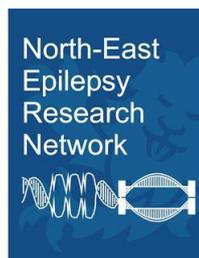


Friday 5<sup>th</sup> September 2014  
Beamish Hall Hotel

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# North East Epilepsy Research Network Meeting



**Sponsors:** Cyberonics, Desitin, Digitimer, Eisai Ltd, Genzyme, Rosemont Pharmaceuticals, Scientifica, UCB Pharma Ltd

## Programme

08.30-09.00 Registration and coffee

09.00-09.15 Introduction – Drs Baker/Cunningham/Hart/Taggart/Trevelyan

### ***Session 1: Epilepsy models: an update of current approaches***

Chair – Dr Mark Cunningham

09.15-9.45 Dr Peter Massey (University of Bath)  
*A chronic model of acquired epilepsy in organotypic rat entorhinal cortex-hippocampal brain slices*

9.45-10.15 Felix Chan (Newcastle University)  
*A novel in vitro brain slice model of mitochondrial epilepsy*

10.15-10.45 Tea/Coffee  
View Exhibition/Posters

### ***Osselton Lecture***

Chair – Dr Andy Trevelyan

10.45-11.45 Professor Kevin Staley (Massachusetts General Hospital, USA)  
*From Gibbs-Donnan to Epilepsy: how impermeant anions establish the neuronal chloride concentration.*

11.45-12.45 Lunch  
View Exhibition/Posters

### ***Session 2: Neuropsychological insights into epilepsy***

Chair – Dr Tom Kelly

12.45-13.15 Professor Gus Baker (University of Liverpool)  
*The impact of anti-epileptic drugs in utero: an unfolding story.*

13.15-13.45 Dr Ingram Wright (North Bristol, NHS Trust)  
*Variability in cognitive functioning and implications for management of childhood epilepsy.*

13.45-14.15 Dr Liam Dorris (University of Glasgow)  
*Accelerated forgetting in children with generalised epilepsies - a phenomena seeking an explanation.*

14.15-14.45 Dr Ryley Parrish (Newcastle University)  
*Methylation Mechanisms in Epilepsy and Associated Memory Deficits.*

14.45-15.05 Tea/Coffee  
View Exhibition/Posters

## Programme (continued)

### **Session 3: Chloride regulation in epilepsy**

Chair – Dr Simon Taggart

- 15.05-15.35 Dr Freek Hoebeek (Erasmus University, Rotterdam, The Netherlands)  
*Cerebellar impact on thalamo-cortical oscillations during absence seizures*
- 15.35-15.55 Dr Gian Michele Ratto (Scuola Normale Superiore, Pisa, Italy)  
*In vivo measurement of intracellular chloride and pH during neuronal development by means of 2-photon spectroscopy.*
- 15.55-16.15 Dr Andrew Trevelyan (Newcastle University)  
*Chloride loading in neurons: just how epileptogenic is it?*
- 16.15-16.35 Dr Andrei Ilie (University of Oxford)  
*Neuronal chloride (Cl<sup>-</sup>) dynamics during seizure activity.*

16.35-17.00 Break

### **Session 4: Peritumoural epilepsy**

Chair – Dr Yvonne Hart

- 17.00-17.30 Dr Mark Cunningham (Newcastle University)  
*Critical mechanisms for epileptogenesis in peritumoural tissue: in vitro human studies*
- 17.35-18.00 Professor Ian Whittle (Division of Clinical Neuroscience, University of Edinburgh)  
*Peritumoural brain hyperfunction: implications for seizure generation*
- 18.00-18.15 Presentation of poster prize and concluding remarks
- 18.15 - Drinks and dinner (*Stables restaurant*)

# A chronic model of acquired epilepsy in organotypic rat entorhinal cortex-hippocampal brain slices

**Dr Peter Massey,**

Department of Pharmacology,  
University of Bath

Epilepsy research relies on animal models of acquired epilepsy *in vivo*. Commonly, an acute epileptogenic insult is delivered leading to a chronic epileptic condition, after which brain tissue is harvested for experimental study *in vitro*. Most studies are conducted after spontaneous seizures emerge, but following network changes longitudinally during the latent period is problematic. To circumvent these difficulties we have developed an alternative model of acquired epilepsy in organotypic slice cultures. Combined slices of entorhinal cortex and hippocampus are prepared from a neonate (P12-15) Wistar rat. After 10-14 days, slices are exposed to a control or an epileptogenic medium (12 mM K<sup>+</sup>/0.5 mM Mg<sup>2+</sup>; HKLM). Matched slices are then examined electrophysiologically for the development of spontaneous epileptiform activity.

Spontaneous paroxysmal-like events (SPLEs) manifest between around 4-10 weeks, and by 8-10 weeks SPLEs are apparent in most HKLM slices. SPLEs in CA3 are interictal-like and regular, whereas those in EC are ictal-like, prolonged (~30-70s) and intense. This epileptiform activity is also glutamate receptor-dependent and sensitive to a number of clinically effective anti-epileptic drugs.

Thus, this new model shows great promise as an alternative to traditional models and could allow longitudinal monitoring of network changes in tissue from a single animal.

