Published in final edited form as:

J Neurosci Methods. 2009 May 30; 180(1): 87. doi:10.1016/j.jneumeth.2009.03.012.

A method for recording resistance changes non-invasively during neuronal depolarization with a view to imaging brain activity with Electrical Impedance Tomography

Ori Gilad^{1,2}, Anthony Ghosh¹, Dongin Oh¹, and David S Holder¹

¹ Departments of Clinical Neurophysiology and Medical Physics, University College, London, UK

² Abramson Center for Medical Physics, Sakler Faculty of Exact Sciences, Tel-Aviv University, Tel-Aviv, Israel

Abstract

Electrical Impedance Tomography (EIT) is a recently developed medical imaging method which has the potential to produce images of fast neuronal depolarization in the brain. The principle is that current remains in the extracellular space at rest but passes into the intracellular space during depolarization through open ion channels. As current passes into the intracellular space across the capacitance of cell membranes at higher frequencies, applied current needs to be below 100 Hz. A method is presented for its measurement with subtraction of the contemporaneous evoked potentials which occur in the same frequency band. Neuronal activity is evoked by stimulation and resistance is recorded from the potentials resulting from injection of a constant current square wave at 1 Hz with amplitude less than 25% of the threshold for stimulating neuronal activity. Potentials due to the evoked activity and the injected square wave are removed by subtraction. The method was validated with compound action potentials in crab walking leg nerve. Resistance changes of $-0.85 \pm 0.4\%$ (mean $\pm$ SD) occurred which decreased from $-0.97 \pm 0.43\%$ to $-0.46 \pm 0.16\%$ with spacing of impedance current application electrodes from 2 to 8mm but did not vary significantly with applied currents of 1–10 μ A. These tallied with biophysical modelling, and so were consistent with a genuine physiological origin. This method appears to provide a reproducible and artefact free means for recording resistance changes during neuronal activity which could lead to the long-term goal of imaging of fast neural activity in the brain.

1. Introduction

Functional neuroimaging has improved greatly in the past two decades but the ‘holy grail’ would be to image neuronal activity non-invasively with a time and spatial resolution of about 1 ms and 1 mm respectively. There has recently been a resurgence of interest in this goal, and the techniques of source modelling of the EEG (Baillet et al., 2001) and MEG (Hamalainen et al., 1993; Baillet et al., 2001), their multimodality fusion with MRI (Dale et al., 2001), direct mapping with MRI (Hagberg et al., 2006; Parkes et al., 2007) and diffuse optical tomography

Corresponding author: Dr Ori Gilad, Address: 29 Inbar Street, Zichron Ya'akov 30900 Israel, Phone/fax: +972 775035037, Mobile: +972 525909921, o.gilad@ucl.ac.uk.

Prof David S Holder, Dr Anthony Ghosh and Dr Dongin Oh: Address: Department of Medical Physics & Bioengineering, Malet Place Engineering Building, University College London, Gower Street, London WC1E 6BT, UK

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

(Syre et al., 2003; Steinbrink et al., 2005) have been investigated but currently cannot achieve this goal. Electrical impedance Tomography (EIT) is a novel medical imaging method which has the potential to achieve this revolutionary advance, by imaging electrical impedance changes over milliseconds (Holder, 1987) which occur when neuronal ion channels open during activity and the cell membrane resistivity decreases (Cole et al., 1939). The underlying purpose of this work was to develop a method which could form the basis for tomographic imaging of resistance changes during neuronal depolarization in the brain.

1.1. Introduction to Electrical Impedance Tomography and its potential use in imaging neural activity

EIT provides information regarding the internal electrical properties inside a body based on non-invasive voltage measurements on its boundary. Data acquisition is performed through an array of electrodes which are attached to the boundary of the imaged object. Sequences of small insensible currents, typically about of 1 mA, are injected into the object through these electrodes and the corresponding boundary electric potentials are measured over a predefined set of electrodes. The process is repeated for numerous different configurations of applied current. The internal admittivity (or impedivity) distribution can be inferred using this boundary data. EIT was first proposed as a medical imaging method by Henderson and Webster (1978) and was initially applied to chest imaging (Brown et al., 1985; Brown et al., 1987; Metherall et al., 1996). Potential applications of EIT for imaging brain function and pathology include detection and monitoring of cerebral ischemia and haemorrhage (McArdle et al., 1988; Holder, 1992a; Gibson et al., 2000; Romsauerova et al., 2006; McEwan et al., 2006), localisation of epileptic foci (Bagshaw et al., 2003; Fabrizi et al., 2006), normal haemodynamic brain function (Tidswell et al., 2001) and neuronal activity (Holder, 1987; Boone et al., 1995).

The principle by which EIT could image neuronal activity rests on the application of low frequency currents below about 100 Hz which remain in the extracellular space under resting conditions because they cannot enter significantly into the intracellular space across the capacitative cell membrane. During the action potential or neuronal depolarization, the membrane resistance diminishes by about 80x (Cole et al., 1939) so that the applied current enters the intracellular space as well. Over a population of neurones, this will lead to a net decrease in the resistance during coherent neuronal activity, such as cortical evoked responses, as the intracellular space will provide additional conductive ions (Boone et al., 1995; Liston et al., 2000; Liston, 2004).

An important advantage of this potential application of EIT over inverse source modelling of the EEG or MEG is that this mechanism effectively rectifies the recording of ionic channel opening – resistance across the membrane can only fall. In this way, impedance falls irrespective of whether the neurotransmitter giving rise to the change is excitatory or inhibitory. Neuroelectric or neuromagnetic signals cancel out when measured from a distance unless the neuronal processes are spatially aligned, as in the dendritic tree of the pyramidal cells. The rectified resistance change could capture activity in the entire depolarized tissue, regardless of the spatial arrangement, so the opportunity to record the changes from a distance are much greater.

In addition, the inverse solution for EIT is in principle unique for the idealised case where there is complete and noise free knowledge of all boundary potentials with all possible current injections and sufficient “smoothness” of the unknown conductivity profile (Calderón, 1980; Isaacson et al., 1989) unlike that for inverse source modelling where a variety of constraints of indeterminate validity must be employed to give a solution (Bleistein et al., 1977). However, some constraints are also needed in EIT solutions in practice, where the inverse solution is ill-posed and there are discrete measurements and instrumentation errors. Nevertheless, in

experimental studies, blurred but consistently reliable images may be obtained for test objects in saline filled tanks (Holder, 2005).

