Review

Feasibility of developing a method of imaging neuronal activity in the human brain: a theoretical review

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Abstract—*A theoretical analysis has been performed to suggest directions for research into the development of a device which could image neuronal electrical activity in the human brain in three dimensions. Proposed criteria for the device are a spatial resolution of 1 mm^3 and temporal resolution, after averaging to a repeated stimulus, of 1 ms for events related to the action potential, or 1 s for metabolic changes. It is proposed that, for the rapid changes related to the action potential, electron spin resonance using a potential-sensitive spin label, impedance imaging and NMR are suitable in principle but that only ESR and impedance methods may have sufficient sensitivity and these merit further assessment. For metabolic changes, NMR and PET may be used as at present, and ESR may be developed in time, but images based on these changes would have limited value in that they could only give an indirect index of neuronal discharge. Unique reconstructions based on the EEG or MEG are theoretically impossible, and imaging using X-rays, microwaves, or ultrasound may be possible in principle but these techniques would not be sufficiently sensitive.*

Keywords—*Action potential, Applied potential tomography, Brain, Computerised tomography, Electroencephalography, Electron spin resonance, Impedance imaging, Magnetoencephalography, Microwaves, Nuclear magnetic resonance, Positron emission tomography, Ultrasound*

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1 Introduction

INTEREST IN THE coding of information in the pathways of the brain has long been the subject of interest in the neurosciences, but has been hampered by the lack of methods capable of measuring this activity in a simple and noninvasive fashion. The ideal method would be a device which could produce three-dimensional images of neuronal activity throughout the brain, and with a time course of the order of the action potential, namely milliseconds. While the construction of such a device might have been thought impossible a decade ago, at least the imaging aspect could now be overcome by the use of techniques of computerised reconstruction which have been developed for X-ray computerised tomography and other imaging techniques. This study is an attempt to estimate if these imaging techniques could be coupled to a suitable spectroscopic modality which would enable images to be produced which represented neuronal discharge in the human brain. The purpose of the study is to review the field and suggest directions for research.

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The most accurate index of activity would be measurement of the rate of discharge in neurones and their processes. The basis of each such discharge is the action potential, during which there is a depolarisation of the neuronal membrane by about 110 mV. It lasts approximately 2 ms, and is due to the transfer of micromolar quantities of ions such as sodium and potassium across the membrane (AIDLEY, 1978; HOLDER, 1986). Any technique which could detect these, even when averaged over the population of a selected voxel, would need to be very sensitive in view of the time scale and small magnitude. However, there are also metabolic recovery processes which accompany action potential activity, and act to restore ionic gradients to normal. These are related to accumulation of ions in the extracellular space, which acts as a sink. This has the advantage that the changes are approximately a thousand times larger, with changes of the order of millimolar (HOLDER, 1986), and so are far easier to measure. Unfortunately they have the disadvantage that they have a correspondingly longer time scale of seconds, and so can only be at best an indirect guide to neuronal activity.

It is necessary to propose criteria for the device to assess feasibility, and these are based on the above considerations. The spatial resolution would be 1 mm^3, to allow resolution of neuroanatomical pathways in the brain, and

temporal resolution would be 1 ms for imaging related to the action potential, or 1 s for that related to metabolic recovery processes. In addition, to improve sensitivity, there would be repeated averaging to a stimulus, much as for evoked responses in clinical neurology. Total data collection time would be 20 min or so. It is envisaged that the machine would physically resemble a CT scanner. It would produce a similar set of images, except in that each voxel would represent summated neuronal activity for that volume and time period, and there would be a set of such images for each period (millisecond or second) of the averaged trace.

The assessment has been performed by reviewing all possibly relevant techniques, both those already in use in imaging and others currently used for spectroscopy in the basic sciences. They include a variety of forms of electromagnetic radiation and ultrasound. Modalities of electromagnetic radiation between 3 GHz or 12 400 eV have been excluded, as they would not have sufficient penetration for the human head (LIN and CLARKE, 1982; JOHNS and CUNNINGHAM, 1980). In each case, the technique was assessed for a means by which it could register neuronal activity, for its imaging possibilities, and then the sensitivity was estimated with respect to the above criteria for both changes related to the action potential and metabolic recovery processes. Where appropriate, allowance was made for technical advances that could reasonably be expected in the next few years.

For some of the calculations below, it has been necessary to use a figure for the ratio of neuronal membrane per gram of brain tissue. While figures are not directly available for white matter in mammalian brain, the spectrum of fibre sizes is similar to that of the vagus nerve of the rabbit (KEYNES and RITCHIE, 1965; WAXMAN and SWADLOW, 1977), and so the ratio may be taken as similar at $6000\,cm^2\,g^{-1}$ (KEYNES and RITCHIE, 1965). The ratio for grey matter in the cerebral cortex is higher at $50\,000\,cm^{-2}\,g^{-1}$ (MCILWAIN and BACHELARD, 1971).

The assessments for each technique are presented in ascending order of frequency below. It is proposed that two techniques, electron spin resonance (ESR) and impedance imaging, merit more assessment for imaging changes related to the action potential, and three, nuclear magnetic resonance (NMR), ESR and positron emission tomography (PET) appear suitable for imaging related metabolic changes.

2 Electromagnetic fields below the microwave range

2.1 *Impedance imaging (applied potential tomography)*

Impedance measurements in a nonimaging mode have been in clinical use for many years. Measurement is made between 20 and 100 kHz to reduce electrode artefact (BARBER and BROWN, 1984). The signal is mainly related to the conductivity of the intervening tissue (BROWN, 1983). In this way, impedance has been used to measure cardiac stroke volume, gastric emptying, bladder fullness, thoracic volume and intraventricular haemorrhage in the neonatal head (ISKANDER and DURNEY, 1980; BROWN *et al.*, 1985). In the technique of 'rheoencephalography' multichannel measurements of impedance across the adult head are made (MCHENRY, 1965). The signals detected are related to the ECG, and are probably due to changing proportions of cerebrospinal fluid and blood with the arterial pulse (BOSTEN *et al.*, 1982). This technique is performed without averaging; there have been no reports of signals related to neuronal activity when used in this way.

