Wada and Penninger, 2004). The targets of ERK1/2 include transcription factors, cytoskeletal proteins, protein kinases and Ca^{2+} / cAMP response element-binding protein (CREB)/CRE.

It has been suggested that ERK1/2 activation protects against neuronal damage (Hetman and Gozdz, 2004;Impey et al., 1999;Xia et al., 1995;Hetman et al., 1999) and generates LTP (English and Sweatt, 1997;Impey et al., 1999). Stimulation of the ERK1/2-signaling pathway promotes neuronal survival. ERK2 phosphorylates pro–caspase-9 at the Ser 125 site, which inhibits caspase-9 processing and subsequently prevents caspase-3 activation (Allan et al., 2003;Cheung and Slack, 2004). ERK1/2 activity increases in neuronal cultures after induction of NMDA-mediated excitotoxicity, seizure-like activity, exposure to okadaic acid, or after oxidative stress (Irving and Bamford, 2002;Chu et al., 2004), possibly indicating an endogenous neuroprotective mechanism following neuronal activity.

ERK activation dramatically decreases mortality during seizures in the pilocarpine model of SE (Berkeley et al., 2002) suggesting that a neuroprotective role for ERK1/2. However, recent studies suggest that ERK1/2 can also be epileptogenic. Activation of ERK1/2 through a tyrosine kinase-dependent process by group I mGluRs is necessary for the induction of prolonged epileptiform discharges in the hippocampus (Zhao et al., 2004). ERK1/2 phosphorylation increases by up to 80% about 20 minutes after treatment with 4-AP, which induces epileptiform activity in CA3 region in hippocampus slices. Application of the ERK pathway inhibitors U0126 or PD98059 which inhibit phosphorylation of ERK1/2 reduce 4AP-induced epileptiform discharges in the CA3 area (Merlo et al., 2004). Additionally, a constitutively active form of MEK1

(caMEK1), resulting in ERK activation, induces spontaneous epileptic seizures, possibly due to stimulation of NMDA receptor activity (Nateri et al., 2007).

The complex role of ERK1/2 in SE may be due to its dual roles in neuroprotection and neurodegeneration. It has been suggested that ERK1/2 activation can promote neuronal death. Inhibiting the activation of ERK1/2 can lead to reduced neuronal injury and loss of function in animals (Chu et al., 2004). Inhibition of ERK1/2 activity significantly reduced neurological deficits in ischemic brain injury in mice (Wang et al., 2003) and led to neuroprotection from brain injury resulting from occlusion of the middle cerebral artery (Alessandrini et al., 1999). The mechanism of the opposing roles for ERK1/2, prosurvival and cell-death-promoting activity, has been suggested to be through effects on glutathione metabolism to limit oxidative stress, and serving as a necessary signal to trigger cell death when cellular defence against oxidative stress is exhausted (Luo and DeFranco, 2006).

Akt or protein kinase B has three isoform in mammals, termed Akt1, Akt2 and Akt 3. All three Akt/PKB isoforms contains an amino terminal pleckstrin homology domain, a central kinase domain and a carboxyl-terminal regulatory domain that contains the hydrophobic motif. All Akt isoforms possess two regulatory phosphorylation sites, Thr308 in the activation loop within the kinase domain and Ser473 in the C-terminal regulatory domain. The PH domain of Akt binds to PIP3, produced by activated PI3K; this alters its conformation to allow subsequent phosphorylation by the phosphoinositide-dependent kinase-1, phosphorylation of Thr308 and phosphorylation of Ser473. Akt is thus activated in a PI3-kinase-independent manner. The Akt signalling pathway is important for regulating cell survival. Akt promotes cell survival by

mediating growth factors both directly and indirectly (Song et al., 2005). Akt (protein kinase B) is able to modulate BAD (Bcl-2-associated X protein) activity by phosphorylating BAD at the Ser136 residue that is critical for sequestration to 14-3-3 (Masters et al., 2001). Akt activity is regulated by phosphorylation via phosphatidylinositol 3 kinase (Burgering and Coffer, 1995;Datta et al., 1997;Dudek et al., 1997).

Recent research indicates that the activation of Akt protects cells in animal models of SE. Seizures, induced by microinjection of kainic acid into the amygdale, induced the dissociation of BAD from 14-3-3 and the subsequent dimerization of BAD with BCL-Xl, resulting in cell damage in hippocampus, whereas undamaged cortex exhibited Akt phosphorylation. Blocking the activation of Akt exacerbated the damage (Henshall et al., 2002). Furthermore, in rats with seizures induced by administration of kainic acid, inhibition of Akt activity resulted in an increase in apoptotic cell death (Côté et al., 2005).

1.7.2.2 Antiepileptic drugs provide neuroprotective effects during epileptogenesis

Treatment with AEDs during or after brain injury in chronic epilepsy models, such as status epilepticus and kindling, can reduce cell loss and neuronal death. Such neuroprotection could contribute to modification of or delay in the development of epilepsy. Antiepileptic drugs are aimed at preventing and suppressing seizure activity. Some may ameliorate necrotic and apoptotic neuronal death. Injection of dizocilpine (MK-801) and retigabine after kainate-induced SE prevented neurodegeneration and expression of markers of apoptosis in limbic brain regions and significantly reduced the damage in limbic regions (Ebert et al., 2002;Brandt et al., 2003a). In the lithium-

pilocarpine model, topiramate decreased cell death in CA1 and CA3 in hippocampus (Rigoulot et al., 2004). Valproate has a powerful neuroprotective effect in the hippocampal formation and the dentate hilus in SE models (Brandt et al., 2006). Vigabatrin protected the hippocampus from brain damage efficiently in Ammon's horn, and to a lesser extent in the hilus following pilocarpine-induced SE (Andre et al., 2001). Carbamazepine reduced damage of the hippocampal formation in pilocarpine-induced SE model (Capella and Lemos, 2002). Treatment with atipamezole started 1 week after the induction of SE and continued for 9 weeks reduced hilar cell damage and mossy fibre sprouting (Pitkänen et al., 2004). The severity of hippocampal cell loss was milder after diazepam treatment in amygdala-kindled rats (Pitkänen et al., 2005).

However, it is still questionable whether neuroprotection during or after brain injury will be able to prevent the process of epileptogenesis. Indeed, a number of studies have concluded that preventing neurodegeneration or neuronal cell loss does not always prevent epileptogenesis (Ebert et al., 2002;Rigoulot et al., 2004).

1.8 Antiepileptogenesis

The definition of an antiepileptogenic compound is one that prevents or modulates the process of developing epilepsy. Several experimental studies have aimed at preventing epileptogenesis in animal models, including status epilepticus and kindling models, by administering antiepileptic drugs. (Table 1.3)

Table 1.3: The summary the effect of antiepileptic drugs on preventing epileptogenesis

Treatment	Model	Mortality	Seizure				Cell Damage	references
			behavioral Severity	duration	frequency	amplitudes		
Atipamezole	Kindling		⇩	NE	⇩		⇩	Pitkanen et al. (2004)
Carbamazepine	Pilocarpine			⇩	⇩		⇩	Capella et al. (2002)
Diazepam	kindling	⇩	⇩	NE	⇩		⇩	Pitkanen et al. (2005)
	Kindling		⇩		⇩	⇩		Brandt et al. (2003)
	Pilocarpine	⇩			⇩	⇩		Rigoulot et al. (2003)
Gabapentin	Kainate				⇩			Cilio et al. (2001)
Levetiracetam	Kindling		⇩	⇩				Husum et al. (2004)
	Kindling		⇩	NE				Gu et al. (2004)
	pilocarpine					⇩	⇩	Klitgaard et al. (2001)
	Kindling		⇩	⇩				Loscher et al. (1998)
Phenobarbital	pentylenetetrazol		⇩					Silva Burm et al. (2000)
Phenytoin	Flurothyl		NE					Applegate et al. (1997)
	Electrical	NE	⇩			NE		Prasad et al. (2002)
Topiramate	pilocarpine	NE			NE	NE	⇩	Rigoulot et.al (2003)
	Electrical	NE	⇩			⇩		Prasad et al. (2002)
Valproate	Pilocarpine					NE	NE	Klitgaard et al. (2001)
	Flurothyl		⇩					Applegate et al. (1997)
Vigabatrin	Pilocarpine	NE	NE			⇧	⇩	Andre et al. (2001)
	Electrical	NE		NE	NE		NE	Halonen et al. (2001)
YM90K	Kindling		⇩	⇩			⇩	Kodama et al, (1999)

1.9 Valproic acid

Valproate (VPA, valproic acid), also referred as di-n-propylacetic acid, is an eight-carbon branched-chain fatty acid with anti-convulsant properties (Figure. 1.5). VPA or more usually its sodium salt is widely used to treat epilepsy, bipolar disorders and migraine.

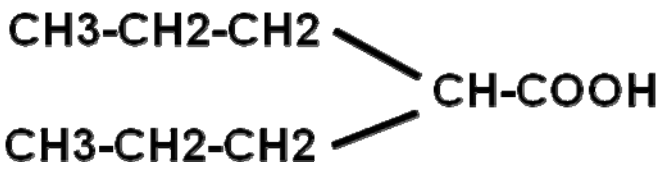


Figure 1.5: The structure of Valproic acid ($C_8H_{16}O_2$). Valproic is a short chain fatty acid with anti-convulsant properties.

VPA, first synthesized by Burton in 1882, was not used clinically for over 80 years. The anti-convulsive action was first described by Pierre Eymard in 1962. In 1967, VPA was first marketed as ‘‘Depakine’’ in France. There are now more than 100 countries in which VPA is used for treating epilepsy, and VPA has established itself as one of the foremost antiepileptic drugs, especially for generalized epilepsies.

VPA also exerts powerful neuroprotective effects and prevents part of the behavioural alterations in *in vivo* and *in vitro* epilepsy models. The effect of VPA on epileptogenesis is still controversial. Chronic treatment with VPA after kainate-induced SE (VPA 600 mg/kg twice daily after kainic acid induced SE and lasted for 40 day, follow by 300 mg/kg twice daily for 10 days) prevented spontaneous recurrent seizures (during the treatment period), deficits in learning in the Morris water maze test, and reduced histological lesions in the hippocampus (Bolanos et al., 1998). Administration of VPA

(400mg/kg) immediately after SE followed by three times daily administration of 200 mg/kg for 4 weeks, however did not prevent the later occurrence of spontaneous seizures but did prevent locomotor hyperactivity and reduced neuronal damage in the hippocampal formation (Brandt et al., 2006). VPA reduced the incidence and intensity of convulsions and prolonged the duration of the latency period. However, VPA (100 mg/kg) did not eliminate epileptiform activity which was induced by metaphit, a proposed phencyclidine receptor activator (Stanojlovic' et al., 2007).

In contrast, there is evidence that VPA has a neuroprotective effect in *in vivo* and *in vitro* models. VPA significantly reduces cell death in hippocampal slices exposed to cytotoxic levels of extracellular ATP (Wilot et al., 2007). VPA (0.07–1.4 mM) prevented apoptotic morphological and biochemical changes, such as cell shrinkage, mitochondria damage, increase of lactate dehydrogenase (LDH) activity, and caspase-3 protein expression, in a model of potassium efflux-induced neuronal death in SH-SY5Y human neuroblastoma cells (Li and El-Mallahk, 2000). VPA (1-2 mM) promoted survival against low-K^+-induced apoptosis in primary cultures of cerebellar granule cells (Mora et al., 1999). VPA (300 mg/kg immediately after ischemia followed by repeated injections every 12 hours) also markedly decreased brain infarct volumes and ischemia-induced neurological deficit scores following cerebral ischemia and suppressed ischemia-induced caspase-3 activation (Ren et al., 2004).

1.9.1 Mechanisms of action

It has been suggested that the acute actions of anti-anticonvulsant effects of VPA involve interference with the GABA system and sodium-channels. VPA (30-100 nM) increases levels of GABA, and enhances GABAergic inhibition (Balding and Geller,

1981). This may result from the inhibition of succinic semialdehyde dehydrogenase (the enzyme responsible for degradation of succinic semialdehyde to succinic acid) and GABA transaminase (the enzyme which catalyses the degradation of GABA to succinic semialdehyde). The inhibition of succinic semialdehyde dehydrogenase increases the level of succinic semialdehyde, which inhibits GABA transaminase and so prevents GABA catabolism (Johannessen, 2000; Löscher, 1999). VPA also modulates voltage-gated sodium channels and voltage dependence of sodium current steady-state inactivation, resulting in reducing cellular excitability and suppressing high-frequency firing of neurons (McLean and Macdonald, 1986;Vreugdenhil and Wadman, 1999;Van den Berg et al., 1993). Moreover, chronic treatment with VPA (0.6 mM) up-regulates cell surface expression of sodium channels via transcription/translation-dependent mechanisms (Yamamoto et al., 1997).

GABA enhancement in brain and in synaptic may only be a secondary action of valproate and the Na^+ channel inhibition is controversial; it has been proposed that other actions form the primary basis for the antiepileptic activity of valproate (Perucca, 2005). Moreover, it is unclear whether these theoretical mechanisms of action contribute to VPA's neuroprotective or antiepileptogenic effect.

1.9.1.2 The action of VPA on cAMP/PKA signalling system

The effects of VPA on AMPA receptors and other work (see below) indicate that PKA-cAMP is a major target for VPA. cAMP, a cyclic nucleotide, serves as a second messenger which is important for diverse physiological effects via cascades of intracellular messengers. The intracellular cAMP level is regulated by the balance between the activities of Adenylyl Cyclase and the cyclic nucleotide Phosphodiesterase.

VPA inhibits forskolin-stimulated cAMP accumulation in intact cells in a concentration-dependent manner. The valproate (1 mM and 2mM) induced inhibition of cAMP accumulation was partially reversed by the phosphodiesterase (PDE) inhibitor isobutylmethyl xanthine (IBMX). This result suggested that the inhibition of forskolin-stimulated cAMP accumulation by VPA was not a direct effect by inhibition of adenylyl cyclase activity, but an effect on cAMP metabolism (Gallagher et al., 2004). Other studies have demonstrated that prolonged exposure of C6 glioma to VPA (0.5 mM) influenced multiple components of the beta-adrenergic receptor-coupled cyclic adenosine monophosphate (cAMP) generating system, including receptor complement, cAMP production, 3H forskolin binding sites, and G protein activation. Chronic treatment with VPA (0.5 mM) significantly attenuated receptor- and postreceptor-stimulated cAMP production. The basal levels of cAMP in cells were reduced by approximately 20% in intact cells and by 35% in cell membranes after prolong incubation in VPA. Also, chronic VPA incubation decreased forskolin- stimulated cAMP production in intact cells by 60% (Chen et al., 1996). This result is supported by further studies. Cortical neurons treated with VPA had decreased cAMP level induced by stimulation of adenylyl cyclase with forskolin and stimulation of β-adrenergic receptors which are linked to activation of adenylyl cyclase with isoproterenol compared to control neurons. An *in vivo* study, using microdialysis to measure extracellular levels of cAMP in the prefrontal cortex of freely moving animals, demonstrated that forskolin-induced cAMP rises were significantly attenuated by VPA treatment. Furthermore, chronic administration of VPA (0.5 mM) decreased the density of β-adrenoreceptors which was reflected by a decrease in isopreterenol-evoked, receptor-mediated cAMP production in the rat prefrontal cortex (Montezinho et al., 2006;Montezinho et al., 2007).

1.9.1.3 VPA's effect on PKA-cAMP can also influence the extracellular signal-regulated kinase (ERK) pathway.

Treatment with VPA not only activates ERK pathway, but also increases BDNF in rat hippocampus. Treatments with VPA (400 mg/dg/day) increase the DNA binding activity of transcription factor polyomavirus enhancer-binding protein (PEBP) and robustly increased the levels of bcl-2 which is transcriptionally regulated by PEBP2 in frontal cortex (Chen et al., 1999). In addition, treatment with VPA (20 gm/kg containing rodent chow for 9 days or 28 days) increases the activation of phospho-ERK44/42. Phosphorylation of ERK44 at Thr202/Tyr204 and ERK42 at Thr183/Tyr185 by MAP/ERK kinase (MEK) corresponds to ERK activation. VPA also increases the level of activation of phospho-ribosomal protein S6 kinase-1 (RSK1) which is activated by phosphorylation of phospho-ERKs. Phospho-RSKs phosphorylate Bcl-2 antagonist of cell death (BAD) at Ser112, and cAMP response element-binding protein (CREB) at Ser 133, increasing CREB transcriptional activity (Einat et al., 2003).

It has been shown that VPA enhances cellular functions induced by ERK activation, such as neurogenesis, neurite growth and neuronal survival. VPA (20 gm/kg containing rodent chow) enhances ERK pathway-dependent cortical neuronal growth and promotes neural stem cell proliferation-maturation (neurogenesis), and hugely increased the number of BrdU-positive cells in the dentate gyrus (Hao et al., 2004). Using a biomedical model system Dictyostelium discoideum, VPA caused a transient increase in the activation of the MAPK signalling pathway, as shown by ERK2 phosphorylation. In Dictyostelium, the enhancement of intracellular cAMP by binding to cAR1 modulates ERK2 phosphorylation. Activation of PKA via stimulation by cAMP reduced ERK2 phosphorylation. PKA blocks upstream (MEKK)-dependent ERK2 phosphorylation. Increased pERK2 levels are also present following VPA (1mM) treatment, suggesting a

role for VPA in the up-regulation of MEKK activity through the inhibition of the PKA signalling pathway. Therefore, it has been suggested that the rise in pERK2 level result from VPA attenuation of cAMP-dependent signalling (Figure 1.6) (Boeckeler et al., 2006). Other evidence showed that VPA (1mM)-induced activation of ERK was blocked by the mitogen-activated protein kinase/ERK kinase inhibitor PD098059. VPA increased the expression of genes regulated by the ERK pathway, such as growth cone-associated protein 43 and Bcl-2, promoted neurite growth and cell survival, and enhanced norepinephrine uptake and release (Yuan et al., 2001).

1.9.1.4 VPA's effect on PKA-cAMP can also influence the AMPA receptor

There is also evidence that VPA can interfere with AMPA receptor trafficking. Modification of the levels of synaptic AMPA receptors by receptor subunit trafficking, insertion, and internalization, is important for regulating postsynaptic responses, learning, and various forms of neural and behavioural plasticity (Malinow and Malenka, 2002;Rumpel et al., 2005;Hayashi et al., 2000;Jeon et al., 2006).

AMPA receptor subunit GluR1 trafficking is regulated by protein kinase A (PKA), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and protein kinase C (PKC) via phosphorylation of specific sites on GluR1 (Lee et al., 1998;Malinow and Malenka, 2002;Esteban et al., 2003;Blackstone et al., 1994;Roche et al., 1996;Mammen et al., 1997;Glade-McCulloh et al., 1993). Phosphorylation of the receptor subunits regulation is important for intrinsic channel properties of the receptor and the interaction of the receptor with associated proteins, modulating the membrane trafficking and synaptic targeting of the receptors (Malinow and Malenka, 2002).

Chronic treatment with VPA (1mM) reduced surface expression of GluR1 and GluR2. Phosphorylation of GluR1 at the PKA (cAMP-dependent protein kinase) site (s845) which is critical for AMPA receptor insertion is reduced in VPA-treated neurons. In addition, GluR1 phosphorylation was attenuated in hippocampus VPA-treated animals *in vivo*. These results suggest that VPA down-regulates GluR1/2 by inhibiting phosphorylation of GluR1 at its PKA site as a drug-specific "chronic mechanism". (Vandenberghe et al., 2005;Du et al., 2008;Du et al., 2006;Du et al., 2004).

1.9.2 The side effect of VPA

Even though, VPA is widely used in clinical as treatment in epilepsy (complex partial and absence seizures) schizoaffective disorders (Puzyn'ski and Kłosiewicz, 1984), mania (Freeman et al., 1992) and migraine prophylaxis (Yurekli et al., 2008), using VPA has potentially serious side effects. VPA is a potent teratogen that most notably induces neural tube defects (NTDs) in human, mouse, and other vertebrate embryos. Exposure of the human embryo to VPA is also associated with risk of autism which is a brain development disorder characterized by impaired social interaction and communication, and by restricted and repetitive behaviour (Arndt et al., 2005) and at risk for significantly lower IQs (Meador et al., 2009). The other serious side effect of VPA therapy is hepatotoxicity (Powell-Jackson et al., 1984) and blood dyscrasia resulting in thrombocytopenia, and prolonged coagulation times. VPA may also cause acute hematological toxicities, especially in children, including rare reports of myelodysplasia and acute leukemia-like syndrome (Williams et al., 2008;Coyle et al., 2005). Long-term treatment with VPA cause cognitive and motor dysfunction, for example Parkinsonian symptoms (Armon et al., 1996), and even pseudoatrophic brain changes which is reversible shrinkage of the brain (McLachlan, 1987)

1. 10 Summary

VPA exerts a powerful neuroprotective effect in a variety of animal models including *in vivo* status epilepticus models (Bolanos et al., 1998;Brandt et al., 2003).One mechanism underlying VPA's neuroprotective effect has been suggested to be attenuation of protein kinase A (PKA) activity, which results in an increase in an increase in the activated (phosphorylated) form of ERK2 (Boeckeler et al., 2006). It is, however, unclear whether VPA directly affects Protein kinase A or indirectly by inhibiting the formation (or increasing the breakdown) of cyclic adenosine monophosphate (cAMP) (Chen et al., 1996;Gallagher et al., 2004). In support of this, an *in vivo* microdialysis study demonstrated that increases in cAMP levels in the prefrontal cortex of freely moving animals induced by an infusion of the adenylyl cyclase activator forskolin were attenuated by treatment with VPA (Montezinho et al., 2006;Montezinho et al., 2007)

Modulation of the cAMP-PKA pathway by VPA has other implications. VPA's antimanic effect has been suggested to be secondary to attenuation of the surface and synaptic expression of AMPA receptors through a decrease in AMPA receptor subunit GluR1 phosphorylation at a specific PKA site (Du et al., 2004). Moreover, the cAMP-PKA pathway is critical for the expression of NMDA receptor-independent long term potentiation (LTP) at the mossy fibre to CA3 pyramidal cell synapse in the hippocampus (Huang et al., 1994;Weisskopf et al., 1994;Nicoll and Malenka, 1995), and has been suggested to be involved in Schaffer collateral-CA1 LTP. Therefore inhibition of this pathway could impact upon LTP at these synapses. Moreover this pathway has also been proposed to play an important role in hyperexcitability and epileptogenesis. PKA activity increases in the neocortex and hippocampus of rats during epileptogenesis (Yechikhov et al., 2001). Activation of the cAMP-PKA pathway increases excitability and enhances epileptiform activity in rat hippocampal slices and human dentate gyrus

cells (Boulton et al., 1993;Higashima et al., 2002;Chen and Roper, 2003), and, *in vivo*, results in a potentiation of epileptic activity and status epilepticus (Üre and Altrup, 2006). Therefore, the question was asked whether VPA through an action on PKA activity can modify synaptic plasticity and whether such a mechanism of action can explain its antiepileptic effect.

Although VPA is used extensively for treating various kinds of epilepsy, the use of VPA is, however, limited by severe adverse effects. Its efficacy in epilepsy treatment and serious side effects associated with its therapy, provide an excellent incentive for developing a second-generation VPA which would possess broad-spectrum antiepileptic activity, better potency than VPA, and would lack side effects such as the teratogenicity and hepatotoxicity. 4-Methyloctanoic acid is, similar to valproate, a branch chain fatty acid, but in contrast to valproate it lacks any teratrogenic effect in animal models (Ambroso et al., 1999;Narotsky et al., 1994) and has a long safety histroy as a flavouring in foods. We therefore tested this compound in in vitro and in vivo animal models. If it is in efffective, the plan was to test further similarly structurered compounds.

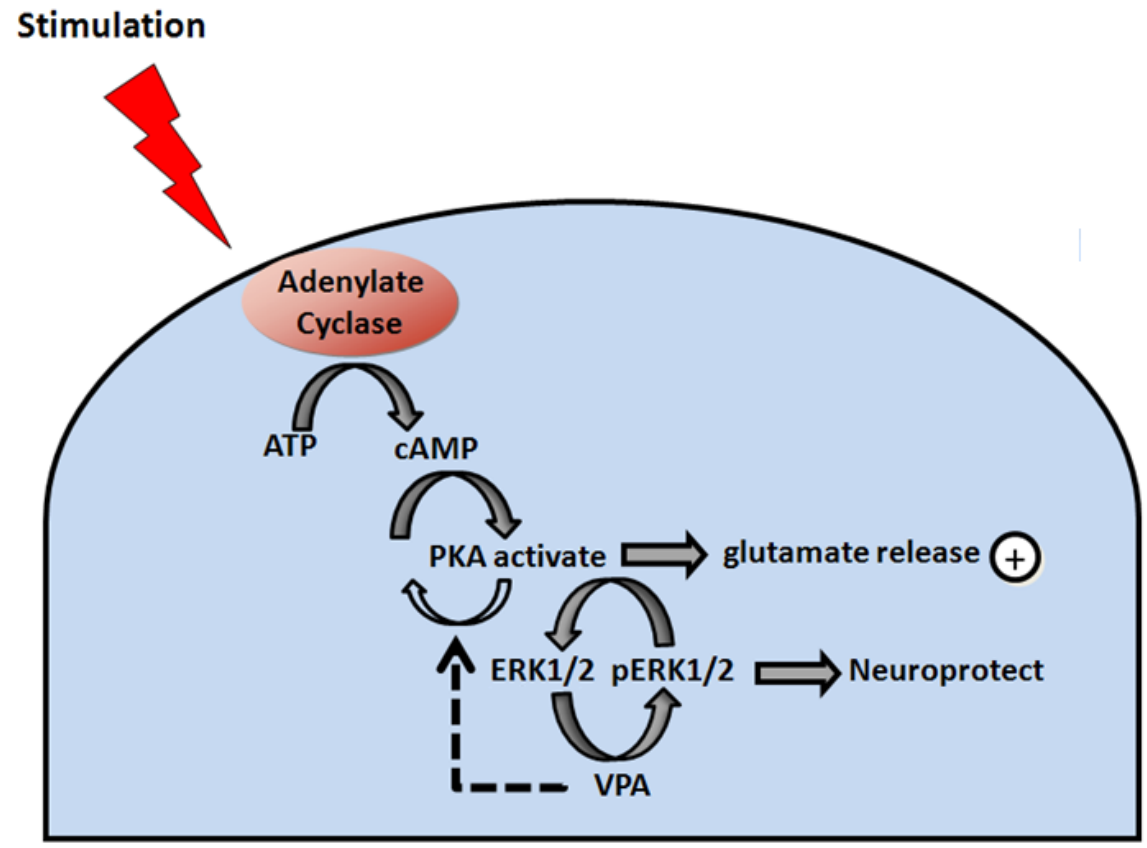


Figure 1.6: VPA regulates the mitogen-activated protein kinase pathway which may be through modulation of cAMP/PKA signalling. Activation of PKA via increase of cAMP leads to inhibit the activation of ERK1/2 which contributes to neuroprotective effect. VPA, however, increase the activities form pERK 1/2, and this may be through the inhibition the activity of PKA.

1.11 Aims

1. To test the effect of VPA on PKA dependent long-term potentiation at the mossy fibre to CA3 synapse.

2. To test whether VPA has a direct effect on PKA activity or an indirect effect through cAMP modulation.

3. To test whether the effect of VPA on PKA/cAMP signalling system can explain the anticonvulsant effect of VPA.

4. To test whether the effect of VPA differs between epileptic and non-epileptic animals.

5. To determine whether a VPA analogue that has less teratogenic effect has a similar physiological and anticonvulsant profile to VPA

Chapter 2: General materials and methods

2.1 Introduction

In this thesis, various epilepsy models were used. The choice of the appropriate model, either *in vitro* model or *in vivo* model, depends on the question being asked. Using *in vitro* models allows greater control of the physiological environment, drug concentrations, and minimizes contamination by other drug such as anesthetic drugs that are necessary in an anesthetized *in vivo* preparation. Further, it is possible to selectively block specific pathways in a controlled fashion, enabling a better dissection of mechanisms of action. *In vitro* models are designed to generate fast, initial and reproducible data that will give general insight as to the action of drug that may be expected in *in vivo*. However, *in vitro* models lack the complexity of the *in vivo* models and by definition can only reproduce epileptiform activity not seizures (which are clinically discernible events). Acute *in vivo* seizure models have provided the evidence of efficacy for most of our presently used antiepileptic drugs (White, 1997). Using *in vivo* animal models allow for studying the progress of disease and collecting more clinically relevant data that is able to provide greater insight into the human disease. Acute models, are advantageous in that it is possible to induce a seizure and so know when the seizure will occur (this is of benefit when testing drug effects). However, it does not reproduce faithfully the human condition in which spontaneous seizures occur; for this chronic epilepsy models are required. For chronic models of epilepsy with pathological findings similar to human such as neuronal loss, glial activation and gliosis in epileptic tissue, and mossy fiber sprouting, it is useful to study the development of epilepsy from the initial insult to the brain to the occurrence of spontaneous seizures. (Löscher, 2002;Cole and Dichter, 2002). In addition, chronic epilepsy animal models are useful for identifying and characterizing the cellular and molecular mechanisms of the progressive consequences of epilepsy, which may present targets for therapeutic interventions (Cole and Dichter, 2002).

2.2 *In vivo* experiments

2.2.1 Animals

Male Sprague–Dawley rats, weighing between 250 g to 330 g, were used. Rats were kept under controlled environmental conditions (24–25 °C; 50–60% humidity; 12 h light/dark cycle) with free access to food and tap water. All experiments followed the Home Office regulations under the Animal (Scientific Procedures) Act, 1986.

2.2.2 Anesthesia

Male Sprague–Dawley rats were placed in a plastic chamber with 4% isoflurance in O_2 (isoflurance was purchased from Merial Animal Health Ltd. Harlow, Essex, UK). The rats were moved from the chamber to the stereotaxic frame (David Kopf stereotaxic frame) after a surgical plane of anaesthesia was achieved, which was determined by lack of movement and withdrawal to foot or tail pinch. The plane of anaesthesia was maintained with 2% isoflurance in O_2 (at a flow rate of 2 litres /minutes) during surgery.

2.2.3 Use of stereotaxic frame for rats

Once anaesthetised, the hair on the rat's head was clipped and the rats were then moved to a stereotaxic frame. The rat's incisors were placed over a tooth bar and the non-traumatic ear bars were placed such that the head was fixed and did not move with gentle downwards pressure. The height of the tooth bar was then adjusted so that the top of the skull was horizontal.

2.2.4 The electrical stimulation model

2.2.4.4 Electrode preparation

The recording electrodes were prepared from intracranial Electrode (25 mm, Plastics One E363/1) by stripping the end insulation for < 0.5 mm using scalpel blade.

The stimulation electrodes were constructed by twisting 2 intracranial Electrode (25 mm, Plastics One E363/2). The 2 stainless wires were separated vertically by 0.5 mm. and the insulation was removed at the electrode tips by using a scalpel blade.

A silver wire (about 4 cm), soldered to a female socket contact (Plastics One E363/0) was used as reference and earth electrode.

2.2.4.2 Electrode implantation

Three scull screws (plastics one) were placed in scull before implantation in order to anchor the headset, two anterior to bregma and one posterior to bregma (Figure 2.1). The skull screws were also used for securing the silver wire served as earth electrode which was wound around skull screws for approximately 2 cm, and was placed subcutaneously. The earth was additionally used as the indifferent reference electrode.

The recording electrode was stereotaxically implanted into the dentate gyrus (coordinates: lateral 2.5 mm from midline, antero-posterior -4 mm from bregma, dorso-ventral -3 mm from brain surface). Bipolar stimulation electrodes were implanted in the perforant pathway (coordinates: lateral 4.4 mm from midline, antero-posterior -8.1 mm from bregma, dorso-ventral -4 mm from brain surface) (Figure 2.1). These stereotaxic coordinates relative to bregma were based on the atlas of Paxinos and Watson (1998).

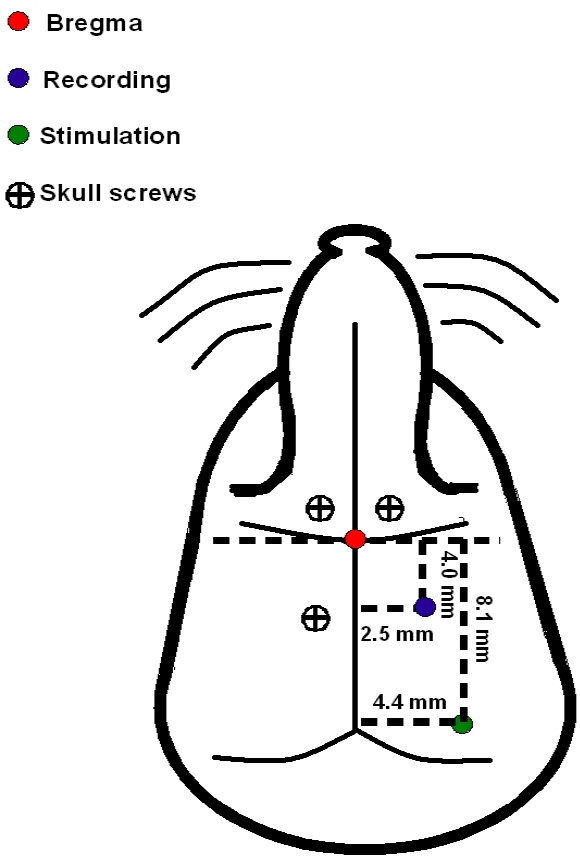


Figure 2.1: Schematic diagram of the skull with position of recording electrode, stimulation electrode and anchor screws. The recording electrode (blue) was stereotaxically implanted into the dentate gyrus (coordinates: lateral 2.5 mm from midline, antero-posterior -4 mm from bregma, dorso-ventral -3 mm from brain surface). Bipolar stimulation electrodes (Green) were implanted in the perforant pathway (coordinates: lateral 4.4 mm from midline, antero-posterior -8.1 mm from bregma, dorso-ventral -4 mm from brain surface). The three scull screws (⊕) were placed in scull before implantation in order to anchor the headset, two anterior to bregma (red) and one posterior to bregma.

Test stimulations were delivered every 10 second with 4-5 mV, 50 μs monopolar pulses via a Neurolog stimulator. The depth of electrodes was adjusted to find the maximal population spike amplitude of the field potential (in the range 5-10mV)

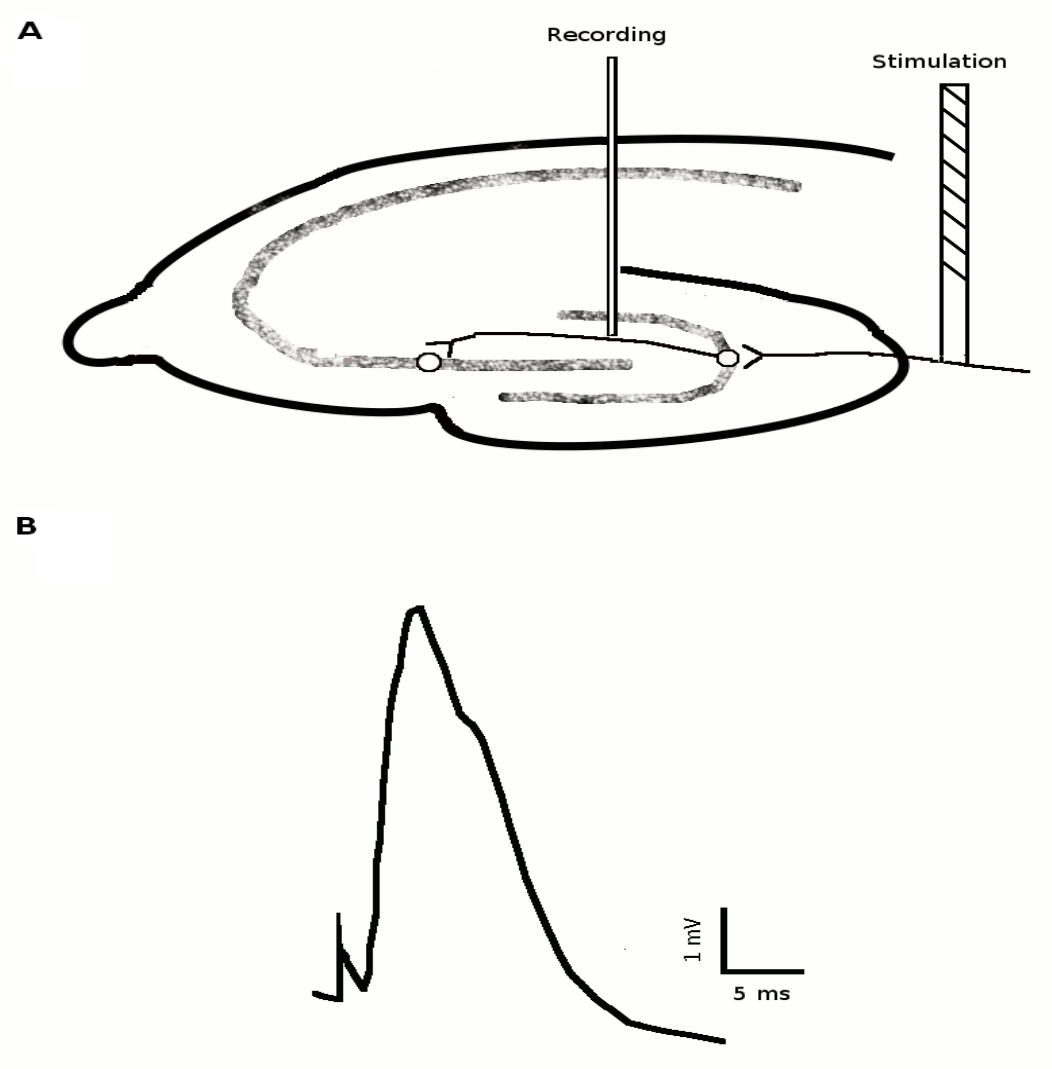


Figure 2.2: Stimulation of the perforant pathway. (A) *The bipolar stimulation was place in perforant pathway and the recording electrode was placed into the dentate gyrus.* (B) *The population spike in DG was elicited by stimulation in perforant pathway.*

Once electrode positions were optimized, the entire headset was fixed and anchored using dental acrylic cement (Kemdent, Swindon, UK). The animals were allowed to recover for a period of 7 days after surgery

2.2.4.3 Electrical induction of self-sustained status epilepticus (SSSE)

Seven days after electrode implantation, the rats were electrically stimulated via the perforant pathway with 4-5 mA, 50 μs monopolar pluses at 20 Hz for 2 hours to induce SSSE (figure 2.3). For the stimulation, the trains was generated by a pulse generator (NL310, Digitimer, Herts) and delivered via stimulus isolator (NL800 Digitimer; Herts). During the experiment, the EEG was recorded during stimulation and for up to 3 hours afterwards, using a neurolog system (Digitimer; Herts), consisting of a NL101 headstage connected to a NL104 amplifier and a NL125 filter unit (for settings see figure 2.4), the signal was digitalized using CED micro1410 (Cambridge Electronic design, UK) and stored using "Spike 2". The electrophysiological data were saved on a laptop computer for post-hoc analysis.

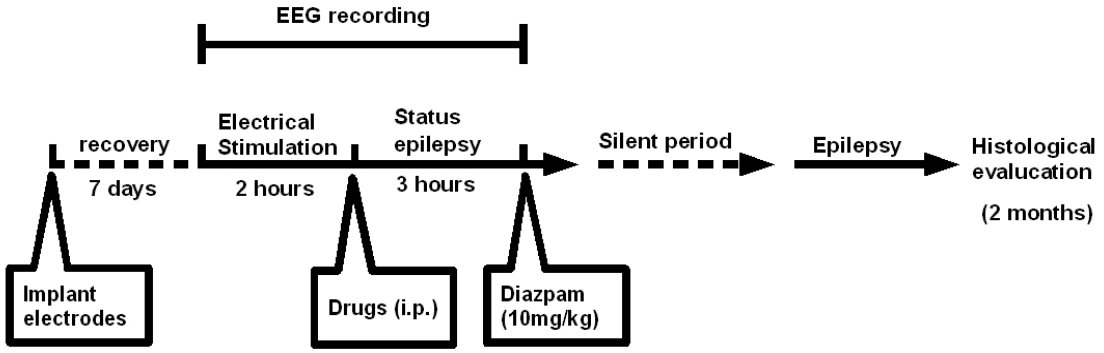


Figure 2.3: Summary of the procedure - electrical induction of self-sustained status epilepticus. Rats were electrically stimulated via the perforant pathway with 4-5 mA, 50 μs monopolar pluses at 20 Hz for 2 hours to induce self-sustained-status epilepticus (SSSE) seven days after electrode implantation. Three hours after the induction of the SSSE, the rats were given diazepam (10 mg/kg) by intraperitoneal injection to terminate the seizure activity. Two months after induction of status epilepticus in rat, the brain was moved and the histopathological examination was performed

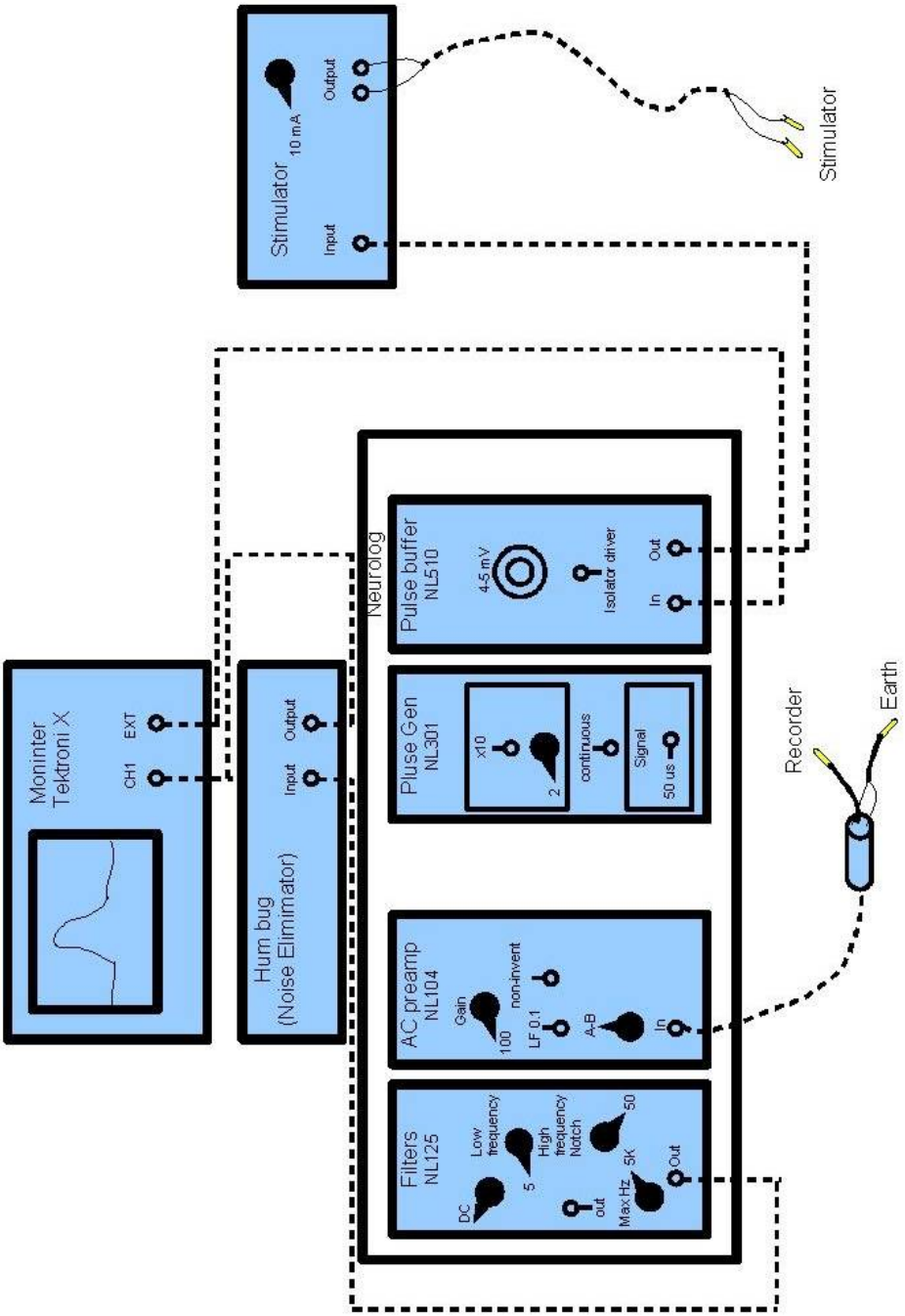


Figure 2.4: Summary of the settings for electrical induction of self-sustained status epilepticus. During experiment, the stimulation was generated by a pulse generator (NL310, Digitimer, Herts) and delivered via stimulus isolator (NL800 Digitimer; Herts). The electroencephalography (EEG) was recorded using a neurolog system (Digitimer; Herts). The signal was digitalized using CED micro1410 (Cambridge Electronic design, UK) and stored using "Spike 2". The electrophysiological data were saved on a laptop computer.

Behavioural seizures were monitored every 10 minutes and classified using the Racine scale (Racine, 1972) (Table 2.1)

Table 2.1: Behavioural rating of induction of a self-sustained status epilepticus based on Racine scale (1972)

stage 1	Mouth and facial movements
stage 2	Head nodding and more serve facial and mouth movements (jaw-opening)
stage 3	Forelimb clonus
stage 4	Rearing and bilateral forelimb clonuses
stage 5	Rearing and falling, with loss of postural control, full motor seizure.

Three hours after the induction of the SSSE, the rats were given diazepam (10 mg/kg) (Hameln Pharmaceuticals Ltd. Gloucester, UK) by intraperitoneal injection to terminate the seizure activity. If necessary, this dose of diazepam was repeated until motor and EEG seizure activity ceased in all rats. Rats developed SSSE after stimulating perforant pathway. Two months after induction of status epilepticus in rats, the brain was moved and was analysed histologically, in order to verify the location of the recording and stimulation electrodes, and to perform histopathological examination for the effect of drugs (VPA and 4-methyloctanoic acid) on neurodegeneration caused by status epilepticus.

2.2.5 Pilocarpine model

Sustained seizures were induced in experimental animals by the administration of pilocarpine (320 mg/kg, ip) (Sigma), a muscarinic cholinergic agonist. Twenty minutes before and after pilocarpine administration, animals were injected with a low dose of

the cholinergic antagonist Scopolamine methyl nitrate (1 mg/kg, ip) (Sigma) to reduce peripheral muscarinic effect and lower mortality rate. Diazepam (10 mg/kg, ip) was administered to the animals 90 minutes after the administration of pilocarpine to terminate behavioural seizures.

After the pilocarpine injection, all animals were assessed for severity of behavioural seizures. Excessive salvation and seizures were classified based on Racine scale (see Table 2.1). Only the occurrence of behavioural seizures reaching at least Racine stage 3 was considered as successful induction of SE. After SE, all animals were injected with 1-2 ml normal saline subcutaneously and given rodent food soaked with water for 24 hours. The animals experienced SE were kept for two weeks, prior to *in vitro* electrophysiological experiments (Figure 2.5).

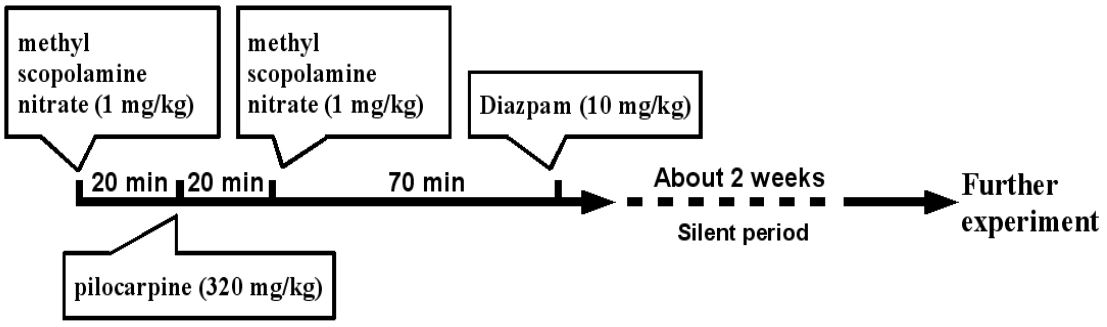


Figure: 2.5: Summary of the procedure of the pilocarpine model. Application of pilocarpine (320 mg/kg) induces sustained seizures in experimental animals. Twenty minutes before and after pilocarpine administration, animals were injected with scopolamine methyl nitrate (1 mg/kg) to reduce peripheral muscarinic effect and lower mortality rate. Diazepam (10 mg/kg) was administered to the animals 90 minutes after the administration of pilocarpine to terminate behavioural seizures. The in vitro electrophysiological experiment was carried out 2 weeks after induction of status epilepticus.

2.2.6 Brain Histology

2.2.6.1 Brain fixation

Rats were killed with an overdose pentobarbital sodium (500mg/kg, ip) (Fort dodge Animal Health, Southampton, UK). After injection, the skull was opened and the brain removed by severing it from the upper cervical cord. The brain was immediately immersed in 4% paraformaldehyde (Sigma) in 0.1 M phosphate buffer (pH 7.4) (Sigma) for over 24 hours, prior to transfer to phosphate buffer solution with 30% sucrose in 0.1 M phosphate buffer (pH 7.4) as a post-fixation solution.

2.2.6.2 Sliced preparation:

Brain sections (40-μm thick thickness) were cut using a vibroslicer (Leica VT 1000S Microsystems, Wetzlar, Germany). The far rostral and caudal regions of brain were removed. The caudal side was glued on to the centre of the rotating specimen holder (figure 2.7). An agar block (4% or 7% agar, Sigma) was glued adjacent to the brain, to support the brain tissue during slicing. The brain and holder were transferred to a tray filled with phosphate buffer. A slow speed and high frequency (0.12 mm/s and 90 Hz, respectively) for the blade were used to obtain brain slices. Tissue sections were carefully transferred to a tray filled with phosphate buffer. They were further transferred onto microscope slides (SuperForst®Plus, VWR international).

