Neuroimaging of animal models of brain disease

Mark F Lythgoe*, Nicola R Sibson† and Neil G Harris‡

*RCS Unit of Biophysics, Institute of Child Health, University College London, London, UK, †MRC Biochemical & Clinical Magnetic Resonance Unit, Department of Biochemistry, University of Oxford, Oxford, UK and ‡Academic Neurosurgery, Centre for Brain Repair & Wolfson Brain Imaging Centre, University of Cambridge, Cambridge, UK

The main aim of this review is to describe some of the many animal models that have proved to be valuable from a neuroimaging perspective. This paper complements other articles in this volume, with a focus on animal models of the pathology of human brain disorders for investigations with modern non-invasive neuroimaging techniques. The use of animal model systems forms a fundamental part of neuroscience research efforts to improve the prevention, diagnosis, understanding and treatment of neurological conditions. Without such models it would be impossible to investigate such topics as the underlying mechanisms of neuronal cell damage and death, or to screen compounds for possible anticonvulsant properties. The adequacy of any one particular model depends on the suitability of information gained during experimental conditions. It is important, therefore, to understand the various types of animal model available and choose an appropriate model for the research question.

This review covers a wide selection of experimental research studies that combine animal models with neuroimaging. Some of the topics (e.g. stroke) have seen a great deal of imaging activity, and others (e.g. epilepsy or inflammatory disorders) are given as examples of emergent neuroimaging disciplines. The purpose is to illustrate that by selecting combinations of appropriate imaging techniques and suitable animal models of disease, experimental imaging can contribute greatly to elucidating the mechanisms of human brain disease and to develop improved treatment strategies.

Cerebral ischaemia

The use of animal models in neuroimaging stroke research has focused on a number of areas which include: (i) defining and understanding the concept of the penumbra1,2; (ii) development of new imaging techniques

Correspondence to:
Dr MF Lythgoe, RCS Unit of Biophysics, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK
for the diagnosis and prognosis of stroke\textsuperscript{3,4}; and (iii) the investigation both of the underlying processes that lead to cell death\textsuperscript{5,6}, and of possible therapies for the treatment of stroke\textsuperscript{7,8}. Over the last decade, comprehensive reviews have described the numerous possible animal models in the investigation of cerebral ischaemia\textsuperscript{9–12} and their use in nuclear magnetic resonance (NMR) investigations\textsuperscript{13}. In addition, the relevance of these animal models to human disease has also been considered\textsuperscript{14–16}. The following sections contain an overview of the types of procedures (based on the widely used rat model) and model classifications used in experimental stroke research, together with a brief account of various animal models which highlight the possibilities for neuroimaging in the investigation of cerebral ischaemia.

Experimental models may be broadly classified by a reduction in cerebral blood flow (CBF), as either global or focal models, which may, in turn, be permanent or reversible in nature. In 1975, Robinson and colleagues developed a model of \textit{permanent focal ischaemia} in the rat, in which the middle cerebral artery (MCA) was coagulated distal to the rhinal fissure, the procedure being performed through a craniotomy\textsuperscript{17}; this model has been used extensively with magnetic resonance (MR) to determine the temporal evolution of ischaemic damage\textsuperscript{18} and assess the efficacy of drug therapies\textsuperscript{19}. Permanent MCA occlusion models do not permit the investigator to monitor the effects of reperfusion following the initial insult. To address this problem, experimenters have developed a number of methods for occluding and reperfusing the MCA. In 1986, Koizumi \textit{et al}\textsuperscript{20} developed a new \textit{reversible focal ischaemic} model. The MCA was occluded by a silicone rubber cylinder attached to a thread inserted through the internal carotid artery in Wistar rats. Re-circulation was accomplished by pulling the thread out of the artery\textsuperscript{21–23}. This model has the advantage of avoiding craniotomy and has played an important MR role in investigating reversible ischaemic injury\textsuperscript{24}, novel therapeutic strategies\textsuperscript{25}, the relationship of CBF to metabolic disturbances\textsuperscript{26}, delayed neuronal death\textsuperscript{27} and the diffusion-perfusion mismatch phenomena\textsuperscript{28}. Permanent occlusion of the MCA may also be performed using the intraluminal suture approach which has been used with MR for the investigation of novel contrast mechanisms at high field\textsuperscript{29} and the relationship between CBF, the apparent diffusion coefficient (ADC) of water and hypoxia\textsuperscript{30}. Models of \textit{focal oligaemia} are not common, although they are finding increasing use in the investigation of the pathophysiology of penumbra, in which regions of oligaemic misery perfusion are present\textsuperscript{30}. The aim of these experimental models is to produce a large focal lesion in which the CBF is moderately reduced throughout the MCA territory. The reduction in CBF is produced by MCA stenosis\textsuperscript{31}, hypotension\textsuperscript{32} or partial obstruction of the MCA\textsuperscript{33}. Further methods of inducing focal ischaemia which avoid a craniotomy...
include: (i) injection of homologous blood clot fragments \(^{24-36}\); (ii) systemic injection of Rose Bengal dye, followed by photochemical activation to induce focal thrombosis and cerebral ischaemia \(^{37}\); or (ii) a local application of endothelin-1, a vasoconstrictive agent, which produces a blood flow decrease with a half-time of 45–60 min \(^{38}\). An animal model of subarachnoid haemorrhage (SAH) has been developed using a modification of the intraluminal suture model, which allows perforation of the circle of Willis and subsequent production of a SAH \(^{34,39}\). Models of **global ischaemia** are usually transient, affecting wide-spread areas of the brain, leading to neuronal damage in selectively vulnerable regions. A period of 10–30 min of ischaemia in the four-vessel occlusion model in the rat produces cell changes that provide evidence of selective vulnerability and delayed neuronal damage \(^{40-44}\).

