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

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HIGHLIGHTS

- Electrophysiology in human epilepsy samples gives unique insights into pharmacoresistant epilepsy.
- A number of technical and logistical considerations associated with preparing human epileptic slices.
- Interictal activity seen more routinely than ictal discharges in human epileptic slices.
- Much still to be gleaned by the application of organotypic culture techniques, imaging and mutlielectrode array recordings.

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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.

Contents

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1. 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 Komlósi et al., 2012; Molnár 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 Köhling 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 (Köhling and Avoli, 2006), and discuss what the future may hold for experimental studies involving human epileptic brain slices.

2. 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., 2015), 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 optimised 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.