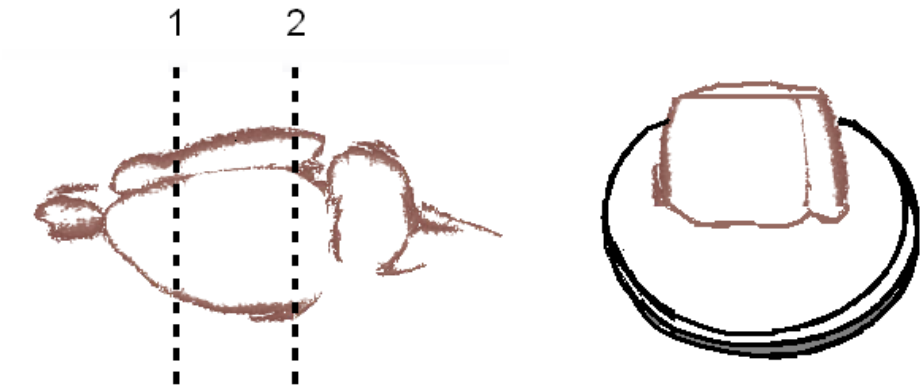


Figure 2.6: *The preparation of brain slices. Whole brain was glued to the centre of the rotating specimen holder. An agar block is fixed adjacent to the brain to support for the tissue during slicing.*

2.2.6.3 Nissl stain

Nissl stain was use for checking the position of electrodes and examining the degree of neurodegeneration after inducing status epilepticus by electrical stimulation. First, sections were hydrated in xylene, 100% alcohol, and 95% alcohol, 70% alcohol and rinsed in water. The sections then were stained in warmed cresyl violet solution (37-50 ºC) in order to improve penetration and enhance even staining. These were rinsed quickly in distilled water. The sections were dehydrated in 70 % alcohol, 95% alcohol, and 100% alcohol and washed with xylene. Finally, they were mounted with permanent mounting medium (DPX mountant, VWR International) (procedure see table 2.2). Nissl stain stains nuclei blue and the nissl bodies violet; the background is colourless under microscope (Alcohol was purchased from Merck, Xylene was purchased from VWR internal, and Cresyl violet was purchased from Sigma).

Table 2.2: Procedure for nissl stains

procedure	Time (minutes)
Xylene	5
Xylene	5
100% alcohol	5
100% alcohol	5
95% alcohol	5
95% alcohol	5
70% alcohol	5
Water	Drip
Cresyl violet solution	15-30
Water	3-5
70% alcohol	3-5
95% alcohol	3-5
100% alcohol	3-5
100% alcohol	3-5
Xylene	5
Xylene	5
DPX moountant	mounted

2.3 *In Vitro* experiments

2.3.1 Slice preparation

2.3.1.1 Procedure

1. Brain preparation

Rats were decapitated after killing by intraperitoneal injection with an overdose of pentobarbitone (500 mg/kg). The whole brain was removed immediately after the skull was opened, and placed in oxygenated ice-cold sucrose solution (NaCl 87 mM, KCl 2.5 mM, $MgCl_2$ 7 mM, $CaCl_2$ 0.5 mM, NaH_2PO_4 1.25 mM, sucrose 75mM and glucose 3 mM) (All compounds required for ice-cold sucrose solution were purchased from Sigma).

2. Separating the brain/hippocampus:

Isolated-hippocampus preparation: Isolated-hippocampus preparation: The cerebellum and hindbrain were removed from the whole brain. The cortex was then

separated from the hippocampi. The hippocampi were each straightened and placed length-wise into grooves of a previously prepared agar block. The agar block with two hippocampi was glued to the centre of the rotating specimen holder (As figure 2.8). This was then rapidly transferred into the buffer tray filled with oxygenated ice-cold sucrose solution (In mM: NaCl 87, KCl 2.5, $MgCl_2$ 7, $CaCl_2$ 0.5, NaH_2PO_4 1.25, sucrose 75, glucose 3).

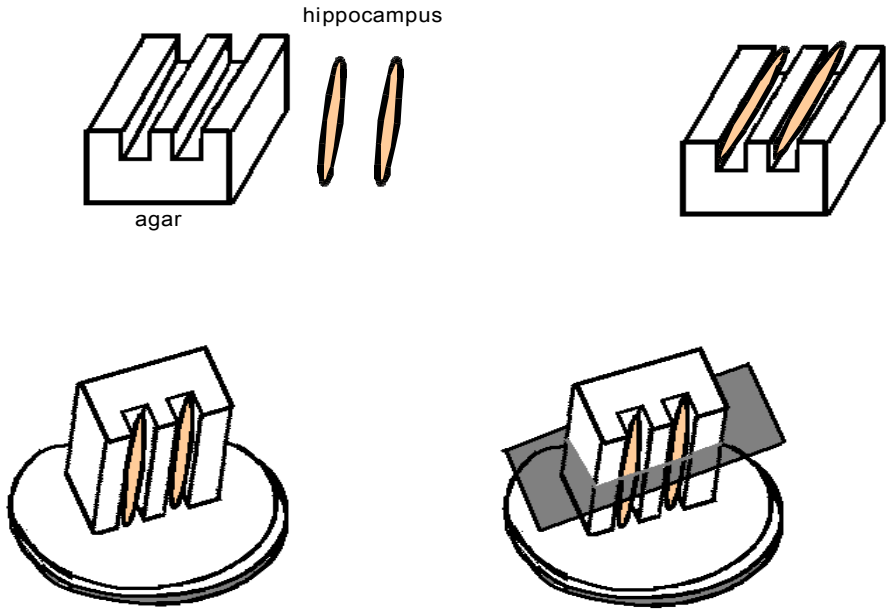


Figure 2.7: The preparation of isolated-hippocampus. The cortex was separated from the hippocampi after cerebellum and hindbrain were removed from the whole brain. The hippocampi were each straightened and placed length-wise into grooves of agar block. The agar block with two hippocampi was glued to the centre of the rotating specimen holder.

Combined horizontal entorhinal cortex-hippocampus slices: For this preparation, the whole brain was sectioned as in figure 2.9 and glued to the centre of the rotating specimen holder. An agar block (4% or 7%) is fixed adjacent to the brain. This provided support for the tissue during slicing.

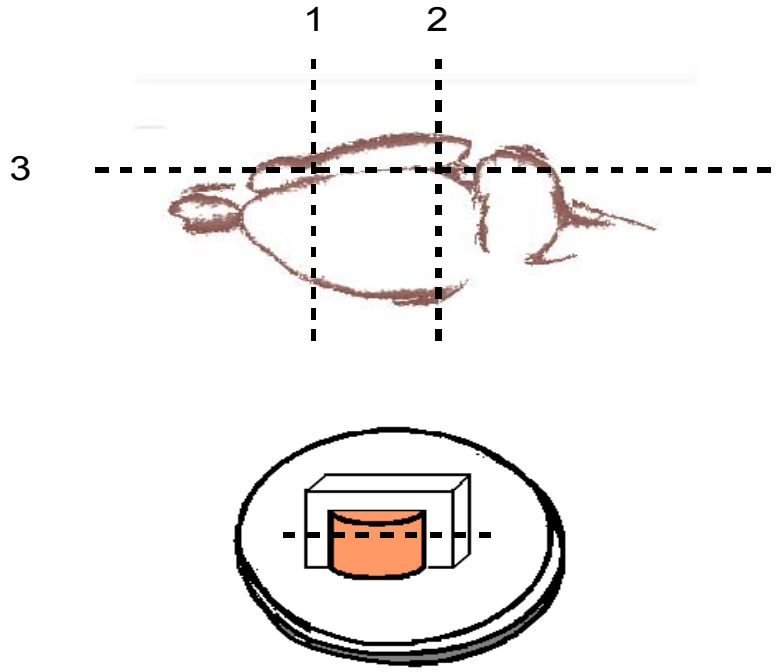


Figure 2.8: The preparation of combined horizontal entorhinal cortex-hippocampus slices. Whole brain was sectioned in order to remove cerebellum and hindbrain and glued to the centre of the rotating specimen holder. An agar block is fixed adjacent to the brain to support the tissue during slicing.

3. Slicing

During slicing, a slow speed and high frequency was used. The frequency was set between 0.12 to 0.16 mm/sec speed and the frequency of blade oscillation from 60 to 90 Hz. 350-400 μm slices were cut depending on experiment. Cut slices were transferred to a Petri dish filled with oxygenated ice-cold sucrose solution.

4. Storage hippocampal slices

Slices, then, were collected and placed in an interface chamber contained aCSF (In mM: NaCl 119, KCl 2.5, $MgSO_4$ 1.3, $CaCl_2$ 5, NaH_2PO_4 1, glucose 16.6) (All compounds required for aCSF were purchased from Sigma) over one hour before transfer to submersion recording chamber (Figure 2.10). The aCSF solution was gassed with 95% O_2 mixed with 5% CO_2.

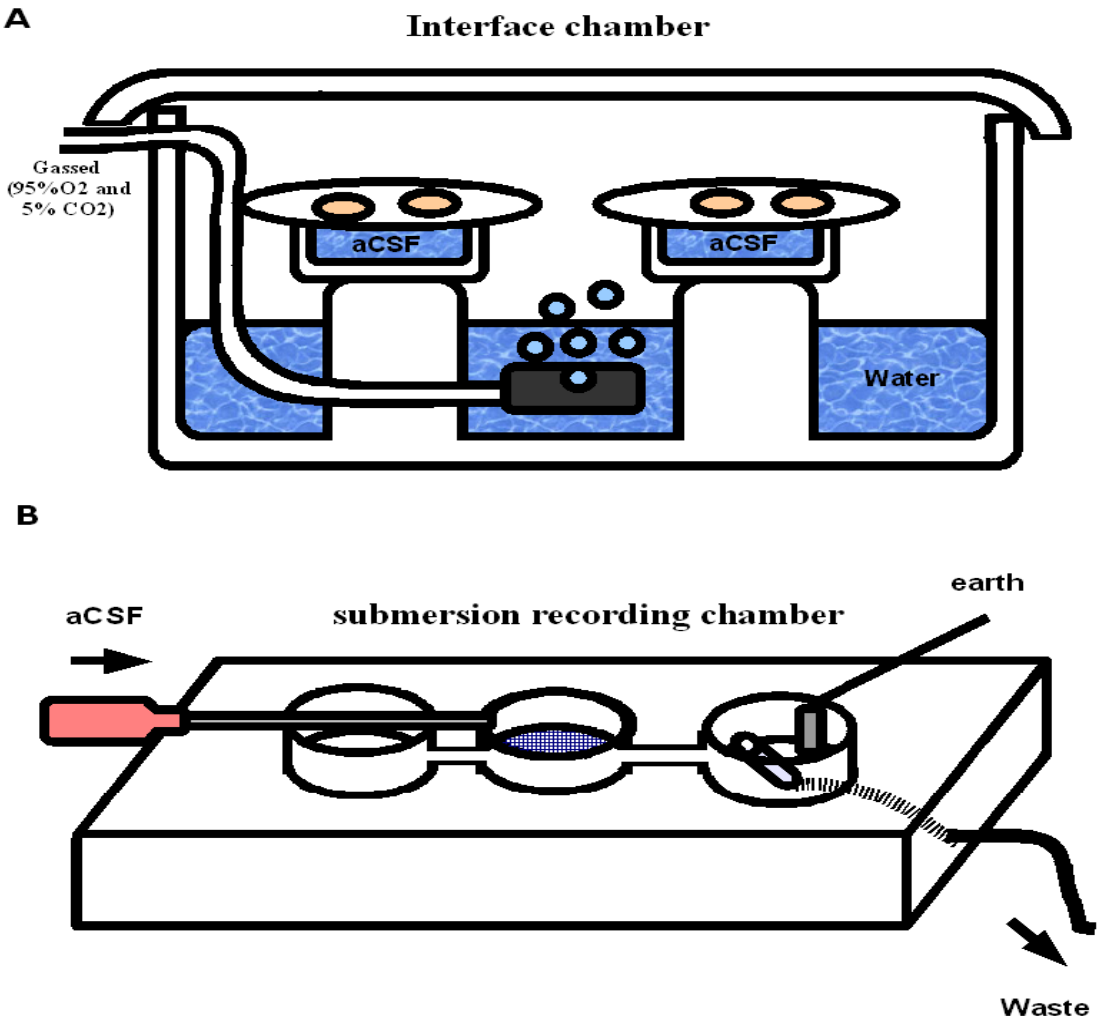


Figure 2.9: The illustration of types of chamber used in in vitro experiment. (A) *The interface chamber was used for storage hippocampus slices.* (B) *The submersion recording chamber was used during recording.*

2.3.1.2 Electrophysiology

Extracellular recordings give information about the synchronous activity of a population of neurons (field potentials) which reveal the activity of many neurons at the same time (Suter et al., 1999). The typical field excitatory postsynaptic potentials (fEPSPs) waveform includes stimulus artifact, fibre volley which represents the action potentials firing in presynaptic fibres and the summation of post-synaptic potentials (Figure 2.10)

Field excitatory postsynaptic potentials (fEPSPs) were recorded with an axonpatch-1D amplifier connected to a Hum Bug Eliminator (Digitimer), which effectively eliminates 50/60Hz noise and harmonics without altering the frequency characteristics of the input signal. The output was connected via a National Instruments A/D board to a computer running Labview software and stored for offline analysis. For stimulation I used a Digitimer constant current isolated stimulator (model DS3) connected to the National Instrument board output. (For settings see Figure 2.11).

To record fEPSPs, a hippocampal slice was placed into a submersion recording chamber (Figure 2.10). The slice was gently immobilised by a platinum wire with attached nylon thread. Fresh carbogenated aCSF was perfused through the submersion recording chamber at > 2ml/min.

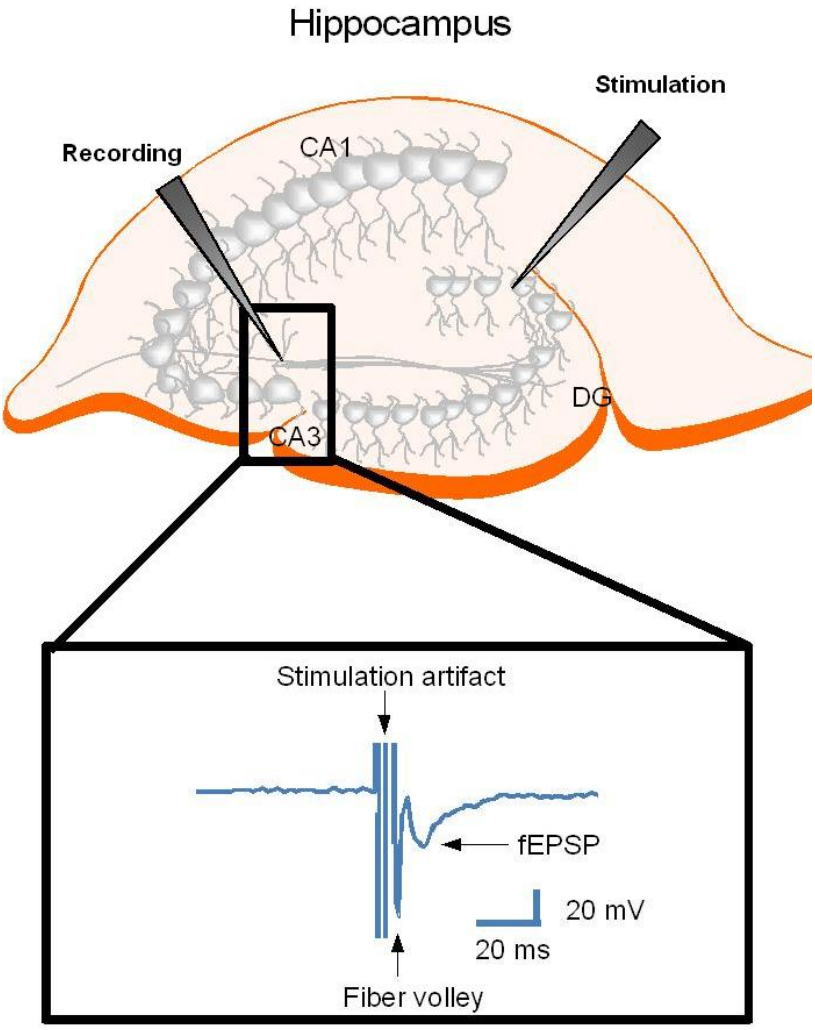


Figure 2.10: Illustration of typical field excitatory postsynaptic potentials (fEPSPs) recorded in mossy fiber synapse into CA3 region. The waveform includes stimulus artifact, fibre volley which represents the action potentials firing in presynaptic fibres and fEPSP

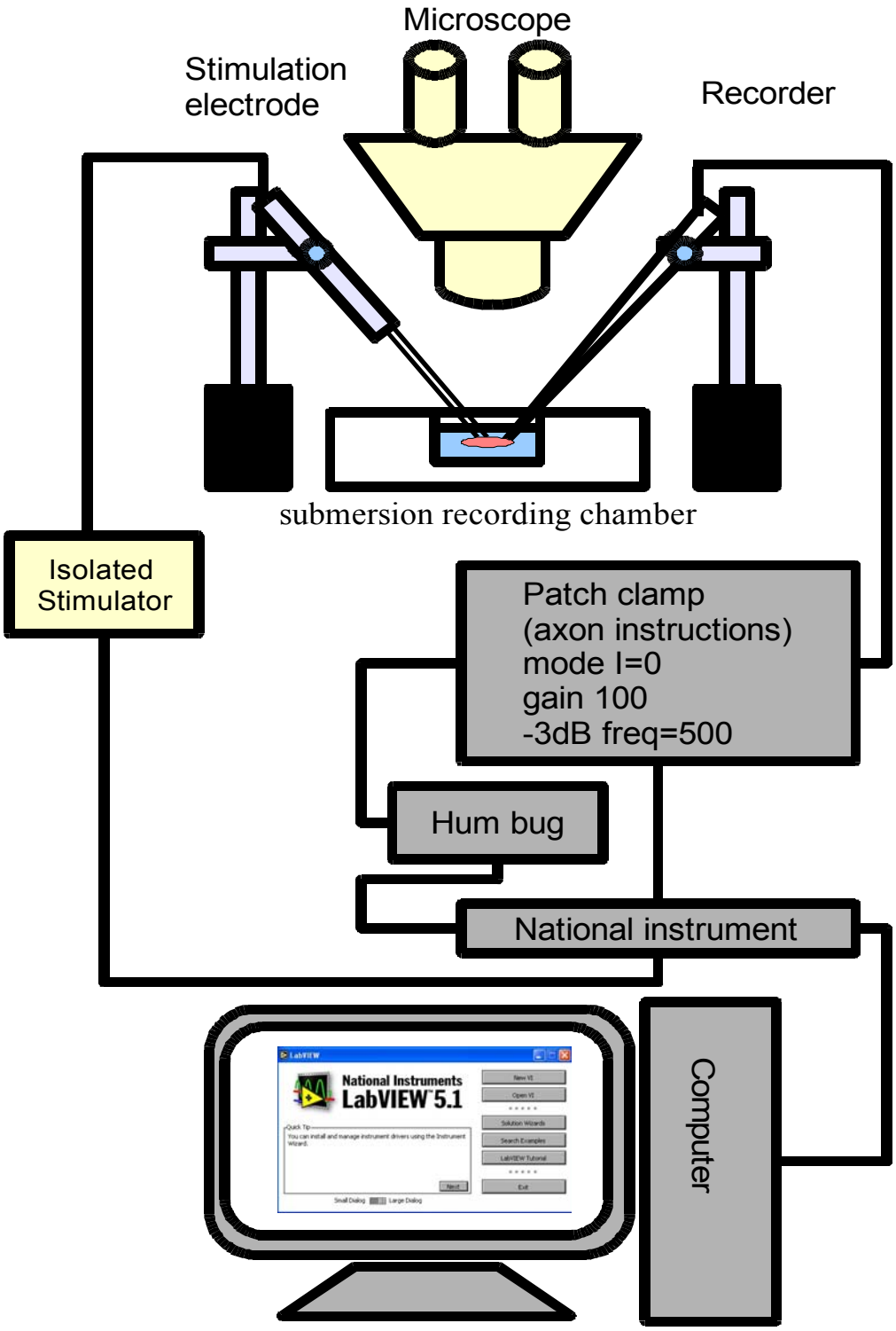
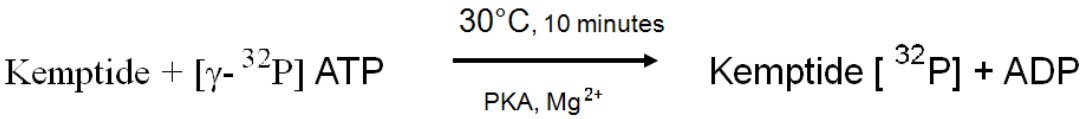


Figure 2.11: The settings for electrophysiological experiment. To record Field excitatory postsynaptic potentials (fEPSPs), a hippocampal slice was placed into a submersion recording chamber. fEPSPs were recorded with an axonpatch-1D amplifier connected to a Hum Bug Eliminator (Digitimer). The output was connected via a National Instruments A/D board to a computer running Labview software and stored for offline analysis. Digitimer constant current isolated stimulator (model DS3) which is connected to the National Instrument board was used to deliverer stimulation.

2.4 PKA assay method

This assay is designed to measure the phosphotransferase activity of PKA (cAMP-dependent Protein Kinase) by phosphorylation of a specific substrate (kemptide) using the transfer of the γ-phosphate of adenosine-5'-[^{32}P] triphosphate ([γ-^{32}P] ATP).



The phosphorylated substrate is separated from the residual [γ-^{32}P] ATP using P81 phosphocellulose paper and quantitated by using a scintillation counter. Phosphocellulose paper is able to bind phosphorylate and phosphorylated peptide quantitatively, whereas untreated [γ-^{32}P] ATP and free [^{32}] P phosphate are washed away by dilute phosphoric acid.

In brief, the assay solution consisted of 20 mM 3-(N-morpholino) propanesulfonic acid (MOPS), pH 7.2, 25 mM β-glycerophosphate, 5 mM ethylene glycol tetraacetic acid (EGTA), 1 mM sodium orthovanadate and 1 mM dithiothreitol, cAMP, Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly), ATP-mix (1 part of gamma -^{32}P ATP with 9 parts of cold ATP) and Bovine heart PKA (The PKA analysis kit, including emptide, cAMP, PKC/CaMK inhibitor cocktail, PKA inhibitor Peptide, assay dilution buffer, magnesium/ATP cocktail, and P81 phosphocellulose paper, was purchased from Upstate, Millipore; gamma- ^{32}P ATP was purchased from PerkinElmer).

The whole experiment is divided into seven different groups with different treatments. 1)

The enzyme background, calculated from parallel reactions which did not contain kemptide, was subtracted from all groups. 2) The control group. 3) The PKA inhibition group, PKA inhibitor peptide was added to the assay solution. This acted as a positive control group. 4) The four treatment groups with different concentrations of VPA (VPA 50 μM, VPA 100 μM, VPA 500 μM, and 1000 μM). The VPA pre-prepared from 1 M stock solution. During the experiment, the VPA was diluted to desired concentrations using assay dilution buffer.

Reactions were carried out at 30 °C for 10 min (water bath). Aliquots were spotted onto P-81 phosphocellulose square paper and the radio labelled substrate was allowed to bind to P-81 paper for 30 seconds. The papers were washed in 0.75 % H_3PO_4 three times in a beaker with a stirrer under a small sieve. The papers were rinsed in acetone. The assay squares were transferred to a scintillation vial (PerkinElmer) and 2ml scintillation cocktail (PerkinElmer), which is a solution of a beta particle emitter. This is then analysed in an organic solvent along with a fluor emitting a scintillation of light, which was read by a scintillation counter (Beckman Coulter, LA6500) (Figure 2.12).

The data were calculated as the percentage of PKA activity. Enzyme background data were subtracted from the raw CPM data and all the data were normalised by dividing by the data from control treatment.

$$\text{PKA activity} = \frac{CPM_{data} - CPM_{background}}{CPM_{control}} 100\%$$

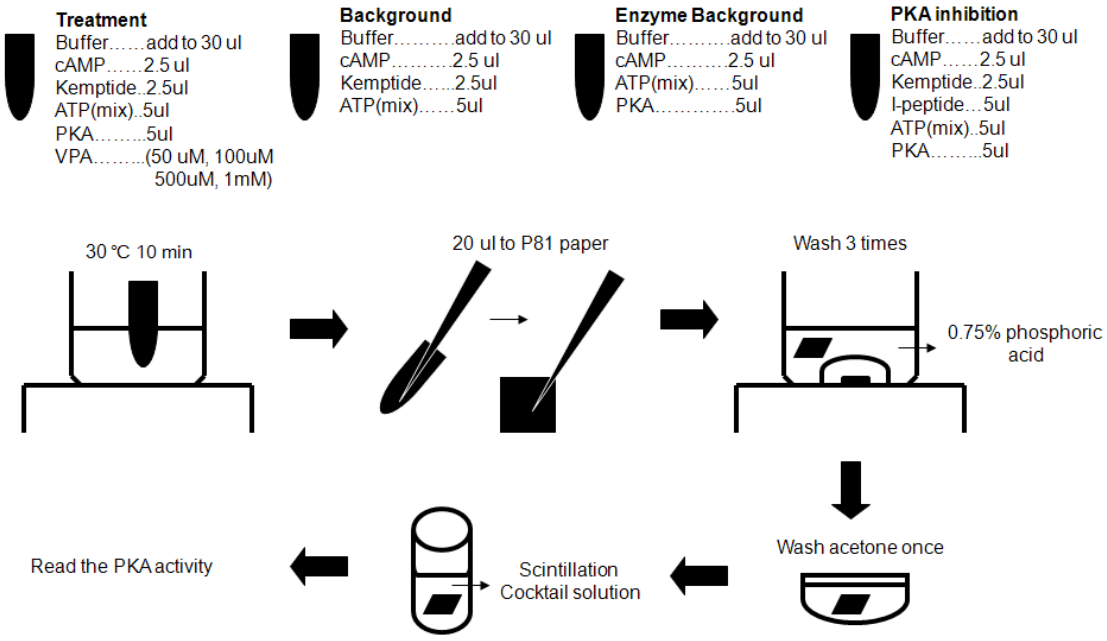


Figure 2.12: The protocol of measurement the PKA activity. Reactions were carried out at 30 °C for 10 min (water bath). Aliquots were spotted onto P-81 phosphocellulose square paper and the radio labelled substrate was allowed to bind to P-81 paper for 30 seconds. The papers were washed in 0.75 % H_3PO_4 three times in a beaker with a stirrer under a small sieve. The papers were rinsed in acetone. The assay squares were transferred to a scintillation vial and 2ml scintillation cocktail, which is a solution of a beta particle emitter. This is then analysed in an organic solvent along with a fluor emitting a scintillation of light, which was read by a scintillation counter.

2.5 cAMP Assays

This assay principle is based on enzyme fragment complementation (EFC). This assay takes advantage of a genetically engineered E. coli β-galactosidase (β-gal) enzyme. The β-gal enzyme consists of two fragments, a large protein fragment as an enzyme acceptor (EA) and a small peptide fragment as an enzyme donor (ED). The enzyme lacks activity when the two fragments are separated. In the working solution, the two fragments tend to recombine spontaneously by a complementation process to form active enzyme.

Once the β-gal recombines, it hydrolyzes luminescent or fluorescent substrates and produces a signal.

The ED fragment is not only able to reassociate with EA to form active enzyme, but also recognized by anti-cAMP antibody. In this assay, cAMP from cell lysates and ED-labelled cAMP (ED-cAMP) compete for antibody binding sites. Unbound ED-cAMP is free to complement EA to form active enzyme, which subsequently hydrolyzes fluorescent substrate for EFC detection by a microplate reader. Based on this principle, the competing intracellular cAMP and the impact of enzyme, such as adenylyl cyclise, can be measured (Figure 2.13).

2.5.1 Preparation

The cAMP hithunter was purchased from GE Healthcare and stored in fridge -20 °C. Kit Components : (1) cAMP Lysis Buffer; (2) cAMP EA Reagent; (3) cAMP ED Reagent; (4) cAMP Antibody; (5) cAMP Standard; (6) FL Substrate Reagent and FL Substrate Diluent; (7) BKG buffer.

Standard solution: cAMP Standard stock (0.25mM) series diluted 9 fold in eppendorf tubes (The range of cAMP concentration of standard is from 62.5 to 9.54 x 10^{-3} mM) by using Lysis Buffer/Ab Working Solution (1 part of cAMP plus 3 parts of Lysis Buffer/Ab Working Solution)

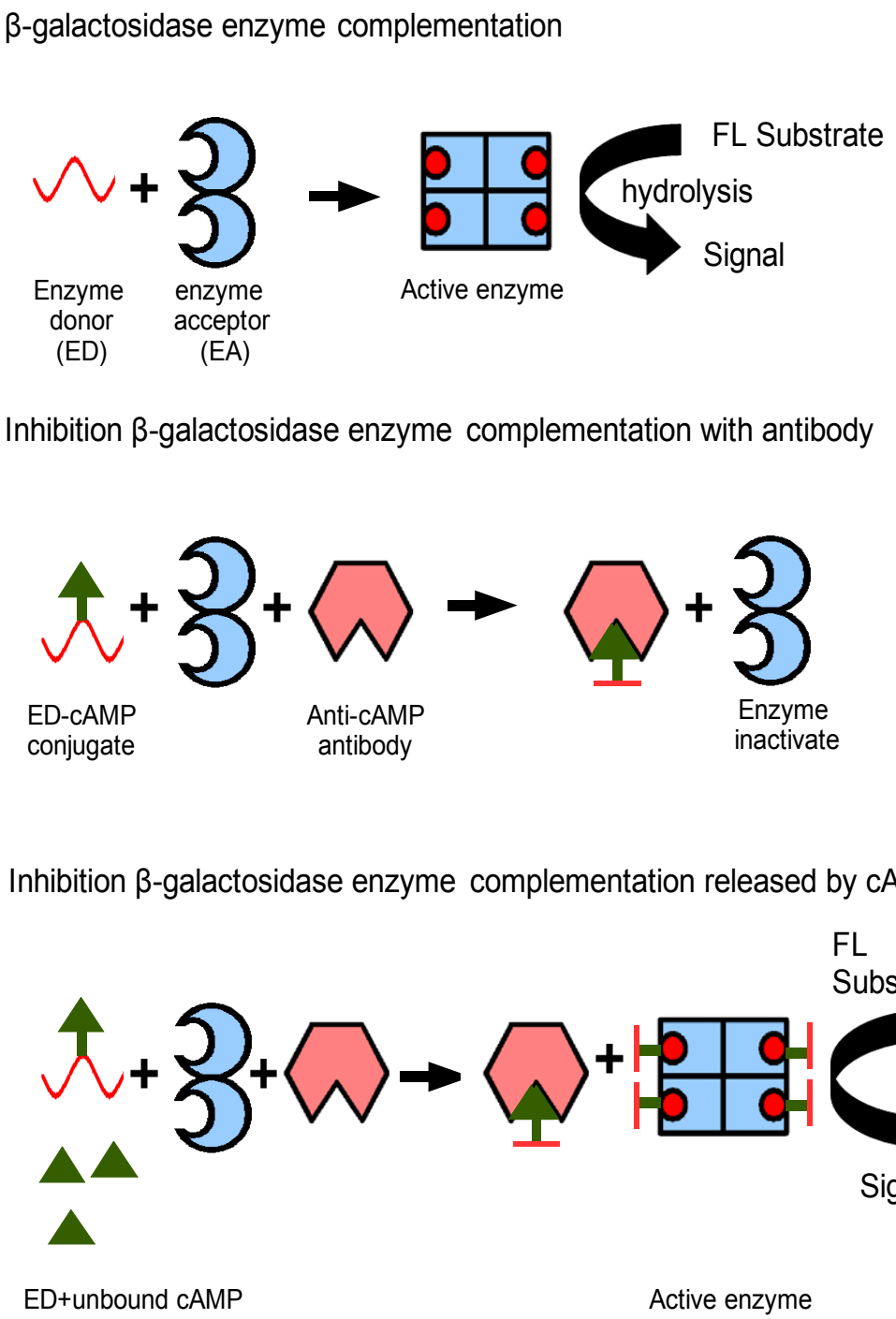


Figure 2.13: The principle of cAMP assays-hithunter cAMP assay. Two fragments of E. coli β-galactosidase (β-gal): a large protein fragment (enzyme acceptor, EA) and a small peptide fragment (enzyme donor, ED). Separately, these fragments are inactive. The recombining of EA and ED actives enzyme, which is able to hydrolyze substrate to produce a fluorescent signal. In this assay, cAMP from cell lysates and ED-labelled cAMP (ED-cAMP) compete for antibody binding sites. Unbound ED-cAMP is free to bind to EA to form active enzyme, which is able to produce a fluorescent signal. The amount of signal produced is proportional to the amount of cAMP in the cell lysate.

2.5.2 Procedure

Cell preparation: The hippocampus acquired from newborn rat (P0) was dissected out from heads of fetuses and placed in ice cold CMF-HBSS (Invitrogen). 2.5% trypsin (Invitrogen) was added in order to dissociate dissected cells. Once the cells were dissociated, the cell density was determined by cell counting, and cell viability was determined by the trypan blue exclusion method (Tolnai, 1975). During cell counting, cell suspension was diluted 1:5 in 0.4% trypan blue solution (Sigma). After being stained with trypan blue, the cells should be counted within 3 minutes. Stained cell suspension (20 µl) was placed onto hemacytometer (Hawksely, BS748), which was placed under a microscope for cell counting. Then, 150 µL(hippocampal cell number is 30,000) /tube of cells in medium with Neurobasal A (Invitrogen) was dispensed in 1.5 ml in eppendorf tubes. The media was gently aspirated after spinning down at 10,000 rpm and dispensed in 120 µL/tube of Hanks' Buffer (Invitrogen). These were incubated for 10 minutes at room temperature. 40 µL of cells were dispensed into a 384-well microtiter plate (10,000 cells/well). Then, hippocampal cells were treated with forskolin (50 µM) with different concentrations of VPA (10 µM, 100 µM, and 1 mM). The reaction was allowed to continue for 30 minutes, and the supernatant was removed. The cAMP extracted from the hippocampal cells was mixed with cAMP antibody and lysis solution, and incubated at room temperature for 60 minutes. The fluorescent substrate and the enzyme donor were mixed together and incubated for 60 minutes at room temperature. The enzyme acceptor was added and incubated for 60 minutes at room temperature. The relative fluorescent units were determined using a fluorescent reader (Molecular Devices, Model: GEMINI XS). Samples were excited at 530 nm, and an emission spectrum was collected from 540 nm to 640 nm (Table 2.3).

Table 2.3: The procedure of cAMP assays with different treatment by using cAMP hit hunter kit. The treatment including, BKG- back ground contro1 , standard-cAMP standard solution ranged from 62.5 to 9.54 x 10-3 mM, cell-experiment group(10,000 cells/well) with forskolin (50 μM) and different VPA concentration(10 μM, 100 μM and 1 mM)), and zero-experimental control(10,000 cells/well) with only forskolin (50 μM) .

Condition	BKG	Standard	Cells	Zero (control)
Step 1: Forskolin/PBS			10 μL agonist (or drug)	10 μL PBS
			Incubate at 37°C for 30 minutes	
Step 2: Std/Lysis/Ab or Lysis Ab	20 μL Lysis/Ab	20 μL STD/Lysis/Ab	20 μL Lysis/Ab	20 μL Lysis/Ab
60 minutes incubation at room temperature				
Step 3: ED/substrate/ BKG	13 μL BKG/substrate	13 μL ED/FL substrate	13 μL ED/FL substrate	13 μL ED/FL substrate
60 minutes incubation at room temperature				
Step 4: EA	10 μL EA			
60 minutes incubation at room temperature				
Read on a fluorescent reader Excitation λ = 530 nM ± 25 nm, Emission λ = 610 nM ± 20 nm				

Chapter 3: The effect of VPA on mossy fibre long-term potentiation

3.1 Introduction

VPA, di-n-propylacetic acid, is an eight-carbon branched-chain fatty acid. It is widely used clinically as an AED and a mood-stabilizing drug in bipolar disorder. It is also used to treat migraine headaches and schizophrenia.

VPA exerts a powerful neuroprotective effect in a variety of models including *in vivo* epilepsy models. Chronic treatment with VPA prevents the deficits in learning and prevents SE-induced hippocampal damage. Following kainic acid-induced SE, animals treated with VPA did not have deficits in learning in Morris water maze test, and had fewer histological lesions in the hippocampus (Brandt et al., 2003a). Treatment with VPA after SE induced by electrical stimulation of the basolateral amygdala prevented hyperexcitability and locomotor hyperactivity. Furthermore, treatment with VPA decreased hippocampal damage including the dentate hilus, and prevented some of the behavioural alterations (Brandt et al., 2006).

Previous studies suggest that the neuroprotective effect of VPA may involve the mitogen –activated protein pathway, through modulation of protein kinase A signalling pathway (Boeckeler et al., 2006). VPA activates extracellular signal-regulated kinase (ERK) which is known to be important for neuronal survival and long term neuronal plasticity. Furthermore, VPA increased the expression of genes regulated by the ERK pathway, and promotes neuronal survive (Yuan et al., 2001). Furthermore, an *in vivo* microdialysis study demonstrated that increases in cAMP levels in the prefrontal cortex of freely moving animals induced by an infusion of the adenylyl cyclase activator forskolin were attenuated by treatment with VPA (Montezinho et al., 2006;Montezinho et al., 2007).

Modulation of the cAMP/PKA pathway by VPA could have other implications. The cAMP/PKA pathway is critical for the expression of NMDA receptor independent LTP at the mossy fibre- CA3 synapse in the hippocampus (Huang et al., 1994;Nicoll and Malenka, 1995;Tzounopoulos et al., 1998;Weisskopf et al., 1994), and has been suggested also to be involved at Schaffer collateral-CA1 LTP. At this latter synapse, cAMP-mediated gene expression and synthesis of new protein, resulting in the growth of new synaptic connections, are required for LTP expression (Montarolo et al., 1986;Glanzman et al., 1990). Therefore inhibition of the cAMP pathway could impact upon long-term potentiation at these synapses. Moreover these pathways have also been proposed to play an important role in hyperexcitability and epileptogenesis.

Therefore, it has been hypothesized that the impact of VPA on cAMP/PKA signalling which contribute to VPA's neuroprotective effect would affect mossy fibre LTP (Figure 3.1).

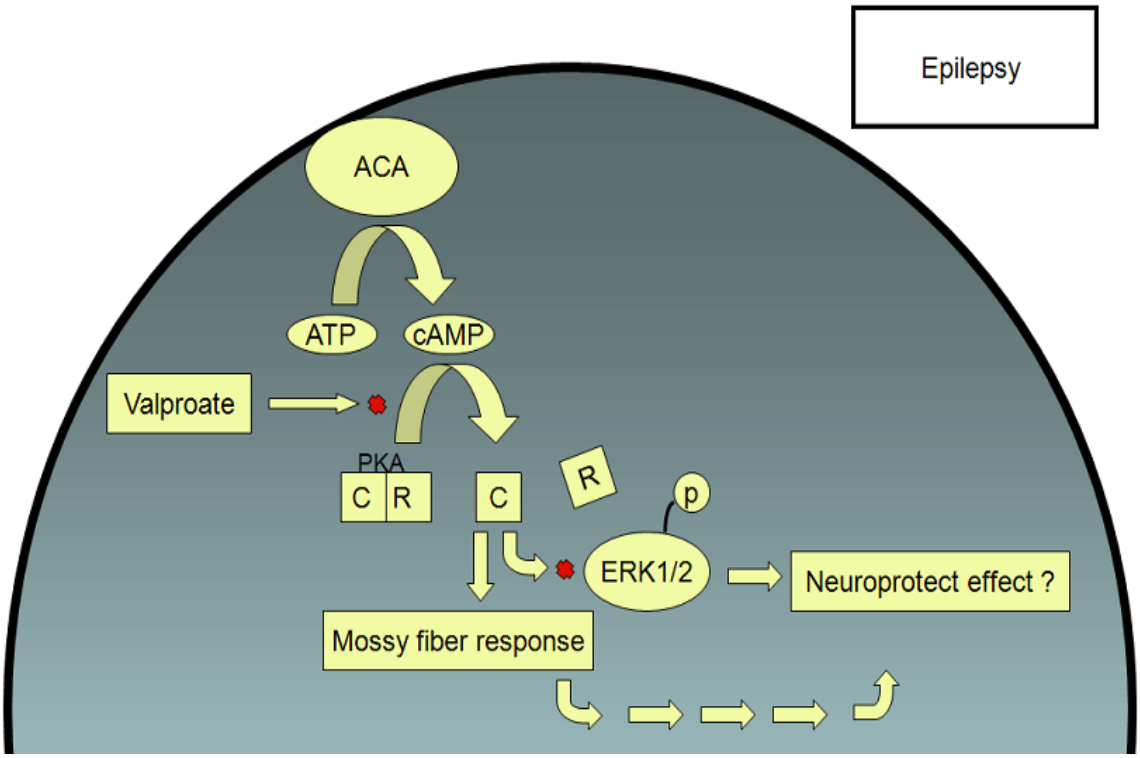


Figure 3.1: Activation of adenylyl cyclase (ACA) catalyzes the transformation of ATP to cAMP, and further activates protein kinase A (PKA). PKA phosphorylates other proteins, altering their function such as decrease the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) which is widely believed that contributes neuroprotective in epileptic condition. PKA also contributes to the enhancement of mossy fibre responses which may be related to neuroprotective effect. VPA, however, may inhibit the activation of PKA and provides the neuroprotective effect (Red Cross indicates the inhibition of the activity).

3.2 Methods

3.2.1 Electrophysiology

Isolated hippocampus slices were used in thesis experiment. Field excitatory postsynaptic potentials (fEPSP) were recorded using glass microelectrodes (approximately 2MΩ resistance) filled with aCSF solution positioned in stratum lucidum in CA3, the mossy fibre termination zone. Bipolar stainless steel stimulating electrodes were positioned in the dentate granule cell layer, and 0.6 mA pulses (400 µs duration) were applied with constant current stimulators (Figure 3.2). The electrode positions and stimulus intensities were adjusted until mossy fibre fEPSPs of maximal amplitude were recorded. Mossy fibres were identified by marked frequency facilitation which is a marked increase in the amplitude of the responses with an increase in the frequency of stimulation (> 2 fold increase in amplitude on increasing stimulation frequency from 0.05 Hz to 1 Hz).

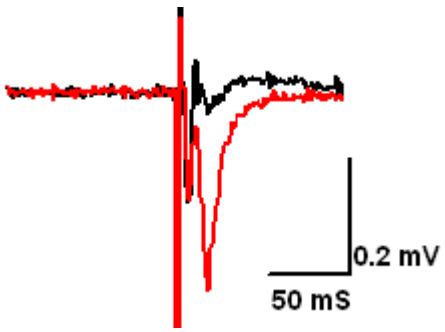


Figure 3.2: The trace recording of mossy fibre responses stimulated with 0.05 Hz and 1 Hz. The waveform of this recording includes stimulus artifact, fibre volley, and the amplitude of fEPSP increase twice bigger once the frequency of stimulation increases from 0.05 Hz to 1 Hz. The black trace recording shows the baseline recording of mossy fibre response which is stimulated with 0.05 Hz; the red trace recording shows that mossy fibre response which was stimulated with 1Hz.

All the drugs were applied by bath perfusion. Valproate (1 mM) (Sigma), pentenoic acid (1 mM) (Sigma), APV-5 (50 μM)(Ascent), NBOX sodium (25 μM) (Sigma) and H89 (10 μM) (Sigma) dissolved in distilled water, and forskolin (50 μM) (Sigma) dissolved in dimethyl sulfoxide (DMSO) (Sigma) were prepared as 1000 times stocks and stored at -20°C. All drugs were directly dissolved in aCSF to achieve their final concentrations during experiments and applied by gravity. All experiments were carried out at room temperature.

3.2.2 High-frequency stimulation induced enhancement of mossy fibre responses

In order to induce maximal mossy fibre synapse to CA3 LTP, three 1 second trains of high frequency stimuli (100 Hz) every 10 seconds was applied in the presence of the NMDA receptor antagonist APV (50μM).

Recording was then continued for 100 minutes after the induction protocol. VPA (1 mM) was applied before the LTP induction protocol and throughout the experiment. At the end of the experiment AMPA/kainate receptors were blocked with NBQX (25 μM). Although definitions of the timing of LTP and short term potentiation differ, for these studies LTP was considered to be a potentiation lasting greater than 30 minutes. Therefore, to assess LTP, the magnitude of potentiaion was calculated after 30 minutes after stimulation and the VPA and control slices were compared using a Student's t test.

3.2.3 Forskolin- induced enhancement of mossy fibre responses

For the purpose of this experiment, fEPSP potentiation was elicited by application of the adenylyl cyclase activator forskolin (50 μM). Forskolin is commonly used to raise levels of cyclic AMP (cAMP) by activating the enzyme adenylyl cyclase. Application of

forskolin at mossy fibre–CA3 synapses induces LTP by increasing presynaptic adenylyl cyclase and so activating cAMP-dependent protein kinase (PKA). There were four different treatment groups: control, VPA (1mM), pentenoic acid (1 mM) (a straight chain fatty acid with no anticonvulsant activity) and H89 (10 μM) (a PKA inhibitor).

3.2.4 Data analysis

Because mossy fibre fEPSPs have a complex fibre volley that can contaminate the recording, the fibre volley (response after blocking AMPA/kainate receptors) was subtracted from all fEPSPs. The fEPSPs were then normalized to baseline (prior to LTP induction or application of forskolin). Data are shown as mean ± SEM. The mean fEPSP amplitudes were compared between groups using either Student's Test or one way ANOVA with Tukey test as post hoc test. $P < 0.05$ was considered as significant.

The equation of normalization of fEPSP:

$$\frac{fEPSP_{data} - \text{average of } fEPSP_{NBQX}}{\text{Average } (fEPSP_{baseline} - \text{average of } fEPSP_{NBQX})}$$

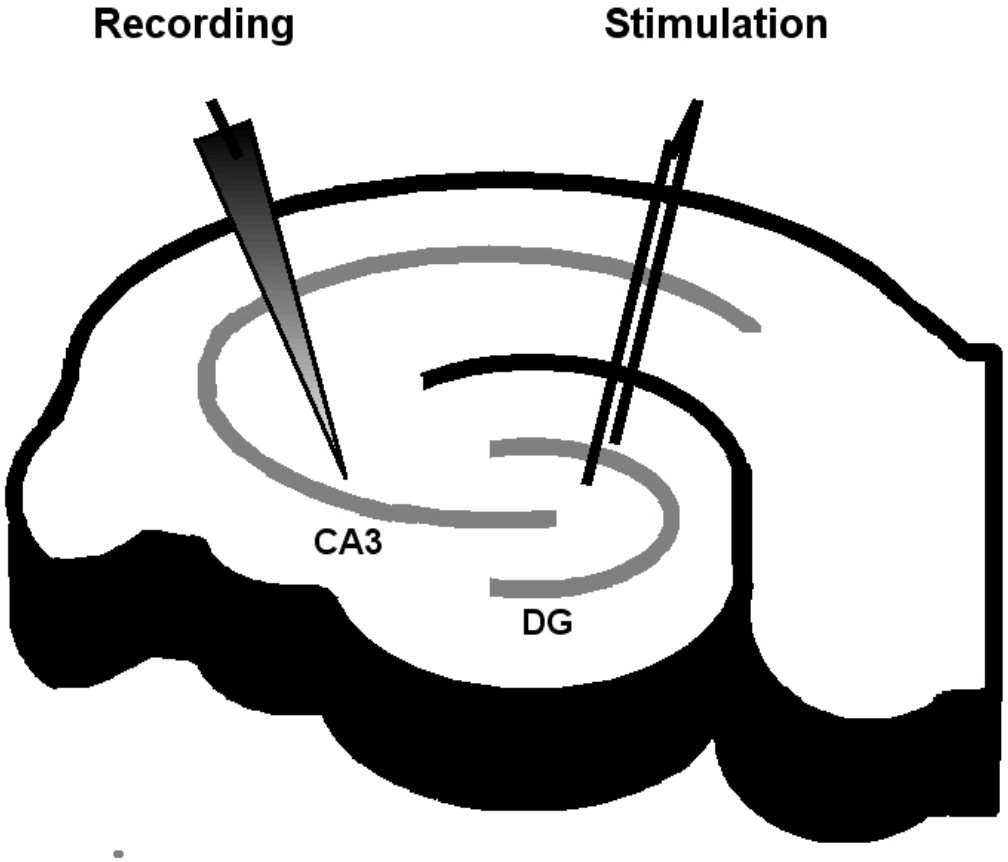


Figure 3.3: The arrangement of recording in mossy fibre field. A recording electrode is placed in stratum lucidum. A bipolar stimulating electrode is placed in dentate granule cell layer.

3.3 Results

3.3.1 VPA and pentenoic acid did not interfere with the short-term potentiation and baseline neurotransmission in mossy fibres synapse CA3.

The effect of VPA (1 mM) and pentenoic acid (1mM), a short- straight chain fatty acid (see Table 3.1), on short-term potentiation and baseline neurotransmission in the mossy fibre synapse CA3 was first determined. The advantage of a specific feature of mossy fibres is their identity- marked frequency facilitation (Schmitz et al., 2001). So once a candidate mossy fibre fEPSP was found, the stimulus frequency was increased from 0.05 Hz to 1 Hz and at least a two fold increase in fEPSP amplitude was used to confirm that the response was indeed a mossy fibre response.

After baseline recording, in half the experiments VPA was added to the perfusate. The frequency facilitation was repeated in slices with and without VPA. There is no significant effect of VPA on fEPSP amplitude or short-term plasticity (Before VPA 1mM: 313.56±23.64% of baseline, after VPA 1mM: 293.46±35.47% of baseline) (Figure 3.4).

Similarly, there is no significant effect on the enhancement of mossy fibre after stimulation with 0.05 Hz and 1 Hz in pentenoic acid treated group (Before pentenoic acid 1mM: 313.56±23.64%, after pentenoic acid 1mM: 293.46±35.47%). Furthermore, application with VPA or pentenoic acid didn’t affect baseline neurotransmission in mossy fibres synapse CA3 (Figure 3.5).