Global ischaemia induced by unilateral occlusion of the CCA with hypoxic ventilation, produces ischaemic damage predominantly in the hemisphere ipsilateral to the occlusion and allows the region-specific responses to a global insult to be monitored \(^{45}\).

Following experimental studies in the late 1970s, the term ‘penumbra’ was introduced to designate a zone of brain tissue with moderate ischaemia and impaired neuronal function, with the neuronal paralysis being fully reversed upon reperfusion \(^{46-48}\). Currently, the term has a wider definition and is used to describe a region of ischaemic tissue peripheral to the core where viable neurons may be found, and thus may be potentially salvageable with suitable intervention \(^{49,50}\). Thus, the penumbra is an important target for acute stroke therapy. Since the penumbra can be considered as a temporary phase of potential viability through which ischaemic tissue progresses into infarction, the therapeutic time-window is limited, possibly to a few hours \(^1,50\); therefore, early detection is essential. Following occlusion of the MCA in the cat, sequential positron emission tomography (PET) studies demonstrated that CBF was decreased to < 30% of control, cerebral metabolic rate of oxygen consumption (CMRO\(_2\)) was less diminished and oxygen extraction fraction (OEF) was increased, indicating misery perfusion. During permanent occlusion, the ischaemic penumbra advanced with time from the centre to the borders of the MCA territory (Fig. 1). If the MCA was reperfused after 30 min and OEF returned to normal by 24 h, no infarct was noted. However, if the initial increase in OEF diminished during the occlusion phase, the tissue would proceed on to irreversible damage \(^2\). Other PET studies have confirmed the presence of the penumbra during prolonged periods of misery perfusion, which further suggest a window of opportunity for therapy \(^{51}\). Penumbral zones can be delineated using several different imaging modalities, each of which have definitions that rely on the underlying mechanisms of that technique \(^{50}\). In 1990, Moseley et al \(^{52}\) observed the first diffusion-
weighted imaging (DWI) changes in an animal model of cerebral ischaemia and this ADC reduction of tissue water diffusion was attributed to an osmotically obliged shift of extracellular water to intracellular compartments, as a result of a disruption of ion homeostasis and formation of cytotoxic oedema. Following this initial paper, the relationship of DWI to the penumbra has been the topic of many studies. Early work suggested that the region of signal intensity change in DWI corresponds closely to the region of peri-infarct acidosis, but also encompasses the area of ATP depletion (infarct core). Therefore, it was postulated that the outer margin of the DWI visible lesion corresponds with that of the penumbra. However, this is only part of the picture, as it is now acknowledged that regions of DWI change that normalise on reperfusion may proceed on to infarction at a later time point and that the areas of so-called diffusion-perfusion mismatch need to be considered.

The occurrence of transient waves of membrane depolarisation known as spreading depression (SD) emanating from an infarct core has been...
suggested as one mechanism for the expansion of tissue injury into the penumbral zone. During SD, the metabolic rate of the tissue increases in response to the greatly enhanced energy demands of the activated ion exchange pumps. In the penumbra, flows are suppressed and, as a result, the increased metabolic demand is not compensated by an increase in oxygen and glucose. Eventually, ATP stores will be depleted, followed by the cascade of pathophysiological events leading to tissue infarction. Using DWI and ADC maps to monitor cell volume change, and gradient-echo MRI to following apparent changes in blood flow, both of these consequences can be imaged. The first observation during focal ischaemia of transient abnormalities on DWI, co-incident with transient depolarisations, was described by Gyngell et al. The change in ADC associated with SD has been used to study the pathological basis by which SD leads to infarct growth and the mechanism whereby neuroprotective drugs may have their therapeutic effect.

Epilepsy

The epilepsies are a group of disorders with a wide range of presentations and multiple aetiologies. In order to model such a diverse condition, several different and quite diverse approaches have been developed, and currently no single model is appropriate. In 1972, Purpura et al. produced a comprehensive guide covering many experimental models of epilepsy used in research, which still is an extremely useful guide to the various methods used to induce epilepsy or seizures. Clinically, the epilepsies are characterised by spontaneous recurrent epileptic seizures, which are caused by generalised paroxysmal or partial (focal) discharges in the brain. Various animal models have been selected to highlight the classification of epilepsy disorders, questions related to epileptogenic mechanisms, brain injury associated with seizures and assessment of the efficacy of anti-epileptic drugs.

