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Review

Diffusion, confusion and functional MRI

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ABSTRACT

Diffusion MRI has been introduced in 1985 and has had a very successful life on its own. While it has become a standard for imaging stroke and white matter disorders, the borders between diffusion MRI and the general field of fMRI have always remained fuzzy. First, diffusion MRI has been used to obtain images of brain function, based on the idea that diffusion MRI could also be made sensitive to blood flow, through the intravoxel incoherent motion (IVIM) concept. Second, the IVIM concept helped better understand the contribution from different vasculature components to the BOLD fMRI signal. Third, it has been shown recently that a genuine fMRI signal can be obtained with diffusion MRI. This "DfMRI" signal is notably different from the BOLD fMRI signal, especially for its much faster response to brain activation both at onset and offset, which points out to structural changes in the neural tissues, perhaps such as cell swelling, occurring in activated neural tissue. This short article reviews the major steps which have paved the way for this exciting development, underlying how technical progress with MRI equipment has each time been instrumental to expand the horizon of diffusion MRI toward the field of fMRI.

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Introduction

Diffusion MRI was born in 1985, before functional MRI (fMRI). Diffusion MRI has had a very successful life on its own (more than 290000 entries in Google Scholar for 'diffusion MRI' at time of writing) and its main clinical domain of application has been neurological disorders, especially for the management of acute stroke patients (Schellinger et al., 2000). It is also rapidly becoming a standard for white matter disorders, as Diffusion Tensor Imaging (DTI) can reveal abnormalities in white matter fiber structure (Le Bihan, 2003) and allow outstanding maps of brain connectivity to be obtained (Hagmann et al., 2007), which is of great potential to establish the Human Brain Connectome and evaluate connectivity disorders (Le Bihan and Johansen-Berg, 2012). However, the borders between diffusion MRI and the general field of fMRI field have always remained fuzzy. First, one of my initial goals in using diffusion MRI was to obtain images of brain function, based on the idea that diffusion MRI could also been made sensitive to blood flow, through the intravoxel incoherent motion (IVIM) concept which I had introduced. However, the first trials were not so successful and, in 1992, BOLD fMRI came in as a much easier and more sensitive approach. Second, the IVIM concept, although sometimes controversial, helped better understand the contribution from different vasculature components to the BOLD fMRI signal. Third, it has been shown recently than a genuine fMRI signal can be obtained with diffusion MRI, notably different from the BOLD fMRI signal, especially for its much faster response to brain activation both at onset and offset. Although the mechanisms of this new "DfMRI" approach are again a subject of controversy, DfMRI seems to reveal structural changes in the neural tissues, such as cell swelling, which occur when neural tissue gets activated, a significant departure to current imaging methods based on the neurovascular coupling mechanism. This short article will review the major steps which have paved the way for this exciting development, underlying how technical progress with MRI equipment has each time been instrumental to expand the horizon of diffusion MRI toward the field of fMRI.

In my hands diffusion MRI started in 1984 (I was then a medical resident and a graduate student in Physics). I came up with some

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fuzzy intuition that, perhaps, a molecular diffusion measurement would result in low values in solid tumors because of molecular movement restriction, while diffusion would be somewhat enhanced in flowing blood. Georges Wesbey and Michael Moseley had suggested that same year that diffusion could perhaps be imaged by using regular MR imaging sequences (playing with the slice selection gradient pulses), but it was clear to me that clean measurements of diffusion would require special treatment. Based on the pioneering work of Hahn, Carr and Purcell, and, most importantly, Stejskal and Tanner in the 1960s (Stejskal and Tanner, 1965), I thought specific magnetic gradient pulses should be used for diffusion encoding, but the problem was to mix such pulses with those used in the MRI sequence for spatial encoding. This was not trivial. The potential was to *localize* the diffusion measurements, that is, to obtain *maps* of the diffusion coefficients in tissues, which had never been done before, especially *in vivo*. I was very excited about this potential, and in a matter of weeks, diffusion MRI as we know it was conceived, born, implemented, and patented.

