Human brain slices for epilepsy research: pitfalls, solutions and future challenges.

Roland S.G. Jones[1], Anderson Brito da Silva[2], Roger G. Whittaker[2] Gavin L. Woodhall[3], and Mark O. Cunningham[2]

1) Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom

2) Institute of Neuroscience, The Medical School, Framlington Place, Newcastle University, Newcastle upon Tyne, UK

3) School of Life and Health Sciences, Aston University, Aston Triangle, Birmingam, B4 7ET, UK

Address for correspondence: *Mark O. Cunningham*

Institute of Neuroscience

The Medical School

Framlington Place

Newcastle University,

Newcastle upon Tyne

NE2 4HH, UK

Tel: +44 191 2088935

Abstract

Increasingly, neuroscientists are taking the opportunity to use live human tissue obtained from elective neurosurgical procedures for electrophysiological studies *in vitro*. Access to this valuable resource permits unique studies into the network dynamics that contribute to the generation of pathological electrical activity in the human epileptic brain. Whilst this approach has provided insights into the mechanistic features of electrophysiological patterns associated with human epilepsy, it is not without technical and methodological challenges. This review outlines the main difficulties associated with working with epileptic human brain slices from the point of collection, through the stages of preparation, storage and recording. Moreover, it outlines the limitations, in terms of the nature of epileptic activity that can be observed in such tissue, in particular, the rarity of spontaneous ictal discharges, We discuss manipulations that can be utilised to induce such activity. In addition to discussing conventional electrophysiological techniques that are routinely employed in epileptic human brain slices, we review how imaging and multielectrode array recordings could provide novel insights into the network dynamics of human epileptogenesis. Acute studies in human brain slices are ultimately limited by the lifetime of the tissue so overcoming this issue provides increased opportunity for information gain. We review the literature with respect to organotypic culture techniques that may hold the key to prolonging the viability of this material. A combination of long-term culture techniques, viral transduction approaches and electrophysiology in human brain slices promotes the possibility of large scale monitoring and manipulation of neuronal activity in epileptic microcircuits.

Introduction

The use of animal models has been at the forefront of basic research in epilepsy. In particular, *in vitro* brain slice preparations from rodents have provided insights into the pathophysiology of epilepsy by the use of electrophysiological, optical imaging, molecular and cell biology techniques. Nonetheless, as revealing as 40 years of *in vitro* brain slice preclinical research have been, many fundamental neurobiological issues surrounding epilepsy remain. From a translational perspective, an evaluation of the clinical relevance of *in vitro* rodent models of epilepsy is of paramount important if shortcomings in current treatment approaches are to be overcome. There is no doubt that a best alternative to animal models would be to conduct basic functional and mechanistic studies in a completely homologous 'model' i.e. living human brain tissue. This is becoming increasingly possible using tissue resected during neurosurgery for refractory epilepsy. As noted, many human epilepsies are refractory to current pharmacological intervention, leaving resective surgery to remove the seizure focus as the most viable alternative treatment. Tissue excised during this process is often discarded, but, with a willing collaborative effort between clinicians and basic scientists, it can be retained for *in vitro* functional physiological investigation.

That it is not used more widely is due to a combination of issues: it requires a very close and cooperative collaboration between an interested and motivated surgical *team* (neurophysiologists, neurologists, neurosurgeons) and basic scientists; moral and ethical permissions are complex and require careful planning and operation; close proximity between operating theatre and research laboratory is highly desirable, to maintain viability of the live tissue once it is excised; tissue is often available on a sporadic basis and often at short-notice; the origin and orientation of the tissue is

often different from sample to sample depending on seizure focus; limited availability and sample variability mean that accumulation of sufficient observations is often slow. Another major impediment cited by many is the lack of adequate control tissue since it is essentially the epileptic tissue that is being removed. However, this is compensated for, to some extent, by surgical resections usually extending beyond the focal region to ensure adequate removal of dysfunctional tissue, and/or removal of non-epileptic tissue from overlying regions to approach a deep tumour or lesion (see also Komlosi et al, 2012; Molnar et al, 2008; Szabadics et al, 2006). Indeed, at both Aston and Newcastle, non-epileptic control tissue samples are available as frequently as those from pathologically altered regions. However, if the issues are addressed appropriately then use of living tissue provides a priceless source of information concerning human epilepsy. As well as providing the most relevant information about the human clinical condition, this information can cross-validate and guide the production of better, more refined and realistic animal models.

Evidence has been emerging for some time that patients suffering from intractable epilepsy (in particular mesial temporal lobe epilepsy (mTLE)) would benefit from referral for surgery much earlier than is currently the case (deTisi et al., 2011; Engel et al., 2003, 2012; Wiebe et al., 2001) to improve the chance of a successful clinical outcome. An increased uptake of resective surgery for epilepsy should be viewed as a unique opportunity for neuroscientists to undertake *ex vivo* research on epileptic human tissue. However, it should be noted that patients undergoing surgery have established epilepsy, exhibit heterogeneous histories, and have been exposed to prolonged periods of various anti-epileptic drugs that have failed to provide adequate seizure control. Thus, tissue obtained from these patients is advantageous from the point of view of probing potential mechanisms underlying pharmacoresistance but

less so for gaining insight into the process of epileptogenesis in the human brain. Using approaches initially developed for rodent brain slices by McIlwain and others (Collingridge, 1995), the ability to prepare and maintain slices of epileptic human cortical tissue gives unprecedented access to human brain tissue for subsequent interrogation with a variety of tools. At the time of this publication, there have been approximately 110 novel papers reporting on the use of human brain slices *in vitro.* In 2006, the methodological approaches that can be applied to human brain slices to gain insight into epilepsy were extensively reviewed by Kohling and Avoli. Rather than re-review the literature up to 2006, the current article aims to provide an update on progress in this field that has followed in the ten years since the previous review (Kohling and Avoli, 2006), and discuss what the future may hold for experimental studies involving human epileptic brain slices.

1. Resection, collection and preparation of human epileptic material: critical steps for maintaining functional viability

The bulk of human brain tissue that is removed during elective neurosurgery is ultimately used for diagnostic purposes. Previously, this has meant that limited scientific information has been derived from this resource as standard histopathological stains are primarily used to examine such samples. However, in recent years more sophisticated molecular biological techniques have been employed to examine the contribution of epigenetics (Kobow and Blümcke, 2014; Miller-Delaney et al., 2014), gene expression (Beaumont et al., 2012; Rakhade et al., 2005) and regulation (Johnson et al., 2015) in human epilepsy. Whilst this is useful in providing a molecular basis for observed phenotypic variations in the pathological condition, it tells us very little regarding the functional changes that correspond to cellular, synaptic and network activity in the human epileptic brain. From a functional

perspective, epilepsy remains a disease of the brain that arises due to excessive neuronal activity. As such the use of electrophysiological techniques to study the condition either *in vivo* (e.g. EEG) or using brain slices *in vitro* (e.g. extracellular local field potential recordings) remains the 'gold standard' scientific approach.

In the same way that ensuring an optimized protocol is used for obtaining high-quality brain slices for animal studies, this also applies to human epileptic brain slices. An important aspect of this type of work is the ability to ensure that the neurosurgeon resects the tissue quickly and with minimal traumatic damage to the tissue. If the sample is physiologically compromised at this stage then conducting electrophysiological studies in slices prepared from a sub-optimal sample will be fruitless. This can be avoided through clear communication with the neurosurgical team with regard to the manner in which the tissue should be obtained, before embarking on such a programme of research

In the majority of epilepsy centres around the world, all patients selected for surgery will have undergone an exhaustive array of presurgical examinations that include, neuropsychological tests, surface EEG, intra-cranial electrocorticography (ECoG) (sub-dural grid electrodes and/or depth electrodes), neuroimaging (positron-emission tomography (PET) and magnetic resonance imaging (MRI)) and neurological assessment. As such, all these modalities will not only provide detailed anatomical information to the neurosurgeon on the location in the brain that contains the seizure onset zone, but also vital background information that can be correlated with functional studies in the excised tissue. Using the presurgical information, with the aid of a neuro-navigation system, the surgical team can ascertain the location of the aberrant region (neocortex or hippocampus) to be removed. A craniotomy is performed under general anaesthesia and, following the opening of the dura, the

pathological cortex can be removed by subpial resection. It is important that the white matter is cut to ensure that the sample is resected "en bloc" as the white matter and vasculature on the pial surface serve as important landmarks critical for orientation of the sample for subsequent slice preparation. Following successful resection it is imperative that the neurosurgeon manipulates the tissue bloc with the utmost care to ensure maintenance of functional viability in the slices to be obtained. Using forceps, the sample should be picked up by a corner of the white matter, taking care not to compress the gray matter, and placed/dropped into a small beaker containing artificial cerebrospinal fluid (aCSF).

For electrophysiological studies the tissue should be sliced with a vibratome into thinner sections (400-450 μm) as quickly as possible. However, it is likely that the operating theatre may be located at a distance from the laboratory, and the option of preparing slices in proximity to theatre may not be possible. It is therefore essential that the collection aCSF remains chilled and continually carbogenated (95% O_2/5% CO_2) to ensure that the tissue does not become anoxic. Portable chambers for the transport of prepared slices have been previously described (Hsiao et al., 2015; Kohling et al., 1996). It is also relatively straightforward to construct a system in which an icebox and a small (34L) disposable carbogen cylinder can be placed within a human tissue transportation box. In our experience, using this system we have easily kept human cortical samples healthy for periods up to 30 minutes, as verified by subsequent viable electrophysiological recordings.