Animal models of generalised seizures include the animals affected by genetic reflex epilepsy, such as the baboon (Papio papio), mouse (DBA/2J), genetically epilepsy-prone rats (GEPRs), rabbits, and the Fayoumi chicken. Seizures may be induced by intermittent light stimulation, noise, movement, and stress. Another animal model that exhibits generalised seizures is the maximal electroshock model which involves electrical stimulus to evoke a tonic extension of the hind-limbs. The model is predominately used to test possible therapies for primary and secondary generalised epilepsies. Models for simple partial seizures include focal micro-application of topical convulsants such as penicillin, bicuculline, picrotoxin, strychnine, and kainic acid on
the cerebral cortex. Acute direct repetitive electrical stimulation can lead to discharges that persist for seconds or minutes after the electrical stimuli cease. Chronic seizure models develop following the application of metals such as alumina hydroxide, cobalt, tungsten or zinc. Spontaneous recurrent seizures may appear 2 months after the injection, and persist in some cases for several years. Models of complex partial seizures may be induced via injection of tetanus toxin into the hippocampus; seizures occur at 1–7 days post-injection and then chronically. Administration of kainic acid, an excitotoxic analogue of L-glutamate, leads to spontaneous recurrent seizures and hippocampal damage even when injected systemically. A now widely used animal model for the study of epileptogenesis is the kindling model. One technique for induction is regular stimulation of chronically implanted electrodes until spontaneous generalised seizure is achieved. The ubiquitous pilocarpine model presents with status epilepticus usually within 1–2 h of injection, which may last for up to 12 h and then finally progresses to spontaneous recurrent seizures. Animal models of status epilepticus (SE) may be produced via techniques such as the administration of chemical convulsant or electrical stimulation.

Although the majority of experimental epilepsy research has been performed outside the neuroimaging field, there has been increasing interest following some early nuclear magnetic resonance (NMR) spectroscopy investigations, and more recently imaging studies using magnetic resonance. Broadly speaking, the present goal of imaging is to characterise both the metabolic derangement and brain injury associated with seizures, using functional (spectroscopy, diffusion and perfusion imaging) and structural (T1 and T2) neuroimaging. The first in vivo MR experiment in an animal model of seizures revealed a decrease in phosphocreatine, without a change in ATP levels during seizure activity. This and several other spectroscopy studies demonstrated the feasibility of combing animal models of epilepsy and NMR. Non-invasive imaging of animal models of epilepsy started in the early 1990s. A preliminary imaging study provided evidence for tissue damage detectable by MRI following kainate injection in the rat brain. Although the procedure did not induce seizures in this study, the T2-weighted strategy provided a better image contrast for the kainic acid lesion than the T1 approach, and the diffusion-weighted images showed improved contrast for oedematous tissue. Another early study imaged blood flow changes via the MR blood oxygenation level dependent (BOLD) contrast in the kainic acid model of seizures in rats. Increases in blood flow were associated with minor behavioural seizure signs; but, as seizure activity progressed, signal intensity remain near control values possibly due to the increased oxygen extraction of the tissue.
In 1993, Zhong et al. published a important paper using diffusion-weighted MR imaging to investigate changes associated with SE. Following intraperitoneal injection of bicuculline in the rat, the ADC in the brain decreased 14–18% during seizures. No changes occurred in T₁ or T₂. This result demonstrated that, during a seizure, the ADC changed in a similar fashion to that reported in ischaemia, but under different circumstances as the blood flow is increased and the ATP stores are only modestly reduced. This finding was also confirmed during flurothyl exposure, which is thought to act on the sodium channel to alter the responsiveness of the GABA and glutamate receptors, causing convulsions at high doses and during frontal cortical electroshock. This ADC decrease during seizure activity leads to the provisional hypothesis that the ADC change may be due to perturbations in intracellular cytosolic streaming. Although this study has been supported by more recent work, there is still no complete explanation for the ADC change in stroke or epilepsy. Magnetic resonance spectroscopic imaging (MRSI) together with T₂-weighted and diffusion-weighted imaging (DWI) was first used in experimental epilepsy to investigate selective hippocampal and piriform cortex damage following kainite-induced status epilepticus in the rat. Decreased N-acetyl aspartate (NAA), increased lactate and decreased ADC were observed at 12 h with little evidence of histological and T₂-weighted changes, and it was suggested that such changes may provide a diagnostic measure for evaluating risk of neuronal damage after SE.

The long-term temporal evolution of lesion development, investigation of epileptic activity and tissue damage are well suited to the non-invasive nature of MRI. Several studies have now monitored T₂, ADC and blood brain barrier (BBB) breakdown following SE. In the piriform and entorhinal cortices, and in the amygdala area, the T₂ was increased by 24 h, then progressively normalised by 5–9 days and finally increased again in the chronic phase (9 weeks; Plate XV see end of file p.). The chronic T₂ increase corresponds to gliosis, and characterises the initial step leading to development of epilepsy that could result from spontaneous seizures.

