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Visualising Microglial Activation In Vivo

RICHARD B. BANATI*

Department of Neuropathology, Departments of Psychiatry, Molecular Neuropsychiatry, Charing Cross Hospital, Imperial College School of Medicine, and MRC Clinical Sciences Centre (PET Neurology), Hammersmith Hospital, London, United Kingdom

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ABSTRACT In health, microglia reside as quiescent guardian cells ubiquitously, but isolated without any cell-cell contacts amongst themselves, throughout the normal CNS. In disease, however, they act as swift "sensors" for pathological events, including subtle ones without any obvious structural damage. Once activated, microglia show a territorially highly restricted involvement in the disease process. This property, peculiar to microglia, confers to them diagnostic value for the accurate spatial localisation of any active disease process, acute or chronic. In the brain, the isoquinoline PK11195, a ligand for the peripheral benzodiazepine binding site (PBBS), binds with relative cellular selectivity to activated, but not resting, microglia. Labelled with carbon-11, (R)-PK11195 and positron emission tomography (PET) have been used for the study of inflammatory and neurodegenerative brain disease in vivo. These studies demonstrate meaningfully distributed patterns of regional [^{11}C](R)-PK11195 signal increases that correlate with clinically observed loss of function. Increased [^{11}C](R)-PK11195 binding closely mirrors the histologically well-described activation of microglia in the penumbra of focal lesions, as well as in the distant, anterograde, and retrograde projection areas of the lesioned neural pathway. There is also some indication that in long-standing alterations of a neural network with persistent abnormal input, additional signals of glial activation may also emerge in transsynaptic areas. These data suggest that the injured brain is less static than commonly thought and shows subtle glial responses even in macroanatomically stable appearing regions. This implies that glial activation is not solely a sign of tissue destruction, but possibly of disease-induced adaptation or plasticity as well. Whilst further technological and methodological advances are necessary to achieve routine clinical value and feasibility, a systematic attempt to image glial cells in vivo is likely to furnish valuable information on the cellular pathology of CNS diseases and their progression within the distributed neural architecture of the brain. *GLIA 40:206–217, 2002.*

INTRODUCTION

An important limitation of many clinical in vivo brain-imaging techniques is the difficulty to relate the measured signal to a specific cellular source or event and thus gain a more detailed understanding of its pathophysiological significance. For example, magnetic resonance imaging (MRI) allows measurement of local signal perturbations in diseased brain tissue with high sensitivity but, depending on the particular spin-echo sequences used, the signal often cannot be readily associated with any specific cellular response: histopathologically very different tissue, such as neuronal vacuolation or dense astrogliosis, can produce indistinguishable MR signal intensities (Chung et al., 1999). The use of paramagnetic contrast agents, such as dime-

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*Correspondence to: Richard B. Banati, Department of Neuropathology, Molecular Neuropsychiatry, Division of Neuroscience and Psychological Medicine, Faculty of Medicine, Imperial College, Charing Cross Campus, Fulham Palace Road, London, W6 8RF, UK. E-mail: richard.banati@csc.mrc.ac.uk

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glumine gadopentate (Gd-DPTA), which provides a measure of blood-brain barrier leakage, can partly overcome this lack of cellular specificity.

Another complementary approach is magnetic resonance spectroscopy (MRS), which allows the highly specific detection of spectra from various brain metabolites. Its ability to detect pathology in "normal-appearing" white matter (Rovaris and Fillipi, 2000) has been demonstrated in patients with multiple sclerosis. However, MRS, too, averages the signals from several cellular subpopulations of different physiological and pathological significance and, therefore, the identification of the cell-specific components within the averaged spectra MR can be difficult. Currently, the sensitivity and with it the spatial resolution of MRS is relatively poor and remains to be improved.

An alternative method is the use of tracer molecules that provide the needed specificity on the basis of defined receptor-ligand kinetics, such as the use of positron emission tomography (PET) and specific radiolabelled molecules. This brain-imaging approach is based primarily on the known distribution of, e.g., a neuroreceptor or metabolic pathway in the CNS of normal individuals, for example, the normal signal pattern of [^{18}F] FDG (2-fluoro-2-deoxy-D-glucose) in the healthy cortex. Against this normal standard, the pathological abnormalities in patients with brain disease are usually defined as deficits, i.e., relative losses of regional signal. There are some notable exceptions, such as in tumours, where the relevant information of the image lies in an increase of the regional signal. In neurodegenerative diseases, however, current clinically used neuroimaging with radiotracers mainly provides "deficit-images" that are more or less closely correlated with a clinically observed deficit in brain function. The number of in vivo probes that would permit detection and measurement of de novo expressed molecules specifically associated with the cellular changes that constitute the neuropathological tissue reaction, i.e., the "positive phenomenology" of the disease, is still very limited.

This review outlines the rationale for employing [^{11}C](R)-PK11195 PET to detect activated microglia in vivo and use their presence as a generic marker of active disease. Apart from being closely linked to disease activity, microglia are a particularly interesting target for clinical imaging because their distribution occurs in a confined, anatomically meaningful way, i.e. at the primary sites of tissue pathology and secondarily in the remote projection areas of the injured neurons.

THE PK11195 BINDING SITE

The isoquinoline PK11195 was originally described as a compound that partially displaces certain benzodiazepines, such as diazepam. It was, however, subsequently found to bind to a site that is unrelated pharmacologically, structurally, functionally, and in anatomical distribution to the central benzodiazepine receptor associated with γ-aminobutyric acid (GABA)-regulated channels. Particularly abundant in peripheral organs and haematogenous cells, but present in the normal CNS only at very low levels, the binding site for PK11195 was named peripheral benzodiazepine binding site (PBBS) (for review, see Hertz, 1993). The PBBS is the 18-kDa subunit of an at least trimeric complex, the two other subunits of which are the 32-kDa voltage-dependent anion channel and the 30-kDa adenine nucleotide carrier in the outer membrane of mitochondria (Anholt et al., 1986), hence its other name, mitochondrial benzodiazepine receptor. However, PK11195 binding may also be present in nonmitochondrial fractions of brain extracts, and mitochondria-free erythrocytes (Olson et al., 1988; Hertz, 1993; Cahard et al., 1994). Immunocytochemical staining suggests the presence of PBBS in and around the nuclei of tumour cells (Hardwick et al., 1999). Amongst others, the PBBS plays an important role in steroid synthesis and regulates immunological responses in mononuclear phagocytes. The numerous other putative functions of the PBBS, that still have to merge into a coherent theory of is biological role, have recently been reviewed by Gavish et al. (1999).