Reconstruction of images based on impedance measurements is difficult, because the current is volume conducted, and conventional projection reconstruction techniques cannot be used. Recently, many of these problems have been overcome by the use of a four-electrode system. Two inner electrodes produce a constant current, and the potential difference between the outer two is recorded. A ring of electrodes is placed around the subject, and then potential changes for all possible permutations of source and recording pairs are recorded by rapid multiplexing. Imaging is achieved by weighted back projection of the lines of isopotential which are curved. This technique overcomes many of the problems encountered by previous methods which attempted to measure current transmission and, indeed, the only published images *in vivo* have been produced by this method. They include images of the forearm, thorax, and stomach (BROWN *et al.*, 1985; see Fig. 1).

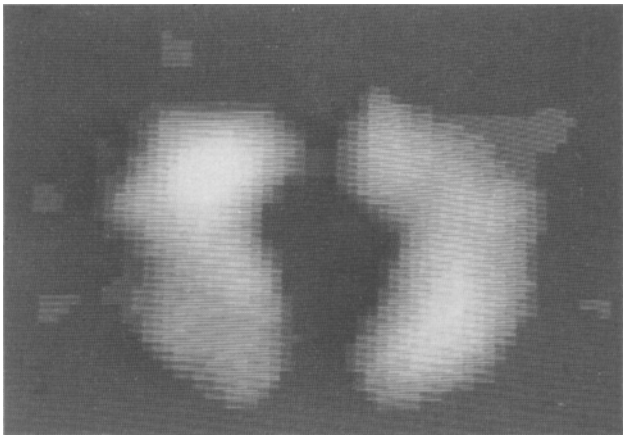

Fig. 1 *Impedance image of the increase in resistivity on inspiration, achieved by the placement of 16 electrodes around the chest at the level of the nipples* (BROWN et al., *1985*). (*By kind permission of Professor B. H. Brown*)

The resistance of the neuronal membrane falls from 1000 to 25 Ω cm during the action potential (AIDLEY, 1978), and this could in principle be measured as a change in impedance by scalp electrodes. The change may be difficult to detect, as most current is likely to pass in the extracellular space and be unaltered by changes in membrane resistance. Calculations based on Maxwell's equations for the impedance of uniform spheres in a homogeneous medium suggested that a change of 3 per cent may be expected (VAN HARREFELD and OCHS, 1958). Several experimenters have recorded a change of this magnitude using intracerebral electrodes in animals. A decrease of 3 per cent with the time course of the action potential was recorded after local electrical stimulation in the cerebral cortex of the cat (FREYGANG and LANDAU, 1955), and changes of 1·4–1·8 per cent but with a time course of seconds have been recorded from the hippocampus, amygdala and septal nuclei of the cat after physiological stimuli such as exposure to milk (ADEY *et al.*, 1962). There do not appear to be any reports in the literature of measurement of such changes by external scalp electrodes, and it is difficult to arrive at an estimate of the size of the signal at the surface of the scalp. Van Harrefeld and Ochs estimated that 15–20 per cent of the fall in impedance following circulatory arrest was due to cerebrospinal and other extracellular fluid, and a further 11 per cent due to intracranial blood (VAN HARREFELD and OCHS, 1958). The signal will be further degraded by the scalp and cranium. Overall, therefore, the maximum change in impedance that could be produced by simultaneous discharge of all the neurons in a selected volume could be 2 per cent, but in practice may well be much smaller.

The sensitivity of present images is still fairly low at about 1 per cent change in impedance, with a spatial resolution of 10 per cent, for a 16-electrode system (BROWN *et al.*, 1985). However, estimates for a future system with 128 electrodes in a circular array suggest a spatial resolution of 1·5 per cent (equivalent to 2 mm for the human head) and data collection time of 57 ms (BARBER and BROWN, 1984). Without averaging, the smallest changes in impedance which can be used to reconstruct an image are 0·1 per cent (BROWN *et al.*, 1985), but this could be greatly improved by averaging; for instance, a single-channel instrument in use in this department with averaging of up to 1024 times can detect changes in impedance of 0·005 per cent.

In view of the uncertainty as to the magnitude of the impedance changes at the scalp with physiological stimuli in practice, it is difficult to arrive at a definite conclusion. However, the sensitivity of modern impedance measuring devices would permit single-channel measurement of changes some 400 times lower than the estimate for *in vivo* change above, and that of a projected imaging device could well be 20 or more times lower, so there is a considerable margin of error even given large inaccuracies in the estimate. In addition, the short recording time of 57 ms would permit the collection of many sets of data which could be averaged to reduce noise and eliminate artefacts. It is therefore concluded that this technique is possible in principle and, while verification awaits investigation of the size of the impedance signal when measured at the scalp, appears to be promising and merits further investigation.

2.2 *Electromagnetic fields generated by the brain*

The electric potentials generated by nervous activity may be measured externally by scalp electrodes, and this has formed the basis for the technique of electroencephalography. Magnetic fields are generated in a similar way, and can be measured externally by the sensitive superconducting quantum interference device (SQUID) technique. While some localisation is possible by the use of assumptions of source nature and number, rigorous reconstruction is not theoretically possible from external measurements alone (NUNEZ, 1981; WILLIAMSON and KAUFMAN, 1981), and so it is unlikely that either technique could be used for imaging to the above criteria.

2.3 *Microwave imaging*

Microwaves can be used for imaging by the use of conventional projection reconstruction algorithms. This has been achieved on a phantom resembling a human breast which contained ethylene glycol. Carried out at 10 GHz, a spatial resolution of 2 cm was achieved (RAO *et al.*, 1980). However, image quality is seriously degraded by reflection and diffraction at interfaces, and the technique is still experimental.