A relatively small body of work has been undertaken in MR investigations of animal models of epilepsy when compared with other neurological conditions such as stroke. The published studies have established magnetic resonance of epilepsy models as a valuable and developing tool.

**Traumatic brain injury**

Traumatic brain injury (TBI) results in a complex, heterogeneous pathology that varies not only with the severity of insult but
considerably in both spatial and temporal dimensions and with regard to
the location of the initial impact. A number of experimental models
have been developed to simulate brain trauma and the most commonly
used are weight-drop\textsuperscript{103}, impact acceleration\textsuperscript{104}, fluid percussion
(FP)\textsuperscript{105,106}, and cortical contusion injury (CCI)\textsuperscript{107} (for reviews of these
models see Gennarelli et al\textsuperscript{108} and Lighthall et al\textsuperscript{109}). Each model is
designed to simulate closely specific components of the clinical
pathology so that no single model is necessarily the best. Although the
nature of the physical force differs between the models, the resulting
acute and chronic destruction of gross tissue elements is somewhat
similar and can be varied by adjusting the physical force used to produce
the injury.

Autoradiography is the most widely used imaging modality for TBI
research, particularly for mapping cerebral blood flow or cerebral
metabolic rate of glucose utilisation, but it has also been used to
determine calcium accumulation\textsuperscript{110} and microglial/macrophage
activation or infiltration\textsuperscript{111} after injury. Time-course analysis after CCI
injury details a unilateral drop in CBF lasting for days post-injury\textsuperscript{112}
with a concomitant hyper- and subsequent hypo-metabolic state inferred
by CMRG measurements\textsuperscript{113}. Recent advances in PET scanner
technology\textsuperscript{114} has enabled non-invasive studies of metabolism to image
sequentially the same rat following head-trauma\textsuperscript{115}. It is likely that this
methodology is set to be exploited much further for TBI research in the
coming years with the increasing availability of new tracers to map
different receptor distributions and gene expression\textsuperscript{114}.

The continual development of MRI techniques to assess physiology is
particularly advantageous to TBI research since the now routine high
spatial and temporal resolution that is achieved with this technique is
particularly suited to study the highly heterogeneous nature of this
condition. \textit{T}_1 and \textit{T}_2-weighted sequences have been employed to
monitor contusion volume\textsuperscript{116–119} and brain anatomy at various stages
after injury\textsuperscript{120}. A model has also been proposed to map brain water
content based on the signal contrast in \textit{T}_2-weighted images\textsuperscript{121}, although
this methodology has yet to gain wide acceptance by the MR
community. The high sensitivity afforded by diffusion imaging has been
used to detect very early decreases in the apparent diffusion coefficient
(ADC) indicating cytotoxic oedema followed by a later increase
indicative of a vasogenic component\textsuperscript{122–127}. Not all groups have
documented this initial ADC decrease\textsuperscript{128–130} and this may be related to
the model used, the injury severity or, most likely, to the placement of
the measurement region-of-interest. Contrast-enhanced imaging has
been used to map regions of increased blood-brain barrier
permeability\textsuperscript{131–133} and initial decreases in cerebral blood volume\textsuperscript{125}
following injury. However, using contrast agents to map either blood
volume or perfusion in regions where the BBB is permeable is not valid for quantitative analysis, and this may be reflected in this latter study that showed CBV maps were not well correlated with histological damage together with large inter- and intra-subject variability. Using the continuous arterial spin-labelling (CASL) technique to map CBF circumvents this problem since it relies on an endogenous tracer that is formed by magnetically labelling water protons flowing into the brain. Early reductions in CBF have been reported after CCI using CASL together with loss of vascular reactivity. Monitoring CBF at different times after injury in the same rat using this technique demonstrates hypoperfusion in core (Plate XVI see p.xix) and contusion boundary regions followed by a return to normal values at the chronic stage. These findings are in good agreement with autoradiographic data.

Diffuse axonal injury, a hallmark of TBI, is considered to be an important determinant of functional outcome. Magnetisation transfer (MT) imaging, a technique that generates contrast based on the amount and ratio of free and bound water protons, has been shown to be sensitive to axonal injury in a number of disease states. The quantitative measure of MT imaging – magnetisation ratio (MTR) – is significantly decreased in regions of axonal injury after rotational acceleration injury in the pig and in core regions after contusion injury in the rat. While the good agreement between MTR abnormalities and axonal pathology assessed by histology together with its reported high sensitivity for detecting relatively mild injury presumably indicates continued use of this method. Future studies may be limited to large animals since the rat contains a much lower ratio of white:grey matter making detection of white matter pathology difficult at best.

Sub-dural haemorrhage that is present immediately after contusion injury is highly visible on \(T_2^*\) images due to the susceptibility differences relative to the surrounding tissue, although this resolves with time. While this presents few problems for echo-planar spin echo data, it will certainly produce large image distortions for gradient echo image acquisition, for example in a functional imaging paradigm. Thus early time-point functional studies may be limited to PET or autoradiographic studies.