1.2. Magnitude of impedance changes in the brain due to fast neural activity

The magnitude of such fast changes in the brain has been investigated by modelling and animal studies in our group. Mathematical modelling, based on cable theory, estimated local resistivity changes near DC to be 2.8–3.7% for peripheral nerve bundles and 0.06–1.7% for the cortex during Evoked Potentials (EP) (Boone et al., 1995; Liston et al., 2000; Liston, 2004). These predictions are in broad agreement with measured near DC decreases of 0.5–1.0% in crab peripheral nerve (Holder, 1992b; Boone, 1995; Liston, 2004) and 0.01–0.03% from preliminary measurements on the surface of rabbit cortex during median nerve evoked responses (Boone, 1995). The measured changes on the rabbit cortex are about $\times 10$ lower than the estimated local change probably due to partial volume effect.

There are several reports in the literature of attempts to record impedance changes during evoked activity in neural tissue. The frequency of applied current is a critical factor as the modelling above indicates that, in theory, the magnitude of changes expected falls off rapidly with increasing frequency over about 100 Hz. This is because, above this frequency, current starts to pass into the intracellular space at rest by crossing the capacitance of cell membranes; there is therefore a much smaller change when ion channels open during activity and current starts to pass into the intracellular compartment by this resistive route. Resistance decreases of $3.1\% \pm 0.8$ (SE) have been recorded during direct cortical stimulation in the cat, using a four electrode system and square wave pulses 0.3–0.7 ms in duration (Freygang et al., 1955) which were ascribed to membrane resistance changes of dendrites. However, no calibration data were presented, so it is unclear if these unexpectedly large changes were artefactual. Using a two electrode system (which may underestimate the changes) and a Wheatstone bridge operating at 35 kHz, a decrease of 0.03 – 0.1% was reported in frog sciatic nerve (Chailakhian et al., 1957). Their experimental technique was subsequently used by others (Burlakova et al., 1959; Prudnikova, 1959) to investigate the time relationship between the action potential and the putative impedance change. During visual and auditory evoked responses in the cat, and with a similar recording system operating at 10 kHz, a decrease of 0.003% in the cortex (Klivington et al., 1967; Klivington et al., 1968), and of 0.03% in subcortical nuclei (Galambos et al., 1968) was observed. The difference in results may partly be because evoked responses (ER), with natural stimulation, activate fewer fibres than the electrical stimuli used by Freygang and Landau (Freygang et al., 1955). However, a later attempt on rats using direct electrical stimulation of the cortex and measurement at 50 kHz did not show any changes larger than the sensitivity of the measurement of 0.01% (Holder et al., 1988).

Holder (1989) recorded impedance changes at 50 kHz with scalp electrodes in human subjects during somatosensory, auditory and visual evoked responses, and was unable to detect any reproducible changes larger than 0.002 or 0.02% (depending on electrode configuration and period of measurement). The negative results could be ascribed to the measurement at 50 kHz instead of low frequencies below 100 Hz, shown in later studies to be essential (Boone et al., 1995; Liston et al., 2000; Liston, 2004).

1.3. Propagation of local resistance changes to the scalp

The amplitude of the changes locally in the brain is therefore not accurately known but a reasonable value, based principally on the biophysical cable theory modelling, could be taken as a 1% decrease during physiologically evoked activity if recorded with applied current below 100 Hz (Boone et al., 1995; Liston et al., 2000; Liston, 2004). For non-invasive recording of related changes with scalp electrodes, this change will be diluted by partial volume effects and by diversion of applied current by the resistive skull. This has been modelled using an

anatomically realistic Finite Element (FE) model of the head, validated with a sponge perturbation in a saline filled tank and standing scalp voltages measured in humans (Gilad et al., 2009b). For the estimated resistance change of 1% in a 9 cm³ of grey matter in the primary visual cortex (Andrews et al., 1997) with 10% of active dendrites (Liston, 2004) during visual evoked responses and the maximal permitted applied current of 1 mA at 1Hz, the peak scalp voltage changes were $0.0039 \pm 0.0034\%$ ($1.03 \pm 0.75 \mu\text{V}$; Mean $\pm$ 1SD). The SD reflects the uncertainty in the literature over the conductivity selection for the different head compartments. The study also suggested that the greatest changes would be recorded with current injection with a pair of electrodes 5 or 10 cm apart and recording immediately lateral to these over the occiput (Gilad et al., 2009b).

1.4. Purpose

The ultimate purpose of this work was to develop a method for recording resistance changes during neuronal depolarization in the brain, with the intention of using it to form EIT images of neural activity in the future. Such neural images might be with epicortical arrays or scalp electrodes, and in humans or animal studies. The advantage would be that images would be formed without the use of electrodes which penetrate into the substance of the brain.

Biophysical modelling has shown that recording should be below 100 Hz (Boone et al., 1995; Liston et al., 2000; Liston, 2004), so we have used a method based on current injection with a square wave at 1 Hz. Unfortunately, the resulting induced voltage changes which may be ascribed to impedance changes are in the same frequency band as the spontaneous EEG and evoked potentials. It was therefore necessary to develop a robust method for recording the intrinsic resistance changes without contamination with these other signals.

The purpose of this paper is to present this method (termed “LF-EIT” (low frequency EIT)). It has been developed and refined using the compound action potential in crab peripheral nerve, in which the resistance change occurs contemporaneously with the compound action potential. Experimental results for this preparation are presented to demonstrate use of the method and provide evidence that the changes observed appear to be physiological and are not artefactual. The method has in part been presented before (Holder, 1992b; Boone et al., 1995; Liston, 2004); the objective of this manuscript is to present it completely and in detail and with the new additions of using a bi-polar square wave with paired control recording and linear model formulation for calculating the resistance changes detailed below. Presentation of human recordings is outside the scope of this detailed presentation of the method; preliminary measurements in human subjects with scalp electrodes during visual evoked responses (VER) are reported elsewhere (Gilad et al., 2009a).

