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NMR microscopy — beginnings and new directions

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Abstract

In this paper we briefly review the origins of NMR microscopy, and in the spirit of the Sir Peter Mansfield Symposium of which this presentation was a part, point out especially Sir Mansfield and his co-workers contributions in this area. We then review some recent studies applying magnetic resonance (MR) microscopy focusing on our own contributions in these regards, in particular with reference to imaging of single neurons and more recent microimaging studies on isolated perfused brain slices. Finally we briefly describe recent preliminary studies on the feasibility of spectroscopic experiments that may be performed at the single cell level, further illustrating the growing scope and potential of magnetic resonance imaging (MRI) in general as a tool for examining biological systems non-invasively

Keywords: NMR microscopy; Microimaging; Spectroscopic experiments

1. Beginnings

After the invention of magnetic resonance imaging (MRI), the primary drive behind the development of MRI was understandably toward clinical applications. The inherently poor sensitivity of MR limited the spatial resolutions that could be achieved on relatively large objects to the macroscopic. However, even in those early days it was quickly realized that MR may have application at microscopic resolutions [1–4], especially on small objects; i.e. MR microscopy was feasible.

Early published discussions in these regards were presented in a book on NMR imaging by Mansfield and Morris [4]. Based on a combination of approximations, assumptions and empirical measurements they generated the following expression;

$$t_{vol} \approx \left(\frac{S}{N}\right)^2 a^2 \left(\frac{T_1}{T_2^*}\right) \frac{1.418 \times 10^{-15}}{\upsilon^{7/2} C} \left(\frac{1}{\Delta x}\right)^6$$

where t_{vol} is the imaging time, S/N is the signal to noise ratio, a is the radius of the rf detector coil, T_1 and T_2^* are the relaxation times, υ is the operating frequency, c is a factor related to the imaging sequence efficiency, and Δx the isotropic spatial resolution. The authors repeatedly stress the approximate nature of this formulation since many factors are difficult to approximate and there are several practical considerations that may degrade its accuracy. They then generate plots at different imaging scales to estimate the acquisition times that would be required to achieve a range of spatial resolutions. Fig. 1 is taken directly from the book and shows the estimated relationship in the microscopic regime, i.e. for objects around 1 mm in size. Looking directly at the graph an isotropic resolution of 10 μm should be achievable in just a few minutes with a S/N of 10. Adjusting for the assumption that the coil size is not usually matched exactly to the sample size, and bearing in mind that no account is taken of potential losses because of diffusion, this estimate is well within an order of magnitude. This is quite remarkable if one takes into account the timing of this work. Since the book was published in 1982 these estimates were made very early in the 1980s. To put this in perspective, at that time MRI was still not considered by many as a viable clinical tool, the magnet system of choice was still an electromagnet (the utility of superconducting

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magnets was still an issue), experiments based on animal tissues had predicted that imaging on humans above 10 MHz would be impossible, and even fMRI did not exist!

Studies to develop an MR microscope were being undertaken around that time by Dr Kyle Hedges, a student of Dr Paul Lauterbur. Dr Hedges generated a thesis in which he used small rf coils to obtain isotropic resolutions of tens of micrometres on an ex-vivo water snail [3]. Unfortunately this work remained unpublished beyond a short reference to the feasibility of these techniques in a meeting abstract [2]. In the mid 1980s several groups made forays into MR microscopy, including studies on rat brain by Johnson et al [5] in 1986, and an examination of a plant stem by Eccles and Callaghan [6] also in 1986. Over the next decade MR microscopy developed rapidly with applications in the biological and materials sciences [7,8]. It is beyond the scope of this paper to review this extensive and rapidly growing literature; rather the reader is referred to the literature with the text by Callaghan an excellent starting point [7]. In the rest of this paper the discussion is focused to work performed primarily by the authors.