**Parkinson’s disease**

The disease is characterised by degeneration of the nigrostriatal dopaminergic pathway between the cell bodies in the substantia nigra and the projections in the striatum. Striatal dopamine depletion results in progressive loss of motor control and the characteristic symptoms of the disease.
disease. Experimental models of Parkinson’s disease fall into two major categories: (i) pharmacological, for example reserpine or amphetamine administration to deplete dopamine, a largely reversible treatment; and (ii) lesioning using neurotoxins which is permanent, for example intraparenchymal injection of 6-hydroxy-dopamine (6-OHDA) or systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as reviewed by Hantraye. Imaging experimental models has largely been in the domain of PET imaging with the availability of radioligands to monitor both pre- and postsynaptic function using 1-[^18F]-fluoro-l-dopa, [^11C]-CFT and [^11C]-raclopride to monitor dopamine turnover, dopamine transporter densities and D2-receptor densities, respectively, in various models with and without neural transplants. Cerebral metabolic mapping with autoradiography has been used to determine the acute increase and chronic decrease in brain activity, reflecting cell activation followed by cell loss in regions targeted by MPTP treatment in the non-human primate. The technique has also been used to map regions responsive to levodopa in the 6-OHDA lesioned rat. This methodology has now been largely supplanted by functional MRI or pharmacological MRI techniques with activity induction by levodopa, D1, D2 receptor agonists, amphetamine or dopamine transporter agonists. This methodology has also been used to assess neural transplantation. Using more classical anatomical MRI sequences, temporal signal changes in T1 and T2 have been used to map regions of degeneration after lesioning in non-human primates and attempts have been made to correlate T1 relaxation times with the abnormal accumulation of iron in degenerating dopaminergic neurones in the 6-OHDA rat model.

**Huntington’s disease**

This inherited disease is characterised by degeneration of GABAergic neurones localised mainly to the caudate and putamen with projections to the globus pallidus and substantia nigra. Experimental models are based on acute intrapallidal lesioning using GABAergic antagonists or N-methyl-D-aspartate-selective agonists or chronic lesioning using systemic administration of 3-nitropropionic acid. Since the discovery of the gene mutation for the disease, new transgenic mouse models are being developed. Imaging data are largely PET-based, using markers described for Parkinson’s disease with more experimental data utilising fluorodeoxyglucose to map regions of reduced metabolism indicative of cell loss. Striatal excitotoxic lesions are visible with T2-weighted protocols, although at acute time-points only diffusion-weighted imaging provides sufficient sensitivity.
Multiple sclerosis

Despite a wealth of studies, the pathogenesis of multiple sclerosis (MS) is still not fully understood. These difficulties in understanding MS stem partly from the highly complex nature of the lesion (involving demyelination, re-myelination, T-cell and macrophage recruitment, BBB breakdown and oedema) and in part from the inaccessibility of neuropathological correlates. A primary goal of animal studies is to determine the relationship between the histopathology of a disease and the MRI signal changes. Experimental allergic encephalopathy (EAE) is an autoimmune CNS disorder that can be induced in susceptible species and strains by peripheral sensitisation to white matter homogenate or myelin basic protein in adjuvant. Models of EAE have been developed in mice, rats, guinea pigs and non-human primates (for reviews see Gold et al. and Brok et al.), and all except the murine model have been studied extensively using MRI. However, a major problem with these models is that few of them adequately represent all of the features of human MS. In addition to which, the lesions evolve spontaneously at any site within the brain, and often exhibit varying temporal progressions. An alternative model that has recently emerged is the delayed-type hypersensitivity (DTH) model in the rat, which involves sensitisation of the immune system to a non-CNS antigen (heat-killed BCG) previously deposited in the brain. The DTH response exhibits a highly reproducible temporal progression, encompassing all of the primary features of MS lesions; T-cell and macrophage infiltration, BBB breakdown, oedema and tissue damage, including primary demyelination. A major advantage of this model for longitudinal MRI studies is that the site of the lesion is precisely dictated by the location of the intracerebral BCG injection.

Early MRI findings in the EAE models included increased T1, increased T2 and contrast enhancement, thus corresponding broadly to those found most commonly in MS patients. However, only a relatively small number of studies have investigated correlations between MRI and histopathology obtained at the same time point. An increase in T2 has been found to correspond to regions of macrophage recruitment and oedema in both guinea pig and rat EAE models. However, T2 changes were also associated with demyelination in the rat, but not in the guinea pig. In the marmoset model of EAE, increased proton density and T2 appear to be associated with regions of either perivascular cuffing, demyelination or perivascular gliosis. The variability of these findings suggests that T2-weighted MRI alone is not a reliable method of distinguishing purely inflammatory lesions from either demyelinating or re-myelinating lesions. Contrast-enhancing lesions in MS patients are generally considered to be a sensitive marker for disease activity. In both guinea pig and rat models of EAE, BBB breakdown has been found to correlate
with macrophage recruitment. In contrast, in the marmoset EAE model, which arguably provides the most accurate representation of the relapsing-remitting form of MS, contrast-enhancing areas correlated solely with acute, actively demyelinating lesions. However, recent work in the rat DTH model has demonstrated that axonal injury and inflammatory events occurring within a lesion are not restricted to the period of BBB breakdown and contrast enhancement. These findings suggest that MS disease progression may persist behind an intact BBB, and, consequently, contrast-enhancement may not be an accurate marker of disease activity.