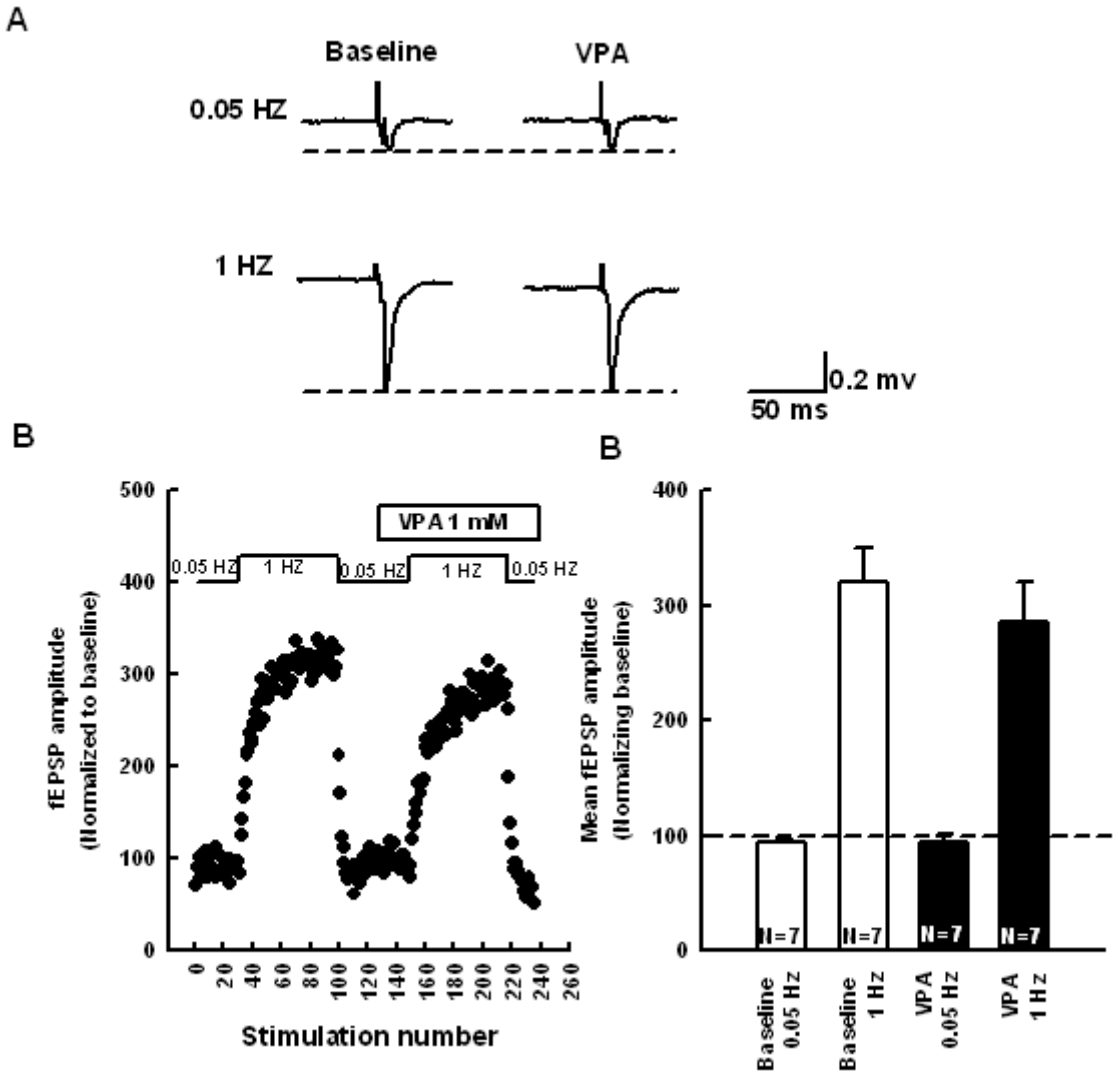


Figure 3.4: The effect of VPA on STP at the mossy fibre synapse to CA3 region in hippocampus. (A) *Trace of average EPSCs stimulated in dentate granule cell layer with frequency 0.05 Hz and 1 Hz present with/without VPA.* (B) *Example of the change of fEPSP amplitude in mossy with different stimulation frequency (0.05 Hz and 1 Hz). fEPSP amplitudes recordings plotted against stimulation number. fEPSP amplitudes normalized to the baseline; baseline values were set at 100%.* (C) *Comparison of the mean amplitude of fEPSP in control condition and present with VPA. Graphs show means ± SEM. There is no significant difference in control condition and VPA-treated condition with either 0.5 Hz or 1 Hz stimulation.*

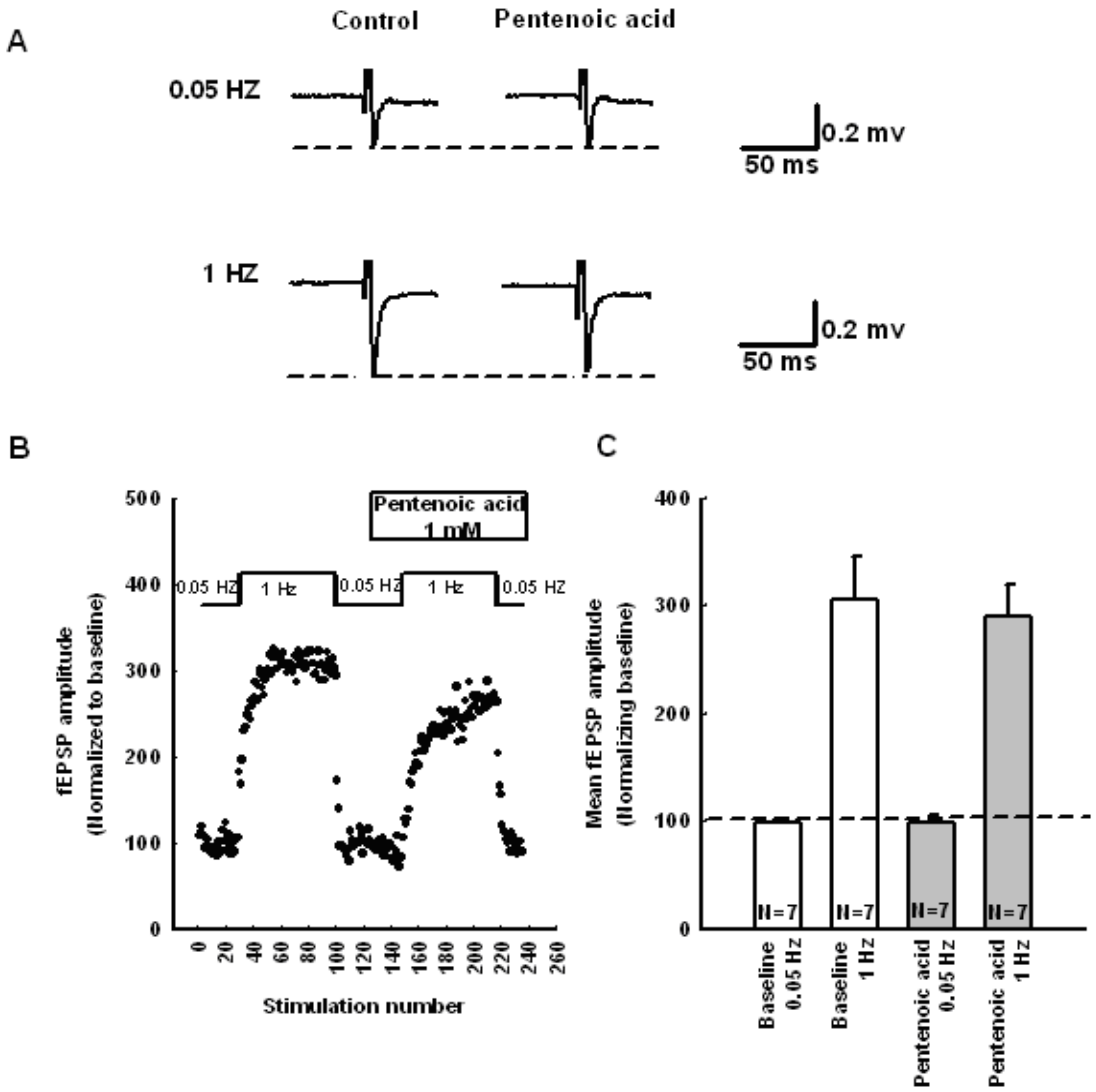


Figure 3.5: The effect of pentenoic acid on STP at the mossy fibre synapse to CA3 region in hippocampus. (A) *Trace of average EPSCs stimulated in dentate granule cell layer with frequency 0.05 Hz and 1 Hz present with/without pentenoic acid.* (B) *Example of the change of fEPSP amplitude in mossy with different stimulation frequency (0.05 Hz and 1 Hz). fEPSP amplitudes recordings plotted against stimulation number. fEPSP amplitudes normalized to the baseline; baseline values were set at 100%.* (C) *Comparison of the mean amplitude of fEPSP in control condition and present with pentenoic acid. Graphs show means ± SEM. There is no significant difference between control condition and pentenoic acid-treated condition.*

3.3.2 VPA decreases the enhancement of mossy fibre responses induced by high-frequency stimulation

Activation of cAMP-dependent protein is required to induce mossy fibre LTP which is also known as a NMDA-independent LTP. The question whether there is an effect of VPA on NMDA receptor independent, PKA dependent LTP at mossy fibre to CA3 pyramidal cell synapses was asked. The experiments were performed in isolated hippocampal slices; a bipolar stimulating electrode was placed in dentate granule cell layer; a recording electrode was placed in stratum lucidum (mossy fibre termination zone).

Once the mossy fibre response was confirmed by the identification of short-term frequency-dependent facilitation, mossy fibre fEPSPs were recorded for 30 minutes as baseline with or without VPA 1mM. LTP was then induced as detailed above (Figure 3.6). To prevent NMDA receptor mediated LTP, the experiments were carried out in the presence of APV-5 (50 μM) to block NMDA-mediated responses.

The NBQX, AMPA/kainate receptor antagonist, was applied at the end of each experiment. The NBQX blocked the EPSCs, leaving the fibre volley which is subtracted from all traces (Figure 3.7).

In the control group, the LTP protocol results in rapid short term potentiation and then the amplitude decreases over 10 minutes or so to reveal LTP. In the VPA treated group, the initial short-term potentiation was unaffected, arguing against an effect of VPA on the induction protocol. However, LTP was significantly reduced (Figure 3.8).

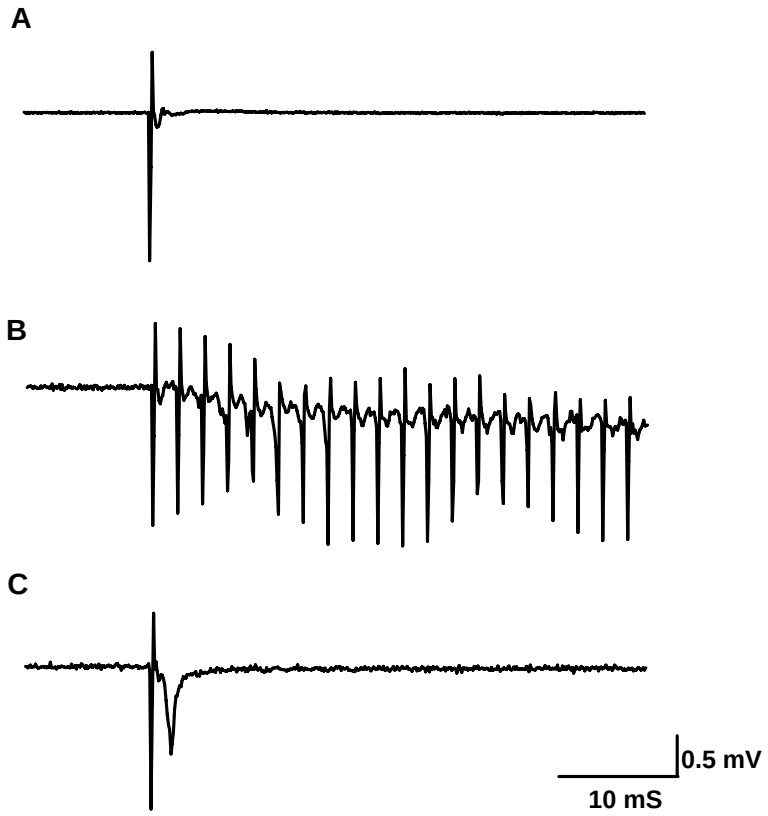


Figure 3.6: Marked facilitation following LTP induction. High frequency stimulation (three times 100 Hz/ second for every 10 seconds) enhances mossy fibre response. This enhancement of mossy fibre can last for hours (A) *The mossy fibre response recorded before stimulation.* (B) *The mossy fibre response recorded during Stimulation* (C) *the mossy fibre response recorded immediately after high frequency stimulation.*

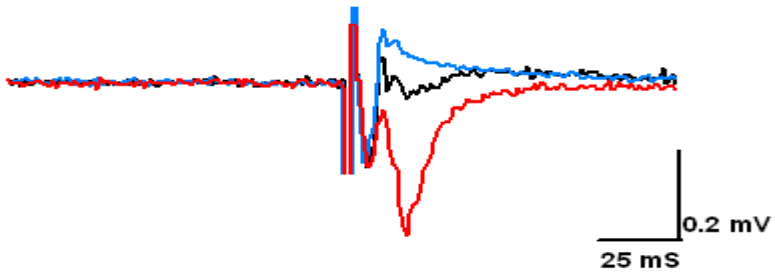


Figure 3.7: The identification of mossy fibre response. The black trace shows the baseline recording of mossy fibre response; The red trace shows that mossy fibre response was increase as twice bigger as baseline while stimulus intensity was increased from 0.05 Hz to 1 Hz; The blue trace shows the complex fibre volley following block of ionotropic glutamate receptors.

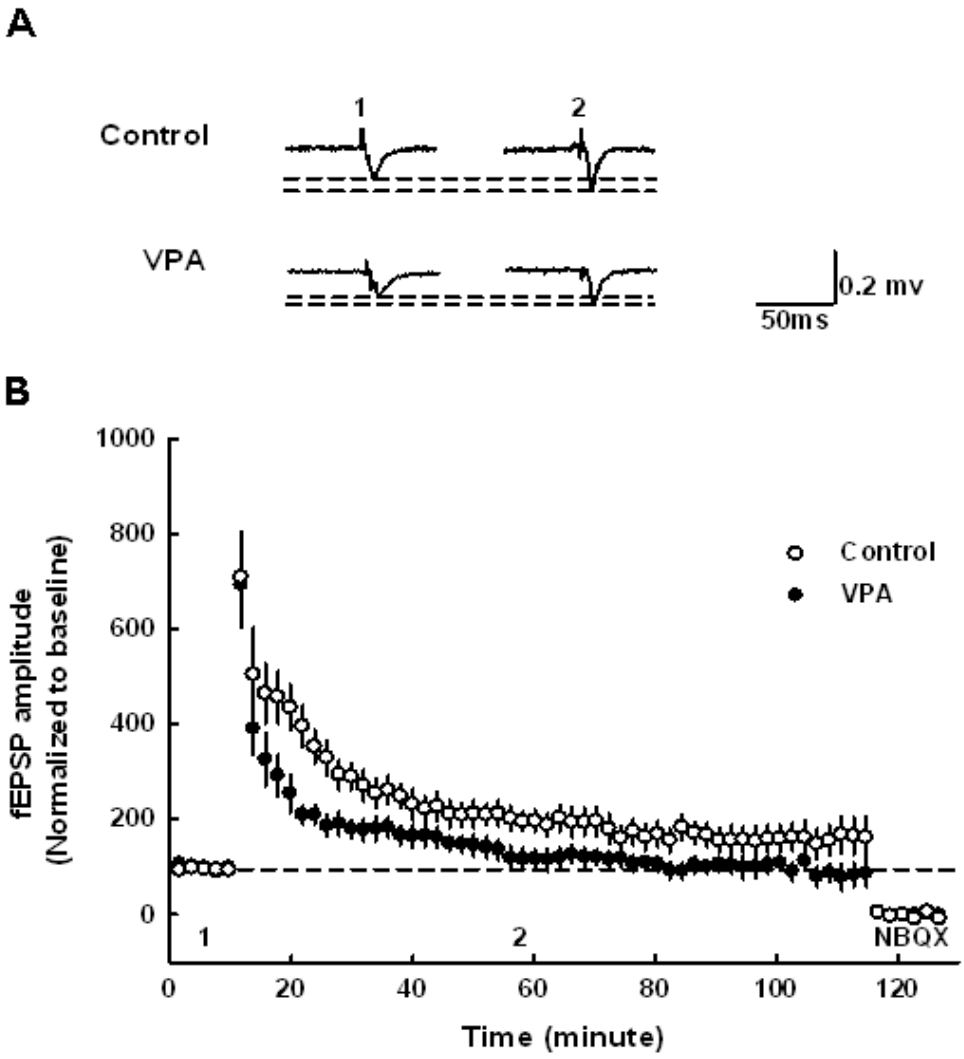


*Figure 3.8: The effect of VPA on LTP at the mossy fibre synapse to CA3 region in hippocampus after high frequency stimulation. (*A*) Trace of average fEPSCs before and after tetanus in control condition and VPA-treated condition respectively. (*B*) Summary the data for mossy fibre LTP induced by tetanus. fEPSC amplitudes recordings plotted against time. fEPSC amplitudes normalized to the baseline values.*

To compare VPA and control group, the amplitude of LTP after high frequency stimulation over 30 minutes was averaged. The mean amplitude of mossy fibre LTP was significantly suppressed in the group treated with VPA (148 ± 20 %), compared to control group (231 ± 22% n=6) (p=0.02 by Student's t test) (Figure 3.9)

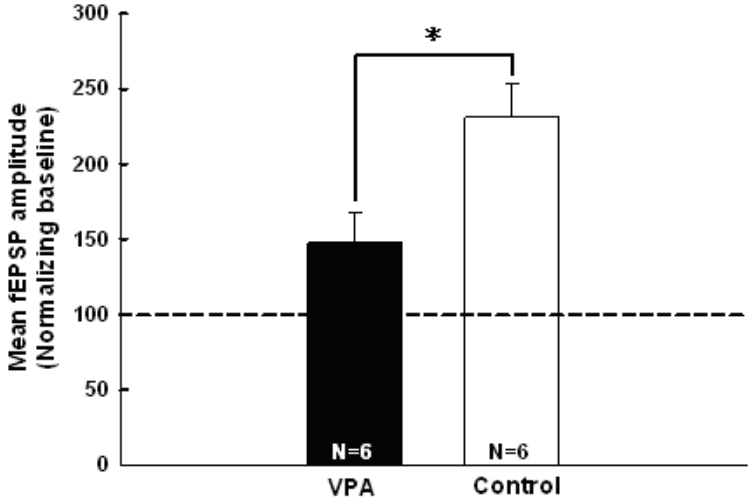


*Figure 3.9: Comparison of the mean fEPSP of amplitude after tetanus stimulation in control group and VPA-treated group. Each column shows the mean value of the fEPSPs expressed as a percentage of the baseline value (during the 10 min before tetanus). Graphs show means ± SEM. Statistical analysis was performed by Student's t-test. * Indicates a significant difference between the two groups (P<0.05). The magnitude of the LTP in VPA-treated group is significant smaller than in the control group.*

3.3.3 VPA decreases the enhancement of mossy fibre responses induced by application of forskolin

To further evaluate whether the effect of VPA on mossy fibre LTP is mediated by cAMP/PKA signalling system, mossy fibre LTP was elicited by application of the adenylyl cyclase activator, forskolin (50 μM) (Figure 3.10).

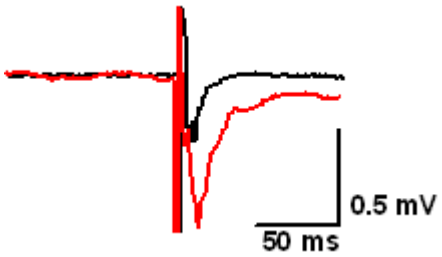


Figure 3.10: The trace recording of enhancement of mossy fibre responses by treatment with forskolin (50 μM). The black trace shows the baseline recording of mossy fibre response; the red trace shows that mossy fibre response following application of forskolin.

As in the previous experiment, APV-5 was applied throughout. The mossy fibre excitatory postsynaptic potentials (EPSPs) elicited by this method were identified by marked frequency facilitation and sensitivity to the group II metabotropic glutamate agonist (2S, 1'R, 2'R, 3'R)-2-(2,3-dicarboxylcyclopropyl)-glycine (DCG-4) (1 μM) at the end of the experiment. The group II metabotropic glutamate receptor (mGluR2) suppresses synaptic transmission and reduces the synaptic excitation at the hippocampal mossy fibre-CA3 synapse by presynaptic mechanisms.(Yoshino et al., 1996;Kamiya and Yamamoto, 1997).

In control conditions, 30 minutes after application of forskolin, the fEPSP was reach a maximum and entered a steady state (mean amplitude of fEPSPs after application of forskolin was 458.56±53.33% of baseline, n=8) (Figure 3.11). In order to confirm that the enhancement of mossy fibre responses is mediated by activation of PKA, a PKA inhibitor H89 (10 μM) was added. There was a significant effect on the enhancement by forskolin (458.56 ± 53.33% of baseline, n=8) (Figure 3.11). Post-hoc analysis indicated that forskolin-induced enhancement of transmission was significantly attenuated in the presence of H89 (281.65 ±37.60% of baseline, $n = 5$, $p < 0.05$)

Forskolin (50 μM) were added in the presence of VPA (1mM), or a straight chain fatty acid pentenoic acid (1 mM) as a further control (Figure 3.12). As has been previously observed, forskolin enhanced the fEPSP amplitude (458.56 ± 53.33% of baseline, n=8). There was a significant effect of treatment on the enhancement by forskolin ($p =0.014$, single factor ANOVA) (Table 3.2). Post-hoc analysis indicated that this enhancement was significantly attenuated in the presence of VPA (259.49 ± 25.99% of baseline, $n = 7$, $p < 0.05$), but not in the presence of pentenoic acid (470.86 ± 76.69 % of baseline, n=6) (Table 3.3).

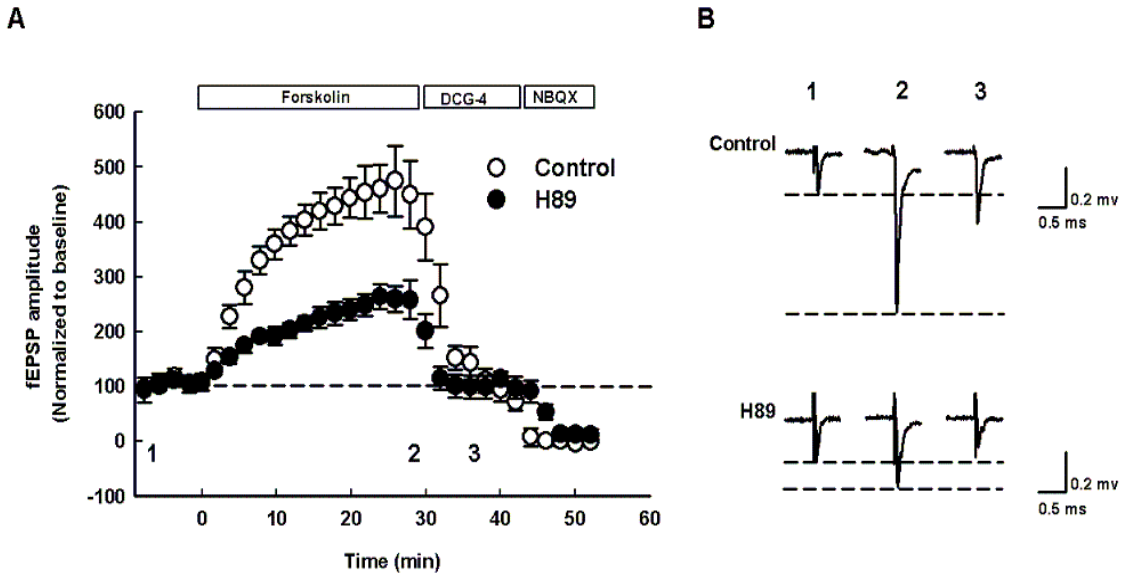


Figure 3.11: PKA contributes to LTP at the mossy fibre synapse to CA3 region in hippocampus after application of forskolin (50 μM), adenylyl cyclase activator. (A) *Summary data for mossy fibre LTP induced by treatment with forskolin. fEPSP amplitudes recordings plotted against time. EPSC amplitudes normalized to the baseline (during 10 minutes before treatment with forskolin) were set at 100% for each individual experiment).* (B) *Trace of average EPSCs before, during, and after application of forskolin.*

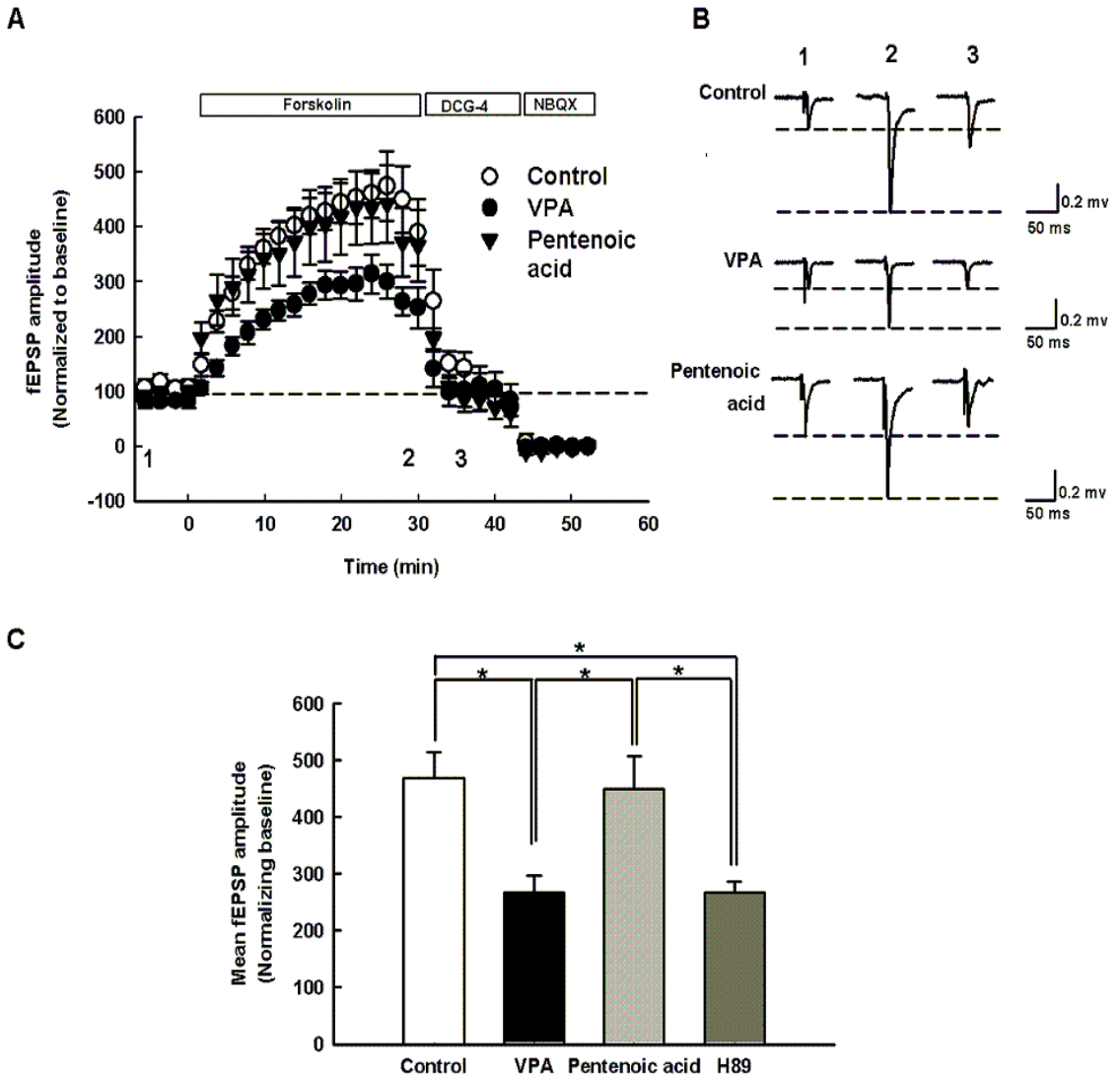


*Figure 3.12: VPA suppressed LTP at the mossy fibre synapse to CA3 region in hippocampus after application of forskolin (50 μM), adenylyl cyclase activator. (*A*) Summary the data for mossy fibre LTP induced by treatment with forskolin. fEPSP amplitudes recordings plotted against time. EPSC amplitudes normalized to the baseline (during 10 minutes before treatment with forskolin) were set at 100% for each individual experiment). (*B*) Trace of average EPSCs before, during, and after application with forskolin. (*C*) Comparison of the mean fEPSP of amplitude after treatment with forskolin. Each column shows the average value of the fEPSPs expressed as a percentage of the baseline value. Graphs show means ± SEM. Statistical analysis was performed by post hoc test-tukey test, following by ANOVA. * Indicates a significant difference between the two groups (P<0.05).*

Table 3.1: The result of ANOVA test of groups with different treatments (control, H89, VPA, and pentenoic acid)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24.167	3	8.056	6.504	0.003
Within Groups	27.247	22	1.238		
Total	51.414	25			

* **Sum of Squares:** The estimate of variance corresponds to the numerator of the variance ratio.

Df: Degrees of freedom for each estimate of variance. The degrees of freedom associated with the sources of variance

Mean Square: Each mean square is calculated by dividing the sum of square by its degrees of freedom.

F: F ratio is calculated by dividing mean square between-groups by mean square within groups (F = Mean square Between-groups / Mean square Within-groups)

Sig.: Sig. gives the significance of the F ratio. This is the p value. If the p value is less than or equal α level, then you can reject H0 that all the means are equal. That is, there is insufficient evidence to claim that some of the means may be different from each other.

Table 3.2: Post-hoc comparison of multiple comparisons of treatments (Control, H89, VPA, and pentenoic acid) by Tukey test

Comparisons of groups	Mean Difference (Between groups)	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Control VS VPA	2.01372(*)	.59486	0.013	0.3619	3.6655
Control VS Pentenoic acid	0.18872	.61915	0.990	-1.5306	1.9080
Control VS H89	2.00717(*)	.61915	0.018	0.2879	3.7264
VPA VS Pentenoic acid	-1.82501(*)	.61915	0.035	-3.5443	-0.1057
VPA VS H89	-0.00655	.61915	1.000	-1.7258	1.7127
Pentenoic acid VS H89	1.81845(*)	.64252	0.045	0.0343	3.6026

* **Mean Difference:** difference between the means

Std. Error: standard error of the mean these are the standard errors associated with the coefficients.

Sig.: p value for the multiple comparisons used in testing whether a given coefficient is significantly different. P<0.05 consider as significant difference consider:

95% Confidence interval for Lower Bound and Upper Bound: gives an estimated range of values, usually calculated as percentage 95% confidence intervals for the coefficients. The upper and lower bounds of a 95% confidence interval are the 95% confidence limits. The confidence intervals are related to the p-values such that the coefficient will not be statistically significant if the confidence interval includes 0

3.4 Discussion

These results demonstrate that application of VPA had no effect on single fEPSPs or on short-term potentiation. However, VPA markedly impaired mossy fibre LTP induced by high frequency stimulation or through application of forskolin.

Activation-dependent synaptic potentiation can be divided into several distinct temporal phase, including short-term potentiation (STP) and long-term potentiation (LTP). STP plays a fundamental role in information processing and rapid regulation of presynaptic input. STP enhancement reflects an increase in the probability of release of available quanta, or an increase in the number of release sites which are capable of releasing a quantum (Zucker and Regehr, 2002). Unlike associational/commissural synapses which are relatively insensitive to frequency of stimulation, marked frequency facilitation at low stimulation frequencies is a characteristic of mossy fibre to CA3 pyramidal cell synapses (Salin et al., 1996). VPA had no effect on single fEPSPs or on short-term potentiation indicates that VPA does not have any effect on neurotransmitter release probability.

A further characteristic of mossy fibres is a robust NMDA receptor independent LTP. This LTP is dependent on the activation of protein kinase A via cAMP. NMDA receptor-independent LTP in the mossy fibre pathway is blocked by inhibitors of PKA or genetic manipulation of PKA (Weisskopf et al. 1994; Huang et al. 1994; Huang et al. 1995; Huang, 1996). In addition, application of adenylyl cyclase activator, forskolin, or membrane-permanent analogous of cAMP causes a long lasting presynaptic enhancement of mossy fibre responses (Huang et al., 1994; Nicoll and Malenka, 1995; Tzounopoulos et al., 1998; Weisskopf et al., 1994).

In this study, the effect to VPA on mossy fibre LTP by High-frequency stimulation (HFS) was first verified. HFS also induced a large PTP (post-tetanic potentiation)/STP that declined to a stable value in approximate 10-20 minutes. However, STP and LTP are expressed via different mechanisms, occluding LTP does not occlude STP expression, occluding STP does not occlude LTP expression (Schulz and Fitzgibbons, 1997). Therefore, in order to eliminate contamination of PTP/STP, LTP data were obtained more than 30 min after HFS. Application of VPA impaired induction of LTP in mossy fibre synapse to CA3 which is similar to application of PKA antagonist, H89, indicating that VPA may impair the formation of LTP in mossy fibre by inhibiting the activity of PKA. Inhibition of mossy fibre LTP and forskolin enhancement of transmission is consistent with an action of VPA on the cAMP/PKA pathway but it does not distinguish between an effect on cAMP accumulation or a direct action on PKA (this will be investigated in subsequent chapters). Furthermore, VPA could be acting downstream of PKA.

In these experiments, the pentenoic acid was used as an experimental control. Pentenoic acid, a short straight chain fatty acid (an analogue of VPA), has little or no anticonvulsant properties. As application of pentenoic acid lacked an effect on the enhancement of mossy fibre responses induced by forskolin, then this raises the question whether an action of VPA on the cAMP/PKA pathway may be involved in its antiepileptic effect (This question will be addressed in subsequent chapters).

In contrast to its effect on LTP, VPA had no significant effect on baseline transmission or short term plasticity. What is the possible physiological consequence of VPA's effect on LTP? LTP has been proposed to be the cellular basis of memory formation in the

CNS, and so an action on inhibiting mossy fibre LTP may be relevant to the cognitive impact of high dose VPA (Lamberty et al., 2000). Further, the inhibition of long term enhancement of excitatory transmission may play a part in the antiepileptogenic effect of VPA (Silver et al., 1991).

Are the concentrations used here relevant to human use? The concentrations used in these studies were 1 mM. The therapeutic plasma level of VPA in human is 50 mg/L-150 mg/L (Covanis et al., 1982). The ratio of total and unbound plasma VPA concentrations is around 10 % (Cloyd et al., 2003), indicating the pharmacologically active concentration is relatively low. However, the concentration range of VPA necessary to have an antiepileptic effect in animals is orders of magnitude higher than in humans (about 200-500 mg/L) (Nau et al., 1981;Chapman et al., 1982), and the reason for this remains unclear. The VPA concentration in rat brain tissue that mediates an antiepileptic effect is about 150-200 μg/g (Chapman et al., 1982; Löscher et al., 1989), and these concentrations are consistent with a molar concentration of VPA of 1 mM. Therefore, the concentration here is directly relevant to the therapeutic concentration of VPA in rat brain.

Chapter 4: The effect of VPA on cAMP/PKA signalling system

4.1 Introduction

In chapter 3, it has showed that the VPA decreased the enhancement of mossy fibre responses either induced by high frequency stimulation or by application of forskolin. These results strongly suggest a mechanism of VPA action may involve modulation of the cAMP/PKA signalling system.

cAMP is derived from adenosine triphosphate (ATP) and is used for intracellular signal transduction. cAMP is involved in the regulation of several signalling pathways such as extracellular signal-regulated kinases 1 and 2 (ERK1/2) (Hafner et al., 1994;Enserink et al., 2002;de Rooij et al., 1998a;Kawasaki et al., 1998). The cAMP signalling pathway is also important in many biological processes, ranging from growth, differentiation, and gene expression to secretion and neurotransmission. It acts to stimulate protein kinases, such as protein kinase A (PKA) and it is rapidly broken down in cells by phosphodiesterases to terminate the signal.

cAMP binds to regulatory units of protein kinase A, and causes dissociation between the regulatory and catalytic subunits. Activation of catalytic units results in the phosphorylation of substrate proteins. The activation of PKA catalyzes the transfer of phosphate from ATP to specific serine or threonine residues of protein substrates. This may activate or inhibit protein activity. For example, PKA phosphorylation has a direct action on regulating synaptic AMPA receptors in the rat hippocampus (Esteban et al., 2003;de Rooij et al., 1998b;Roche et al., 1996;Carvalho et al., 1999) which may contribute to synaptic plasticity. PKA can also phosphorylate specific proteins that bind to promoter regions of DNA, resulting in increased expression of specific genes. Abnormal cAMP signalling system has been suggested to contribute to the increased excitability of neurons in epileptic animals (Tehrani and Barnes, 1995b;Yechikhov et

al., 2001).

It is not clear from previous studies from chapter 3, whether VPA has a direct effect on PKA or an indirect effect by decreasing cAMP accumulation. In order to set out to distinguish these two possibilities in this chapter, biochemical assays were used.

4.2 Method

4.2.1 cAMP assay

The hit hunter cAMP assay kit used in this experiment was purchased from GE Healthcare, UK. HitHunter cAMP Assays are based on using β-galactosidase (β-gal) enzyme consisting of two fragments, enzyme acceptor (EA) and enzyme donor (ED). These fragments are inactive separately, but in solution they rapidly complement to form active ß-gal enzyme which can hydrolyze substrate to produce fluorescent signal. For competing antibody binding sites by cAMP from cell lysates and ED-labelled cAMP (ED-cAMP), unbound ED-cAMP is free to bind to EA to form active enzyme. The amount of signal produced is proportional to the amount of cAMP in the cell lysate (for detail, see chapter 2 general methods).

For these experiments, hippocampal cells (10,000 cells/ well) were acquired from newborn rats (P0) and stored in medium neurobasal, containing 2% B27 and 25 µM glutamine. Then the hippocampal cells were transferred into black 384-well microtiter plates (PerkinElmer). After removal of the medium, cells were stimulated with 50 µl of forskolin (50 µM) or forskolin plus VPA at different concentrations (10 µM, 100 µM, or 1 mM) in HBSS buffer for 30 minutes. Once the supernatant media was removed, 20 µl of anti-cAMP antibody in lysis buffer was added. After an incubation time of 60 minutes at room temperature, 10 µl of ED-cAMP (with substrate) was added. After

incubation at room temperature for 60 minutes, 10 µl EA was added and incubated for a further 60 minutes at room temperature, prior to reading the value of fluorescence, using software "SOFTmax pro".

4.2.2 PKA assay

An alternative explanation for the action of VPA illustrated in chapter 3 is a direct effect on protein kinase A. To test this, an *in vitro* assay of PKA activity by measuring PKA phosphorylation of Kemptide substrate (see general methods) was used. In brief, the assay for PKA activity was carried out in a 30 µl reaction volume containing kemptide substrate, PKA, magnesium/ATP cocktail containing [γ-^{32}P]ATP and without/ with different concentrations of VPA (50 µM, 100 µM, 500 µM, and 1 mM). Then, the reaction solution was incubated at 30 °C for 10 min. A 20 µl aliquot of the reaction solution was spotted on P81 phosphocellulose paper, washed with 0.75% phosphoric acid. The P81 paper (millpore) was washed with acetone, left to dry, followed by adding scintillation cocktail solution and counting in a scintillation counter.

4.2.3 Data analysis

For cAMP assay, the fluorescence value was normalized to the control treatment. For the PKA assay, the CPM value from the scintillation counter was normalized to control treatment. Data are shown as mean ± SEM. The mean values in groups were compared by one way ANOVA and tukey test as post hoc test. $P < 0.05$ was considered as significant.

4.3 Results

4.3.1 The effect of VPA on cAMP accumulation after stimulation with forskolin

In this experimental paradigm, an assay is first performed with various concentrations of standard solution. The standard cAMP solution (0.25 mM) was serially diluted 9 times (concentration range was 62.5 to 9.54 x 10-3 mM). cAMP standard detection reagents were added according to the assay protocols and fluorescence was read one hour after the addition of the EA reagent. These data are used to make the standard curve by using four-parameter logistic model, plotting concentration on the X axis, and assay measurement on the Y axis displayed a pronounced sigmoidal or S shape (Figure 4.1). The values obtained from experimental groups were accepted when the values were located between the extremes of the standard curve.

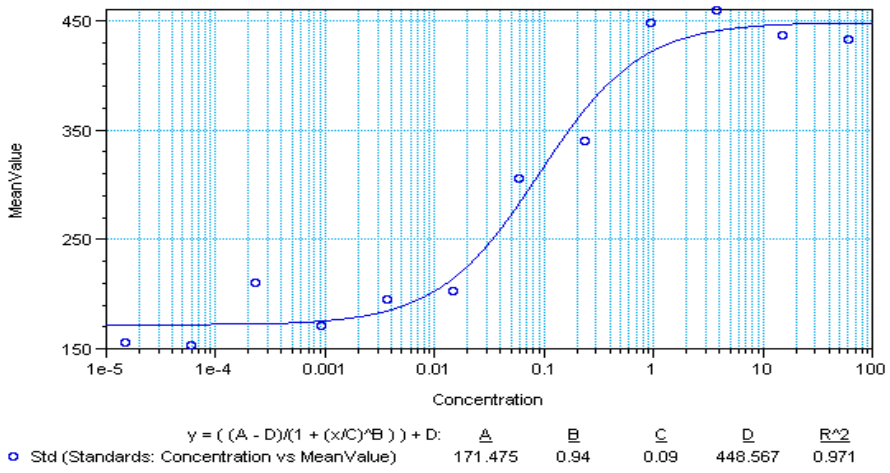


Figure 4.1: Example of a plotted standard curve

Forskolin, 50 µM, was added without/with different concentrations of VPA. The fluorescence in the presence of different concentrations of VPA were compared with vehicle-control group and expressed as percent responses over cAMP level in vehicle-control group. The hippocampal cells treated with cAMP analogue, forskolin, elicited a

robust activation of cAMP production (139.28 ±5.79%, n=13). This was significantly different from the vehicle-control group ($P < 0.01$; $n = 14$). VPA 1 mM suppressed the forskolin-mediated activation to 120.38±3.43%, n=15, P=0.012, compared to forskolin treated group) (Table 4.1 and Table 4.2). This effect was dose dependent with a low concentration of VPA (10 μM) having no discernible effect on cAMP production (Figure 4.2). This result suggests that the effect of VPA on mossy fibre LTP may through an action on cAMP accumulation.

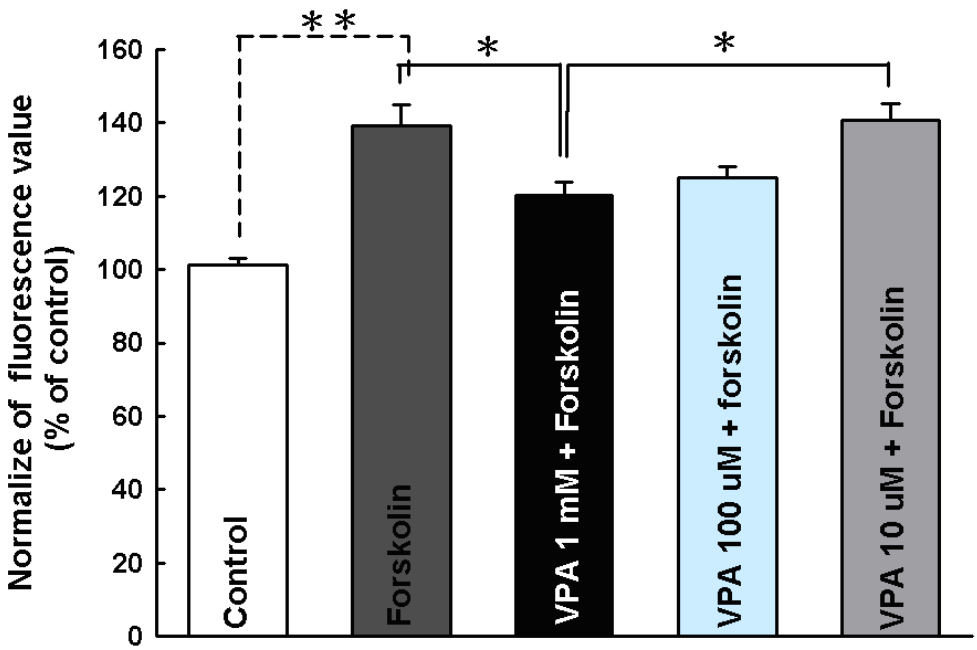


*Figure 4.2: The effect of VPA on cAMP accumulation induced by forskolin (50 μM) in hippocampal cells. Application with forskolin significantly increased the cAMP accumulation. The high concentration of VPA (1mM) significantly suppressed the cAMP accumulation mediated by forskolin. The low concentration of VPA (10 μM) shows a lack of effect on suppression of cAMP accumulation induced by forskolin. Graphs show means ± SEM. Statistical analysis was performed by post hoc test-tukey test, following by ANOVA. * Indicates a significant difference between the two groups (P<0.05), and ** Indicates a significant difference between the two groups (P<0.01).*

Table 4.1: The ANOVA test result of cAMP accumulation with different treatment (Control, forskolin 50μM, VPA 10 μM, VPA 100 μM, and VPA 1mM)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.418	3	0.139	5.781	0.002
Within Groups	1.229	51	0.024		
Total	1.647	54			

Table 4.2: Post-hoc comparison of cAMP accumulation with different treatment by Tukey Test (Control, forskolin 50 μM, VPA 10 μM, VPA 100 μM, and VPA 1mM)

	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Forskolin (50 μM) -VPA (1mM)	0.18900(*)	0.05882	0.012	0.0328	0.3452
Forskolin (50 μM) - VPA (100 μM)	0.14064	0.05882	0.092	-0.0156	0.2969
Forskolin (50 μM) - VPA (10 μM)	-0.01312	0.06214	0.997	-0.1782	0.1519
VPA (1mM) - VPA (100 μM)	-0.04835	0.05668	0.829	-0.1989	0.1022
VPA (1mM) - VPA (10 μM)	-0.20212(*)	0.06012	0.008	-0.3618	-0.0424
VPA (100 μM) - VPA (10 μM)	-0.15376	0.06012	0.063	-0.3134	0.0059

* The mean difference is significant at the .05 level.

4.3.2 The effect of VPA on PKA activity

For this assay, kemptide phosphorylation (see methods) was measured. Adding the PKA inhibitor peptide (1mM) significantly inhibited the activity of PKA (44.32±5.35% of control group n=8, $p<0.01$, compared to control group) (Table 4.3 and Table 4.4). VPA, on the other hand, had no or little effect on the inhibition of PKA activity, even at high concentrations (Figure 4.3). These data indicate that there is no direct inhibitory effect of VPA on PKA phosphotransferase activity.

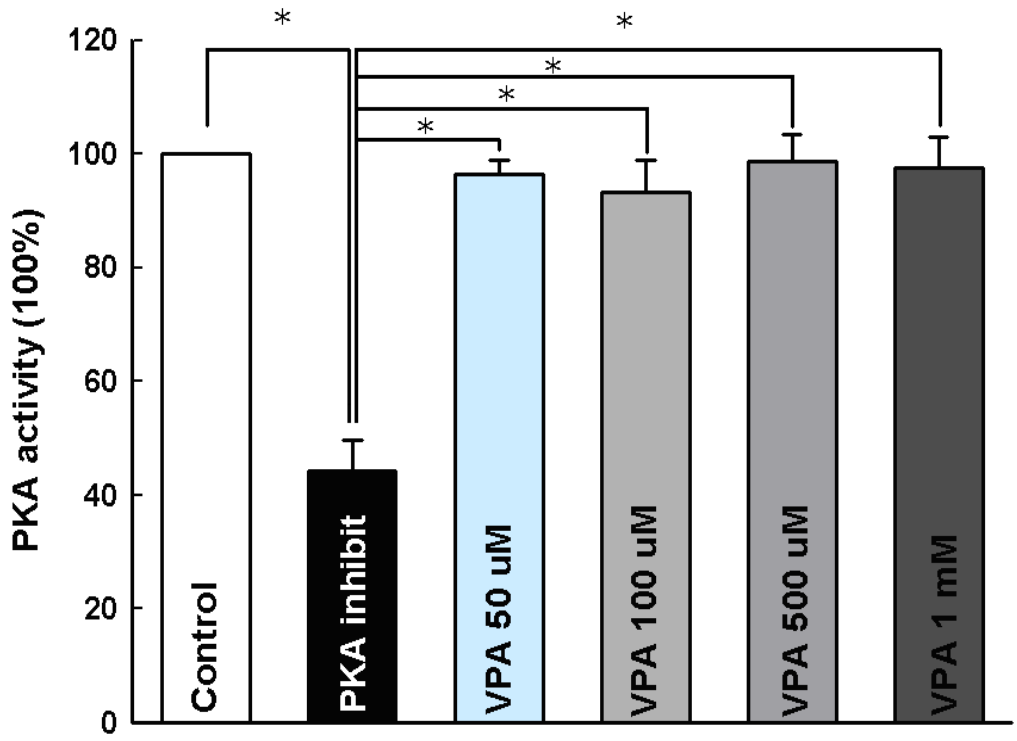


*Figure 4.3: The effect of VPA on PKA activity. In this experiment, the effect of VPA on PKA activity was measured by using kemptide, a selective substrate for PKA. Effect of VPA at different concentrations (VPA 50 µM, VPA 100 µM, VPA 500 µM and VPA 1mM) on PKA activation in PKA assay is compared with control condition. The result shows that VPA does not significantly suppress PKA activity, even at high concentrations (1 mM). Graphs show means ± SEM. Statistical analysis was performed by post hoc test-Tukey test, following by ANOVA. * Indicates a significant difference between the two groups ($P<0.05$).*

Table 4.3: The AVOVA test result of PKA activity with different treatment (Control, PKA inhibit peptide, VPA 50 μM, VPA 100 μM, VPA 500 μM and VPA 1mM)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.886	5	0.377	24.291	0.000
Within Groups	0.652	42	0.016		
Total	2.538	47			

Table 4.4: Post-hoc comparison of PKA activity with different treatment by using Tukey test (Control, PKA inhibit peptide 50 μM, VPA 10 μM, VPA 100 μM, and VPA 1mM)

Comparison	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
Control - PKA inhibit peptide	0.55684(*)	0.06231	0.000	0.3708	0.7428
Control - VPA (50 μM)	0.03558	0.06231	0.992	-0.1504	0.2216
Control - VPA (100 μM)	0.06758	0.06231	0.885	-0.1184	0.2536
Control - VPA (500 μM)	0.01271	0.06231	1.000	-0.1733	0.1987
Control - VPA (1 mM)	0.02398	0.06231	0.999	-0.1620	0.2100
PKA inhibit peptide - VPA (50 μM)	-0.52127(*)	0.06231	0.000	-0.7073	-0.3353
PKA inhibit peptide - VPA (100 μM)	-0.48927(*)	0.06231	0.000	-0.6753	-0.3033
PKA inhibit peptide - VPA (500 μM)	-0.54413(*)	0.06231	0.000	-0.7301	-0.3581
PKA inhibit peptide - VPA (1 mM)	-0.53287(*)	0.06231	0.000	-0.7189	-0.3469
VPA (50 μM) - VPA (100 μM)	0.03200	0.06231	0.995	-0.1540	0.2180
VPA (50 μM) - VPA (500 μM)	-0.02287	0.06231	0.999	-0.2089	0.1631
VPA (50 μM) - VPA (1 mM)	-0.01160	0.06231	1.000	-0.1976	0.1744
VPA (100 μM) - VPA (500 μM)	-0.05487	0.06231	0.949	-0.2409	0.1311
VPA (100 μM) - VPA (1 mM)	-0.04360	0.06231	0.981	-0.2296	0.1424
VPA (500 μM) - VPA (1 mM)	0.01127	0.06231	1.000	-0.1747	0.1973

* The mean difference is significant at the .05 level.