The first brain images were obtained on an almost homemade 0.5 T scanner called "Magniscan" by then CGR (Compagnie Générale de Radiologie), a French company located in Buc near Versailles in France (this company was sold to General Electric Medical Systems in July 1987 at the time I joined NIH). The first trials were not always successful. First, the MRI scanner operated at 0.5 T and the signal was very low (most brain diffusion MRI studies are performed today at 3 T). Second, gradient hardware barely allowed strengths beyond 8 or 10 mT/m to be reached (and still with large eddy currents), and "*b* values" larger than 100 or 200 s/mm² were not even in sight. (The *b* value is a central concept to diffusion MRI (Le Bihan et al., 1986; Le Bihan and Breton, 1985). It is calculated based on the intensity and the time course of the magnetic field gradient pulses used to make the MRI images sensitive to diffusion. The higher the *b* value, the larger is the sensitivity to diffusion. Plain MRI images have a native *b* value less than 5 mm²/s (contribution to the gradient pulses used to imaging). With contemporary scanners *b* values as high as 1000–3000 s/mm² are the norm). Third, there was no echo-planar imaging (EPI), just plain spin-echo sequences. Acquisition times necessary for diffusion encoding were very long (close to 10 min per scan) and, as respiratory gating was also not available, motion artifacts were sometimes atrocious. However, when it worked diffusion images of the brain were stunning, revealing unknown contrast features. I used my own brain and those of some of my colleagues before scanning patients: The potential of diffusion MRI to evaluate neurological disorders was established, and the rest is history.

The world's first diffusion images of the brain were made public in August 1985 at the Society of Magnetic Resonance in Medicine (SMRM) meeting in London. That year there were only three abstracts on diffusion imaging at the SMRM meeting (the other two dealt with diffusion measurements in a chicken egg (Taylor and Bushell, 1985) and in a human hand (Merboldt et al., 1985)). In 2011, more than 1100 abstracts (out of 4600) at the ISMRM meeting could be retrieved with *diffusion* as a key word. My first diffusion MRI paper appeared in 1985 in French in the journal of the Academy of Sciences (Le Bihan and Breton, 1985). However, this paper did not get much attention, clearly because it was written in French. My next paper in *Radiology* (Le Bihan et al., 1986) was much better received, with more than 1500 citations to date. Still, diffusion MRI was extremely sensitive to motion artifacts, to the point that some colleagues (sometimes prominent ones, so I'll keep their names secret) kept telling me and others after my talks, that it was not possible to measure diffusion in the brain, despite my efforts to explain that incoherent molecular motion and coherent macroscopic motion could be sorted out. It was very discouraging, but, fortunately for diffusion MRI, I sticked to my ideas and maintained my efforts and it paid off, as diffusion MRI progressively gained momentum (I had two SMRM abstracts in 1986, three in 1987). To my delight, some of the early detractors of diffusion MRI later started to work full time on it.

Meanwhile, during those years, I was investigating the idea that diffusion MRI could indeed provide information on perfusion. I came up with this view that, perhaps, the movement of the blood in the microvasculature could be modelled as a pseudo-diffusion process at a macroscopic scale. In the true (molecular) diffusion process, molecules move because of their own thermal energy and can be considered as colliding with each other (actual diffusion of water is, indeed, much more complex, see (Le Bihan, 2007) for a review). Each collision results in a change in the motion direction of each molecule, and the overall process is well described by a random walk, as first realized by Einstein (Einstein, 1905). Similarly, one may consider, at a macroscopic level, that in blood, in addition to diffusion, water molecules follow the stream and change direction between each capillary segment. If those segments are disposed in space in a pseudo-random manner, the overall movement mimics a random walk and the mathematical model used for diffusion should work as well. Although the difference in spatial scale between the processes of diffusion (nanometers) and pseudodiffusion (tens of micrometers) extends across five orders of magnitude, it is amazing to observe that the respectively associated diffusion and pseudo-diffusion coefficients only differ by roughly one order of magnitude (*D*, the molecular diffusion coefficient of water in tissues, is about 1×10^{-3} mm²/s, while D^*, the pseudo-diffusion coefficient associated with blood flow, is about 10×10^{-3} mm²/s in the brain). This is because those coefficients combine effects of elementary particle velocity and distance (Le Bihan and Turner, 1992). Molecular diffusion is a very fast process as far as molecular distances are concerned, while blood flow pseudo-diffusion is comparatively much slower, but involves distances of tens of micrometers. In any case, the proximity in values between *D* and D^* allow them to be evaluated together with the same diffusion MRI sequence, which is good news (Fig. 1), but it also means that diffusion MR images are prone to contamination by blood microcirculation effects.