CELLULAR SOURCE OF PK11195 BINDING IN THE CNS

There are a number of discrepancies in the published literature as to the exact cellular source of the upregulated PK11195 binding in CNS pathology. These appear to have resulted largely from the extrapolation of in vitro data to the situation in vivo, differences in experimental models, i.e., lesions with or without blood-brain barrier damage and the finding that there is only partial overlap of the cellular source of PK11195 binding data with immuncytochemical staining patterns against the PBBS using a polyclonal antibody (Kuhlmann and Guilarte, 2000). Further, recent data point to another potential source of variability that has not yet systematically been accounted for; i.e., the possibility that changes in the interaction between the subunits of the PBBS, rather than a change in the number of binding sites as such, may result in subsequent changes in the PK11195 binding capacity (Golani et al., 2001). In this review, the reported observations pertain exclusively to PK11195 or its enantiomers.

In vitro, astrocytes are found to have high binding of PK11195 (Hertz, 1993; Itzhak et al., 1993). However, observations made in a number of experimental lesion models and diseases with blood-brain barrier damage suggested that focally increased PK11195 binding is due to binding to infiltrating haematogenous cells (Benavides et al., 1988; Dubois et al., 1988; Price et al., 1990). Peripheral benzodiazepine binding sites are highly expressed by haematogenous cells, such as T lymphocytes and peripheral monocytes (Cahard et al., 1994). Subsequent experimental in vivo studies found

that the distribution pattern of increased PBBS expression more closely matches the distribution of activated microglia than that of reactive astrocytes (Dubois et al., 1988; Myers et al., 1991a,b; Stephenson et al., 1995; Conway et al., 1998; Raghavendra Rao et al., 2000). Additional in vitro studies have shown that the increase of microglial (R)-PK11195 binding in response to an activating stimulus is due to an increase in the number of binding sites and not a change in affinity (Banati et al., 2000). Observations using axotomy models, where confounding blood-brain barrier damage does not occur, demonstrated (Banati et al., 1997) that activated microglia are the main source of (R)-PK11195 binding in vivo (Fig. 1a–f) and that the increase in binding marks the transition from the resting to the activated state (Fig. 2a). Further transformation of microglia into ameboid phagocytes, as seen in lethal motor neuron injury, does not appear to lead to a further increase in [^{3}H](R)-PK11195 binding (Banati et al., 1997).

In support of these findings, high-resolution microautoradiography with [^{3}H](R)-PK11195 combined with immunohistochemical cell identification, and performed on the same tissue section in inflammatory disease, such as multiple sclerosis and experimental allergic encephalomyelitis, has shown that increased binding of [^{3}H](R)-PK11195 is found on infiltrating blood-borne cells and on activated microglia (Banati et al., 2000) (Fig. 1g–j). The latter appear to become the dominant source of binding in areas without any obvious histopathology and remote from the primary pathological focus. Some discrepancy, however, remains: in a neurotoxic lesion model, immunoreactivity primarily in and around the nucleus of reactive hippocampal astrocytes was detected by a polyclonal antibody against the peripheral benzodiazepine receptor (Kuhlmann and Guilarte, 2000). The failure to find a complete match of the reported immunocytochemical stain for the peripheral benzodiazepine receptor with the cellular distribution of the microautoradiographic PK11195 label may either have technical reasons, such as the differing sensitivity and specificity of the various detection methods, or indicate that the immunocytochemically detected site within the heteromeric PBBS is not completely identical with the autoradiographically detected PK11195 binding sites. Also, with respect to the above microautoradiographic double-labelling data (Banati et al., 2000), it may be noteworthy that the relative cellular selectivity for activated microglia has been established using the R-enantiomer of PK11195, which has a higher affinity for the PK11195-binding site than the commonly used racemate (Shah et al., 1994). It may thus be advisable not to view (R)-PK112195 binding as necessarily synonymous with the PBBS complex, as the latter is possibly associated with other yet to be fully characterised proteins (Blahos et al., 1995; Galiegue et al., 1999).

The lack of significantly increased [^{11}C](R)-PK11195 binding in astrocyte-rich tissue, such as in patients with hippocampal sclerosis (Banati et al., 1999), lends further support to the view that microglia are the dominant site of (R)-PK11195 binding. Importantly, these patients had low seizure frequency, as one might expect frequent seizures to induce pathological changes with activation of microglia and consequently increase in PK11195-binding sites. Likewise, long-established nonactive multiple sclerosis lesions identified as hypointense areas in the MRI and known to be surrounded by reactive astrogliosis do not necessarily display an increased [^{11}C](R)-PK11195 PET signal (Banati et al., 2000). The observation that tissue surgically removed from patients with hippocampal sclerosis and temporal lobe epilepsy contains a high number of PK11195-binding sites, increasing with disease severity, is only an apparent contradiction (Kumlien et al., 1992; Sauvageau et al., 2002). Particularly in patients who require surgery, the disease is not otherwise controllable; i.e., it is progressive (Sutula and Pitkänen, 2001; Fuerst et al., 2001), and the resected tissue, albeit dominated by reactive astrocytes, contains a significant portion of activated microglia (Beach et al., 1995) (Fig. 1k) and thus a microglial source of increased PK11195 binding. High-resolution [^{3}H](R)-PK11195 autoradiography of such epilepsy tissue shows small accumulations of silver grains overlying activated microglia (Fig. 1 l-n). Although reactive astrocytes are obviously found within the area of increased PK11195 binding and appear to have immunoreactivity for a subunit of the PBBS complex (Sauvageau et al., 2002), no data are available demonstrating that immunocytochemically identified reactive astrocytes in vivo actually carry the autoradiographic label signifying [^{3}H](R)-PK11195 binding.