We thank the National Centre for the Replacement Refinement and Reduction of Animals in Research (NC3Rs) for financial support.

# A novel in vitro brain slice model of mitochondrial epilepsy

**Felix Chan,**

Institute of Neuroscience,  
Newcastle University

**Introduction:** Mitochondrial disease often presents with an epileptic phenotype – ‘mitochondrial epilepsy’. It is extremely difficult to control and drug development for this condition is lagging due to a lack of a good functional model. Post-mortem neuropathological studies of temporal neocortex from patients with mitochondrial epilepsy have shown deficiency in mitochondrial respiratory chain complexes I and IV along with a pattern of astrogliosis.

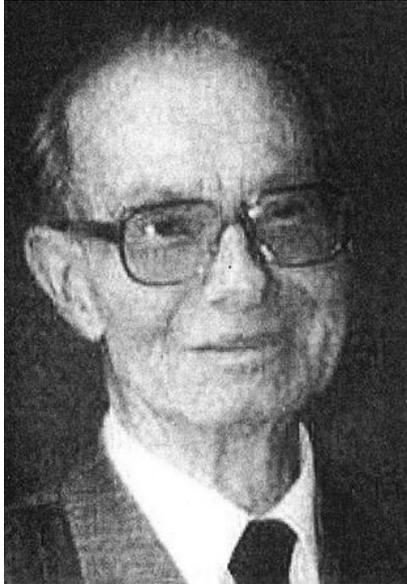
**Aims:** Building on these neuropathological results, we aim to develop a functional model of mitochondrial epilepsy utilizing various mitochondrial inhibitors; rotenone (complex-I inhibitor), potassium cyanide-KCN (complex-IV inhibitor), and fluorocitrate (astrocytic specific aconitase inhibitor).

**Methods:** Hippocampal-temporal neocortex slices are prepared from adult Wistar rats. Extracellular local field potential recordings are taken from CA3 of the hippocampus or layer II of temporal neocortex. Various inhibitor combinations are tested to induce epileptiform activity in vitro.

**Results:** Epileptic activity was readily generated in both the hippocampus (CA3) and temporal neocortex by adding fluorocitrate (0.1 mM) followed by co-application of rotenone (500nM) and KCN (10 $\mu$ M). Carbamazepine, lamotrigine, and levetiracetam – common antiepileptic drugs - were tested and all drugs fail to suppress the epileptic activity. Post-hoc immunohistochemistry showed a pattern of astrogliosis and a significant reduction of parvalbumin-expressing interneurons in the hippocampus CA3 (n=6).

**Conclusion:** We have successfully developed a novel in vitro brain slice model for mitochondrial epilepsy. The model replicates most features seen in the human neuropathology and demonstrate signs of pharmacoresistance. It is hoped that this can be a robust model to help elucidate the mechanisms behind mitochondrial epilepsy generation and for novel drug development studies.

## John “Os” Walkinshaw Osselton (1928-2009)



John Osselton, or “Os” as he was generally known, spent his entire academic career at Newcastle University, where his lifelong research interest in EEG contributed significantly to our understanding of epilepsy.

Oz was born into a medical family in Newcastle in 1928. His mother had been something of a medical pioneer; not only was she the third female to qualify in medicine from Newcastle University (then part of Durham University), graduating in 1911, but one of the first female consultant anaesthetists in the UK. It was natural therefore that Oz, despite graduating from Newcastle University in 1949 with a BSc in Electrical Engineering, should embark upon a career in medical research.

His first appointment in 1949 was as Research Assistant in the Department of Psychological Medicine at the Royal Victoria Infirmary, in the section of “Applied Electrophysiology”. The then head of department, Professor Alexander Kennedy, keen to embrace the new technology of EEG dispatched Oz to the Burden Neurological Institute in Bristol, to learn about EEG from his former colleague William Grey Walter (Kennedy and Walter had been colleagues at the Maudsley Hospital before the war). Grey Walter had already built the first EEG system in the UK in the 1930s after visiting Hans Berger’s laboratory, improving greatly on the original design. With their shared interest in refining and improving EEG technology, Walter and Oz were destined to be lifelong friends. Oz was promoted to Lecturer in 1954 and then Senior Lecturer in EEG in 1965. He retired in 1984 and died after a long illness in 2009.

Oz published numerous teaching articles and scientific papers, but is probably best remembered internationally as the co-author of two of the first EEG textbooks: “clinical EEG” and “EEG technology” and co-editing both volumes of the more comprehensive textbook “Clinical Neurophysiology”. He is remembered locally in Newcastle for being an outstanding teacher, having trained many generations of neurologists, neurophysiologists and technicians in the mystery of EEG interpretation.

# From Gibbs-Donnan to Epilepsy: how impermeant anions establish the neuronal chloride concentration

**Professor Kevin Staley,**

Massachusetts General Hospital,  
USA

Humans pay a high price for the size of the brain, yet 25% of brain volume is extracellular “space”. This space is filled with a gelatinous array of polyanionic macromolecules, no two of which are alike. Why are these macromolecules so unique? We will delve into the wonderful world of Donnan equilibria to show that signaling by the inhibitory transmitter GABA is strongly influenced by cytoplasmic and extracellular polyanionic macromolecules. We will discuss the possibility that the individuality of these polyanions could support information storage, and that long-standing observations regarding the cytoplasmic and extracellular milieus in epilepsy may doom patients to intractability until we learn to alter this macromolecular code.