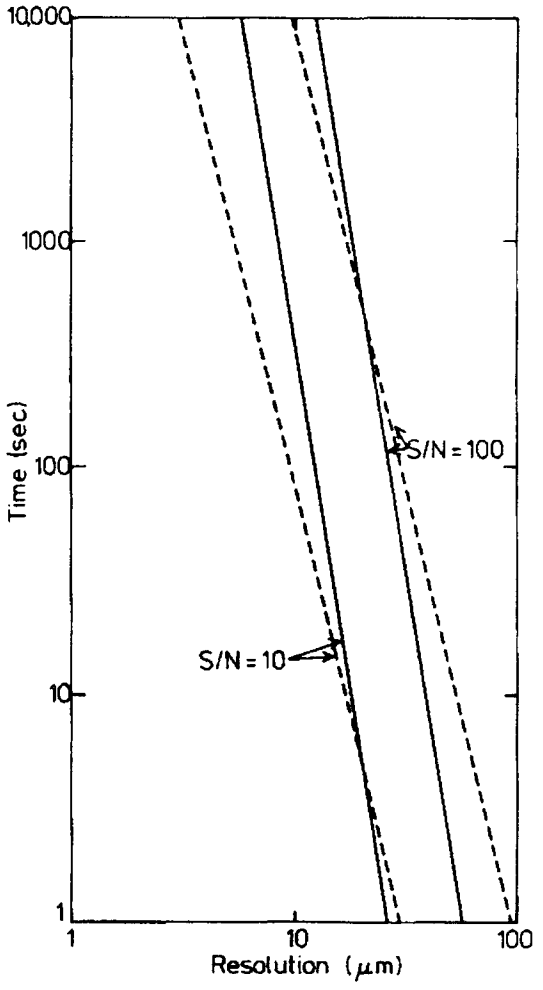

Fig. 1. An approximate relationship between SNR and data acquisition time at various spatial resolutions. The figure is taken from Ref. [4] by permission.

Also in 1986 a study reporting microscopic resolution MR images of single cells was published [9] that helped ignite the interest in the potential for MR microscopy. Although the cell was large (a frog ovum) the images clearly distinguished the cell nucleus and cytoplasm. For the first time the MR microscope was able to peer into biological tissues at the cellular level. Immediately these data offered promise for the determination of the origins of signals in whole tissues, and possibly another way to study basic cell biology. However the studies in themselves were limited — although the frog ovum is a bona fide single cell, it is a very large cell (around 1 mm in diameter), and also an oocyte, making it not typical of most other cells. The MR images show primarily just two compartments (cytoplasm and nucleus) at present spatial resolutions, and in the ovum there is a large lipid signal in the cytoplasm. Great improvements of spatial resolution of an order of magnitude or more (in all three spatial dimensions, i.e. a SNR improvement of more than three orders of magnitude) would be required to see other subcellular structures without the use of signal enhancing exogenous agents. Immediately it is thus difficult to see what could be learnt about single cells themselves using these techniques.

Nevertheless, attempts were made to broaden the scope of the cell systems that could be examined, but the requirement that they were large limited most to the examination of plant cells [10], with the studies performed primarily in a radiological fashion. However among these studies, Sir Mansfield and co-workers performed the first and we believe only attempt to date to perform dynamic studies on single plant cells, illustrating the potential of MR microscopy to follow functional processes in plants at the cellular level. Building on their first studies on plant cells [11], Mansfield and co-workers placed onion skin cells in a hypertonic solution and measured the rate of cell shrinkage, allowing the rate of cell plasmolysis to be determined [12]. A novel technological feature of the same work was the use of a small rf coil mounted on a microscope slide that could be removed from the rf probe [13]. In this way, the same sample could be examined optically to provide accurate correlative image data.

To date, the only other animal cell examined is also relatively large (≈ 300 μm in diameter) and is the L7 neuron isolated from the sea hare *Aplysia californica* [10]. Although still large compared to most cells, it is worth noting that the neuron is only $\approx 3\%$ the volume of the frog ova, indicating the technical improvements that were made. In the neuron, the nucleus and cytoplasm were again distinguished as shown in Fig. 2, exhibiting very different NMR characteristics [14].

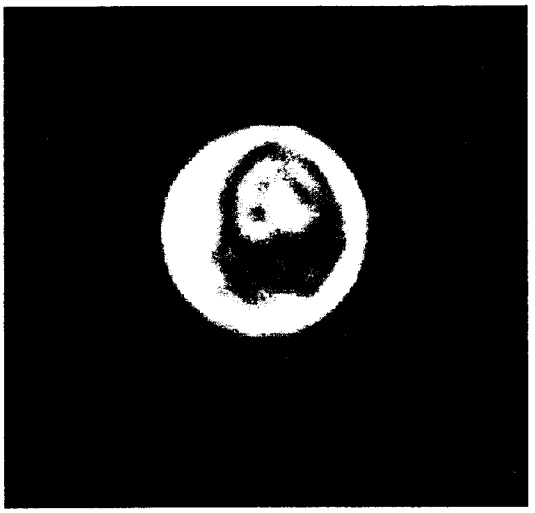

Fig. 2. NMR microimage of a single *Aplysia* neuron. The central bright area is the nucleus surrounded by a dark cytoplasm on this T_2 weighted image ($20 \times 20 \times 100$ μm resolution). The bright signal around the cell arises from artificial sea water.

