MR imaging in the non-human primate: studies of function and of dynamic connectivity
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Since its early development in the late 1940s, nuclear magnetic resonance has become a powerful tool for applications ranging from chemical analysis or the study of the structure of solids to biomedical investigations. In the early 1990s the potential of this technique for functional brain mapping was demonstrated, causing unprecedented excitement in both basic and clinical neuroscience. It was shown that by using the appropriate pulse sequences the so-called functional magnetic resonance imaging (fMRI) technique can be made sensitive to local magnetic susceptibility alterations produced by changes in the concentration of deoxyhemoglobin in venous blood vessels. This blood-oxygenation-level-dependent (BOLD) contrast mechanism was successfully implemented in awake human subjects, in small animals, and recently in the non-human primate — the experimental animal of choice for the study of cognitive behavior. Simultaneous imaging and electrode recordings promise new insights into the mechanisms by which large-scale networks in the brain contribute to the local neural activity recorded at a given cortical site. Moreover, the use of MRI-visible tracers and of electrical microstimulation applied during imaging proves to be ideal for the study of connectivity in the living animal.

Introduction

Understanding brain function requires comprehension of the physiological workings of its cellular components, as well as a detailed knowledge of their structural and functional connectivity. Moreover, the functional plasticity of the brain, reflected in its capacity for anatomical reorganization, means that a mere ‘snapshot’ of its architecture is not enough. Desirable, instead, are repeated anatomical and physiological observations of its connectivity patterns at different organizational levels. In vivo neuroimaging, especially the spatiotemporally resolved magnetic resonance imaging (MRI), together with its functional variant, functional magnetic resonance imaging (fMRI), is therefore of obvious importance, and it is currently one of our best tools for linking cognition and action with their neural substrates in humans.

At the moment, brain fMRI relies mainly on the blood-oxygen-level-dependent (BOLD) contrast mechanism, which was first described in rat studies using high magnetic fields [1]. BOLD contrast is derived from the field inhomogeneities induced by deoxyhemoglobin (dHb), which is confined in the intracellular space of the red blood cells that in turn are restricted to the blood vessels. Magnetic susceptibility differences between the dHb-containing compartments and the surrounding space generate magnetic field gradients across and near the compartment boundaries. Pulse sequences designed to be sensitive to such susceptibility differences alter the signal whenever the concentration of dHb changes.

Upon neural activation, an increase in dHb concentration would be expected to enhance the field inhomogeneities, reducing the BOLD signal. Yet, a few seconds after the onset of stimulation the BOLD signal actually increases. This enhancement reflects an increase in cerebral blood flow (CBF) that overcompensates for the increased oxygen consumption, and ultimately delivers an oversupply of oxygenated blood [2,3]. BOLD imaging has been successfully applied in humans [4–6] and was recently applied in the non-human primate [7–9,10–12].

An example of BOLD imaging in the monkey is shown in Figure 1a, a high resolution functional scan obtained from...
an anesthetized monkey in a moderately high field (4.7 Tesla) scanner [11]. A gradient-recalled multi-shot, multi-slice echo planar imaging method, optimized for both the field and the subject-species, was used to acquire volumes of 13 slices every 6 seconds while a polar-transformed rotating checkerboard was presented to the monkey through a fiber-optic system. The image shows maps of the activation’s statistical significance (Z-scores from a T-test) superimposed on T1-weighted anatomical scans; whereby T1 refers to the time constant describing the tissue’s magnetization re-growth. Significant clusters of activation can be seen in the lateral geniculate nucleus (LGN) and the striate cortex. The LGN of the monkey is only about 6 mm across in the rostro-caudal direction and approximately 5 mm in the dorso-ventral and mediolateral directions. The precise anatomical localization of its activation is therefore important evidence for the spatial specificity of fMRI in high magnetic fields.