2. Materials and Methods

2.1. Rationale

The principle of the method is to inject a constant current square wave and estimate the resulting resistance changes from the recorded voltages at different nearby electrodes. Averaging is necessary to improve the SNR, so the signal is time locked to repeated evoked neurophysiological activity (in practice a compound action potential in crab nerve or, eventually, cortical evoked responses in humans). The resulting voltage will contain three elements – 1) The square wave carrier 2) The evoked neurophysiological potential and 3) A change in the square wave due to a transient resistance change. In principle, the evoked neurophysiological activity may be subtracted by making a paired recording without the square wave carrier signal (Holder, 1992b) but a more efficient method is to use a bipolar square wave; addition of the two phases yields the compound action potential and subtraction yields the square wave with the resistance change (Boone, 1995). In principle, the resistance change could

then be simply produced by subtraction of the square wave. However, in practice, the carrier itself was distorted by use of a coupling high pass filter and non-idealities at the electrodes, and became a decaying exponential which varied unpredictably. This was therefore removed by making a paired control recording with the square wave carrier but no neurophysiological activity.

The principle of removing the evoked neurophysiological activity by subtraction rests on the assumption that it remains constant and is unaffected by the current used to record the impedance change. An essential part of the development of the method was therefore to establish the current level which gave as high a signal to noise ratio as possible but did not affect the evoked activity. In practice, this was set at about 10% of the threshold for initiation of a neurophysiological effect.

The endogenous potentials generated by neuronal activity were termed v and in this study were in the form of compound action potentials (CAP) produced during crab nerve stimulation. These stimuli were applied during both cycles of the square wave. The baseline voltage produced by the passage of square wave current was termed b and during neuronal activity, a resistance decrease will produce a deflection δ on the square wave baseline. The resulting signal V recorded was a composite signal which comprised three components: neuronal activity signal v , bi-polar square wave baseline b , and a deflection in the baseline due to resistance decrease δ (Fig. 1).

In practice, the square wave b was 2 orders of magnitude larger than δ and the non-ideal nature of the square wave obscured the transient signals. This waveform therefore varied from experiment to experiment and we elected to subtract it empirically by making a paired control recording without stimulation so that v and δ were not present. This provided us with a reference recording for b which was used to subtract the non-ideal square wave from the active recording with the stimulation (Fig. 2). The non-idealities had a low bandwidth ($<3\text{Hz}$) as they originated from capacitive coupling at the electrodes and changes in contact impedance which slightly changed the magnitude of the square wave voltage signal. These slow variations were reproducible between the active and control recordings while any rapid non-idealities which could obscure the rapid resistance changes were negligible.

An LF-EIT recording consisted of a 1 minute “control” recording with only a 1 Hz square wave with $n = 60$ cycle periods followed by an “active” 1 minute recording with stimulation of compound action potentials at 2 Hz (120 stimuli) in addition to the square wave. 1 minute was chosen as a reasonable period for prevention of drying of the nerve before it was irrigated between recordings with bathing solution. The estimation of the v and δ signals and their variances was obtained by averaging all square wave cycles through the above subtraction and summation procedure (Fig. 2) and a linear model derived for this purpose (see Appendix A).

Another effect which interfered with the separation of the different components was the difference in the square wave amplitude between the control and active recordings due to drying of the nerve during recording, which increased the total resistance. This effect was included in the linear model by setting a different gain for the control and active data (see Appendix A).

In order to remove ringing artefacts caused by the polarity transitions of the square wave, the 500 ms cycles of the square waves had sections from 0 to 100 ms and 400 to 500 ms removed so that the final trace from each cycle was 300 ms long.

2.2. Potential artefact due to perturbation of the compound action potential or evoked response by applied current

The main possible source of artefact when using this technique was the possibility that the injected measuring current altered neuronal activity. This could have resulted in a different compound action potential in the presence of positive and negative phases of the square wave current which would have caused an artefactual apparent resistance change after subtraction of the two phases. In principle, it could be difficult to distinguish this artefactual change from a genuine resistance change, as they occur in the same bandwidth.

The most convincing method for disproving the presence of such artefact appears to be to examine the relationship of the observed resistance change with respect to applied current level. When the current level is high enough to cause such artefact, increasing current (in either direction) might be predicted to cause an increasing artefactual change but this would not be linear, as the opening or closing of ion channels is non-linear. However, the potential difference due to any genuine resistance change would be linearly related to the applied current i.e. the proportional change would be independent of applied current. This effect could be obviated in the case of 1D crab nerve recording by placing the first recording electrode several space constants (the distance along an axon at which the potential due to applied current falls to $1/e$ of its value) proximal to the measuring current electrodes, so that the measured CAP would be unaffected by any such effect of the applied current, and a virtual monopolar recording was achieved by placing the second differential recording electrode very distal so that the CAP had dispersed (Holder, 1992b). Further validation is to record the resistance changes using different spacing between the current drive electrodes. For larger spacing, the proportion of non-activated tissue between the drive electrodes increases and proportional resistance change is expected to decrease (Holder, 1992b). In addition, the delay between the CAP and the resistance change increases for larger spacing since the location of the measured resistance change is between the drive electrodes. For larger spacing, this becomes more distal to the site of CAP measurement.

2.3. Crab nerve preparation

Nerves were extracted from the walking leg of the edible crab (Holder, 1992b), *Cancer pagurus*, and were placed within an electrode array (Fig. 3). The nerve was placed in a square groove 1.5 mm wide by 1.5 mm deep in a block of Perspex (20×4 cm). Electrodes comprised platinum or chloride silver foil, (1.5 mm wide), placed in perpendicular grooves, 1.5 mm wide by 3 mm deep, except for the ground electrode which was 2 mm wide. Since silver is toxic to nerves, all electrodes were made of platinum apart from the measurement electrodes R1 and R2 which were silver/silver chloride in order to yield low noise from the electrode-electrolyte interface. The central 1 cm or so of the electrode grooves near the nerve were filled with Agar equilibrated with Crab Ringer's solution, in order to avoid direct contact of the electrode metal with the nerve. The remaining part of the groove was filled with silicone rubber glue to cover the remaining electrode wire and copper wires soldered to them which led away to the recording apparatus. The entire array was kept at a temperature of 4°C by bathing in ice water.

