Final Report: 10 May 2010

WMRF 157: Testing the effect of temperature and polyethylene glycol on neocortical slice viability

Aims

There were two main aims of this project: to quantify the effect of artificial cerebrospinal fluid (ACSF) temperature and polyethylene glycol on tissue slice viability/health; and to describe how electrophysiologically recorded slice activity changes as tissue health deteriorates. The purpose of the latter is to provide an objective means of assessing slice health, using electrophysiological parameters.

Results

The effect of ACSF temperature changes on seizure-like activity has been recorded from 24 cortical slices. Seizure-like activity has been reliable generated by removing magnesium from the ACSF – this is a commonly used seizure model in brain slice experiments. Three temperatures have been trialled, 26ºC, 31ºC and 36ºC, representing the broad range of temperatures utilised by different research groups. The results demonstrate that the pattern of low-magnesium seizure-like activity changes consistently with time across all three temperatures; the amplitude and length of seizure-like events gradually reduce and event frequency increases. Importantly, the changes in event frequency and length occur earlier than the effect on event amplitude. Overall, the results indicate that the healthiest tissue (i.e. at the beginning of the recording period) generate spontaneous seizure-like events that are relatively infrequent, long and of high amplitude. Thus, a combination of frequency, length and amplitude of spontaneously occurring seizure-like events is likely to be a more sensitive measure of slice status than amplitude alone.

In 11 slices, an assessment of slice health (following recording at 36ºC for 3 hours) has been made using a live-dead cell fluorescent stain; and compared with slices maintained for the equivalent time period at room temperature. No differences in slice health have been found between the different temperatures, suggesting that the deterioration in slice health with time occurs independently of ACSF temperature. This is consistent with the findings above that electrophysiological changes with time are consistent across all three temperatures. However, some problems have been encountered with the fluorescence assessment of slice viability (see below). We are currently working

on refining these methods to provide a more robust measure. Until then, these findings remain preliminary. For this reason the effect of polyethylene glycol on slice viability has not yet been tested.

These results have been written up and submitted for publication in the journal Neuroscience Methods. The paper is currently being revised for resubmission.

Problems encountered

Fluorescent imaging and quantification of tissue health has proved more difficult than anticipated. The methods employed utilise two fluorescent stains, one of which enters and stains living cells and other which enters and stains dead cells. The tissue is imaged by photographing through an epifluorescent microscope. The main issue is that epifluorescent microscopy does not "see" very deeply into tissue (even though the slices we use are only 400μm thick, they are surprisingly opaque). Because the slicing procedure unavoidably damages the outer 10-20 μm of each slice, comparisons between slices exposed to different conditions are nullified somewhat because the superficial regions where imaging is possible are largely dead before the experiments are commenced. Because of these drawbacks, it has only been possible to qualitatively describe the effects of temperature on slice health using fluorescence imaging. To more accurately quantify live-dead cell parameters, it may be necessary to investigate confocal imaging, which allows greater depth of penetration into the healthier regions of the tissue.

Equipment purchases

A thermostatic temperature controller (Harvard Warner Solution In-Line Heater and Heater Controller Single Channel) was purchased at a cost of $3880. This apparatus allows the temperature of our tissue slice perfusion bath to be adjusted and maintained up to approximately 37ºC.