4.4 Discussion

By using biochemical methods to test whether VPA affects the production of cAMP or the activity of PKA, the result showed that VPA decreased the cAMP level in a high concentration whereas there is a lack effect of VPA on PKA activity in lower concentration (10 μM and 100 μM). Besides, there is a lack of an inhibitory effect of VPA on PKA phosphotransferase activity, even at high concentration (VPA 1 mM). This result is consistent with previous work on VPA on PKA activity at lower VPA concentrations (VPA range from 1 to 200 μM) by using kemptide (100 mM) as the substrate for PKA. 2-ene-VPA (2-ene-valproate, 2-propyl-2-pentenoic acid), which is the major VPA metabolite in humans, has a lack of an inhibitory effect on PKA phosphotransferase activity at concentrations ranging from 100 to 400 μM (Mirnikjoo et al., 2001). These results suggest that the mechanism of VPA involves modulation of cAMP accumulation induced by application with forskolin, but there is no direct effect on PKA. The effect on cAMP could be secondary to an effect on phosphodiesterases or adenylyl cyclase (my experiments do not distinguish these possibilities).

In the experiment of using the cAMP assay, the hippocampal cells were acquired from newborn rat by using primary hippocampal cell culturing methods. Primary hippocampal cell culturing, a well developed method, is used by neuroscientists to examine the activity and properties of neurons at the individual cell and single synapse level. The hippocampus may be isolated from each newborn animal in as short as 2 to 3 minutes, and the cultures can be maintained for up to two weeks. One possible confounder is that the data acquired from hippocampal cells from newborn rats may be different from adult hippocampus. Indeed, it has been shown that adenylyl cyclase system in the rat brain is more susceptible to aging processes. For example, [3H] forskolin binding shows a conspicuous reduction in various brain areas in 18-month-old

rats, compared to adult animals. In 24-month-old rats, the reduction of [3H]forskolin binding was observed in the cerebral cortex, hippocampal CA3 pyramidal cell layer, dentate gyrus, thalamus and molecular layer of cerebellum (Araki et al., 1995). This may partly provide an explanation that the enhancement of cAMP accumulation by application of forskolin is 139.28 ±5.79% of control, whereas the enhancement of mossy fibre response induced by application of forskolin is 458.56±53.33%. This may also be explained by a non-linear relationship between cAMP concentration and neurotransmitter release. Despite differences in absolute enzyme activity between immature brain and adult brain, this assay still provides a qualitative insight into the action of VPA on modulation of cAMP accumulation.

Recent studies in the rat prefrontal cortex have shown that 48 hours of VPA treatment reduced β-adrenergic receptor activation of cAMP production and direct adenylyl cyclase stimulation via forskolin (Montezinho et al., 2006;Montezinho et al., 2007). However, it was not clear from these experiments if this occurred through transcriptional regulation, or if this effect was adenylyl cyclase isoenzyme specific. One recent report has suggested that VPA works through elevated phosphodiesterase activity in addition to reduced adenylyl cyclase activity, in C6 glioma cells (Gallagher et al., 2004). In another study, chronic treatment of these cells with VPA for 6 days showed reduced basal, and forskolin-induced cAMP production, in both intact cells and cell membranes (Gallagher et al., 2004;Chen et al., 1996). The data shown here suggest, for the first time, an acute biochemical action of VPA in synaptic signalling. Due to the rapid timing of these effects, they are therefore independent of teratogenic (transcription-regulated) effects of VPA.

VPA shows a powerful neuroprotective effects *in vitro* and *in vivo* model of various degenerative disorders; however, the mechanism is still unclear. It has been suggested that VPA up-regulates the activation of ERK1/2 through an inhibitory effect on PKA pathways in Dictyostelium discoideum (Boeckeler et al., 2006). Other studies also provide evidence that VPA increases phosphorylation of ERK1/2 (Yuan et al., 2001;Boeckeler et al., 2006). ERK1/2 is a member of mitogen activated protein kinases which can be activated by various growth factors and cAMP-signalling pathway (Vossler et al., 1997;Hetman and Gozdz, 2004). Therefore, VPA's activity on the cAMP/PKA pathway may also contribute to its neuroprotective effect.

This action of VPA on the cAMP/PKA signalling system may also contribute to other effects of VPA on receptors and cellular pathways. GABA(A) and GABA(B) receptors are modulated by PKA mediated phosphorylation (Moss et al., 1992;Couve et al., 2002). The cAMP/PKA signalling system also modulates Na^+ channel slow inactivation (Chen et al., 2006). Moreover, cAMP/PKA signalling can interact with other signalling pathways, such as the calmodulin-dependent protein kinase cascade (DeBernardi and Brooker, 1996) and the PI3K pathway (Namkoong et al., 2009), possibly contributing to the diverse pharmacological actions of VPA.

Chapter 5: The mechanisms of action of VPA related to cAMP/PKA signalling in *in vitro* epilepsy models

5.1 Introduction

There is considerable evidence that cAMP/PKA activity has an impact on the development of seizure activity in both *in vivo* and *in vitro* models.

The level of cAMP is significantly increased in epileptogenic cortical foci produced by freezing and application of penicillin (Walker et al., 1973; Krivánek and Mares, 1977). Repeated injections of subconvulsive doses of cAMP into rats' amygdale produced progressive seizure development (Kihara et al., 1989). In *in vitro* seizure models (PTZ model and an electrical stimulation model) in cortical slices, cAMP accumulation is also observed (Lewin et al., 1976;Kakiuchi et al., 1969). Furthermore, activation of adenylyl cyclase with forskolin or inhibition of phosphodiesterase with rolipram enhanced the ictal-like afterdischarges (ADs) induced by high-frequency stimulation in rat hippocampal slices (Higashima et al., 2002). In epileptic patients, the cAMP concentration in the cerebrospinal fluid is also elevated after a seizure (Myllylä et al., 1975).

PKA is one of the primary targets of cAMP. PKA has been proposed to contribute to the development of epilepsy. In rats which are prone to epilepsy, PKA activity increases in neocortex and hippocampus (Yechikhov et al., 2001). Activation of the cAMP/PKA signalling system increases excitability and enhances epileptiform activity in rat hippocampal slices and human dentate gyrus (Boulton et al., 1993;Higashima et al., 2002;Chen and Roper, 2003). It has also been shown that application of forskolin can block endogenous antiepileptic processes; moreover, activation of PKA can prolong epileptic activity and induce status epilepticus (Üre and Altrup, 2006).

In previous chapters, it has been demonstrated that VPA decreases the enhancement of mossy fibre response either induced by electrical stimulation, or through application of forskolin. Moreover, in hippocampal cells, VPA decreased cAMP accumulation after forskolin application. These experiments indicate that VPA can modulate cAMP/PKA signalling through decreasing cAMP accumulation. Does this explain or contribute to the anticonvulsant action of VPA? To address this, well-established *in vitro* seizure models were used: PTZ model and low magnesium model. The advantage of using these two *in vitro* acute seizure models is that they are both sensitive to VPA , but are induced by different methods (Armand et al., 1998). Efficacy in both, therefore, adds weight to the antiepileptic effects of a drug.

PTZ-model: Exposure of hippocampal slices (in an interface chamber) to PTZ results in the appearance of spontaneous interictal-like discharges in CA1 and CA3 subfields (Piredda et al., 1985;Leweke et al., 1990). Similar results can be obtained with submerged slices treated with PTZ once extracellular K^+ concentration is increased (Heinemann et al., 1977). However, it has also been shown to increase voltage-gated potassium conductances, permitting rapid action potential firing (Madeja et al., 1996). In addition, PTZ induces calcium releases from calcium stores, and increases intracellular calcium, contributing to the generation of bursting activity (Sugaya and Onozuka, 1978).

Low Mg^{2+} model: application of low Mg^{2+} aCSF generates rhythmic short recurrent discharges activity in CA1 and CA3 area in hippocampus in rats (Mody et al., 1987). It has been suggested that low-Mg^{2+}-induced seizure-like events originate from the entorhinal cortex and propagate towards the temporal neocortex and the hippocampal

formation (Buchheim et al., 2000). This may be due to enhancement of NMDA receptor currents by releasing the NMDA receptor from the inhibitory action of Mg^{2+} (Mody et al., 1987;Senatorov et al., 1995).

Indeed there are limitations of using *in vitro* slice models of acute seizure activity, such as the massive loss intrinsic and extrinsic connections; furthermore the main problem of using *in vitro* slice models is interpretation of the result obtained in epileptogenic condition in intact system. However, slice models can provides useful information about seizure activity modification by application of drugs.

In this study, the aims are to investigate whether cAMP/PKA signalling is involved in epileptiform activity, and whether the effect of VPA on modulation of cAMP/PKA signalling contributes to the anticonvulsant action of VPA. In order to distinguish the role of cAMP and PKA in epileptiform activity, H89 (an inhibitor of protein kinase A) and SQ22536 (a cell-permeable adenylyl cyclase inhibitor) were used during the experiments. Further, in order to elucidate whether the anticonvulsant action of VPA may be via modulation of cAMP/PKA signalling system, pentenoic acid, a VPA analogy lacking anticonvulsant effect, was used as an experimental control.

5.2 Methods

Combined horizontal entorhinal cortex-hippocampus slices from male Sprague-Dawley rats were used in these *in vitro* epilepsy models. The method of induction of epileptiform discharges was modified from previously described protocols (Heinemann 1994, 1998). During the experiment, the slices were transferred from an interface chamber to a submersion recording chamber and continuously perfused with prewarmed

(about 33 °C) oxygenated (95% O_2, 5% CO_2) aCSF for one hour prior to the start of recording. fEPSP recording was made by placing a glass microelectrode filled with aCSF solution in stratum radiatum of CA1. A bipolar stimulating electrode was positioned in the Schaffer collateral/commissural fibre pathway in stratum radiatum to confirm slice viability. Following stimulation of Schaffer collateral/commissural pathway, a clear field response at the pyramidal cell layer was observed. This field response consisted of positive waves, the population excitatory post-synaptic potential (pEPSP), and a brief negative wave, the population spikes (PS) (Figure 5.1).

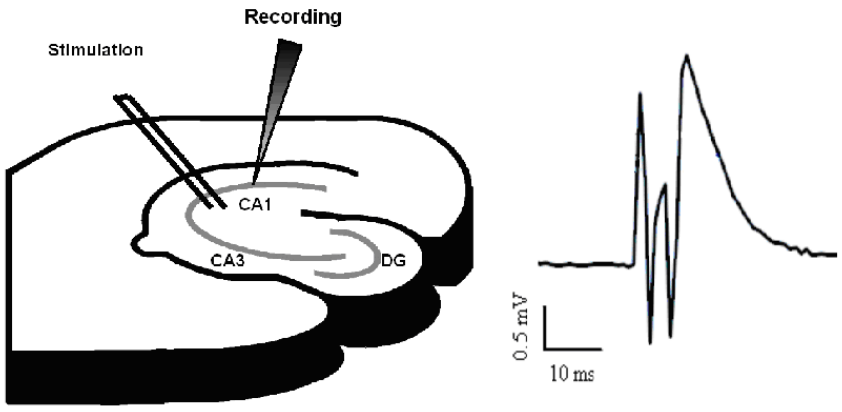


Figure 5.1: The arrangement of electrodes in hippocampus slices for in vitro epilepsy model. Left, the position of electrodes for recording was placed in CA1 pyramidal cell, and stimulation electrode was placed in Schaffer collateral/commissural fibre pathway in combined horizontal entorhinal cortex-hippocampus slices. Right, the fEPSP response in CA1 was evoked by stimulation Schaffer collateral/commissural fibre pathway. This waveform of a recording from the stratum pyramidale is initially a positive-going fEPSP that at times displayed a downward population spike.

Pentylenetetrazole model: PTZ (2 mM) (sigma) was added to the perfusate and K^+ was increased to 6 mM in order to induce epileptiform activity. Drugs were applied once the frequency and amplitude of the epileptiform discharges were stable for at least 10 minutes. Anticonvulsant effects were evaluated by measuring the variation of frequency of the discharges every minute.

Low-Mg^{2+} Model: Low Mg^{2+}-induced epileptiform activity was elicited by using Mg^{2+}-free aCSF. After the short discharges in hippocampal area CA1 were stable for at least 10 minutes, drugs were applied for 40 minutes. Discharge frequency was measured every minute.

All the drugs were applied by bath perfusion. VPA (1mM) (sigma), pentenoic acid (1mM) (sigma), H89 (10 μM) (sigma) were dissolved in distilled water, and SQ 22536 (100 μM) (sigma) was dissolved in DMSO. All the drugs were prepared as 1000 times stocks and stored at -20°C. All drugs were directly dissolved in aCSF to achieve their final concentrations during experiments. The concentration of SQ22536 (100 μM) and H89 (10 μM) were chosen to have a maximal but specific effect in the hippocampus (Otmakhova and Lisman, 1998;Komagiri and Kitamura, 2003;Alle et al., 2001;Huang et al., 2002).

The baseline consisted of the average discharge frequency over 10 minutes prior to drug application. The discharge frequency was then averaged every 5 minutes during the experiment and normalised to baseline. All data were expressed as mean±SEM. The mean discharge frequencies were compared by one way ANOVA and tukey test as post hoc test. Differences were considered as significant at $P < 0.05$.

5.3 Results

5.3.1 The role of cAMP/PKA signalling system and the effect of VPA in PTZ model

PTZ-induced positive field potentials appeared 10-30 minutes after application of PTZ plus increase of [K^+] (to 6 mM) (Figure 5.2). The epileptiform discharge consisted of a positive field potentials on which population spikes were superimposed. At steady state, the mean frequency of recurrent bust was 14.92± 0.84 per minute, n=25.

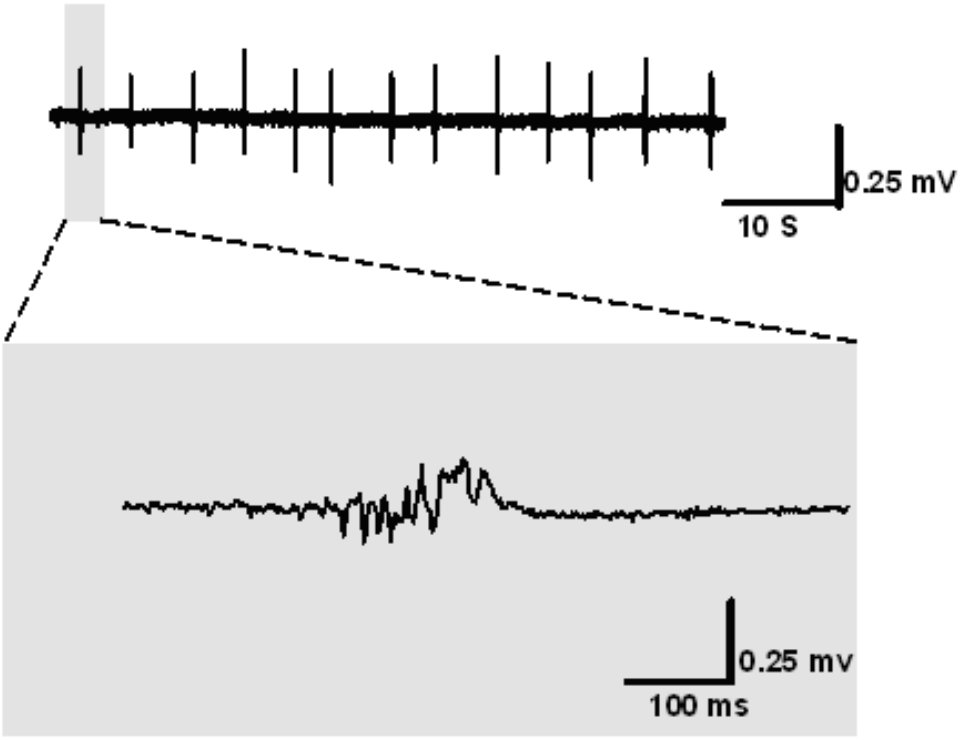


Figure 5.2: Examples of CA1 pyramidal cell layer responses following PTZ application. Lower panel illustrates the trace on an expanded time scale.

Application of VPA (1 mM) significantly suppressed the frequency of burst discharges in this PTZ model to 75.06±1.68 % of control in CA1 region at 30-40 minutes after application (P<0.01) (Figure 5.3 and Figure 5.5). In contrast, application of pentenoic acid (1 mM), a straight chain analogue of VPA, only had a slight effect on the frequency of burst discharges (90.28± 2.01% of baseline) (Table 5.1 and Table 5.2)

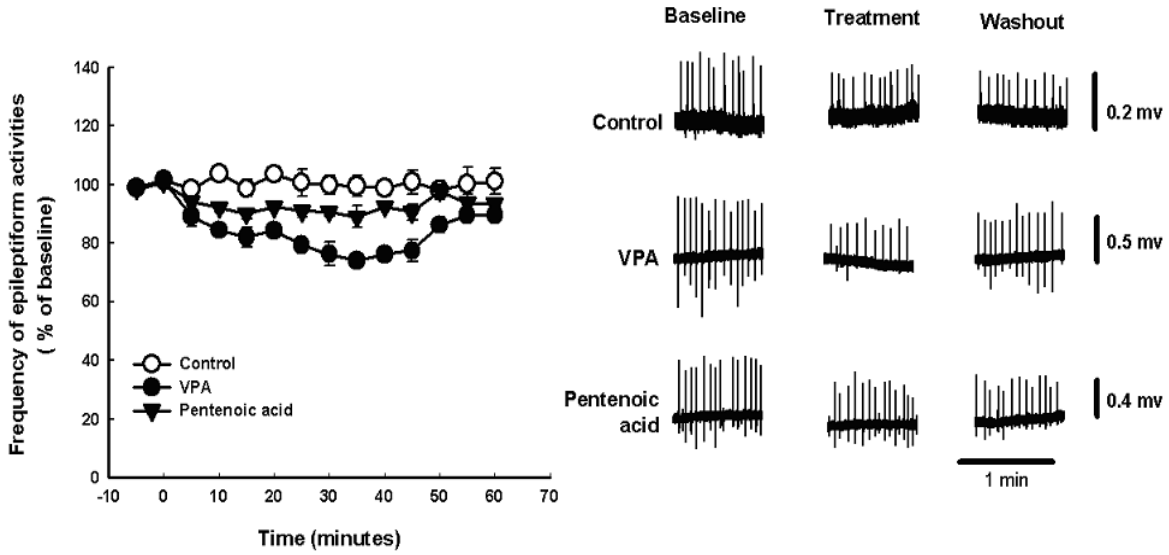


Figure 5.3: Summary of the change in the frequency of PTZ-induced burst discharges following application of VPA and pentenoic acid. The frequency of PTZ-induced burst discharges plotted against time. Drugs were applied between 0-40 minutes. Examples of trace recordings of PTZ-induced burst discharges are in right panels. Top-the trace recordings obtained in control condition; the middle-trace recordings obtained before, during and after application of VPA (1 mM); the lower- trace recordings obtained before, during and after application of pentenoic acid (1mM).

In order to address the question of whether cAMP/PKA signalling can directly influence the burst discharges in PTZ model, H89 (10 μM), a specific PKA inhibitor, was applied. Application of H89 had a non-significant effect on the frequency of epileptiform activity (88.47± 4.25% of baseline) (Figure 5.4 and Figure 5.5). Surprisingly, application of SQ 22536 (100 μM), an adenylyl cyclase inhibitor, significantly increased the frequency of burst activity to 127.39± 3.45 %, n=5, in 30-40 minutes after application in this PTZ model (P<0.01, compared to control group) (Table 5.1 and Table 5.2). These effects were reversible after wash out (Figure 5.4).

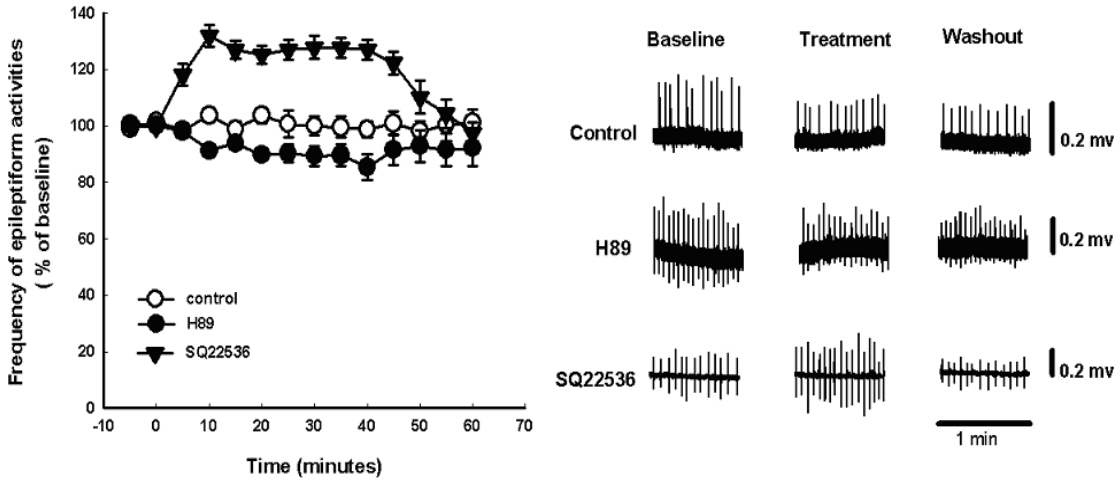


Figure 5.4: Summary of the changes in the frequency of PTZ-induced burst discharges following application of H89 and SQ22536. The frequency of PTZ-induced burst discharges plotted against time. Drugs were applied between 0-40 minutes. Example traces are illustrated in the right-hand panels. Top-the trace recordings obtained in control condition; the middle-trace recordings obtained before, during and after application of H89 (10 μM); the lower- trace recordings obtained before, during and after treatment with SQ22536 (100 μM).

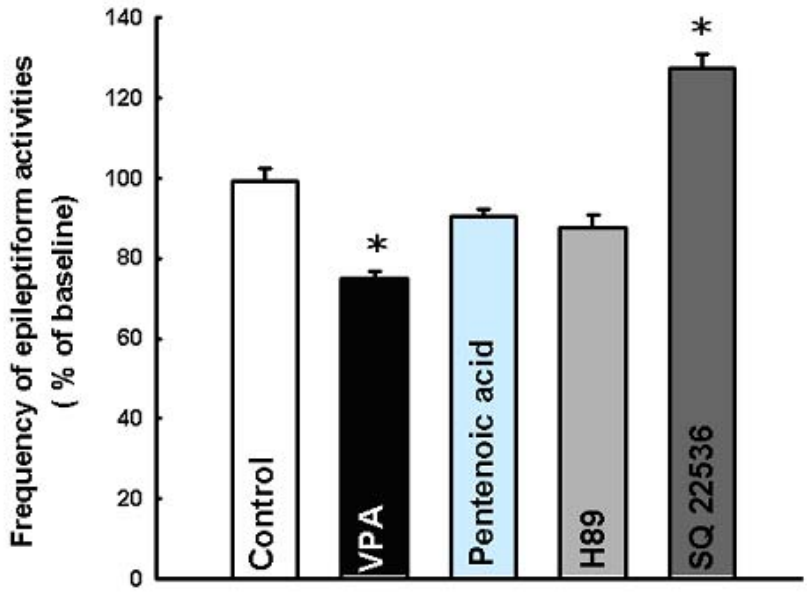


*Figure 5.5: Comparison of the mean frequency of PTZ-induced burst discharges with different treatments. Each column shows the mean value of the frequency of PTZ-induced burst discharges expressed as a percentage of the baseline value. Graphs show means ± SEM. Statistical analysis was performed by post hoc test-Tukey test, following by ANOVA. * Indicates a significant difference (P<0.05), compared to control group.*

Table 5.1: The result of ANOVA analysis with different treatment group (Control condition, VPA 1mM, pentenoic acid 1mM, SQ22536 100 μM, H89 10 μM) the main factor in PTZ model.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.762	4	0.190	40.664	0.000
Within Groups	0.094	20	0.005		
Total	0.855	24			

Table 5.2: Post-hoc comparison of different treatments in PTZ model by Tukey test

	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Control VS. VPA (1 mM)	0.24121(*)	0.04328	0.000	0.1117	0.3707
Control VS. Pentenoic acid (1 mM)	0.08912	0.04328	0.276	-0.0404	0.2186
Control VS. H89 (10 μM)	0.10725	0.04328	0.136	-0.0223	0.2368
Control VS. SQ22536 (100 μM)	-0.28203(*)	0.04328	0.000	-0.4115	-0.1525
VPA (1 mM) VS. Pentenoic acid (1 mM)	-0.15209(*)	0.04328	0.017	-0.2816	-0.0226
VPA (1 mM) VS. H89 (10 μM)	-0.13396(*)	0.04328	0.040	-0.2635	-0.0045
VPA (1 mM) VS. SQ22536 (100 μM)	-0.52324(*)	0.04328	0.000	-0.6527	-0.3937
Pentenoic acid (1 mM) VS. H89 (10 μM)	0.01813	0.04328	0.993	-0.1114	0.1476
Pentenoic acid (1 mM) VS. SQ22536 (100 μM)	-0.37114(*)	0.04328	0.000	-0.5006	-0.2416
H89 (10 μM) VS. SQ22536 (100 μM)	-0.38927(*)	0.04328	0.000	-0.5188	-0.2598

* The mean difference is significant at the .05 level.

Although non-significant, there may be a small effect of H89, to address whether the effect of VPA on PKA system contributes to the efficacy of VPA in this model, the efficacy of VPA in the presence of H89 (10 μM) was tested. As shown in Figure 5.6, VPA (1mM) suppressed the frequency of bursts to75.24±3.89% of baseline, n=6, $p<0.01$ compared to baseline by paired-sample t test). However, the effect of VPA on suppression of frequency of burst activity was unaffected by the presence of H89 (without H89: 75.06±1.68 % n=5; with H89: 75.24±3.89%, n=6), indicating that the anticonvulsant effect of VPA is not through inhibition of PKA activity.

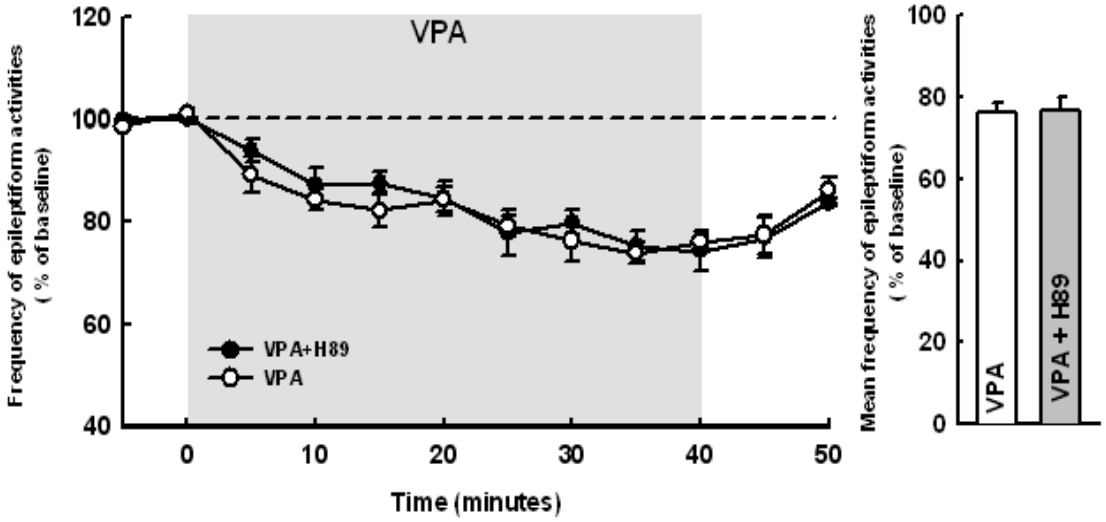


Figure 5.6: Summary data for the effect of VPA (1 mM) on frequency of PTZ-induced burst discharges in area CA1 recorded from the stratum pyramidale without or without H89 (10 μM). The frequency of PTZ-induced burst discharges plotted against time. Drugs were applied between 0-40 minutes. Comparison of the mean frequency of PTZ-induced burst discharges showed on the right panel. There is no significant difference on the effect of VPA in the frequency of PTZ-induced burst discharges in the present without or without H89.

5.3.2 The role of cAMP/PKA signalling system and the effect of VPA in low Mg^{2+} model

The anticonvulsant action of VPA and modulation of the cAMP/PKA signalling system was further tested in a separate *in vitro* epilepsy model induced by Mg^{2+} free aCSF in combined entorhinal- hippocampal slices.

Omission of Mg^{2+} from the perfusate induced epileptiform recurrent short discharges in CA1 area after 20-40 minutes. After about one hour, the epileptiform discharges attained a stable rate of 3.16±0.29 per minutes, n=25. Each discharge comprised 2 to 5 population spikes on a positive filed potential (Figure 5.7).

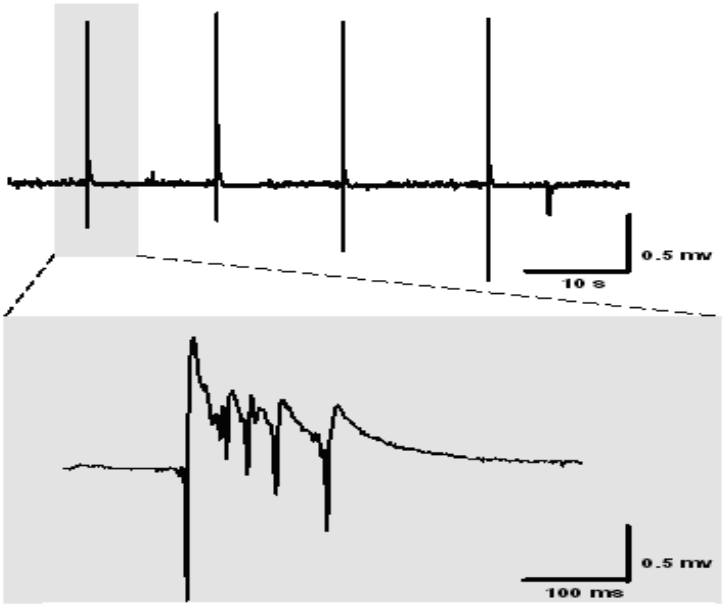


Figure 5.7: Examples of pyramidal cell layer responses evoked by low-Mg^{2+} aCSF application. Application of low-Mg^{2+} aCSF induced epileptiform activity- recurrent short discharges. Lower panel illustrates expanded time scale. The epileptiform activity evoked by low-Mg^{2+} aCSF application is comprised of population spikes

VPA (1 mM) suppressed the frequency of recurrent short discharges to 72.65±3.59 % of baseline, n=5 at 30-40 minutes after application (p=0.033, compared to control) (Table 5.3 and Table 5.4) and this effect is reversible after wash out. Application of pentenoic acid, in contrast, has no significant effect on the frequency of recurrent short discharges in 30-40 minutes after application (92.51± 6.49 % of baseline, respectively, n=5) (Figure 5.8 and Figure 5.10).

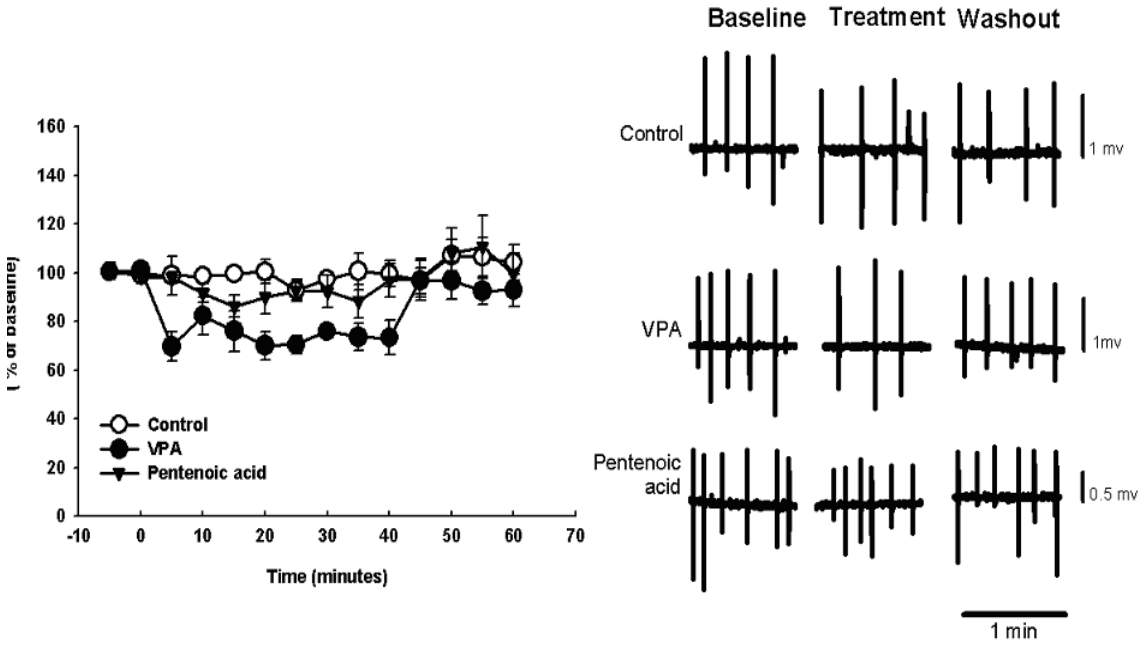


Figure 5.8: Summary of the change of frequency of low Mg^{2+}-induced epileptiform discharges in area CA1 recorded by application with VAP and pentenoic acid. Right panel shows the frequency of epileptiform activity induced by low Mg^{2+} aCSF plotted against time. Drugs were applied between 0-40 minutes. Right panel shows the example of the trace recording of low Mg^{2+}-induced epileptiform discharges in area CA1. Top- the trace recordings obtained in control condition; the middle-trace recordings obtained before, during and after application of VPA (1 mM); the lower- trace recordings obtained before, during and after application of pentenoic acid (1 mM).

Similar to the PTZ model, application of H89 has no significant effect on the frequency of burst discharges (100.38± 4.38 %, n=5). Application of SQ22536 significantly increased the frequency of epileptiform discharges repetition in 30-40 minutes after treatment (149.99±8.60% of baseline, n=5, $p<0.01$ compared to control) (Table 5.3 and Table 5.4). The effect of SQ22536 on epileptiform activity was reversible after washout (Figure 5.9 and Figure 5.10).

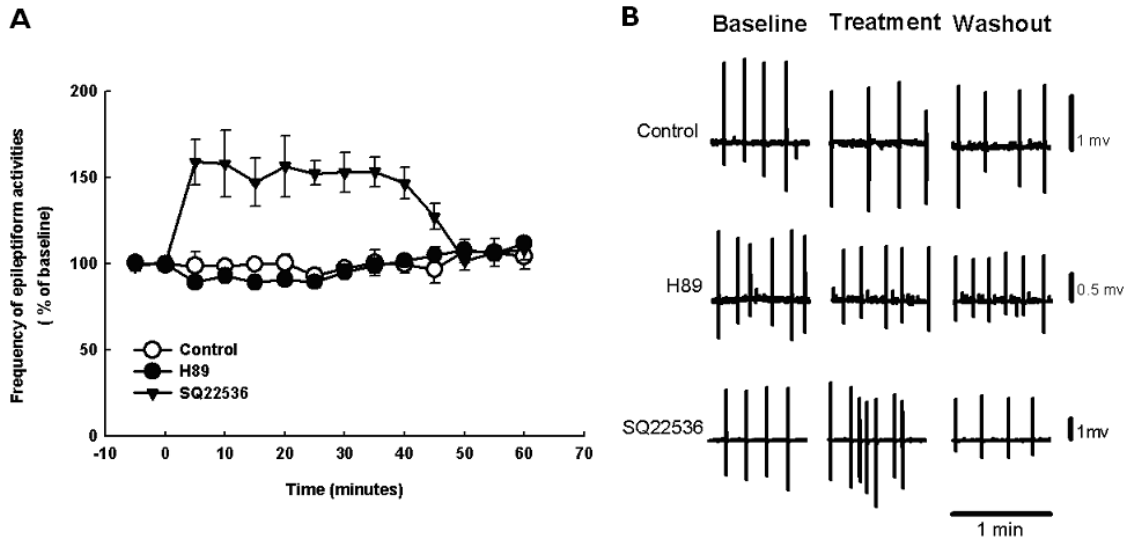


Figure 5.9: Effect of cAMP/PKA signalling system on epileptiform activity induced by low Mg^{2+} aCSF in combined rat entorhinal cortex-hippocampus slices. (A) *Summary the data for the change of frequency of low Mg^{2+}-induced epileptiform discharges in area CA1 recorded from. The frequency of epileptiform activity induced by low Mg^{2+} aCSF plotted against time. Drugs were applied between 0-40 minutes.* (B) *Sample trace recordings of low Mg^{2+}-induced epileptiform discharges. Top-the trace recordings obtained in control condition; the middle-trace recordings obtained before, during and after application of H89 (10 μM); the lower- trace recordings obtained before, during and after application of SQ22536 (100 μM).*

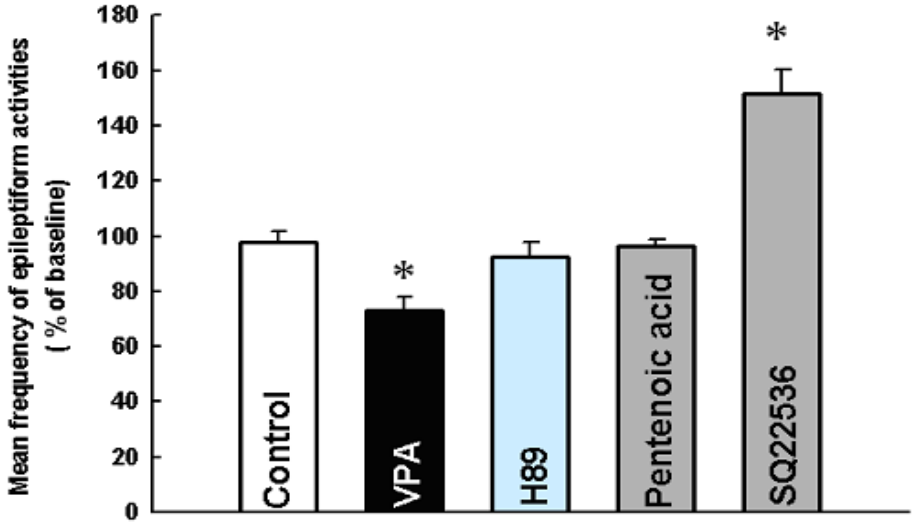


*Figure 5.10: Comparison of the mean frequency of epileptiform activity with different treatments. Each column shows the mean value of the frequency of epileptiform- activity expressed as a percentage of the baseline value. N =5 for each drug. Graphs show means ± SEM. Statistical analysis was performed by post hoc test-Tukey test, following by ANOVA. * Indicates a significant difference (P<0.05).*

Table 5.3 The ANOVA result in for effect of treatment (Control condition, VPA 1mM, pentenoic acid 1mM, SQ22536 100 μM, H89 10 μM) in low Mg^{2+} model.

	Sum of	df	Mean Square	F	Sig.
Between Groups	1.627	4	0.407	21.851	0.000
Within Groups	0.372	20	0.019		
Total	2.000	24			

Table 5.4: Multiple comparisons of treatments in low Mg^{2+} model by Tukey-test.

	Mean Difference	Std. Error	Sig.	95%	
				Lower	Upper
Control VS. VPA (1mM)	0.27517(*)	0.08630	0.033	0.0169	0.5334
Control VS. Pentenoic acid (1mM)	0.07658	0.08630	0.898	-0.1817	0.3348
Control VS. H89 (10 μM)	-0.00218	0.08630	1.000	-0.2604	0.2560
Control VS. SQ22536 (100 μM)	-.49828(*)	0.08630	0.000	-0.7565	-0.2400
VPA (1mM) VS. Pentenoic acid (1mM)	-0.19859	0.08630	0.186	-0.4568	0.0596
VPA (1mM) VS. H89 (10 μM)	-0.27735(*)	0.08630	0.032	-0.5356	-0.0191
VPA (1mM) VS. SQ22536 (100 μM)	0-.77345(*)	0.08630	0.000	-1.0317	-0.5152
Pentenoic acid (1mM) VS. H89 (10 μM)	-0.07876	0.08630	0.889	-0.3370	0.1795
Pentenoic acid (1mM) VS. SQ22536 (100 μM)	-0.57486(*)	0.08630	0.000	-0.8331	-0.3166
H89 (10 μM) VS. SQ22536 (100 μM)	-0.49610(*)	0.08630	0.000	-0.7543	-0.2379

* The mean difference is significant at the .05 level.

5.4 Discussion

In this study, the question whether the effect of VPA on modulation of cAMP/PKA signalling system could explain the antiepileptic effect of VPA was asked. I took advantage of two acute *in vitro* seizure models, PTZ model and low Mg^{2+} model, in which VPA has previously been shown to be effective (Armand et al., 1998;Sokolova et al., 1998). By using these two well-established models, it showed that VPA has a significant depressive effect on epileptiform activities, whereas pentenoic acid lacks effect. Furthermore, the effect of VPA on depression of frequency of burst spikes is not directly through modulation PKA. In these experiments, it shows that application of SQ22536 significantly increased the frequency of bursts in these two models, whereas H89, a PKA inhibitor, had no significant effect, and could not occlude the effects of VPA.

These experiment, combined hippocampal-entorhinal-cortex slice was used, instead of isolated hippocampal slices. Unlike isolated hippocampal slices, hippocampal-entorhinal cortex slices preserve the connectivity between the entorhinal cortex and hippocampus. The entorhinal cortex is the major source of afferents to the hippocampus via the perforant path. The hippocampal-entorhinal cortex slice therefore enables recurrent excitation between the hippocampus and entorhinal cortex and so is better able to maintain epileptiform activity.

Alterations in neuronal metabolism plays an important role in epileptogenesis (Arabadzisz et al., 2005;Raza et al., 2004) and protein phosphorylation has been reported to increase in status epilepticus (Funke et al., 1998;Moussa et al., 2001). There

is also considerable evidence that potentiating the production of cAMP increases epileptic activity (Higashima et al., 2002; Üre and Altrup, 2006), and that cAMP production increases with seizure activity (Hattori et al., 1987;Hattori et al., 1993). This and the persistent increase in the expression level of type II adenylyl cyclase mRNA after generalized seizure (Iwasa et al., 2000) has led to the proposal that increases in cAMP and/or activation of PKA have a proepileptic effect. Since a robust action of VPA on cAMP/PKA signalling and a lack of effect of pentenoic acid a compound that has no antiepileptic activity *in vivo* was observed (Chapman et al., 1983;Keane et al., 1983), the question whether this could explain the antiepileptic effect of VPA was asked. The advantage of two acute *in vitro* seizure models in which VPA has previously been shown to be effective were taken (Armand et al., 1998;Sokolova et al., 1998).

Consistent with previous studies, the result was observed an antiepileptic effect of VPA; importantly, pentenoic acid had no effect in these models, consistent with the hypothesis. To further test this hypothesis, the effects of decreasing cAMP production through inhibition of adenylyl cyclase with SQ22536 or inhibition of PKA activity with H89 were tested. Inhibition of PKA activity had no effect on seizure activity and did not occlude the effects of VPA, indicating that VPA was reducing epileptiform activity through a different mechanism. The results do not imply that the action of VPA on adenylyl cyclase/PKA has no antiepileptic effect, but that this action cannot explain VPA's efficacy in these slice models

Surprisingly, inhibition of adenylyl cyclase with SQ22536 increased the epileptiform activity. One possible explanation, which was hypothesised by Ferrendelli et al. (1980),

is that accumulation of cAMP may be involved in mechanisms of inhibiting the spread and/or duration of seizure discharges but not in seizure induction. Pre-treatment with drugs decreasing accumulation of cAMP resulted in augmented seizure severity (Gross and Ferrendelli, 1979). The role of cAMP is further confounded by the observation that under some circumstances cAMP analogues can have an antiepileptic effect (Apland et al., 1997). This suggests a biphasic effect in which increases in cAMP (such as following administration of forskolin), and marked reductions in cAMP levels can both promote seizure activity.

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Chapter 6: The effect of VPA on mossy fibre response in status epilepticus rat

6.1 Introduction

As detailed in chapter 1, there are numerous mechanisms that contribute to the development of epilepsy. In addition to the development of epilepsy, there are changes that occur in synaptic plasticity, in particular the occurrence of metaplasticity- a change in the ability of the system to undergo synaptic plasticity (Goussakov et al., 2000). This has been observed at the mossy fibre to CA3 synapse; the ability to elicit mossy fibre LTP is compromised in post status epilepticus rats (Goussakov et al., 2000). This is associated with a decrease in paired-pulse facilitation lasting at least several weeks after SE; this implies that release probability at this synapse is increased during epileptogenesis so that LTP cannot occur (Goussakov et al., 2000). One possible explanation is that the cAMP/PKA signalling system is overstimulated during epileptogenesis, so occluding mossy fibre LTP. If this is the case, then VPA should have an effect on baseline transmission at the mossy fibre-CA3 synapse in epileptic rats. In this chapter, this hypothesis is set up to be tested.

The pilocarpine model of epilepsy was used to address this hypothesis. There are a number of advantages of using the pilocarpine model of epilepsy to address the effect of VPA on the mossy fibre -CA3 response following SE. Unlike the electrical stimulation model in which electrodes are implanted into brain, the pilocarpine model of epilepsy does not require implantation or prior anaesthesia, reducing both animal suffering and experimental time. More importantly, the pilocarpine model is a well characterised chronic epilepsy model which shares many of the clinical and pathophysiological characteristics of partial-complex or temporal-lobe epilepsy in humans (Babb et al., 1991;Houser, 1990;Strowbridge et al., 1992;Sutula et al., 1989), such as hippocampal sclerosis, mossy fibre sprouting (Mello et al., 1993), neuronal hyperexcitability (Isokawa and Mello, 1991), altered GABA(A) receptor function (Rice et al., 1996), and

interictal spike patterns on EEG (Leite et al., 1990).

Administration of pilocarpine to rats results in progressive worsening seizures, leading to long-lasting SE (Turski et al., 1989). The behavioural and EEG alterations, which are induced by administration pilocarpine in rats, are similar to those observed in human temporal lobe epilepsy (Cavalheiro et al., 1996;Cavalheiro, 1995;Curia et al., 2008). After onset of SE, pilocarpine-treated animals enter seizure-free period, which is known as the latent period. During the latent period, pilocarpine-treated animals generally show normal behaviour and EEG activity. Recurrent spontaneous seizure occurs after latent period.

In this model, days to weeks following SE, animals begin having spontaneous recurrent seizures (SRSs) and continue to have seizures for the lifetime of the animal (Mello et al., 1993;Priel et al., 1996). Thus, the pilocarpine model with its similarities to human epilepsy provides a relevant model to investigate the molecular mechanisms underlying the transition from a normal to an epileptic brain following a brain insult such as SE.

Using the pilocarpine model, the question whether the effect of VPA on the mossy fibre -CA3 response was altered following SE was asked.

6.2 Methods

6.2.1 Seizure induction

Details of the experimental procedure are described in general methods chapter. In brief, adult male Sprague–Dawley rats were administrated scopolamine methyl nitrate (1 mg/kg, ip), 20 min before and after induction of SE by injection of pilocarpine (320

mg/kg). Seizures were monitored behaviourally. Seizures were terminated with diazepam (10 mg/kg, ip) 90 min after the injection of pilocarpine, and diazepam dosing was repeated as necessary to prevent recurrence of seizure behaviour. Following termination of seizures, all animals were kept hydrated with subcutaneous saline. Only animals that displayed continuous convulsive seizure activity reached stage three were used in the experimental ‘‘seizure’’ group.

This protocol results in animals progressing on to spontaneous recurrent seizures (Mello et al., 1993) (Figure 6.1). and optimises the number of animals developing epilepsy against mortality from SE (Curia et al., 2008).