Gradient echo sequences, like those used for the acquisition of the images in Figure 1, are sensitive to both small and large vessels [13,14]. The significant contribution of the large vessels can lead to erroneous mapping of the activation site, as the flowing blood will generate BOLD contrast downstream of the actual tissue with increased metabolic activity. Thus, the extent of activation will appear to be larger than it really is. The contribution of large vessels depends on both the field strength and the parameters of the pulse sequences [15–18], and they can be de-emphasized in stronger magnetic fields, because the strength of the BOLD that originates in the parenchyma (extravascular) increases more rapidly for small
vessels than it does for large ones (for review see [19]). Thus, with a sufficiently high signal-to-noise ratio (SNR), signals originating from the capillary bed are discernable in strong magnetic fields and better reflect the site of actual neural activation [20,21]. This property can be exploited to obtain precise maps of areas defined on the basis of functional architecture [22].

Specifically, cortex consists of specialized, separate areas, which can be identified by their cyto- and myelo-architecture, physiological properties and connectivity (e.g. Felleman and Van Essen [23]). In the visual system, the boundaries of some areas can be determined by exploiting their retinotopic organization, and several laboratories have already developed methods for measuring visual field maps in the human brain (for a review see Wandell [24]). Retinotopic maps were also recently obtained in monkeys [25], and were found to register well with those derived from the same species using anatomical and physiological techniques. In contrast to these techniques, however, non-invasive mapping of the retinotopic areas of individual monkeys is likely to prove a valuable tool for several longitudinal investigations, including studies of learning, plasticity and reorganization.

When imaging of the entire brain volume is not the primary interest, then small surface radiofrequency (RF) coils can be used in either human or animal studies to obtain the highest possible SNR in spatially resolved images. The coils are often used as transceivers, namely both for exciting the tissues and for receiving the RF signals transmitted by them. Image quality and resolution can be further improved by geometrically matching the coils to a specific tissue region and, in animal experiments, by implanting the coils in the body (for references see [26,27]). The small RF surface coil used in Figure 1b–d was implanted within the bone (intraosseally). The achieved resolutions in this case were 125 × 125 × 720 μm³, 125 × 125 × 720 μm³ and 75 × 150 × 300 μm³.

The contrast sensitivity of the image in Figure 1b is sufficient to reveal the characteristic striation of the primary visual cortex. The dark line shown by the white arrow is the well-known, approximately 200 μ thick Gen
nari line formed by the axons of pyramidal and spiny stellate cells contained in middle cortical layer (lamina IVb). Figure 1c shows fMRI correlation coefficient images (in color) superimposed on the actual echoplanar (EPI) (T₂-weighted) images. T₂ refers to the transverse relaxation time constant in the presence of field inhomogeneities. The two most commonly varied MRI parameters are the relaxation time constants T₁ and T₂. As mentioned above, T₁ is the tissue’s magnetization re-growth. T₂, on the other hand, refers to the so-called transverse relaxation in an ideal homogenous magnetic field. In actuality the transverse relaxation is more rapid because of local field inhomogeneities, including those within the imaged tissue itself. It is then characterized by the decay constant T₂ rather than T₂. In the T₁-weighted image of Figure 1c robust activation and good anatomical detail can be discerned. Voxels of this size reflect the activity of as few as 600–1200 cortical neurons, providing us with the opportunity to study how neural networks are organized, and how small cell assemblies contribute to the activation patterns revealed in fMRI. Furthermore, they enable direct comparisons between the imaging signals and those signals obtained in intracortical recordings.

Intracortical recordings and fMRI

The successful application of BOLD fMRI in human or animal brain research ultimately depends on a comprehensive understanding of the relationship between the hemodynamic signal and the underlying neuronal activity. Functional MRI has already been combined with optical imaging recordings of intrinsic signals [28] and electroencephalography (EEG; e.g. [29]). However, the first method also measures hemodynamic responses [30] and thus can offer very little direct evidence of the underlying neural activity, whereas EEG studies typically suffer from poor spatial resolution and imprecise localization of the electromagnetic field patterns associated with neural current flow. Recently, combined intracortical recordings and BOLD fMRI were successfully applied in anesthetized and conscious monkeys. The BOLD response was found to directly reflect an increase in neural activity, best correlating with those electrical signals that are thought to represent synaptic inputs and local intracortical processing [31]. This conclusion was derived from the detailed study of different components of the digitized comprehensive mean extracellular field potential (mEFP), which encompasses time-varying spatial distributions of action potentials superimposed on relatively slow varying field potentials.