Thus, (R)-PK11195 binding in the brain in vivo is not, strictly speaking, cell-specific but has a relative cellular selectivity for activated microglia and, in the

Fig. 1. **a:** High-resolution microautoradiography with [^{3}H](R)-PK11195 shows no discernible cellular location of the autoradiographic signal in the normal facial nucleus. **b–e:** In the same animal, the operated contralateral facial nucleus 4 days after facial nerve axotomy reveals dense accumulations of silver grains, indicating [^{3}H](R)-PK11195 binding, in cells that lie in close apposition to blood vessels (b), most likely perivascular cells or perivascular microglia, (c) in parenchymal cells with the characteristic, bean-shaped nucleus of microglia and (d,e) most prominently in perineuronal microglia as identified by positive Ox-42 immunostaining (f) (Banati et al., 1997). **g–j:** Double-labelling with [^{3}H](R)-PK11195 microautoradiography and Ox-42 immunostaining performed on the same section (here shown in a white matter tract from an animal with experimental allergic encephalitis (Banati et al., 2000.) demonstrates that the identifiable cellular sources of [^{3}H](R)-PK11195 binding are activated microglia, most of which have retained their ramified morphology. The latter indicates, that increased microglial [^{3}H](R)-PK11195 binding does not require their transformation into ameboid phagocytes. n, neuron; b.v, blood vessel. ×40. **k:** Area CA1 of a surgical specimen from a patient with hippocampal sclerosis and loss of neurons, clinically presenting with intractable epilepsy. Immunostaining against CR3/43 reveals the presence of activated microglia (arrows) throughout the hippocampus in the molecular (left), pyramidal (middle) and polymorphic layer (right) and in adjacent areas as previously reported by others (Beach et al., 1995). Left arrow shows an activated microglia with typical rod-cell morphology. **l–m:** High-resolution autoradiography with [^{3}H](R)-PK11195 of a fresh-frozen section from a case with hippocampal sclerosis shows a diffuse background of silver grains without obvious cellular location and small focal accumulations of silver grains overlying activated microglia (immunostained against CD68). Magnification: ×40.

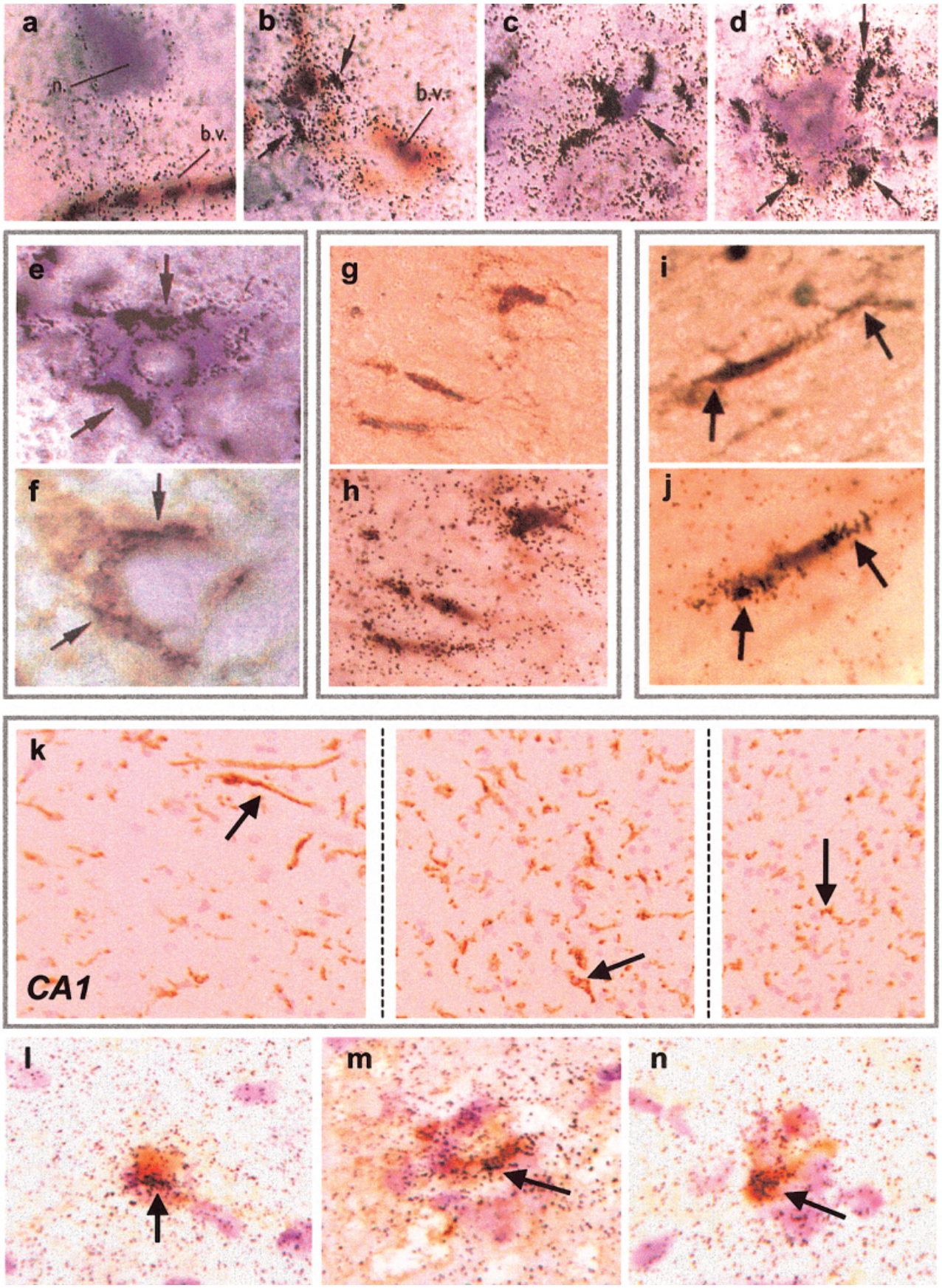

Figure 1.