There are some grounds for expecting that microwaves could interact with discharging neurones. Absorption is related to free tissue water, proteins or protein-bound water, or by the charging of cell membranes depending on frequency (FOSTER and SCHEPPS, 1981). There appears to be an interaction with nerves while discharging, as the action potential in frog sciatic nerve or the ganglia of Aplysia has been shown to be affected by microwaves, and this effect is increased if the preparations are actively discharging during the exposure (MCREE and WACHTEL, 1982; WACHTEL *et al.*, 1975). There is one report of a change in microwave impedance at 10 GHz in mouse brain or cockroach spinal cord with nervous activity (AABY, 1983).

Unfortunately, there are theoretical limitations on the spatial resolution that could be achieved using microwave imaging. This is due to the application of Abbe's law, which predicts that the maximum resolution is equal to the wavelength, for a parallel beam configuration which would be used. The maximum wavelength that could be used to overcome this problem is limited by attenuation in body tissues which becomes significant at higher microwave frequencies. The maximum attenuation that may be used is related to the highest safe exposure, which may be taken as $10\,mW\,cm^{-2}$ according to the American National Standards Institute (WILKENING, 1978), and the highest sensitivity of a conventional diode/dipole measuring system is $2\,\mu W\,cm^{-2}$ (NRCP, 1981). Taking the diameter of the human head as 16 cm, this corresponds to an attenuation coefficient of $0{\cdot}53\,cm^{-1}$, which in turn corresponds to a wavelength of 8·2 mm (5·4 GHz) for cat brain (from published figures for attenuation coefficients, frequency and wavelength for body tissues (NRCP, 1981; KRASZEWSKI *et al.*, 1982)). Even allowing for maximum permissible exposure and recording sensitivity each ten times better, this would still only correspond to a resolution of 7 mm. In practice, resolution is likely to be considerably worse, due to the degrading effects on imaging mentioned above.

Therefore, it appears that, even if a change in microwave transmission change with neural activity did occur and was measurable, the spatial resolution of reconstructed images would be unacceptable.

3 Magnetic resonance techniques

3.1 *Nuclear magnetic resonance (NMR)*

This technique has been in use for many years for spectroscopy in the physical sciences, and has more recently been developed to produce reconstructed three-dimensional images for medical purposes. Its main advantage lies in its versatility: in addition to imaging, it can also be used to identify particular isotopes by their resonant frequency, or different populations of an isotope according to the position within a molecule by chemical shift measurement, to image velocity of moving fluids within an image (BUDINGER and LAUTERBUR, 1984), or to give images with a high temporal resolution of tens of milliseconds by the technique of gating (LANZER *et al.*, 1984). Its main disadvantage is its relative insensitivity, as only approximately one-millionth of available nuclear particles contribute to the signal (BUDINGER and LAUTERBUR, 1984). As a result conventional use of imaging has been largely restricted to measurement of the protons in body water which have a high concentration of $85\,mol\,l^{-1}$ (HILAL *et al.*, 1985).

NMR could be used in a variety of ways to measure neuronal activity. However, the limiting factor is sensitivity, and so, first, the lowest concentration of different isotopic markers that could be imaged for a reasonable resolution needs to be estimated. In this case let us relax the criteria specified above, which are too stringent for this technique, and propose a spatial resolution of $3 \times 3 \times 10$ mm, and total recording time of 1 h.

The relationship of the essential NMR imaging variables signal-to-noise ratio S/N, volume of each voxel V (mm^3), concentration C ($mmol\,l^{-1}$), recording time T (min) and relative sensitivity of the isotope S is shown in eqn. 1 (from HOULT, 1980).

$$S/N \propto VC\sqrt{T}S \quad (1)$$

This may be simply modified to eqn. 2, which permits calculation of the concentration which could be measured

for a specified imaging protocol (suffix s) by comparison to an existing one for which data are available (suffix e). In addition, a factor R has been introduced which represents the improvement in performance that could reasonably be expected in the next few years. At present the highest field for which there are published results *in vivo* is about 1·5 T. It is reasonable to expect that fields of 6 T should be achievable for whole-body or head magnets (BUDINGER and LAUTERBUR, 1984); magnet technology is improving rapidly, and fields over 10 T are now available in commercially produced laboratory NMR spectrometers (WEHRLI, 1982). There is considerable controversy over the relationship between field and sensitivity: theoretically this should be to the power of 7/4 (HOULT, 1980), but other estimates predict no improvement (MANSFIELD and MORRIS, 1982). Nevertheless, a roughly linear improvement has been measured in practice from 5·1 to 63·1 MHz for proton imaging (BOTTOMLEY *et al.*, 1984), and it is reasonable to expect this for higher fields (BUDINGER and LAUTERBUR, 1984). In addition, an improvement of up to four times may be expected from technical improvements (KAUFMAN and CROOKS, 1983), although these may be offset at higher fields. Taking these factors together, it may be estimated that an improvement in sensitivity of ten times could be achieved. This is similar to an estimate from a group operating a device at 1·5 T that it should be possible to halve spatial resolution from 4 to 2 mm or better, which corresponds to an improvement in sensitivity of eight times or more (HILAL *et al.*, 1985).

$$C = \frac{S/N_s V_e \sqrt{T_e} C_e}{S/N_e V_s \sqrt{T_s} R} \quad (2)$$

tial. This could be achieved by the use of a shift reagent coupled to saturation transfer, which would enable distinction of a population of ions which had just moved between extracellular and intracellular compartments. Unfortunately, the movement of such ions is very small: the flux of sodium per impulse has been measured in the walking leg nerve of the crab and is 3·5 pmol per cm^2 of membrane (KEYNES and LEWIS, 1951). Taking the area of neuronal membrane per unit volume of white matter in the brain as discussed above as $6000\,cm^2\,g^{-1}$, and assuming unit flux is similar in these different species, this gives a total movement of 21 μmol. This is for the unmyelinated crab nerve; the magnitude of change is over 1000 times less for myelinated nerves (ASANO and HURLBUT, 1958). These changes are over two orders of magnitude less than the sensitivity discussed above, and so it is clear that these changes could not be imaged using NMR.