Neural signals and their cellular origin

The mEFP represents the weighted sum of all sinks (e.g. negativities caused by Na⁺ or Ca²⁺ moving from the extracellular to the intracellular space) and sources (positivities) along multiple cells. If a microelectrode with a small tip is placed close (within about 50 μm–100 μm) to the soma or the axon of a neuron, then the measured mEFP directly reports the spike traffic of that neuron, and frequently that of its immediate neighbors as well [32].

The firing rate of such well isolated neurons has been the usual measure for comparing neural activity to sensory processing or behavior ever since the earliest development of microelectrodes. A great deal has been learned since then, and the single-electrode single-unit recording technique still remains the method of choice in many behavioral experiments with conscious animals. Like any other method, however, it also has drawbacks, providing information mainly on single receptive fields but no
access to subthreshold integrative processes or the associational operations taking place at a given site. In addition, it suffers from an element of bias towards certain cell types (for a review and references see [33,34]). Spikes generated by large neurons remain above the noise level over a greater distance from the cell than spikes from small neurons, so microelectrodes sample their somas or axons preferentially, a prediction supported by experimental work.

When the activity of a single neuron is not the primary concern of an investigation, microelectrodes of lower impedance and appropriate tip geometry can be constructed that are less inundated by spikes and capture the totality of the potentials in a given region. The mEFP recorded under these conditions is related to both local integrative processes (dendritic events) and spikes generated by several hundred neurons.

A large number of experiments have presented data that indicate that the two different processes can be segregated by subjecting the mEFP to frequency-band separation. A high-pass filter cutoff of approximately 300–400 Hertz is used in most recordings to obtain multiple-unit spiking activity (MUA), and a low-pass filter cutoff of about 200–300 Hz to obtain the so-called local field potentials (LFPs).

**Fast and slow components of the mean extracellular field potential**

The MUAs are a weighted sum of the extracellular action potentials of all neurons within a sphere of approximately 140–300 μm radius, with the electrode at its center. Spikes produced by the synchronous firings of many cells can, in principle, be enhanced by summation and thus detected over larger distances [33,34].

The LFPs are slow fluctuations reflecting cooperative activity in neural populations. Until recently, these signals were thought to represent exclusively synaptic events. Evidence for this came from combined EEG and intracortical recordings that showed that the slow wave activity in the EEG is largely independent of neuronal spiking [33,34]. They also showed that, unlike the multiunit activity, the magnitude of the slow field fluctuations is not correlated with cell size, but instead reflects the extent and geometry of dendrites in each recording site. The pyramidal cells, with their apical dendrites running parallel to each other and perpendicular to the pial surface, form an ideal open field arrangement and contribute maximally to both the macroscopically measured EEG and the LFPs. The LFPs are the weighted average of synchronized dendro–somatic components of the synaptic signals originating from within 0.5–3 mm of the electrode tip (e.g. [35]).

Today we know that LFPs represent both synaptic events and other types of slow activity, including voltage-dependent membrane oscillations (e.g. [36]) and spike afterpotentials. The soma–dendritic spikes in the neurons of the central nervous system (CNS) may be followed by afterpotentials, a brief delayed depolarization, the afterdepolarization, and a longer lasting after-hyperpolarization, which are thought to play an important role in the control of excitation-to-frequency transduction (e.g. [37,38]). Afterpotentials, which were shown to be generated by calcium-activated potassium currents (e.g. [39,40]), have a duration in the order of 10’s of milliseconds and most likely contribute to the generation of the LFP signals (e.g. [41]).

**BOLD reflects the input and local processing of a studied brain area**

To understand the contribution of such different cellular events to the generation of the BOLD signal, we examined the correlation of LFPs, MUA and single neuron activity with the hemodynamic response in a large number of combined imaging-physiology experiments in the striate and extrastriate areas of the monkey visual system [31**]. Figure 2 shows the neural and BOLD signals recorded simultaneously from an alert, fixating monkey. At first sight, they all seemed to be correlated with the BOLD response, although increases in the LFP range were consistently greater in both spectral power and reliability (SNR). Furthermore, correlation analysis showed that LFPs are better predictors of the BOLD response than multiunit spiking.