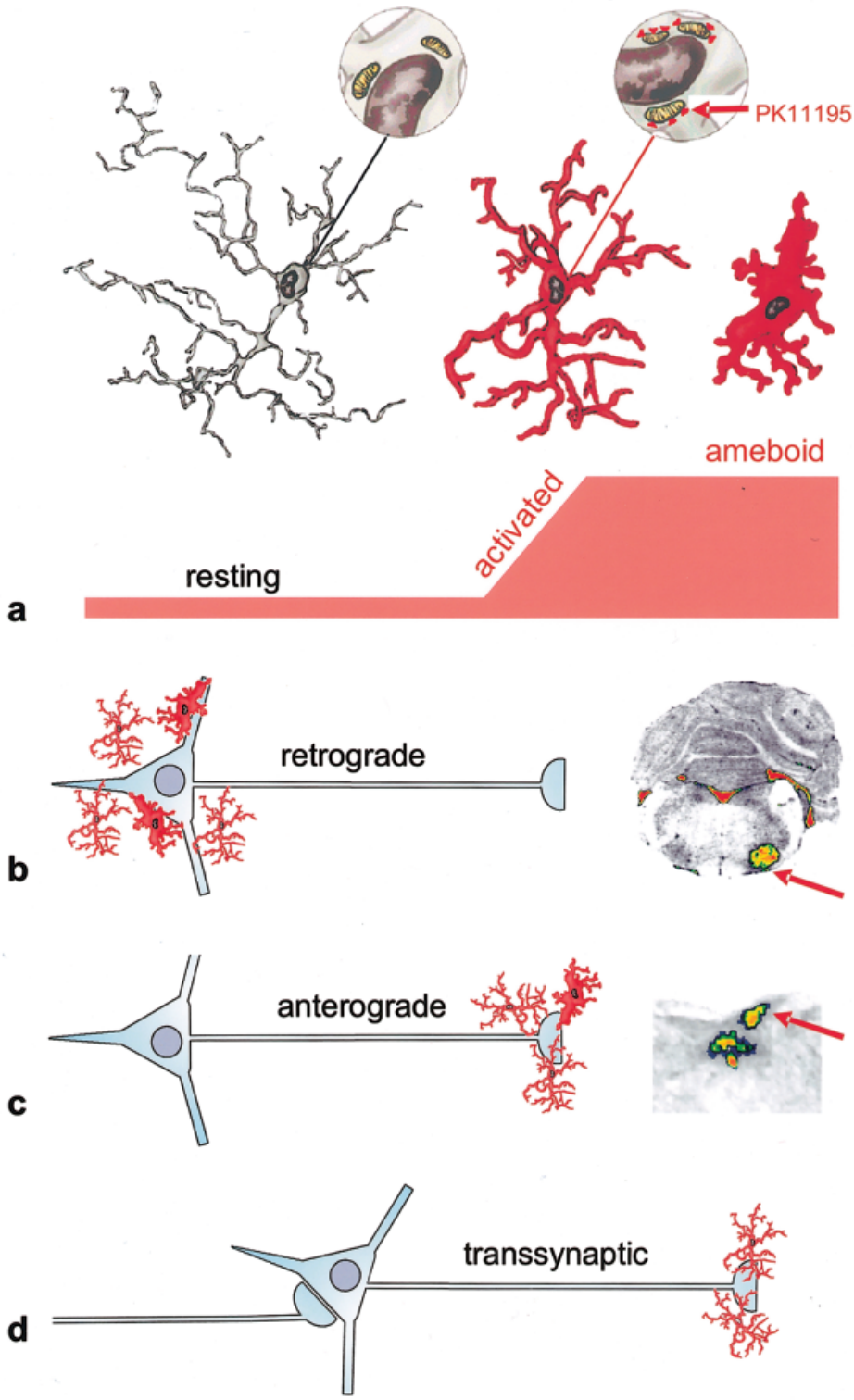

Fig. 2. **a:** Increase in the expression of (R)-PK11195 binding sites marks the transition form the resting state of microglia to the activated state. Further stimulation, such as seen after injuries involving neuronal cell death (Banati et al., 1997), does not appear to lead to any further increase in the binding of (R)-PK11195. **b:** Spatial pattern of microglial activation is largely determined by the distributed neural architecture of the CNS. Microglial activation with the concomitant increase in the (R)-PK11195 binding after injury to a nerve fibre tract (demonstrated by [^{3}H](R)-PK11195 film autoradiography) can be observed as a retrograde reaction, e.g., in the facial nucleus (red arrow) after facial nerve axotomy or (**c**) as an anterograde microglial reaction in the gracile nucleus after sciatic nerve injury (Banati et al., 1997). The additional area next to the gracile nucleus is the central canal and the area postrema, both areas in which microglia are not entirely quiescent. **d:** Transsynaptic microglial activation is not regularly seen in the common animals models of acute brain injury but might be relevant in human brain diseases where pathological states can persist for decades, possibly leading to glial responses in the projection areas of the (uninjured) second-order neuron.

absence of blood-brain barrier damage, microglia appear to be the predominant source of upregulated in vivo binding. PBBS expression appears to be particularly high in proliferating cells and a regulatory influence of the PBBS on cell proliferation has been reported (Alho et al., 1994). The later might also underlie the discrepant observation in astrocytes: in vitro, they have a high rate of proliferation and show significant PK11195 binding which is not the case in vivo where cell division of astrocytes is extremely rare.