In contrast, there are a number of larger changes which accompany action potential recovery processes which have a concentration approaching that of the sensitivity of NMR outlined above. Unfortunately, their time course is correspondingly slower, and the maximum temporal resolution would be seconds.

The largest such change is that of blood flow, which can alter with neural activity by up to 100 per cent (ROSENTHAL *et al.*, 1979; SIESJO, 1978), and has a concentration of $85\,mol\,l^{-1}$ of protons (HILAL *et al.*, 1985). This can be imaged by several different algorithms. *In vivo* human images have been produced (SINGER and CROOKS, 1983) by a method which is based on the selective excitation of a plane of protons, and the proportion which then passes into a plane of interest is related to flow. At present,

Table 1 Performance of NMR imaging for different isotopes

Isotope	Sensitivity*	Field, T	Concentration, $mmol\,l^{-1}$	Resolution, mm	Time, min	S/N	TR s	Reference
1H	1	0·35	85000	1·7 × 1·7 × 7	8·5	75	2·0	FEINBERG *et al.*, 1985
23Na	0·092	1·5	45	10 × 4 × 4	34		0·1	HILAL *et al.*, 1985
31P	0·066	2·7	30	1·5 × 7 × 7	240		2·0	MAUDSLEY *et al.*, 1984

* sensitivity at constant field relative to hydrogen

Published figures for performance for three commonly utilised isotopes are shown in Table 1. The lowest concentration which could be imaged for the above criteria, taking R as 10, can then be calculated from eqn. 2: it is $14\,mmol\,l^{-1}$ for 1-H, $1\,mmol\,l^{-1}$ for 23-Na and $6{\cdot}4\,mmol\,l^{-1}$ for 31-P. Similar figures may be expected for 19-F, 43-Ca and 13-C, which have, respectively, relative sensitivities of 0·83, 0·064 and 0·016 (KRAMER, 1981). A further improvement in these figures might be obtainable by the use of spin-spin coupling, which has already achieved an increase in sensitivity of 11 times for ^{13}C-^{1}H coupling (ROTHMAN *et al.*, 1985). However, if gating to a stimulus is used, this will degrade sensitivity further, according to the number of bins used for each stimulus interval.

Although a detailed analysis of the differences between different isotopes and techniques is outside the scope of this discussion, it may be concluded that it should be possible to image isotopes at a concentration of the order of $10\,mmol\,l^{-1}$ while retaining the capability to distinguish different isotopes or compounds by the use of chemical shift. The smallest changes in NMR measurements are limited by the Lorentzian waveform, but it should be possible to detect changes down to as little as 5 per cent of the total concentration (GADIAN, 1982).

In principle, NMR could be used to detect neuronal firing by measuring the flux of ions such as sodium which occurs across the cell membrane with each action potential. This could be achieved by the use of a shift reagent coupled to saturation transfer, which would enable distinction of a population of ions which had just moved between extracellular and intracellular compartments. Unfortunately, the movement of such ions is very small: the flux of sodium per impulse has been measured in the walking leg nerve of the crab and is 3·5 pmol per cm^2 of membrane (KEYNES and LEWIS, 1951). Taking the area of neuronal membrane per unit volume of white matter in the brain as discussed above as $6000\,cm^2\,g^{-1}$, and assuming unit flux is similar in these different species, this gives a total movement of 21 μmol. This is for the unmyelinated crab nerve; the magnitude of change is over 1000 times less for myelinated nerves (ASANO and HURLBUT, 1958). These changes are over two orders of magnitude less than the sensitivity discussed above, and so it is clear that these changes could not be imaged using NMR.

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A variety of metabolites such as ATP, glucose, NADH and amino acids are present in concentrations of approximately $1\,mmol\,l^{-1}$ in the brain, and change concentration by up to 100 per cent with neural activity (SIESJO, 1978). Sodium and potassium accumulate with repetitive activity to give relatively large changes in concentration. Potassium is unfortunately unsuitable because of its low relative sensitivity at constant field of 0·001 (MANSFIELD and MORRIS, 1982). The increase in intracellular sodium concentration with neural activity is technically difficult to measure but, as ionic equilibrium is maintained (RITCHIE, 1973), should change by the same amount as potassium, which has been extensively measured, and increases from a baseline of $3\,mmol\,l^{-1}$ to $10\,mmol\,l^{-1}$ in mammalian cerebral cortex on stimulation (NICHOLSON, 1980). This is supported by the findings of an increase in intracellular sodium concentration of between 4 and $9\,mmol\,l^{-1}$ in rat sympathetic neurones on stimulation by carbachol (BALLANYI *et al.*, 1983), which is superimposed on a resting intracellular concentration of sodium of $12–20\,mmol\,l^{-1}$ (HILAL *et al.*, 1985). Calcium ions transfer across the neural membrane with activity, but in a concentration of only up

to 0·13 mmol l^{-1} (NICHOLSON, 1980). While this is too small for detection directly, the effect could be enhanced by the use of nFBAPTA, which is a fluorine-containing compound which alters its chemical shift as the concentration of calcium alters (SMITH *et al.*, 1983). From the published figures, this could give a chemical shift of 0·3 parts in 10^6 for an extracellular concentration of calcium of 2·4 mmol l^{-1}, and of 4FBAPTA of 3·4 mmol l^{-1}.

These changes therefore lie near the limits of detectability estimated above. If necessary, the problem of detection could be overcome by further relaxing criteria for spatial or temporal resolution.