9 electrodes were used and comprised two for stimulation of the action potential (S1, S2), ground electrode (G), four for injection of the square wave current for resistance measurements (D1-D4 – two were selected at a defined spacing for any one recording) and two for recording the resulting potentials (R1, R2). The spacing between electrode pairs S1-S2, S2-G, G-R1, and D1-D2 was 2 mm; R1-D1 was 3 mm; D1-D3 was 4 mm; D1-D4 was 8 mm and D4-R2 was 100 mm (Fig. 3). These spacing values are between the outer edges of the electrodes.

Once placed within the longitudinal groove, the nerve was immersed in crab Ringer's solution made of 525mM/l NaCl, 13.3mM/l KCl, 12.4mM/l CaCl₂, 24.8mM/l MgCl₂ and 5mM/l

Dextrose (Schei et al., 2008). The nerve was blotted using filter paper at the start of each 1 minute recording and then irrigated again as soon as it was completed.

2.4. Instrumentation

The acquisition system comprised a Neurolog NL900D case (Digitimer, Welwyn Garden City, UK), DC coupled pre-amplifier (NL106, common mode rejection ratio > 68 dB @ 50 Hz and input impedance > 1 M Ω .) set to a gain of 50, low pass filter of 1kHz (NL125/6) and National Instruments (Austin, Texas, USA) analog to digital converter (NI USB 6259) set to ± 1 V dynamic range (covering ± 20 mV after the pre-amp), 10kHz sampling rate and with 16 bits resolution enhanced to 18 bits (76 nV) using x16 oversampling (Fig. 4). A custom made communication box and control units controlled the timings of the square wave current source, pulse buffer (NL510A) and stimulus isolator (NL800A, Digitimer) as in Fig. 1.

The current source was a custom made isolated bipolar square wave current source with maximal current of 100 μ A (Boone, 1995) with a negligible bias of 10^{-5} under typical load of 1k Ω . Prior to each recording, the current source was calibrated so that the DC offset was <1%. A 0.1 μ F capacitor was connected to the output of the current source so that with a typical load of 1 k Ω the bandwidth of the square wave applied to the nerve was limited to approximately 1.5 kHz. Since the power spectrum of a square wave decays with frequency as 1/f, 70% of the power was applied below 100 Hz and the remaining 30% were applied up to 1.5 kHz. This square wave resistance measuring current did not produce neural stimulation as the current levels used were less than 25% of the threshold for stimulating neuronal activity.

2.5. Experimental protocols

The CAP was initiated using 2 stimuli/sec of 3–10 mA and 0.5 ms duration through electrodes S1-S2; the current was adjusted to provide supramaximal stimulation, defined as 50% above the lowest level which produced a maximal largest compound action potential. A dataset of 57 one minute control and active recordings were acquired from 8 nerves. For studying the effect of current level, the D1-D2 spacing of 2 mm was fixed and 8 recordings with currents of 1, 2, 5, 10, 10, 5, 2 and 1 μ A were recorded from 4 nerves (33 recordings; one extra recording at 5 μ A). For studying the effect of current drive spacing, the current level was fixed to 2 μ A and 6 recordings with spacing of 2, 4, 8, 8, 4 and 2 mm were recorded from 4 nerves (24 recordings). The signals were always recorded through electrodes R1 and R2 and the spacing of 2, 4 and 8 mm were obtained using drive electrodes D1-D2, D1-D3 and D1-D4 respectively.

2.6. Data analysis

First, the v (CAP) and δ (resistance changes) waveforms were averaged and derived from each 1 minute recording using the above method. Then, in addition to the 1 kHz hardware low pass filter applied during the acquisition, an 8th order Butterworth low pass filter with cut off frequency of 500 Hz was applied to the final waveforms to improve the SNR. For preventing phase distortions by this infinite impulse response filter, the data was passed through in both backward and forward directions.

The maximal change (most negative value as resistance changes are negative) was determined for each of the 57 recordings. The delay from the stimulus of these peak changes and the CAPs was also measured. The SNR for each individual recording was estimated as the ratio between the absolute maximal change and rms of the noise during the pre-stimulus time. 4 recordings were excluded due to external interference. An additional 5 pairs of recordings made from the same nerve in close time proximity were excluded due to inconsistency of more than 50% in the magnitudes of the resistance changes. These inconsistencies were probably due to the delicate process of blotting most of the Ringer's solution before each recording which produced

variable shunting of measuring current through the solution instead of mainly flowing through the nerve.

The waveforms of the remaining 43 recordings are presented. Significance of changes over time was determined with Student's t-test for negative mean with a critical value of $p < 0.05$ (Appendix A). One way analysis of variance (ANOVA) was used to test whether the means and delays for different current or spacing conditions were different. Unless stated otherwise, data are presented as mean $\pm$ 1 SD.

3. Results

Significant resistance changes of -0.85 ± 0.4 % (range -0.2 to -2.0 %) were observed across the 43 valid recordings with a similar timing to the CAPs which had a peak amplitude of 10 ± 2.4 mV (range 5.8 to 16.1 mV) (Fig. 5). The SNR of individual recordings was 80 ± 50 (range 17 to 194). There was no significant difference in the resistance changes with different applied currents ($p = 0.67$; $F = 0.53$; $df = 28$; Fig. 6a). There was a significant decrease in the resistance change with increasing separation of current drive electrodes – it decreased from -0.97 ± 0.43 % at 2 mm separation of D1 and D2 to -0.46 ± 0.16 % at 8 mm separation ($p = 0.02$; $F = 4.73$; $df = 25$; Fig. 6b).

The delay from the stimulus to the peak of the CAP was 3.3 ± 0.2 ms (range 3 to 3.7 ms) and was constant when varying both current levels ($p = 0.37$; $F = 1.07$; $df = 28$; Fig. 7a) and current drive spacing ($p = 0.68$; $F = 0.4$; $df = 25$; Fig. 7b). Assuming that the CAP originated from S2, the conduction velocity was 2.6 ± 0.2 m/s.

The delay from the stimulus to the peak resistance change in δ was 5.7 ± 0.4 ms (range 5 to 6.5 ms) and was constant when varying current level ($p = 0.86$; $F = 0.25$; $df = 28$; Fig. 7a). Assuming that the resistance change occurred between the two drive electrodes, 14.75 mm from the mid-point between S2, the propagation speed of the resistance change was 2.6 ± 0.1 m/s, similar to that of the CAP. When increasing the current drive spacing from 2 to 8 mm, this delay increased from 5.7 ± 0.3 ms to 7.9 ± 0.4 ms ($p = 7e-9$; $F = 47$; $df = 25$; Fig. 7b) as would be expected from further propagation of the CAP before the resistance change is measured as well as dispersion due to different propagation speeds in nerves with variable diameters.