Most startling is the observation that the water transverse relation time (T_2) and diffusion coefficient (D) were both several times larger than that of the cytoplasm, forcing NMR models of tissue compartmentation to consider not only an intra and extracellular compartment, but also separate nuclear and cytoplasmic compartments [10]. Changes in these NMR signals were quantified via the use of a microperfusion chamber that facilitated control of the extracellular environment [15].

Of particular importance in the sequence of studies on single *Aplysia* neurons was one performed in collaboration with Sir Peter Mansfield and co-workers. Previously in Mansfield's group, Sharp et al. developed a line narrowing imaging sequence that eliminated susceptibility distortion in microimages [16]. This was particularly timely, since there existed at that time concern that the contrast observed in single animal cells between the cell nucleus and cytoplasm arose from microsusceptibility effects. Had this been true, it would have been difficult to extrapolate the information acquired to lower, clinically relevant field strengths, since the susceptibility effects are magnetic field dependent. In collaboration with Blackband and co-workers, line narrowing experiments were performed on single neurons at Nottingham University using their home built NMR microscope, demonstrating that microsusceptibility effects were not significant at 500 MHz [17]. Thus the data obtained on single cells at high field strengths could be meaningfully used to interpret clinical MRI.

Taken together, these observations led to deductions regarding the signal origins in tissues and how they may depend on pathology. However the single cell studies are limited in their utility to test these speculations, in that the cell is not surrounded by a realistic extracellular compartment. It was thus necessary to find another controlled model with which our speculations could be tested, leading us to turn to the isolated perfused brain slice.

2. New directions

The brain slice model was pioneered by McIllwain in 1951 [18] and is a well used model for neurobiologists [19]. It has also been used for NMR spectroscopic studies, wherein large numbers of slices are placed in a perfusion chamber and multinuclear spectroscopic studies used to examine basic metabolic issues (for example [20]). We obtained the first MR images of single isolated perfused brain slices [21], an example being shown in Fig. 3. Although the detail is quite exquisite there is no hope with present resolutions of resolving individual cellular structures (without the use of exogenous agents). However it is possible to quantitate the NMR characteristics of the tissue and use this data in combination with the single cell models in an effort to construct a working model of biological tissues. In particular we have not concentrated on obtaining the highest resolution images feasible, since these require long data accumulation times. Rather, we have relaxed the resolution requirements facilitating image acquisitions in minutes so that dynamic processes can be monitored on the tissues in a meaningful physiological manner (see Fig. 3). Driven by the known sensitivity of diffusion weighted MR to ischemia, our early efforts have focused on using the brain slice model to help elucidate the mechanisms of signal changes in brain ischemia. The diffusion coefficient in the brain slice was

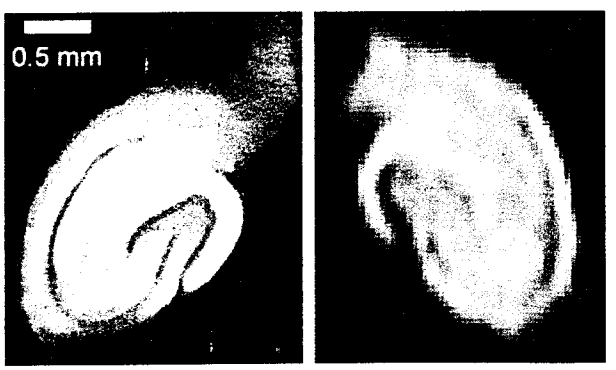

Fig. 3. Left, diffusion weighted NMR microimage of an isolated perfused rat hippocampal brain slice (500 μm in thickness) with a resolution of $20 \times 20 \times 300$ μm collected in 14 h. Right, lower resolution images (120×230 μm) in a few minutes (4.3 min) for dynamic physiological studies. The trade-off between resolution and imaging time is clearly demonstrated by these images.

observed to be non-monoexponetial, fitting well to a biexponential [22]. It has thus been postulated that the long and short diffusion coefficients measured in this way arise from the intra and extracellular compartments. Further, by perturbing the brain tissues with tonicity changes [21], or sodium pump disruption after ouabain application [22], the relative sizes of the two compartments is seen to change, but not the diffusion coefficient itself in each compartment. This observation is consistent with cell swelling data obtained on single neurons [15].