It may therefore be concluded that NMR could not be used to image changes directly related to the action potential with the desired resolution. It is not possible to image changes related to metabolic recovery processes at the present time, but, given technical advances in the near future that might reasonably be predicted, this should become possible. However, the changes are at the limit of detectability, and it may be necessary to sacrifice spatial or temporal resolution to achieve adequate signal-to-noise ratios.

3.2 *Electron spin resonance (ESR)*

This technique is closely related to NMR, but differs in that the electromagnetic field produced by an unpaired electron is measured. It has the advantages that it is more sensitive than NMR, because of the greater charge-to-mass ratio of the electron (MYERS, 1973), and it also can be used to provide information on selected molecular environments within the sample by the use of an artificially introduced probe compound, termed a spin label. This consists of a suitable radical, such as the nitroxide radical, which contains the requisite unpaired electron, and which is coupled to a molecule with the desired biological properties. It is necessary to introduce an artificial probe, as suitable compounds which contain an unpaired electron do not occur naturally. The main disadvantage is that the spin relaxation time is far shorter than NMR, with a T_2 of microseconds (MYERS, 1973), so that data acquisition is a problem, and the majority of ESR is therefore performed in continuous-wave mode. Nevertheless, pulsed Fourier transform ESR has been developed for specialised applications, and the sensitivity is now comparable to that of continuous-wave ESR (THOMANN *et al.*, 1984).

Imaging with ESR could, in principle, be achieved in the same way as NMR by frequency and phase encoding, but is still in an early stage of development. However, experimental continuous-wave one-dimensional imaging has been achieved by a variety of methods (KARTHE and WEHRSDORFER, 1978; OHNO, 1982; HERRLING *et al.*, 1982), and, more recently, a spin echo sequence with pulsed ESR has been developed which considerably increases resolution (MILOV *et al.*, 1985). These studies have been performed in the X-band region (around 9 GHz), which would not be suitable for biological specimens because of the relatively high permittivity of biological tissues (see below). In the more appropriate L-band region of 500–1·6 GHz topical ESR measurements have been performed on phantoms with surface coils, with a comparable sensitivity to X-band ESR (NISHIKAWA *et al.*, 1985), and two-dimensional imaging by filtered back projection has been achieved by similar methods, when two 1·2 mm capillary phantoms were clearly imaged in a 65 × 65 matrix image from six projections (BERLINER and FUJII, 1984). Continuous-wave imaging would be too slow for clinical applications, but, in principle, pulsed ESR could be extended to imaging in the same way as for NMR.

The way in which ESR could be used to measure neuronal firing has already been established in principle *in vitro*, and involves the use of a potential-sensitive spin label probe, an example of which is shown in Fig. 2. This is a charged molecule which has both a polar and nonpolar moiety and so distributes at a lipid/aqueous interface, such as that at the surface of the neuronal membrane. Because it is charged the distribution between the population in the viscous membrane and aqueous bathing solution alters as the potential gradient across the neuronal membrane alters during the action potential. This can be recorded because the highfield ($I = -1$) peak broadens when the spin label is in the viscous neuronal membrane as opposed to the nonviscous aqueous bathing solution. This is due to the broadening effects of local magnetic fields which are largely cancelled out when the spin label rotates faster in the nonviscous medium (Fig. 3). The recorded spectrum is a measure of the sum of these two components, and so reflects the proportions in the two environments, which is in practice proportional to the amplitude of the highfield peak (CAFISO and HUBBELL, 1978). This process has been performed in artificial phospholipid vesicles (CAFISO and HUBBELL, 1978) and purified bovine rod outer segments (CAFISO and HUBBELL, 1980), when changes in peak amplitude of 90 per cent and 5 per cent, respectively, were recorded on depolarisation.

In practice, several problems would have to be overcome: the spin label would have to cross the blood/brain barrier, distribute equally along all suitable membranes and be resistant to degradation in biological tissues. In addition, since the spin label contains a free radical, it might be expected that safety could be a problem. However, recent results using sister chromatid exchange, a sensitive and reliable test for mutagenicity, showed that nitroxides were not mutagenic (AFZAL, 1984), and toxicity results on small animals yielded LD50 values well above 10–22 mmol kg^{-1}, which is almost one hundred times higher than maximum levels used for contrast agents used for NMR. Therefore a recent review concludes that spin labels appear to be safe (BERLINER and FUJII, 1984).

It thus appears that ESR imaging of neuronal activity is

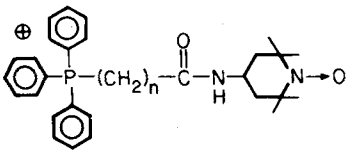

Fig. 2 *Alkyltriarylphosphonium spin label*

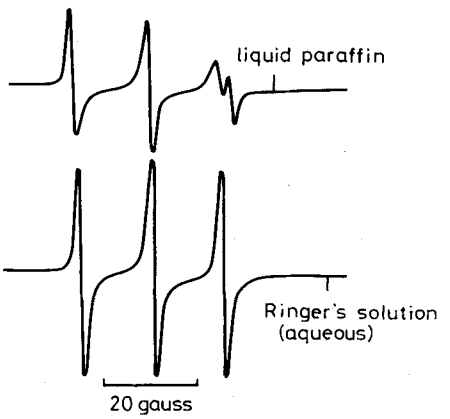

Fig. 3 *ESR spectrum of 1 mmol solution of the spin label TEMPO in a viscous lipid (liquid paraffin) or less viscous aqueous environment (Ringer's solution). It may be seen that the highfield (I = −1) peak is broadened in the viscous environment*

possible in principle but, clearly, the major problem is likely to be one of sensitivity.