The state and composition of the aCSF used for tissue collection and preparation of human slices is also of great importance. Many early reports using human tissue used an ice cold (4˚C) standard aCSF solution that included (in mM) NaCl (124-129), KCl (2-4), $CaCl_2$ (1.6-2.4), $MgSO_4$ (1.3-2), NaH_2PO_4 (1.25), $NaHCO_3$ (21-26) and glucose

(10-12.5). This solution is largely derived from animal slice work, and studies (Aghajanian and Rasmussen, 1989) have suggested that the this form of aCSF leads to a significant degree of neuronal death in human slices and, in particular, causes a dramatic loss of GABAergic mediated inhibition (Kuenzi et al., 2000). To overcome this problem, many groups have utilised a modified aCSF solution to confer neuroprotective properties. This can be achieved in a number of ways. The replacement of sodium with sucrose prevents neuronal toxicity mediated by glutamate (Alvarado-Rojas et al., 2015; Cohen et al., 2002; Cunningham et al., 2012; Huberfeld et al., 2007; Huberfeld et al., 2011; Pallud et al 2014; Roopun et al., 2010; Simon et al., 2014; Wittner et al., 2009). Supplementation of the aCSF with various anti-oxidants (ascorbic acid, alpha-tocopherol (Wozny et al., 2003), n-acetyl cysteine, uric acid and pyruvate) will limit damaging redox reactions occurring at ionic and synaptic receptors. The addition of ascorbic acid (Brahma et al., 2000) and taurine (Kreisman and Olson, 2003) also aids volume regulation and thus limits cell swelling and subsequent neuronal death. A simultaneous reduction of the Mg^{2+} and elevation of the Ca^{2+} extracellular concentrations (Alvarado-Rojas et al., 2015; Cohen et al., 2002; Huberfeld et al., 2007; Huberfeld et al., 2011; Pallud et al 2014; Wittner et al., 2009) can act to diminish overall levels of excitation during the cutting process. In our laboratories we have developed a combination of these approaches to ensure tissue protection and use a modified collection and cutting aCSF solution for human cortical tissue that contains (in mM) sucrose (171), KCl (2.5), $MgCl_2$ (10), $NaHCO_3$ (25), 1.25 NaH_2PO_4, glucose (10), $CaCl_2$ (0.5), N-acetyl cysteine (1), taurine (1) and pyruvate (20). The following anti-inflammatory and antioxidative agents are also added to the solution (final concentration); indomethacin (45 μM), ascorbic acid (300 μM) and uric acid (400 μM) (Prokic et al., 2015; Jones, Cunningham and Woodhall, unpublished

observations). In addition, neuroprotective agents such as Brilliant Blue G and ketamine can be added to prevent neuronal death via overactivation of P_2X_7 (Arbeloa et al., 2012) and NMDA (Aitken et al., 1995; Lench et al., 2015; Lipton et al., 1995) receptors, respectively.

There has been a considerable degree of advancement made in the quality of vibratomes used for cutting brain slices, with a particular emphasis on the reduction of z-axis deflection to reduce damage. The ability to minimise the extent of vertical vibration (Geiger et al., 2002) to levels of <1 μm has been shown, with subsequent light and electron microscopy analysis, to greatly enhance the preservation of cellular elements, dendritic processes and presynaptic terminals in cut brain slices. For example, this has permitted electrophysiologists to conduct patch-clamp recordings directly from rodent and murine mossy fibre presynaptic terminal (Bischofberger et al., 2006). It is now well accepted that the improved preservation of superficial tissue layers of slices by minimized z-axis deflection acts to greatly improve the overall viability and condition of the *in vitro* brain slice preparation.

The prepared human brain slices are transferred for storage to a holding chamber in which they are continually carbogenated and maintained at room temperature in aCSF prepared for the purposes of electrophysiological recordings. In general, most laboratories using human brain slices prepare these with a thickness in the region of 400-450 μm. The ability to record network activity is likely to be comprised by preparing thinner slices. The holding and recording aCSF solution used in a number of published studies varies between the following values (in mM) NaCl (124-129), KCl (2-4), $CaCl_2$ (1.6-2.4), $MgSO_4$ (1.3-2), NaH_2PO_4 (1.25), $NaHCO_3$ (21-26) and glucose (10-12.5). In these holding conditions, it is our experience that slices are

viable for periods of time that exceed those observed in rodent brain slices. Indeed, anecdotal evidence would suggest that the under the same storage conditions the relative viability of human brain slices is greater than that seen in rodent brain slices (Gabor Tamas, personal communication). Whilst unreported by other groups, our experience has been that electrophysiological signals (spontaneous LFP; evoked field EPSPs) are still present in human brain slices up to 20 hours post-resection of the sample. It is interesting to speculate whether epileptic human tissue slices can be kept viable for longer periods of time. In a later section of this review we will explore how organotypic culture techniques could be used to prolong the functionality of tissue for longer periods of time to maximise usage. It is also interesting that recent work with rodent tissue has suggested that the emergence of bacterial infection significantly reduces the viability of acute slices over periods of 6-12 hours following slice preparation. To overcome this problem, Buskila et al. (2014) have developed a brain slice storage system (the Braincubator) which maintains slices at 15-16˚C and constantly filters the aCSF through an ultra violet C (UVC) chamber. This reduces the capacity for the growth conditions for mesophilic gram-negative bacteria through a combination of irradiation and hypothermia. The authors demonstrated, using whole cell patch clamp recordings, that the intrinsic and synaptic properties of neocortical pyramidal cells were viable and unchanged for up to 36 hours post-slice preparation. The usefulness of such a slice storage system for prolonging acute recording sessions with epileptic human brain slices is worth consideration and further exploration.

2. Choosing the optimal in vitro environment for human brain slice studies.

Brain slice experiments have traditionally used interface or submerged slice recording chambers. Both have advantages and disadvantages dependent on the experimental questions to be addressed. Is there any particular advantage to either for human brain

slice experiments?

The interface chamber ("Oslo" or "Haas" chamber) maintains brain slices between an interface of carbogenated (95% O_2/5% CO_2) aCSF and a humidified carbogenated atmosphere and ensures that relatively thick (400-500 μm) sections can be kept in a good physiological condition for prolonged periods (10-20 hours). Disadvantages of this approach are the inability to use visualisation (for e.g. IR-DIC microscopy) to target neurons for single cell electrophysiological recording purposes, and relatively low perfusion rates (1-1.4 $mlmin^{-1)}$) mean that the time course for equilibration of bath applied drugs can be prolonged (up to 30-40 minutes). However, a major advantage over submerged slices is an increased ability to record neuronal *network* activity. This is likely to be due to differences in the degree of oxygenation that occurs in submerged chambers. Submerged chambers essentially only provide superfusion on one (top) side of the slice since the other (bottom) side rests on the base of the chamber. With interface slices oxygen is provided via the flow of aCSF occurs across the bottom of the slice *and* via the gaseous environment above the slice. Thus, submerged slices may suffer altered distribution of not only oxygen, but also additional nutrients and chemicals. It is now generally accepted that neuronal network activity observed in interface slices is difficult to replicate in submerged slice chambers. A common feature of both pathological and physiological network oscillations is a requirement for high levels of neuronal activity. Evidence from PET scans in human epilepsy patients (Alavi et al., 2011; Pan et al., 2008) and metabolic recordings (fluorescent FAD + or NADH) in animal models of epilepsy (Dora, 1983; Heinemann et al., 2002a, 2002b; Ivanov and Zilberter, 2011; Ivanov et al., 2015; Kann et al., 2005; Kovacs et al., 2001; O'Connor et al., 1972, Schuchmann et al., 1999; Zhao et al., 2011) confirm the metabolic demands of interictal and ictal

electrophysiological behaviour. The most profound differences in experiments using interface versus submerged chambers have been reported in those where ensuring adequate levels of neuronal activity (for e.g. neuronal network gamma frequency oscillations) are required (Gloveli et al., 2005; Hajos et al., 2009). As such, the interface chamber offers a more 'physiological-like' environment for electrophysiological studies in human epileptic brain slices, particularly when assessing the cellular and synaptic features of pathological network are the primary focus. Many recent papers (Cohen et al., 2002; Cunningham et al., 2012; Huberfeld et al., 2007; Huberfeld et al., 2011; Pallud et al 2014; Roopun et al., 2010; Simon et al., 2014; Wittner et al., 2009) have used this form of recording chamber when conducting in vitro electrophysiology studies on resected human tissue *in vitro*.

It may be possible to overcame most of the some of the disadvantages of either submerged or interface chambers by employing recent approaches developed for rodent slices to human tissue. Some laboratories have circumvented the oxygenation problem in submerged slices by elevating the perfusion rate (in some cases doubling or tripling that used in interface chambers). Perhaps a better approach is a dual perfusion submerged chamber where laminar flow of aCSF is directed above and below the slices (Hajos et al., 2009; Spampanato and Mody, 2007). Both modifications ultimately lead to increased oxygenation, but increased flow rates introduce the additional issue of reduced mechanical stability that could be detrimental for long-term recordings. That network activity can be sustained for long periods of time using a dual perfusion style chamber with low flow rates (Hajos et al., 2004, 2009) provides a scenario where long-term electrophysiological recordings in human slices are viable. To overcome the difficulty of visualizing neurones in interface recordings, Aivar et al. (2014) have also developed an interface chamber

that can be adapted to an upright microscope providing access for visualised patch clamp recordings using a long working distance objective (x40). Using this approach, allowed simultaneous patch clamp of pyramidal cells and extracellular recording of physiological- and pathological-like high frequency network oscillations (HFOs) in the rodent hippocampal slices.