## The impact of anti-epileptic drugs in utero: an unfolding story

**Professor Gus Baker,**

University of Liverpool

Antiepileptic drugs (AEDs) are associated with teratogenic risk to the development of the fetus, with the prevalence of major congenital malformations differing by treatment type and dose. Determining the association between exposure to AEDs and child cognitive functioning represents a significant challenge and previous research including case studies, retrospective studies and prospective studies have methodological pitfalls. Despite limitations there is growing evidence that exposure to sodium valproate (VPA) in utero is associated with significantly poorer functioning. Prospective studies consistently document that VPA is associated with an increase in risk of cognitive impairment in young children; but any longer term effects are unlikely to be comprehensively documented until the children studied are at least six years of age, where cognitive development is more stable. In a comparison across AED types, Meador and colleagues demonstrated significantly poorer IQ in school aged children exposed in utero to VPA, against those exposed to carbamazepine (CBZ), lamotrigine (LTG) and phenytoin (PHT). However, a comparison against control children was not possible and therefore the effects of CBZ and LTG on school aged child IQ remains inconclusive. Deficits in IQ have significant educational, health and economic implications and therefore risks conveyed to the fetus by medications need to be delineated.

In this presentation the author will draw upon the research of the Liverpool and Manchester neurodevelopment Group and others to highlight the journey of discovering the teratogenic effects of AED's from clinical observation to changes in Clinical Practice.

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# Variability in cognitive functioning and implications for management of childhood epilepsy

**Dr Ingram Wright,**

Consultant Paediatric Neuropsychologist  
(North Bristol NHS Trust and University  
Hospitals Bristol NHS Trust)  
Honorary Senior Lecturer (University of  
Bristol)

Variability in cognitive functioning is commonly reported by parents of children with epilepsy. Alongside colleagues, I have characterised the form of variability reported by parents and link this with the underlying pathophysiology of epilepsy syndromes and broader parental concerns. We describe questionnaire measures developed for assessment of these subjective concerns alongside approaches to the objective assessment of cognitive variability. We draw on evidence for transient cognitive impairment linked to subclinical epileptiform discharges. Specific reference will also be made to the role of sleep in consolidation of learning and sleep disturbance linked to corresponding cognitive problems in childhood epilepsy.

Throughout this presentation we take the view that concerns about variability in performance is a key consideration in the optimal medical management of childhood epilepsy.

# Accelerated forgetting in children with generalised epilepsies - a phenomena seeking an explanation

**Dr Liam Dorris,**

University of Glasgow

Despite typically scoring within the average range on tests of memory and intelligence, the educational attainment of young people with 'genetic generalised epilepsies' continues to show disadvantage relative to a range of normally developing and non-neurological chronic illness groups. The economic, health and social costs of this under-attainment are considerable. There is continuing debate around causation, with explanations featuring the role of stigma, psychosocial adjustment and expectation, the role of cognitive dysfunction, the neurodevelopmental impact of anti-epileptic medications and the impact of seizure severity.

This talk focuses on the cognitive phenomenon of accelerated long-term forgetting (ALF), in which newly acquired memories are intact after short delays but fade over days to weeks, as a potential cause of the learning and attainment problems experienced by children with epilepsy. The concept of accelerated forgetting in epilepsy will be considered alongside findings from the small number of adequately designed paediatric studies. Past and current work from the author's research group will be discussed and audience contributions towards future directions welcomed.

## Background reading:

- Davidson M, Dorris L, O'Regan M, Zuberi SM. (2007). Memory consolidation and accelerated forgetting in children with idiopathic generalized epilepsy. *Epilepsy Behav.* 11(3):394-400
- Gascoigne MB, Barton B, Webster R, Gill D, Antony J, Lah SS. (2012). Accelerated long-term forgetting in children with idiopathic generalized epilepsy. *Epilepsia.* 53(12):2135-40

# Methylation Mechanisms in Epilepsy and Associated Memory Deficits

**Dr Ryley Parrish,**

The Institute of Neuroscience,  
Newcastle University

Temporal lobe epilepsy (TLE) patients exhibit signs of memory impairments even when seizures are pharmacologically controlled. Surprisingly, the underlying molecular mechanisms involved in TLE-associated memory impairments remain elusive. Memory consolidation requires epigenetic transcriptional regulation of genes in the hippocampus; therefore, we aimed to determine how the epigenetic mechanism, DNA methylation affect learning-induced transcription of memory-permissive genes in the epileptic hippocampus. Using the kainate rodent model of TLE and focusing on the brain-derived neurotrophic factor (Bdnf) gene as a candidate of DNA methylation-mediated transcription, we analyzed DNA methylation levels in epileptic rats following learning. After detection of aberrant DNA methylation at the Bdnf gene, we investigated functional effects of altered DNA methylation on hippocampus-dependent memory formation in our TLE rodent model. We found that behaviorally driven Bdnf DNA methylation was associated with hippocampus-dependent memory deficits. Bisulfite sequencing revealed that decreased Bdnf DNA methylation levels strongly correlated with abnormally high levels of Bdnf mRNA in the epileptic hippocampus during memory consolidation. Methyl supplementation via methionine (Met) increased Bdnf DNA methylation and reduced Bdnf mRNA levels in the epileptic hippocampus during memory consolidation. Met administration reduced interictal spike activity, improved theta rhythm power, and reversed memory deficits in epileptic animals. The rescue effect of Met treatment on learning-induced Bdnf DNA methylation, Bdnf gene expression, and hippocampus-dependent memory, were attenuated by DNA methyltransferase blockade. Our findings suggest that manipulation of DNA methylation-mediated gene transcription should be considered as a viable treatment option to ameliorate memory impairments associated with TLE.