This will in part depend on the concentration of spin label that could be obtained in human brain that would be bound to nerve membrane and so contribute to the signal. In experiments to measure potential using optical dyes, it was estimated that a ratio of 10–11 mol cm^{-2} of dye to area of nerve membrane corresponded to one dye molecule per 25 phospholipid molecules in the membrane (WAGGONER and GRINVALD, 1977). Cafiso and Hubbell suggest a slightly lower concentration of 1 spin label molecule per 100 membrane phospholipid molecules in order to prevent interference with membrane behaviour by the spin label (CAFISO and HUBBELL, 1981). Taking this into account, and the ratio of neuronal membrane per unit volume as above, this suggests a concentration of spin label of 15 μmol l^{-1} in unmyelinated white matter and 125 μmol l^{-1} in grey matter, attached to neuronal membranes in the brain.

With respect to the projected sensitivity for ESR imaging, a significant advantage is that the magnetogyric ratio of an electron is 657 times higher than that of a proton (MYERS, 1973), so that imaging could be performed at the optimum frequency, and would not be limited by magnet technology, as is the case at present for NMR. The optimum frequency may be calculated from eqn. 3, which gives the relationship between the relative signal ψ_r, the applied field H_o, the attenuation coefficient α, the depth of tissue traversed x and the power by which signal increases with field q (from HOULT, 1980; JOHNS and CUNNINGHAM, 1980):

$$\psi_r \propto H_o^r e^{-\alpha x} \tag{3}$$

Values for α were obtained for cat brain from Table 5.1 of NRCP, (1981) and Table 1 of KRASZEWSKI *et al.* (1982). x was taken as 8 cm, being the maximum depth of tissue radiation would have to traverse in the human head (LIBOVE and SINGER, 1980), and curves were calculated for values of q of 1, 1·25 and 1·5, in view of the uncertainty of the relationship of signal to field strength. The results are shown in Fig. 4, and indicate that the optimum field is approximately one gigahertz. This corresponds to a field of 0·036 T, which is well within the scope of modern magnet technology. In this respect, there should therefore be a significant increase in sensitivity compared with an NMR device operating at 2 T, which corresponds to approximately 80 MHz for proton NMR.

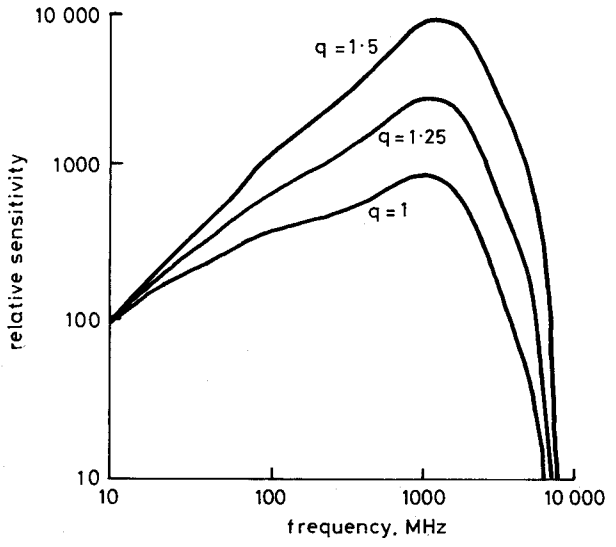

Fig. 4 *Relative sensitivity for magnetic resonance imaging of the human head, calculated from attenuation coefficients for human soft tissue, head diameter of 16 cm and an increase of intrinsic sensitivity of the device with respect to field to the power* 'q' *of between 1 and 1·5 (see text)*

The likely sensitivity of an ESR imaging device may be estimated by comparison with NMR. A laboratory ESR spectrometer can measure concentrations of spin label of 0·1 μmol l^{-1} at 9 GHz, in contrast to 0·1 to 5 mmol l^{-1} concentrations for laboratory NMR at 80 MHz (ILES *et al.*, 1982), which corresponds to a difference in sensitivity of approximately ten thousand times at these frequencies. On the basis that sensitivity is linearly related to field, this suggests that ESR spectrometry at the desired frequency of 1 GHz should have a sensitivity one thousand times greater than NMR at 80 MHz. Naturally, any estimate of the sensitivity of an ESR imaging device must be speculative, but an attempt may be made if it is postulated that the sensitivity of imaging relative to spectrometry will be similar to that of NMR, on the basis that factors such as increase in coil diameter and decrease in filling factor, which decrease sensitivity for NMR imaging, will be comparable. The concentrations of isotopes that can be imaged by NMR in the region of 80 MHz is of the order of 10 mmol l^{-1} (see above), so, taking all these factors into consideration, this suggests that ESR could image concentrations of the order of 10 μmol l^{-1} at 1 GHz. Thus it may be seen that concentration of spin label estimated for this technique certainly can be measured by a laboratory ESR spectrometer, and, on the above basis, lies at the projected limits of detectability for ESR imaging.

ESR imaging could also be used to detect changes in the concentration of metabolites, by the use of a metabolic precursor which was labelled with a suitable radical. The concentrations of the labelled metabolite which would be in the millimolar range should then lie well within the capabilities of an imaging device.

It thus appears that ESR appears promising for the imaging of neuronal activity. Eventual implementation rests on the development both of ESR imaging as a technique and the synthesis of a potential sensitive spin label or metabolic precursor with suitable properties, all of which pose considerable technical problems. Even if this could be achieved, sensitivity would probably lie at the limit of the technique. Nevertheless, none of these problems are insurmountable in principle, and it is concluded that the technique at least merits further investigation.

4 Techniques operating at high frequencies

4.1 *Positron emission tomography (PET)*

This is a sensitive and versatile technique, which has so far been the only method to produce images of metabolic activity *in vivo*. Images of cerebral blood flow, oxgen and glucose utilisation, as well as the distribution of a variety of neurotransmitter receptors, have been produced (PHELPS and MAZZIOTTA, 1985). Imaging is performed by reconstruction from gamma-rays emitted by decaying positrons, so that penetration is not a problem. It is extremely sensitive, and concentrations as low as picomolar can be detected (PHELPS and MAZZIOTTA, 1985). Currently spatial resolution is less than other imaging techniques, and is typically about 9 mm (HOFFMAN *et al.*, 1984), but a resolution of 4·2 mm has been achieved (CHO *et al.*, 1983), and it has been predicted that it should be feasible to image at the theoretical limit of the technique of 2 mm (BUDINGER *et al.*, 1984). Temporal resolution is relatively poor, as it is desirable to image for a substantial part of the life of the radioisotope employed, which is generally many minutes (BARRIO, 1983). A typical imaging time is 10 min for a head scan with 18-fluorodeoxygucose (SANK

et al., 1983), but temporal resolution can be improved by the technique of gating, and a gating window as short as 80 ms has been reported (TER-POGOSSIAN *et al.*, 1984).