3. Epileptic human brain tissue: what it is good for?

The advantage of using human epileptic brain slices is that a number of techniques and manipulations that would be not be possible *in vivo* can be applied to the tissue *ex vivo*. In particular, the use of electrophysiological techniques permits mechanistic insight into signals that underlie electrophysiological signatures in intact patient recordings (for e.g. LFP recordings relevant to EEG markers). A number of publications in human brain tissue have observed spontaneous events that closely resemble interictal spikes and associated HFOs. Understanding the mechanisms that underlie such pathological HFOs is of paramount importance. This activity is strongly associated with epileptogenic networks in patients with mTLE. Recent work has suggested that pathological HFOs may represent a unique biomarker that could aid the localisation of brain regions to be resected during epilepsy surgery (see Staba et al., 2014). Moreover, HFOs associated with interictal events are intimately correlated with seizure onset zone in drug resistant epilepsy patients (Akiyama et al., 2011; Cho et al., 2012; Haegelen et al., 2013; Jacobs et al., 2010; Okanishi et al., 2014; Wu et al., 2010). Given the profound association between HFOs and seizure generation, a better understanding of the neuronal behaviors that generate HFO will provide information that could be used to pharmacologically target HFOs and potentially overcome the issue of drug resistant epilepsy.

In this respect, several laboratories have exploited the use of epileptic human brain slices to study the mechanistic nature of HFOs. First reported by Kohling et al. (1998) in epileptic human neocortical slices, HFOs are strongly correlated with interictal sharp wave events. This initial study focused on the cellular and synaptic features of the sharp wave events concluding that they are mediated primarily through non-NMDA and GABAergic mediated synaptic activity. More recently, Roopun et al. (2010) recorded spontaneous HFOs (100-500 Hz) in association with interictal sharp waves in human neocortical slices obtained from mTLE patients. In combination with a computational network model, this work demonstrated a weak correlation between chemical synaptic conductances and the HFO. They also showed that blockade of gap junctions abolished HFOs whereas the application of a $GABA_A$ receptor antagonist had no effect. In a subsequent paper (Simon et al., 2014), using the same dataset, HFOs were separated into ripple (<200 Hz) and fast ripple (>200 Hz) components. Using multi-electrode UTAH array recordings it was shown that both forms of activity were predominant in the superficial (II/III) layers of the neocortex. Combined extracellular recordings and intracellular measurements of principal cell membrane potential revealed that, whilst excitatory postsynaptic potentials (EPSPs) occur during fast ripple HFOs, there was no correlation between the chemical synaptic activity and the network HFOs. This finding supports the notion that human fast ripple HFOs are generated by a gap junction coupled plexus of axons (Cunningham et al., 2012; Roopun et al., 2010; Simon et al., 2014; Traub et al., 2011, 2014)

HFOs have also been detected in subicular slices obtained from mTLE patients. Alvarado-Rojas et al. (2015) used concurrent extracellular local field potential (LFP) and intra- or juxtacellular recordings, to study cellular and synaptic components underlying spontaneous and induced HFOs (150-250 Hz) in the ripple frequency

range. Spontaneous HFOs associated with interictal discharges were correlated with sparse firing and, in the majority of cases, inhibitory postsynaptic potentials (IPSPs). In contrast, HFOs associated with preictal discharges (elicited by using a pro-convulsant aCSF - reduced $[Mg^{2+}]_o$ and elevated $[K^+]_o$) were associated with principal neuron burst firing and a robust depolarization of the membrane potential. Intriguingly, the authors report that the faster component of HFOs (i.e. fast ripples <200 Hz) were observed. *In vivo* studies in mTLE patients have demonstrated a clear association between fast ripple HFOs in the range of 200-500 Hz with the seizure onset zone, as compared to ripple HFO (<200 Hz). These finding suggests that fast ripple HFOs are a strong biomarker of disease activity. Given the importance of the faster component of HFOs for epileptogenicity is it surprising that an assessment of the neuronal contributions to this activity were not forthcoming in this paper.

3. Spontaneous epileptic activity in human epileptic brain slices

One expectation from recordings in living human brain slices would be to observe spontaneous epileptic activity. However, in the majority of studies reviewed by Kohling and Avoli (2006), pro-convulsant agents (GABA receptor antagonists; 4-aminopyridine), pro-excitable alterations in the aCSF (e.g. increased $[Na^+]_o$ or $[K^+]_o$; lowered $[Mg^{2+}]_o$), or combinations of both, were required to routinely observe interictal events in brain slices from epileptic patients. It has also been reported that ictal activity is elicited by 4-AP application in a subpopulation of samples from patients with focal cortical dysplasia (Avoli et al., 2003). However, over the last ten years, an increasing number of laboratories have shown that pro-epileptic manipulations are not required and have reported that spontaneous network pathological activity can be recorded under 'resting' conditions (Cunningham et al., 2012; Huberfeld et al., 2007; Roopun et al., 2010; Simon et al., 2014; Wittner et al.,

2009) *in vitro*. It is not clear why there is an increasing observation of spontaneous activity in human epileptic brain slices but it seems likely that improvements in technical aspects of slice preparation and preservation have played a role. Most laboratories now attempt to limit neuronal damage during slice preparation, with modified aCSF (e.g. addition of antioxidants, increased magnesium concentration, replacement of sodium ions with sucrose). These factors are likely to ensure that complex pathological network events are preserved in human epileptic brain slices, as a result of general improvements in slice viability. Whilst technical aspects will be critical for preserving the viability of the slices, additional factors will still contribute to inconsistency in the observation of pathological network activity; these may include variations in surgical technique used to remove samples across different centres, variations in the angle used during slice preparation, variations in the regions of the brain the sample has been obtained from, and time taken for transportation/slicing of the sample.

The observation of spontaneous interictal activity in studies principally emanating from Richard Miles's group in Paris (Cohen et al., 2002; Huberfeld et al., 2007; Huberfeld et al., 2011; Pallud et al 2014; Wittner et al., 2009), have demonstrated remarkable consistency. In a series of papers this group have shown that the subiculum and associated parahippocampal structures are capable of generating complex interictal events *in vitro*. One reason for the consistency of these findings may be due to the preservation of the network of limbic structures and the methods by which they are removed. Unlike the neocortex, which can be problematic with respect to orientation for slice preparation, the hippocampus exhibits a well-delineated structure that permits visual orientation of the samples for slicing. Additionally, the surgical method (anterior temporal lobectomy) by which the intact hippocampus and

associated parahippocampal structures are removed ensures ease of orientation of the sample prior to the cutting of the slices. Despite the success of this group and others (Cohen et al., 2002; Cunningham et al., 2012; Huberfeld et al., 2007; Huberfeld et al., 2011; Pallud et al 2014; Roopun et al., 2010; Simon et al., 2014; Wittner et al., 2009;) to record spontaneous interictal activity *in vitro,* there is only a single published report in which spontaneous ictal-like events have been observed (Cunningham et al., 2012). Moreover, as alluded to by Heinemann and Staley (2014) it is also extremely difficult to induce ictal-like events in human hippocampal and neocortical tissue. Recent work has resorted to the strategy of manipulating ionic concentrations in the aCSF (reduced $[Mg^{2+}]_o$ and elevated $[K^+]_o$ in subiculum; Huberfeld et al., 2011) or a combination of ionic and pharmacological manipulation (elevated $[K^+]_o$ and bicuculline in temporal neocortex; Heinemann and Staley, 2014) to elicit seizure like events. However, preliminary data from the Woodhall laboratory demonstrates that ictal events can be observed in human temporal cortical slices obtained from a paediatric patient with a Type II focal cortical dysplasia (Fig. 1). These ictal events can be reliably evoked by bath application of kainate (100 nM) and carbachol (5 μM) and persist for many hours in such conditions. It is worth considering how these findings translate from rodent and human brain slices. Is there equivalence between epileptiform activity induced in human slices by drugs and/or altered ionic environments to that observed in rodent brain slices using similar acute means? Surprisingly, seizure induction (using 4-AP) is known to be more readily achievable in control rodent slices compared to slices obtained from animals with epilepsy (kainate model; Zahn et al., 2008). This finding would certainly align with *in vitro* observations concerning the difficulty of inducing seizure activity in specimens obtained from drug resistant epilepsy patients. However, preliminary findings (M.O. Cunningham) has shown that when slices obtained from

non-epileptic comparison samples (peritumoural tissue) are exposed to reduced $[Mg^{2+}]_o$ and elevated $[K^+]_o$ neither interictal nor ictal-like events are observed. This may suggest that, human cortical tissue that can be considered more "normal" than epileptic tissue is not as susceptible to generate epileptiform activity following acute manipulation as control rodent tissue. These findings may be interpreted as a species difference in terms of the threshold for seizure generation, with human cortical tissue more resistant to seizure generation compared to the rodent brain. Further work should aim to understand the contrast between seizure threshold in the rodent and human brain and the mechanistic features that underlie such differences.

-insert Figure 1 here-

This failure to consistently observe spontaneous seizure events may be frustrating for those wanting to study the *de novo* genesis of seizures in human cortical microcircuits, but the fact that it is difficult to capture such activity in human epileptic slices *in vitro* may, in itself, be a revealing observation. What might explain this scenario? Early reports (Telfeian et al., 1999) illustrated a mismatch between events recorded using perioperative ECoG and neuronal measurements of hyperexcitability (i.e. burst firing in response to electrical stimulation) in neocortical slices obtained from chronic refractory epilepsy patients. This has more recently been further explored by Theyel et al. (2010), who used flavoprotein autofluorescene (FA) imaging to evaluate slice connectivity following electrical stimulation. FA imaging is dependent on alterations in mitochondrial flavoproteins in the electron transport chain and its activation is reliant upon postsynaptic depolarization (Husson et al., 2007). For this reason, it provide a non-invasive surrogate marker of network connectivity. Using epileptic human neocortical slices cut at different angles, this work demonstrated a high degree of variability of both evoked and spontaneous network activity. This

finding potentially supports the view that inconsistencies with respect to observing spontaneous interictal and ictal-like discharges may be related to a low level of connectivity dependent on the angle at which the slices are cut.