It took a great deal of brainstorming with my mentor, Maurice Guéron (at the Ecole Polytechnique, where I was also completing my PhD in physics), to come up with the concept of intravoxel incoherent motion, or IVIM, to cover the overall molecular displacements to which "diffusion" MRI could be sensitive. Hence, it was very clear that the results of diffusion measurements with MRI could include perfusion effects, among other things, not only true diffusion, as demonstrated later by Yamada et al. (Yamada et al., 1999) in the liver, and the term *apparent diffusion coefficient* and acronym *ADC* were defined and introduced (Le Bihan et al., 1986). The theoretical framework for IVIM and the demonstration of the validity of the concepts in phantoms and in vivo were introduced in a seminal *Radiology* paper (Le Bihan et al., 1988), accompanied by a terrific editorial by Thomas Dixon (Dixon, 1988). Interestingly, Tom has told me that this editorial was one of his most cited papers, and indeed, it is fair to say that IVIM has been a great subject of controversy, as almost 20 years have passed from the genesis of the concept to standard application in clinical practice (Le Bihan, 2008).

Back in the middle of the 1980s, my major goal for IVIM imaging was to produce maps of brain perfusion to investigate brain function, given the known coupling between neural activation, metabolism, and blood flow (through the neurovascular coupling hypothesis (Roy and Sherrington, 1890)). At that time fMRI was not yet born and images of brain function were obtained using Positron Emission Tomography (PET). I recall scanning the daughter of my mentor back in 1986 (the visual stimulation was very crude, just a color print from a magazine shaken in front of her eyes; see Fig. 2). It did not work very well, although we got some encouraging results. The reason is that cerebral blood volume represents a very small fraction of brain tissue (around 2–4%), which required high signal:noise ratios in MRI images to be detected. IVIM-based fMRI did not survive competing methods that appeared at about the same time, first one based on contrast agents (Belliveau et al., 1991) which was short-lived, and almost

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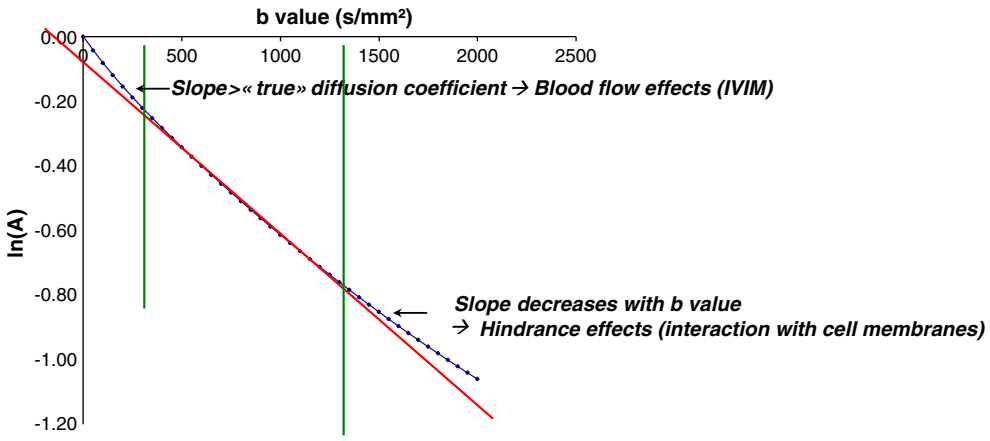

Fig. 1. Attenuation of the MRI signal as a function of the *b* value (diffusion sensitization). The logarithm of the signal attenuation in a diffusion MRI experiment is expected to be a straight line for free diffusion (red line). The slope is the diffusion coefficient. In experimental conditions in the brain the signal attenuation presents 2 levels of curvature. At very low *b* values (below 300 s/mm²) the deviation from a straight line comes from blood flow in randomly oriented vessels (IVIM effect). At high *b* values (above 1500 s/mm²), the curvature comes from the non-Gaussian (hindered and not free) diffusion behaviour. A pool of water molecules has shorter diffusion displacements than expected (slow diffusion, decrease in ADC), because they interact with obstacles, predominantly membranes.

immediately replaced with the blood oxygen level dependant (BOLD) concept (Ogawa et al., 1992). BOLD fMRI was clearly much easier to implement and much more sensitive, as it artificially increases the weight of the blood flow component to the MRI signal (please see the review by S. Ogawa in this issue), so there was no real room for the challenging IVIM method.