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## Cerebellar impact on thalamo-cortical oscillations during absence seizures

**Dr Freek Hoebeek,**

Erasmus University, Rotterdam,  
The Netherlands

Cerebellar output neurons are directly connected to relay neurons in a variety of thalamic nuclei and are therefore likely to mediate thalamo-cortical activity patterns. I will present how the cerebellar activity is modulated during generalized spike-and-wave discharges in the thalamo-cortical network of murine models for absence epilepsy. Our data show that neuromodulation of the cerebellar output is highly effective in stopping thalamo-cortical oscillations, which advocates the therapeutic potential of the cerebellar output to control absence epilepsy.

## In vivo measurement of intracellular chloride and pH during neuronal development by means of 2-photon spectroscopy

**Dr Gian Michele Ratto,**

Scuola Normale Superiore, Pisa, Italy

The intracellular concentration of chloride is regulated by the expression of two cation Cl<sup>-</sup>-co-transporters, NKCC1 and KCC2, which move Cl<sup>-</sup> in opposite directions. Interestingly, their expression is strongly regulated during development: NKCC1 is highly expressed in the pre and neonatal life, whereas KCC2 is mostly expressed in the adult; this is predicted to cause a shift in [Cl<sup>-</sup>] between neonatal and adult brain. Although several studies in slice and cell cultures have suggested that Cl<sup>-</sup> is depolarizing in the perinatal period in immature neurons, an in vivo absolute measurement of the [Cl<sup>-</sup>] shift has never been performed.

Here, we have devised a method for absolute measurement of [Cl<sup>-</sup>]<sub>i</sub> (and pH) in vivo in rodents by using a modified genetically encoded sensor (ClpHensor2). This sensor is formed by the fusion between a Cl<sup>-</sup> and pH sensitive GFP (E2GFP) and an insensitive RFP (msKate). First, we characterized the 2-photon spectral properties of the sensor, showing that increasing [Cl<sup>-</sup>]<sub>i</sub> causes a decrease of the green/red fluorescence. A shift in pH causes a strong alteration of the 2-photon excitation spectra of E2GFP. Then, the sensor was targeted to the visual or somatosensory cortex by means of in utero electroporation at E15.5 (mouse) or E17.5 (rat). We demonstrated the integrity and stoichiometry of the sensor by in situ fluorescence correlation spectroscopy and by measuring trafficking across the nuclear membrane. Then, we found that brain tissue has a strongly wavelength-dependent effect on the propagation of excitation and emission. These effects were very strong in vivo, but they were also present in slices and affected Chloride measures. By means of analytical spectral decomposition we could measure and compensate for the spectral distortion caused by the tissue. We determined that [Cl<sup>-</sup>]<sub>i</sub> is very high until P8 ( $50.6 \pm 21.5$  mM) and then strongly decreases ( $12.9 \pm 8.2$  mM) after P18. These data are the first direct demonstration of the developmental regulation of Chloride concentration in vivo.

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## Chloride loading in neurons: just how epileptogenic is it?

**Dr Andrew Trevelyan,**

Institute of Neuroscience,  
Newcastle University

Altered inhibitory function is an important facet of epileptic pathology. A key concept is that GABAergic activity can become excitatory, arising from raised intraneuronal chloride. It has proved difficult, however, to relate such cellular explanations to measures that can be recorded in humans and which thus have clinical relevance. High frequency oscillations (HFOs) fall into this category, because they are believed to identify sites of epileptic pathology. We asked therefore what patterns of pathological activity are associated with chloride dysregulation. We made novel use of Halorhodopsin, to load clusters of pyramidal cells artificially with Cl<sup>-</sup>. This produced a transient rise in network excitability, with many distinctive features of epileptic foci, but notably, did not actually trigger full ictal events. I will discuss what this tells us about the "chloride dysfunction hypothesis" of ictogenesis.