The relation of the two types of signals to BOLD was best appreciated, however, in cases of a complete dissociation between slow waves and spikes. Sites exhibiting a strong neural response adaptation were characterized by MUA that returned to the baseline a few seconds after stimulus onset, and local field potentials that remained elevated for the entire duration of the visual stimulus. In such cases, LFPs were the only neural signal to be associated with the BOLD response. This striking result suggests that spikes are a ‘fortuitous’ predictor of the BOLD signal, simply because the firing of neurons usually happens to correlate with the LFPs. A similar dissociation between spikes and CBF has also been demonstrated in microstimulation studies in the cerebellar cortex (for a review of this work see Lauritzen and Gold [42]).

The differential contribution of LFPs and MUA to the BOLD response can also be demonstrated by neuropharmacological manipulations that permit the selective modulation of interneuronal and pyramidal activity. Figure 3 shows the effects of intracortical serotonin injections into the primary visual cortex of the monkey during simultaneous acquisition of BOLD and neural responses (N Logothetis, unpublished data). Using a triple pipette (electrode, saline and the neuromodulator) 20 micro-liters of 0.01 Mol 5HT (5-hydroxytryptamine hydrochloride; serotonin) were injected over a period of 10 minutes. A
couple of minutes after the injection, a profound suppression of the MUA was observed (Figure 3a). The LFP signal showed a slight increase and returned to baseline within a few minutes. However, no significant change was discernible in the BOLD response. Spectrograms obtained before and after the 5HT injection during visual stimulation showed that the stimulus-induced spikes were entirely eliminated, whereas LFP activity was moderately increased (Figure 3b). BOLD response to the stimuli was unaltered, indicating once again the possibility of a total dissociation between spiking activity and hemodynamic responses. On the basis of all of these dissociations, we conclude that the LFP signal is the key variable for the BOLD response.

Taken together, these results suggest that the BOLD signal mainly reflects the incoming specific or association inputs into an area, and the processing of this input information by the local cortical circuitry (including excitatory and inhibitory interneurons). Usually, the incoming subcortical or cortical input to an area will generate the kind of output activity that is typically measured in intracortical single-unit recordings, and the recorded spike rate will be correlated to the BOLD signal. If it does not, perhaps because the activity of projection neurons is shunted by concurrent modulatory input, hemodynamic responses will still be generated, but the spiking activity measured with microelectrodes will—in such cases—be a poor predictor of the hemodynamic response. Several experiments demonstrate the plausibility of this explanation.

In a recent study, Tolias and co-workers [43] used an adaptation technique to study the brain areas that process motion information [44]. They repeatedly imaged a monkey’s brain while the animal viewed continuous motion in a single unchanging direction. Under these conditions, the BOLD response adapts. When the direction of
motion reverses abruptly, the measured activity immediately shows a partial recovery toward the initial activity levels, or rebound. The extent of this rebound was considered to be an index of the average directional selectivity of neurons in any given activated area. The results confirmed previous electrophysiological studies that had identified a distributed network of visual areas (V1, V2, V3, V5/MT) in the monkey that process information about the direction of visual motion.

Surprisingly, however, strong activation was also observed in area V4, which is only weakly involved in motion processing (e.g. [45]). Such a discrepancy can be explained on the basis of the arguments developed earlier. Areas V4 and MT are interconnected [46]. Although they process separate stimulus properties, each area may influence the sensitivity of the others by providing some kind of ‘modulatory’ input, which in itself is insufficient to drive the pyramidal cells recorded in a typical electrophysiology experiment. In such cases, BOLD fMRI will reveal significant activation of an area whose output may be only indirectly related to the stimulus or task, providing results that do not match those of neurophysiology experiments. Similarly, attentional effects on the neurons of striate cortex have been very difficult to measure in monkey electrophysiology experiments [47,48]. Yet for similar tasks strong attentional effects are readily measurable with fMRI in the human primary visual cortex (V1) (e.g. [49]).

