Preparation of rodent and human cerebral cortex slices for acute and organotypic culture investigations

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Introduction

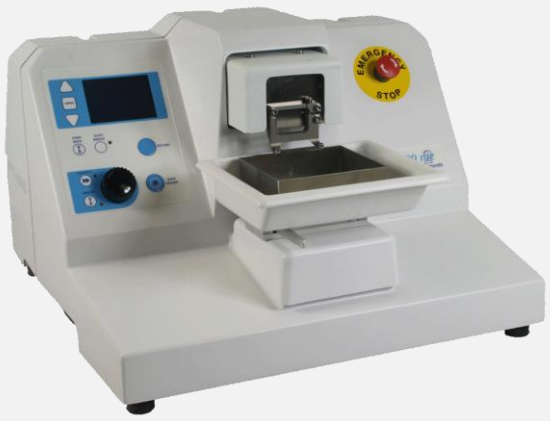
The cerebral cortex is one of the most complex biological systems known to science. Although columnar and laminar structure is preserved throughout much of the neocortex, differences in cellular connectivity and type mean that each cortical area presents unique challenges in preserving network function and cellular health in experimental preparations. What is more, inter-species differences in anatomy and connectivity present a translational challenge in medical research. Using acutely prepared brain slices allows for higher throughput and a wider variety of recording techniques than *in vivo* investigations, and enables the use of human tissue obtained with consent from surgical patients undergoing surgical resections of epileptic foci. However, the production of slices from both humans and rodents needs to be performed to the highest standard in order to maintain as much connectivity and cellular health as possible. This is especially important if the slices are to be used in longer-term experiments such as organotypic culture, which allows further manipulations such as optogenetic construct transfection or longitudinal monitoring of network function.

Procedures

Solution Preparation: Our laboratory uses one general recipe for artificial cerebrospinal fluid (aCSF) for recording acute slice preparations, whether from rodent or human tissue and regardless of recording type (e.g. patch clamp, field potential or multi-electrode array). However, the cutting solution used to prepare the slices differs between human and rodent tissue. For *ex vivo* experiments using rodents, we use a sucrose-based cutting solution (Johnson *et al*, 2017) and for human tissue work an optimised choline-based solution is used (Wright *et al*, 2020). For organotypic culture, this solution is filter-sterilised before use in slice preparation. All aCSF is constantly bubbled with carbogen (95% O_2 5% CO_2) to maintain pH and supply the tissue with adequate oxygen. We use a variety of antioxidant and neuroprotectant compounds in our slicing and incubation solutions, including N-acetyl-cysteine, aminoguanidine. taurine, ethyl pyruvate and ascorbic acid (for discussion see Johnson *et al*, 2017).

Vibrotome Blade: Many laboratories use disposable carbon steel razor blades to prepare their slices – these can produce good results if care is taken to remove any lubricant or protectant oil on the blades by washing with dilute ethanol. More durable stainless-steel blades are available, however our laboratories have produced the most consistent and high-quality slices by fitting our vibratomes with re-usable zirconium-ceramic blades such as the Campden **7550-1-C**. As long as the blades are kept clean and the leading edge is not damaged by forceps or

Product Focus: 7000smz-2 Vibrotome



Our top of the range high precision, vibrating microtome (vibrotome for short) is the tissue slicer of choice for preparations for visual patch clamping or high-resolution imaging.

Using our vibrotomes, research detailing sectioning for visual patching of neurological tissue, heart, lung, and tissue scaffolds have all been published. The **7000smz-2** vibrotome represents significant advances with higher precision at a lower cost.

Application Parameters	
Amplitude	2.0 – 2.5mm
Frequency	60Hz
Advance Speed	0.04-0.12mm/s

For more information click visit https://campdeninstruments.com/products/7000smz-2-vibrotome

other tools the blades should remain sharp for several months.

Slicing Speed: The speed of slice cutting depends on the age of the subject and the cortical area of interest. Human tissue is very variable in terms of white/grey matter composition and care must be taken when mounting the sample to maximise the amount of grey matter. Slicing should start off at slow speed and increased gradually whilst ensuring that the tissue does not ruck up or catch on the blade – this is usually due to the presence of meninges or significant areas of white matter. Rodent tissue is more predictable and should be sliced at a consistent speed – generally the fastest that can be performed without causing cell loss or striations on the tissue surface. This can be assessed under a patch-clamp rig microscope, success often depends on the Z-axis stability of the slicer and poor slices will appear 'ridged' under magnification. Some areas such as the entorhinal cortex should be sliced at a slower speed to preserve vulnerable cells.