Other imaging modalities have been used less frequently in animal studies, but have yielded some interesting findings. It has been suggested that directional changes in diffusion may distinguish between acute and chronic EAE lesions, such that in acute lesions diffusion increases in all directions, probably as a consequence of oedema, whilst in chronic lesions diffusion only increases perpendicular to the main axon axis, possibly reflecting demyelination. Recent measurements of magnetisation transfer ratio (MTR) in EAE have suggested that decreases in MTR, which are frequently assumed to reflect demyelination in human MS, may in fact result from inflammatory related changes to white matter structure rather than myelin loss per se. In contrast, the short component of tissue water $T_2$ may more accurately reflect myelin content. New contrast agents have recently provided an alternative approach to imaging EAE. A recent study has shown that the use of a superparamagnetic iron oxide contrast agent enables macrophage recruitment to the CNS to be followed in vivo. In addition, PET studies of EAE models have demonstrated that inflammatory processes such as up-regulation of nitric oxide and microglial activation can be identified using labelled agents.

Thus, correlation of MRI and histopathological findings in animal models of MS is beginning to aid our understanding of MS and interpretation of human MS data. However, the data from these models currently exhibit a number of conflicts. These apparent discrepancies may be resolved partly on the basis of interspecies differences. However, separation of the underlying histopathological events, either with simpler models of inflammation or manipulation of the available models of MS-like lesions, may enable more accurate identification of the MRI-neuropathology correlates. In addition, the integration of more MRI modalities and novel contrast agents may more easily differentiate between the underlying histopathological events.

**Acute CNS inflammation**

The inflammatory response is part of the hosts’ defence to injury and infection, but can exacerbate tissue injury when excessive or
inappropriate. It has become clear that inflammation contributes not only to the archetypal CNS inflammatory disease, MS, but also to a wide variety of acute neurological and chronic neurodegenerative diseases, such as stroke, head trauma, Alzheimer’s disease, prion disease and HIV-related dementia. Despite this, little is known of the effects of inflammatory processes within the CNS, or their contribution to MR images of human neuropathologies. However, recent work has begun to investigate systematically the effects of well-defined aspects of the inflammatory process within the CNS. The approach used is to micro-inject focally specific mediators of inflammation, cytokines, into the rat brain and to investigate their individual actions using multimodel MRI. Two of the pro-inflammatory cytokines that have been most widely associated with CNS pathologies are interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α). IL-1β is believed to exacerbate neuronal loss, but the mechanisms of its action remain unresolved. Similarly, elevated cerebral TNF-α expression appears to correlate with disease progression in a number of neuropathologies, but the consequences of this expression are not known.

The first MRI study of cytokine-induced CNS inflammation demonstrated that IL-1β induces an increase in cerebral blood volume (CBV), shortly followed by a neutrophil-dependent breakdown of the blood-brain barrier (BBB). A subsequent reduction in the apparent diffusion coefficient (ADC) of tissue water long outlived the neutrophil-restricted leukocyte recruitment to the CNS, and neutrophil-depletion studies indicated that both this ADC reduction and the early increase in CBV were only partially-dependent on recruited neutrophils. This study suggests that exacerbation of neuronal injury may be a result of both a direct cytotoxic action of IL-1β on neurones and secondary by-stander damage caused by recruited leukocytes. The reduction in ADC was not associated with any indicators of ischaemia, and in fact was shortly preceded by an increase in CBV. However, IL-1β is known to be up-regulated during cerebral ischaemia, and these findings suggest that IL-1β may initiate events that contribute to reductions in ADC that are associated with ischaemic lesions.

In contrast to IL-1β, TNF-α has recently been shown to cause an acute reduction in CBV (Fig. 2A,D) that is mediated by the vasoconstrictor endothelin (Fig. 2D). There are two TNF-α receptors in the brain (TNFR1 and TNFR2) and selective activation of TNFR1 alone did not elicit the reduction in CBV (Fig. 2D), indicating that the CBV decrease is dependent on activation of the TNFR2 pathway. As with IL-1β, breakdown of the BBB (Fig. 2B) with concomitant reduction in brain water ADC (Fig. 2C) were also observed, although these were delayed relative to the changes induced by IL-1β. In contrast to IL-1β, TNF-α induces solely macrophage recruitment to the CNS. Both TNF-α receptor pathways were found to contribute to BBB breakdown, ADC
reduction and macrophage recruitment. These results suggest that either or both of these MRI-visible effects may be linked to macrophage recruitment. Interestingly, the delayed reduction in ADC was not dependent on the early decrease in CBV, and this study identifies further factors that may contribute to reductions in tissue water ADC observed in neuropathologies.