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# Neuronal chloride (Cl<sup>-</sup>) dynamics during seizure activity

**Dr Andrei Ilie,**

University of Oxford

How neurons control their intracellular Cl<sup>-</sup> concentration ([Cl<sup>-</sup>]<sub>i</sub>) is a fundamental question that has direct relevance to hyperexcitability conditions such as epilepsy. When [Cl<sup>-</sup>]<sub>i</sub> is relatively low, the reversal potential of the Cl<sup>-</sup> permeable GABA<sub>A</sub> receptors (GABA<sub>A</sub>R) is more negative than the membrane potential and GABA<sub>A</sub>R activation will have a hyperpolarising and inhibitory effect upon the postsynaptic neuron. In contrast, if [Cl<sup>-</sup>]<sub>i</sub> is relatively high, the GABA<sub>A</sub>R reversal potential will be more positive and GABA<sub>A</sub>R activation will have a depolarising effect. The [Cl<sup>-</sup>]<sub>i</sub> can be dynamically shifted and regulated over both short timescales (such as during a single seizure episode) and longer timescales (such as during brain development or chronic epilepsy) with important consequences for GABAergic signalling and excitability. In this talk I will discuss some of the mechanisms and factors that are responsible for regulating fast dynamic changes in [Cl<sup>-</sup>]<sub>i</sub> and postsynaptic GABA<sub>A</sub>R signalling in the context of seizure activity.

## Critical mechanisms for epileptogenesis in peritumoural tissue: in vitro human studies

**Dr Mark Cunningham,**

Institute of Neuroscience,  
Newcastle University

Approximately 4300 people a year are diagnosed with a brain tumour in the UK and around 0.1% of the UK population will have a symptomatic brain tumour at any one time. Seizures are the presenting symptom in up to 40 % of patients with brain tumours. Although low-grade gliomas are associated with a much better prognosis than other types of brain tumour, seizures are a much more common feature, occurring in 72-89% of patients. Half of these patients will experience recurrent seizures and 50% of this group will suffer from medically refractory seizures.

Novel pharmacological approaches are required to overcome this shortfall in therapeutic efficacy. Recent work using an animal model of brain tumour has suggested a role for elevated glutamatergic drive in epileptogenesis. This is due to increased release of glutamate from glioma cells via the system xc- cysteine-glutamate transporter and impaired glutamate uptake by glial cells. Moreover, in humans with tumour-associated seizures, high glutamate concentrations have been observed.

We have tested the efficacy of an antagonist of system xc-, sulfasalazine (SAS), on spontaneous epileptic activity in tissue obtained from adult human patients with gliomas. In spontaneously active peritumoural slices, inter-ictal discharges were significantly reduced ( $P < 0.05$ ;  $n=3$ ) using concentrations in the range of 10-50  $\mu\text{M}$  SAS. Previous studies have observed subtle effects of SAS on epileptiform (zero magnesium induced) activity only at higher concentrations (250  $\mu\text{M}$ ) in slices from an animal model of brain tumour. However, at this concentration SAS is known to be potent noncompetitive antagonist of NMDA receptors. We therefore tested the impact of SAS on zero magnesium induced epileptic activity in human brain slices. There was no effect at lower concentrations ( $< 50 \mu\text{M}$ ) but the induced activity was significantly reduced at higher concentrations ( $> 100 \mu\text{M}$ ). This effect on induced epileptic activity is likely to be due to NMDA receptor blockade. Peritumoural slices exhibiting spontaneous epileptic activity demonstrated strong co-localisation of antibodies staining for the intermediate filament glial fibrillary associated protein (GFAP) and system xc-.

These data demonstrate that in human peritumoural cortical regions excessive glutamate release from human glioma cells contributes to epileptic activity. Furthermore, molecules that target system xc- could be useful therapeutic targets for tumour epileptogenesis. Abstract

# Peritumoural brain hyperfunction: implications for seizure generation

**Professor Ian Whittle,**

Forbes Professor Emeritus in Surgical Neurology,  
Centre for Clinical Brain Sciences, University of Edinburgh

Seizures are a common clinical manifestation of intracranial tumours. In many patients they are the only symptom. Their incidence varies considerably with brain tumour type being around 80% in oligodendrogliomas (Glioma WHO II) and 30% in Glioblastomas (Glioma WHO IV). They are also common in meningiomas, an intracranial extracerebral tumour. As well as tumour type factors such as age, tumour size and tumour location in the brain influence seizure incidence. Therefore the mechanisms of peritumoural brain hyperfunction that result in seizures must be multifactorial although there may well be common pathways in epileptogenesis. Many of the pathophysiological changes in the peritumoural brain around gliomas are well characterised and very different between low grade (WHO I-II; well differentiated tumour cell invasion, axodendritic changes, intact BBB) and high grade (WHO III-IV; brain tissue destruction, extensive malignant cell invasion of brain, hypoxic changes, loss of BBB characteristics, peritumoural brain edema) gliomas. The peritumoural changes seen with meningiomas and metastatic brain tumours are also complex and very different in their dynamic evolution.

Given this pathophysiological heterogeneity what are the critical mechanistic changes involved in epileptogenesis? Is the peritumoural brain resting membrane potential altered? If so does it arise as a result of changes in brain pH, membrane receptor and ion channel dysfunction or imbalance, or is it the effects of factors secreted into the peritumoural brain by the tumour that effect membrane homeostasis.

Investigating peritumoural factors involved in seizure generation should ideally be done by a multidisciplinary team involving neurosurgeons, clinical neurophysiologists, neuropathologists and basic scientists with access to proteomic and genetic tools, cell culture techniques and experimental in vivo and in vitro neurophysiological recording techniques. Experiments need to be critically designed so that the results can translate to, and be relevant to the clinical problem Ideally they should involve both clinical and in vitro studies that are complementary.