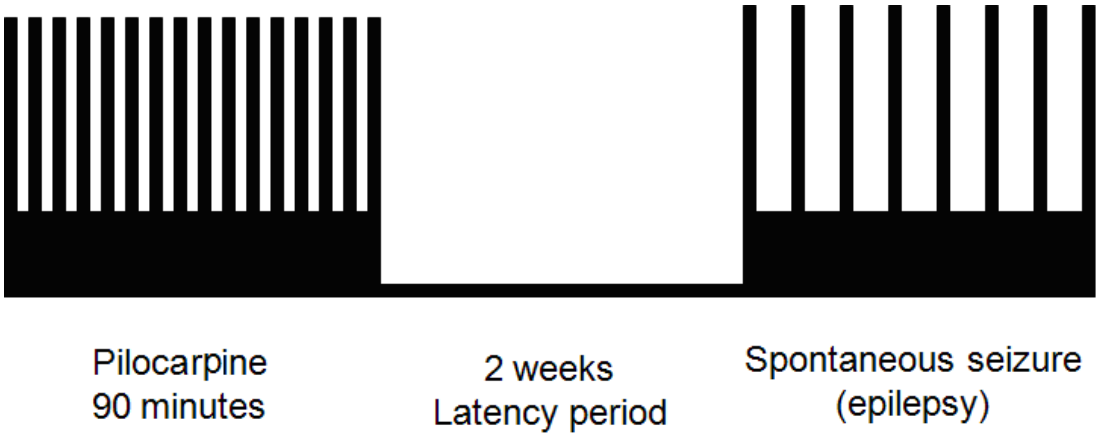

Figure 6.1: The progress of developing epilepsy in pilocarpine model.

6.2.2 Electrophysiology

Two weeks after induction of epilepsy by administration of pilocarpine, the rats were decapitated after killing by injection with an overdose of pentobarbitone (500 mg/kg, ip). Brains were removed and preserved in oxygenated ice-cold sucrose solution. Transverse slices (350 μm) were prepared and were then stored in an interface chamber containing artificial cerebrospinal fluid solution (aCSF) (detail see general methods). The slices were stored for over one hour before being transferred to a submersion recording chamber continually perfused with aCSF for recording.

fEPSP were recorded using glass microelectrodes (approximately 2MΩ resistance) filled with aCSF solution positioned in the stratum lucidum in CA3, the mossy fibre termination zone. Bipolar stainless steel stimulating electrodes were positioned in the dentate granule cell layer, and 0.6 mA pulses (400 μs duration) were applied with constant current stimulators. The electrode positions and stimulus intensities were adjusted until mossy fibre fEPSPs of maximal amplitude were recorded.

6.2.3 Data analysis

As in chapter 3, NBQX 25 μM (sigma), AMPA/kainate receptor antagonist, was applied at the end of each mossy fiber experiment to assess the amplitude of the fiber volleys, and was subtracted from fEPSP responses. The amplitude of the fiber volleys were normalised to baseline. Data are shown as mean ± SEM. The mean fEPSP amplitudes in groups were compared by paired T-test. Differences were considered as significant at $P < 0.05$.

6.3 Results

6.3.1 Behavioural alterations after administration of pilocarpine

The pilocarpine model is a well documented animal model to investigate the development of epilepsy. After pilocarpine administration (320 mg/kg, ip), rats demonstrate consistent behavioural changes. These began with akinesia, ataxia, lurching, peripheral cholinergic signs (such as piloerection, bloody tears, and diarrhoea), stereotyped movements (continuous sniffing, paw licking, rearing), clonic movements of forelimbs, head bobbing and tremors and finally evolved to the general motor seizure which are classified as stage 5 by the Racine scale (Racine, 1972). In this study, 80 % of rats developed stage 3 seizures following administration of pilocarpine. Only these rats were used in subsequent experiments (Figure 6.2).

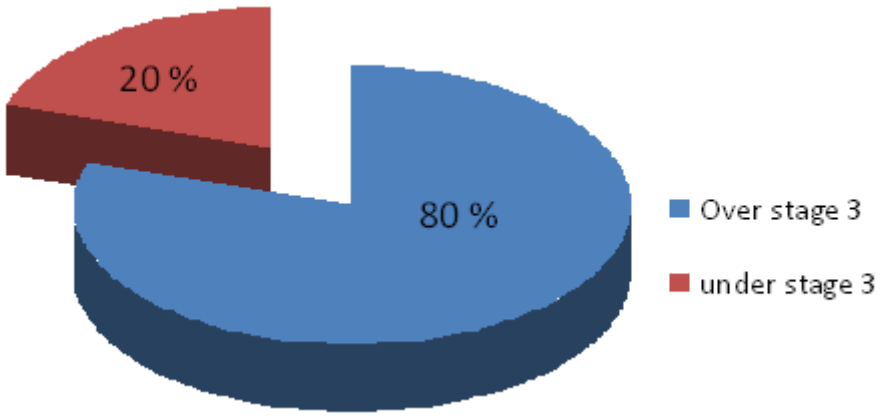

Figure 6.2: The percentage of the rats treated with pilocarpine developing stage three seizures during status epilepticus.

The time for rats to develop stage 3 seizures are varied. The mean time for rats to develop seizure reach stage 3 is 23±4.56 minutes. A box-whisker plot which is helpful to understand the distribution of the time needed for rats to develop different stage seizures were used (Figure 6.3). The line in the middle of the box indicates the median values which is 29 minutes. The value of right and left lines of each "box" (25th and 75th percentiles) are 29 minutes and 35 minutes, respectively. The "whiskers" (lines extending right and left the box) show the extent minimum value and maximum value which are 15 minutes and 56 minutes, respectively. Because of the whisker to the right of the box is much longer than the one to the left indicates that the distribution is positively skewed.

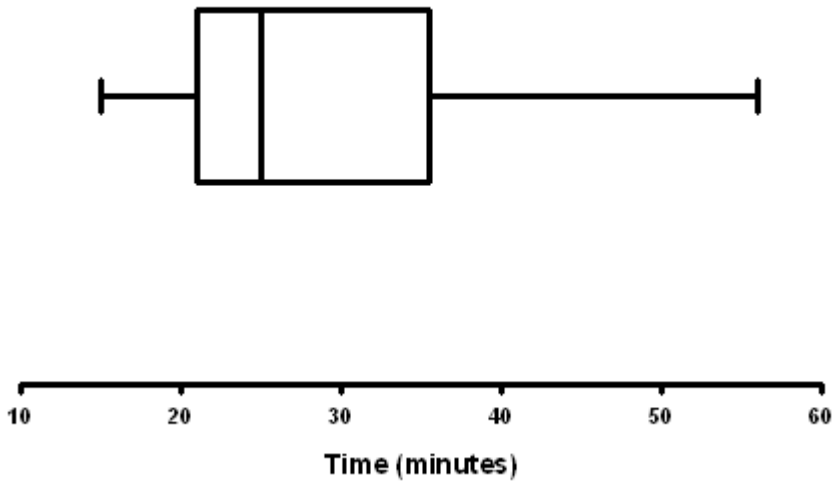

Figure 6.3: Box-and-whiskers plot showing that the time developing to stage 3 seizures, following intraperitoneal injection with pilocarpine (320 mg/kg). The box Represents ± 25% percentile and the whiskers represent full range.

6.3.2 The alteration of effect of VPA on short-term potentiation in mossy fibres synapse CA3 in rat with status epilepticus

Rats following SE were killed two weeks after SE at a time when metaplasticity of mossy fibre responses has been previously described. Slices form these animals were compared against those from control (non-SE) animals from chapter 3. The effects of VPA (1 mM) on baseline neurotransmission and on short-term potentiation in the mossy fibre synapse CA3 (previously in chapter 3) were first tested. The result showed that in control animals VPA had no effect on these). The experiments were performed in isolated hippocampal slices, a bipolar stimulating electrode were placed in dentate granule cell layer; a recording electrode was placed in stratum lucidum (as previously described). Once a candidate mossy fibre fEPSP was found, the stimulus frequency was increased from 0.05 Hz to 1 Hz. (See chapter 3).

After baseline recording with 0.05 Hz and 1 Hz electrical stimulation, VPA was added to the perfusate and the frequency facilitation was repeated. Similar to chapter 3, there was no effect of VPA on fEPSP amplitude during stimulation at 0.05 Hz (after VPA 1mM: 87.35±8.06 % of baseline) (Table 6.1). However, VPA altered short-term plasticity. Frequency facilitation was significantly decreased (Before VPA 1mM: 349.32±47.08% of baseline, after VPA 1mM: 255.96±30.69% of baseline, p=0.03 paired t test) (Figure 6.4 and Table 6.2).

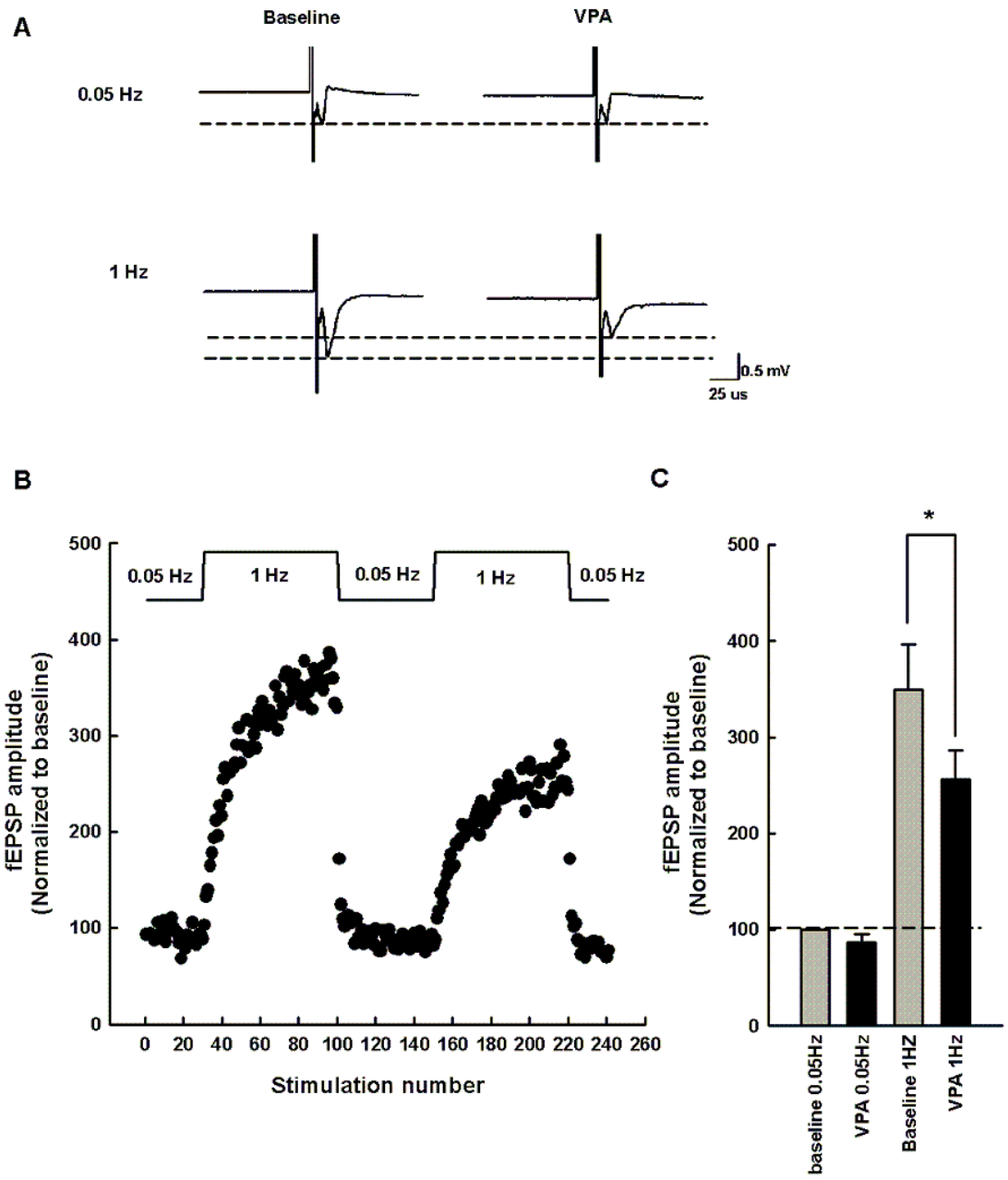

Figure 6.4: The effect of VPA on STP at the mossy fibre synapse to CA3 region in hippocampi acquired from post status epilepticus rats. The hippocampal slices were acquired from the rats 2 weeks after induction of status epilepticus. (A) *Example fEPSPs at 0.05 Hz and 1 HZ without and with VPA.* (B) *Example experiment of fEPSP amplitude at different stimulation frequency (0.05 Hz and 1 Hz) before and then after addition of VPA.* (C) *Comparison of the mean amplitude of fEPSP before and after administration with VPA (1mM) at two stimulation frequencies. Graphs show means ± SEM. * Indicates a significant difference between the two groups (P<0.05).*

Table 6.1 Paired Samples T-Test of the baseline transmission before and after treated VPA in mossy fibre synapse to CA3 in hippocampus acquired from status epilepticus rats.

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Rat in control condition (Before VPA Vs After VPA	0.12651	0.18018	0.08058	-0.09721	0.35024	1.570	4	0.191

Table 6.2 Paired Samples T-Test of STP before and after treated VPA in mossy fibre synapse to CA3 in hippocampus acquired from status epilepticus rats.

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
status epilepticus (Before VPA Vs After VPA)	0.93364	0.63872	0.28565	0.14056	1.72672	3.269	4	0.031

6.4 Discussion

In this experiment, a post-status epilepticus epilepsy model, the "Pilocarpine model", was used to investigate whether VPA could reverse the mossy fibre metaplasticity observed with epileptogenesis. Unexpectedly, VPA had no effect on baseline transmission but decreased short term potentiation in epileptic animals.

It is suggested that the cAMP/PKA signalling system as mossy fibres during epileptogenesis may be "over activated". Genetically altered animals which display spontaneous seizure activity show an abnormal elevation of protein kinase A activity (Tehrani and Barnes, 1995a;Yechikhov et al., 2001). If this were the case VPA would be expected to reduce baseline transmission in the epileptic animals through inhibition of cAMP accumulation. However, no such change was observed. Instead, VPA decreased short term potentiation (STP) at the mossy fibre synapse. This contrasts with the effects of VPA in slices from healthy animals (chapter 3) where VPA had no effect on STP. What is the mechanism of this? Normally STP is the result of increasing presynaptic calcium accumulation and is partly dependent on the activation of pre-synaptic kainate receptors (Contractor et al., 2001). The absence of an effect of VPA on mossy fibre STP in naive animals suggests that VPA does not act on these mechanisms. Also it suggests that VPA is not causing a use dependent block of sodium channels at the low frequency (1 Hz) necessary to induce STP at this synapse. That VPA having an effect in epileptic animals results in two new hypotheses: 1) the mechanism underlying STP at mossy fibres has changed in epileptogenesis, and 2) this new mechanism is sensitive to inhibition by VPA. Indeed, it has been previously established that SE can lead to a long-lasting modification of release probability in mossy fibre synapses. Paired-pulse facilitation and augmentation in mossy fibres are potently reduced several weeks after SE (Goussakov et al., 2000). Mossy fibre LTP is also severely diminished. In addition,

potentiation of transmission by activation of either adenylyl cyclase or protein kinase A is abolished in SE animals (Goussakov et al., 2000).

Further testing of these hypotheses was beyond the scope of this thesis. Nevertheless, the effect of VPA on STP in only epileptic animals indicates that such a mechanism may selectively decrease higher frequency transmission specifically in epileptic tissue and could contribute to the antiepileptic effect of VPA.

Chapter 7: The effect of 4-methyloctanoic acid, an analogue of VPA, in animal models of epilepsy

7.1 Introduction

Although valproic acid (VPA) is used extensively for treating various kinds of epilepsy, its use has been hampered by its teratogenic potential. It may result in multiple birth defects, dysmorphic facies, developmental delay, learning difficulties and/or behavioural problems in humans (Kini, 2006), and in animals (Narotsky et al., 1994;Menegola et al., 1998). In addition, therapeutic use of the VPA has been associated with severe and often fatal hepatotoxicity. Patients usually present with lethargy, anorexia, and vomiting with rapid progression to coma (Stephens and Levy, 1992). Therefore, it would be advantageous to develop a more potent and safer drug.

There have been previous studies of the efficacy of branched chain fatty acids in sound induced epilepsy mice (Chapman et al., 1983). Previous studies have indicated that branched chain fatty acids have activity against seizure induced by pentylenetetrazole, whereas straight chain fatty acids have limited effect on the suppression of seizure activity. Furthermore, branched chain fatty acids increase brain GABA levels, whereas straight chain fatty acids do not (Keane et al., 1983).

4-methyloctanoic acid, an analogue of VPA, is a branched short-chain fatty acid (Figure 7.1). It is responsible for the sweaty/sour flavour in mutton (Brennand and Lindsay, 1992). During cooking, 4-methyloctanoic is released from lamb and mutton (Brennand et al., 1989). Even though, milk fat of cows only contain low concentrations of 4-methyloctanoic acid, milk fat of sheep and goat contains significant amounts of 4-methyloctanoic, which contributes to mutton-like and goat-like flavours. In addition, 4-methyloctanoic acid from goat and sheep milks provides distinguishing flavours to various cheese (Ha and Lindsay, 1991). Importantly, fatty acids with a methyl side chain have been shown to lack teratogenic effects (Narotsky et al., 1994).

In this chapter, the potency of VPA and 4-methyloctanoic acid, a branched-chain analogue of VPA was first tested, for their anti-acute seizure activity in *in vitro* PTZ and low Mg^{2+} model. Further, VPA and 4-methyloctanoic were tested for their anticonvulsant activity in a SE *in vivo* model (the perforant pathway stimulation model). Two months later, the neuroprotective effect of VPA and 4-methyloctanoic acid were evaluated by histological method. Finally, in order to understand whether 4-methyloctanoic shares similar mechanisms with VPA on the modulation of cAMP, the effect of 4-methyloctanoic on the enhancement of mossy fibre responses induced by application with forskolin was also tested.

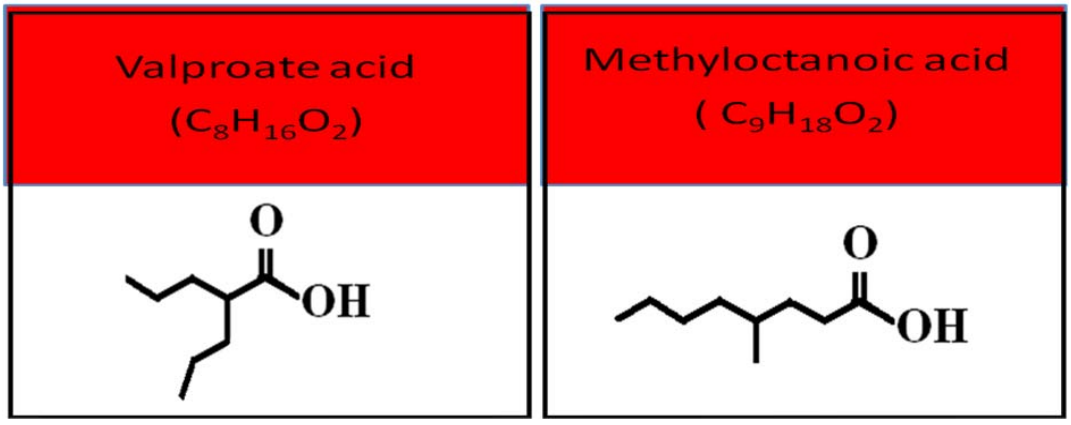

Figure 7.1: Comparison of the structures of VPA and 4-methyloctanoic acid

7.2 Methods

7.2.1 *In vitro* seizure models

Hippocampus slides preparation

Horizontal combined entorhinal cortex-hippocampus slices (350-μm) were prepared from SD rat and storage in an interface chamber contained artificial cerebrospinal fluid solution (aCSF) gassed with 95% O2 / 5% CO2 (See general method).

PTZ model

Pentelenetetrazole (PTZ) (2 mM) was added to the perfusate and $[K^+]$ was increased to 6 mM in order to induce epileptiform activity.

Low Mg^{2+} model

Application with Mg^{2+} free aCSF generated a rhythmic short recurrent discharges activity in CA1 and CA3 area in hippocampus in rats.

(For more detail methods see general method and chapter)

7.2.2 *In vivo* experiments

In vivo status epilepticus: This method has been described in detail in *in vivo* status epilepticus.

The depths of the electrodes were adjusted to maximize the slope of the dentate granule cell field potential. During surgery, the location of recording electrode can be recognized by the patterns of field potential responses (Figure 7.2). When the recording electrode entered CA1 region, the field potential shows a negative wave superimpose a positive wave. When recording electrode close to the dentate gyrus, the pattern of field potential changed, the latency was decreased and the positive wave was deceased. When

the electrode entered molecular layer of the dentate gyrus, it started to show population spikes. The optimist positions of recording electrode (especially regarding depth) is the amplitude of maximum population spike reached 4-5 mV.

The electrodes were held in place with dental acrylic and skull screws. The animals were allowed to recover from anaesthesia. Seven days later, the perforant path was electrically stimulated with 4-5 mA 50 µsec monopolar pulses at 20 Hz for 2 hr; this induced self-sustaining SE. After 10 min of self-sustaining SE, compounds (VPA or 4-methyloctanoic acid) or vehicle were administered and the behavioural seizures and EEG were monitored for 3 hours. At this point diazepam (10 mg/kg) was administered to all animals to stop the status epilepticus. Groups were compared by ANOVA followed by post-hoc testing using Tukey test.

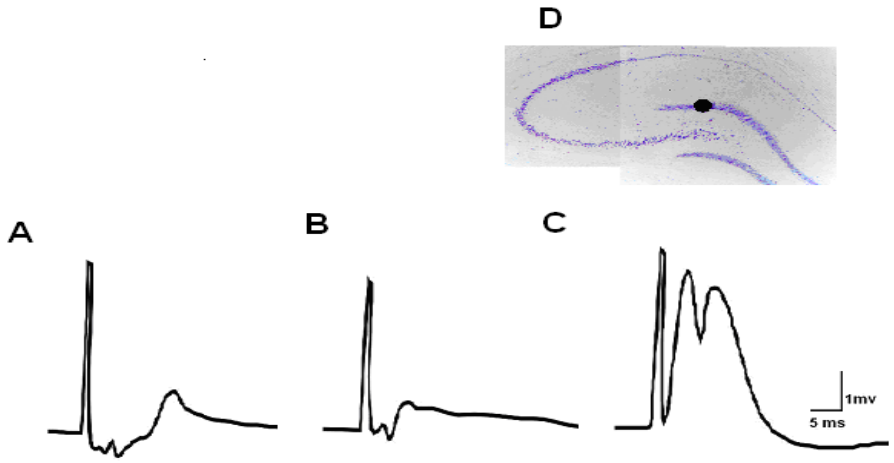

Figure 7.2: Histological localization and of chronically implanted electrodes. (A)*The field potential recording in CA1 shows a negative wave superimposes a positive wave.* (B)*The latency was decreased and the positive wave was deceased when electrode recording close to dentate gyrus.* (C)*The field potential recording in molecular layer of the dentate gyrus shows population spikes.* (D) *The photo of hippocampal slices. The black dock is the location of recording electrode in dentate gyrus*

7.3 Result

7.3.1 The effect of 4-methyloctanoic acid on acute seizure *in vitro* model-PTZ model

Application with branched chain fatty acid, 4-Methyloctanoic acid (1 mM), significantly suppressed the frequency of burst discharge induced by administration with PTZ (49.14±4.49 %) in the CA1 region 30-40 minutes after application ($P<0.01$, compared to control group). The effect of 4-methyloctanoic was greater than that of VPA (1mM) (VPA: 75.06±1.68 % of baseline, n=5, $p<0.01$ compared to 4-Methyloctanoic acid) (Figure 7.3, Table 7.1, and Table 7.2).

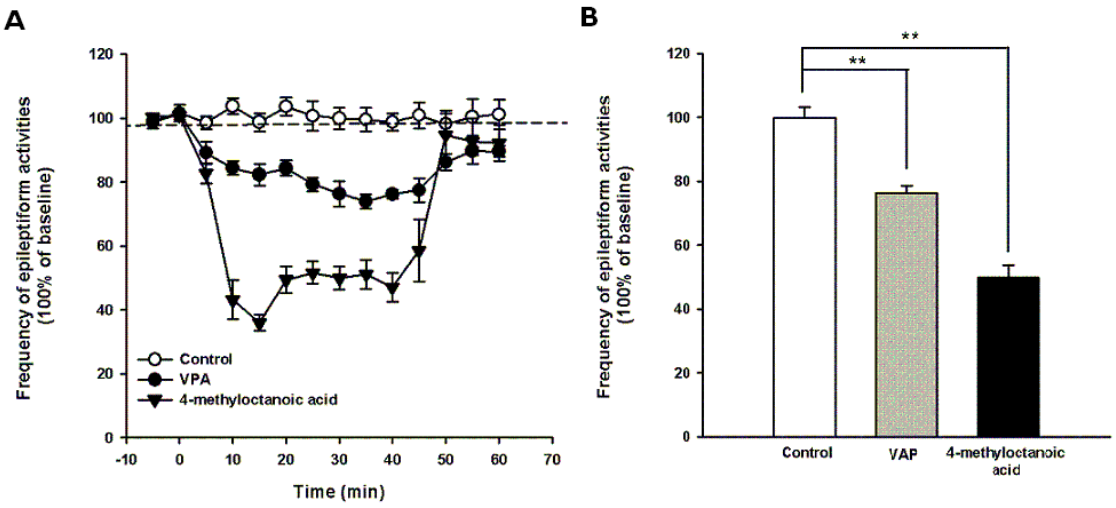

*Figure7.3: The comparison of the effect of 4-methyloctanoic acid and VPA on the frequency of epileptiform activity induced by application of PTZ in combined entorhinal cortex-hippocampus. (A) Summary of the frequency of PTZ-induced burst discharges following application of 4-methyloctanoic acid or VPA. The frequency of epileptiform activity induced by PTZ plotted against time. The drugs were application from time=0 to 40 minutes. (B) Comparison of the mean frequency of PTZ-induced burst discharges with different treatments. Graphs show means ± SEM. * Indicates a significant difference at P<0.05, ** Indicates a significant difference at P<0.01*

Table 7.1: The ANOVA result of frequency of burst-discharge in different treatmenst (Control, 4-methyloctanoic acid 1mM, VPA 1mM) in in vitro model of epilepsy-PTZ model.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.627	2	0.313	57.009	0.0000007
Within Groups	0.066	12	0.005		
Total	0.693	14			

Table 7.2: Post-hoc comparison of frequency of burst-discharge with treatments (Control, 4- methyloctanoic acid 1mM, VPA 1mM) in in vitro PTZ model by Tukey test

	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Control VS 4-methyloctanoic acid	0.50056(*)	0.04689	0.000	0.3755	0.6257
Control VS VPA	0.24121(*)	0.04689	0.001	0.1161	0.3663
4-Methyloctanoic acid VS VPA	-0.25935(*)	0.04689	0.000	-0.3844	-0.1343

* The mean difference is significant at the .05 level.

7.3.2 The effect of 4-methyloctanoic *in vitro* low Mg^{2+} model

In order to confirm the potency of 4-methyloctanoic, the effect in a different *in vitro* model induced by low-Mg^{2+} in combined entorhinal-hippocampal slices was also tested. VPA (1 mM) suppressed the frequency of recurrent short discharges to 72.65±3.59 % of baseline, n=5 30-40 minutes after application and the effect of suppression is reversible after wash out. Application of 4-methyloctanoic acid almost abolished the frequency of recurrent short discharges30-40 minutes after application to (1.60± 3.12 % of baseline, n=5, $p<0.01$compared to control; $P<0.01$ compared to VPA) (Figure 7.4, Table 7.3, and Table 7.4).

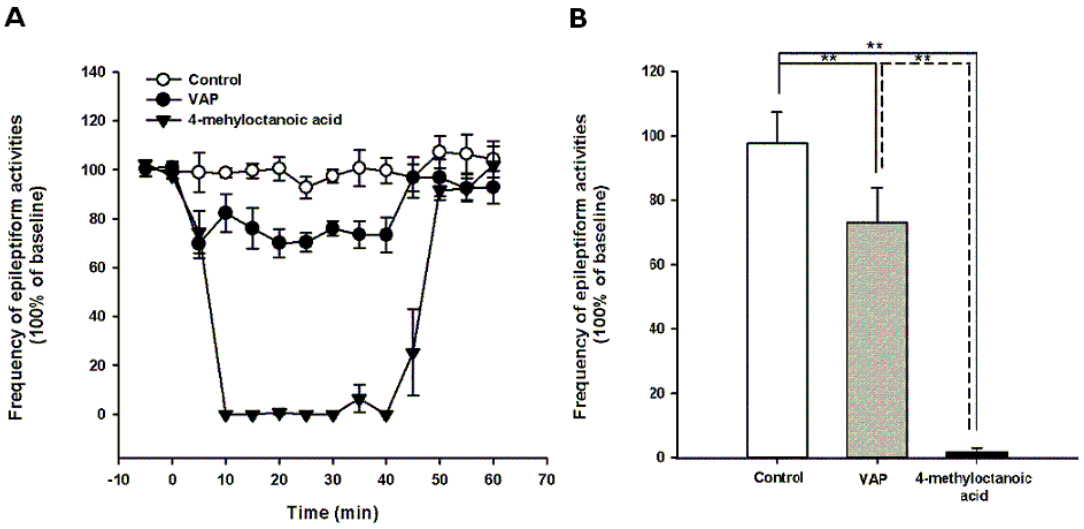

*Figure 7.4: The comparison of the effect of 4-methyloctanoic acid and VPA on the frequency of epileptiform activity induced by low Mg^{2+} aCSF in combined entorhinal cortex-hippocampus. (A) Summary the change in the frequency of low Mg^{2+} -induced burst discharges following application of 4-methyloctanoic acid or VPA. The frequency of epileptiform activity induced by Mg^{2+}-free aCSF plotted against time. The drugs were given from time = 0 to 40 minutes. (B) Comparison of the mean frequency of low Mg^{2+} -induced burst discharges with different treatments. Graphs show means ± SEM. * Indicates a significant difference P<0.05, ** Indicates a significant difference P<0.01*

Table 7.3: The ANOVA result of frequency of burst-discharge in different treatment groups (Control, 4-methyloctanoic acid 1mM, VPA 1mM) in in vitro low Mg^{2+} model.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24898.944	2	12449.472	169.752	0.000
Within Groups	880.070	12	73.339		
Total	25779.014	14			

Table 7.4: Post-hoc comparison of burst-discharge of different treatments (Control, 4-methyloctanoic acid 1mM, VPA 1mM) in in vitro low Mg^{2+} model by Tukey test

	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Control VS VPA	24.61214(*)	5.41624	0.002	10.1623	39.0619
Control VS 4-methyloctanoic acid	96.06384(*)	5.41624	0.000	81.6141	110.5136
VPA VS 4-methyloctanoic acid	71.45171(*)	5.41624	0.000	57.0019	85.9015

* The mean difference is significant at the .05 level.

7.3.3 The effect of 4-methyloctanoic in *in vivo* electrical stimulation model of status epilepticus

To further demonstrate efficacy in epilepsy with these compounds, 4-methyloctanoic acid in an *in vivo* model of status epilepticus induced by perforant pathway stimulation was tested. This model is resistant to a number of antiepileptic drugs and does not involve the application of an exogenous convulsant.

First, the effective concentration of VPA in this *in vivo* model was tested. VPA is effective in this model at 600 mg/kg which abolished the seizure activity (not only the EEG activity but also behavioural seizures) in all animals. This contrasted with lower dose VPA (400 mg/kg) which was ineffective (Figure 7.5).

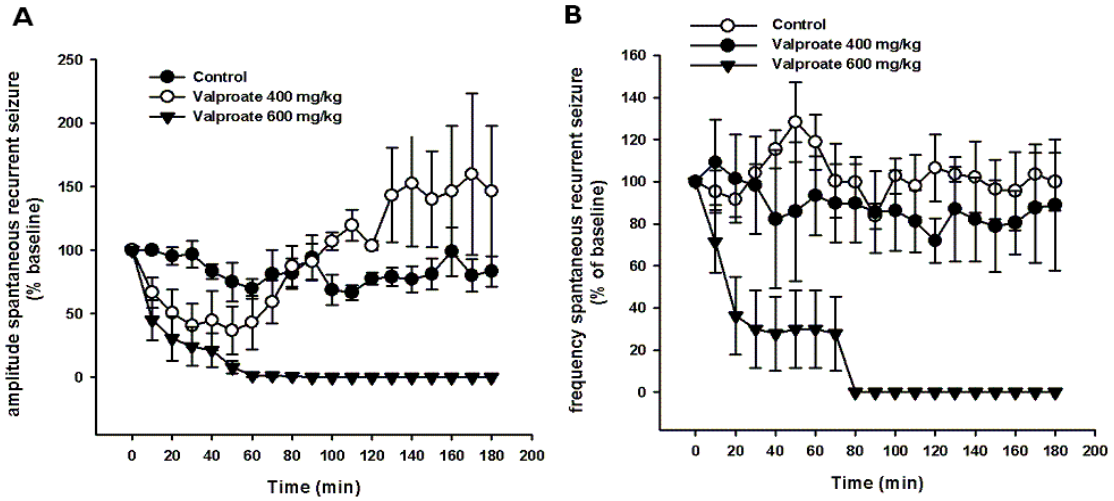

Figure 7.5: Comparison of the effects of VPA in different concentration (400 mg/kg and 600 mg/kg) on spontaneous spikes activity, including spike amplitude and spike frequency, induced by perforant path stimulation. Rats given saline were used as control group (n=5). (A) *A summary of the change of amplitude after administration with VPA (n=5 at each dose) in SE rats.* (B) *A summary of the change of frequency after administration with VPA in SE rats.*

The efficacy of 4-methyloctanoic acid (400 mg/kg) against VPA (400 mg/kg) and vehicle control (DMSO) group (Summary in Table 7.5 and Table 7.6) were compared. Administration of 4-methyloctanoic reduced the mean spike frequency to 39.33±11.28 % in the first hour, 18.06±10.28% in the second hour, 26.85±12.31% in the third hour. 4-methyloctanoic terminated status epileptius (defined as a spike frequency of less than 1 Hz) in status epilepticus animals. Administration with VPA has less effective on spike frequency than administration with 4-methyloctanoic acid (75.16±9.23 % in the first hour, 61.05±8.33% in the second hour, 55.08±6.64% in the third hour). In VPA group, status epilepticus was not stopped in any of the animals investigated. In this experiment, the DMSO which is the solvent of 4-methyloctanoic acid was also tested; administration with DMSO shows no effect on the spike frequency (100.44±5.17 % in the first hour, 103.32±9.79% in the second hour, 97.56±4.98% in the third hour) (Figure 7.6; Figure 7.7)

As result shown in Figure 7.6; Figure 7.8, 4-Methyloctanoic acid also shows a significant effect on suppression of the spikes amplitude (to 27.17±9.79 % in the first hour, 18.87±1.15% in the second hour, 44.45±17.13% in the third hour). Interestingly, administration with VPA suppressed spike amplitude which increased at later time points (40.06±7.74 % in the first hour, 49.02±15.59% in the second hour, and 92.22±36.88% in the third hour) (Summary Table 7.7 and Table 7.8).

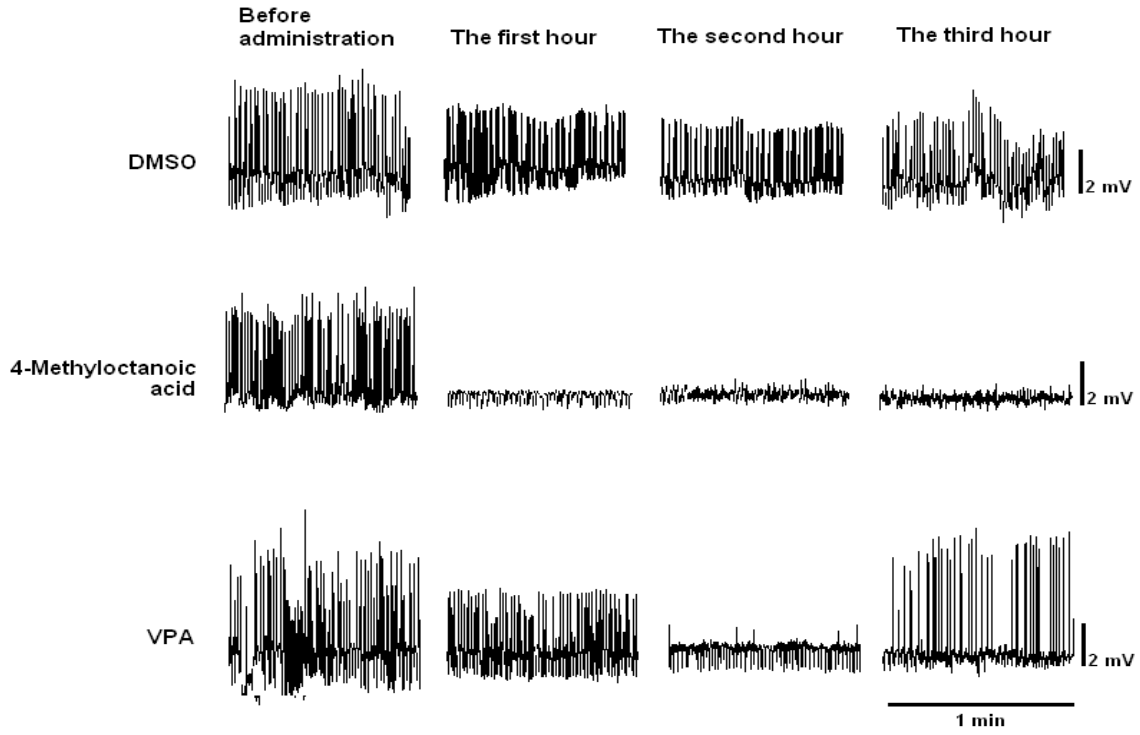

Figure 7.6: Illustrative trace samples of EEG from status epilepticus animal. Administration with VPA (400 mg/kg) or 4-methyloctanoic acid (400 mg/kg) shows attenuation of seizure activity by VPA and termination of seizure activity by 4-methyloctanoic acid, whereas the DMSO has no effect on seizure activity.

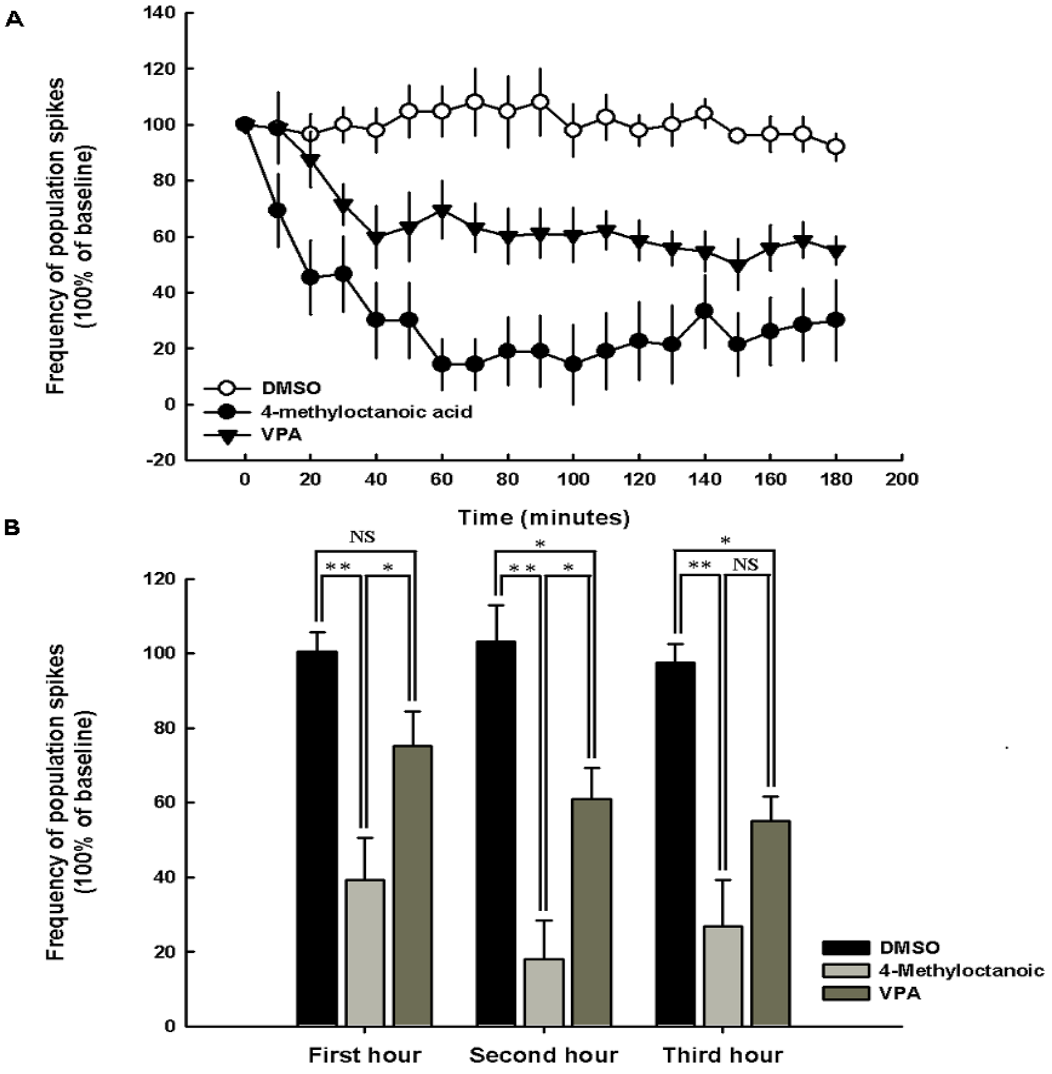

Figure 7.7: Comparison of the effects of 4-methyloctanoic acid (400 mg/kg) and VPA (400 mg/kg) on the frequency of the spontaneous spikes activity, induced by perforant path stimulation. (A) *Time course of the effects on spontaneous spike frequency following administration of DMSO (n=5), VPA (n=7) or 4-methyloctanoic acid (n=7) in 10 minutes after stop perforant path stimulation.* (B) *Summary data for the effects of DMSO, VPA and 4-methyloctanoic acid in one, two and three hour after administration. . * Indicates a significant difference P<0.05, ** Indicates a significant difference P<0.01 and NS indicates non-significant.*

Table7.5: The ANOVA result of spikes frequency in status epilepticus rats with different treatment (DMSO, 4-methyloctanoic acid 400 mg/kg, VPA 400 mg/kg.)

ANOVA	The first hour	The second hour	The third hour
F Values	9.607	18.472	13.387
Sig.	0.002	0.00007	0.0004

Table 7.6: The result of Post Hoc Tests-Tukey (Multiple Comparisons), compared the spike frequency in one hour, two hour and three hour after different treatment (DMSO, 4-methyloctanoic acid 400 mg/kg, VPA 400 mg/kg) in status epilepticus rats

One hours after administration drugs	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
4-methyloctanoic acid VS DMSO	-0.61106(*)	0.14241	0.002	-0.9785	-0.2436
4-Methyloctanopic acid VS VPA	-0.35825(*)	0.13000	0.036	-0.6937	-0.0228
DMSO VS VPA	0.25280	0.14241	0.209	-0.1147	0.6203

* The mean difference is significant at the .05 level.

Two hours after administration drugs	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
4-Methyloctanoic acid VS DMSO	-0.85167(*)	0.14094	0.00005	-1.2153	-0.4880
4-Methyloctanoic acid VS VPA	-0.42999(*)	0.12866	0.011	-0.7620	-0.0980
DMSO VS VPA	0.42168(*)	0.14094	0.022	0.0580	0.7854

* The mean difference is significant at the .05 level.

Three hours after administration drugs	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
4-Methyloctanoic acid VS DMSO	-0.70704(*)	0.13667	0.0003	-1.0597	-0.3544
4-Methyloctanoic acid VS VPA	-0.28228	0.12476	0.091	-0.6042	0.0397
DMSO VS VPA	0.42475(*)	0.13667	0.018	0.0721	0.7774

* The mean difference is significant at the .05 level.

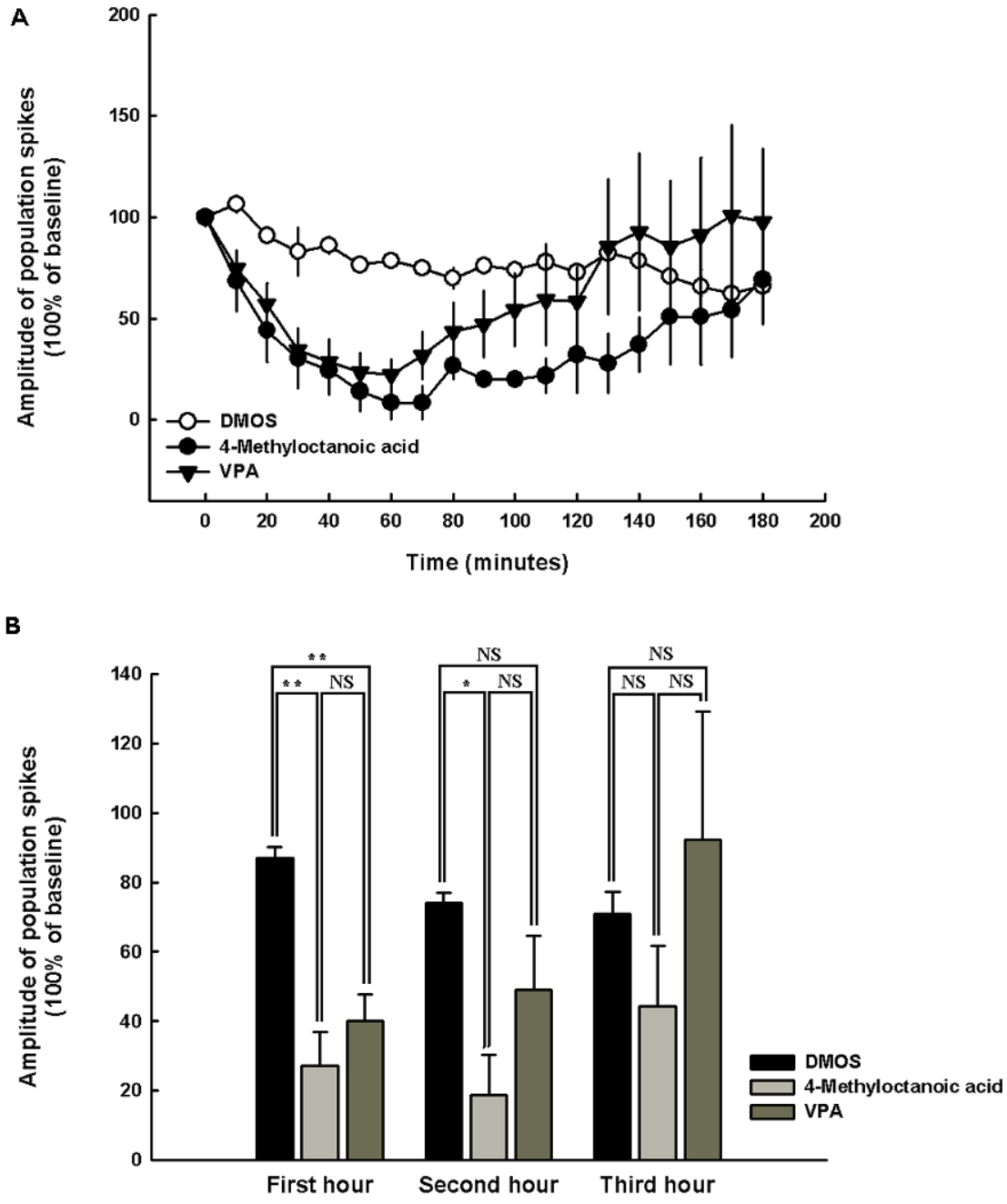

Figure 7.8: Comparison of the effects of 4-methyloctanoic acid (400 mg/kg) and VPA (400 mg/kg) on the amplitude of the spontaneous spikes activity, induced by perforant path stimulation. (A) *Time course of the effects on spontaneous spike amplitude following administration of DMSO (n=5), VPA (n=7) or 4-methyloctanoic acid (n=7) in 10 minutes after stop perforant path stimulation.* (B) *Summary data for the effects of DMSO, VPA and 4-methyloctanoic acid in one, two and three hour after administration. * Indicates a significant difference P<0.05, ** Indicates a significant difference P<0.01 and NS indicates non-significant.*

Table 7.7: ANOVA result of spikes amplitude in status epilepticus animals with different treatments (DMSO, 4-methyloctanoic acid 400 mg/kg, VPA 400 mg/kg)

	The first hour	The second hour	The third hour
F Values	13.119	4.601	0.912
Sig.	0.0004	0.026	0.422

Table 7.8: The result of Tukey (Multiple Comparisons), comparing the spike amplitude in one hour, two hour and three hour after different treatments (DMSO, 4-methyloctanoic acid 400 mg/kg, and VPA 400 mg/kg) in status epilepticus animal.

One hours after administration drugs	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
4-Methyloctanoic acid VS DMSO	-0.59742(*)	0.12015	0.00039	-0.9074	-0.2874
4-Methyloctanoic acid VS VPA	-0.12886	0.10968	0.484	-0.4119	0.1541
DMSO-VPA	0.46856(*)	0.12015	0.003	0.1585	0.7786

* The mean difference is significant at the .05 level.

Two hours after administration drugs	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
4-Methyloctanoic acid VS DMSO	-0.55298(*)	0.18456	0.022	-1.0292	-0.0767
4-Methyloctanoic acid VS VPA	-0.30150	0.16848	0.205	-0.7362	0.1332
DMSO-VPA	0.25147	0.18456	0.383	-0.2248	0.7277

* The mean difference is significant at the .05 level

Three hours after administration drugs	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
4-Methyloctanoic acid VS DMSO	-0.26464	0.38799	0.777	-1.2658	0.7365
4-Methyloctanoic acid VS VPA	-0.47764	0.35418	0.390	-1.3915	0.4363
DMSO-VPA	-0.21300	0.38799	0.848	-1.2141	0.7881

* The mean difference is significant at the .05 level

Effects on behavioural seizures

Continuous perforant path stimulation initially elicits a series of wet dog shakes and later increasingly severe seizures including tonic-clonic seizures. By the end of the stimulation, all animals had reached stage 5 seizures (full motor seizure, rearing and failing). After stimulation, behavioural seizures continued at Racine stage 2 to stage 5 in untreated animals.

Following stimulation, the behavioural seizures was assessed in control, VPA and 4-methyloctanoic animals (Table 7.9 and Figure 7.9). In the first hour both VPA and 4-methyloctanoic acid significantly decreased seizure severity compared to control ($P < 0.05$ and $P < 0.01$ respectively). By two hours, there was also a significant difference between VPA and 4-methyloctanoic acid groups ($P < 0.05$) but by three hours, there was no significant difference between groups. Impressively, by two hours 4-methyloctanoic acid has stopped behavioural seizures in all animals, over half of which did not have any behavioural seizure recurrence by 3 hours.