A problem remains with the brain slice data in that the calculated volumes of the two compartments based on the diffusion measurements do not agree with estimates of the size of those compartments as measured using other non-NMR techniques, in that the size of the intracellular compartment is greatly underestimated [22]. However this discrepancy could arise from several sources, the most prominent being that a correction has not been applied to allow for T_2 differences between the compartments as again implied by the single cell work. Estimates indicate that this would correct the discrepancy and corroborative T_2 measurements will soon be made. Additionally, we have demonstrated that contrast agents (Gd-DTPA-BMA) can be introduced to the brain slice preparation to independently measure the cell volume fraction in the brain slice model, and that this method is also sensitive to cell volume changes, this time induced by the addition of mannitol [23]. A direct correlation of the diffusion measured cell volume fraction and that determined by the exogenous agent should prove informative.

Recently, our work on the brain slice model has also extended beyond the gross tonicity changes or sodium channel disruption to examine the effect of a neurotransmitter on the images [24]. Application of the neurotransmitter NMDA also caused measurable cell swelling (as implied by biexponential diffusion measurements) that was not observed after the NMDA was applied in the presence of MK-801, a neurotransmitter blocking agent. These data illustrate that the MR images can be sensitive to direct neurochemical changes further broadening the potential of the model. Future experiments will involve quantitation of other functional processes in the brain slices, attempts to make regionalized measurements, and correlation with data obtained using other modalities. As stated, our long term goal is then to use these data to help construct a working model of MR signals from biological tissues. Consequently we believe that this may lead to an improved and more quantitative understanding of MRI which in turn will help improve the clinical utility of MRI.

3. Newer directions

So, where to go from here? NMR microscopy is a new modality and we expect great progress in the next few years with respect to its utility for understanding the origins of MR signals in tissues, and potentially for gleaning new information on the behavior of cells themselves. As an indicator, recent studies performed with co-workers at the Universities of Illinois (Dr Webb), Chicago (Sam Grant), Florida (Dr T. Mareci and Dan Pant) and the National High Magnetic Field Laboratory (Dr S. Gibbs), have employed so-called MR microcoils at 600 MHz to further improve the SNR in the microimaging experiments on single neurons. Using these technologies we have obtained the first spatially localized ^{1}H spectra on single neurons [25]. In just a few minutes resonances arising from highly concentrated osmolytes (betaine and choline at 100–300 mM) can be observed in these aquatic cells, with indications that metabolite levels could be measured in under 2 h. These data indicate for the first time the feasibility of NMR spectroscopy at the single cell level, and have been submitted for publication. Additionally we have also succeeded in generating the first sodium images of these single neurons using double tuned microcoils [26]. Resolutions of $80 \times 80 \times 300$ μm were achieved in 5.5 h (mistakenly the abstract states 32 min because of an acquisition error). These are long acquisition times prohibiting useful and potentially exciting dynamic studies at this point, but we are hopeful that technological advances will facilitate reductions in scan times. The images demonstrate a hyperintense nuclear signal intensity and little if any signal from the cytoplasm with the imaging parameters used. Although it is early in these studies (in particular measurement of T_2 is required), we speculate that the nuclear and cytoplasmic sodium T_2's differ in an analogous fashion to that of the water T_2, reflecting decreased sodium mobility in the cytoplasm. Together, these single cell proton spectroscopy and sodium imaging experiments serve to increase the incredible scope of NMR experiments. Both NMR imaging and spectroscopy are now feasible from man, through animal and excised tissues and perfused assemblies of cells, down to the single cell level.

4. Conclusion

The potential of NMR microscopy was recognized very early by the pioneers of MRI, with Sir Mansfield playing a major role in this regard. MR microscopy has now been available in a practical form for over a decade; indeed, it is now possible to buy very effective microscopy attachments to conventional spectrometers from commercial vendors for a relatively small addi-

tional cost. As these units become more widely available and exploited the full utility of NMR microscopy will progressively be realized. Although it does not yet offer the resolutions available with most other microimaging modalities, it does provide non-invasive measurements and the ability to perform dynamic functional studies on live biological tissues. We believe that our studies on single cells and brain slices demonstrate this emerging potential, providing quantitative information on the origins of signals from biological tissues that ultimately will help improve the clinical utility of magnetic resonance imaging and spectroscopy.

The limits of NMR microscopy, particularly in terms of spatial resolution, also require exploration. This short paper has not discussed potential technological developments for NMR microscopy, but several possibilities exist, including smaller rf coils, cooled or superconducting coils and higher magnetic field strengths. The full potential of exogenous agents as labels or signal enhancers remains to be explored for MR microscopy. The future potential of MR microscopy is wide open, and we can be grateful that the pioneering efforts of visionaries such as Sir Peter Mansfield will make studies of NMR microscopy an exciting endeavor as we step into the next millenium.

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