The specific contributions that intraoperative neurosurgical and neurophysiological studies and tissue harvesting can provide; the use and limitations of experimental in vivo models; findings from dynamic in vitro studies, proteomic profiling and immunocytochemical studies of peritumoural brain will be outlined. The potential of multidisciplinary research using tissue slice electrophysiology, membrane dyes, tissue culture with co-cultured neural and glioma stem cells will be discussed.

## Posters

- **A novel in vitro brain slice model of pharmacoresistant mitochondrial epilepsy: ‘a dual neuronal – astrocytic hit hypothesis’.**  
Felix Chan, Newcastle University
- **Characteristics of evolving epileptiform activity in brain slices.**  
Neela Codadu, Newcastle University
- **Unbalanced peptidergic inhibition in superficial neocortex underlies spike and wave seizure activity.**  
Stephen Hall, University of York
- **Are GABAergic interneurons key perpetrators in the development of epilepsy in mitochondrial disease?**  
Nicola Lax, Newcastle University
- **Abatement of epileptic spike-wave discharges through single pulse stimulation**  
Peter Taylor, Newcastle University
- **The effect of parvalbumin expressing interneurons on the propagation speed of epileptic seizures in vitro.**  
Chris Thornton, Newcastle University
- **Dynamic mechanisms of neocortical focal seizure onset.**  
Yujiang Wang, Newcastle University
- **Assessing seizure susceptibility using visual psychophysical tests.**  
Partow Yazdani, Newcastle University

# A novel in vitro brain slice model of pharmacoresistant mitochondrial epilepsy: ‘a dual neuronal – astrocytic hit hypothesis

**Felix Chan,**

Newcastle University

Epilepsy is among the most common neurological manifestations of mitochondrial disease. Patients with mitochondrial epilepsy have extremely poor prognosis and a difficult to control epilepsy. Drug development in this field has been lagging due to a lack of good functional models. Post-mortem neuropathological studies of temporal neocortex from patients with mitochondrial disease have shown deficiency in mitochondrial respiratory chain complexes I and IV in both GABAergic interneurons and astrocytes, with a pattern of astrogliosis. Building on these observations, we aim to develop a novel model of mitochondrial epilepsy utilizing various mitochondrial inhibitors; rotenone (complex-I inhibitor), potassium cyanide-KCN (complex-IV inhibitor), and fluorocitrate (astrocytic specific aconitase inhibitor). Using in vitro electrophysiological techniques, we conducted extracellular local field potential recording from acutely prepared hippocampal-temporal cortex brain slices from adult rats.

Epileptic activity was readily generated in both the hippocampus (CA3) and temporal neocortex by adding fluorocitrate (0.1 mM) followed by co-application of rotenone (500nM) and KCN (10 $\mu$ M). Interictal sharp wave discharges started to emerge after 30-60 minutes of the rotenone/KCN application followed by ictal seizure-like discharges at prolonged application (up to 120 minutes). Applying either fluorocitrate or rotenone-KCN alone did not generate any epileptic activity. We have also replicated these experiments in surgically resected human temporal neocortical slices obtained from patients undergoing amygdalohippocampectomy for pharmacoresistant epilepsy (n=4). Three commonly used conventional antiepileptic drugs – carbamazepine, lamotrigine, and levetiracetam - were tested and all drugs fail to suppress the epileptic activity, even at suprathreshold concentration. Post-hoc immunohistochemistry of these epileptic brain slices showed a similar pattern of astrogliosis as seen in the human neuropathological studies. Interestingly, there was also a significant reduction of parvalbumin-expressing interneurons in the hippocampus CA3 (n=6).

In conclusion, we have successfully developed a novel in vitro brain slice model for mitochondrial epilepsy. It is the first functional model developed for mitochondrial epilepsy. The model replicates most of the features seen in the human neuropathology and also show signs of pharmacoresistance as is what is observed in the clinical setting. We hypothesized that there is a selective vulnerability of inhibitory interneurons towards respiratory chain inhibition that when coupled with astrocytic mitochondrial impairment can lead to epileptic activity generation. It is hoped that this can be a robust model to help elucidate the mechanisms behind mitochondrial epilepsy generation and can also be used for novel drug development studies.

## Characteristics of evolving epileptiform activity in brain slices

Neela Codadu,  
Newcastle University

Organised and structured neuronal activities are qualities of normal brain function. However, then there is a tendency for the network to become hyperexcitable if the network tips over some apparent threshold level of excitation, or below some threshold level of inhibition, although this process, termed ictogenesis is not understood. This hyperexcitable state of the network underlies the pathological condition of seizures. It is important therefore to distinguish between different pathological mechanisms that contribute to the hyperexcitable state.

Epileptiform activity can be readily induced in brain slices prepared from wild-type mice by bathing in different pro-epileptic bathing media, including 0 Mg<sup>2+</sup>, and 4-aminopyridine (4-AP). However, the distinctive patterns of activity do not develop instantly, but rather evolve over a period of many minutes to hours. We therefore developed a set of measurements to characterise the evolution of epileptic activity when bathed in different pro-epileptic bathing media. Post hoc analysis of these different measures has been performed.