Table 7.9: Numbers of animals with each behavioural seizure severity after status epilepticus induction (severity is given as maximum seizure severity in that time period)

	First hours			Second hour			Third hour		
	DMSO	VPA	4-MO	DMSO	VPA	4-MO	DMSO	VPA	4-MO
Stage 0			2			7			4
Stage 1			1						1
Stage 2		5	4		7		2	3	1
Stage 3	1	2		3			2	4	
Stage 4	1								
Stage 5	3			2			1		1

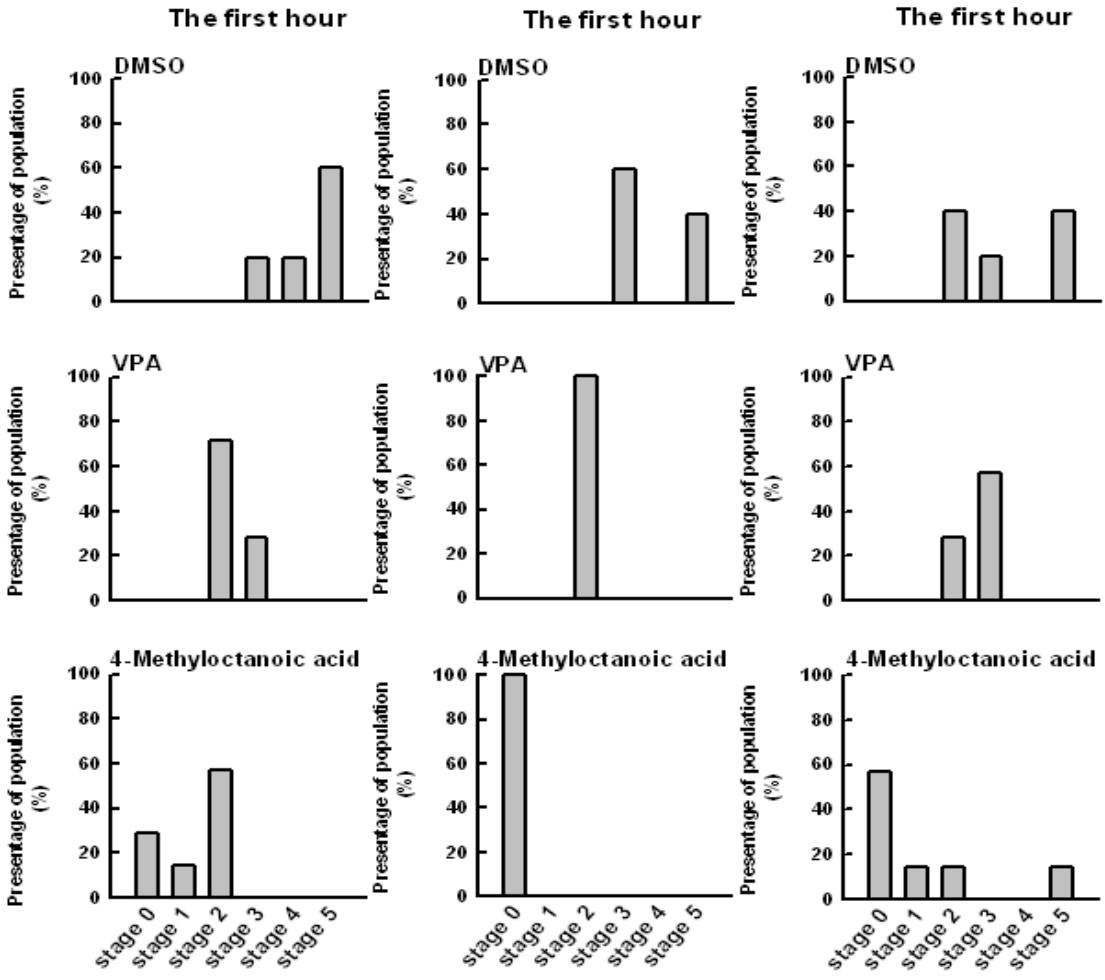

Figure 7.9 Comparison of the severity of seizure activity in the group application of 4-methyloctanoic acid (400 mg/kg) and VPA (400 mg/kg) induced by perforant path stimulation. Percentage of animals with each seizure severity.

7.3.4 Degeneration of the hippocampus following status epilepticus

Figures (Figure 7.10; Figure 7.11; Figure 7.12) illustrate nissl stain sections of the hippocampal region, dentate hilus, CA3, CA1, of non-epileptic controls and the three groups of epileptic animal. All slices containing hippocampus (40 μM) were collected, and the neurodegeneration was evaluated in each slices. Even though, the degrees of neurodegernation in each treatment (DMSO, VPA, 4-methyloctanoic acid) were not quantified, qualitative histology was visually assessed and graded as mild, moderate or severe. Visual inspection of these sections indicates severe neuronal damage of dentate hilus and CA3 area, and milder loss in CA1 area in DMSO-treated epileptic rats. In VPA-treated epilepticus rats, there was a moderate cell loss in the hippocampus formation, including dentate hilus, CA1, CA3. The result indicates that VPA protected cell from neurodegeneration in hippocampus formation. In 4-methyloctanoic acid, however, there is a severe cell loss in the area of the hilus. The region CA1 and CA3 show less extended cell damage, indicating that 4-methyloctanoic acid protects cell from neurodegeneration in area CA1 and CA3, but not in dentate hilus.

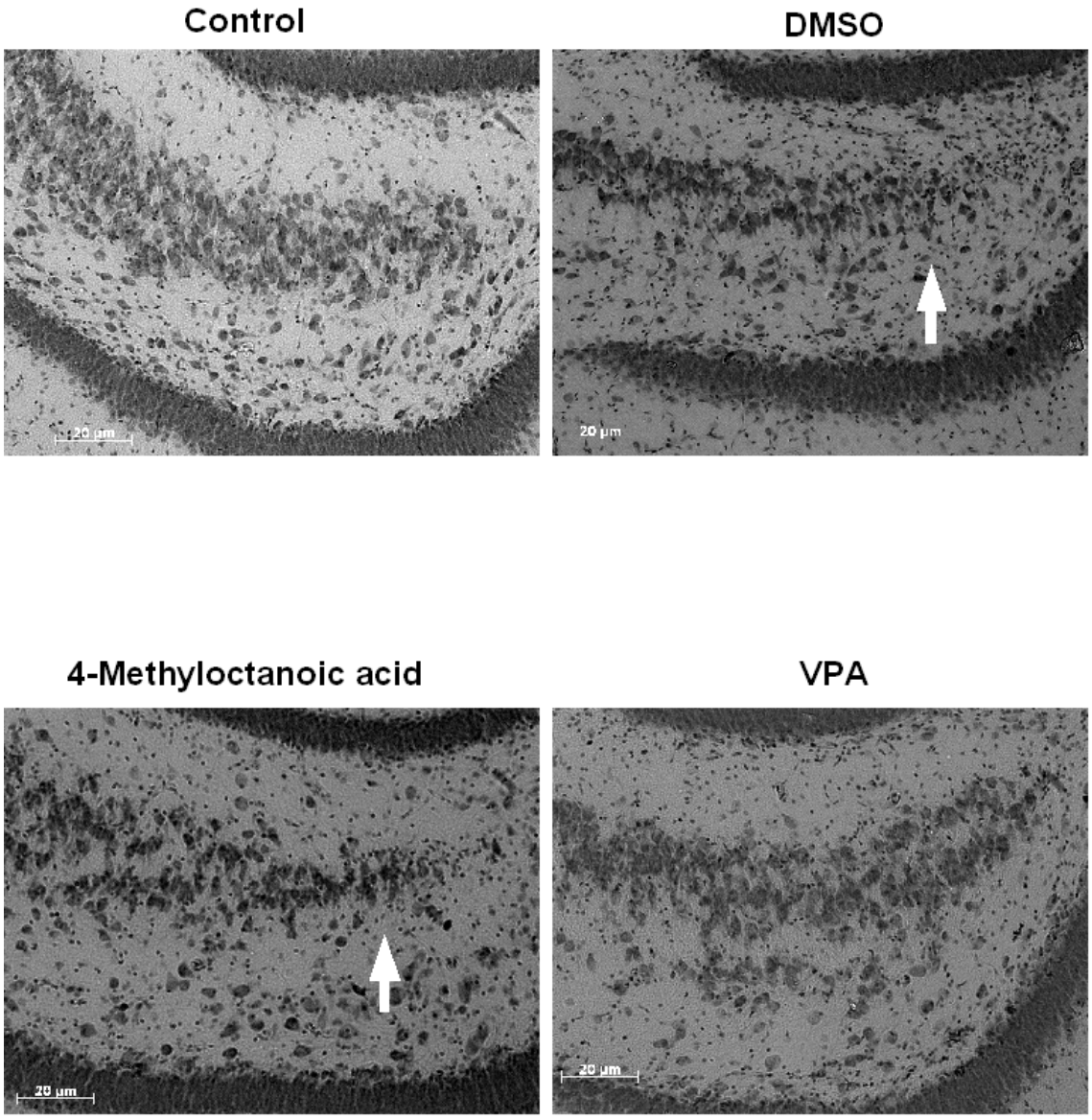

Figure 7.10: The example of representative nissl-stained horizontal sections of the dentate hilus in hippocampus. The animals were divided into non-SE animal as control animal, DMSO-treated SE animal, VPA-treated SE animal, and 4-methyloctanoic acid-treated SE animal. The DMSO-and 4-methyloctanoic acid treated epileptic rat showed severe neuronal damage in dentate hilus in the hippocampal formation. In contrast, the VPA-treated epileptic rat shows a less extended cell loss. (Arrow indicates the neurodegeneration)

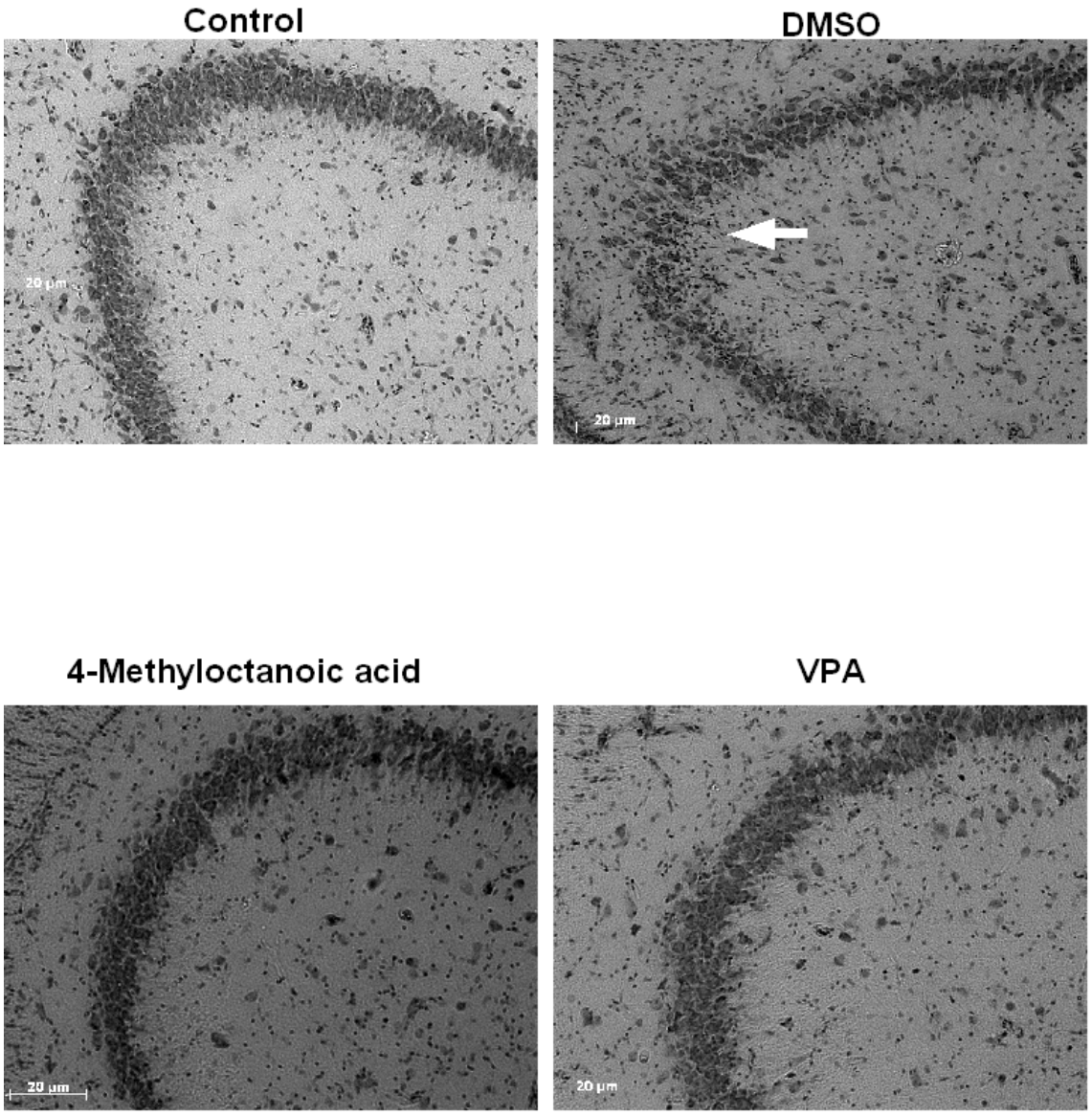

Figure 7.11: The example of representative nissl-stained horizontal sections of the CA3 in hippocampus. The animals were divided into non-SE animal as control animal, DMSO-treated SE animal, VPA-treated SE animal, and 4-methyloctanoic acid-treated SE animal. The DMSO-and treated SE animal showed severe neuronal damage in CA3 region in the hippocampus. In contrast, the VPA-treated and -4methyloctanoic acid-treated SE animal shows moderated cell damage compared to non-epileptic rats. (Arrow indicates the neurodegeneration)

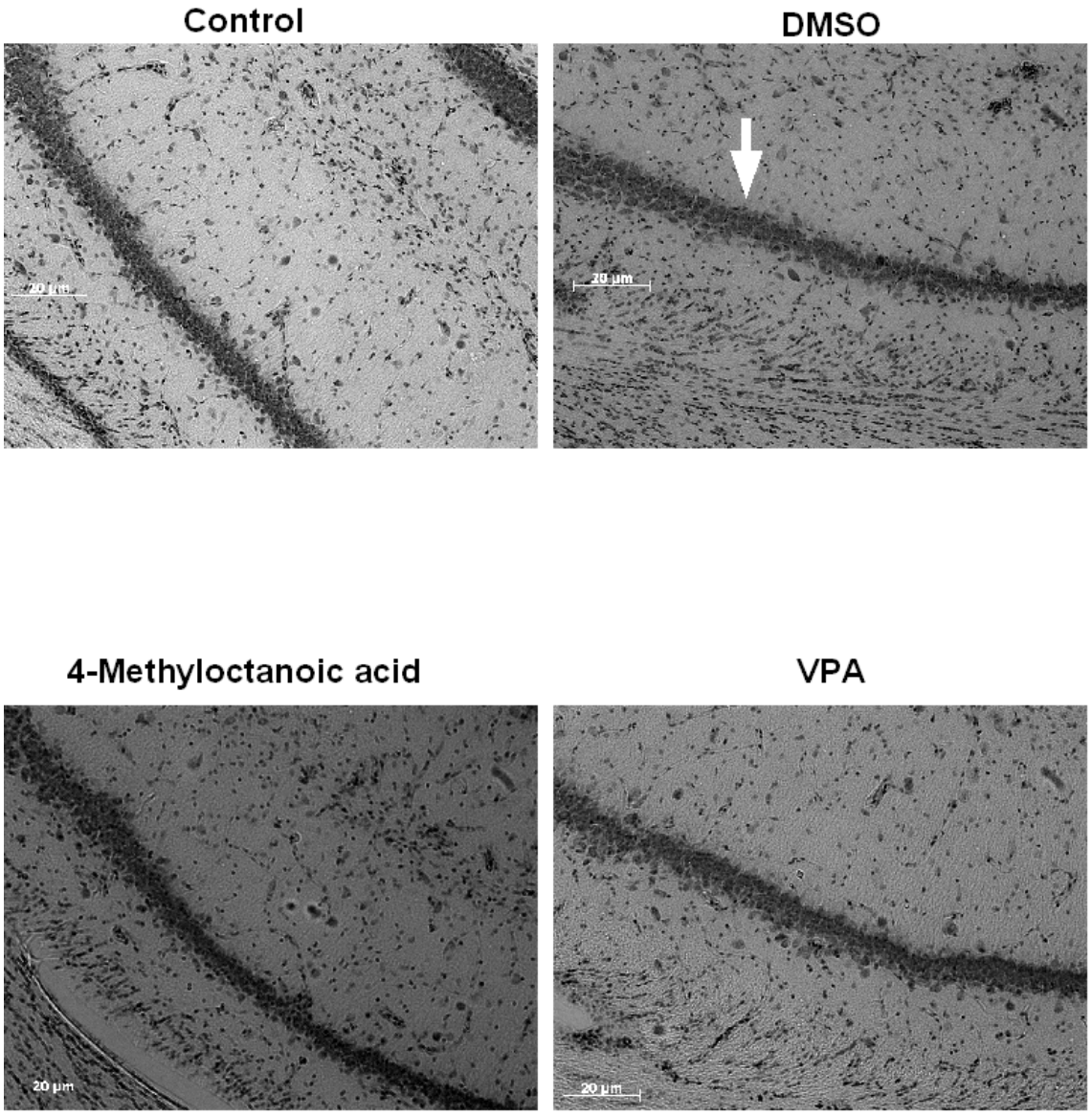

Figure 7.12: The example of representative nissl-stained horizontal sections of the CA1 in hippocampus. The animals were divided into non-SE animal as control animal, DMSO-treated SE animal, VPA-treated SE animal, and 4-methyloctanoic acid-treated SE animal. The SE animal received administration with DMSO shows a mild neural loss in CA1. The animals received VPA and 4-methyloctanoic acid has no difference compared to control animal. (Arrow indicates the neurodegeneration)

7.3.5 The effect of 4-methyloctanoic acid on forskolin induced mossy fibre potentiation

The question whether 4-methyloctanoic had similar effects to VPA on mossy fibre to CA3 transmission was asked. The effect of 4-methyloctanoic acid on short-term potentiation and baseline neurotransmission at the mossy fibre to CA3 synapse were tested in hippocampus slices acquired from normal rats. The normal brain slices were chosen for following reasons. First, in order to elucidate whether the action of mechanism of the 4-methyloctanoic shares the similar action of mechanism of VPA on mossy fibre LTP, the experiments were conduct in the same conduction as previous experiments (Chapter 3). Second, the network of hippocampus formation and the property of synapse may alter during epileptogenesis. The result shows that there is no effect of 4-methyloctanoic acid short-term potentiation (Before 4-methyloctanoic acid 1mM: 268.41±18.05% of baseline, after 4-methyloctanoic acid 1mM: 263.78±31.68% of baseline) or baseline transmission (Before 4-methyloctanoic acid 1mM: 97.38±3.70% of baseline, after 4-methyloctanoic acid 1mM: 94.55±7.29% of baseline) (Figure 7.13)

Then, the effect of administration of 4-methyloctanoic acid on enhancement of mossy fibre responses induced by forskolin was test. Thirty minutes after forskolin application, the fEPSP was 231.67 ± 53.56% of baseline in 4-methyloctanoic acid treated group (n = 6). This was significantly less than control conditions (458.56±53.33% of baseline P = 0.005). Using the values obtained with VPA in chapter 3, and comparing using ANOVA and post-hoc testing, it can be seen that the effect of 4-methyloctanoic acid is similar to that of VPA (Figure 7.14, Table 7.10, and Table 7.11).

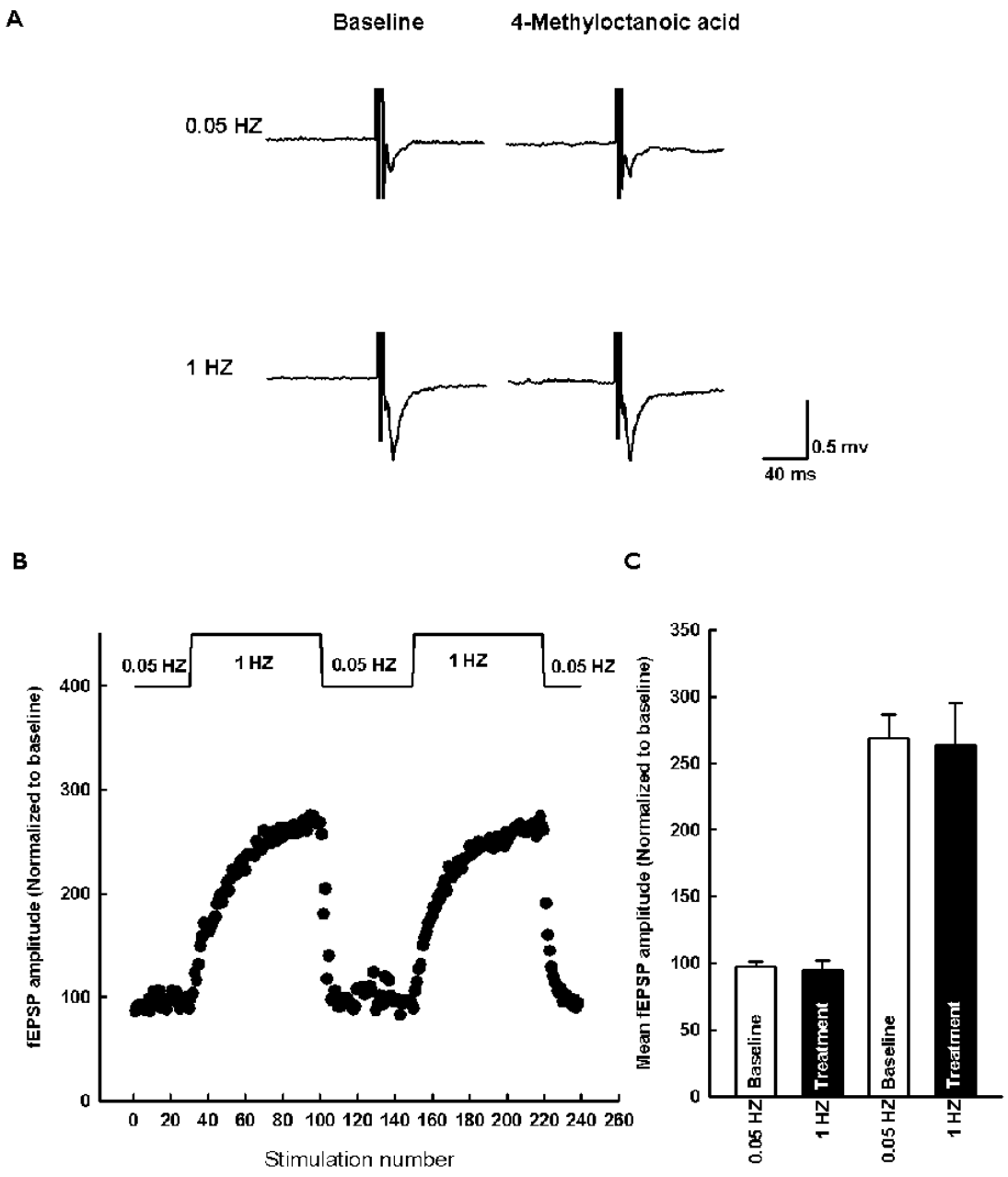

Figure 7.13: Summary the effect of 4-methyloctanoic acid (1mM) on baseline transmission and short term potentiation at the mossy fibre synapse to CA3 region in hippocampus. (A) *Example of average traces EPSCs stimulated in dentate granule cell layer with frequency 0.05 Hz and 1 HZ.* (B) *Example of the change of fEPSP amplitude in mossy fibre with different stimulation frequency (0.05 Hz and 1 Hz). fEPSP amplitudes recordings plotted against stimulation number.* (C) *Comparison of the mean amplitude of fEPSP before after administration 4-methyloctanoic acid. Graphs show means ± SEM. There is no significant difference in control conditions and 4-methyloctanoic acid-treat condition at either 0.5 Hz or 1 Hz.*

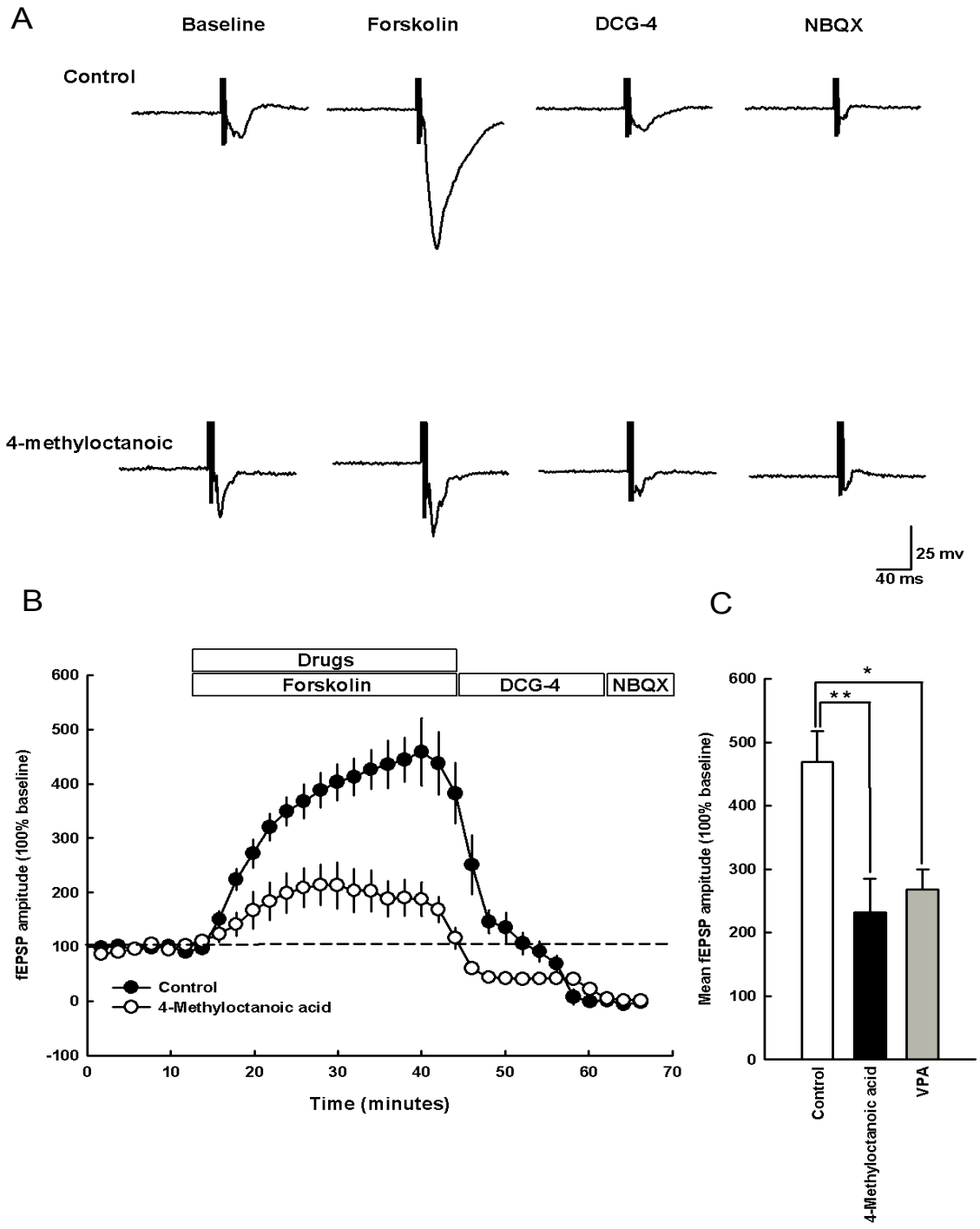

Figure 7.14: Summary the effect of 4-methyloctanoic acid (1 mM) on enhancement of mossy fibre responses induced by application of forskolin (50 µM). (A) *Examples of average traces of mossy fibre responses recorded in control condition and application of 4-methyloctanoic.* (B) *Summary the effect of 4-methyloctanoic on enhancement of mossy fibre responses induced by application of forskolin.* (C) *Comparison of the mean fEPSP amplitude after application of forskolin. Graphs show means ± SEM. Groups were compared using post-hoc Tukey test. * Indicates a significant difference between the two groups (P<0.05). ** Indicates a significant difference between the two groups (P<0.01)*

Table 7.10: The ANOVA for the fEPSP amplitude after application of forskolin in different treatment groups (control, VPA, and 4-methyloctanoic acid).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21.981	2	10.990	8.171	0.003
Within Groups	22.866	17	1.345		
Total	44.847	19			

Table 7.11: The result of Tukey test (Multiple Comparisons), for each treatment group

	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Control VS VPA	2.01372(*)	0.61993	0.012	0.4234	3.6041
Control VS 4-MO	2.36856(*)	0.64524	0.005	0.7133	4.0238
VPA VS 4-MO	0.35484	0.64524	0.848	-1.3004	2.0101

* The mean difference is significant at the .05 level.

7.4 Discussion

Here it has shown that 4-methyloctanoic acid is more potent than VPA in two *in vitro* seizure models, and an *in vivo* model of SE. Furthermore, similar to VPA, application with 4-methyloctanoic acid suppressed the forskolin enhancement of in mossy fibre to CA3 synaptic transmission.

The dose of VPA 400-600 mg/kg *in vivo* was used in this study. And the dose of 4-methyloctanoic acid (400 mg/kg) was decided upon the effective dose of VPA in this *in vivo* model. VPA is effective in this model at 600 mg/kg which abolished the seizure activity in all animals wherease the lower dose VPA (400 mg/kg) which was ineffective. In order to compare the effect of 4-methyloctanoic acid and VPA, the lower dose of 400 mg/kg in VPA and 4-methyloctanoic acid was used.

It could be argued that the dose is much higher than the normal clinical dose, indicating that my results may not be clinically relevant. However, the "therapeutic range" of VPA in animal models of seizure states is much higher than in humans (Nau et al., 1981;Chapman et al., 1982) (Table 9.1). The minimum VPA dose which has an anticonvulsant effect in experimental animal models *in vivo* is 100-700 mg/kg P.O. (40-400 mg/kg ip.) (Chapman et al., 1982). The result indicates that the doses of VPA necessary for antiepileptic activity in human are about ten times lower than those necessary in rodent. The reasons for this are unclear; however, high plasma protein binding and active outward transport of VPA at the blood-brain barrier may be involved. The pharmacokinetic parameters of VPA in the human are drastically different from those in animals. Due to the high metabolic rates of VPA in rodents, VPA has a very short half life and a high plasma clearance rate (Nau et al., 1981;Nau and Löscher, 1984). In animal models, steady state concentrations are difficult to maintain for a

period of time, because of the very high metabolic rate (Nau et al., 1981).

4-methyloctanoic acid, an analogue of VPA, is a natural substance existence in daily food product such as cheese and lamb (Brennand and Lindsay, 1992), indicating that it may be safer than VPA, even though its toxicity has not been determined, including oral toxicity , dermal toxicity , and inhalation toxicity. The concentration in subcutaneous adipose is much greater than its flavour threshold (Brennand et al., 1989). Similarly, the cheeses made from goat milk and sheep milk contain significant amounts of 4-methyloctanoic acid (from 0.09 μg/g to 9.70 μg/g) (Ha and Lindsay, 1991). These findings suggest that 4-methyloctanoic acid is likely to be safe in humans at low doses.

4-methyloctanoic acid has not been investigated extensively. The anticonvulsant effect of 4-methyloctanoic acid had not yet been studied and described before.
The teratogenic effects of VPA have been proposed to be secondary to its effect as a histone deacetylation inhibitor (Gurvich et al., 2005;Eikel et al., 2006;Phiel et al., 2001). Fatty acid with methyl side chains lack this activity and so are likely to have a much lesser teratogenic effect (Narotsky et al., 1994). 4-methyloctanoic acid has great effect on suppression of epileptiform activity in two vitro model (PTZ model and Mg^{2+} model) and on decreasing spontaneous spike activity and behavioural seizures *in vivo* (perforant path stimulation model). Furhter, 4-methyloctanoic acid also provides neuroprotective effect in hippocampus after SE. These preliminary data presented here suggest that 4-methyloctanoic acid may be more potent than VPA in treating seizures and therefore may represent an alternative. Its putative action on the cAMP/PKA signalling pathway predicts that 4-methyloctanoic acid may have neuroprotective and antiepileptic effects. There was some evidence of neuroprotection in the animal SE model. This effect may

also mean that it shares with VPA some of the cognitive side-effects. Further testing of this drug is, however, warranted.

Chapter 8: General discussion

8.1 Results summary

Chapter 3: VPA inhibits PKA-dependent, long-lasting enhancement of synaptic transmission elicited by high-frequency stimulation or application of forskolin at mossy fibre to CA3 pyramidal cell synapses.

Chapter 4: VPA has no direct effect on PKA phosphotransferase activity, even at high concentrations (VPA 1 mM). However, VPA decreases forskolin-induced cAMP accumulation in a high concentration (1mM).

Chapter 5: VPA's anticonvulsant action in two *in vitro* models of epileptiform activity (PTZ model and low-Mg^{2+} model) is not secondary to its effect on cAMP/PKA activity. Surprisingly, SQ22536, an adenylyl cyclase inhibitor, increases the frequency of epileptiform bursts in these two models.

Chapter 6: Application of VPA significantly decreases mossy fibre frequency facilitation (short-term plasticity) in epileptic, but not control- rats.

Chapter 7: 4-methyloctanoic acid, a VPA analogue, is more potent than VPA in two *in vitro* models of epileptiform activity, PTZ model and Mg^{2+} model. Moreover, 4-methyloctanoic acid was significantly more effective than VPA at reducing the spike frequency in an *in vivo* model of status epilepticus, the perforant path stimulation model. Similar to VPA, 4-methyloctanoic acid also inhibits mossy fibre LTP.

8.2 The effect of VPA on cAMP/PKA signalling may have an impact on learning and memory, but may prevent epileptogenesis.

In this thesis, it has been showed that VPA has no effect on baseline transmission or on short term potentiation at the mossy fibre to CA3 pyramidal cell synapses but decreased forskolin mediated enhancement of mossy fibre responses. Consequently VPA inhibits NMDA receptor-independent, long-term potentiation at the mossy fibre to CA3 synapse. Furthermore, VPA impaired the enhancement of cAMP accumulation whereas there was no direct effect on PKA activity.

The action of VPA on cAMP signalling system may also provide a possible explanation for the observation that VPA has been shown to act at many receptors and different cellular pathways such as glutamate receptors (Gean et al., 1994;Rinaldi et al., 2007), inositol metabolic pathway (Shaltiel et al., 2004;Williams et al., 2002;Tokuoka et al., 2008), wnt signals (Hall et al., 2002), histone Deacetylation (Göttlicher et al., 2001;Phiel et al., 2001;Li et al., 2008). cAMP is a second messenger important in many biological processes used for intracellular signal transduction, conveying the cAMP-dependent pathway. GABA(A) and GABA(B) receptors are modulated by PKA mediated phosphorylation, suggesting that the intracellular cAMP levels may modulate the responses of neurons to GABA and consequently have profound effects on synaptic excitability (Moss et al., 1992;Couve et al., 2002). cAMP/PKA signalling system also modulates Na^+ channel slow inactivation contributing to cellular plasticity controlling the firing behaviour of central neurons (Chen et al., 2006). Furthermore, AMP/PKA signalling serves as a second messenger, and is able to crosstalk to other signalling pathways such as ERK pathway (Houslay and Adams D.R., 2003), calmodulin-dependent protein kinase cascade (DeBernardi and Brooker, 1996), PI3K pathway (Namkoong et al., 2009), etc, resulting in diverse physiological effects.

The result further showed that this effect does not explain VPA's antiepileptic action. One alternative possible mechanism of action of VPA is through modulation the inositol metabolic pathway. VPA has been suggested to reduce phospholipid signalling processes and cause a rapid attenuation of phosphatidylinositol (PI) turnover in Dictyostelium. Furthermore, PI turnover is not dependent upon inositol recycling, but is regulated through de novo inositol biosynthesis which is PI3K-dependent (Orabi et al., 2010). This research also indicates that attenuation of PI signalling through inhibition of de novo inositol biosynthesis in Dictyostelium predicts seizure control activity in animal models of epilepsy (Orabi et al., 2010)

Even though the effect of VPA on modulation of cAMP/PKA signalling system does not explain VPA's antiepileptic action; it may, however, contribute to other actions of VPA, including effects on neuroprotection, epileptogenesis, and the impact of VPA on learning and memory.

9.2.1 The effect of VPA on epileptogenesis through modulation cAMP/PKA signalling system

There is increasing interesting in the effect of VPA in preventing epileptogenesis. However, the effect of VPA on epileptogenesis is still controversial. VPA has been suggested to be anti-epileptogenic in the kainic acid epilepsy model of TLE (Bolanos et al., 1998). In another epilepsy model, the kindling model, VPA profoundly inhibited kindling development. VPA inhibited the steady increase in after-discharge duration and behaviour seizure class, which were observed in the vehicle-treated animals, in a dose-dependent manner during 9 days of its administration (Silver et al., 1991). However, VPA did not prevent epileptogenesis in rats with SE induced by prolonged electrical stimulation of the basal amygdale, but it did prevent hyperexcitability and locomotor

hyperactivity (Brandt et al., 2006). Disappointedly, VPA does not prevent post-traumatic epilepsy in patients with head trauma (Temkin et al., 1999).

Even though the effect of VPA on epileptogenesis is uncertain, VPA exerts powerful neuroprotective effects in varied *in vitro* and *in vivo* models. Treatment with VPA after status epilepticus induced by electrical stimulation of the anterior basolateral amygdala prevented the loss of principal cells in the hippocampus formation, including the dentate hilus (Brandt et al., 2006). In the kainate model, treatment with VPA 24 h after SE onset protectedCA1 from damage and to a lesser degree CA3 (Bolanos et al., 1998). VPA has also been found to be neuro-protective in cultured cerebellar granule cells of rat against apoptosis induced by low K^+ concentration (Mora et al., 1999). Hippocampal slices prepared from rats chronically treated with VPA had a significant reduction in cell death in the presence of cytotoxic extracellular ATP (Wilot et al., 2007).

It has been suggested that VPA's neuroprotective effect involves its modulation of extracellular signal-regulated kinase (ERK). ERK regulates AP-1 function and is utilized by neurotrophins to mediate their diverse effects, including neuronal differentiation, neuronal survival, long term neuroplasticity, and potentially learning and memory. VPA can activate the MAPK/ERK signalling pathway by increased phosphorylation of ERK1 and ERK2 in rat hippocampus and frontal cortex (Einat et al., 2003). VPA-induced activation of ERK was blocked by the mitogen-activated protein kinase/ERK kinase inhibitor PD098059 and dominant-negative Ras and Raf mutants but not by dominant-negative stress-activated protein kinase/ERK kinase and mitogen-activated protein kinase 6 mutants. VPA also increased the expression of genes regulated by the ERK pathway, including growth cone-associated protein 43 and Bcl-2,

promoted neurite growth and cell survival, and enhanced norepinephrine uptake and release. These data demonstrate that VPA is an ERK pathway activator and produces neurotrophic effects (Yuan et al., 2001).

Recent research has suggested that VPA can activate ERK via cAMP/PKA signalling system. Application with forskolin blocked ERK phosphorylation with an EC50 of less than 3 μM, and application with the PKA inhibitor H-89 enhanced ERK phosphorylation, implicating inhibition of PKA as the predominant pathway for ERK activation (Boeckeler et al., 2006). However, my data indicate that VPA acts not directly on PKA but by inhibiting the accumulation of cAMP.

9.2.2 VPA and learning and memory

LTP has been proposed to be the cellular basis of memory formation in the central nervous system. It has been demonstration that VPA induces cognitive impairments in Morris maze performance since the treated animals (VPA, 200-300 mg/kg, in normal rats and VPA, 100 mg/kg, in amygdala-kindled rats) required a longer time to find the hidden platform. Also, VPA (1mM) caused a decrease in posttetanic potentiation and LTP in CA1 area of rat hippocampal slices (Lamberty, 2000). Other studies also indicated that VPA (0.1 mg/ml) abolished the expression of NMDA receptor-dependent LTP, even though it did not inhibit NMDA receptor-independent LTP in hippocampal CA1 area (Lee, 1996).

The cAMP/PKA signalling pathway is crucial for both the late phase of LTP in the hippocampus and in the late, de novo brain protein synthesis-dependent phase of memory formation (Tully, 1997;Scharf et al., 2002). PKA mutant mice show

impairments in CA1 Late-LTP elicited by spaced stimulation (Abel et al., 1997). Late LTP transforms newly learned information into a permanent and stable state. It depends on a crucial phase of gene expression and de novo protein synthesis in rodents that requires phosphorylation/activation by PKA and ERK/MAP of the transcription factor CREB (cAMP response element-binding protein)(Gonzalez and Montminy, 1989;Dash et al., 1991;Bernabeu et al., 1997;Scharf et al., 2002;Impey et al., 1998;Roberson et al., 1999). VPA could therefore have an impact on memory and learning through its action on PKA. Is there evidence for such an action in humans? There is certainly growing evidence that children exposed in utero to VPA may have later learning difficulties (Meador et al., 2009). In adults, there are well recognised reports of cognitive impairment associated with VPA use. Patients receiving long-term VPA therapy can a syndrome of reversible Parkinsonism and cognitive impairment. The signs of parkinsonism includes the 4 to 7 Hz tremor, rigidity or cogwheeling, decreased alternate motion rates (extremities, tongue) or hypokinetic speech, abnormalities of posture or gait (instability, shuffling), and bradykinesis. Furthermore, long term using VPA can cause memory dysfunction and personality changes (Armon et al., 1996). Since the anticonvulsant effect of VPA does not depend upon its action on PKA, it may be possible to design drugs in future that are anticonvulsant but have a much improved cognitive profile.

9.3 The effect of 4-methyloctanoic acids, an analogue of VPA on animal models of epilepsy

4-methyloctanoic acids, also known as hircinoic acid, appears to contribute mutton and goat meat odour and responsible for the "billy-goat" flavour of goat milk cheese. The study of this compound is mainly in food industry and agriculture. The property of its anti-epileptic effect has not been previously explored and discussed.

Application of 4-methyloctanoic acid in an *in vivo* model of SE demonstrates efficacy in animal seizure models. SE was induced by stimulation of the perforant path in awake, freely moving rats. This model is resistant to a number of antiepileptic drugs and does not involve the application of an exogenous convulsant. The efficacy of 4-methyloctanoic acid (400 mg/kg) against valproic acid (400 mg/kg) and vehicle (DMSO) was compared. 4-methyloctanoic acid was significantly more effective at reducing the spike frequency, spikes amplitude, and behavioural seizures. 4-methyloctanoic acid protected neurodegeneration in CA3 and CA1 in hippocampus after SE, suggesting that it may share the same mechanism with VPA on modulation of cAMP/PKA signalling system. Indeed administration with 4-Methyloctanoic acid suppressed the enhancement of mossy fibre responses, suggesting that this compound may act on modulation of cAMP signalling. These finding, therefore provide a novel insight into the effectiveness of this compound in the treatment of epilepsy, suggesting that this compound has a potential to develop as an anticonvulsant drug which is more efficient with less teratogenic potential.

9.4 Using animal model to investigate Human disease- epilepsy

In this thesis, varied animal models were used to study the epilepsy which is a human neurological disease. The ability to reproduce human diseases in animal models is a great advantage for modern experimental medicine. Animal models, such as genetically modified animals, and experimental animal model involving chemical or electrical stimulation can mimic the human condition, both pathophysioloigcally and pharmacologically. *In vitro* models involving manipulations conducted in organs, tissues, cells, and biomolecules in a controlled, artificial environment are useful tools to elucidate the disease pathology, the mechanism of pathology, and evaluate the effect of

drugs (Figure 8.1). Unfortunately it is rarely possible to reproduce all aspects of human disease in animals. Therefore, the relevance of animal models to human disease is not always clear cut.

Indeed, the validity of animal epilepsy models has been challenged for the following reasons. (1) Many animal studies are carried out in "normal" rather than "epileptic" brains. (2) The behavioural expression of seizures in animals is different from that in humans. (3) There may be different anatomical and physiological substrates for seizures in animals (Sarkisian, 2001). Nevertheless, notwithstanding their limitations, animal models have given us considerable insight into the pathogenesis of human epilepsy, and have enabled drug development (Pitkänen al., 2007; Löscher, 2002).

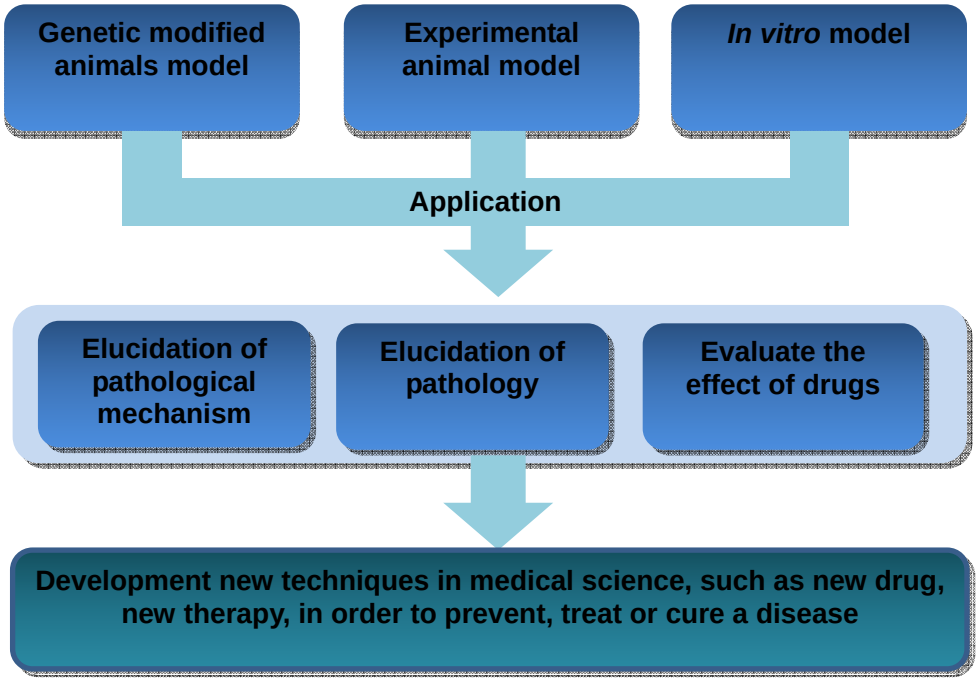

Figure 8.1: Research of animal models for human diseases. The animal models, such as genetic modified animals, experimental animal model, and in vitro model are useful tool to elucidate the disease pathology, the mechanism of pathology and evaluate the effect of drugs. Furthermore, the benefit of using animal's model to study human disease is to develop the new technique or new therapy in order to prevent, treat and cure disease, such as epilepsy

9.6 Future work

Many questions have been raised in my thesis. Does the effect of VPA on the cAMP/PKA signalling system translate to a neuroprotective and antiepileptogenic effect *in vivo*? To address this I would like to show that VPA attenuates PKA activation during SE and that specific targeting of the PKA signalling cascade can prevent neuronal death and epileptogenesis. Ideally, I would like to screen VPA analogues through a cAMP assay to identify putative antiepileptic compounds that lack this effect. The neuroprotective and anticonvulsant properties of those compounds (and also their impact on memory and learning) need to be further tested.

In this thesis, a new putative antiepileptic drug has been identified. Much further work is required before this compound could be considered for human trials, including toxicity testing and testing in other animal epilepsy models (e.g. the NIH screening program). Lastly, we have now developed a wireless telemetry unit. This requires testing in larger numbers of animals over longer periods of time. If it lives up to its present promise, it may provide an ideal way to test the antiepileptogenic and antiepileptic affect of drugs *in vivo* both acutely and with more chronic administration.

Appendix: Method development Wireless transmitter

A.1 Introduction

There is a growing need for long term continuous EEG recordings in animals in order to monitor seizure frequency and the process of epileptogenesis more accurately. Moreover, it is critical to have such a system in place to monitor the impact of treatments on both seizures and epileptogenesis.

Conventionally, physiological data, such as EEG, heartbeat, blood pressure, gained from animals are recorded by a direct connection of animals to the recording apparatus. The EEG recording, for example, is through a cable which connects between EEG electrodes on the animal's head and the EEG recording amplifier. There are a number of disadvantages to this: (1) tethering of an animal can cause a certain amount of distress (2) cables form the animal amplify any movement artefact (3) Cables from the animal act as an antenna and so can "pick up" extraneous electrical noise, in particular "mains hum". Therefore, there are considerable advantages in developing wireless telemetric recording systems.

In order to develop a useful and stable wireless transmitter which can be implanted into experimental animals, there are some aspects needed to be addressed. First of all, the wireless transmitter should be as small and light as possible in order to ensure that awake experimental animals are as comfortable as possible, to minimize the suffering of animals and to permit subcutaneous placement. Secondly, the wireless transmitter should be fully bio-stable and biocompatible in order to permit prolonged implantation (months). Thirdly, battery life should be compatible with continuous recording over weeks to months. Fourthly, it is important to have a switching system which can be operated from outside of the body. Furthermore, a high sampling frequency is necessary in order to be able to record fast activity associated with seizures, yet the energy

consumption of the implanted wireless transmitter should be as low as possible to preserve battery life. Finally, in order to increase flexibility, transmitter data should be able to be acquired by remote computer or laptop directly, or via the local area network.

The object of this part of the thesis was the development of a telemetric system in order to test novel treatment strategies. The design and testing of the system was in collaboration with Open Source Instruments, MA, USA, who designed and built the electronic circuits. I implanted these wireless transmitters into rats and tested their ability to record EEG accurately and over prolonged periods of time in adult freely moving rats under normal conditions, during and following SE.

A.2 Methods

A.2.1 Overview

This system consists of an implantable transmitter which amplifies and converts the recorded signal and communicates with an antenna via radio transmission over a distance ranged from 50-300 cm. The receiver antenna is connected to a data receiver via a cable connection. The data are displayed and saved by software developed by open instruments for this purpose through Long-Wire Data Acquisition (LWDAQ) hardware (Figure A.1).