Recent findings from combined ECoG and microelectrode recording in epilepsy patients suggest that pathological seizure activity may arise from small patches of neocortex (Schevon et al., 2008, 2012; Stead et al., 2010;). Using clinical macroelectrodes and research microelectrodes *in vivo*, other groups have described localized electrographic activity termed, 'micro-seizures' (Truccolo et al., 2011, 2014). Based on these observations, it seems likely that, unless the resected sample is obtained from directly within the seizure onset zone or very close to it, the chances of recording spontaneous seizure activity in the slices will be low. Indeed, there is anatomical and gene expression evidence that supports the notion of microlesions in human epileptic cortex. Dachet et al. (2014) obtained resected cortical tissue identified as either high or low-spiking (interictal discharges recorded using long-term ECoG) and used transcriptomic techniques to produce a cellular interactome, i.e. a cell specific set of molecular interactions. The prediction of the cellular interactome suggested an increase in vascularity and microglia and the presence of microlesions in the deep layers of the cortex. Using histological methods, in high spiking samples, they demonstrated a loss of NeuN staining, a reduction in MAP2 and a local infiltration of microglia in the deep layers. It is worth considering then, that localized patches of epileptic activity observed *in vivo* and *in vitro* (Simon et al., 2014) may be the functional correlate of such anatomical microlesions. Further studies should consider a combination of *in vitro* electrophysiological spatial mapping with *post-hoc* neuroanatomical examination.

It is likely then the difficulty of observing spontaneous ictal activity may be linked to

combination of variation in cortical connectivity and the presence of microlesions in the epileptic cortex, and it may be the exception rather than the rule that spontaneous activity is seen in human slices *in vitro*

4. Monitoring small/large scale network dynamics in human brain slices in vitro

To understand how human epileptic circuits generate the pathological activity described above it is necessary to record how events occurring on a large scale (i.e. LFP) correlate with the small scale activity of local neuronal populations (i.e. single cells - neurons and/or glia). There are a variety of ways in which this can be achieved. Experimental work using the magnesium free model of acute seizures in rodent brain slices has demonstrated that fast calcium imaging of interictal and ictal events in combination with whole cell patch clamp recordings can be achieved (Trevelyan et al., 2006, 2007a, 2007b; Sabolek et al., 2012; Schevon et al., 2012). This approach has provided insight with respect to correlating the behavior of individual neurons to the propagation of ictal events in rodent neocortical networks and for examining the relative contributions of pyramical cells and interneuron to seizure generation (Lillis et al., 2012). An alternative approach is to utilise multi-electrode arrays that allow the extraction of both LFP data and single unit recordings. Conventional (60 channel) planar arrays of microelectrodes have been used to look at initiation and propagation of activity in rodent slices using the magnesium-free model (Hill et al., 2010) and the 4-AP/bicuculline model (Chang et al., 2011; Gonzalez-Sulser et al., 2011). More recently, an active-pixel sensor MEA chip system with 4096 electrodes has been developed and used in combination with voltage sensitive dye imaging to examine 4-AP induced epileptic events in rodent entorhinal-hippocampal slices (Ferrea et al., 2012). This high-resolution high-density system permits high sampling (>5 kHz) rates on an array covering an area of 2.6 x 2.6mm^2 with an electrode pitch of 21μm.

Using the approaches outlined above, initial attempts have been made to examine coordinated interactions between large groups of astrocytes and neurons in the human epileptic circuits. Navarette et al. (2013) have reported the use of Fluo-4-AM and Sulforhodamine 101 to image astrocytes in human hippocampus and cortex resected from mTLE patients. Using this approach, concurrently with patch clamp recordings, they demonstrated that human astrocytes are capable of releasing glutamate that acts on neuronal NMDA receptors to regulate excitability. The combination of cellular electrophysiology and fast calcium imaging approaches, successfully used in acute rodent epilepsy studies, unfortunately, have not yet been attempted in human epileptic tissue.

However, there is increasing use of MEA recordings in human epileptic brain slices. Hsiao et al. (2012) employed a planar 60-channel MEA system, to make recordings from hippocampal slices obtained from patients with mTLE. They confirmed the viability of the preparation by eliciting extracellular field responses in the dentate gryus to electrical stimulation of the perforant path. A combination of high $[K^+]_o$ (8mM), low $[Mg^{2+}]_o$ (0.25mM) and 4-AP induced the emergence of asynchronous interictal activity in the dentate gryus, CA1 and subiculum. Whilst pathological activity was recorded in these various regions no attempts were made to examine features such as the initiation and/or propagation of the epileptic events. Dossi et al. (2014) have also used a planar MEA approach in epileptic human cortical slices. Using a zero $[Mg^{2+}]_o$ and high $[K^+]_o$ (6mM) aCSF they were able to transform the slices from a state where only spontaneous interictal activity occurred to one where ictal events were also seen. Such evoked ictal activity was observed in samples from 75% of patients and in 66.7% of the slices. Both these studies demonstrate the ease with which network LFP recordings can be reliably obtained from epileptic human

brain slices. Dossi et al. (2015) also report that high pass filtering of the LFP signal allows the extraction of multi-unit signals. In order to relate the network events to the small scale, i.e., the activity of individual neurons, it is necessary that multiple single extracellular action potentials (units) can be detected. This is now possible with the advent of silicon probes with multiple recording sites in close proximity on the probe shanks. The advantage of this approach is that it permits precise temporal recordings of isolated signals from individual neurons in tandem with population network activity. Using silicon probes, preliminary data from our laboratory (see Fig. 2) has demonstrated the ability to record both extracellular ictal events (induced with modified ACSF) in the human neocortex and to extract individual signal units using principal component analysis.

-insert Figure 2 here-

This type of recording has allowed a detailed examination of the phase relationship of the spiking of single unit clusters during pathological HFOs (ripples and fast ripples) associated with the evoked epileptic activity. The ability to monitor large numbers of single units simultaneously with LFP/EEG signals of interictal and ictal discharges has been previously demonstrated using *in vivo* recordings in human epilepsy patients (Bower et al., 2012; Cash and Hochberg, 2015 Keller et al., 2010; Schevon et al., 2012; Truccolo et al., 2011). The advantages of this approach are clear; high-resolution temporal recordings from individual neurons in combination with network measurements in the epileptic brain in a clinical environment. However, from a scientific perspective the main disadvantage is an inability to extract information regarding the spatial resolution of neuronal activity across the cortical mantle. Ninety six channel Utah multielectrode arrays used *in vivo*, penetrate the cortex to depths of 1-1.5 mm, sampling neuronal activity predominantly from layers 3 and 4 of the

human neocortex. Using the same multielectrode arrays in human slices *in vitro* (Fig. 2) permits orientation of the array in a tangential manner and means that neural signals can be sampled across all cortical layers. Laminar recordings of rodent neocortical epileptiform activity in a variety of acute pharmacological models (4-AP, bicuculline, zero $[Mg^{2+}]_o$) have observed heterogeneity in terms of the source of events (Borbély et al., 2006). Using current source analysis techniques, 4-AP induced activity was shown to arise from layer V whereas bicuculline and magnesium free evoked events emanated in the superficial (II) and deep layers (IV-V) of the neocortex, respectively. However using voltage sensitive dye (VSD) imaging approaches, magnesium-free evoked epileptic activity was capable of arising from either superficial or deep layers of the rodent neocortex (Tsau et al., 1999). More recently a combination of LFP recordings and VSD imaging has revealed 4 distinct types of interictal events induced by pharmacological dis-inhibition in rodent cortical-hippocampal slices (Adams et al., 2015). One type of event was generated in the hippocampus but failed to propagate from this structure. For 2 of these cases, the events originated in the hippocampus and projected towards the primary auditory cortex via intermediate structures. A subtype of one of these events was observed to propagate back to the hippocampus. Finally, a form of interictal event was generated in the neocortex locally and was non-propagating. The local non-propagating cortical interictal event was arose in the superficial layers whereas interictal events projecting in from the hippocampus were initiated in the deep layers. CSD studies of interictal spikes in human cortex *in vivo* (Ulbert et al., 2004) and *in vitro* (Kohling et al., 1999) have demonstrated that layer V is the predominant generator of this pathological activity. Given the discordance regarding the elucidation of layer specific generation of interictal patterns of activity, the use of multielectrode arrays in human epileptic

brain slices will aid in clarifying this particular question and provide a mechanistic understanding of this characteristic feature of human epilepsy.

5. Organotypic culture techniques in human epileptic tissue

A major drawback to the use of living human tissue obtained through brain resection is the limited and often sporadic availability of tissue. We have been considering various ways of accelerating the progress of research using living human tissue. Although relatively robust if maintained in optimal conditions, slices produced from each sample have a limited acute viability of around 12-14 hours, meaning that much of the fresh tissue is not available for use in functional physiological studies. As a means of maximising the utility of valuable human tissue, one obvious approach would be to prolong its viable lifetime through organotypic culture. The use of organotypic culture has become an established experimental procedure to maintain lifetime and viability of animal tissue. Whilst such an approach in human tissue has received only sporadic attention, particularly in the last two decades, it provides an attractive possibility to maximise the use of human living brain slices.