In summary, combined physiology and fMRI experiments suggest that the BOLD signal primarily reflects the input of neuronal information to the relevant area of the brain and its processing there, rather than the output signals transmitted to other regions of the brain by the principal neurons; the cells that are most easily accessible in single cell recordings in the behaving animal. These findings are in agreement with several observations from experiments on the brain’s energy metabolism and the mechanisms that couple neural activation with metabolic demands.
Selective neurovascular and neurometabolic coupling

Basics of brain energy metabolism

The brain’s demand for substrate requires adequate delivery of oxygen and glucose through elaborate mechanisms regulating CBF. Early experimental evidence that suggested a regional coupling between these mechanisms and neural activity [50] was later verified by means of the deoxyglucose autoradiographic technique that enabled spatially resolved measurements of glucose metabolism [51].

In humans, the first quantitative measurements of regional brain blood flow and oxygen consumption were performed using radiotracer techniques, which were followed by the introduction of positron emission tomography (PET; for a historical review see Raichle [52]). PET showed that maps of activated brain regions can be produced by detecting the indirect effects of neural activity on variables, such as cerebral blood flow [53], cerebral blood volume (CBV) [2], and blood oxygenation [2,3]. At the same time, optical imaging of intrinsic signals demonstrated the spectacular precision of neurovascular coupling (the strong correlation between neural and vascular changes) by constructing detailed maps of cortical microarchitecture in both the anesthetized and the alert animal [30].

Although the existence of a regional coupling between neural activity, metabolism, and hemodynamic changes is now established, the nature of the links among these processes remain largely unknown. One important question is whether changes in CBF are driven directly by energy demand or by neurotransmitter related signaling (see review by Attwell and Iadecola [54]). If energy demand is indeed the trigger for CBF changes, then which are the cellular processes and sites that dominate the energy consumption? The structural and functional organization of the neuro-vascular system provides some insights into these questions.

Structural neurovascular coupling

The density of the vascular network in a given region largely correlates with its average activity. Most importantly, however, the spatial correlations reported by a large number of investigators have been mainly between vascular density and the number of synapses rather than the number of neurons. For instance, on the basis of its density the human cortical vascular network can be subdivided into four layers parallel to the surface [55]. These layers systematically overlap with certain portions of the cytoarchitectonically defined Brodmann’s laminae. Notably, the first Duvernoy layer, which consists of vessels oriented approximately parallel to neural fibers, is entirely within the lower part of the molecular layer (Layer 1), which in the rodent has the lowest concentration of cell bodies and highest density of synapses [56]. Similarly, in the primate, this layer has the lowest concentration of neurons, the highest concentration of astrocytes, and a high density of synapses [57]. Its vascularization is therefore suggestive of the contribution of these cellular types and components in the hemodynamic response.

The spatial correlation of blood vessels and neural components reported for the human brain were later confirmed in animal studies [58]. The vascularization was found to be lowest in Lamina I and highest in IVc, with an average IVc/I ratio (across animals) of approximately 3:1. Interestingly, the IVc/I ratio of synaptic density in the striate cortex of macaque is 2.43:1, that of astrocytes is 1.2:1, and that of neurons is 78.8:1. Assuming some similarity in the distribution of neurons, synapses, and astrocytes between squirrel monkeys and macaques, the recent data would also support the notion that vascular density is correlated with the density of persynaptic elements rather than that of neuronal somata.

Functional neurovascular coupling

The cerebral metabolic rate (CMR), is commonly expressed in terms of oxygen consumption (CMRO2) because glucose metabolism is about 90% aerobic and therefore parallels oxygen consumption (e.g. [59]). The CMRO2 varies with neuronal shape, size and firing properties, whereby large projection neurons, which maintain energy-consuming processes such as ion pumping over a large membrane surface, may have larger energy requirements.