The cause of the relative preference of PK11195 binding to mononuclear-phagocytes, including micro-

glia, might be related to the particular state of activation of these cell-types leading not only to a particularly high density of mitochondria but also a relatively higher number of mitochondria expressing the PBBS as compared to e.g. lymphocytes (Cahard et al., 1994). The dependence of microglial (R)-PK11195 binding on the state of activation has been demonstrated in vivo and in vitro, the latter showing that the increase in binding is not solely due to a higher number of microglia but also an at least two-fold increase in the binding per cell upon stimulation (Banati et al., 2000).

Finally, it is important to note that a number of areas in the brain have relatively high constitutive binding of PK11195, primarily those in which microglia are known to be constitutively more active than in the healthy brain parenchyma. These include all regions lacking the blood-brain barrier, such as the floor of fourth ventricle, and extraparenchymal structures, such as the central canal, choroid plexus, ependymal cell layers, larger blood vessels, walls of the venous sinuses, and meninges. High PK11195 binding is also found in the olfactory bulbs and the pituitary gland, regions in which the cellular or synaptic turnover is comparatively high. Low-level PK11195 binding, i.e., slightly above background and white matter, is seen in grey matter structures, but high-resolution autoradiography has not shown any identifiable cellular source (Banati et al., 2000). These low-level sources of PK11195 binding are currently, however, unlikely to reach the detection threshold for in vivo imaging and, in any case, the resultant error would be made consistently across groups (see below).

[^{11}C](R)-PK11195 PET IMAGING: METHODOLOGICAL ASPECTS

The potentially useful clinical application of the specific ligand PK11195 is based on three observations: (1) normal brain shows only minimal binding of PK11195; (2) in CNS pathology, in vivo PK11195 binding is predominantly found on activated microglia; and (3) when labelled with carbon-11, PK11195 can be used as a ligand for PET (Benavides et al., 1988; Junck et al., 1989; Cremer et al., 1992; Ramsay et al., 1992; Sette et al., 1993; Myers et al., 1991a,b, 1999; Banati et al., 1999). In addition, PK11195 has a number of kinetic properties that permit its use as an in vivo ligand: the extraction PK11195 from blood to brain is rapid and high (>90%) and is unimpeded by the blood-brain barrier; i.e., tracer delivery is similar in areas with and without a blood-brain barrier (Price et al., 1990; Cremer et al., 1992; Dumont et al., 1999).

Ligand-based in vivo imaging of cellular responses associated with tissue pathology faces a number of problems reminiscent of those encountered when using histological and immunocytochemical staining methods on tissue sections, the most important being specificity and quantification of the signal. For example, there may be cross-reactivity of the immunocytochemical stain and nonlinearity of the signal, particularly if secondary antibodies are used. The latter usually only allows for a semiquantitative measurement, such as counting numbers of positive cells but not, strictly speaking, the number of available binding sites. The most important confounds when attempting to secure specificity and quantification of an in vivo PET signal, particularly in inflammatory brain disease, include regional increases in blood flow, changes in blood-brain barrier permeability, and in the case of microglia, the recruitment of peripheral mononuclear phagocytes, i.e., cells with similar receptor profile. Biomathematical modelling, such as compartmental modelling, can be used to define the parameter of interest, e.g., specific binding, and correct for some confounds, such as regional variations in blood circulation, and thus variations in the availability and delivery of the ligand. The 'binding potential' of a ligand is one measure of specific binding and represents the ratio of the rate constant with which the ligand binds to the target molecule and the rate constant of dissociation from the target (k3/k4) (Fig. 3e).

Using a simplified reference tissue model, the binding potential of [^{11}C](R)-PK11195 can be arrived at by calculating for each voxel (= volume representing a point in the three-dimensional image space) the delivery ratio (between the area in which increased binding is expected and a disease-free reference region) and the wash-out rate constant of the ligand (Lammertsma and Hume, 1996; Gunn et al., 1997). The disease-free reference region from which to draw the normal kinetic behaviour is often defined anatomically. However, microglia are distributed ubiquitously throughout the brain and, given the fact that their activation occurs along projection pathways into healthy appearing tissue, such an anatomically defined reference region may be contaminated by activated [^{11}C](R)-PK11195 binding microglia. An alternative approach is the use of cluster analysis to classify the tissue on the basis of the kinetic behaviour of the ligand and then extract all those voxels, in which the ligand exhibits the kinetic behaviour normally seen in healthy brain tissue, irrespective of their spatial distribution within the brain (Gunn et al., 1998) (Fig. 3a–d). Once an appropriate cluster has been identified, it can serve as the ligand input function for a compartmental model, and binding can be calculated for each image voxel. Thus, the resulting binding potential map (i.e., the PET-image) is essentially the pictorial representation of those local tracer kinetics that fulfil the requirement laid out above and can, therefore, be assumed to relate to the underlying interaction of the radiolabelled PK11195 with its glial binding site. Such binding potential maps have to be interpreted with caution since the described model makes a number of important simplifying assumptions concerning the kinetic behaviour of the ligand. For example, in the case of [^{11}C](R)-PK11195, the normal tracer kinetic observed in the cortex of disease-free brains is treated as entirely free of any significant baseline binding.