Work in the 1940s demonstrated that brain tissue of foetal and new-born origin could be maintained in roller tube culture relatively easily for extended periods (e.g. Hogue, 1946, 1947). Early attempts to culture adult tissue were largely unsuccessful, but optimisation of culture conditions produced the forerunner of studies to produce organotypic human cultures in the early 1950s. Costero and Pomeroy (1951) made roller tube cultures of cortical explants from adult (and occasionally juvenile) human brains (samples approximately 1mm square) from patients undergoing surgery for intracranial tumours or relief of psychiatric disorders or intractable pain. In cultures maintained for up to 4 weeks, large pyramidal neurones seem to survive reasonably

well in this tissue, although they showed clearly morphological changes, and migrated only slowly from their point of origin. Smaller neurones seemed to undergo more rapid migration and were much more susceptible to degeneration and morphological change to a gliaform phenotype. Very similar studies were reported by Hogue in 1953 who made explant cultures of frontal, parietal and temporal cortical and cerebellum form patients ranging from 4-68 years old undergoing neurosurgical procedures. Both studies reported neurones surviving for many months, although technical limitations mean that the 'normality' of the tissue was very hard to assess.

Following these early studies there was little in the way of enthusiasm for developing organotypic human cultures until the early 1990s, when there was a renewed interest in the potential use of human foetal tissue from elective terminations to study neurodevelopment. For example, Lyman et al. (1991, 1992) established organotypic explants of both forebrain (14-21 weeks gestation) and spinal tissue (21-23 weeks), with monitored survival times of up to 12 weeks plus, and were able to follow neuronal migration and synaptogenesis. Letinic et al. (2001, 2002) maintained foetal (15-25 weeks) forebrain slices (300-350 μm thick) in organotypic culture for 48-72 hours. They were able to follow the migration of GABAergic neurones into the dorsal thalamus and neocortex using either slices containing the whole forebrain structure, or by use of co-culture of different regions. More recently, Javocevski et al. (2007) used fetal forebrain slices (interface method) (5-24 weeks) to examine the role of PSA-NCAM in development of myelination in over 5 days in culture. None of these studies make any comment on the level of preservation of 'normal' structure or the temporal viability of the cultured tissue. However, an even more recent study (Martinez et al., 2011) of foetal (7-18 weeks) forebrain slices (400 μm) used a Live/Dead assay (calcein and propidium iodide) to assess neuronal viability. Neuronal proliferation

was monitored with 5-ethynyl-2-deoxyuridine. The study concluded that, with optimisation of the culture conditions, good viability could be maintained for up to 28 days, with significant neuronal death only being observed from 21 days onwards.

There has been sporadic interest in the use of living postnatal human tissue for organotypic culture. Jung et al. (2002) developed a human organotypic brain slice model to study the migration of tumour cells in cortical tissue. They obtained excised temporal and frontal cortex from patients (presumably juvenile/adult) undergoing surgery for arteriovenous malformation, trauma, or epilepsy and made 1 mm thick slices held in interface-type wells (Stoppini et al., 1991) initially for 10 days in culture, before the insertion of tumour cells or tumour spheroids to monitor migration over subsequent weeks. Unfortunately they provide little detail or illustration of the structure or viability of the human slices, other than to say "We were able to maintain the anatomical structure of the brain for a 4- to 6-week period, but after that the cultured brain becomes necrotic. Organotypic culture of brain was well preserved histologically for a week…" and "the cytoarchitecture of the brain tissue remains viable and intact without showing histopathological signs of obvious necrosis for 14 days".

In 2002, Verwer's group in Amsterdam reported the surprising observation that neurones in adult cortical tissue sampled post-mortem could survive in organotypic slices for extended culture periods. They obtained motor cortex samples from control patients with no neurological or psychiatric disease and from a number of patients with a variety of degenerative disorders (principally Parkinson's and Alzheimer's disease. Patients were aged 45-93, and tissue was sampled from 2-8 hours post-mortem. Slices (200 μm thick) were cultured free floating and maintained for up to 50 days with occasional preparation kept for up to 78 days. Various measures of cell

number, morphology, cytoarchitecture and viability indicated that many neurones and glia survived post-mortem and were in relatively good health in about 50% of cultures even after 30 days. Slices from patients with neurodegenerative disorders, particular those with Alzheimer's often exhibited pathological signs of the underlying disorder (Verwer et al., 2002a, 2003). The same group also reported similar success with post-mortem culture of slices from hippocampus, cerebellum and nucleus basalis of Meynert (Verwer et al., 2002b). More recently, they have provided evidence that neuronal survival in organotypic slices obtained post mortem from precentral gyrus of both control and Alzheimer's patients (age range 55-93) is greatly improved by co-culture with rat embryonic stem cells (Wu et al., 2008). The rat and human tissue was co-cultured with a separating filter membrane suggesting that diffusible factors from the stem cells were responsible for slowing degeneration. A similar enhanced survival in post mortem slices by anti-inflammatory cytokines has also been described (Bsibi et al., 2006).

Perhaps the first study directly relevant to our currently considered approach was that of Chaichana et al., (2007), who compared the preservation of structure in human tumour and non-tumour cortical tissue from paediatric and adult patients (age ranges not given) undergoing surgery for tumor, trauma, arteriovenous malformation, aneurysm or epilepsy. Cortical tissue was obtained from frontal and temporal cortex, and slices were cut at 350 μm and cultured in interface Millicell inserts for up to 14 days. Slices showed good preservation of astrocyte morphology over 11 days with a decline after that, although there was a steady decline in the number of astrocytic process over this period. However, neuronal somata showed a relatively rapid degeneration over the culture period, with most being lost after 3 days, although dendrites often survived. Remarkably, too, axonal processes and synaptic contacts

were well preserved even after 11 days. Interestingly, whilst EM sections are likely to show contacts with surviving dendritic structures, some synaptic contacts revealed vesicles in both "pre-" and "postsynaptic" structures, which might suggest that these were axo-axonic synapses between terminals that survived the loss of the cell soma.

Organotypic culture of slices from patients undergoing surgery for drug refractory epilepsy was also described by Gonzalez-Martinez et al., (2007). In this study, slices (200 μm) were prepared from temporal, occipital, frontal and parietal cortices, with the majority coming from patients with cortical dysplasias. Slices comprised the cortex, white matter and underlying subventricular zone, but were maintained only over short culture periods (12-48 hours). The paper suggests that cresyl violet staining in control tissue (from patients undergoing tumour removal) shows "normal cytoarchitecture", whereas samples from cortical dysplasia patients (and other malformation associated epilepsies) shows "loss of normal organization". However, there is no detailed information on the general preservation and viability of the cultured tissue presented. What is particular interesting is that markers for neurogenesis reveal the presence of cellular proliferation in the subventricular zones and migration of cells from here to the overlying cortex over 48 hours, but this was restricted to epileptic samples and not seen in controls.

A limited study by Hocke et al., (2007) prepared temporal lobe slices (350 μm) from resections in 3 epileptic patients (no ages given) and examined ^{18}F-deoxglucose (FDG) uptake after 3-days in culture. The authors suggest that histological examination shows hippocampal cell loss that reflects hippocampal sclerosis associated with temporal lobe epilepsy. However, the poor quality of the images and sections combined with the lack of control comparison make it difficult to assess any real significance of the culture approach in this case. The authors did show a

regionally distributed uptake of FDG in the slices after 3 days in culture; they suggested that this reflected the preservation of viable neurones and could be used as a quality control measure for human tissue in organotypic culture. Another limited study briefly described organotypic slices (1 mm thick) of adult spinal cord obtained post-mortem (up to 48 hours; Jeong et al., 2011). These were used as a model to study the potential of using transplantation of bone marrow–derived mesenchymal precursor cells in spinal repair. Organotypic cultures were apparently maintained for up to 21 days, but no information on viability and cellular preservation is available.

Sebollela et al., (2012) used organotypic culture of temporal cortical slices to study the molecular effects of soluble oligomers of amyloid-beta peptide. Tissue was obtained fresh from adult patients undergoing hippocampal removal for drug refractory epilepsy, and was limited to overlying anterior temporal cortex. This tissue was debulked as an approach to the affected hippocampus and was assessed as "healthy" by prior MRI scan so was considered as genuine control tissue to asses the effect of treatments without the complication of underlying pathology. Slices (300 µm) were cultured using an interface approach and viability monitored with a Live/Dead Assay (calcein plus ethidium homodimer) for up to 25 days in vitro. This assay showed that cell viability was maintained at around 50% or greater over the culture period. NeuN and GFAP staining after 4 days in culture showed the presence of morphologically normal and healthy neurones and astrocytes. Interestingly, although 24-hours incubation with amyloid-beta peptides caused significant gene expression changes, there were no observable changes in neuronal viability in the cultures.

The most comprehensive study to date of the use of organotypic culture to facilitate the study of living human tissue was published recently by Eugene et al., (2014).

Slices were prepared from fresh tissue resected from hippocampus and temporal cortex from drug-refractory adult epilepsy patients (20-55 years old). This study is the first with the primary aim of developing and optimizing the culture approach to specific preserve the living tissue for research into basic mechanisms of epilepsy. Slices from both hippocampus and temporal cortex were 300 µm thick and maintained in culture by the interface method (Stoppini et al., 1991) for up to 4 weeks. The authors used some immunostaining and EM approaches to examine the morphology and ultrastructure of cultured slices after 6-10 days in vitro in acutely fixed tissue, but only sparse details are given. There is some indication that granule cells in the dentate gyrus show dispersal in culture, but it is not clear whether this is a feature of the epileptic tissue, or as a result of the culture. EM studies suggest good preservation of synaptic structure after 6-10 days in culture.

What the study gives us, for the first time, is good solid electrophysiological evidence for functional integrity of neurones and synaptic networks in cultured slices. Extracellular recording revealed spontaneous interictal-like activity in the hippocampus and subiculum, which developed around 6-10 days in culture but was absent prior to this. It peaked around 15 days and declined thereafter. Such activity was not seen the temporal cortex but could be readily induced by a variety of convulsant treatments. A limited number of whole cell recordings from subicular neurones showed a relatively healthy action potential, and the ability to burst fire that was similar to rodent tissue and in acutely prepared human tissue. Overall this paper has made the technical advance of defining optimal conditions and incubation procedures for maintaining organotypic human tissue slices and shown that functional connectivity and integrity may be achieved over relatively long culture periods.