The major disadvantage of the technique is that the signal cannot be directly coupled to physiological activity except by the binding or distribution of the compound which contains the isotope in the tissue, as it is simply the concentration of isotope which is imaged. As a result, maximum temporal resolution is limited to the transport of the radioisotope to and from the sample by blood flow, which is of the order of seconds (ROSENTHAL *et al.*, 1979). In addition, many techniques rely on serial measurements of arterial concentration of the radioisotope in order to extrapolate cerebral concentration, which further limits temporal resolution.

Thus PET cannot in principle be used to image changes directly related to the action potential with a resolution of milliseconds, but it is suitable for changes related to metabolic recovery processes. Of these, the most suitable is that of regional blood flow, which can be measured by the use of 15-O_2. A temporal resolution of 40 s has been achieved with a method employing serial arterial measurements (RAICHLE *et al.*, 1984), but a more suitable method would be one which employs steady-state conditions (FRACKOWIAK and LENZI, 1982), with which it should be possible to achieve a resolution of seconds by the use of gating. Oxygen consumption can also be measured in a similar fashion (FRACKOWIAK and LENZI, 1982; RAICHLE *al.*, 1984), although such evidence as is available, from rabbit vagus (RITCHIE, 1967, corrected to body temperature), suggests that the time course is much longer at 79 s. This method requires prior knowledge of blood flow, but this problem could be overcome by measuring this previously, and extrapolating to further images, which should be valid if a repeatable evoked response protocol is used. The half life of deoxyglucose is 7·7 h in grey matter and 9·9 h in white matter (SOKOLOFF, 1981), so that imaging of glucose consumption by the use of 19-fluorodeoxyglucose (SANK *et al.*, 1983) would not be suitable.

PET should therefore be suitable for imaging metabolic changes such as blood flow or oxygen consumption with a temporal resolution limited by the physiological changes themselves which are of the order of seconds, and with a spatial resolution of a few millimetres, but could not, in principle, be used to image changes related directly to the action potential.

4.2 *Single-photon-emission computed tomography (SPECT)*

This is a technique which is closely related to PET, but uses isotopes which emit a single photon. Selection of the direction of the origin of the radiation is achieved by the use of a collimator, which unfortunately excludes 999 out of each 1000 photons which are emitted, so that the efficiency of use of radiation is 10–100 times less than PET (KNOLL, 1983). Spatial resolution may be as much as 8·5 mm (ROGERS *et al.*, 1984), but is typically 17–20 mm (BUDINGER, 1982). It has the practical advantage that isotopes can be obtained by secondary decay, so that immediate access to a cyclotron facility is not needed, but the poor resolution makes it unsuitable for the criteria specified above.

4.3 *X-rays*

In the basic sciences, X-ray spectroscopy and diffraction have been extensively used for the determination of chemical structure, and X-ray diffraction has been used to measure changes in physiological systems such as structural changes in muscle on contraction (HUXLEY and FARUQI, 1983) or changes in the width of the extracellular space between myelin sheaths in peripheral nerve (BLAISIE *et al.*, 1972). Imaging of internal anatomy using computerised reconstruction is now well established and can be achieved with a contrast resolution of 0·5 per cent (HENDEE, 1983), spatial resolution of 0·1 mm and temporal resolution of 33 ms (BOYD and LIPTON, 1983).

X-rays could possibly be used to image neuronal activity by the use of a probe which altered its absorption of X-rays as the neuronal membrane depolarised. A similar process which employs fluorescent dyes irradiated with light at wavelengths between 570 and 840 nm has been reported, and changes in absorption of as much as 0·5 per cent for the olfactory bulb of the salamander with neuronal activity have been recorded (ORBACH and COHEN, 1983). The mechanism is unclear, but includes changes in monomer-dimer equilibrium, dye rotation and dye binding (COHEN and SALZBERG, 1978). Such a technique could give a measurable change with X-rays if a similar probe could be designed which would shift the wavelength at which its k edge occurred as depolarisation occurred. This could then be detected by the use of a highly monochromatic X-ray source, set at a wavelength near to the k edge, such that the frequency of the k edge of the probe altered to either side of the frequency of the source when depolarisation occurred. This could then be registered as a change in absorption as a several-fold change in absorption occurs around the k edge. This shift in k edge occurs in other biological compounds with changes in molecular conformation; for instance, the k edge of iron in cytochrome c shifts by 2 eV as the pH changes from 7 to 10 (SHULMAN *et al.*, 1976), and so, in theory at least, it might be feasible to design a probe with suitable properties. In practice, provision of a suitable source would also pose a considerable problem, as a cyclotron or a radioisotope which may not be able to produce the required intensities would need to be used.