4. Discussion

4.1. Summary of method and results

A method has been developed for the non-invasive measurement of rapid resistance changes due to synchronous ion channel opening in neuronal membranes during depolarization. Modelling studies and consideration of the underlying physiology unfortunately suggested that recording needed to be below 100 Hz (Boone et al., 1995; Liston et al., 2000; Liston, 2004). As mentioned above, this is because the mechanism of the resistance change is that current is restricted to the extracellular space at rest but flows into the intracellular compartment through ion channels as they open during depolarization. Modelling suggested that significant current starts to flow into the intracellular compartment through the membrane capacitance at frequencies above 100 Hz, which would decrease the amplitude of the expected change. Unfortunately, this requires recording in the band of the endogenous cortical EEG or evoked potentials. In principle, the endogenous activity therefore had to be subtracted as it could not be removed by filters directly as it was in the same frequency band. In principle, this could be accomplished by simple paired stimulus and subtraction method (Holder, 1992b) but more efficiently achieved using a scheme of summation and subtraction of two phases of a bi-polar square wave carrier (Boone, 1995). The original method of Boone was extended in this study

by introducing an additional control recording to compensate for variable baseline decay. In addition, a linear model was introduced for deriving the model parameter, their standard error and testing for significant changes.

In this study, the method has been validated in a set of recordings on crab nerves during the compound action potential which showed appropriate changes which did not appear to be artefactual and were of the same direction and similar magnitude as those measured previously (Holder, 1992b; Boone, 1995; Liston, 2004) and predicted by a biophysical model (Boone et al., 1995; Liston et al., 2000; Liston, 2004).

Peak changes of $-0.85 \pm 0.4\%$ ($n = 43$) during CAP of 10 ± 2.4 mV were measured with high SNR (80 ± 50). These did not vary with different currents ($p = 0.67$) and decreased with increasing drive electrode spacing ($p = 0.02$).

The peak of the resistance changes occurred 2.4 ms after the peak of the CAP since the resistance change measurement was more distal than the CAP measurement. This delay was fixed for different current levels ($p = 0.86$) but increased when the current drive spacing increased ($p = 7e-9$) as the resistance change measurement site became more distal.

4.2. Technical issues

Although the resistance change in individual 1 minute recordings had a high SNR, they were variable between repeated recordings under the same conditions (Fig. 5 and Fig. 6). The main uncontrolled variable which appeared to contribute to this variability was the difficulty with blotting a similar amount of Ringer's solution prior to each recording. Any remaining solution might be expected to carry some of the applied current instead of it passing through the nerve. This decreased the observed resistance change. Another possible uncontrolled variable was deterioration in the change of the nerve health during the recording session so repeated recordings from the same nerve would gradually decrease as more nerve fibres die. This was partly overcome when we applied a reversed protocol within each nerve (e.g. 2, 4, 8, 8, 4, 2 mm spacing) and excluded pairs of identical recordings which had >50% difference in their peak changes. Although methods have been described in which a nerve may be placed in a humidified chamber (Shanes, 1949) or liquid paraffin (Chapman, 1966), we elected to use this method as nerve survival appeared to be much better with repeated irrigation with Ringer's solution. Unfortunately, continuous irrigation was not practicable as this would have formed a much greater shunt path for applied current.

Work in progress is to implement an analytical baseline correction which could discard the need for a control recording and reduce the total recording time by 50% (Oh et al., 2008). This would be important for human measurements when long averaging would be required to overcome background EEG noise.

4.3. Possible artefact due to alteration of the compound action potential by the applied measuring current

The main possible source of artefact when using this technique was the possibility that the injected measuring current altered neuronal activity which could have resulted in a distorted evoked response or compound action potential which could yield an artefactual apparent resistance changes. In these crab nerve recordings, the first measuring electrode was proximal to the measuring current electrodes, so that the measured CAP would be unaffected by any such effect of the applied current and a virtual monopolar recording was achieved by placing the second differential recording electrode very distally so that the CAP had dispersed (Holder, 1992b). Furthermore, the proportional resistance changes were not significantly different when varying the applied current ($p = 0.67$). This supports the assumption that the applied current

did not alter neuronal activity and that the changes were not artefactual. This result is consistent with previous studies which employed a simpler subtraction method (Holder, 1992b).

Further support for the validity of the results was the significant decrease of the resistance change with increasing current drive spacing ($p = 0.02$, $n = 25$). For larger spacing, the proportion of non-activated tissue between the drive electrodes increases and proportional resistance change is expected to decrease (Holder, 1992b). Furthermore, the resistance changes were delayed relative to the CAP as they were measured more distally and this delay increased with increasing current drive spacing ($p = 7e-9$). This suggests that the measured changes originated more distally where the current drive electrodes were located and were not due to artefact in the CAP which occurred more proximally and measured at electrode R1.

4.4. Implications for Electrical Impedance Tomography of neural depolarization in the brain

The original purpose of developing this method was to enable tomographic imaging of fast neural activity in the brain. At present, it is not clear if this method could yield images with scalp electrodes which are sufficiently accurate for clinical or experimental routine use. In a modelling study using an anatomically realistic FE model of the head, peak scalp voltage changes of $0.0039 \pm 0.0034\%$ ($1.03 \pm 0.75 \mu\text{V}$; Mean $\pm$ 1SD) were estimated to occur during local resistance change of 1% in a 9 cm^3 of grey matter in the primary visual cortex during visual evoked responses (Gilad et al., 2009b). In the human case, long averages are required for sensing such changes which are of the order of $1 \mu\text{V}$. Direct validation that any measured changes are genuine by comparing the changes measured by different current levels would be difficult since the current levels which could be used are limited and the measuring electrodes could not be realistically placed proximal to the active neuronal processes.

The method is presented in the hope of stimulating further development with which technical innovations might improve the signal to noise ratio. Even if non-invasive imaging with scalp electrodes is not possible, this method could be used for recording from peripheral nerve, which would give different information to nerve conduction studies, or else be used for EIT with intracranial electrodes in animal studies or the available special situation of epilepsy patients with implanted subdural electrodes. The latter studies are in progress (Gilad et al., 2008). If reliable images can be produced in these research applications, this would still constitute a significant addition to neuroscience technology.