High-resolution autoradiography studies showed that the uptake of deoxy-glucose is higher in the neuropil, with its axonal, dendritic, and astrocytic processes, than in the cell bodies. These results were confirmed by others, which suggests that synaptic activity dominates energy consumption (for example, Sokoloff [60]). They are further corroborated by microstimulation experiments, in which the increase in glucose utilization was assessed during orthodromic and antidromic stimulation, the former activating both pre- and post-synaptic terminals and the latter activating only postsynaptic terminals. Increases were only observed during orthodromic stimulation ([61–63]; for a review see Jueptner and Weiller [64]), which suggests that coupling occurs primarily between energy metabolism and activity in the presynaptic terminals.

The notion that perisynaptic activity (synaptic events and restoration of gradients disturbed by postsynaptic potentials) consumes a greater proportion of the available energy receives support from the metabolic requirements of other cellular components of the brain. Neurons are not the only elements contributing to the energy metabolism of the brain; glia and vascular endothelial cells do so as well. In fact, research suggests a tightly regulated glucose metabolism in all brain cell types (for detailed references see Volterra and co-workers [65]).
An interesting case is the glial cell known as the astrocyte, which forms a massive bridge system between neurons and the brain’s vasculature. The structural and functional characteristics of astrocytes make them ideal viaducts between the neuropil and the intraparenchymal capillaries. It has been suggested that for each synaptically released glutamate molecule taken up by an astrocyte (with two to three Na⁺ ions), one glucose molecule enters the same astrocyte, two ATP molecules are produced through glycolysis, and two lactate molecules are released and consumed by neurons to yield 18 ATPs through oxidative phosphorylation. In other words, synaptic activity may be tightly coupled to glucose uptake through the neuron–astrocyte system (e.g. [66,67]).

The neuron–astrocyte system is compatible with the notion that neuronal signals, which are mediated by fast neurotransmitters or by ions or molecules that are transiently released in the extracellular space upon neural excitation, can trigger receptor-mediated glycogenolysis in astrocytes in anticipation of or at least parallel to excitation, can trigger receptor-mediated glycogenolysis in astrocytes in anticipation of or at least parallel to excitation (for a review see [68]).

Atwell and Laughlin [69**] proposed that the greater part of energy expenditure is attributable to the postsynaptic effects of glutamate (about 34% of the energy in rodents and 74% in humans is attributable to excitatory postsynaptic currents). They formed this proposal on the basis of computations of the number of vesicles released per action potential, the number of postsynaptic receptors activated per vesicle released, the metabolic consequences of activating a single receptor and changing ion fluxes, and neurotransmitter recycling [54**,69**]. Yet others have suggested that most of the brain’s energy is consumed for the generation and propagation of action potentials [70]; according to these estimates, the cost of an action potential would permit only 1% of the neurons in any area to be active concurrently. This prediction seems to be at odds with experimental and theoretical studies that suggest that the cortical neurons operate on a high-input regime with a well-controlled balance between excitation and inhibition [71–74].

In summary, the neurovascular coupling may be mediated by fast neurotransmitters, other signaling molecules (nitric oxide, prostaglandins etc.) or by any other mechanism able to sense the increasing or decreasing energy demand rapidly enough. In the former case, research suggests that a glutamate-evoked Ca²⁺ influx in postsynaptic neurons activates the signaling-relevant molecules that in turn produce a vasodilatation. This vasodilatation reflects both the activity of neurons presynaptic to the cells that have released the signaling molecules and the level of depolarization of the postsynaptic cell, which will typically alter the Mg²⁺-block of NMDA receptors and the resulting Ca²⁺ influx [54**]. In the latter case, overwhelming evidence suggests that energy demand will be driven to a lesser degree by the need to recycle neurotransmitters and to a greater degree by the processes of restoring perturbed gradients through postsynaptic currents. Both pre- and postsynaptic currents are dominant elements of the local field potentials, which — as mentioned above — were in fact found to correlate best with the hemodynamic changes in the cerebral and cerebellar cortex.