Further work, however, has proven the validity of the IVIM concept, with an increase in the IVIM perfusion parameters in brain activated regions (*increase* of ADC in activated voxels), and the potential of the approach to aid in our understanding of the different vascular contributions to the fMRI signal (Gangstead and Song, 2002; Jin and Kim, 2008; Song et al., 1996), see also the review article by A. Song in this issue). Interestingly, IVIM MRI has also been used in the context of fMRI in a negative way. A limitation of BOLD fMRI is its spatial resolution, as flow increase in somewhat large arteries or veins feed or drain large neuronal territories. By inserting "diffusion" gradient pulses in the MRI sequence (corresponding to low *b* values), one may crush the contribution of the largest vessels (with high D^* values associated with fast flow) in the BOLD signal and improve the spatial resolution of the activation maps (Boxerman et al., 1995; Duong et al., 2003; Lee et al., 2002; Michelich et al., 2006; Song and Li, 2003). Signal from large vessels with rapid flow disappears quickly with very low *b* values, while smaller vessels with slower flow might still contribute to the IVIM signal acquired with *b* values larger than 200 s/mm². Several groups have relied on this trick, though not always adequately acknowledging to the IVIM concept. For instance, the IVIM concept has been proposed to suppress signal from extracellular flowing fluid in perfused cell systems (Van Zijl et al., 1991; Zhao et al., 2008). It remains that there are genuine potential applications for IVIM MRI. Perfusion is a very important surrogate marker of many physiological or pathological processes. With MRI perfusion parameters can be obtained using gadolinium-based contrast agents, either injected as a bolus (to determine blood flow, transit times) or in a steady-state mode (to address blood volume, vessel permeability). With the rising concern of Nephrogenic Systemic Fibrosis (NSF), some patients cannot be explored with contrast agents. IVIM MRI may then appear as an interesting alternative to provide crucial clues on perfusion in tissues (Luciani et al., 2008; Posse, 1992; Tsuda et al., 1997; Yamada et al., 1999) (Moore et al., 2000a,b). The Arterial Spin Labelling (ASL) approach which was introduced in the early 1990s (Williams et al., 1992) also does not make use of contrast agents, but the rapid T1 decay of the magnetically labelled water makes it particularly challenging to study slow flow, which is not a limitation for IVIM MRI. Indeed, the IVIM concept has also been combined with the ASL technique to sort out the amount of labelled water present in the tissue and the vasculature (Kim and Kim, 2006; Silva et al., 1997).

Probably because of my Radiology article, diffusion MRI has been associated for many years with perfusion imaging, hence the many

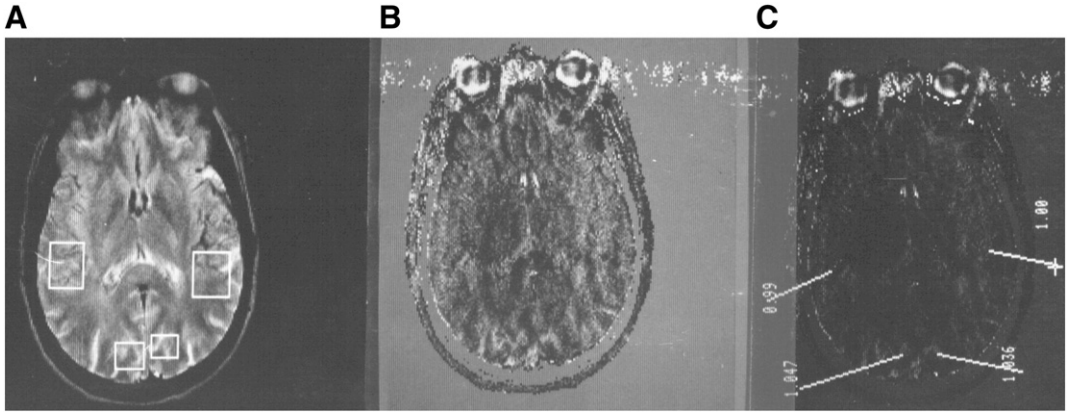

Fig. 2. Early IVIM fMRI study (scanned Polaroid photos). Left: T2w (b = 0) image, middle: calculated IVIM image during visual activation, right: Subtraction between IVIM images obtained during activation and rest (experiment dated October 20th, 1986). ROIs used for measurements are shown in A. The activated IVIM image shows stripes in the back of the brain which could be activated visual cortex (and many artifacts…). The subtraction image seems to indicate a 4% ADC decrease during activation in the visual cortex, but not elsewhere, at least with faith eyes.