Fig. 2 MRI data acquired following focal micro-injection of 1.5 μg (in 1 μl) recombinant rat TNF-α (rrTNF-α) into the left striatum of rat brain. (A) CBV map acquired 1.5 h after injection of rrTNF-α. An area of reduced CBV can be seen in the injected (left) striatum. (B) T₁-weighted image acquired 10 min after administration of an intravenous contrast agent in the same animal 24 h after focal striatal injection of rrTNF-α. The region of hyperintensity indicates loss of BBB integrity in regions corresponding to those showing reduced ADC. (C) ADC map acquired 24 h after focal striatal injection of rrTNF-α. A wide-spread area of reduced tissue water diffusion is visible in the injected striatum and surrounding cortical regions (left side of image). (D) Graph of the CBV measurements made at 1.5 h after intracerebral TNF-α injection. Values are expressed as ratios of CBV obtained from ROIs in the treated (left) striatum versus the untreated (right) striatum. The effects of an ET receptor antagonist (Ro 46-2005) and activation of the TNF-α type 1 receptor alone, by intrastriatal injection of recombinant human (rhuTNF-α), are demonstrated. Values close to 1.0 indicate no change in striatal rCBV, and all values are mean ± SD. ***P < 0.005, unpaired t-test. Adapted from Sibson et al.183.
These MRI studies of cytokine-induced CNS inflammation identify alterations in CBV, tissue homeostasis and the BBB as potentially adverse effects of IL-1β and TNF-α within the brain, which may contribute to neuropathologies. Manipulation of the experimental models has enabled some of the underlying mechanisms of the MRI-visible effects to be determined and has identified potential therapeutic targets in neuropathologies associated with expression of these cytokines.

Conclusions

The combination of appropriate imaging techniques and suitable animal models of disease can greatly elucidate our understanding of human brain pathologies. This experimental imaging partnership has contributed to the development of novel imaging techniques, to the promotion of better diagnostic and prognostic measures, and to elucidation of the basic mechanisms of cellular injury leading to improved therapies.

Acknowledgements

The authors acknowledge the Wellcome Trust for their support of the work carried out at the Radiology and Physics Unit, Institute of Child Health and Great Ormond Street Hospital, London, and the Medical Research Council for their support of the work carried out at the MRC Biochemical and Clinical Magnetic Resonance Unit, Oxford. We are grateful to David Gadian, Rod Scott and Robyn Haselfoot for their helpful comments and suggestions. The authors also thank Prof. HD Heiss for the contribution of a figure to this article.

References

1 Obrenovitch TP. The ischaemic penumbra: twenty years on. Cerebrovasc Brain Metab Rev 1995; 7: 297–323
5 Hoehn-Berlage M. Diffusion-weighted NMR imaging: application to experimental focal cerebral ischemia. NMR Biomed 1995; 8: 345–58
7 Fisher M. Characterizing the target of acute stroke therapy. *Stroke* 1997; 28: 866–72
37 Van Bruggen N, Cullen BM, King MD et al. T2- and diffusion-weighted magnetic resonance imaging of a focal ischemic lesion in rat brain. Stroke 1992; 23: 576–82
44 Grohn OHJ, Kettunen MI, Penttinen M, Van Zijl PCM, Kauppinen RA. Cerebral blood flow, T1, relaxation time and the trace of the diffusion tensor in graded ischemia in the rat [Abstract]. Proc ISMRM, 7th Annual Meeting, Philadelphia 1999; 2: 329
47 Astrup J, Symon L, Branston NM. Cortical evoked potentials and extracellular K+ and H+ at critical levels of brain ischemia. Stroke 1977; 8: 51–7
51 Young AR, Sette G, Touzani O et al. Relationships between high oxygen extraction fraction in the acute stage and final infarction in reversible middle cerebral artery occlusion: an


Li F, Liu K-A, Silva MD et al.Transient and permanent resolution of ischemic lesions on diffusion-weighted imaging after brief periods of focal ischemia. Stroke 2000; 31: 946–54


Seyfried TN, Glaser GH. A review of mouse mutants as genetic models of epilepsy. Epilepsia 1985; 26: 143–50


Campbell AM, Holmes O. Bicuculline epileptogenesis in the rat. Brain Res 1984; 323: 239–46
253

Neuroimaging of animal models

74 Usunoff G, Atsev E, Tchavdarov D. On the mechanisms of picrotoxin epileptic seizure (macro- 


76 Cavalheiro EA, Riche DA, Le-Gal-La-Salle G. Long-term effects of intrahippocampal kainic 
acid injection in rats: a method for inducing spontaneous recurrent seizures. *Electroencephalography 

77 A poner-Marsan C. Focal electrical stimulation. In: Purpura DP, Penry JK, Woodbury DM, 
Tower DB, Walter RD. (eds) *Experimental Models of Epilepsy – A Manual for the Laboratory 
Worker*. New York: Raven, 1972; 147–72

78 Ward A. Topical convulsant metals. In: Purpura DP, Penry JK, Woodbury DM, Tower DB, 
Walter RD. (eds) *Experimental Models of Epilepsy – A Manual for the Laboratory Worker*. 
New York: Raven, 1972; 13–35