The study of networks with MRI
Connectivity studies with paramagnetic tracers

Neuroanatomical cortico–cortical and cortico–subcortical connections have been examined mainly by using degeneration methods and anterograde and retrograde tracer techniques (e.g. [23,75]). Although such studies have demonstrated the value of the information gained from the investigation of the topographic connections among different brain areas, they do require fixed processed tissue for data analysis, and therefore cannot be applied to an animal participating in longitudinal studies, in which consecutive studies examining an entire circuit are carried out in the same subject.

MRI visible tracers that are infused into a specific brain region and are transported anterogradely or retrogradely along axons may enable us to study neuronal connectivity in the living animal. Such paramagnetic tracer studies may also be used to validate and further develop non-invasive fiber tracking techniques, such as diffusion tensor MRI, which permits the study of connectivity even in the human brain.

Manganese (Mn²⁺) is an interesting example of an MRI-visible contrast agent. The axonal transport of its radioactive isotope (⁵⁴Mn²⁺) was first studied using histological methods [76,77]. Although these studies were carried out with the goal of understanding the regional specificity of Mn²⁺ distribution, they indicated the usefulness of Mn²⁺ as an anterograde neuronal tract tracer. Mn²⁺ distribution and transport has been also studied with MRI in rats and mice [78,79]. Injection of manganese (manganum chloride, MnCl₂) in to a nostril or an eye yields a clear signal enhancement in the olfactory and visual pathways [78,79]. Furthermore, the possibility that the transport of manganese may pass across synapses was suggested by some studies [77,78]. Pautler et al. [78] indicated that Mn²⁺ must have traversed a synapse to explain the enhancements detected in the olfactory cortex of the mouse following the injection of its olfactory bulb. By contrast, Watanabe and co-workers [79] reported that the signal enhancement they observed in their rat study was confined to regions known to receive direct projections from the retina, and concluded that it did not constitute evidence for trans-synaptic crossing of Mn²⁺.

An example of trans-synaptic transfer of manganese is displayed in Figure 4 (N Logothetis, unpublished data).
Following intravitreal (into the vitreal body of the eye ball) manganese injection a series of anatomical scans were acquired that illustrate the transfer of the substance along the retinogeniculate–striate pathway. Signal enhancement is clear along the optic nerve and tract, the dorsal LGN, superior colliculus, optical radiation and striate cortex. Similar results were obtained in a recent study that provided a detailed account of both the specificity and the trans-synaptic transfer of manganese in the basal ganglia of the monkey. Injections were made into the striatum. Its projections were confirmed histologically by injecting wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) into the same sites that had previously been injected with MnCl₂. The size and location of the projection foci in the striatal targets were comparable to those found in both the magnetic resonance and the histology images. By injecting WGA-HRP at the same sites as MnCl₂, we also confirmed for each animal the absence of a direct connection from the injection sites to various brain structures (e.g. thalamic nuclei). In this study, manganese was actually found in several structures receiving no direct projections from the injected sites.

**MR imaging and electrical microstimulation**

Our knowledge of connectivity and functional organization could profit a great deal from the combination of MRI with electrical microstimulation. Microstimulation is established as an important neurobiological tool for the study of areal representation and the functional properties of CNS output structures. A new method was recently developed that combines this technique with fMRI for the detailed study of neural connectivity in the alive animal. Specially constructed microelectrodes were used to directly stimulate a selected subcortical or cortical area while simultaneously measuring changes in brain activity, which was indexed by the BOLD signal [81]. The exact location of the stimulation site was determined by using anatomical scans, as well as by the study of the physiological properties of neurons. Electrical stimulation was delivered using a biphasic pulse generator attached to a constant-current stimulus isolation unit. The compensation circuit, designed to minimize interference generated...
by the switching gradients during recording, was always active, minimizing the gradient-induced currents in the range of the stimulation current. Local microstimulation of the striate cortex yielded both local BOLD signals and activation of areas V2, V3, and MT. Microstimulation of dorsal LGN resulted in the activation of both striate cortex and areas V2, V3, and MT. Figure 5 shows an example of V1 activation after microstimulation of LGN. The findings show that microstimulation combined with fMRI can be an exquisite tool for finding and studying target areas of electrophysiological interest.

