Measuring the bulk impedance of brain tissue *in vitro*

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Abstract

Theoretical and numerical models of brain activity suggest a link between seizures and electrical connectivity. We have therefore been motivated to measure electrical conductivity in brain tissue. Such measurements *in vitro* are difficult; it is necessary to use a conductive inorganic salt solution, artificial cerebrospinal fluid (ACSF), to keep the tissue alive. We have attempted to provide a robust method to make such measurements. Mouse brain tissue was sliced (400 μm) using established methods. Half the slices were placed in standard ACSF; half were placed in ACSF devoid of magnesium ions. The latter case promotes seizure activity. Electrical activity was measured with a tungsten electrode at various places on the slices. Sixty-nine samples of cortex (2 mm × 2 mm) were cut with a razor. Their areas were measured with a calibrated microscope. Each sample was placed between two flat Ag/AgCl electrodes in a Perspex sandwich. Excess ACSF was removed with filter paper. The impedance was measured at 25°C from 20 Hz to 2 MHz with an Agilent E4980A four-point impedance meter in a shielded room, using a low current. Between 1 kHz and 100 kHz the conductivity was approximately 0.2 S m^{-1}; outside this range dispersion occurred. Samples prepared in the magnesium-free ACSF had a conductivity about 10% lower. The Cole-Cole model of conductivity was fitted. There were few significant differences between the parameters for the different groups measured.

1. Introduction

Measuring electrical conductivity is a well-established process in the solid-state. The method of van der Pauw can be used for a thin, 2-dimensional sample with point-electrodes [1,2], or a 1-dimensional method (e.g. with flat guarded electrodes) can be implemented [3,4].

The measurements are harder to do in biological tissue. There has been some motivation to do this for measuring meat quality [5]. Additionally, from a neuroscience perspective there is interest in terms of describing electrical properties of tissue (e.g. for epilepsy research, seizure prediction and modelling purposes). Our motivation for this research is to use electrical conductivity as an indirect measure of the connectivity between brain cells, thus informing the development and interpretation of models of brain activity.

Often nonlinear capacitor-resistor circuit models showing dispersion are used as a model for the conductivity, for example the Cole-Cole model [6]. Several dispersive regimes are usually present [7]. However, it is hard to identify dispersive regimes with particular processes and make meaningful conclusions about the nature of the material from measurement of circuit parameters [8].

Curiously, more work done has been done in live animals rather than in tissue samples; for example Logothetis *et al.* measured conductivity with four point-electrodes – current was injected and extracted current through two electrodes, and the potential difference measured between the other two. Impedance could then be calculated with electrostatics [9].

Work with brain tissue *in vitro* is difficult because tissue is easily damaged, degrades with time, and needs to be bathed in artificial cerebrospinal fluid (ACSF), an inorganic salt solution, which itself has an appreciable conductivity. Elbohouty tried many approaches to account for the presence of ACSF in measurements of conductivity, but concluded that the best method was simply to remove the ACSF and measure the tissue's characteristics quickly [10,11,12].

Brain slices are two-dimensional; this restricts us to 2-d or 1-d measurements. Both have been established [11,12]. With a two-dimensional method, exploiting van der Pauw's theorem, there is a need to switch voltage and current electrodes making a time-consuming process to make measurements at several frequencies. We have therefore been restricted to 1-d measurements if we require a broad frequency band.

In this work we have optimised the 1-d method for brain tissue. We have been able to make fast (low tissue degredation), low-noise, low-inductance measurements from 20 Hz - 2 MHz at a controlled temperature. Chemical effects at the electrodes have been minimized. We also have shown that a Cole-Cole element fits the high-frequency (>10 kHz) dispersion well.

2. Method

2.1 Electrodes and equipment

Silver-silver chloride Electrodes have been used since they provide a low noise biologically compatible interface with the tissue. Silver rod of 5 mm diameter was cut into several cylinders. A pair of these were polished manually and chloride chemically. They were mounted in a bespoke Perspex sandwich device that ensures the electrodes are d = 400 μm apart when closed. Contacting wires were kept short. The device is shown in Figure 1.