The sensitivity of such a technique would also pose a problem, and may be estimated as follows. The fraction of radiation that is transmitted, A, is related to the fractional change in contrast that would occur with a change in absorption from downfield of the k edge, when absorption is low (suffix '), to upfield of the k edge, when absorption is high (suffix ''), CR, by

$$CR = \frac{A'' - A'}{A'}$$

Since (from JOHNS and CUNNINGHAM, 1980)

$$A = e^{-\mu x}$$

where μ is the attenuation coefficient and x is the sample thickness; then, substituting for attenuation coefficients for a body tissue such as muscle (suffix m) and the probe (suffix p)

$$CR = \frac{\exp^{-}(\mu_p'' + \mu_m)x}{\exp^{-}(\mu_p' + \mu_m)x} - 1$$

$$= \exp(\mu_p' - \mu_p'') - 1 \qquad (4)$$

To compare different isotopes, it is necessary to express μ in terms of (μ/ρ), the mass attenuation coefficient, where ρ is density in g ml^{-1}, C is concentration in mol l^{-1} and Z is the atomic number of the probe. Then

$$\mu = (\mu/\rho)ZC \times 10^{-3} \qquad (5)$$

The lowest concentration that could be imaged may then be estimated by combining eqns. 4 and 5 and rearranging:

$$C = \frac{\ln (CR + 1)}{((\mu/\rho)''_p - (\mu/\rho)'_p)Zx \times 10^{-3}} \quad (6)$$

Taking the minimum detectable contrast resolution as 0·005 as above, x as 0·1 cm, the width of one pixel, (μ/ρ) for iodine as 26·1 and 4·9 $cm^2 g^{-1}$ either side of the k edge, and as 7·3 and 1·86 $cm^2 g^{-1}$ for lead (JOHNS and CUNNINGHAM, 1980), this gives a minimum detectable concentration of 44 $mmol l^{-1}$ for a probe containing iodine, or 110 $mmol l^{-1}$ for one containing lead. The concentration of probe employed in the fluorescent dye experiments above was estimated as 10^{-11} $mol cm^{-2}$ of membrane, which corresponds to 60 $\mu mol l^{-1}$ for brain, taking the value of 6000 cm^2 of neuronal membrane per gram of brain as above. This is clearly far lower than the above limit of detectability. This limit approximates more closely to the concentration of metabolites, which is of the order of millimolar. While this could be possible for a lower spatial resolution, this would have no advantage over other methods for imaging metabolites described above. Taking both technical and sensitivity problems into account, it seems most unlikely that imaging of neuronal activity based on transmission of X-rays could work.

One other way in which it could be conceived that X-rays could be used for this purpose would be by an extension of the methods of X-ray diffraction mentioned above. However, these were for small systems with a regular repeating pattern of nerve or muscle fibres. Passage of X-rays across the entire brain would encounter fibres at all orientations, and so it is also unlikely that this method could work.

5 Ultrasound

Ultrasound imaging by reflection at tissue interfaces is well established as an imaging method in medical practice. More recently, there has been interest in imaging with transmitted ultrasound. Unfortunately, there are considerable technical problems due to reflection and diffraction in tissue. This is particularly true for the adult head; although it has been possible to produce a computed ultrasound image of the infant head, where the skull is not calcified, with a spatial resolution of approximately 5 mm, the image of the adult head was unsatisfactory (DINES *et al.*, 1981). Development of improved algorithms is under way (SCHOMBERG *et al.*, 1984), and it has been predicted that imaging of the adult head should become possible with technical improvements (DINES *et al.*, 1981).

The mechanism of interaction of ultrasound with tissues is not well understood, though proposed interactions include protein conformational change, alterations in the hydration layer of macromolecules and absorption by cellular structural components (DUNN, 1974; EDMONDS, 1982). No reports of modification of ultrasound by neural activity exist, but the converse interaction appears to exist in that ultrasound at therapeutic levels has been reported to cause reversible axonal block in frog and cat peripheral nerve, increased reflex and spontaneous discharge in mammalian spinal cord (WILLIAMS, 1983) and changes in the EEG of the squirrel monkey (HU and ULRICH, 1976), although this latter change was considered by other investigators to be at least partly artefactual (AMIN *et al.*, 1981). It therefore seems possible that there could be a difference in absorption of ultrasound with neural activity. However, the maximum contrast resolution of ultrasound is approximately 1 per cent (STEWART, 1974), and possibly related changes are relatively small: the volume of periaxonal water probably changes by a factor of 10^{-6} (COHEN and KEYNES, 1971), and the concentration of ion channels, which undergo a conformational change with neural activity, may be estimated from saxitoxin measurements for rabbit vagus (CHIU and RITCHIE, 1981) and the ratio of neural membrane to volume of brain above, to be approximately 0·1 $\mu mol l^{-1}$.

It therefore appears most unlikely that ultrasound could be used to image neural activity with the required resolution.

6 Conclusions

Two techniques, impedance imaging and electron spin resonance, appear to be suitable in principle for imaging neuronal discharge in the human brain and cannot be ruled out on the basis of sensitivity. While there are formidable technical problems that would have to be overcome to achieve imaging with these in human subjects, these are not insurmountable in principle, and it is concluded that these techniques merit further investigation and development.

Imaging of the larger but slower metabolic changes which accompany neuronal activity is already possible using PET in a time-independent manner. It should be possible to extend this to imaging of metabolic changes such as blood flow or metabolites using PET or NMR with a temporal resolution of the order of seconds in the relatively near future, and it may be possible to do this with ESR imaging in the more distant future. While these measurements will be of interest from a metabolic point of view, they are only indirectly related to neuronal discharge and so will be a less satisfactory index of neural activity.

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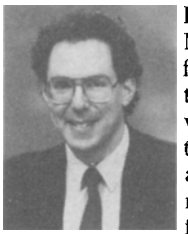
Author's biography



David S. Holder received his first degree in Medical Sciences, Physiology and Biophysics from Cambridge University (first class) and then graduated in Clinical Medicine from University College Hospital, London, in 1978. He then underwent training in general medicine and neurology, including posts at the Hammersmith Hospital and the National Hospital for Nervous Diseases in London. From 1982 to 1984 he studied biophysics and imaging technology at the University of California, Berkeley, USA. He then carried out preliminary research into ESR and impedance imaging of cerebral activity while Clinical Registrar in Neurology at St. James's University Hospital in Leeds, and is now performing this on a full-time basis in the Department of Physiology at University College, London.