Slice Incubation: The preservation of slice health and network function is especially important for experiments involving coupled cortical oscillations, and we have found that this emergent network activity is more reliably generated if slices have been stored in an interface chamber rather than a submerged storage chamber. To that end, for local field potential recordings of oscillatory activity our slices (400-650µm) are stored resting on filter paper in a sealed interface chamber system until required for recording. Slices prepared for patch clamp experiments (350-400µm) are stored submerged in a 250ml beaker on a custom 3D-printed slice holder (plans available on request).

Specific examples

We have deployed local field potential recordings in rodent cortical slices displaying phase-amplitude coupled theta and gamma oscillations, evoked using a combination of kainic acid (150nM) and carbachol (10µM) to replace lost neuromodulatory tone. This approach has allowed us to investigate the nature of cross-frequency coupling across the layers of single cortical areas (e.g. M1) and between distinct cortical areas (e.g. M1 and S1) and perform a range of manipulations to test the receptor and cellular drivers of this network activity (Johnson *et al*, 2017). We have also used this preparation to investigate the network-level actions of a range of anti-epileptic drugs.

Our human tissue slices have allowed us to use both patch-clamp and field recordings to investigate the pathologies of a range of epilepsies, and explore new avenues for pharmacological intervention including cannabinoid-based compounds and ketogenic diet metabolites (e.g. Wright *et al*, 2020). Expanding this work to encompass organotypic culture has meant that we can investigate long-term drug interventions in hard-to-treat epilepsies and gain more insight into the pathophysiology of these conditions.

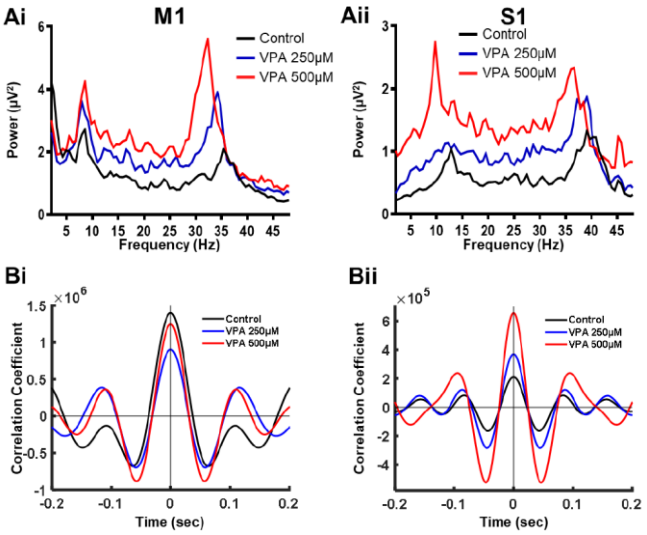


Fig.1. Slice preparations with coupled oscillatory activity can be used to investigate the network actions of antiepileptic drugs. In this example sodium valproate (VPA) causes power and frequency shifts in theta and gamma ranges in a dose- and area-dependent manner

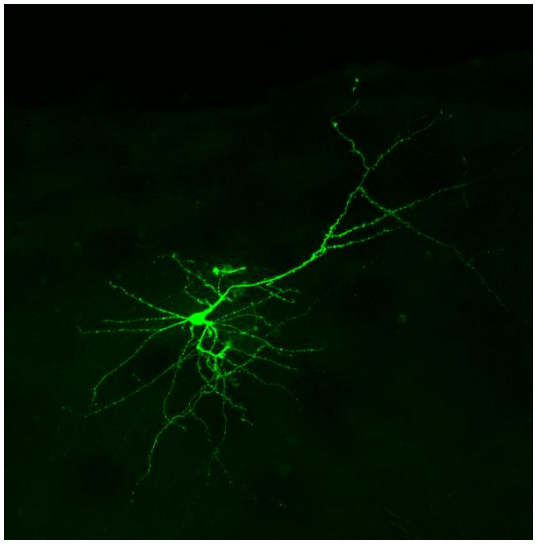


Fig.2. Human cortical pyramidal cell from paediatric epilepsy patient still displays normal morphology and dendritic spine density after 7 DIV organotypic culture. Patch-clamp recorded cell filled with biocytin and processed with Alexa-Streptavidin

References

Johnson, N.W., Özkan, M., Burgess, A.P., Prokic, E.J., Wafford, K.A., O'Neill, M.J., Greenhill, S.D., Stanford, I.M. & Woodhall, G.L. (2017) Phase-amplitude coupled persistent theta and gamma oscillations in rat primary motor cortex *in vitro*. *Neuropharmacology*, **119**, 141-156. DOI: https://doi.org/10.1016/j.neuropharm.2017.04.009

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