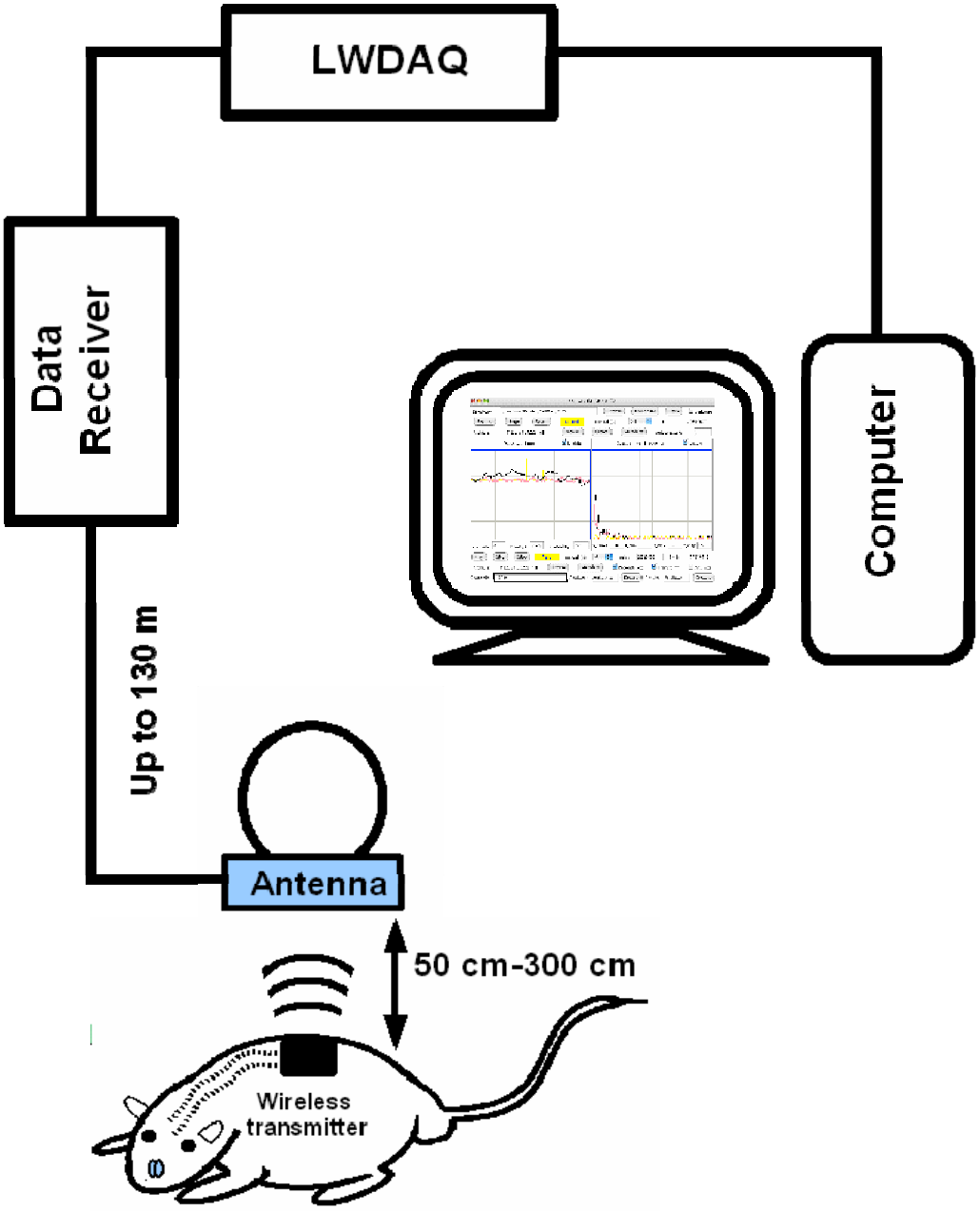

Figure A.1: Schematic of the main components of the telemetric system. The recording electrodes are implanted on the cortical surface and the reference electrodes are place on the surface of skull. The electrodes are connected to a transmitter placed in the back area of the rat. The transmitter is switched ON/OFF by the magnet. The recorded signal is directly amplified and digitized by the transmitter, emitted in all directions and detected by the receiver at a distance of up to 300 cm. The data are transferred from the receiver to a computer via LWDAQ system.

A.2.2 Subcutaneous transmitter design

A number of different encapsulating processes were tested. Silicone covering alone was penetrated by fluid within a week in five out of five animals. This led to the use of an initial epoxy coating to prevent water penetration. The Subcutaneous Transmitter (Figure A.2) was therefore encapsulated in epoxy and silicone, which proved to be water resistant in all animals tested up to 2 months. Further complicating factors were the wires from the transmitter to the electrodes. Coiled steel wire provided the only wire tested that was durable, yet strong enough to not break over such long recording periods (other wires tested were silver n = 3, copper n = 2 and uncoiled steel n = 5). The Subcutaneous Transmitter detects biometric signals with thin wires and transmits them with another wire acting as an antenna. The Subcutaneous Transmitter transmits signals from the body of a freely-moving rat to a receiving RF antenna at ranges of 50 cm to 300 cm. (for summary of subcutaneous Transmitter see Table A.1)

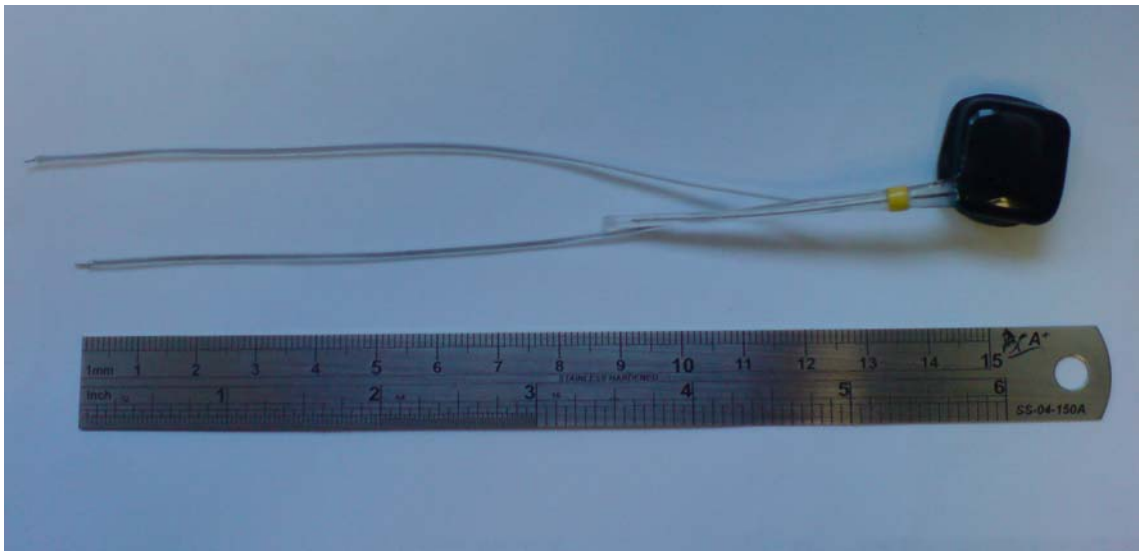

Figure A.2: The example of subcutaneous transmitter

Table A.1: Properties of the transmitter unit

Property	
Weight	5 g (with batteries and wires)
Dimensions	20 mm × 20 mm × 10 mm
Power supply	3-V 120 mA-hr Li
Switch	a magnet
Data Transmission Rate	400 samples per second
Data Format	16-bit digital with 24-bit ID
Life time battery	over 4 months

In order to conserve the battery, the transmitter transmits information in short bursts at RF frequency (915 MHz). These bursts contain information about the transmitter (which number) and the EEG signal. In this way many transmitters can be recorded simultaneously by the same receiver (each transmitter transmits at different times for only a fraction of time).

A.2.3 RF Antenna

The RF Antenna is a loop of wire with diameter 328 mm and with impedance 102Ω which is optimised to record othe915-MHz signal. The RF antenna has to be positioned near to the cage and away from reflecting surfaces which result in “collisions” causing interference. A collision is the interaction of a signal with its reflection, resulting in corruption of the signal.

A.2.4 Data Receiver

The data Receiver consists of a demodulating Receiver, saw oscillator, and data recorder with BNC connection for an RF Antenna. The demodulating receiver combines the RF input passes with the local oscillator input. The demodulated output is passed via an ethernet cable to LWDAQ. The receiver has a buffer, so that if the connection to the LWDAQ is broken data is still stored.

A.2.5 LWDAQ hardware

The LWDAQ is a TCPIP-based hardware which permits data transmission via a local network or the internet.

The LWDAQ software has been developed by Open Source Instruments Inc (USA) is a combination of analysis routines written in Pascal and communication routines written in TclTk. The core of LWDAQ Software is a TclTk interpreter. This interpreter executes the TclTk scripts that control the LWDAQ over TCPIP and creates the graphical interface. It loads the analysis library “lwdaq” which is created for the LWDAQ software only, and it makes its routines available to the scripts as TclTk commands.

A.2.6 Surgery

All experiments were conducted in accordance with the Home Office regulations under the Animal (Scientific Procedures) Act, 1986. Male Sprague–Dawley rats weighed between 300 g to 400 g were used. The animals were housed individually in standard plastic cages (42 cm × 26 cm × 20 cm) under a 12 h light–dark cycle. The room temperature was maintained at 24–25 °C and relative humidity at 50–60%. Standard rodent food and tap water were provided.

The surgery was performed in deeply anaesthetised animals, maintained with 2% isoflurance in O_2 (at a flow rate of 2 litres/minutes) on a stereotaxic frame with heating pad (David Kopf stereotaxic frame)

The transmitter was placed subcutaneously on the back of the animal and secured with a suture. The wires from the transmitter were tunnelled under the skin to the exposed skull. One wire was connected to an exposed silver wire ground. The other wire was insulated with ~ 1 mm of wire exposed. This was held in place extradurally with a skull screw. The whole assembly was held in place with dental cement (Simplex Rapid, Acrylic Denture Polymer, UK) (Figure A.3).

A.2.7Data recordings

The recording started immediately after surgery. The rats were placed in a plastic cage. The subcutaneous transmitter was switched on with the magnet and the signal was observed in real-time on a computer's screen. The signal was recorded with LWDAQ software and saved on the hard-disk. Data were then replayed or further analysed.

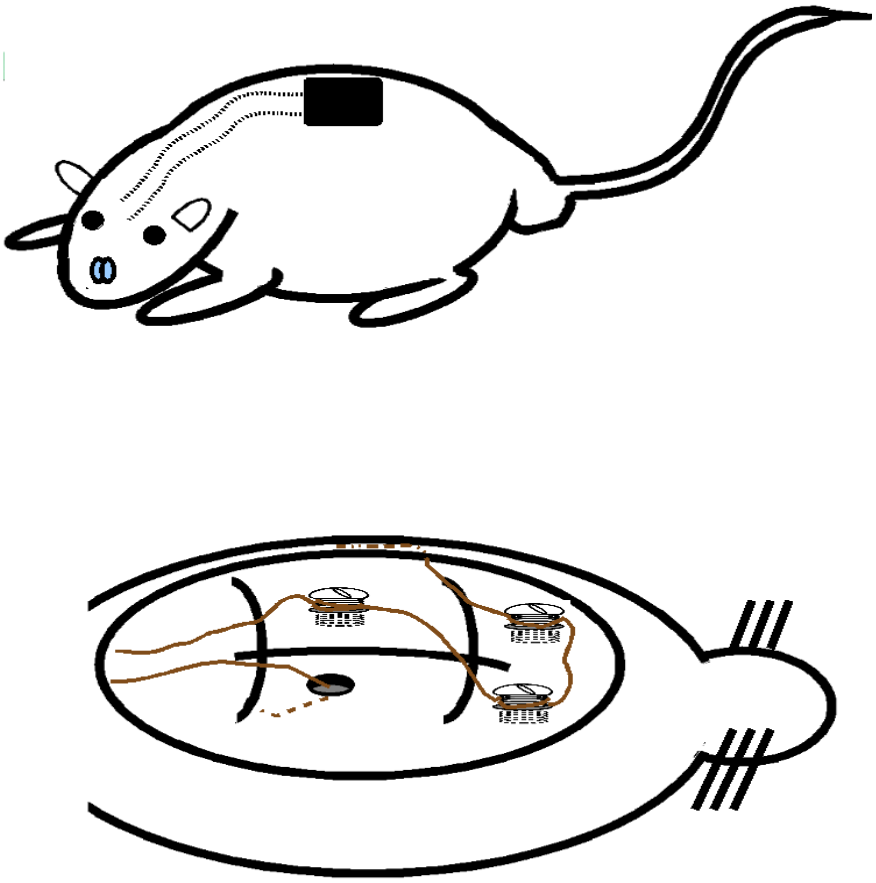

Figure A.3: Implantation of wireless transmitter (A) *Placement of the transmitter in the back, the leads were tunnelled under the skin from the back to the skull and holes were drilled in the skull to place the recording electrode, which were fixed to the skull with dental cement.* (B) *The reference electrodes were placed above the skull, and the recording electrode recording electrode was placed above the cortex (extradurally).*

A.2.8 Kainic acid induced Status epilepticus

SE was induced with a single dose of 10 mg/kg of Kainic acid given intraperitoneally (ip) to Sprague-Dawley rats (300-400 g), which were then carefully observed to detect signs of seizures. One hour after the first Racine stage 5 seizure, 10 mg/kg of diazepam or 30 mg/kg pentobarbital was injected (ip) to stop the seizures. The wireless transmitter was used to continuously record EEG.

A.3 Result

After implantation of the transmitter, rats were placed in standard plastic cages individually, and EEG was recorded continuously (Figure A.4). Continuous EEG recordings were achievable for over 1 month before battery failure.

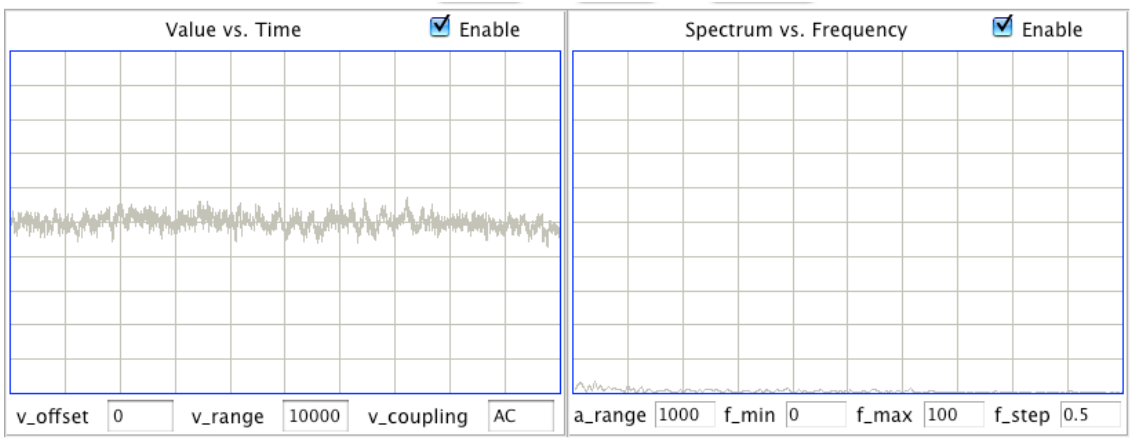

Figure A.4: Example of EEG recording from wireless transmitter in a control rat one week after transmitter implantation. Voltage versus time is on the left, 140 μV/div and 800 ms/div. Spectrum versus frequency (fast Fourier analysis) is on the right, with 15 μV/div and 10 Hz/div.

A.3.1 The EEG record during status epilepticus induced by administration with Kainic acid

In two animals, I have been able to undertake long term recordings following kainic acid injection. An example EEG recording during the kainic acid injection is given below. Injection of kainic acid (10 mg/kg, ip) induced convulsive SE. Pentobarbital (30 mg/kg) was given to reduce the mortality, 1 hour after the first stage 5 seizure.

Ten minutes after injection, spikes were apparent, even though the rat had no sign of behaviour seizure. The frequency of spikes ranges from 0-30 Hz. The spike amplitudes were 560-700 μV. As for spectrum analysis, the peak amplitude was about 22.5-30 μV between 5-10 Hz (Figure A.5).

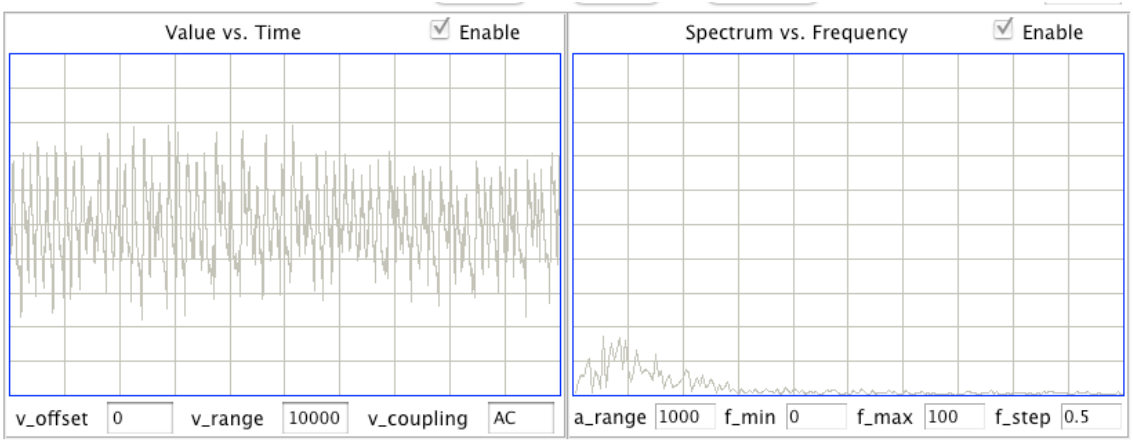

Figure A.5: Example of EEG recording from wireless transmitter in rat 10 minutes after injected kainic acid. Voltage versus time is on the left, with 140 µV/div and 800 ms/div. Spectrum versus frequency is on the right, with 15 µV/div and 10 Hz/div.

Twenty minutes after injection, the rat showed head nodding which is classified as a stage two seizure. Spike amplitudes ranged from 420-560 µV. The spectrum analysis demonstrated three sharp peaks, 22.5 µV at 2.5HZ, 37.5 µV at 7.5 HZ, and 30 µV at 12.5 Hz. (Figure A.6).

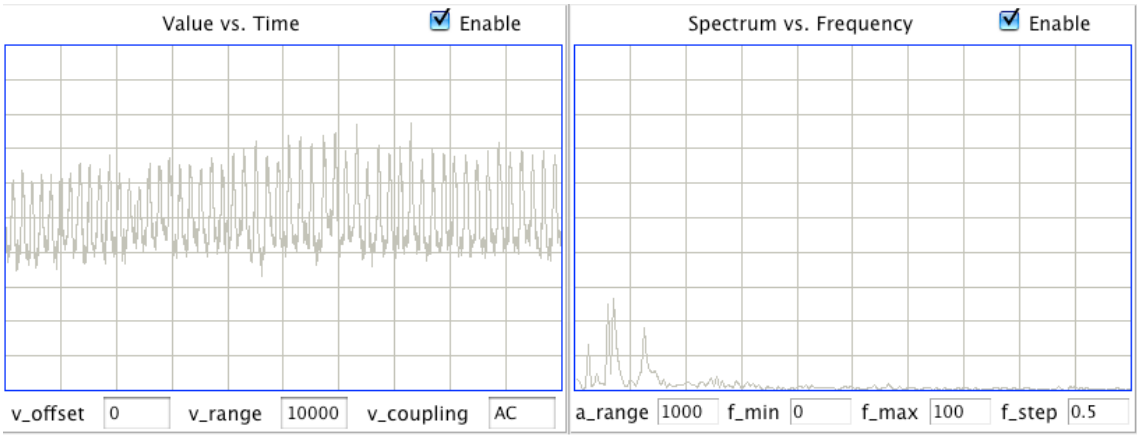

Figure A.6: Example of EEG recording from wireless transmitter in rat with stage two behavioural seizures induced by injected kainic acid. Voltage versus time is on the left, with 140 µV/div and 800 ms/div. Spectrum versus frequency is on the right, with 15 µV/div and 10 Hz/div.

The behavioural seizure progressed to stage 3 one hour after injection. The amplitude of the spikes increased to 500 µV. There was a peak in the frequency spectrum at 15-16 Hz with amplitude 35-37.5 µV.

The behavioural seizure progressed to stage 3 one hour after injection. The amplitude of the spikes increased to 500 µV. There was a peak in the frequency spectrum at 15-16 Hz with amplitude 35-37.5 µV.

About 90 minutes after injection the seizure reached stage 5, with rearing and failing. The amplitude of the EEG spikes dramatically increased to 1400-1500 µV. In the frequency spectrum, there was a sharp peak at frequency 2.5-7.5 Hz with amplitude over 80-90 µV (Figure A.7).

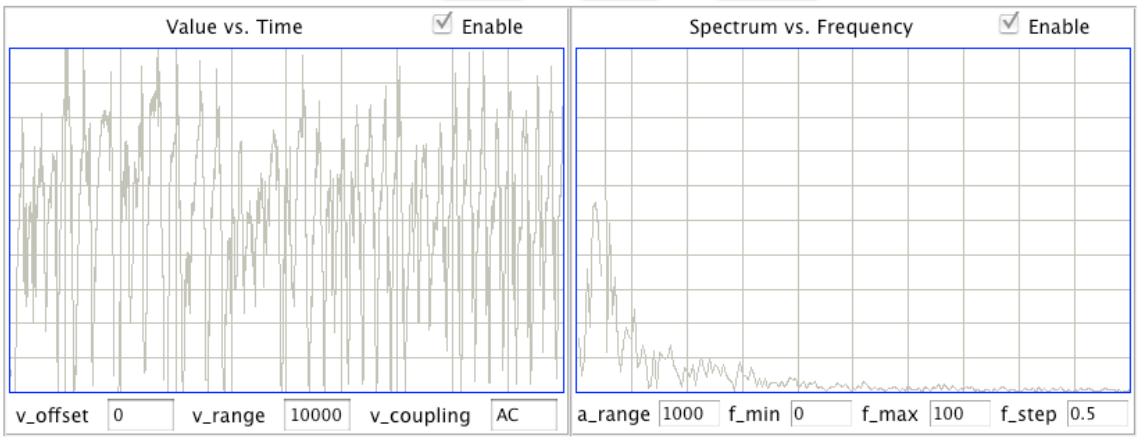

Figure A.7: Example of EEG recording from wireless transmitter in rat with stage 5 behavioural seizure. Voltage versus time is on the left, with 140 µV/div and 800 ms/div. Spectrum versus frequency is on the right, with 15 µV/div and 10 Hz/div

A.4 Discussion

In collaboration with Opensource Instruments we developed a long-term wireless EEG monitoring system that offers several advantages over currently available telemetric systems. The relatively small size and weight of our fully implantable device is very well accepted by adult rats which do not show any kind of behavioural impairments or sign of discomfort. Furthermore, the transmitter can be easily switched on and off from outside the body. The LDWAQ software is easy to use and can be used to analyse the signal. The system is inexpensive, portable and easy to use. Another advantage of this system is the transmitting data can be displayed and recorded via internet.

The system has only so far undergone preliminary testing and a larger study is still required. Some problems still, however, need to be addressed. There are silent transmission areas such that when the rat gets into certain positions reception of the signal is poor, and in some animals the subcutaneous wires broke.

Another problem is that the broad-band low power signals can easily be overwhelmed by ambient interference, such as mobile telephones. Besides, in the laboratory, there are many extraneous signals that can cause interference (Figure A.8).

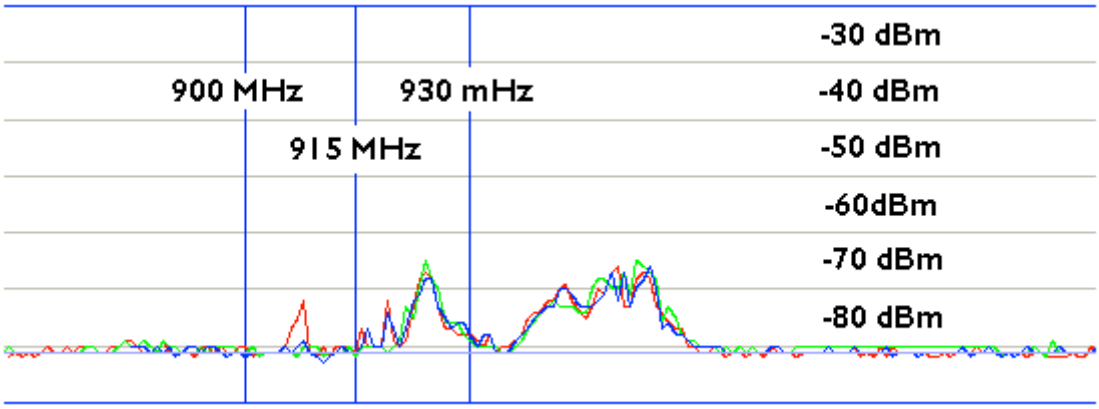

Figure A.8: Spectrum analysis of environmental noise. Blue lines are 900 MHz, 915 MHz, and 930 MHz. The horizontal lines mark relative power density in +10 dB increments. In the recording room, there is residual power in the Fixed Mobile band, 942 MHz - 960 MHz. The peaks between 920MHz-930 MHz are another fixed mobile band. Below that we have several power peaks in the range 910 MHz to 925 MHz.

In order to address these problems, we have started using Faraday cages to prevent interference. The faraday cages can also isolate transmitters from one to another, so that transmitters with the same channel number will not interfere with each other. By enclosing the transmitters and receiver in a faraday cage, we hope to decrease ambient interference and improve reception of the transmitter signals.

Reference List

Abel T, Nguyen PV, Barad M, Deuel TAS, Kandel ER, Bourtchouladze R (1997) Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. Cell 88:615-626.

Adams B, Sazgar M, Osehobo P, Van der Zee CE, Diamond J, Fahnestock M, Racine RJ (1997) Nerve growth factor accelerates seizure development, enhances mossy fiber sprouting, and attenuates seizure-induced decreases in neuronal density in the kindling model of epilepsy. J Neurosci 17:5288-5296.

Adams JM, Cory S (1998) The Bcl-2 protein family: Arbiters of cell survival. Science 281:1322-1326.

Alessandrini A, Namura S, Moskowitz MA, Bonventre JV (1999) MEK1 protein kinase inhibition protects against damage resulting from focal cerebral ischemia. PNAS 96:12866-12869.

Alexander GM, Godwin DW (2006) Metabotropic glutamate receptors as a strategic target for the treatment of epilepsy. Epilepsy Res 71:1-22.

Allan LA, Morrice N, Brady S, Magee G, Pathak S, Clarke PR (2003) Inhibition of caspase-9 through phosphorylation at Thr 125 by ERK MAPK. Nat Cell Biol 5:647-654.

Alle H, Jonas P, Geiger JR (2001) PTP and LTP at a hippocampal mossy fiber-interneuron synapse. PNAS 98:14708-14713.

Ambroso JL, Frank ER, Keller LH, Trimmer GW, Waterman SJ (1999) Developmental toxicity assessment of C8 iso acid in CD rats: relevance to embryotoxic aliphatic carboxylic acids. Toxicol Sci 52:240-247.

Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J (2007) The hippocampus book. Oxford.

Andre V, Ferrandon A, Marescaux C, Nehlig A (2001) Vigabatrin protects against hippocampal damage but is not antiepileptogenic in the lithium-pilocarpine model of temporal lobe epilepsy. Epilepsy Res 47:99-117.

Apland J, Helekar S, Lebeda F (1997) Suppression of drug-induced epileptiform discharges by cyclic AMP in rat hippocampus. Mol Chem Neuropathol 31:265-278.

Applegate CD, Samoriski GM, Ozduman K (1997) Effects of valproate, phenytoin, and MK-801 in a novel model of epileptogenesis. Epilepsia 38:631-636.

Arabadzisz D, Antal K, Parpan F, Emri Z, Fritschy JM (2005) Epileptogenesis and chronic seizures in a mouse model of temporal lobe epilepsy are associated with distinct EEG patterns and selective neurochemical alterations in the contralateral hippocampus. Exp Neurol 194:76-90.

Araki T, Kato H, Fujiwara T, Itoyama Y (1995) Age-related changes in bindings of second messengers in the rat brain. Brain Res 704:227-232.

Armand V, Louvel J, Pumain R, Heinemann U (1998) Effects of new valproate derivatives on epileptiform discharges induced by pentylenetetrazole or low Mg2+ in rat entorhinal cortex-hippocampus slices. Epilepsy Res 32:345-355.

Armon C, Shin C, Miller P, Carwile S, Brown E, Edinger JD, Paul RG (1996) Reversible parkinsonism and cognitive impairment with chronic valproate use. Neurology 47:626-635.

Arndt TL, Stodgell CJ, Rodier PM (2005) The teratology of autism. Int J Dev Neurosci 23:189-199.

Artemowicz B, Sobaniec W (2005) Neuroprotection possibilities in epileptic children. Rocz Akad Med Bialymst 50 Sl:91-95.

Arundine M, Tymianski M (2003) Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. Cell Calcium 34:325-337.

Arzimanoglou A, Hirsch E, Nehlig A, Castelnau P, Gressens P, de Vasconcelos AP (2002) Epilepsy and neuroprotection: an illustrated review. Epileptic Disord 4:173-182.

Asprodini EK, Rainnie DG, Shinnick-Gallagher P (1992) Epileptogenesis reduces the sensitivity of presynaptic gamma- aminobutyric acid B receptors on glutamatergic afferents in the amygdala. J Pharmacol Exp Ther 262:1011-1021.

Attwell PJE, Singh Kent N, Jane DE, Croucher MJ, Bradford HF (1998) Anticonvulsant and glutamate release-inhibiting properties of the highly potent metabotropic glutamate receptor agonist (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV). Brain Res 805:138-143.

Babb TL, Kupfer WR, Pretorius JK, Crandall PH, Levesque MF (1991) Synaptic reorganization by mossy fibers in human epileptic fascia dentata. Neuroscience 42:351-363.

Balding FJ, Geller HM (1981) Sodium valproate enhancement of gamma-aminobutyric acid (GABA) inhibition: electrophysiological evidence for anticonvulsant activity. J Pharmacol Exp Ther 217:445-450.

Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, Langer SZ (1998) International union of pharmacology. XV. subtypes of gamma -aminobutyric acid A receptors: classification on the basis of subunit structure and receptor function. Pharmacol Rev 50:291-314.

Bartolomei F, Khalil M, Wendling F, Sontheimer A, Régis J, Ranjeva JP, Guye M, Chauvel P (2005) Entorhinal cortex involvement in human mesial temporal lobe epilepsy: an electrophysiologic and volumetric study. Epilepsia 46:677-687.

Beck H, Goussakov IV, Lie A, Helmstaedter C, Elger CE (2000) Synaptic plasticity in the human dentate gyrus. J Neurosci 20:7080-7086.

Ben-Ari Y, Gho M (1988) Long-lasting modification of the synaptic properties of rat CA3 hippocampal neurones induced by kainic acid. J Physiol 404:365-384.

Ben-Ari Y, Tremblay E, Ottersen OP, Naquet R (1979) Evidence suggesting secondary epileptogenic lesions after kainic acid: pretreatment with diazepam reduces distant but not local brain damage. Brain Res 165:362-365.

Ben-Ari Y, Cossart R (2000) Kainate, a double agent that generates seizures: two decades of progress. Trends Neurosci 23:580-587.

Bengzon J, Kokaia Z, Elmér E, Nanobashvili A, Kokaia M, Lindvall O (1997) Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. PNAS 94:10432-10437.

Berkeley JL, Decker MJ, Levey AI (2002) The role of muscarinic acetylcholine receptor-mediated activation of extracellular signal-regulated kinase 1/2 in pilocarpine-induced seizures. J Neurochem 82:192-201.

Bernabeu R, Bevilaqua L, Ardenghi P, Bromberg E, Schmitz P, Bianchin M, Izquierdo I, Medina JH (1997) nvolvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning inrats. PNAS 94:7041-7046.

Bikson M, Ghai RS, Baraban SC, Durand DM (1999) Modulation of Burst Frequency, Duration, and Amplitude in the Zero-Ca2+ Model of Epileptiform Activity. J Neurophysiol 82:2262-2270.

Blackstone C, Murphy TH, Moss SJ, Baraban JM, Huganir RL (1994) Cyclic AMP and synaptic activity-dependent phosphorylation of AMPA- preferring glutamate receptors. J Neurosci 14:7585-7593.

Blair RE, Sombati S, Lawrence DC, McCay BD, DeLorenzo RJ (2004) Epileptogenesis causes acute and chronic increases in GABAA receptor endocytosis that contributes to the induction and maintenance of seizures in the hippocampal culture model of acquired epilepsy. J Pharmacol Exp Ther 310:871-880.

Bliss TVP, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31-39.

Blümcke I, Behle K, Malitschek B, Kuhn R, Knöpfel T, Wolf HK, Wiestler OD (1996) Immunohistochemical distribution of metabotropic glutamate receptor subtypes mGluR1b, mGluR2/3, mGluR4a and mGluR5 in human hippocampus. Brain Res 736:217-226.

Boeckeler K, Adley K, Xu X, Jenkins A, Jin T, Williams RSB (2006) The neuroprotective agent, valproic acid, regulates the mitogen-activated protein kinase pathway through modulation of protein kinase A signalling in Dictyostelium discoideum. Eur J Cell Biol 85:1047-1057.

Bolanos AR, Sarkisian M, Yang Y, Hori A, Helmers SL, Mikati M, Tandon P, Stafstrom CE, Holmes GL (1998) Comparison of valproate and phenobarbital treatment after status epilepticus in rats. Neurology 51:41-48.

Boulton CL, McCrohan CR, O'Shaughnessy CT (1993) Cyclic AMP analogues increase

excitability and enhance epileptiform activity in rat neocortex in vitro. Eur J Pharmacol 236:131-136.

Brandt C, Potschka H, Löscher W, Ebert U (2003a) N-methyl--aspartate receptor blockade after status epilepticus protects against limbic brain damage but not against epilepsy in the kainate model of temporal lobe epilepsy. Neuroscience 118:727-740.

Brandt C, Gastens AM, Sun Mz, Hausknecht M, Löscher W (2006) Treatment with valproate after status epilepticus: Effect on neuronal damage, epileptogenesis, and behavioral alterations in rats. Neuropharmacology 51:789-804.

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

Brennand CP, Lindsay RC (1992) Distribution of volatile branched-chain fatty acids in various lamb tissues. Meat Sci 31:411-421.

Brennand.C.P., Ha JK, Lindsay RC (1989) Aroma properties and thresholds of some branched-chain and other minor volatile fatty acids occurring in milkfat and meat lipids. J Sens Stud 41:105-120.

Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA (1998) Selective changes in single cell GABAA receptor subunit expression and function in temporal lobe epilepsy. Nat Med 4:1166-1172.

Buchheim K, Schuchmann S, Siegmund H, Weissinger F, Heinemann U, Meierkord H (2000) Comparison of intrinsic optical signals associated with low Mg2+– and 4-aminopyridine–induced seizure-like events reveals characteristic features in adult rat limbic system. Epilepsia 41:635-641.

Buhl EH, Otis TS, Mody I (1996) Zinc-induced collapse of augmentedinhibition by GABA in a temporal lobe epilepsy model. Science 271:369-373.

Burgering BM, Coffer PJ (1995) Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. Nature 376:599-602.

Capella HM, Lemos T (2002) Effect on epileptogenesis of carbamazepine treatment during the silent period of the pilocarpine model of epilepsy. Epilepsia 43:110-111.

Cartmell J, Schoepp DD (2000) Regulation of neurotransmitter release by metabotropic glutamate receptors. J Neurochem 75:889-907.

Carvalho AL, Kameyama K, Huganir RL (1999) Characterization of phosphorylation sites on the glutamate receptor 4 subunit of the AMPA receptors. J Neurosci 19:4748-4754.

Castillo PE, Weisskopf MG, Nicoll RA (1994) The role of Ca2+ channels in hippocampal mossy fiber synaptic transmission and long-term potentiation. Neuron 12:261-269.

Cavalheiro E (1995) The pilocarpine model of epilepsy. Ital J Neurol Sci 16:33-37.

Cavalheiro EA, Santos NF, Priel MR (1996) The pilocarpine model of epilepsy in mice. Epilepsia 37:1015-1019.

Cavazos JE, Das I, Sutula TP (1994) Neuronal loss induced in limbic pathways by kindling: evidence for induction of hippocampal sclerosis by repeated brief seizures. J Neurosci 14:3106-3121.

Cavazos JE, Sutula TP (1990) Progressive neuronal loss induced by kindling: a possible mechanism for mossy fiber synaptic reorganization and hippocampal sclerosis. Brain Res 527:1-6.

Chandler KE, Princivalle AP, Fabian-Fine R, Bowery NG, Kullmann DM, Walker MC (2003) Plasticity of GABA(B) receptor-mediated heterosynaptic interactions at mossy fibers after status epilepticus. J Neurosci 23:11382-11391.

Chang BS (2003) Mechanisms of disease: epilepsy. N Engl J Med 349:1201.

Chapman A, Keane PE, Meldrum BS, Simiand J, Vernieres JC (1982) Mechanism of anticonvulsant action of valproate. Prog Neurobiol 19:315-359.

Chapman A, Meldrum BS, Mendes E (1983) Acute anticonvulsant activity of structural analogues of valproic acid and changes in brain GABA and aspartate content. Life Sci 32:2023-2031.

Chen G, Manji HK, Wright CB, Hawver DB, Potter WZ (1996) Effects of valproic acid on [beta]-adrenergic receptors, G-proteins, and adenylyl cyclase in rat C6 glioma cells. Neuropsychopharmacology 15:271-280.

Chen G, Zeng WZ, Yuan PX, Huang LD, Jiang YM, Zhao ZH, Manji HK (1999) The mood-stabilizing agents lithium and valproate robustlIncrease the levels of the neuroprotective protein bcl-2 in the CNS. J Neurochem 72:879-882.

Chen HX, Roper SN (2003) PKA and PKC enhance excitatory synaptic transmission in human dentate gyrus. J Neurophysiol 89:2482-2488.

Chen Y, Yu FH, Surmeier DJ, Scheuer T, Catterall WA (2006) Neuromodulation of Na+ channel slow inactivation via cAMP-dependent protein kinase and protein kinase C. Neuron 49:409-420.

Cheung ECC, Slack RS (2004) Emerging role for ERK as a key regulator of neuronal apoptosis. Sci STKE 2004:e45.

Chevaleyre V, Castillo PE (2002) Assessing the role of Ih channels in synaptic transmission and mossy fiber LTP. PNAS 99:9538-9543.

Chu CT, Levinthal DJ, Kulich SM, Chalovich EM, DeFranco DB (2004) Oxidative neuronal injury: The dark side of ERK1/2. Eur J Biochem 271:2060-2066.

Cilio MR, Bolanos AR, Liu Z, Schmid R, Yang Y, Stafstrom CE, Mikati MA, Holmes GL (2000) Anticonvulsant action and long-term effects of gabapentin in the immature brain. Neuropharmacology 40:139-147.

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

Cloyd JC, Dutta S, Cao G, Walch JK, Collins SD, Granneman GR (2003) Valproate unbound fraction and distribution volume following rapid infusions in patients with epilepsy. Epilepsy Res 53:19-27.

Cole AJ, Dichter M (2002) Neuroprotection and antiepileptogenesis: overview, definitions, and context. Neurology 59:1S-2.

Colwell CS, Levine MS (1999) Metabotropic glutamate receptor modulation of excitotoxicity in the neostriatum: role of calcium channels. Brain Res 833:234-241.

Contractor A, Swanson G, Heinemann SF (2001) Kainate receptors are involved in short- and long-term plasticity at mossy fiber synapses in the hippocampus. Neuron 29:209-216.

Côté A, Chiasson M, Peralta MR, Lafortune K, Pellegrini L, Tóth K (2005) Cell type-specific action of seizure-induced intracellular zinc accumulation in the rat hippocampus. J Physiol 566:821-837.

Couve A, Moss SJ, Pangalos MN (2000) GABAB receptors: a new paradigm in G protein signaling. Mol Cell Neurosci 16:296-312.

Couve A, Thomas P, Calver AR, Hirst WD, Pangalos MN, Walsh FS, Smart TG, Moss SJ (2002) Cyclic AMP-dependent protein kinase phosphorylation facilitates GABAB receptor-effector coupling. Nat Neurosci 5:415-424.

Covanis A, Gupta AK, Jeavons PM (1982) Sodium valproate: monotherapy and polytherapy. Epilepsia 23:693-720.

Coyle TE, Bair AK, Stein C, Vajpayee N, Mehdi S (2005) Acute Leukemia Associated with Valproic Acid Treatment: A Novel Mechanism for Leukemogenesis? ASH Annual Meeting Abstracts 104:4453.

Curia G, Longo D, Biagini G, Jones RSG, Avoli M (2008) The pilocarpine model of temporal lobe epilepsy. J Neurosci Methods 172:143-157.

Curtis MA, Faull RLM, Eriksson PS (2007) The effect of neurodegenerative diseases on the subventricular zone. Nat Rev Neurosci 8:712-723.

Dalby MO, Mody I (2001) The process of epileptogenesis: a pathophysiological approach. Curr Opin Neurol 14:187-192.

Dash PK, Karl KA, Colicos MA, Prywes R, Kandel ER (1991) cAMP response element-binding protein is activated by Ca2+/calmodulin- as well as cAMP-dependent protein kinase. PNAS 88:5061-5065.

Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME (1997) Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91:231-241.

de Deyn PP, Marescau B, MacDonald RL (1990) Epilepsy and the GABA-hypothesis a brief review and some examples. Acta Neurol Belg 90:65-81.

de Lanerolle NC, Eid T, von Campe G, Kovacs I, Spencer DD, Brines M (1998) Glutamate receptor subunits GluR1 and GluR2/3 distribution shows reorganization in the human epileptogenic hippocampus. Eur J Neurosci 10:1687-1703.

de Rooij J, Zwartkruis FJT, Verheijen MHG, Cool RH, Nijman SMB, Wittinghofer A, Bos JL (1998a) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. Nature 396:474-477.

de Rooij J, Zwartkruis FJT, Verheijen MHG, Cool RH, Nijman SMB, Wittinghofer A, Bos JL (1998b) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. Nature 396:474-477.

de Vasconcelos AP, Baldwin RA, Wasterlain CG (1995) Nitric Oxide Mediates the Increase in Local Cerebral Blood Flow During Focal Seizures. PNAS 92:3175-3179.

DeBernardi MA, Brooker G (1996) Single cell Ca2+/cAMP cross-talk monitored by simultaneous Ca2+/cAMP fluorescence ratio imaging. PNAS 93:4577-4582.

Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The Glutamate Receptor Ion Channels. Pharmacol Rev 51:7-62.

Doi T, Ueda Y, Tokumaru J, Mitsuyama Y, Willmore LJ (2001) Sequential changes in AMPA and NMDA protein levels during Fe(3+)-induced epileptogenesis. Brain Res Mol Brain Res 92:107-114.

Draguhn A, Traub RD, Schmitz D, Jefferys JGR (1998) Electrical coupling underlies high-frequency oscillations in the hippocampus in vitro. Nature 394:189-192.

Du J, Creson TK, Wu LJ, Ren M, Gray NA, Falke C, Wei Y, Wang Y, Blumenthal R, hado-Vieira R, Yuan P, Chen G, Zhuo M, Manji HK (2008) The role of hippocampal GluR1 and GluR2 receptors in manic-like behavior. J Neurosci 28:68-79.

Du J, Gray NA, Falke CA, Chen W, Yuan P, Szabo ST, Einat H, Manji HK (2004) Modulation of synaptic plasticity by antimanic agents: the role of AMPA glutamate receptor subunit 1 synaptic expression. J Neurosci 24:6578-6589.

Du J, Suzuki K, Wei Y, Wang Y, Blumenthal R, Chen Z, Falke C, Zarate CA, Jr., Manji HK (2006) The anticonvulsants lamotrigine, riluzole, and valproate differentially regulate AMPA receptor membrane localization: relationship to clinical effects in mood disorders. Neuropsychopharmacology 32:793-802.

Dudek FE, Obenaus A, Tasker JG (1990) Osmolality-induced changes in extracellular volume alter epileptiform bursts independent of chemical synapses in the rat: Importance of non-synaptic mechanisms in hippocampal epileptogenesis. Neurosci Lett 120:267-270.

Dudek FE, Yasumura T, Rash JE (1998) 'Non-synaptic' mechanisms in seizures and epileptogenesis. Cell Biol Int 22:793-805.

Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, Greenberg ME (1997) Regulation of neuronal survival by the serine-threonine protein kinase Akt. Science 275:661-665.

Ebert U, Brandt C, Löscher W (2002) Delayed sclerosis, neuroprotection, and limbic epileptogenesis after status epilepticus in the rat. Epilepsia 43:86-95.

Eikel D, Lampen A, Nau H (2006) Teratogenic effects mediated by inhibition of Histone deacetylases: evidence from quantitative structure activity relationships of 20 valproic acid derivatives. Chem Res Toxicol 19:272-278.

Einat H, Yuan P, Gould TD, Li J, Du J, Zhang L, Manji HK, Chen G (2003) The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. J Neurosci 23:7311-7316.

Elmer E, Kokaia M, Ernfors P, Ferencz I, Kokaia Z, Lindvall O (1997) Suppressed kindling epileptogenesis and perturbed BDNF and TrkB gene regulation in NT-3 mutant mice. Exp Neurol 145:93-103.

Elmer E, Kokaia M, Kokaia Z, Ferencz I, Lindvall O (1996) Delayed kindling development after rapidly recurring seizures: relation to mossy fiber sprouting and neurotrophin, GAP-43 and dynorphin gene expression. Brain Res 712:19-34.

English JD, Sweatt JD (1997) A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. J Biol Chem 272:19103-19106.

Enserink JM, Christensen AE, de Rooij J, van Triest M, Schwede F, Genieser HG, Doskeland SO, Blank JL, Bos JL (2002) A novel Epac-specific cAMP analogue demonstrates independent regulation of Rap1 and ERK. Nat Cell Biol 4:901-906.

Esteban JA, Shi SH, Wilson C, Nuriya M, Huganir RL, Malinow R (2003) PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. Nat Neurosci 6:136-143.

Faber DS, Korn H (1989) Electrical field effects: their relevance in central neural networks. Physiol Rev 69:821-863.

Ferkany JW, Zaczek R, Coyle JT (1982) Kainic acid stimulates excitatory amino acid neurotransmitter release at presynaptic receptors. Nature 298:757-759.

Ferrendelli JA, Blank AC, Gross RA (1980) Relationships between seizure activity and cyclic nucleotide levels in brain. Brain Res 200:93-103.

Fisher RS, Alger BE (1984) Electrophysiological mechanisms of kainic acid-induced epileptiform activity in the rat hippocampal slice. J Neurosci 4:1312-1323.

Frankel WN, Taylor BA, Noebels JL, Lutz CM (1994) Genetic Epilepsy Model Derived From Common Inbred Mouse Strains. Genetics 138:481-489.

Freeman TW, Clothier JL, Pazzaglia P, Lesem MD, Swann AC (1992) A double-blind comparison of valproate and lithium in the treatment of acute mania. Am J Psychiatry 149:108-111.

French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, Spencer DD (1993) Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. Ann Neurol 34:774-780.

Frey U, Frey S, Schollmeier F, Krug M (1996) Influence of actinomycin D, a RNA synthesis inhibitor, on long-term potentiation in rat hippocampal neurons in vivo and in vitro. J Physiol 490:703-711.

Fritschy JM, Kiener T, Bouilleret V, Loup F (1999) GABAergic neurons and GABAA-receptors in temporal lobe epilepsy. Neurochem Int 34:435-445.

Frotscher M, Soriano E, Misgeld U (1994) Divergence of hippocampal mossy fibers. Synapse 16:148-160.

Funke MG, Amado D, Cavalheiro EA, Naffah-Mazzacoratti MG (1998) Tyrosine phosphorylation is increased in the rat hippocampus during the status epilepticus induced by pilocarpine. Brain Res Bull 47:87-93.

Gajda Z, Gyengesi E, Hermesz E, Ali KS, Szente M (2003) Involvement of gap junctions in the manifestation and control of the duration of seizures in rats in vivo. Epilepsia 44:1596-1600.

Gajda Z, Szupera Z, Blazso G, Szente M (2005) Quinine, a blocker of neuronal Cx36 channels, suppresses seizure activity inrat neocortex in vivo. Epilepsia 46:1581-1591.

Gallagher HC, Bacon CL, Odumeru OA, Gallagher KF, Fitzpatrick T, Regan CM (2004) Valproate activates phosphodiesterase-mediated cAMP degradation: relevance to C6 glioma G1 phase progression. Neurotoxicol Teratol 26:73-81.

Gasparini F, Bruno V, Battaglia G, Lukic S, Leonhardt T, Inderbitzin W, Laurie D, Sommer B, Varney MA, Hess SD, Johnson EC, Kuhn R, Urwyler S, Sauer D, Portet C, Schmutz M, Nicoletti F, Flor PJ (1999) (R,S)-4-Phosphonophenylglycine, a potent and selective group III metabotropic glutamate receptor agonist, is anticonvulsive and neuroprotective in vivo. J Pharmacol Exp Ther 289:1678-1687.

Gean PW, Huang CC, Hung CR, Tsai JJ (1994) Valproic acid suppresses the synaptic response mediated by the NMDA receptors in rat amygdalar slices. Brain Res Bull 33:333-336.

Ghai RS, Bikson M, Durand DM (2000) Effects of applied electric fields on low-calcium epileptiform activity in the CA1 region of rat hippocampal slices. J Neurophysiol 84:274-280.

Gibbs JW, Shumate MD, Coulter DA (1997) Differential epilepsy-associated alterations in postsynaptic GABA(A) receptor function in dentate granule and CA1 neurons. J Neurophysiol 77:1924-1938.

Glade-McCulloh E, Yamamoto H, Tan SE, Brickey DA, Soderling TR (1993) Phosphorylation and regulation of glutamate receptors by calcium/calmodulin-dependent protein kinase II. Nature 362:640-642.