Thus, there is optimism that organotypic culture can realistically be applied to human

brain slices. This will give us the opportunity to prolong the tissue life over several days and allow maximum use of this incredibly valuable resource for mechanistic studies of epileptic activity in the most valid "model" of the human condition.

6. Concluding remarks

In this review we emphasize the critical steps required to ensure the viability of slices prepared from surgical tissue samples obtained from elective epilepsy surgery cases. We also outline the optimal methods required to obtain recordings of neurophysiological activity associated with epilepsy. We draw attention to the difficulty in observing ictal patterns of activity spontaneously but summarise the methods that can be used to artificially elicit this behaviour. We propose that large-scale recording techniques (Ca^{2+} imaging and/or multielectrode array recordings) be routinely utilised in order to maximize the data obtained from this resource and assess questions concerning circuit dynamics in the human epileptic brain. As with all acute *in vitro* brain slice work, the longevity of neuronal tissue is a limiting factor. Whilst previously thought to be only achievable in juvenile tissue, the observation that adult human epileptic slices can be cultured using organotypic approaches, presents numerous experimental opportunities. A consensus on the methodologies concerning *in vitro* epileptic human brain slice studies would ensure consistency and reproducibility of findings in laboratories conducting this type of work. Such agreement would be critical when considering the formation of a multi-centre network to conduct studies on resected epileptic material.

Acknowledgements

Work discussed in this review has been supported by grants from Dr Hadwen Trust (MOC), Birmingham Children's Hospital & Aston Brain Centre (GLW), GW Pharma (GLW), Epilepsy Research UK (PGE1504; RSJG, GLW, MOC), the Wellcome Trust/EPSRC (102037; RGW, MOC) and a CAPES (Brazil) funded PhD studentship (BEX-0437-14-0; ABDS, RGW, MOC).

References

Adams C, Adams NE, Traub RD, Whittington MA. Electrographic waveform structure predicts laminar focus location in a model of temporal lobe seizures in vitro. PLoS One. 2015;10:e0121676.

Aitken PG, Breese GR, Dudek FF, Edwards F, Espanol MT, Larkman PM, Lipton P, Newman GC, Nowak TS Jr, Panizzon KL, et al. Preparative methods for brain slices: a discussion. J Neurosci Methods. 1995;59:139-149.

Aivar P, Valero M, Bellistri E, Menendez de la Prida L. Extracellular calcium controls the expression of two different forms of ripple-like hippocampal oscillations. J Neurosci. 2014; 34:2989-3004.

Aghajanian GK, Rasmussen K. Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneurons in adult rat brain slices. Synapse. 1989;3:331-338.

Akiyama T, McCoy B, Go CY, Ochi A, Elliott IM, Akiyama M, Donner EJ, Weiss SK, Snead OC 3rd, Rutka JT, Drake JM, Otsubo H. Focal resection of fast ripples on extraoperative intracranial EEG improves seizure outcome in pediatric epilepsy. Epilepsia. 2011;52:1802-1811.

Alavi A, Yakir S, Newberg AB. Positron emission tomography in seizure disorders.

Ann N Y Acad Sci. 2011;1228:E1-E12.

Alvarado-Rojas C, Huberfeld G, Baulac M, Clemenceau S, Charpier S, Miles R, de la Prida LM, Le Van Quyen M. Different mechanisms of ripple-like oscillations in the human epileptic subiculum. Ann Neurol. 2015;77:281-290.

Arbeloa J, Pérez-Samartín A, Gottlieb M, Matute C. P2X7 receptor blockade prevents ATP excitotoxicity in neurons and reduces brain damage after ischemia. Neurobiol Dis. 2012;45:954-961.

Beaumont TL, Yao B, Shah A, Kapatos G, Loeb JA. Layer-Specific CREB target gene induction in human neocortical epilepsy. J Neurosci 2012;32:14389-14401.

Bischofberger J, Engel D, Li L, Geiger JR, Jonas P. Patch-clamp recording from mossy fiber terminals in hippocampal slices. Nat Protoc. 2006;1:2075-2081.

Borbély S, Halasy K, Somogyvári Z, Détári L, Világi I. Laminar analysis of initiation and spread of epileptiform discharges in three in vitro models. Brain Res Bull. 2006;69:161-167.

Brahma B, Forman RE, Stewart EE, Nicholson C, Rice ME. Ascorbate inhibits edema in brain slices. J Neurochem. 2000;74:1263-1270.

Bsibsi M, Persoon-Deen C, Verwer RW, Meeuwsen S, Ravid R, Van Noort JM. Toll-like receptor 3 on adult human astrocytes triggers production of neuroprotective mediators. Glia 2006;53:688-695.

Buskila Y, Breen PP, Tapson J, van Schaik A, Barton M, Morley JW. Extending the viability of acute brain slices. Sci Rep. 2014;4:5309.

Chaichana KL, Capilla-Gonzalez V, Gonzalez-Perez O, Pradilla G, Han J, Olivi A,

Brem H, Garcia-Verdugo JM, Quiñones-Hinojosa A. Preservation of glial cytoarchitecture from ex vivo human tumor and non-tumor cerebral cortical explants: A human model to study neurological diseases. J Neurosci Methods 2007;164:261-270.

Cho JR, Joo EY, Koo DL, Hong SC, Hong SB. Clinical utility of interictal high-frequency oscillations recorded with subdural macroelectrodes in partial epilepsy. J Clin Neurol. 2012;8:22-34.

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

Collingridge GL. The brain slice preparation: a tribute to the pioneer Henry McIlwain. J Neurosci Methods. 1995;59(1):5-9.

Costero I, Pomerat CM. Cultivation of neurons from the adult human cerebral and cerebellar cortex. Am J Anat. 1951;89:405–467.

Cunningham MO, Roopun A, Schofield IS, Whittaker RG, Duncan R, Russell A, Jenkins A, Nicholson C, Whittington MA, Traub RD. Glissandi: transient fast electrocorticographic oscillations of steadily increasing frequency, explained by temporally increasing gap junction conductance. Epilepsia. 2012;53:1205-1214.

de Tisi J, Bell GS, Peacock JL, McEvoy AW, Harkness WF, Sander JW, Duncan JS. The long-term outcome of adult epilepsy surgery, patterns of seizure remission, and relapse: a cohort study. Lancet. 2011;378:1388-1395.

Dora E. Glycolysis and epilepsy-induced changes in cerebrocortical NAD/NADH redox state. J. Neurochem., 1983;41:1774–1777

Dossi E, Blauwblomme T, Nabbout R, Huberfeld G, Rouach N. Multi-electrode array recordings of human epileptic postoperative cortical tissue. J Vis Exp. (92):e51870.

Engel J Jr, Wiebe S, French J, Sperling M, Williamson P, Spencer D, Gumnit R, Zahn C, Westbrook E, Enos B; Quality Standards Subcommittee of the American Academy of Neurology; American Epilepsy Society; American Association of Neurological Surgeons. Practice parameter: temporal lobe and localized neocortical resections for epilepsy: report of the Quality Standards Subcommittee of the American Academy of Neurology, in association with the American Epilepsy Society and the American Association of Neurological Surgeons. Neurology. 2003;60:538-547.

Engel J Jr, McDermott MP, Wiebe S, Langfitt JT, Stern JM, Dewar S, Sperling MR, Gardiner I, Erba G, Fried I, Jacobs M, Vinters HV, Mintzer S, Kieburtz K; Early Randomized Surgical Epilepsy Trial (ERSET) Study Group. Early surgical therapy for drug-resistant temporal lobe epilepsy: a randomized trial. JAMA. 2012;307:922-930.

Eugène E, Cluzeaud F, Cifuentes-Diaz C, Fricker D, Le Duigou C, Clemenceau S, Baulac M, Poncer JC, Miles R. An organotypic brain slice preparation from adult patients with temporal lobe epilepsy. J Neurosci Meth. 2014;235:234-244.

Geiger JR, Bischofberger J, Vida I, Fröbe U, Pfitzinger S, Weber HJ, Haverkampf K, Jonas P. Patch-clamp recording in brain slices with improved slicer technology. Pflugers Arch. 2002;443:491-501.

González-Martínez JA, Bingaman WE, Toms SA, Najm IM. Neurogenesis in the postnatal human epileptic brain. J Neurosurg. 2007;107:628-635.

Gloveli T, Dugladze T, Rotstein HG, Traub RD, Monyer H, Heinemann U,

Whittington MA, Kopell NJ. Orthogonal arrangement of rhythm-generating microcircuits in the hippocampus. Proc Natl Acad Sci U S A. 2005;102:13295-13300.

Haegelen C, Perucca P, Châtillon CE, Andrade-Valença L, Zelmann R, Jacobs J, Collins DL, Dubeau F, Olivier A, Gotman J. High-frequency oscillations, extent of surgical resection, and surgical outcome in drug-resistant focal epilepsy. Epilepsia. 2013;54:848-857.

Hájos N, Pálhalmi J, Mann EO, Németh B, Paulsen O, Freund TF. Spike timing of distinct types of GABAergic interneuron during hippocampal gamma oscillations in vitro. J Neurosci. 2004;24:9127-9137.

Hájos N, Ellender TJ, Zemankovics R, Mann EO, Exley R, Cragg SJ, Freund TF, Paulsen O. Maintaining network activity in submerged hippocampal slices: importance of oxygen supply. Eur J Neurosci. 2009;29:319-327.