We made extracellular field recordings from hippocampal CA1 and multiple locations in neocortex, in horizontal slices kept in interface chambers, and prepared from young adult mice (1-3months). We analysed the times to first and subsequent ictal events, the pattern of inter-ictal activity, and the transition to a late pattern that has been likened to status epilepticus (Heinemann et al., 1994). We also measured changes in the speed of the ictal wavefront propagation, which is determined by efficacy of feedforward inhibition ahead of the ictal wavefront (Trevelyan et al. 2007). We found marked differences in the patterns of evolution of these various patterns of activity.

In both 0Mg<sup>2+</sup> and 4AP models, the first full ictal event (rhythmic, large amplitude discharges persisting for over 10s) occurred after a relatively long time, 670.1±71s and 910.91±99s, respectively. The second event occurred at far shorter latency in both models. There was a small subsequent increase in the rate in the 0 Mg model, but in the 4AP model, subsequent events occurred at a steady rate. The speed of the wavefront showed a more gradual progressive increase with each successive event. In contrast, the rate of occurrence of interictal events was remarkably stable, at 0.14±0.02Hz, although they did increase in amplitude. This stable rate is at odds with the gradual increase in wavefront propagation and other documented gradual changes in synaptic connectivity, suggesting that the rate is set by factors that, unlike synaptic properties, are invariant. We speculate that glial behaviour may underlie these events. Experiments will be carried out further to explore the role of glial cells and glial-neuron interactions in epilepsy.

# **Unbalanced peptidergic inhibition in superficial neocortex underlies spike and wave seizure activity**

**Stephen Hall,**

University of York

Spike and wave (SpW) discharges manifest on a continuum of pathologies, increasing in severity from occasional rolandic spikes, through to continuous spike and wave discharges during sleep (CSWS). They are associated with a broad range of diagnoses such as absence epilepsies, Lennox-Gastaut syndrome and similar presentations, Landau-Kleffner syndrome, Rett syndrome, autistic regression and fragile-X mutations. Many animal models give clues as to the mechanistic origin of SpW. For example, pharmacological manipulation which selectively boosts GABAB receptor-mediated inhibition robustly generates SpW (Snead, 1991). In addition, manipulation of nicotinic acetylcholine receptors also reveals a role in SpW generation. Nicotinic receptor agonists reduce or abolish on-going SpW generation in a rat genetic model of absence seizures (Danober et al., 1993) and the nicotinic receptor non-competitive antagonist d-tubocurarine generates SpW de novo when injected intracerebroventricularly (Dajas et al., 1983). Here we use an isolated association neocortex slice preparation, in conjunction with in vivo recordings and computational modelling to address the mechanisms behind SpW discharges.

We highlight that SpW discharges are a neocortical phenomenon which can be generated both in vivo and in isolated parietal cortex. We also demonstrate that SpW activity can be generated through the antagonism of nicotinic receptors, serotonergic receptors or a combination of the two. Furthermore we highlight specific interneurons involved in the generation of SpW activity, which are peptidergic by nature. The results suggest a mechanism of SpW generation where a 'double' disinhibition in superficial neocortical layers plays a major role. We propose that this disinhibition, when driven by delta wave activity, generates a hyper-excitable neocortex, resulting in the characteristic spike and wave discharge pattern.

Dajas et al. *Exp Neurol*. 1983. 79: 1: 160-7.

Danober et al. *Eur. J. Pharmacol*. 1993. 234: 2: 263-268.

Snead. *Neuropharmacology*. 1991. 30: 2: 161-167. Abstract

# Are GABAergic interneurons key perpetrators in the development of epilepsy in mitochondrial disease?

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Mitochondrial disease is an important cause of epilepsy, particularly in patients who harbour mitochondrial DNA point mutations, m.3243A>G and m.8344A>G, and defects in the nuclear-encoded polymerase  $\gamma$ . In these patients, epilepsy might be the defining feature of disease, such as myoclonus in MERRF, or present on a background of neurological deficits. Recent evidence from the UK MRC Mitochondrial Disease Patient Cohort identifies epilepsy in at least 20% of patients; specifically affecting 26% and 70% of patients with m3243A>G or m8344A>G mutations, respectively. This highlights that epilepsy is major contributor to disease burden. Despite the high prevalence and severity of epilepsy in mitochondrial disease, we know very little about the mechanism of how mtDNA defects contribute to neuronal dysfunction and lead to an epileptic event.

We performed an extensive neuropathological investigation of nineteen patients with clinically and genetically confirmed mitochondrial disease to dissect out the mechanisms contributing to the development of epilepsy. Since previous studies have suggested a specific vulnerability of interneurons to mitochondrial respiratory chain impairment, particularly complex I, we focused our attention on the GABAergic interneuron populations in our patient tissues to determine whether there was a loss of inhibition due to impaired mitochondrial function.

To determine whether interneurons play a key role in the development of seizures, we developed a quantitative immunofluorescent method which demonstrates significant deficiency for complexes I and IV within GABAergic interneurons within the occipital and temporal cortex (brain regions frequently implicated in seizure activity). We also show that GABAergic interneuron density is reduced, while the density of pyramidal cells is maintained indicating that the balance of excitation to inhibition may have shifted to promote excitability.