Glanzman DL, Kandel ER, Schacher S (1990) Target-dependent structural changes

accompanying long-term synaptic facilitation in Aplysia neurons. Science 249:799-802.

Goddard GV (1967) Development of epileptic seizures through brain stimulation at low intensity. Nature 214:1020-1021.

Gonzalez GA, Montminy MR (1989) Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell 59:675-680.

Göttlicher M, Minucci S, Zhu P, Krämer OH, Schimpf A, Giavara S, Sleeman JP, Coco FL, Nervi C, Pier GP, Heinzel T (2001) Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J 20:6969-6978.

Goussakov IV, Fink K, Elger CE, Beck H (2000) Metaplasticity of mossy fiber synaptic transmission involves altered release probability. J Neurosci 20:3434-3441.

Gross RA, Ferrendelli JA (1979) Effects of reserpine, propranolol, and aminophylline on seizure activity and CNS cyclic nucleotides. Ann Neurol 6:296-301.

Gu J, Lynch BA, Anderson D, Klitgaard H, Lu S, Elashoff M, Ebert U, Potschka H, LöscherW (2004) The antiepileptic drug levetiracetam selectively modifies kindling-induced alterations in gene expression in the temporal lobe of rats. Eur J Neurosci 19:334-345.

Gurvich N, Berman MG, Wittner BS, Gentleman RC, Klein PS, Green JBA (2005) Association of valproate-induced teratogenesis with histone deacetylase inhibition in vivo. FASEB J 19:1166-1168.

Ha JK, Lindsay RC (1991) Contributions of cow, sheep, and goat milks to characterizing branched-chain fatty acid and phenolic flavors in varietal cheeses. J Dairy Sci 74:3267-3274.

Hafner S, Adler HS, Mischak H, Janosch P, Heidecker G, Wolfman A, Pippig S, Lohse M, Ueffing M, Kolch W (1994) Mechanism of inhibition of Raf-1 by protein kinase A. Mol Cell Biol 14:6696-6703.

Hall AC, Brennan A, Goold RG, Cleverley K, Lucas FR, Gordon-Weeks PR, Salinas PC (2002) Valproate regulates GSK-3-mediated axonal remodeling and synapsin I clustering in developing neurons. Mol Cell Neurosci 20:257-270.

Halonen T, Nissinen J, Pitkänen A (2001) Chronic elevation of brain GABA levels beginning two days after status epilepticus does not prevent epileptogenesis in rats. Neuropharmacology 40:536-550.

Hamilton SE, Loose MD, Qi M, Levey AI, Hille B, McKnight GS, Idzerda RL, Nathanson NM (1997) Disruption of the m1 receptor gene ablates muscarinic receptor-dependent M current regulation and seizure activity in mice. PNAS 94:13311-13316.

Hao Y, Creson T, Zhang L, Li P, Du F, Yuan P, Gould TD, Manji HK, Chen G (2004) Mood stabilizer valproate promotes ERK pathway-dependent cortical neuronal growth and neurogenesis. J Neurosci 24:6590-6599.

Harris EW, Cotman CW (1986) Long-term potentiation of guinea pig mossy fiber

responses is not blocked by N-methyl d-aspartate antagonists. Neurosci Lett 70:132-137.

Harvey BD, Sloviter RS (2005) Hippocampal granule cell activity and c-Fos expression during spontaneous seizures in awake, chronically epileptic, pilocarpine-treated rats: implications for hippocampal epileptogenesis. J Comp Neurol 488:442-463.

Hattori Y, Moriwaki A, Hayashi Y, Hori Y (1993) Involvement of Adenosine-Sensitive Cyclic AMP-Generating Systems in Cobalt-Induced Epileptic Activity in the Rat. J Neurochem 61:2169-2174.

Hattori Y, Moriwaki A, Yasuhara H, Hori Y (1987) Noradrenaline-Sensitive Cyclic AMP-Generating System of Rat Cerebral Cortex with Iron-Induced Epileptiform Activity. Jpn J Physiol 37:161-167.

Hayashi Y, Shi SH, Esteban J, Piccini A, Poncer JC, Malinow R (2000) Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science 287:2262-2267.

Heinemann U, Lux HD, Gutnick MJ (1977) Extracellular free calcium and potassium during paroxysmal activity in the cerebral cortex of the cat. Exp Brain Res 27:237-243.

Henshall DC, Araki T, Schindler CK, Lan JQ, Tiekoter KL, Taki W, Simon RP (2002) Activation of Bcl-2-associated death protein and counter-response of Akt within cell populations during seizure-induced neuronal death. J Neurosci 22:8458-8465.

Henshall DC, Simon RP (2005) Epilepsy and apoptosis pathways. J Cereb Blood Flow Metab 25:1557-1572.

Henze DA, Urban NN, Barrionuevo G (2000) The multifarious hippocampal mossy fiber pathway: a review. Neuroscience 98:407-427.

Herman ST (2002) Epilepsy after brain insult: targeting epileptogenesis. Neurology 59:S21-S26.

Hetman M, Gozdz A (2004) Role of extracellular signal regulated kinases 1 and 2 in neuronal survival. Eur J Biochem 271:2050-2055.

Hetman M, Kanning K, Cavanaugh JE, Xia Z (1999) Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase. J Biol Chem 274:22569-22580.

Higashima M, Ohno K, Koshino Y (2002) Cyclic AMP-mediated modulation of epileptiform afterdischarge generation in rat hippocampal slices. Brain Res 949:157-161.

Hochman DW, D'Ambrosio R, Janigro D, Schwartzkroin PA (1999) Extracellular chloride and the maintenance of spontaneous epileptiform activity in rat hippocampal slices. J Neurophysiol 81:49-59.

Hochman DW, Baraban SC, Owens JWM, Schwartzkroin PA (1995) Dissociation of Synchronization and Excitability in Furosemide Blockade of Epileptiform Activity. Science 270:99-102.

Holmes GL, Ben-Ari Y (2001) The neurobiology and consequences of epilepsy in the developing brain. Pediatr Res 49:320-325.

Houser CR (1990) Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. Brain Res 535:195-204.

Houslay MD, Adams D.R. (2003) PDE4 cAMP phosphodiesterases: modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization. Biochem J 370:1-18.

Huang CC, Chen YL, Lo SW, Hsu KS (2002) Activation of cAMP-dependent protein kinase suppresses the presynaptic cannabinoid inhibition of glutamatergic transmission at corticostriatal synapses. Mol Pharmacol 61:578-585.

Huang CC, Hsu KS (1999) Protein tyrosine kinase is required for the induction of long-term potentiation in the rat hippocampus. J Physiol 520:783-796.

Huang YY, Li XC, Kandel ER (1994) cAMP contributes to mossy fiber LTP by initiating both a covalently mediated early phase and macromolecular synthesis-dependent late phase. Cell 79:69-79.

Husum H, Bolwig TG, Sanchez C, Mathe AA, Hansen SL (2004) Levetiracetam prevents changes in levels of brain-derived neurotrophic factor and neuropeptide Y mRNA and of Y1- and Y5-like receptors in the hippocampus of rats undergoing amygdala kindling: implications for antiepileptogenic and mood-stabilizing properties. Epilepsy Behav 5:204-215.

Impey S, Obrietan K, Storm DR (1999) Making new connections: role of ERK/MAP kinase signaling in neuronal plasticity. Neuron 23:11-14.

Impey S, Obrietan K, Wong ST, Poser S, Yano S, Wayman G, Deloulme JC, Chan G, Storm DR (1998) Cross talk between ERK and PKA is required for Ca2+ stimulation of CREB-dependent transcription and ERK nuclear translocation. Neuron 21:869-883.

Irving EA, Bamford M (2002) Role of mitogen- and stress-activated kinases in ischemic injury. J Cereb Blood Flow Metab 22:631-647.

Isokawa M, Mello LE (1991) NMDA receptor-mediated excitability in dendritically deformed dentate granule cells in pilocarpine-treated rats. Neurosci Lett 129:69-73.

Iwasa H, Kikuchi S, Mine S, Miyagishima H, Sugita K, Sato T, Hasegawa S (2000) Up-regulation of type II adenylyl cyclase mRNA in kindling model of epilepsy in rats. Neurosci Lett 282:173-176.

Jeffrey GT, Dudek FE (1991) Electrophysiology of GABA-mediated synaptic transmission and possible roles in epilepsy. Neurochem Res 16:251-262.

Jensen F (1995) An animal model of hypoxia-induced perinatal seizures. Ital J Neurol Sci 16:59-68.

Jensen MS, Yaari Y (1997) Role of intrinsic burst firing, potassium accumulation, and electrical coupling in the elevated potassium model of hippocampal epilepsy. J

Neurophysiol 77:1224-1233.

Jeon GS, Park SH, Lee KJ, Lee MS, Chun BG, Shin KH (2006) Valproate prevents MK801-induced changes in brain-derived neurotrophic factor mRNA in the rat brain. Eur J Pharmacol 545:142-146.

Johannessen CU (2000) Mechanisms of action of valproate: a commentatory. Neurochem Int 37:103-110.

Johnson MR, Ssnder JWAS (2001) The clinical impact of epilepsy genetics. J Neurol Neurosurg Psychiatry 70:428-430.

Johnston D, Williams S, Jaffe D, Gray R (1992) NMDA-receptor-independent long-term potentiation. Annu Rev Physiol 54:489-505.

Jones LS (1991) Naloxone blocks antiepileptogenic properties of an in vitro electroconvulsive shock model. Brain Res 564:336-340.

Jutila L, Immonen A, Partanen K, Partanen J, Mervaala E, Ylinen A, Alafuzoff I, Paljarvi L, Karkola K, Vapalahti M, Pitkänen A (2002) Neurobiology of epileptogenesis in the temporal lobe. Adv Tech Stand Neurosurg 27:3-22.

Kakiuchi S, Rall TW, McIlwain H (1969) The effect of electrical stimulation upon the accumulation of adenosine 3',s'-phosphate in isolated cerebral tissue. J Neurochem 16:485-491.

Kamiya H, Yamamoto C (1997) Phorbol ester and forskolin suppress the presynaptic inhibitory action of group-II metabotropic glutamate receptor at rat hippocampal mossy fibre synapse. Neuroscience 80:89-94.

Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, Housman DE, Graybiel AM (1998) A family of cAMP-binding proteins that directly activate Rap1. Science 282:2275-2279.

Keane PE, Simiand J, Mendes E, Santucci V, Morre M (1983) The effects of analogues of valproic acid on seizures induced by pentylenetetrazol and GABA content in brain of mice. Neuropharmacology 22:875-879.

Kelsey JE, Sanderson KL, Frye CA (2000) Perforant path stimulation in rats produces seizures, loss of hippocampal neurons, and a deficit in spatial mapping which are reduced by prior MK-801. Behav Brain Res 107:59-69.

Kerr DI, Ong J (1995) GABAB receptors. Pharmacol Ther 67:187-246.

Khalilov I, Holmes GL, Ben-Ari Y (2003) In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. Nat Neurosci 6:1079-1085.

Kharatishvili I, Nissinen JP, McIntosh TK, Pitkänen A (2006) A model of posttraumatic epilepsy induced by lateral fluid-percussion brain injury in rats. Neuroscience 140:685-697.

Kihara S, Kubo M, Ikeda N, Yokoyama S, Matsuzawa Y, Tarui S, Yamamoto A, Hostetler KY (1989) Inhibition of purified human postheparin lipoprotein lipase by beta-adrenergic blockers in vitro. Biochem Pharmacol 38:407-411.

Kini U (2006) Fetal valproate syndrome: a review. Paediatr Perinat Drug Ther 7:123-130.

Klitgaard HV, Matagne AC, Vanneste-Goemaere J, Margineanu DG (2001) Effects of prolonged administration of levetiracetam on pilocarpine-induced epileptogenesis in rat. Epilepsia 42:114-115.

Kodama M, Yamada N, Sato K, Kitamura Y, Koyama F, Sato T, Morimoto K, Kuroda S (1999) Effects of YM90K, a selective AMPA receptor antagonist, on amygdala-kindling and long-term hippocampal potentiation in the rat. Eur J Pharmacol 374:11-19.

Kohling R, Gladwell SJ, Bracci E, Vreugdenhil M, Jefferys JGR (2001) Prolonged epileptiform bursting induced by 0-Mg2+ in rat hippocampal slices depends on gap junctional coupling. Neuroscience 105:579-587.

Komagiri Y, Kitamura N (2003) Effect of intracellular dialysis of ATP on the hyperpolarization-activated cation current in rat dorsal root ganglion neurons. J Neurophysiol 90:2115-2122.

Konnerth A, Heinemann U, Yaari Y (1986) Nonsynaptic epileptogenesis in the mammalian hippocampus in vitro. I. Development of seizurelike activity in low extracellular calcium. J Neurophysiol 56:409-423.

Krivánek J, Mares P (1977) Cyclic adenosine 3',5'-monophosphate in epileptogenic foci induced by penicillin. Neurosci Lett 6:329-332.

Kunz WS (2002) The role of mitochondria in epileptogenesis. Curr Opin Neurol 15:179-184.

Lamberty Y, Margineanu DG, Klitgaard H (2000) Absence of negative impact of levetiracetam on cognitive function and memory in normal and amygdala-kindled rats. Epilepsy Behav 1:333-342.

Lauri SE, Bortolotto ZA, Bleakman D, Ornstein PL, Lodge D, Isaac JTR, Collingridge GL (2001a) A critical role of a facilitatory presynaptic kainate receptor in mossy fiber LTP. Neuron 32:697-709.

Lauri SE, Delany C, Clarke J, Bortolotto ZA, Ornstein PL, Isaac TR, Collingridge GL (2001b) Synaptic activation of a presynaptic kainate receptor facilitates AMPA receptor-mediated synaptic transmission at hippocampal mossy fibre synapses. Neuropharmacology 41:907-915.

Lee HK, Kameyama K, Huganir RL, Bear MF (1998) NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. Neuron 21:1151-1162.

Lehmann TN, Gabriel S, Eilers A, Njunting M, Kovacs R, Schulze K, Lanksch WR, Heinemann U (2001) Fluorescent tracer in pilocarpine-treated rats shows widespread

aberrant hippocampal neuronal connectivity. Eur J Neurosci 14:83-95.

Leite JP, Bortolotto ZA, Cavalheiro EA (1990) Spontaneous recurrent seizures in rats: an experimental model of partial epilepsy. Neurosci Biobehav Rev 14:511-517.

Lemos T, Cavalheiro EA (1995) Suppression of pilocarpine-induced status epilepticus and the late development of epilepsy in rats. Exp Brain Res 102:423-428.

Lerma J (2003) Roles and rules of kainate receptors in synaptic transmission. Nat Rev Neurosci 4:481-495.

Leroy C, Poisbeau P, Keller AF, Nehlig A (2004) Pharmacological plasticity of GABAA receptors at dentate gyrus synapses in a rat model of temporal lobe epilepsy. J Physiol 557:473-487.

Leung LS, Wu C (2003) Kindling suppresses primed-burst-induced long-term potentiation in hippocampal CA1. NeuroReport 14:211-214.

Leweke FM, Louvel J, Rausche G, Heinemann U (1990) Effects of pentetrazol on neuronal activity and on extracellular calcium concentration in rat hippocampal slices. Epilepsy Res 6:187-198.

Lewin E, Golden T, Walker JE (1976) Stimulation of cyclic AMP accumulation by pentylenetetrazol. Exp Neurol 50:422-426.

Li H, Prince DA (2002) Synaptic activity in chronically injured, epileptogenic sensory-motor neocortex. J Neurophysiol 88:2-12.

Li P, Yuan Z, Liu B, Sailhamer EA, Shults C, Velmahos GC, deMoya M, Alam HB (2008) Prevention of hypoxia-induced neuronal apoptosis through histone deacetylase inhibition. J Trauma 64:863-871.

Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X (1997) Cytochrome c and dATP-dependent formation of apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91:479-489.

Li R, El-Mallahk RS (2000) A novel evidence of different mechanisms of lithium and valproate neuroprotective action on human SY5Y neuroblastoma cells: caspase-3 dependency. Neurosci Lett 294:147-150.

Liou AKF, Clark RS, Henshall DC, Yin XM, Chen J (2003) To die or not to die for neurons in ischemia, traumatic brain injury and epilepsy: a review on the stress-activated signaling pathways and apoptotic pathways. Prog Neurobiol 69:103-142.

Lledo PM, Hjelmstad GO, Mukherji S, Soderling TR, Malenka RC, Nicoll RA (1995) Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. PNAS 92:11175-11179.

Lopes da Silva FH, Witter MP, Boeijinga PH, Lohman AH (1990) Anatomic organization and physiology of the limbic cortex. Physiol Rev 70:453-511.

Löscher W (2002) Animal models of epilepsy for the development of antiepileptogenic

and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. Epilepsy Res 50:105-123.

Löscher W, Fisher JE, Nau H, Honack D (1989) Valproic acid in amygdala-kindled rats: alterations in anticonvulsant efficacy, adverse effects and drug and metabolite levels in various brain regions during chronic treatment. J Pharmacol Exp Ther 250:1067-1078.

Löscher W (1999) Valproate: a reappraisal of its pharmacodynamic properties and mechanisms of action. Prog Neurobiol 58:31-59.

Löscher W, Honack D, Rundfeldt C (1998) Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. J Pharmacol Exp Ther 284:474-479.

Lujan R, Nusser Z, Roberts JDB, Shigemoto R, Somogyi P (1996) Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. Eur J Neurosci 8:1488-1500.

Luo Y, de Franco DB (2006) Opposing roles for ERK1/2 in neuronal oxidative toxicity: distinct mechanisms of ERK1/2 action at early versus late phases of oxidative stress. J Biol Chem 281:16436-16442.

Lynch MA (2004) Long-term potentiation and memory. Physiol Rev 84:87-136.

Madeja M, Musshoff off U, Lorra C, Pongs O, Speckmann EJ (1996) Mechanism of action of the epileptogenic drug pentylenetetrazol on a cloned neuronal potassium channel. Brain Res 722:59-70.

Malinow R, Malenka RC (2002) Synaptic plasticity and AMPA receptor trafficking. Ann N Y Acad Sci 25:103-126.

Mammen AL, Kameyama K, Roche KW, Huganir RL (1997) Phosphorylation of the alpha -amino-3-hydroxy-5-methylisoxazole4-propionic acid receptor GluR1 subunit by calcium/ calmodulin-dependent Kinase II. J Biol Chem 272:32528-32533.

Margerison JH, Corsellis JAN (1966) Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. Brain 89:499-530.

Martín ED, Pozo MA (2006) Animal models for the development of new neuropharmacological therapeutics in the status epilepticus. Curr Neuropharmacol 4:33-40.

Masters SC, Yang H, Datta SR, Greenberg ME, Fu H (2001) 14-3-3 Inhibits Bad-induced cell death through interaction with Serine-136. Mol Pharmacol 60:1325-1331.

Mathern GW, Pretorius JK, Mendoza D, Lozada A, Leite JP, Chimelli L, Fried I, Sakamoto AC, Assirati JA, Adelson PD (1998) Increased hippocampal AMPA and NMDA receptor subunit immunoreactivity in temporal lobe epilepsy patients. J Neuropathol Exp Neurol 57:615-634.

McLachlan RS (1987) Pseudoatrophy of the brain with valproic acid monotherapy. Can

J Neurol Sci 14:294-296.

McLean MJ, Macdonald RL (1986) Sodium valproate, but not ethosuximide, produces use- and voltage- dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. J Pharmacol Exp Ther 237:1001-1011.

Meador KJ (2007) The basic science of memory as it applies to epilepsy. Epilepsia 48:23-25.

Meador KJ, Baker GA, Browning N, Clayton-Smith J, Combs-Cantrell DT, Cohen M, Kalayjian LA, Kanner A, Liporace JD, Pennell PB, Privitera M, Loring DW, the NEAD Study Group (2009) Cognitive Function at 3 Years of Age after Fetal Exposure to Antiepileptic Drugs. N Engl J Med 360:1597-1605.

Meldrum BS (1997) First Alfred Meyer Memorial Lecture. Epileptic brain damage: a consequence and a cause of seizures. Neuropathol Appl Neurobiol 23:185-202.

Mello LEAM, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, Finch DM (1993) Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. Epilepsia 34:985-995.

Mellor J, Nicoll RA (2001) Hippocampal mossy fiber LTP is independent of postsynaptic calcium. Nat Neurosci 4:125-126.

Mellor J, Nicoll RA, Schmitz D (2002) Mediation of hippocampal mossy fiber long-term potentiation by presynaptic Ih channels. Science 295:143-147.

Menegola E, Broccia ML, Prati M, Giavini E (1998) Stage-dependent skeletal malformations induced by valproic acid in rat. Int J Dev Biol 42:99-102.

Merlin LR (1999) Group I mGluR-mediated silent induction of long-lasting epileptiform discharges. J Neurophysiol 82:1078-1081.

Merlin LR (2002) Differential roles for mGluR1 and mGluR5 in the persistent prolongation of epileptiform bursts. J Neurophysiol 87:621-625.

Merlin LR, Wong RK (1997) Role of Group I metabotropic glutamate receptors in the patterning of epileptiform activities in vitro. J Neurophysiol 78:539-544.

Merlo D, Cifelli P, Cicconi S, Tancredi V, Avoli M (2004) 4-Aminopyridine-induced epileptogenesis depends on activation of mitogen-activated protein kinase ERK. J Neurochem 89:654-659.

Mirnikjoo B, Brown SE, Kim HFS, Marangell LB, Sweatt JD, Weeber EJ (2001) Protein kinase inhibition by omega -3 fatty acids. J Biol Chem 276:10888-10896.

Mody I, Lambert JD, Heinemann U (1987) Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. J Neurophysiol 57:869-888.

Moldrich RX, Jeffrey M, Talebi A, Beart PM, Chapman AG, Meldrum BS (2001) Anti-

epileptic activity of group II metabotropic glutamate receptor agonists (-)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268) and (-)-2-thia-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY389795). Neuropharmacology 41:8-18.

Molnar P, Nadler JV (1999) Mossy fiber-granule cell synapses in the normal and epileptic rat dentate gyrus studied with minimal laser photostimulation. J Neurophysiol 82:1883-1894.

Monaghan DT, Cotman CW (1982) The distribution of [3H]kainic acid binding sites in rat CNS as determined by autoradiography. Brain Res 252:91-100.

Montarolo PG, Goelet P, Castellucci VF, Morgan J, Kandel ER, Schacher S (1986) A critical period for macromolecular synthesis in long-term heterosynaptic facilitation in Aplysia. Science 234:1249-1254.

Montezinho LP, Castro MM, Duarte CB, Penschuck S, Geraldes CFGC, Mork A (2006) The interaction between dopamine D2-like and beta-adrenergic receptors in the prefrontal cortex is altered by mood-stabilizing agents. J Neurochem 96:1336-1348.

Montezinho LP, Mork A, Duarte CB, Penschuck S, Geraldes CF, Castro MMC (2007) Effects of mood stabilizers on the inhibition of adenylate cyclase via dopamine D2-like receptors. Bipolar Disord 9:290-297.

Mora A, Gonzalez-Polo RA, Fuentes JM, Soler G, Centeno F (1999) Different mechanisms of protection against apoptosis by valproate and Li+. Eur J Biochem 266:886-891.

Morgan SL, Teyler TJ (2001) Epileptic-like activity induces multiple forms of plasticity in hippocampal area CA1. Brain Res 917:90-96.

Morimoto K, Fahnestock M, Racine RJ (2004) Kindling and status epilepticus models of epilepsy: rewiring the brain. Prog Neurobiol 73:1-60.

Moss SJ, Smart TG, Blackstone CD, Huganir RL (1992) Functional modulation of GABAA receptors by cAMP-dependent protein phosphorylation. Science 257:661-665.

Moussa RC, Ikeda-Douglas CJ, Thakur V, Milgram NW, Gurd JW (2001) Seizure activity results in increased tyrosine phosphorylation of the N-methyl--aspartate receptor in the hippocampus. Brain Res Mol Brain Res 95:36-47.

Murashima YL, Yoshii M, Suzuki J (2000) Role of nitric oxide in the epileptogenesis of EL mice. Epilepsia 41 S6:S195-S199.

Murashima YL, Yoshii M, Suzuki J (2002) Ictogenesis and epileptogenesis in EL mice. Epilepsia 43 Suppl 5:130-135.

Myllylä VV, Heikkinen ER, Vapaatalo H, Hokkanen E (1975) Cyclic AMP Concentration and Enzyme Activities of Cerebrospinal Fluid in Patients with Epilepsy or Central Nervous System Damage. Eur Neurol 13:123-130.

Namkoong S, Kim CK, Cho YL, Kim JH, Lee H, Ha KS, Choe J, Kim PH, Won MH,

Kwon YG, Shim EB, Kim YM (2009) Forskolin increases angiogenesis through the coordinated cross-talk of PKA-dependent VEGF expression and Epac-mediated PI3K/Akt/eNOS signaling. Cell Signal 21:906-915.

Narkilahti S, Pitkänen A (2005) Caspase 6 expression in the rat hippocampus during epileptogenesis and epilepsy. Neuroscience 131:887-897.

Narkilahti S, Pirttila TJ, Lukasiuk K, Tuunanen J, Pitkänen A (2003) Expression and activation of caspase 3 following status epilepticus in the rat. Eur J Neurosci 18:1486-1496.

Narotsky MG, Francis EZ, Kavlock RJ (1994) Developmental toxicity and structure-activity relationships of aliphatic acids, including dose-response assessment of valproic acid in mice and rats. Toxicol Sci 22:251-265.

Nateri AS, Raivich G, Gebhardt C, Da Costa C, Naumann H, Vreugdenhil M, Makwana M, Brandner S, Adams RH, Jefferys JG, Kann O, Behrens A (2007) ERK activation causes epilepsy by stimulating NMDA receptor activity. EMBO J 26:4891-4901.

Nau H, LöscherW (1984) Valproic acid and metabolites: pharmacological and toxicological studies. Epilepsia 25:S14-S22.

Nau H, Zierer R, Spielmann H, Neubert D, Gansau Ch (1981) A new model for embryotoxicity testing: Teratogenicity and pharmacokinetics of valproic acid following constant-rate administration in the mouse using human therapeutic drug and metabolite concentrations. Life Sci, 29:2803-2814.

Nicoll RA, Malenka RC (1995) Contrasting properties of two forms of long-term potentiation in the hippocampus. Nature 377:115-118.

Notenboom RGE, Hampson DR, Jansen GH, van Rijen PC, van Veelen CWM, van Nieuwenhuizen O, de Graan PNE (2006) Up-regulation of hippocampal metabotropic glutamate receptor 5 in temporal lobe epilepsy patients. Brain 129:96-107.

Nusser Z, Hajos N, Somogyi P, Mody I (1998) Increased number of synaptic GABAA receptors underlies potentiation at hippocampal inhibitory synapses. Nature 395:172-177.

O'Keefe J, Nadel L (1978) The hippocampus as a Cognitive Map. Oxford University Press.

Oprica M, Eriksson C, Schultzberg M (2003) Inflammatory mechanisms associated with brain damage induced by kainic acid with special reference to the interleukin-1 system. J Cell Mol Med 7:127-140.

Orabi B, Chang P, Deranieh R, Dhaml M, Yagen B, Bialer M, Greenberg M, Walker MC, Williams RSB (2010) Seizure control predicted by acute phosphoinositide signalling attenuation in Dictyostelium. Nature (submitted).

Otmakhova NA, Lisman JE (1998) D1/D5 Dopamine receptors inhibit depotentiation at CA1 Synapses via cAMP-Dependent Mechanism. J Neurosci 18:1270-1279.

Otmakhova NA, Otmakhov N, Mortenson LH, Lisman JE (2000) Inhibition of the cAMP pathway decreases early long-term potentiation at CA1 hippocampal synapses. J Neurosci 20:4446-4451.

Palmer RMJ, Ferrige AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524-526.

Parent JM, Elliott RC, Pleasure SJ, Barbaro NM, Lowenstein DH (2006a) Aberrant seizure-induced neurogenesis in experimental temporal lobe epilepsy. Ann Neurol 59:81-91.

Parent JM, von dem Bussche N, Lowenstein DH (2006b) Prolonged seizures recruit caudal subventricular zone glial progenitors into the injured hippocampus. Hippocampus 16:321-328.

Parent JM, Janumpalli S, McNamara JO, Lowenstein DH (1998) Increased dentate granule cell neurogenesis following amygdala kindling in the adult rat. Neurosci Lett 247:9-12.

Parent JM, Valentin VV, Lowenstein DH (2002) Prolonged seizures increase proliferating neuroblasts in the adult rat subventricular zone-olfactory bulb pathway. J Neurosci 22:3174-3188.

Patel MN (2002) Oxidative stress, mitochondrial dysfunction, and epilepsy. Free Radical Research 36:1139-1146.

Perez Velazquez JL, Carlen PL (2000) Gap junctions, synchrony and seizures. Trends Neurosci 23:68-74.

Perucca E (2001) Clinical pharmacology and therapeutic use of the new antiepileptic drugs. Fundam Clin Pharmacol 15:405-417.

Perucca E (2005) An introduction to antiepileptic drugs. Epilepsia 46:31-37.

Phiel CJ, Zhang F, Huang EY, Guenther MG, Lazar MA, Klein PS (2001) Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. J Biol Chem 276:36734-36741.

Piredda S, Yonekawa W, Whittingham TS, Kupferberg HJ (1985) Potassium, pentylenetetrazol, and anticonvulsants in mouse hippocampal slices. Epilepsia 26:167-174.

Pitkänen A, Kharatishvili I, Karhunen H, Lukasiuk K, Immonen R, Nairismägi J, Gröhn O, Nissinen J (2007) Epileptogenesis in experimental models. Epilepsia 48:s13-s20.

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

Pitkänen A (2002) Efficacy of current antiepileptics to prevent neurodegeneration in epilepsy models. Epilepsy Res 50:141-160.

Pitkänen A, Kharatishvili I, Narkilahti S, Lukasiuk K, Nissinen J (2005) Administration

of diazepam during status epilepticus reduces development and severity of epilepsy in rat. Epilepsy Res 63:27-42.

Pitkänen A, Narkilahti S, Bezvenyuk Z, Haapalinna A, Nissinen J (2004) Atipamezole, an [alpha]2-adrenoceptor antagonist, has disease modifying effects on epileptogenesis in rats. Epilepsy Res 61:119-140.

Popescu G (2005) Mechanism-based targeting of NMDA receptor functions. CMLS, Cell Mol Life Sci 62:2100-2111.

Porter BE, Cui XN, Brooks-Kayal AR (2006) Status epilepticus differentially alters AMPA and kainate receptor subunit expression in mature and immature dentate granule neurons. Eur J Neurosci 23:2857-2863.

Powell-Jackson PR, Tredger JM, Williams R (1984) Hepatotoxicity to sodium valproate: a review. Gut 25:673-681.

Prasad A, Williamson JM, Bertram EH (2002) Phenobarbital and MK-801, but not phenytoin, improve the long-term outcome of status epilepticus. Ann Neurol 51:175-181.

Priel MR, Albuquerque EX (2002) Short-term effects of pilocarpine on rat hippocampal neurons in culture. Epilepsia 43:S40-S46.

Priel MR, dos Santos NF, Cavalheiro EA (1996) Developmental aspects of the pilocarpine model of epilepsy. Epilepsy Res 26:115-121.

Prince HK, Conn PJ, Blackstone CD, Huganir RL, Levey AI (1995) Down-regulation of AMPA receptor subunit GluR2 in amygdaloid kindling. J Neurochem 64:462-465.

Proper EA, Oestreicher AB, Jansen GH, Veelen CWM, van Rijen PC, Gispen WH, de Graan PNE (2000) Immunohistochemical characterization of mossy fibre sprouting in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. Brain 123:19-30.

Prosser HM, Gill CH, Hirst WD, Grau E, Robbins M, Calver A, Soffin EM, Farmer CE, Lanneau C, Gray J (2001) Epileptogenesis and enhanced prepulse inhibition in GABA(B1)-deficient mice. Mol Cell Neurosci 17:1059-1070.

Puzyn'ski S, Kłosiewicz L (1984) Valproic acid amide as a prophylactic agent in affective and schizoaffective disorders. Psychopharmacol Bull 20:151-159.

Racine RJ (1972) Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol 32:281-294.

Rafiq A, Zhang YF, DeLorenzo RJ, Coulter DA (1995) Long-duration self-sustained epileptiform activity in the hippocampal-parahippocampal slice: a model of status epilepticus. J Neurophysiol 74:2028-2042.

Rashid K, Van der Zee CE, Ross GM, Chapman CA, Stanisz J, Riopelle RJ, Racine RJ, Fahnestock M (1995) A nerve growth factor peptide retards seizure development and inhibits neuronal sprouting in a rat model of epilepsy. PNAS 92:9495-9499.

Raza M, Blair RE, Sombati S, Carter DS, Deshpande LS, DeLorenzo RJ (2004) Evidence that injury-induced changes in hippocampal neuronal calcium dynamics during epileptogenesis cause acquired epilepsy. PNAS 101:17522-17527.

Reid IC, Stewart CA (1997) Seizures, memory and synaptic plasticity. Seizure 6:351-359.

Ren M, Leng Y, Jeong M, Leeds PR, Chuang DM (2004) Valproic acid reduces brain damage induced by transient focal cerebral ischemia in rats: potential roles of histone deacetylase inhibition and heat shock protein induction. J Neurochem 89:1358-1367.

Rice A, Rafiq A, Shapiro SM, Jakoi ER, Coulter DA, DeLorenzo RJ (1996) Long-lasting reduction of inhibitory function and gamma-aminobutyric acid type A receptor subunit mRNA expression in a model of temporal lobe epilepsy. PNAS 93:9665-9669.

Riedel G, Reymann KG (1996) Metabotropic glutamate receptors in hippocampal long-term potentiation and learning and memory. Acta Physiol Scand 157:1-19.

Rigoulot MA, Koning E, Ferrandon A, Nehlig A (2004) Neuroprotective properties of topiramate in the lithium-pilocarpine model of epilepsy. J Pharmacol Exp Ther 308:787-795.

Rinaldi T, Kulangara K, Antoniello K, Markram H (2007) Elevated NMDA receptor levels and enhanced postsynaptic long-term potentiation induced by prenatal exposure to valproic acid. PNAS 104:13501-13506.

Roberson ED, English JD, Adams JP, Selcher JC, Kondratick C, Sweatt JD (1999) The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. J Neurosci 19:4337-4348.

Robinson JH, Deadwyler SA (1981) Kainic acid produces depolarization of CA3 pyramidal cells in the in vitro hippocampal slice. Brain Res 221:117-127.

Roche KW, O'Brien RJ, Mammen AL, Bernhardt J, Huganir RL (1996) Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron 16:1179-1188.

Rogers BC, Barnes MI, Mitchell CL, Tilson HA (1989) Functional deficits after sustained stimulation of the perforant path. Brain Res 493:41-50.

Rose K, Christine CW, Choi DW (1990) Magnesium removal induces paroxysmal neuronal firing and NMDA receptor-mediated neuronal degeneration in cortical cultures. Neurosci Lett 115:313-317.

Rumpel S, LeDoux J, Zador A, Malinow R (2005) Postsynaptic receptor trafficking underlying a form of associative learning. Science 308:83-88.

Salin P, Scanziani M, Malenka R, Nicoll R (1996) Distinct short-term plasticity at two excitatory synapses in the hippocampus. PNAS 93:13304-13309.

Sarkisian MR (2001) Overview of the current animal models for human seizure and

epileptic disorders. Epilepsy Behav 2:201-216.

Scharf MT, Woo NH, Lattal KM, Young JZ, Nguyen PV, Abel T (2002) Protein synthesis is required for the enhancement of long-term potentiation and long-term memory by spaced training. J Neurophysiol 87:2770-2777.

Scharfman HE, Goodman JH, Sollas AL (2000) Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: functional implications of seizure-induced neurogenesis. J Neurosci 20:6144-6158.

Schmitz D, Mellor J, Nicoll RA (2001) Presynaptic kainate receptor mediation of frequency facilitation at hippocampal mossy fiber synapses. Science 291:1972-1976.

Schulz PE, Fitzgibbons JC (1997) Differing mechanisms of expression for short- and long-term potentiationn. J Neurophysiol 78:321-334.

Schwarzer C, Tsunashima K, Wanzenbock C, Fuchs K, Sieghart W, Sperk G (1997) GABAA receptor subunits in the rat hippocampus II: Altered distribution in kainic acid-induced temporal lobe epilepsy. Neuroscience 80:1001-1017.

Scimemi A, Schorge S, Kullmann DM, Walker MC (2006) Epileptogenesis is associated with enhanced glutamatergic transmission in the perforant path. J Neurophysiol 95:1213-1220.

Senatorov VV, Vilbgi I, Tarnawa I, Banczerowski-Pelyhe I, Fülöp Z (1995) Low extracellular magnesium unmasks N-methyl--aspartate-mediated graft-host connections in rat neocortex slice preparation. Neuroscience 64:443-458.

Shaltiel G, Shamir A, Shapiro J, Ding D, Dalton E, Bialer M, Harwood AJ, Belmaker RH, Greenberg ML, Agam G (2004) Valproate decreases inositol biosynthesis. Biological Psychiatry 56:868-874.

Shumate MD, Lin DD, Gibbs III JW, Holloway KL, Coulter A (1998) GABAA receptor function in epileptic human dentate granule cells: comparison to epileptic and control rat. Epilepsy Res 32:114-128.

Silva Brum LF, Elisabetsky E (2000) Antiepileptogenic properties of phenobarbital: behavior and neurochemical analysis. Pharmacol Biochem Behav 67:411-416.

Silver JM, Shin C, McNamara JO (1991) Antiepileptogenic effects of conventional anticonvulsants in the kindling model of epilepsy. Ann Neurol 29:356-363.

Simonato M, Molteni R, Bregola G, Muzzolini A, Piffanelli M, Beani L, Racagni G, Riva M (1998) Different patterns of induction of FGF-2, FGF-1 and BDNF mRNAs during kindling epileptogenesis in the rat. Eur J Neurosci 10:955-963.

Skrede KK, Westgaard RH (1971) The transverse hippocampal slice: a well-defined cortical structure maintainedin vitro. Brain Res 35:589-593.

Sloviter RS (1992) Possible functional consequences of synaptic reorganization in the dentate gyrus of kainate-treated rats. Neurosci Lett 16:91-96.

Sloviter RS (1983) "Epileptic" brain damage in rats induced by sustained electrical stimulation of the perforant path. I. Acute electrophysiological and light microscopic studies. Brain Res Bull 10:675-697.

Sokolova S, Schmitz D, Zhang CL, Löscher W, Heinemann U (1998) Comparison of effects of valproate and trans-2-en-valproate on different forms of epileptiform activity in rat hippocampal and temporal cortex slices. Epilepsia 39:251-258.

Song G, Ouyang G, Bao S (2005) The activation of Akt/PKB signaling pathway and cell survival. J Cell Mol Med 9:59-71.

Sperk G (1994) Kainic acid seizures in the rat. Prog Neurobiol 42:1-32.

Stanojlovic' OP, Hrncic' D.R., Zivanović DP, Susić VT (2007) Anticonvulsant, but not antiepileptic, action of valproate on audiogenic seizures in metaphit-treated rats. Clin Exp Pharmacol Physiol 34:1010-1015.

Stefan H, Lopes da Silva FH, Löscher W, Schmidt D, Perucca E, Brodie MJ, Boon PA, Theodore WH, Moshe SL (2006) Epileptogenesis and rational therapeutic strategies. Acta Neurol Scand 113:139-155.

Stephens JR, Levy RH (1992) Valproate hepatotoxicity syndrome: Hypotheses of pathogenesis. Pharm World Sci 14:118-121.

Strasser A, O'Connor L, Dixit VM (2000) Apoptosis Signaling. Annu Rev Biochem 69:217-245.

Strowbridge BW, Masukawa LM, Spencer DD (1992) Hyperexcitability associated with localizable lesions in epileptic patients. Brain Res 587:158-163.

Sugaya E, Onozuka M (1978) Intracellular calcium: its movement during pentylenetetrazole-induced bursting activity. Science 200:797-799.

Suter KJ, Smith BN, Dudek FE (1999) Electrophysiological recording from brain slices. Methods 18:86-90.

Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L (1989) Mossy fiber synaptic reorganization in the epileptic human temporal lobe. Ann Neurol 26:321-330.

Sutula T, He XX, Cavazos J, Scott G (1988) Synaptic reorganization in the hippocampus induced by abnormal functional activity. Science 239:1147-1150.

Sutula TP (1990) Experimental models of temporal lobe epilepsy: new insights from the study of kindling and synaptic reorganization. Epilepsia 31:S45-S54.

Sweatt JD (2001) The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J Neurochem 76:1-10.

Tehrani MH, Barnes EMJr (1995a) Reduced function of gamma-aminobutyric acidA receptors in tottering mouse brain: role of cAMP-dependent protein kinase. Epilepsy Res 22:13-21.

Tehrani MH, Barnes EM (1995b) Reduced function of [gamma]-aminobutyric acidA receptors in tottering mouse brain: role of cAMP-dependent protein kinase. Epilepsy Res 22:13-21.

Temkin NR, Dikmen SS, Anderson GD, Wilensky AJ, Holmes MD, Cohen W, Newell DW, Nelson P, Awan A, Winn HR (1999) Valproate therapy for prevention of posttraumatic seizures: a randomized trial. J Neurosurg 91:593-600.

Thuault SJ, Davies CH, Randall AD, Collingridge GL (2002) Group I mGluRs modulate the pattern of non-synaptic epileptiform activity in the hippocampus. Neuropharmacology 43:141-146.

Thurber S, Chronopoulos A, Stafstrom CE, Holmes GL (1992) Behavioral effects of continuous hippocampal stimulation in the developing rat. Brain Res Dev Brain Res 68:35-40.

Timofeeva OA, Peterson GM (1999) Dissociation of mossy fiber sprouting and electrically-induced seizure sensitivity: rapid kindling versus adaptation. Epilepsy Res 33:99-115.

Tokuoka SM, Saiardi A, Nurrish SJ (2008) The mood stabilizer valproate inhibits both inositol- and diacylglycerol-signaling pathways in Caenorhabditis elegans. Mol Biol Cell 19:2241-2250.

Tolnai S (1975) A method for viable cell count. Methods Cell Sci 1:37-38.

Traub RD, Pais I, Bibbig A, LeBeau FEN, Buhl EH, Hormuzdi SG, Monyer H, Whittington MA (2003) Contrasting roles of axonal (pyramidal cell) and dendritic (interneuron) electrical coupling in the generation of neuronal network oscillations. PNAS 100:1370-1374.

Tremblay E, Represa A, Ben-Ari Y (1985) Autoradiographic localization of kainic acid binding sites in the human hippocampus. Brain Res 343:378-382.

Tsien JZ, Huerta PT, Tonegawa S (1996) The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. Cell 87:1327-1338.

Tully T (1997) Regulation of gene expression and its role in long-term memory and synaptic plasticity. PNAS 94:4239-4241.

Turski L, Ikonomidou C, Turski WA, Bortolotto ZA, Cavalheiro EA (1989) Review: cholinergic mechanisms and epileptogenesis. The seizures induced by pilocarpine: a novel experimental model of intractable epilepsy. Synapse 3:154-157.

Tzounopoulos T, Janz R, Sudhof TC, Nicoll RA, Malenka RC (1998) A role for cAMP in long-term depression at hippocampal mossy fiber synapses. Neuron 21:837-845.

Üre A, Altrup U (2006) Block of spontaneous termination of paroxysmal depolarizations by forskolin (buccal ganglia, Helix pomatia). Neurosci Lett 392:10-15.

Van den Berg RJ, Kok P, Voskuyl RA (1993) Valproate and sodium currents in cultured hippocampal neurons. Exp Brain Res 93:279-287.

Vandenberghe W, Nicoll RA, Bredt DS (2005) Interaction with the unfolded protein response reveals a role for stargazin in biosynthetic AMPA receptor transport. J Neurosci 25:1095-1102.

Vignes M, Collingridge GL (1997) The synaptic activation of kainate receptors. Nature 388:179-182.

Vissel B, Royle GA, Christie BR, Schiffer HH, Ghetti A, Tritto T, Perez-Otano I, Radcliffe RA, Seamans J, Sejnowski T (2001) The role of RNA editing of kainate receptors in synaptic plasticity and seizures. Neuron 29:217-227.

Vossler MR, Yao H, York RD, Pan MG, Rim CS, Stork PJS (1997) cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. Cell 89:73-82.

Vreugdenhil M, Wadman WJ (1999) Modulation of sodium currents in rat CA1 neurons by carbamazepine and valproate after kindling epileptogenesis. Epilepsia 40:1512-1522.

Wada T, Penninger JM (2004) Mitogen-activated protein kinases in apoptosis regulation. oncogene 23:2838-2849.

Walczak TS (1995) Neocortical temporal lobe epilepsy: characterizing the syndrome. Epilepsia 36:633-635.

Walker JE, Lewin E, Sheppard JR, Cromwell R (1973) Enzymatic regulation of adenosine 3',5'-monophosphate (cyclic AMP) in the freezing epileptogenic lesion of rat brain and in homologous contralateral cortex. J Neurochem 21:79-85.

Walker MC, Perry H, Scaravilli F, Patsalos PN, Shorvon SD, Jefferys JG (1999) Halothane as a neuroprotectant during constant stimulation of the perforant path. pp 359-364.

Walker MC, White HS, Sander JW (2002) Disease modification in partial epilepsy. Brain 125:1937-1950.

Wang X, Wang H, Xu L, Rozanski DJ, Sugawara T, Chan PH, Trzaskos JM, Feuerstein GZ (2003) Significant neuroprotection against ischemic brain injury by inhibition of the MEK1 protein kinase in mice: exploration of potential mechanism associated with apoptosis. J Pharmacol Exp Ther 304:172-178.

Weisskopf MG, Castillo PE, Zalutsky RA, Nicoll RA (1994) Mediation of hippocampal mossy fiber long-term potentiation by cyclic AMP. Science 265:1878-1882.

Wenthold RJ, Petralia RS, Blahos J, Niedzielski AS (1996) Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. J Neurosci 16:1982-1989.

White HS (1997) Clinical significance of animal seizure models and mechanism of action studies of potential antiepileptic drugs. Epilepsia 38:s9-s17.

White HS (2002) Animal models of epileptogenesis. Neurology 59:7S-14.

Williams DC, Massey GV, Russell EC, Riley RS, Ben-Ezra J (2008) Translocation-

positive acute myeloid leukemia associated with valproic acid therapy. Pediatr Blood Cancer 50:641-643.

Williams RSB, Cheng L, Mudge AW, Harwood AJ (2002) A common mechanism of action for three mood-stabilizing drugs. Nature 417:292-295.

Wilot LC, Bernardi A, Frozza RL, Marques AL, Cimarosti H, Salbego C, Rocha E, Battastini AMO (2007) Lithium and valproate protect hippocampal slices against ATP-induced cell death. Neurochemical Research 32:1539-1546.

Wong RKS, Bianchi R, Chuang SC, Merlin LR (2005) Group I mGluR-induced epileptogenesis: distinct and overlapping roles of mGluR1 and mGluR5 and implications for antiepileptic drug design. Epilepsy Currents 5:63-68.

Wuarin JP, Dudek FE (1996) Electrographic seizures and new recurrent excitatory circuits in the dentate gyrus of hippocampal slices from kainate-treated epileptic rats. J Neurosci 16:4438-4448.

Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME (1995) Opposingeffects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270:1326-1331.

Yaari Y, Konnerth A, Heinemann U (1986) Nonsynaptic epileptogenesis in the mammalian hippocampus in vitro. II. Role of extracellular potassium. J Neurophysiol 56:424-438.

Yamakura T, Shimoji K (1999) Subunit- and site-specific pharmacology of the NMDA receptor channel. Prog Neurobiol 59:279-298.

Yamamoto R, Yanagita T, Kobayashi H, Yokoo H, Wada A (1997) Up-regulation of sodium channel subunit mRNAs and their cell surface expression by antiepileptic valproic acid: activation of calcium channel and catecholamine secretion in adrenal chromaffin cells. J Neurochem 68:1655-1662.

Yang E, Zha J, Jockel J, Boise LH, Thompson CB, Korsmeyer SJ (1995) Bad, a heterodimeric partner for Bcl-xL and Bcl-2, displaces bax and promotes cell death. Cell 80:285-291.

Yechikhov S, Morenkov E, Chulanova T, Godukhin O, Shchipakina T (2001) Involvement of cAMP- and Ca2+/calmodulin-dependent neuronal protein phosphorylation in mechanisms underlying genetic predisposition to audiogenic seizures in rats. Epilepsy Res 46:15-25.

Ylinen A, Lahtinen H, Sirviö J, Partanen J, Asikainen A, Gulyas A, Freund TF, Riekkinen P (1991) Behavioural, electrophysiological and histopathological changes following sustained stimulation of the perforant pathway input to the hippocampus: Effect of the NMDA receptor antagonist, CGP 39551. Brain Res 553:195-200.

Yoshino M, Sawada S, Yamamoto C, Kamiya H (1996) A metabotropic glutamate receptor agonist DCG-IV suppresses synaptic transmission at mossy fiber pathway of the guinea pig hippocampus. Neurosci Lett 207:70-72.

Yuan PX, Huang LD, Jiang YM, Gutkind JS, Manji HK, Chen G (2001) The mood

stabilizer valproic acid activates mitogen-activated protein kinases and promotes neurite growth. J Biol Chem 276:31674-31683.

Yurekli V, Akhan G, Kutluhan S, Uzar E, Koyuncuoglu H, Gultekin F (2008) The effect of sodium valproate on chronic daily headache and its subgroups. The Journal of Headache and Pain 9:37-41.

Zalutsky RA, Nicoll RA (1990) Comparison of two forms of long-term potentiation in single hippocampal neurons. Science 248:1619-1624.

Zhao W, Bianchi R, Wang M, Wong RKS (2004) Extracellular signal-regulated kinase 1/2 is required for the induction of group I metabotropic glutamate receptor-mediated epileptiform discharges. J Neurosci 24:76-84.

Zou H, Henzel WJ, Liu X, Lutschg A, Wang X (1997) Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 90:405-413.

Zucker RS, Regehr WG (2002) Short term synaptic plasticity. Annu Rev Physiol 64:355-405.