Conclusions
The suitability of MRI for functional brain mapping has become firmly established over the past decade or so. BOLD fMRI has been successfully implemented in awake human subjects as well as in animals such as rats, cats, and monkeys. The use of high magnetic fields improves both signal specificity and spatial resolution. MRI studies, in which voxels may contain as few as 600–800 cortical neurons, can help us to understand how neural networks are organized, and how small cell assemblies contribute to the activation patterns revealed in fMRI. The combination of this technique with electrophysiology has fully confirmed the longstanding assumption that the regional activations measured in MR neuroimaging do indeed reflect local increases in neural activity. In addition, it has been demonstrated that fMRI responses mostly reflect the input of a given cortical area and its local intracortical processing, including the activity of excitatory and inhibitory interneurons. Finally, MRI visible tracers and microstimulation appear to be ideal for the study of connectivity in the living animal.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
**of outstanding interest


This is a study of BOLD signal changes that are associated with scrambling natural images into different numbers of segments in visually modulated regions of the monkey brain. The authors suggest that the BOLD signal might reflect average activation of local orientation detectors in V1, and sensitivity to more global object representations in higher visual areas. Scrambling causes substantial changes to the spatial frequency content of images.

The authors use fMRI in anesthetized monkeys to study how the primate visual system constructs representations of 3-D shapes from a variety of cues. Computer-generated 3-D objects defined by shading, random dots, structure elements or silhouettes are presented either statically or dynamically (rotating). Results suggest that 3-D shape representations...
are highly localized despite being widely distributed in occipital, temporal, parietal and frontal cortices, and may involve common brain regions regardless of the shape cue.


26. The authors used functional MRI signals to locate the positions and measure the topography of visual areas in a anesthetized macaque monkey. Strong fMRI signals were recorded in striate cortex and the areas V1, V2, V4 and MT revealing the retinotopic organization of these areas. Somewhat weaker signals were observed in other cortical areas, and these provide some insight into the overall organization of visual cortex in more anterior regions of cortex. On the basis of concordance of the fMRI signals with the expected properties of visual areas and topography, the authors conclude that this experimental protocol produces fMRI signals that measure local neural activity. These methods also provide a good foundation for further studies of topology and reorganization in the early cortical pathways.


31. Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A: Neurophysiological investigation of the basis of the fMRI signal. Nature 2001, 412:150-157. The authors present the first simultaneous physiological and fMRI recordings. Local field potentials, single- and multi-unit spiking activity were compared with high spatio-temporal BOLD fMRI responses from the visual cortex of monkeys. The largest magnitude changes were observed in LFPs, which register activity visualized characterized by transient responses were the only signal that significantly correlated with the hemodynamic response. Linear systems analysis on a trial-by-trial basis showed that the impulse response of the neuro-vascular system is animal- and site-specific, and that LFPs yield a better estimate of BOLD than the multunit responses. These findings suggest that the BOLD contrast mechanism reflects the input and intra-cortical processing of a given area, rather than its spiking output.


In this study, an adaptation paradigm was used to study the activity of striate and early extrastriate cortex with fMRI. The representation of directional selectivity was found to be different from estimates formed on the basis of single neuron recordings.


The authors present a review of the literature that suggests that less than 1% of neurons in cortex can be active concurrently. It is suggested that the high cost of spikes requires the brain not only to use representational codes that rely on very few active neurons but also to allocate its energy resources flexibly among cortical regions according to task demand. This constraint may explain the need for mechanisms of selective attention.


80. Saleem KS, Pauls JM, Augath M, Trinath T, Prause EA, Hashikawa T, Logothetis NK: Magnetic resonance imaging of...

The authors present the first application of manganese-enhanced MRI in monkeys. Combined injections of manganese chloride and WGA-HRP were performed in order to evaluate the specificity of the manganese chloride by tracing the neuronal connections of the basal ganglia of the monkey. Mn$^{2+}$ and WGA-HRP yielded remarkably similar and highly specific projection patterns. By showing the sequential transport of Mn$^{2+}$ from striatum to pallidum-substantia nigra and then to thalamus, the authors demonstrated unequivocally MRI visualization of transport across at least one synapse in the CNS of the primate.