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diffusion/perfusion sessions at meetings and workshops, or even books (Le Bihan, 1995), or the journal key-words, although they refer to completely different phenomena, both physically and biologically. This unexpected association has been a little bit puzzling for some of my colleagues which at some point teased me with such aphorisms as "diffusion, perfusion, … confusion" (printed on some tee-shirts during meetings). Anyway, there were real technical issues. Separation of perfusion from diffusion requires good signal-to-noise ratios, which were difficult to reach with low-field MRI systems and limited gradient hardware (Pekar et al., 1991; Wirestam et al., 1996). Although other groups published encouraging results in the brain (Chenevert et al., 1991; Le Bihan et al., 1991; Neil et al., 1994), other researchers expressed concerns about the concepts beyond perfusion measurements with the IVIM method and its ability to measure "classical" perfusion, compared with that of tracer methods. "Perfusion" had to be redefined according to the physiological viewpoint (blood flow) and the radiological viewpoint (vascular density), and the controversy was sometimes fierce (Henkelman, 1990; Le Bihan and Turner, 1992). Indeed, the exact nature of what is measured with IVIM MRI still deserves further investigation.

However, it was not until the availability of EPI on clinical MRI scanners that diffusion and IVIM MRI could really take off (Turner et al., 1990), as results became much more reliable and free of motion artifacts. Newcomers to diffusion MRI should realize how lucky they are to benefit from tremendous advances in MRI technology, combining EPI with parallel imaging using multiple channels, reducing echo times, making acquisitions less vulnerable to motion with respiratory triggering, and enjoying state-of-the art gradient hardware above 40 m/T/m. This move into the clinical field benefited immensely from my collaboration with Robert Turner. With EPI IVIM and diffusion, images could be obtained in a matter of seconds and motion artifacts became history (of course, new types of artifacts came along, such as geometric distortion).

I consider this 1987–1994 period, when I was at NIH, as the golden age of brain MRI. Important concepts were introduced at that time, such as fMRI or the discovery of diffusion anisotropy in brain white matter by Mike Moseley (Moseley et al., 1990), which lead Peter Basser and I, with the help of James Mattiello, coinvent diffusion tensor imaging (DTI) (Basser et al., 1994a,b). This was certainly a time of great excitement. In this context, an important achievement for me was the publication in 1995 of the first textbook on diffusion and functional MRI (Le Bihan, 1995). It was a tremendous success, but, unfortunately, this book quickly went out of print.

Another topic that has recently emerged is the possibility of using diffusion MRI, instead of BOLD, to detect brain activation (Darquie et al., 2001; Le Bihan et al., 2006), despite earlier negative results (Gulani et al., 1999). This time I am referring not to IVIM and perfusion, but to genuine diffusion changes occurring in tissues during neuronal activation. Diffusion in tissues is not free, but largely impeded and restricted by obstacles, mainly cell membranes. Beyond the low *b* value range (above 600 s/mm²), diffusion plots sill present a net curvature, although perfusion effects are no longer contributing (Fig. 1). Using *b* value ranges extending over 3000 s/mm² one may reveal different diffusion behaviors or compartments. The origin of this curvature is still debated (Chin et al., 2003; Stanisz et al., 1997; Yablonskiy et al., 2003), but, as it can be fairly well described by a biexponential law, many groups have suggested that it arises from the presence of two main diffusing water pools in slow or intermediate exchange (Assaf and Cohen, 1998; Niendorf et al., 1996). One is associated with a somewhat fast diffusion coefficient (about $1.3\,10^{-3}$ mm²/s in the brain) and the other with a slow diffusion coefficient (about $0.3\,10^{-3}$ mm²/s), and the ADC depends on the relative contribution of those two compartments in each voxel. The fraction associated with those compartments, 70% and 30% for the fast and the slow components, and the decrease of the slow fraction at short diffusion times invalidate an initial assumption that those pools could correspond to the extra- and the intracellular compartments, respectively. Indeed, there is growing evidence that the slow diffusion pool reflects the interaction of water molecules with cell membranes, although the exact mechanisms remain unclear (Le Bihan, 2007). This view accommodates previous reports linking changes in cell volume (and associated membrane surface) with the ADC, for instance the decreased in ADC observed during cytotoxic edema in acute stroke (Sotak, 2004; Van Der Toorn et al., 1996). Diffusion MRI with very high *b* values may, thus, reflect changes in tissue structure with greater sensitivity.