Understanding the pathogenesis of epilepsy in patients with mitochondrial disease is crucial for improved clinical management and the development of novel targets for therapeutic intervention.

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## Abatement of epileptic spike-wave discharges through single pulse stimulation

**Peter Taylor,**

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Active brain stimulation to abate epileptic seizures has shown mixed success. In spike-wave (SW) seizures, where the seizure and background state were proposed to coexist, single-pulse stimulations have been suggested to be able to terminate the seizure prematurely. However, several factors can impact success in such a bistable setting. The factors contributing to this have not been fully investigated on a theoretical and mechanistic basis. Our aim is to elucidate mechanisms that influence the success of single-pulse stimulation in noise-induced SW seizures.

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# The effect of parvalbumin expressing interneurons on the propagation speed of epileptic seizures in vitro

**Chris Thornton**

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In vitro models have shown that parvalbumin expressing fast spiking (Pv-FS) interneurons provide the bulk of the inhibitory barrages that restrain the propagation of epileptic seizures. We sought to investigate the effect Pv-FS interneurons have on the propagation speed of the ictal wavefront. This has been done by comparing speeds in rat brain slices that have been perfused with sucrose ACSF during preparation, to preserve Pv-FS interneurons, with those that have not. Seizures were induced by washing Mg<sup>2+</sup> ions out of the media bathing the slice. Electrophysiology recordings were performed using a 16 channel multielectrode array and recordings analysed using a custom made program which measures the wavefront based on the spike count. Of the 30 perfused and 12 non-perfused rat slices tested, 5 perfused and 10 non-perfused produced seizures. Of these only 3 perfused and 4 non-perfused slices produced seizure recordings appropriate for analysis. The results from these showed average propagation speeds of 91 mm/s for non-perfused and 64 mm/s for perfused, however the difference was not statistically significant ( $p = 0.14$ ).

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# Dynamic mechanisms of neocortical focal seizure onset

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Recent experimental and clinical studies have provided diverse insight into the mechanisms of human focal seizure initiation and propagation. Often these findings exist at different scales of observation, and are not reconciled into a common understanding. Here we develop a new, multiscale mathematical model of cortical electric activity with realistic mesoscopic connectivity. Relating the model dynamics to experimental and clinical findings leads us to propose three classes of dynamical mechanisms for the onset of focal seizures in a unified framework. These three classes are: (i) globally induced focal seizures; (ii) globally supported focal seizures; (iii) locally induced focal seizures. Using model simulations we illustrate these onset mechanisms and show how the three classes can be distinguished. Specifically, we find that although all focal seizures typically appear to arise from localised tissue, the mechanisms of onset could be due to either localised processes or processes on a larger spatial scale. We conclude that although focal seizures might have different patient-specific aetiologies and electrographic signatures, our model suggests that dynamically they can still be classified in a clinically useful way. Additionally, this novel classification according to the dynamical mechanisms is able to resolve some of the previously conflicting experimental and clinical findings.

# Assessing seizure susceptibility using visual psychophysical tests

Partow Yazdani

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Accurate seizure prediction algorithms would transform the lives of people living with epilepsy. They would also provide information about disease progression. One theory suggests that the timing of seizures reflects fluctuations in the quality of cortical inhibition. Non-invasive assays of cortical inhibition may, therefore, provide clinically useful information. Visual psychophysics assays have been used in schizophrenia (Tadin et al. *J.Neurosci*, 2006), autism (Koldewyn et al. *Brain*, 2010) and major depressive disorders (Golomb et al., *J.Neurosci.*, 2009). To investigate whether such tests may also be useful in assessing seizure risk, we investigated the performance in different visual cortical inhibition assays and whether this co-varied between tasks.

We have recruited 153 volunteer control subjects with no history of neurological disorder and 39 patients with clinically confirmed diagnosis of epilepsy. The control subjects ranged in age from 17.3 to 69.0 (mean 36.6 +/- 17.3years); patients range, 17.0 to 82.3, (mean 42.7 +/- 18.6 years). Some patients were tested soon after diagnosis before anti-epileptic medication was started. Others had long standing epilepsy, and medication was maintained. The duration of epilepsy ranged from a few months to 34 years.

Two different stimulus paradigms were used: (1) a two-choice motion discrimination paradigm (Tadin et al, 2003; left-right choice of movement of sinusoidal gratings“(Gabor patches”); small versus large stimuli, at either high / low contrast) and (2) a four-choice, stationary annulus paradigm (Serrano-Pedraza et al, 2012; identification of location of an oriented sinusoidal, orthogonal or parallel to a background sinusoidal display; high and low contrast). Duration thresholds were derived from fits of psychometric functions, and used to derive surround suppression indices (SSIs) for the two tests. There was a large variance in these measures, but further analysis suggested a clear difference between the two different test paradigms. For instance, age was an important confounding variable in the Tadin SSI, showing a highly significant reduction with increasing age in all groups, but not for the annulus SSI. Also, the Tadin and annulus SSIs were not correlated across populations, or over repeated measures within single individuals. We will also present analyses examining the longitudinal variance in individual patients and control subjects.

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