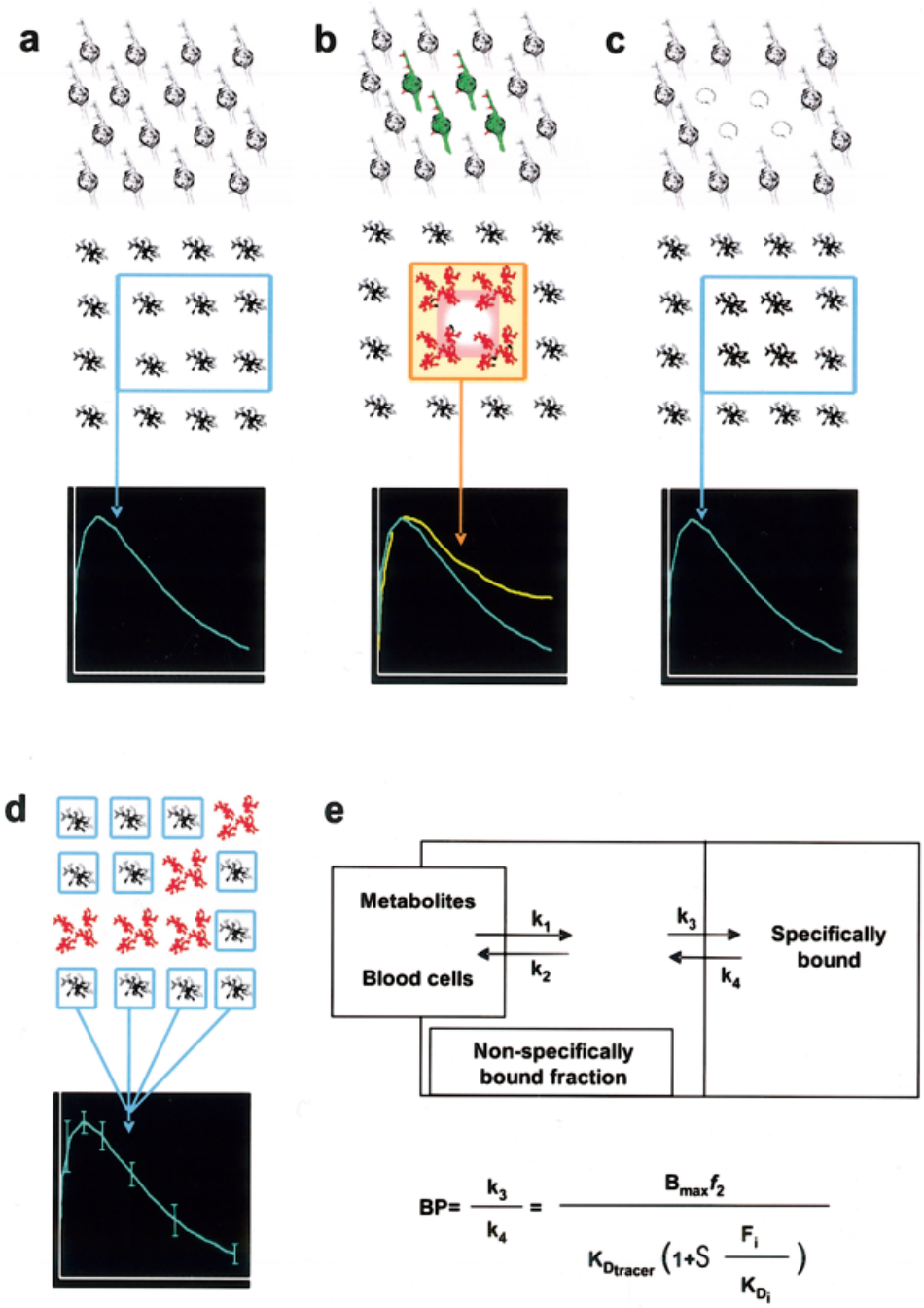

Fig. 3. **a–c:** One approach to measure specific binding of a radioligand in vivo is to compare the kinetic behaviour of the radioligand in an anatomically defined, disease-free reference tissue (curve in a) with the kinetic behaviour in an area of pathology (dotted curve in b) that, due to neuronal injury, contains activated, ligand-binding microglia. Theoretically, once pathological stimuli and secondary responses are no longer active, microglia will revert to their normal resting state and the kinetic behaviour of the radioligand should become again the same as in normal, healthy tissue (c). However, few systematic data are available to show the time span within which microglia return to full quiescence and whether in chronic human brain disease their activation may persist indefinitely. **d:** Due to the ubiquitous presence of microglia throughout the entire CNS and their peculiar activation pattern along large-scale neural networks (Cagnin et al., 2001c), the identification of an anatomically defined, disease-free reference tissue can be difficult. An alternative, nonanatomical tissue classification can be achieved by cluster analysis, whereby the kinetic behaviour of the radioligand is the important discriminant feature, regardless of exact anatomical location (Gunn et al., 1998). This approach permits extraction of only those voxels of the image volume in which the radioligand has a kinetic behaviour not dissimilar to that characteristically seen in normal tissue. All those areas containing activated glial cells and thus abnormal ligand kinetics are excluded. **e:** Once a reference input kinetic has been determined, the specific binding of the radioligand can be calculated as a binding potential (BP), using a (simplified) compartmental model that assumes various rate constants of transfer between the compartments and aims to correct for important confounds, such as regional differences in the delivery of the radioligand. B_{max} is the concentration of specific binding sites, $K_{D\ tracer}$ the equilibrium dissociation constant of the radioligand, f_2 the freely available, unbound radioligand and F_i and K_{Di} the concentration and equilibrium dissociation constants of competing endogenous ligands. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com].