Heinemann U, Buchheim K, Gabriel S, Kann O, Kovacs R, Schuchmann S. Cell death and metabolic activity during epileptiform discharges and status epilepticus in the hippocampus. Prog Brain Res. 2002a;135:197–210

Heinemann U, Buchheim K, Gabriel S, Kann O, Kovacs R, Schuchmann S. Coupling of electrical and metabolic activity during epileptiform discharges. Epilepsia, 2002b;43 (Suppl. 5):168–173

Heinemann U, Staley KJ. What is the clinical relevance of *in vitro* epileptiform activity? Adv Exp Med Biol. 2014;813:25-41.

Hogue MJ. Tissue cultures of the brain: intercellular granules. J Comp Neurol. 1946;85:519-530.

Hogue MJ. Human fetal brain cells in tissue cultures: their identification and motility. J Exp Zool. 1947;106:85–103.

Hogue MJ. A study of adult human brain cells grown in tissue cultures. Am J Anat. 1953;93:397–427.

Hocke C, Blümcke I, Romstöck J, Prante O, Stefan H, Kuwert T, Jeske I. Specific accumulation of 18F-deoxyglucose in three-dimensional long-term cultures of human and rodent brain tissue. Nuklearmedizin. 2007;46:233-238

Huberfeld G, Wittner L, Clemenceau S, Baulac M, Kaila K, Miles R, Rivera C. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. J Neurosci. 2007;27:9866-9873

Huberfeld G, Menendez de la Prida L, Pallud J, Cohen I, Le Van Quyen M, Adam C, Clemenceau S, Baulac M, Miles R. Glutamatergic pre-ictal discharges emerge at the transition to seizure in human epilepsy. Nat Neurosci. 2011;14:627-634.

Hsiao MC, Yu PN, Song D, Liu CY, Heck CN, Millett D, Berger TW. Spatio-temporal inter-ictal activity recorded from human epileptic hippocampal slices. Conf Proc IEEE Eng Med Biol Soc. 2012;2012:5166-5169.

Hsiao MC, Yu PN, Song D, Liu CY, Heck CN, Millett D, Berger TW. An *in vitro* seizure model from human hippocampal slices using multi-electrode arrays. J Neurosci Methods. 2015;244:154-163.

Husson TR, Mallik AK, Zhang JX, Issa NP. Functional imaging of primary visual cortex using flavoprotein autofluorescence. J Neurosci. 2007;27:8665-8675.

Ivanov A, Zilberter Y. Critical state of energy metabolism in brain slices: the

principal role of oxygen delivery and energy substrates in shaping neuronal activity. Front Neuroenergetics. 2011;29:3:9.

Ivanov AI, Malkov AE, Waseem T, Mukhtarov M, Buldakova S, Gubkina O, Zilberter M, Zilberter Y. Glycolysis and oxidative phosphorylation in neurons and astrocytes during network activity in hippocampal slices. J Cereb Blood Flow Metab. 2014;34:397-407.

Jacobs J, Zijlmans M, Zelmann R, Chatillon CE, Hall J, Olivier A, Dubeau F, Gotman J. High-frequency electroencephalographic oscillations correlate with outcome of epilepsy surgery. Ann Neurol. 2010;67:209-220.

Jakovcevski I, Mo Z, Zecevic N. Down-regulation of the axonal polysialic acid-neural cell adhesion molecule expression coincides with the onset of myelination in the human fetal forebrain. Neuroscience. 2007;149:328-337.

Jeong DK, Taghavi CE, Song KJ, Lee KB, Kang HW. Organotypic human spinal cord slice culture as an alternative to direct transplantation of human bone marrow precursor cells for treating spinal cord injury. World Neurosurg. 2011;75:533-539.

Johnson MR, Behmoaras J, Bottolo L, Krishnan ML, Pernhorst K, Santoscoy PL, Rossetti T, Speed D, Srivastava PK, Chadeau-Hyam M, Hajji N, Dabrowska A, Rotival M, Razzaghi B, Kovac S, Wanisch K, Grillo FW, Slaviero A, Langley SR, Shkura K, Roncon P, De T, Mattheisen M, Niehusmann P, O'Brien TJ, Petrovski S, von Lehe M, Hoffmann P, Eriksson J, Coffey AJ, Cichon S, Walker M, Simonato M, Danis B, Mazzuferi M, Foerch P, Schoch S, De Paola V, Kaminski RM, Cunliffe VT, Becker AJ, Petretto E. Systems genetics identifies Sestrin 3 as a regulator of a proconvulsant gene network in human epileptic hippocampus. Nat Commun.

2015;6:6031.

Jung S, Kim HW, Lee JH, Kang SS, Rhu HH, Jeong YI, Yang SY, Chung HY, Bae CS, Choi C, Shin BA, Kim KK, Ahn KY. Brain tumor invasion model system using organotypic brain-slice culture as an alternative to in vivo model. J Cancer Res Clin Oncol. 2002;128:469-476.

Kobow K, Blümcke I. Epigenetic mechanisms in epilepsy. Prog Brain Res. 2014;213:279-316.

Köhling R, Lücke A, Straub H, Speckmann EJ, Tuxhorn I, Wolf P, Pannek H, Oppel F. Spontaneous sharp waves in human neocortical slices excised from epileptic patients. Brain. 1998;121:1073-1087.

Köhling R, Lücke A, Straub H, Speckmann EJ. A portable chamber for long-distance transport of surviving human brain slice preparations. J Neurosci Methods. 1996;67:233-236.

Köhling R, Avoli M. Methodological approaches to exploring epileptic disorders in the human brain in vitro. J Neurosci Meth. 2006;155:1-19.

Köhling R, Qü M, Zilles K, Speckmann EJ. Current-source-density profiles associated with sharp waves in human epileptic neocortical tissue. Neuroscience. 1999;94:1039-1050.

Kovacs R, Schuchmann S, Gabriel S, Kardos J, Heinemann U. Ca2 + signalling and changes of mitochondrial function during low-Mg2 + -induced epileptiform activity in organotypic hippocampal slice cultures Eur J Neurosci. 2001;13:1311–1319

Kreisman NR, Olson JE. Taurine enhances volume regulation in hippocampal slices

swollen osmotically. Neuroscience. 2003;120:635-642.

Kuenzi FM, Fitzjohn SM, Morton RA, Collingridge GL, Seabrook GR. Reduced long-term potentiation in hippocampal slices prepared using sucrose-based artificial cerebrospinal fluid. J Neurosci Meth. 2000;100:117-122

Lench AM, Robson E, Jones RSG. Differential effects of D-cycloserine and ACBC at NMDA receptors in the rat entorhinal cortex are related to efficacy at the co-agonist binding site. PLoS One. 2015;10(7):e0133548.

Letinic K, Zoncu R, Rakic P. Origin of GABAergic neurons in the human neocortex. Nature. 2002;417:645-649.

Letinic K, Rakic P. Telencephalic origin of human thalamic GABAergic neurons. Nat Neurosci 2001;4:931-936

Lipton P, Aitken PG, Dudek FE, Eskessen K, Espanol MT, Ferchmin PA, Kelly JB, Kreisman NR, Landfield PW, Larkman PM, et al. Making the best of brain slices: comparing preparative methods. J Neurosci Meth. 1995;59:151-156.

Lyman WD, Hatch WC, Pousada E, Stephney G, Rashbaum WK, Weidenheim KM. Human fetal myelinated organotypic cultures. Brain Res. 1992;599:34-44.

Martinez R1, Eraso D, Geffin R, McCarthy M. A two-culture method for exposure of human brain organotypic slice cultures to replicating human immunodeficiency virus type 1. J Neurosci Meth. 2011;200:74-79.

Miller-Delaney SF, Bryan K, Das S, McKiernan RC, Bray IM, Reynolds JP, Gwinn R, Stallings RL, Henshall DC. Differential DNA methylation profiles of coding and non-coding genes define hippocampal sclerosis in human temporal lobe epilepsy.

Brain. 2015;13:616-631.

O'Connor MJ, Herman CJ, Rosenthal M, Jobsis FF. Intracellular redox changes preceding onset of epileptiform activity in intact cat hippocampus J. Neurophysiol., 1972;35:471–483

Okanishi T, Akiyama T, Tanaka S, Mayo E, Mitsutake A, Boelman C, Go C, Snead OC 3rd, Drake J, Rutka J, Ochi A, Otsubo H. Interictal high frequency oscillations correlating with seizure outcome in patients with widespread epileptic networks in tuberous sclerosis complex. Epilepsia. 2014;55:1602-1610.

Pallud J, Le Van Quyen M, Bielle F, Pellegrino C, Varlet P, Labussiere M, Cresto N, Dieme MJ, Baulac M, Duyckaerts C, Kourdougli N, Chazal G, Devaux B, Rivera C, Miles R, Capelle L, Huberfeld G. Cortical GABAergic excitation contributes to epileptic activities around human glioma. Sci Transl Med. 2014;6:244ra89.

Pan JW, Williamson A, Cavus I, Hetherington HP, Zaveri H, Petroff OA, Spencer DD. Neurometabolism in human epilepsy. Epilepsia. 2008;49 Suppl 3:31-41.

Prokic EJ, Weston C, Yamawaki N, Hall SD, Jones RS, Stanford IM, Ladds G, Woodhall GL. Cortical oscillatory dynamics and benzodiazepine-site modulation of tonic inhibition in fast spiking interneurons. Neuropharmacology. 2015;95:192-205.

Quiroga RQ, Nadasdy Z, Ben-Shaul Y. Unsupervised spike detection and sorting with wavelets and superparamagnetic clustering. Neural Comput. 2004;16:1661-1687.