79 Jefferys JG, Borck C, Mellanby J. Chronic focal epilepsy induced by intracerebral tetanin toxin. 

80 Mellanby J, Hawkins C, Mellanby H, Rawlins JN, Impey ME. Tetanus toxin as a tool for 

81 Lothman EW, Collins RC, Ferrendelli JA. Kainic acid-induced limbic seizures: 


83 Majkowski J. Kindling: clinical relevance for epileptogenesis in humans. In: Stefan H, 
Lippincott Williams & Wilkins, 1999; 105–13

84 Turski WA, Cavalheiro EA, Schwarz M, Czuczwar SJ, Kleinrok Z, Turski L. Limbic seizures 
induced by pilocarpine in rats: behavioural, electroencephalographic and neuropathological 

85 Roch C, Leroy C, Nehlig A, Namer IJ. Magnetic resonance imaging and diffusion-weighted MRI for 

86 Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: mechanisms and 
relevance to human temporal lobe epilepsy. *Neuroscience* 1985; 14: 375–403


88 Meldrum BS, Brierley JB. Prolonged epileptic seizures in primates. Ischemic cell change and its 
relation to ictal physiological events. *Arch Neurol* 1973; 28: 10–7

89 Lothman EW, Bertram EH, Bekenstein JW, Perlin JB. Self-sustaining limbic status epilepticus 
induced by ‘continuous’ hippocampal stimulation: electrographic and behavioral 

90 Hosford DA. Animal models of nonconvulsive status epilepticus. *J Clin Neurophysiol* 1999; 16: 
306–13

91 Petroff OAC, Pritchard JW, Behar K, Alger JR, Shulman R. *In vivo* phosphorus nuclear 
magnetic resonance spectroscopy in status epilepticus. *Ann Neurol* 1984; 16: 169–77

92 Karlik S, Stavraky R, Taylor A, Fox A, McLachlan R. Magnetic resonance imaging and 
31P spectroscopy of an interictal cortical spike focus in the rat. *Epilepsia* 1991; 32: 446–53

93 Ehsu T, Rooney WD, Graham SH, Mancuso A, Weiner MW, Maudsley AA. MR spectroscopic 
imaging and diffusion-weighted MRI for early detection of kainate-induced status epilepticus 

94 King MD, van-Bruggen N, Ahier RG et al. Diffusion-weighted imaging of kainic acid lesions in 

95 Ogawa S, Lee T. Blood oxygenation level dependence MRI of the rat brain: effects of seizure 

96 Zhong J, Petroff OAC, Pritchard JW, Gore JC. Changes in water diffusion and relaxation 

97 Zhong J, Petroff OAC, Pritchard JW, Gore JC. Barbiturate-reversible reduced of water diffusion 

98 Prichard JW, Zhong J, Petroff OA, Gore JC. Diffusion-weighted NMR imaging changes caused 

99 Duong TQ, Ackerman JHH, Ying HS, Neil JJ. Evaluation of extra- and intracellular apparent diffusion 

Downloaded from https://academic.oup.com/bmb/article-abstract/65/1/235/375346 
by guest 
on 04 June 2018


107 Raghavendra RV, Dogan A, Bowen KK, Dempsey RJ. Traumatic brain injury leads to increased expression of peripheral-type benzodiazepine receptors, neuronal death, and activation of astrocytes and microglia in rat thalamus. Exp Neurol 2000; 161: 102–14


143 Palombo E, Perrino LJ, Bankiewicz KS, Crane AM, Kopin JJ, Sokoloff L. Administration of


164 Matyszak MK, Perry VH. Demyelination in the central nervous system following a delayed-type hypersensitivity response to Bacillus Calmette-Guérin. *Neuroscience* 1993; 64: 967–77

165 Stewart WA, Alvord EC, Hruby S, Hall LD, Paty DW. Early detection of experimental allergic...
encephalomyelitis by magnetic resonance imaging. *Lancet* 1985; 2: 898
182 Blamire AM, Anthony DC, Rajagopalan B, Sibson NR, Perry VH, Styles P. Interleukin-1beta-induced changes in blood-brain barrier permeability, apparent diffusion coefficient, and cerebral blood volume in the rat brain: a magnetic resonance study. *J Neurosci* 2000; 20: 8153–9
Plate XV Comparison between MRI and histopathology 24 h, 14 days and 9 weeks after lithium-pilocarpine-induced SE at the level of the piriform cortex (PIR; scale bar = 200 µm), the entorhinal cortex (ENT; scale bar = 200 µm) and dorsal hippocampus (DH; scale bar = 1 mm). At each time, MRI and histopathology are from the same rat. The early damage (as soon as 24 h after SE) in the piriform and entorhinal cortex correlates well with the early MRI hypersignal (arrow). Conversely, hippocampal sclerosis develops progressively together with the MRI hypersignal (arrows). Image kindly provided by Dr C. Roch.
Plate XVI  Coronal perfusion (CASL) images of a single rat at different time-points after cortical contusion injury demonstrating the continued perfusion deficit in the core region. [Courtesy of NG Harris, MF Lythgoe, DL Thomas, JF Utting, E De Vita, S Chen, DG Gadian, and JD Pickard.]