Acknowledgments

Epilepsy Research Foundation, UK; Ministry of Science & Technology, Israel; and The National Institutes of Health (NIH), US under Grant 5R01EB006597-03 from the National Institute Of Biomedical Imaging And Bioengineering (NIBIB) and the National Eye Institute (NEI).

References

- Andrews TJ, Halpern SD, Purves D. Correlated size variations in human visual cortex, lateral geniculate nucleus, and optic tract. *J Neurosci* 1997;17:2859–68. [PubMed: 9092607]
- Bagshaw AP, Liston AD, Bayford RH, Tizzard A, Gibson AP, Tidswell AT, Sparkes MK, Dehghani H, Binnie CD, Holder DS. Electrical impedance tomography of human brain function using reconstruction algorithms based on the finite element method. *Neuroimage* 2003;20:752–64. [PubMed: 14568449]
- Baillet S, Moshier JC, Leahy RM. Electromagnetic brain mapping. *IEEE Signal Processing Magazine* 2001;18:14–30.
- Bleistein N, Cohen KJ. Nonuniqueness in the inverse source problem in acoustics and electromagnetics. *Journal of Mathematical Physics* 1977;18:194–201.
- Boone, KG. PhD dissertation. University College London; London, UK: 1995. The possible use of applied potential tomography for imaging action potentials in the brain.

- Boone KG, Holder DS. Design considerations and performance of a prototype system for imaging neuronal depolarization in the brain using 'direct current' electrical resistance tomography. *Physiol Meas* 1995;16:A87–A98. [PubMed: 8528130]
- Brown BH, Barber DC, Seagar AD. Applied potential tomography: possible clinical applications. *Clin Phys Physiol Meas* 1985;6:109–21. [PubMed: 4017442]
- Brown BH, Seagar AD. The Sheffield data collection system. *Clin Phys Physiol Meas* 1987;8 (Suppl A): 91–7. [PubMed: 3568577]
- Burlakova, YeV; Vepintsev, BN.; Rass, IT. Lag of the impedance effect behind the action potential in frog nerve. *Biofizika* 1959;4:617–20.
- Calderón, AP. On an inverse boundary value problem. In: Meyer, WH.; Raupp, MA., editors. *Seminar on Numerical Analysis and its applications to Continuum Physics*. Brazilian Math. Society; Rio de Janeiro: 1980. p. 65-73.
- Chailakhian LM, Iur'ev SA. An investigation of the time relations of the action potential and impedance changes on excitation in the frog nerve. *Biophysics* 1957;4:415–24.
- Chapman RA. The Repetitive Responses of Isolated Axons from the Crab, *Carcinus Maenas*. *J Exp Biol* 1966;45:475–88.
- Cole SK, Curtis HJ. Electrical Impedance of the squid giant axon during activity. *J Gen Physiol* 1939;22:649–70. [PubMed: 19873125]
- Dale AM, Halgren E. Spatiotemporal mapping of brain activity by integration of multiple imaging modalities. *Curr Opin Neurobiol* 2001;11:202–8. [PubMed: 11301240]
- Fabrizi L, Sparkes M, Horesh L, Perez-Juste Abascal JF, McEwan A, Bayford RH, Elwes R, Binnie CD, Holder DS. Factors limiting the application of electrical impedance tomography for identification of regional conductivity changes using scalp electrodes during epileptic seizures in humans. *Physiol Meas* 2006;27:S163–S174. [PubMed: 16636408]
- Freygang WH, Landau WM. Some relations between resistivity and electrical activity in the cerebral cortex of the cat. *J Cell Compar Physl* 1955;45:377–92.
- Galambos R, Velluti R. Evoked resistance shifts in unanaesthetized cats. *Exp Neurol* 1968;22:243–52. [PubMed: 5724946]
- Gibson AP, Bayford RH, Holder DS. Two-dimensional finite element modelling of the neonatal head. *Physiol Meas* 2000;21:45–52. [PubMed: 10719998]
- Gilad O, Holder DS. Impedance changes recorded with scalp electrodes during visual evoked responses: implications for Electric Impedance Tomography of fast neural activity. *Neuroimage* 2009a;47(2): 514–22. [PubMed: 19426819]
- Gilad O, Horesh L, Holder DS. A modelling study to inform specification and optimal electrode placement for imaging of neuronal depolarization during visual evoked responses by electrical and magnetic detection impedance tomography. *Physiol Meas* 2009b Jun;30(6):S201–24. [PubMed: 19491442]
- Gilad, O.; OhD; Ghosh, A.; McEvoy, AW.; Akseirod, S.; Holder, DS. Imaging neuronal depolarization in the brain with Electrical or Magnetic Detection Impedance Tomography: a review of experimental studies. In: Hartov, A.; Woo, EJ., editors. *Hanover NH, USA. Proc. of IX Int. Conf. on Electrical Impedance Tomography*; 6-16-2008; 2008. p. 9-12.
- Hagberg GE, Bianciardi M, Maraviglia B. Challenges for detection of neuronal currents by MRI. *Magn Reson Imaging* 2006;24:483–93. [PubMed: 16677955]
- Hamalainen MS, Hari R, Ilmoniemi RJ, Knuutila J, Lounasmaa OV. Magnetoencephalography-theory, instrumentation, and applications to noninvasive studies of the working human brain. *Rev Mod Phys* 1993;65:413–97.
- Henderson RP, Webster JG. An impedance camera for spatially specific measurements of the thorax. *IEEE Trans Biomed Eng* 1978;25:250–4. [PubMed: 680754]
- Holder DS. Feasibility of developing a method of imaging neuronal activity in the human brain: a theoretical review. *Med Biol Eng Comput* 1987;25:2–11. [PubMed: 2826937]
- Holder DS. Impedance changes during evoked nervous activity in human subjects: implications for the application of applied potential tomography (APT) to imaging neuronal discharge. *Clin Phys Physiol Meas* 1989;10:267–74. [PubMed: 2627768]
- Holder DS. Electrical impedance tomography with cortical or scalp electrodes during global cerebral ischaemia in the anaesthetised rat. *Clin Phys Physiol Meas* 1992a;13:87–98. [PubMed: 1563224]