Earlier work on animal models has shown that a decrease in water diffusivity can be visualized using MRI during intense neuronal activation, such as during status epilepticus induced by bicucculine (Zhong et al., 1993) and cortical electroshocks (Zhong et al., 1997). Here, also, the diffusion drop (Hasegawa et al., 1995; Latour et al., 1994; Mancuso et al., 1999) (Latour et al., 1994; Mancuso et al., 1999; Röther et al., 1996) has been correlated to cell swelling (Dietzel et al., 1980; Hansen and Olsen, 1980; Phillips and Nicholson, 1979). Using heavily diffusion-sensitized MRI, a transient increase of the diffusion-weighted signal has been observed in the occipital cortices of human subjects (Le Bihan et al., 2006) and cats submitted to visual stimulation (Yacoub et al., 2008). This diffusion response is characterized by a steep onset and a temporal precedence (time-to-peak, return to baseline) relative to the hemodynamic response detected by blood oxygenation level dependent (BOLD) MRI (Aso et al., 2009) (Fig. 3) suggesting a non-vascular origin, although this hypothesis has been challenged experimentally (Jin and Kim, 2008; Miller et al., 2007) or theoretically (Autio et al., 2011; Kershaw et al., 2009). Although a residual BOLD (vascular) component is clearly present in the overall DfMRI signal (which is also T2 weighted) (Aso et al., 2009), the DfMRI signal increase also reflects a genuine *decrease* of the ADC of water in the cortex undergoing activation (opposite to the small ADC *increase* visible at very low *b* values from IVIM effect, not diffusion), but the exact physiological mechanisms underlying this transient water diffusion response remain, however, not established at this stage. Assuming that the slow diffusion pool could originate from water molecules in interaction with cell membranes, so that variations in ADCs are linked to those of the cell surface, it has been hypothesized (Le Bihan, 2007) that this decreased ADC could result from variations in cell shape and size, such as neural and/or glial cell swelling, occurring during the activation process (Andrew and Macvicar, 1994; Holthoff and Witte, 1996; Takagi et al., 2002; Tasaki et al., 1985). Such variations in cell size linked to neuronal depolarization have also been observed in brain slices where confounding blood effects are removed, using proton-density weighted MRI (Stroman et al., 2008) and diffusion MRI (Flint et al., 2009).

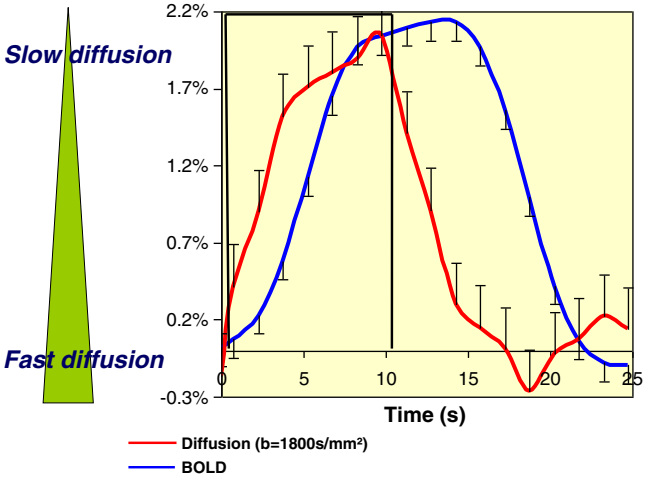

Fig. 3. Time course of the BOLD and diffusion fMRI responses in human visual cortex. The amplitude of the responses is comparable, but the diffusion response is clearly ahead of the BOLD response by several seconds, both at onset and offset. A signal increase in the raw (diffusion-weighted) MRI images reflects decreased diffusion.