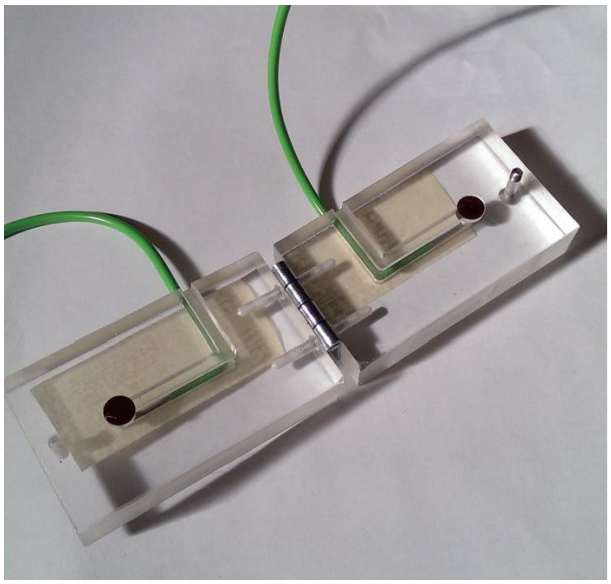

Figure 1: The sandwich device constructed for the measurements

An Agilent E4980A four-point impedance meter was used to measure the impedance Z (Ω) of each sample at two hundred points logarithmically spaced across the frequency range 20 Hz – 2 MHz. Low current was used to prevent chemical effects occurring at the electrodes. A bespoke computer programme was used to control the data acquisition and calculate bulk impedance z (Ω m) via

$$z = Z\,A/d \qquad (1)$$

where A is area of the sample and d its thickness. The temperature of the Perspex sandwich was monitored with a thermocouple mounted at one of the electrodes. Although a PID temperature control system was constructed with a Peltier device and heat pipe to keep the holder at 25°C between measurements, it too cumbersome to use during these measurements.

In setting-up and optimizing the hardware for the experiment, we found parsnip to be a convenient surrogate to brain tissue since it had similar impedance and similar mechanical properties.

2.2 Preparation of tissue

Mouse brains were extracted and sliced using established methods [13]. Three mice were used, one per day. However, technical problems with equipment meant that the quality of slices from the third mouse was much reduced and this data has been excluded from our analysis.

Slices were alternately placed in 'normal' or 'magnesium-free' ACSF [11]. The latter promotes seizure-like activity to occur [14]. For the case of the latter, electrical activity was monitored with a tungsten electrode at six points across the cortex. This allowed identification of which parts of the cortex could be considered 'active'.

Sections (approximately 2 mm × 2 mm) of the cortex of each slice were cut with a razor blade. These sections were photographed with a calibrated microscope and their cross-sectional area determined with computer software. An example photograph is shown in Figure 2.

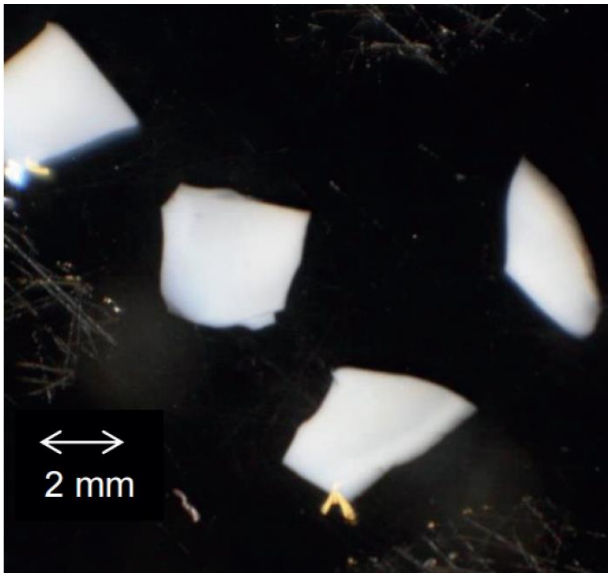

Figure 2: A photograph of cut sections of cortex

2.3 Making measurements

One sample at a time was transferred to the sandwich device. A drop of ACSF was used to wash the sample onto an electrode. Excess fluid was removed with filter paper. The sandwich was closed and a constant pressure applied with a calibrated clamp. Impedance was then measured. After measurement, the sandwich was opened and the sample was flushed away with distilled water. The next sample was then measured.

3. Results

The results showed low electrical noise and low inductance across the whole frequency range. They did not drift when frequency was swept up, down, up then down again. Electrode temperature was within 1°C of 25°C for all measurements.

3.1 Conductivity and impedance measurements

The conductivity of the sections was around 0.2 S m^{-1} in the approximate range 1 kHz – 100 kHz. Outside this

range there was strong frequency-dependence. Figure 3 shows results for bulk impedance z (real and imaginary parts) for the samples prepared in normal ACSF.