However (as described in the previous paragraph), there is diffuse, very low-level, and not obviously cell-associated [^{3}H](R)-PK11195 binding in normal grey matter. As consequence of ignoring this baseline binding, the calculated binding potential maps will slightly underestimate the true binding. Since the error is small and is made consistently across all subjects, the reported relative differences between normal individuals and patients remain essentially unaffected. More important is another assumption, namely, that the amount of nonspecific binding and the volume of distribution of the free/nonspecific compartment is the same for the reference and the target area. Or, in other words, the area of pathology differs in terms of relevant compartments from the reference tissue only in one aspect, i.e. the regional increase in the number of (R)-PK11195 binding sites. However, it is perceivable that in states of severe focal pathology with leakage of the blood-brain barrier, not only blood-borne cells, but also peripheral plasma proteins may enter the brain and thus add an additional compartment of (nonspecific) binding (or an otherwise altered ligand kinetic) that would not be accounted for in the reference tissue model. Therefore, the calculated in vivo binding potential values, particularly in destructive tissue pathology, must be treated as estimates and independent studies using experimental or postmortem tissue remain important for the validation of any in vivo ligand studies. In our own studies, areas of necrosis were found not to show increased autoradiographic [^{3}H](R)-PK11195 binding (unpublished observation), indicating that mere tissue disintegration does not cause increased nonspecific ligand binding. As mentioned above, microglial [^{3}H](R)-PK11195 binding appears to reach a plateau after activation and their transformation into ameboid phagocytes is not accompanied by any further significant increase in binding. Hence, the in vivo [^{11}C](R)-PK11195 signal at the centre of a focal lesion, once appropriately corrected for the blood flow and ligand delivery, is not necessarily expected to be higher than in areas that do not contain full-blown macrophages. In fact, if the overall number of mononuclear phagocytes were low, the focal signal might be lower than in the perifocal penumbra or the connected projection areas. It should be stressed that the main rational for imaging microglia in vivo is the same as for the use of microglial staining in neuropathology. It is the detection of subtle disease processes without destructive tissue pathology or established astrogliosis in regions that lie beyond the obvious primary lesion. In these, regions the assumptions of the model are less likely to be violated.

REMOTE MICROGLIAL ACTIVATION: DISTRIBUTED [^{11}C](R)-PK11195 PET SIGNALS

Peripheral nerve transection experiments consistently demonstrate that "neuroinflammatory" responses are projected bidirectionally along the injured neural fibre tracts. For example, facial nerve transection leads to a retrograde neuronal reaction and to rapid induction of microglial PK11195-binding sites around the somata of lesioned motor neurons facial nucleus, while after sciatic nerve transection, an anterograde response with similar time course occurs in the gracile nucleus in the brain stem, a projection area that contains synaptic terminals from long, ipsilaterally ascending nerve fibres (Kreutzberg, 1996; Banati et al., 1997) (Fig. 2b,c).

This principle can be observed in mutliple sclerosis (Fig. 4a) and stroke patients. In the latter in whom the cortical injury induces a remote microglial response in the ispilateral thalamus, via the damaged corticothalamic connections (Myers et al., 1991b; Sorensen et al., 1996) and thus an increased signal of [^{11}C](R)-PK11195 (Pappata et al., 2000) (Fig. 4b). Similarly, in individuals with hippocampal damage in the wake of herpes simplex encephalitis, [^{11}C](R)-PK11195 PET shows that the distribution pattern of increased [^{11}C](R)-PK11195 binding follows projecting axonal pathways, such as the large association bundles interconnecting mesocortical areas, subicular allocortices, and subcortical amygdaloid nuclei (Cagnin et al., 2001c). Focal damage can thus lead to widespread microglial activation along almost an entire affected neuronal system, in this case, the limbic and associated structures. Does the apparent spread of tissue pathology beyond the primarily affected neural pathways represent merely the delayed, full emergence of the initially sustained damage or, instead, is evidence of more dynamic, transsynaptic knock-on effects on other not directly injured neural networks? In the visual system, chronic transsynaptic changes are well described and can be found in the lateral geniculate neurons after transection of the primary visual afferent pathway (Ghetti et al., 1975; Cowey et al., 1999). Likewise, transsynaptic retrograde degeneration of retinal ganglion cells can be seen after lesions of the striate cortex (Johnson and Cowey, 2000). In experimental lesions or in neurodegenerative disease, transneuronal changes associated with synaptic loss or synaptogenesis occur in the hippocampus and entorhinal cortex (Hoff et al., 1981; Diekmann et al., 1996; Su et al., 1997). Transsynaptic microglial activation as the consequence of neuronal hyperexcitation due to removal of inhibitory regulation was previously reported for the thalamus after intrastriatal nerve cell death (Topper et al., 1993). Middle cerebral artery occlusion, too, can induce transsynaptic neuronal changes associated with microglial activation (Wu and Ling, 1998).

Functionally important, late transsynaptic microstructural changes are also found in the thalamus of limb-amputated primates where they may occur without significant neuronal cell death and are thought to underlie at least in part the cortical plasticity induced by the injury (Jones, 2000). Using [^{11}C](R)-PK11195 PET, it has recently been possible to show long-term transsynaptic glial responses in the human thalamus after peripheral nerve injury (Banati et al., 2001). Such persistence of activated glia may suggest that even in the chronic stages of the posttraumatic recovery pro-