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Although BOLD fMRI has been extremely successful for the functional neuroimaging community, it presents well-known limitations. The degree and mechanism of the coupling between neuronal activation, metabolism, and blood flow are not fully understood and may even fail in some pathological conditions or in the presence of drugs, even though the brain apparently works normally. Also, it has been pointed out that the spatial functional resolution of vascular-based functional neuroimaging might be limited, because vessels responsible for the increase in blood flow and blood volume feed or drain somewhat large territories that include clusters of neurons with potentially different functions. Similarly, the physiological delay necessary for the mechanisms triggering the vascular response to work intrinsically limits the temporal resolution of BOLD fMRI. In contrast to vascular-based approaches, diffusion MRI has the potential to reveal changes in the intrinsic tissue properties during brain activation, which could be more intimately linked to the neuronal activation mechanisms (such as neuronal and/or glial cell swelling accompanying cell depolarization) and lead to an improved spatial and temporal resolution. These changes in the diffusion behavior of water during activation might indeed point out to water movements which have without a doubt a central role in brain physiology (Agre et al., 2004). What is the contribution to brain tissue function of those rapid "mechanical" changes that have been noticed in tissue microstructure (such as the twitching of the dendrite spines (Crick, 1982)? Could we envision that, because laws of nature are most often reversible, neurons or glial cells are also acting as piezoelectric transducers that get depolarized when "feeling" the movements of neighboring cells or submitted to a mechanical pressure, a fast alternative to synaptic transmission for information fluxes? Here, too, diffusion MRI could help our understanding and exploiting of those mechanisms.

In conclusion, diffusion MRI preceded fMRI, but played and is still playing an important role in the development of fMRI. Interestingly, the role of diffusion MRI has highly depended on the intensity of the *b* values which were allowed, following progress in MRI hardware, especially gradient coil hardware (Fig. 4). This role has appeared sometimes confusing to some players, and I hope this short review will have clarified things up. At the beginning, because only very low *b* values (around 100 s/mm²) were possible, ADC measurements were sensitive to blood flow through the IVIM effect. Hence, the possibility to use diffusion MRI or more exactly IVIM MRI to get images of brain function was explored, as a precursor to BOLD fMRI which came soon after. Then, diffusion and IVIM MRI were used in conjunction to BOLD fMRI as a way to suppress signal from flowing blood, so as to sharpen the fMRI images. More recently, while large *b* values (around 1000–3000 s/mm²) became reachable genuine changes in water diffusion in brain activated tissues were observed, opening a new front for fMRI. Whether DfMRI would become a current standard for fMRI or will only be used for specific applications where an accurate temporal resolution is required or to investigate the basic mechanisms underlying neural tissue activation remains to be seen, but diffusion MRI will definitely remain an important component of fMRI.

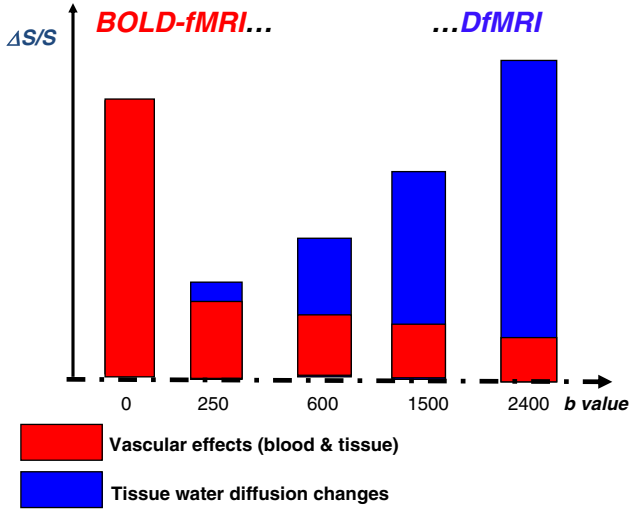

Fig. 4. Theoretical contribution of tissue diffusion and vascular effects to the DfMRI signal change during activation. At $b = 0$, only vascular ($T2^*$) effects are present. This is the usual BOLD effect caused by dephasing of spin magnetization in vessels and in tissues surrounding vessels containing deoxyhemoglobine. When the *b* value is increased the contribution from vessels with fast flow and then slower flow is crushed (IVIM effect). Some genuine diffusion (tissue) effects start to appear. At large *b* values only the residual BOLD effects from tissues remain (slightly decreasing with the *b* value) while the genuine diffusion (tissue) effects predominate.

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