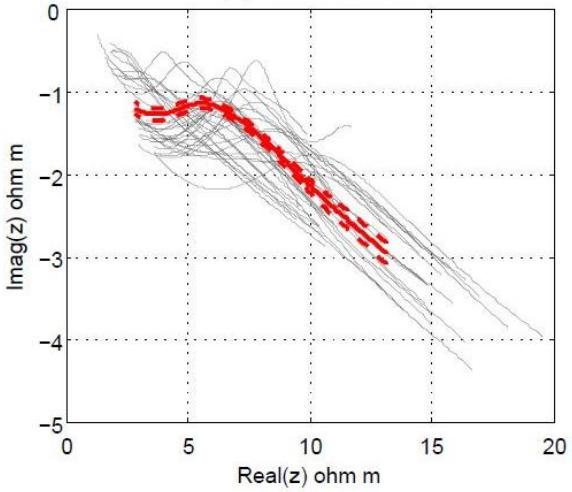

Figure 3: Nyquist plots of bulk impedance [Re(z) against Im(z)] from 20 Hz (right-hand edge) to 2 MHz (left-hand edge) for samples prepared in normal ACSF. Individual samples are shown with thin grey lines, the mean response and the standard uncertainty in the mean are shown by the thick solid line and the thick dotted lines respectively.

There was some variation apparent between conductivities of the three groups (1. samples prepared in normal ACSF, 2. samples prepared in magnesium free ACSF from sections of the cortex where activity was measured, 3. samples prepared in magnesium free ACSF where activity was *not* measured). Figure 4 shows the variation in conductivity [defined as 1/Re(z)] at 10 kHz for the three groups. The conductivity of group 2 samples is significantly lower than those of group 1 and 3 samples.

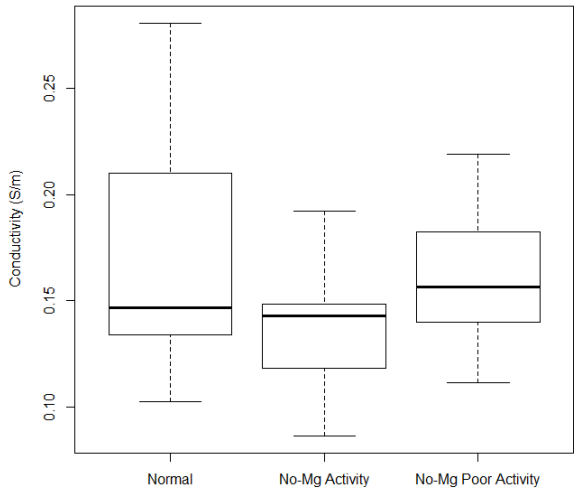

Figure 4: A box-plot of the conductivity from the three sample groups at 10 kHz.

3.2 Fitting the Cole-Cole model

The Cole-Cole model [6,7] is well-used for fitting the dispersive characteristics of biological tissue. It is a simple combination of resistive and non-linear capacitive elements. The circuit diagram is given in Figure 5; the impedance is given by

$$z(\omega) = R_A + \frac{R_B}{1+(j\omega\tau)^\beta}. \qquad (2)$$

where τ is a time-constant, ω the angular frequency and β a parameter ($0 < \beta < 1$) describing the degree of linearity in the response.

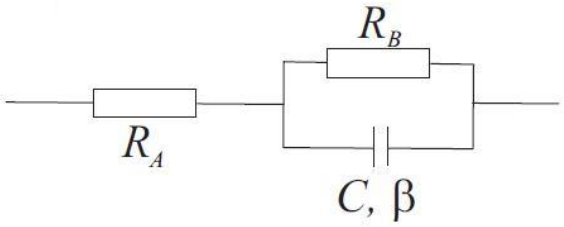

Figure 5: The Cole-Cole model of dispersion in biological material.

Parameters were fitted to the high-frequency dispersive region (> 10 kHz) using a Monte Carlo method. For the majority of samples, the model described the experimental results very well. This allowed distributions of the parameters for the various conditions to be extracted.

4. Discussion

Overall, we have been able to produce results that are low noise, show no indication of stray inductance, and are at consistent temperatures. Results are robust to repeated measurements on the same sample and show no drift with repeated measurements over different samples. High frequency results are consistent with a Cole-Cole model of biological impedance.

However, the method still has several problems. The first is that tissue is sometimes squashed by the sandwich device. That leads to an ill-defined surface area. A guarded electrode set-up [8] with a defined area for the measurement electrode would prevent this but will be difficult to manufacture at such a small scale. Secondly it is not apparent what happens at low frequency – the dispersion 'curve' is a straight line on the Nyquist plot at 20 Hz. Different equipment would be needed to move to lower frequencies. Lower-frequency data would be useful since mouse electroencephalographic rhythms are in the range 1 – 80 Hz [15]. From a neuroscience point of view, we have been unable to establish significant differences between the group 3 samples (magnesium-free ACSF without activity) and the other two groups, meaning we are unable to comment on the nature of the change in conductivity between the seizing and non-seizing samples.

5. Conclusion

We have established a methodology to measure the bulk impedance of samples of brain tissue in vitro, across a broad frequency range. Some development and optimization is still required. We hope to use the

method to inform numerical models of the electrical activity of the cortex and comment on the role of inter-cellular electrical connections in seizures.

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