Rakhade SN, Yao B, Ahmed S, Asano E, Beaumont TL, Shah AK, Draghici S, Krauss R, Chugani HT, Sood S, Loeb JA. A common pattern of persistent gene activation in human neocortical epileptic foci. Ann Neurol. 2005;58:736-747.

Roopun AK, Simonotto JD, Pierce ML, Jenkins A, Nicholson C, Schofield IS, Whittaker RG, Kaiser M, Whittington MA, Traub RD, Cunningham MO. A nonsynaptic mechanism underlying interictal discharges in human epileptic neocortex. Proc Natl Acad Sci U S A. 2010;107:338-343.

Spampanato J, Mody I. Spike timing of lacunosom-moleculare targeting interneurons and CA3 pyramidal cells during high-frequency network oscillations in vitro. J Neurophysiol. 2007;98:96-104.

Schevon CA, Weiss SA, McKhann G Jr, Goodman RR, Yuste R, Emerson RG, Trevelyan AJ. Evidence of an inhibitory restraint of seizure activity in humans. Nat Commun. 2012;3:1060.

Schevon CA, Ng SK, Cappell J, Goodman RR, McKhann G Jr, Waziri A, Branner A, Sosunov A, Schroeder CE, Emerson RG. Microphysiology of epileptiform activity in human neocortex. J Clin Neurophysiol. 2008;25:321-330

Schuchmann S, Buchheim K, Meierkord H, Heinemann U. A relative energy failure is associated with low-Mg^{2+} but not with 4-aminopyridine induced seizure-like events in entorhinal cortex J. Neurophysiol. 1999;81:399–403

Sebollela A, Freitas-Correa L, Oliveira FF, Paula-Lima AC, Saraiva LM, Martins SM, Mota LD, Torres C, Alves-Leon S, de Souza JM, Carraro DM, Brentani H, De Felice FG, Ferreira ST. Amyloid-β oligomers induce differential gene expression in adult human brain slices. J Biol Chem. 2012;287:7436-7345.

Simon A, Traub RD, Vladimirov N, Jenkins A, Nicholson C, Whittaker RG, Schofield I, Clowry GJ, Cunningham MO, Whittington MA. Gap junction networks

can generate both ripple-like and fast ripple-like oscillations. Eur J Neurosci. 2014;39:46-60.

Staba RJ, Stead M, Worrell GA. Electrophysiological biomarkers of epilepsy. Neurotherapeutics. 2014;11:334-346.

Stead M, Bower M, Brinkmann BH, Lee K, Marsh WR, Meyer FB, Litt B, Van Gompel J, Worrell GA. Microseizures and the spatiotemporal scales of human partial epilepsy. Brain. 2010;133:2789-2797.

Stoppini L, Buchs PA, Muller D. A simple method for organotypic cultures of nervous tissue. J Neurosci Meth. 1991;37:173-182

Telfeian AE, Spencer DD, Williamson A. Lack of correlation between neuronal hyperexcitability and electrocorticographic responsiveness in epileptogenic human neocortex. J Neurosurg. 1999;90:939-945.

Theyel BB, Kohrman MH, Frim DM, van Drongelen W. Network variability across human tissue samples in vitro: the problem and a solution. J Clin Neurophysiol. 2010;27:412-417

Traub RD, Cunningham MO, Whittington MA. Chemical synaptic and gap junctional interactions between principal neurons: partners in epileptogenesis. Neural Netw. 2011;24:515-25.

Traub RD, Cunningham MO, Whittington MA. What is a seizure network? Very fast oscillations at the interface between normal and epileptic brain. Adv Exp Med Biol. 2014;813:71-80.

Trevelyan AJ, Sussillo D, Watson BO, Yuste R. Modular propagation of epileptiform

activity: evidence for an inhibitory veto in neocortex. J Neurosci. 2006;26:12447-12455.

Trevelyan AJ, Sussillo D, Yuste R. Feedforward inhibition contributes to the control of epileptiform propagation speed. J Neurosci. 2007a;27:3383-3387.

Trevelyan AJ, Baldeweg T, van Drongelen W, Yuste R, Whittington M. The source of afterdischarge activity in neocortical tonic-clonic epilepsy. J Neurosci. 2007b;27:13513-13519.

Truccolo W, Donoghue JA, Hochberg LR, Eskandar EN, Madsen JR, Anderson WS, Brown EN, Halgren E, Cash SS. Single-neuron dynamics in human focal epilepsy. Nat Neurosci. 2011;14:635-641.

Truccolo W, Ahmed OJ, Harrison MT, Eskandar EN, Cosgrove GR, Madsen JR, Blum AS, Potter NS, Hochberg LR, Cash SS. Neuronal ensemble synchrony during human focal seizures. J Neurosci. 2014;34:9927-9944.

Ulbert I, Heit G, Madsen J, Karmos G, Halgren E. Laminar analysis of human neocortical interictal spike generation and propagation: current source density and multiunit analysis in vivo. Epilepsia. 2004;45 Suppl 4:48-56.

Verwer RW, Hermens WT, Dijkhuizen P, ter Brake O, Baker RE, Salehi A, Sluiter AA, Kok MJ, Muller LJ, Verhaagen J, Swaab DF. Cells in human postmortem brain tissue slices remain alive for several weeks in culture. FASEB J. 2002a;16:54-60.

Verwer RW, Dubelaar EJ, Hermens WT, Swaab DF. Tissue cultures from adult human postmortem subcortical brain areas. J Cell Mol Med. 2002b;6:429-432.

Verwer RW, Baker RE, Boiten EF, Dubelaar EJ, van Ginkel CJ, Sluiter AA, Swaab

DF. Post-mortem brain tissue cultures from elderly control subjects and patients with a neurodegenerative disease. Exp Gerontol. 2003;38:167-172.

Wiebe S, Blume WT, Girvin JP, Eliasziw M; Effectiveness and Efficiency of Surgery for Temporal Lobe Epilepsy Study Group. A randomized, controlled trial of surgery for temporal-lobe epilepsy. N Engl J Med. 2001;345:311-318

Wittner L, Huberfeld G, Clémenceau S, Eross L, Dezamis E, Entz L, Ulbert I, Baulac M, Freund TF, Maglóczky Z, Miles R. The epileptic human hippocampal cornu ammonis 2 region generates spontaneous interictal-like activity in vitro. Brain. 2009:132:3032-3046.

Wozny C, Kivi A, Lehmann TN, Dehnicke C, Heinemann U, Behr J. Comment on "On the origin of interictal activity in human temporal lobe epilepsy in vitro". Science. 2003;301:463.

Wu L, Sluiter AA, Guo HF, Balesar RA, Swaab DF, Zhou JN, Verwer RW. Neural stem cells improve neuronal survival in cultured postmortem brain tissue from aged and Alzheimer patients. J Cell Mol Med. 2008;12:1611-1621.

Wu JY, Sankar R, Lerner JT, Matsumoto JH, Vinters HV, Mathern GW. Removing interictal fast ripples on electrocorticography linked with seizure freedom in children. Neurology. 2010;75:1686-1694.

Wu JY, Guan L, Tsau Y. Propagating activation during oscillations and evoked responses in neocortical slices. J Neurosci. 1999;19:5005-5015.

Zahn RK, Tolner EA, Derst C, Gruber C, Veh RW, Heinemann U. Reduced ictogenic potential of 4-aminopyridine in the perirhinal and entorhinal cortex of kainate-treated chronic epileptic rats. Neurobiol Dis. 2008; 29:186-200

Zhao M, Nguyen J, Ma H, Nishimura N, Schaffer CB, Schwartz TH. Preictal and ictal neurovascular and metabolic coupling surrounding a seizure focus J. Neurosci. 2011;31:13292–13300

Figure Legend

Figure 1. Evoked ictal events in epileptic temporal neocortex in vitro. A. Extracellular local field potential recordings showing slow time course development of the ictal event in the presence of kainate and carbachol. **B.** Morlet wavelet spectrogram of the activity illustrated in **A** shows the characteristic spectral content of a seizure event. **C.** Selected epochs as shown by asterisks in **A** from throughout the ictal event on a faster timescale. In agreement with the spectrogram, small amplitude high frequency oscillations dominate the beginning of the seizure with large amplitude tonic and clonic discharges then developing in the later stages of the ictus.

Figure 2. Multi-electrode array (MEA) recordings in epileptic human cortex. Ai. The image illustrates post-hoc cresyl violet stain of a re-sectioned slice of peritumoural human temporal neocortex (low-grade glioma; grade II astrocytoma) in which recordings were conducted with 64 channel 'Buzsaki' configuration MEA (Neuronexus; Ann Arbor MI, USA). **Aii.** Photomicrograph of a portion of the MEA show the layout of individual contacts on each shank (8 contacts per shank). Each individual contact has a surface area of 160 μm^2, and an impedance of 1–3 MΩ. The inter-shank distance was 200 μm. Recording sites were staggered to provide a two-dimensional arrangement (20 μm vertical separation) **Aiii.** Extracellular recording show local field potential signal of an induced ictal discharge recorded from individual MEA contact. Exposure of the slice to ACSF containing low magnesium (0.25 mM) and high potassium (8mM) produced the emergence of ictal events. A selected event from long time course trace show the seizure onset followed by rhythmic bursts. **Aiv.** Raster plots for 3 simultaneously recorded layer IV-V neurons during the ictal event illustrated in **Aiii.** Note the variation in the firing behavior during the various stages of the seizure event. **B.** Average waveforms for putative

pyramidal cell (**i, ii**) and interneuron (**iii**) and associated plot showing the first and the second coefficient of the wavelet transform (**iv**). Wave_clus algorithm was used to detect and sort the spikes (Quiroga et al., (2004)) using a Wavelet Transform **C.** Phase preference of identified single units during ripple (80-200 Hz) and fast-ripple (200-600Hz) oscillations present during the epoch examined.