- Holder DS. Impedance changes during the compound nerve action potential: implications for impedance imaging of neuronal depolarisation in the brain. *Med Biol Eng Comput* 1992b;30:140–6. [PubMed: 1453778]
- Holder, DS. *Electrical Impedance Tomography: Methods, History and Applications*. 1. Taylor & Francis; 2005.
- Holder DS, Gardner-Medwin AR. Some possible neurological applications of applied potential tomography. *Clin Phys Physiol Meas* 1988;9 (Suppl A):111–9. [PubMed: 3240638]
- Isaacson D, Isaacson EL. Comment on Calderon's Paper: "On an Inverse Boundary Value Problem". *Mathematics of Computation* 1989;52:553–9.
- Klivington KA, Galambos R. Resistance shifts accompanying the evoked cortical response in the cat. *Science* 1967;157:211–3. [PubMed: 17806271]
- Klivington KA, Galambos R. Rapid resistance shifts in cat cortex during click-evoked responses. *J Neurophysiol* 1968;31:565–73. [PubMed: 5709872]
- Liston, AD. PhD dissertation. Middlesex University; London, UK: 2004. *Models and Image Reconstruction in Electrical Impedance Tomography of Human Brain Function*.
- Liston, AD.; Bayford, RH.; Boone, KG.; Holder, DS. Estimation of Impedance Changes Inside the Human Head During Neuronal Depolarisation; Implications for Electrical Impedance Imaging of the Brain. 2000; Chicago. *World Congress on Medical Physics and Biomedical Engineering*; 2000.
- McArdle FJ, Brown BH, Pearse RG, Barber DC. The effect of the skull of low-birthweight neonates on applied potential tomography imaging of centralised resistivity changes. *Clin Phys Physiol Meas* 1988;9(Suppl A):55–60. [PubMed: 3240651]
- McEwan A, Romsauerova A, Yerworth R, Horesh L, Bayford R, Holder D. Design and calibration of a compact multi-frequency EIT system for acute stroke imaging. *Physiol Meas* 2006;27:S199–S210. [PubMed: 16636411]
- Metherall P, Barber DC, Smallwood RH, Brown BH. Three-dimensional electrical impedance tomography. *Nature* 1996;380:509–12. [PubMed: 8606768]
- Oh, D.; Gilad, O.; Ghosh, A.; Holder, DS. Analytic correction for the square wave carrier in low frequency Electrical Impedance Tomography of neuronal depolarization. In: Hartov, A.; Woo, EJ., editors. *Hanover NH, USA. Proc. of IX Int. Conf. on Electrical Impedance Tomography*; 6-16-2008; 2008. p. 5-8.
- Parkes LM, de Lange FP, Fries P, Toni I, Norris DG. Inability to directly detect magnetic field changes associated with neuronal activity. *Magn Reson Med* 2007;57:411–6. [PubMed: 17260380]
- Prudnikova IF. The effects of eserine on the action potential and impedance spike in frog nerve. *Biofiziklia* 1959;4:666–76.
- Romsauerova A, McEwan A, Horesh L, Yerworth R, Bayford RH, Holder DS. Multi-frequency electrical impedance tomography (EIT) of the adult human head: initial findings in brain tumours, arteriovenous malformations and chronic stroke, development of an analysis method and calibration. *Physiol Meas* 2006;27:S147–S161. [PubMed: 16636407]
- Schei JL, McCluskey MD, Foust AJ, Yao XC, Rector DM. Action potential propagation imaged with high temporal resolution near-infrared video microscopy and polarized light. *Neuroimage* 2008;40:1034–43. [PubMed: 18272402]
- Shanes AM. Electrical phenomena in nerve; crab nerve. *J Gen Physiol* 1949;33:75–102. [PubMed: 18139009]
- Steinbrink J, Kempf FC, Villringer A, Obrig H. The fast optical signal--robust or elusive when non-invasively measured in the human adult? *Neuroimage* 2005;26:996–1008. [PubMed: 15961042]
- Syre F, Obrig H, Steinbrink J, Kohl M, Wenzel R, Villringer A. Are VEP correlated fast optical signals detectable in the human adult by non-invasive nearinfrared spectroscopy (NIRS)? *Adv Exp Med Biol* 2003;530:421–31. [PubMed: 14562737]
- Tidswell AT, Gibson A, Bayford RH, Holder DS. Three-dimensional electrical impedance tomography of human brain activity. *Neuroimage* 2001;13:283–94. [PubMed: 11162269]

Appendix A. Linear model and statistical analysis

A linear model was constructed for estimating v (the CAP or EP) and δ (the voltage change due to resistance change). The measured voltage from a single recording channel $V_i^{(j)}$ was defined for the i^{th} time point ($i = 1, 2, \dots, m/2$; $m = 1024$ (SD32R) or $m = 10000$ (NI Board)) and j^{th} square wave cycle. For the active recordings, $j = 1, 2, \dots, n$; $n = 60$ are the square wave cycles during applied stimulation and positive (C_1) or negative (C_2) current injection. For the control recordings, these were marked as C_3 and C_4 for the positive and negative square wave cycles respectively. $V_i^{(j)}$ was then modelled as:

$$V_i^{(j)} = \begin{cases} v_i + \delta_i + b_i + \varepsilon_{ij} & \text{if } j \in C_1 \text{ (stim+positive current)} \\ v_i - \delta_i - b_i + \varepsilon_{ij} & \text{if } j \in C_2 \text{ (stim+negative current)} \\ +Gb_i + \varepsilon_{ij} & \text{if } j \in C_3 \text{ (rest+positive current)} \\ -Gb_i + \varepsilon_{ij} & \text{if } j \in C_4 \text{ (rest+negative current)} \end{cases} \quad (\text{A-1})$$

Where b is the baseline voltage due to the square wave current, ε is a Gaussian noise mainly due to background brain activity and G is a gain factor reflecting the small difference in square wave amplitude between the control and active recordings. Eq. A-1 was abbreviated to:

$$V_i^{(j)} = \alpha_j v_i + \alpha_j \beta_j \delta_i + \gamma_j b_i \quad (\text{A-2})$$

Where α , β , and γ are design indicators:

$$\alpha_j = \begin{cases} 1 & \text{if } j \in C_{1,2} \\ 0 & \text{if } j \in C_{3,4} \end{cases} \quad \beta_j = \begin{cases} +1 & \text{if } j \in C_{1,3} \\ -1 & \text{if } j \in C_{2,4} \end{cases} \quad \gamma_j = \begin{cases} \beta_j & \text{if } j \in C_{1,2} \\ \beta_j G & \text{if } j \in C_{3,4} \end{cases} \quad (\text{A-3})$$

The Maximal Likelihood (ML) estimators of the model parameters v , δ , b and G are given by minimizing the cost function:

$$\text{Cost}(v, \delta, b, G) = \sum_{i,j} \left[V_i^{(j)} - (\alpha_j v_i + \alpha_j \beta_j \delta_i + \gamma_j b_i) \right]^2 \quad (\text{A-4})$$

This cost function was minimized separately for each time point i :

$$\begin{cases} \frac{\partial \text{Cost}}{\partial v_i} = -2 \sum_{j \in C_{1,2}} \left[V_i^j - (v_i + \beta_j (\delta_i + b_i)) \right] = 0 \\ \frac{\partial \text{Cost}}{\partial \delta_i} = -2 \sum_{j \in C_{1,2}} \beta_j \left[V_i^j - (v_i + \beta_j (\delta_i + b_i)) \right] = 0 \\ \frac{\partial \text{Cost}}{\partial b_i} = -2G \sum_{j \in C_{3,4}} \beta_j \left[V_i^j - \beta_j G b_i \right] = 0 \end{cases} \quad (\text{A-5})$$

The partial derivative with respect to G yielded the same equation as obtained for b_i . Since

there was an equal number of positive and negative current injection segments, $\sum_j \beta_j = 0$ and $\beta_j^2 = 1$ which yields

$$\begin{cases} v_i = \frac{1}{2n} \sum_{j \in C_{1,2}} V_i^j \\ \delta_i + b_i = \frac{1}{2n} \sum_{j \in C_{1,2}} \beta_j V_i^j \\ b_i = \frac{1}{2Gn} \sum_{j \in C_{3,4}} \beta_j V_i^j \end{cases} \quad (\text{A-6})$$

For estimating v and δ these become:

$$\begin{cases} v_i = \frac{1}{2n} \sum_{j \in C_{1,2}} V_i^j \\ \delta_i = \frac{1}{2n} \sum_{j \in C_{1,2}} \beta_j V_i^j - \frac{1}{2Gn} \sum_{j \in C_{3,4}} \beta_j V_i^j \end{cases} \quad (\text{A-7})$$

Since the derivatives of the cost function with respect to G yielded the same equation as obtained for b_i an additional criterion was sought for estimating G . The baseline boundary voltage b was about 4–5 orders of magnitude larger than the change δ . Therefore, a tiny mis-registration of G will produce a large bias in δ due to unbalanced baseline between the active and control recordings. Therefore, the gain G was determined by the additional criterion which would zero any DC (or bias) component:

$$\sum_i \delta_i = 0 \quad (\text{A-8})$$

Or

$$\sum_i \left[\frac{1}{2n} \sum_{j \in C_{1,2}} \beta_j V_i^j - \frac{1}{2Gn} \sum_{j \in C_{3,4}} \beta_j V_i^j \right] = 0 \quad (\text{A-9})$$

And finally

$$G = \frac{\sum_i \sum_{j \in C_{3,4}} \beta_j V_i^j}{\sum_i \sum_{j \in C_{1,2}} \beta_j V_i^j}. \quad (\text{A-10})$$

Statistical analysis

using Eq. A-6, G was used to calculate b_i and a series of values was defined for each time point i :

$$\Delta_i^j = \{\beta_j V_i^j - b_i\}; j \in C_{1,2} \quad (\text{A-11})$$

Where

$$\delta_i = \frac{1}{2n} \sum_{j \in C_{1,2}} \Delta_i^j. \quad (\text{A-12})$$

From these series, the standard error (SE_i) was calculated for each time point. During membrane depolarization, the resistivity decreases and the changes in the voltage δ_i are expected to be negative. Therefore, Δ_j^i at each time point i was tested for negative mean using a t-test with a critical value of $p < 0.05$.

Fig. 1.

The recorded signal V from the crab nerve or the scalp was a composite signal which comprised three components: 1) A bi-polar square wave baseline b , 2) Neuronal activity v triggered by visual stimuli (black arrows) or stimulating pulses to the crab nerve and 3) a deflection δ in the baseline due to the resistance decrease. The dotted line in the recorded signal (4) is the VEP as in (2) that would appear in the recorded signal (solid) if the resistance decrease δ was not present. The stimuli were delayed by 150 ms from the beginning of each of the square wave polarities and for the human case were expected to produce the main component of the VEP, the P100, about half way into the 500 ms half square wave cycle.

Fig. 2.

Derivation of the final v and δ waveforms (bottom, red) from the average of the control and active recordings (left) using summation and subtraction of the two polarities (right) followed by a second subtraction between the control and active recordings. For the purpose of explanation, the square wave is shown as square; in practice it was a falling exponential of variable shape. Sum_c, Diff_c and Sum_a, Diff_a are the summation and subtraction of the two polarities of the square wave for the control and active recordings respectively.

Fig. 3.
Electrode array and experimental setup for crab nerve.

Fig. 4.
Instrumentation setup.

Fig. 5.

Group display of all recordings with different current and drive electrode spacing. In each box, the CAP is in black (positive waveforms, mV units on the leftmost axes) and the resistance changes δ are in black or in red for the significant regions (negative waveforms, percentage units on the rightmost axes). Individual waveforms are the estimation after averaging over the 1 minute recording. The stimulus was applied at 0 ms. *a-d*: different current levels for a fixed current drive spacing of 2 mm. *e-g*: different current drive spacing for a fixed current of 2 μ A (*b* and *e* are identical).

Fig. 6. Peak changes in δ (± 1 SE) for (a) the different current levels with $n = 7, 12, 4$ and 6 recordings and (b) the different current drive spacing with $n = 12, 6$ and 8 recordings.

Fig. 7. Delay from stimulus of peak CAP (black, bottom line) or changes in δ (red, top line) (± 1 SE) for (a) the different current levels and (b) the different current drive spacing.