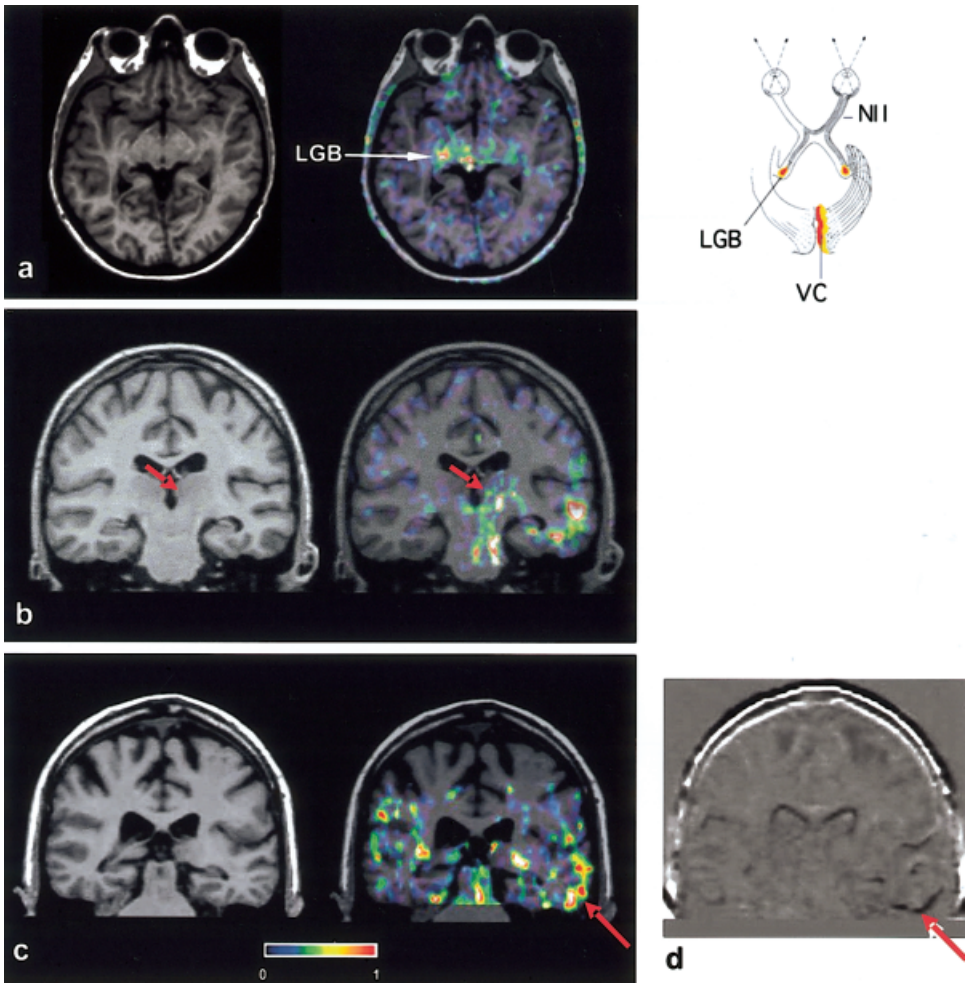

Fig. 4. **a:** In this multiple sclerosis patient with optic neuritis, [^{11}C](R)-PK11195 PET superimposed on the structural magnetic resonance image (MRI) reveals increased signals in the lateral geniculate bodies (LGB, arrow), i.e. the projection areas of the lesioned optic nerves (Banati et al., 2000). An additional signal is seen in the superior colliculus (arrow head). VC=visual cortex. **b:** After an ischemic lesion in the cerebral cortex, increased [^{11}C](R)-PK11195 signals can be seen at the primary lesion site (arrow head) but also in projection areas, such as the thalamus (arrow), where microglial activation occurs in the wake of the injury sustained by the dense reciprocal corticothalamic connections. **c:** In this patient with memory deficits (Cagnin et al., 2001a,b), a regionally increased [^{11}C](R)-PK11195 signal is detected in the left temporal lobe (arrow). **d:** The area of high [^{11}C](R)-PK11195 signal in the left temporal lobe shows progressive atrophic changes detected by MRI difference imaging 12 months after the [^{11}C](R)-PK11195 scan was acquired (arrow; dark areas round the left ventricle and the temporal lobe indicate loss of volume). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com].

cess, structural plasticity continues to occur. Hypothetically, this may provide for continuing functional plasticity well beyond the acute stages of disease (Knecht et al., 1998; Schallert et al., 2000).

WHAT IS THE SIGNIFICANCE OF A PERSISTENT "NEUROINFLAMMATORY" RESPONSE IN VIVO?

The use of [^{11}C](R)-PK11195 PET to study the acute and chronic evolution of primarily noninflammatory brain disease, such as Alzheimer's disease and Parkinson's disease, builds on the concept of neuroinflammation or glial inflammation (Graeber, 2001). The neuronally triggered process of microglial activation is associated with the increased expression of immune molecules, such as MHC II. However, since lymphocytes, infiltrating macrophages, and co-stimulatory are absent, its immunological significance is likely to be distinct from that seen in classic inflammation (Graeber, 2001). Recruitment of peripheral inflammatory cells without concomitant blood-brain barrier damage that leads to the appearance of a classical inflammatory reaction despite the absence any infectious agents can usually only be seen under certain experimental conditions involving neuronal death or dual pathology with simultaneous neuronal injury and autoimmune disease (Raivich et al., 1998; Flugel et al., 2001).

Extending observations from numerous cell culture studies of potentially cytotoxic metabolites released by activated microglia (Giulian and Corpuz, 1993; Banati et al., 1993), such as reactive oxygen intermediates, the "neuroinflammation" hypothesis—reminiscent of earlier "histiogenic autotoxicity" theories (Kraepelin, 1899)—states that microglial activation may cause bystander damage, and thus disease progression. Some epidemiological studies have found a protective effect of antiinflammatory medication against the risk of developing late-onset Alzheimer-type dementia (McGeer and McGeer, 1995; in t' Veld et al., 2001) that lends support to this view. Formal proof that the prolonged presence of activated microglia in vivo is indeed detrimental remains to be established. A tentative conclusion might be drawn from patients after herpes encephalitis, in whom the glial activation pattern, as measured by [^{11}C](R)-PK11195 PET, was somewhat predictive of the subsequent anatomical pattern of at-

rophy, as shown by MR-difference imaging (Cagnin et al., 2001c). Likewise, in a recent study of Alzheimer patients, areas of increased [^{11}C](R)-PK11195 signal were found to become progressively atrophic (Cagnin, et al., 2001b) (Fig. 4c,d). Yet, at this stage the exact causal relationship between regional microglial activation and evolving atrophy remains undecided. It has to be noted that increased [^{11}C](R)-PK11195 binding in areas, such as the thalamus, has not been associated with obvious evolution of structural abnormalities (Banati et al., 2001). With respect to interpreting the physiological significance of increased (R)-PK11195 binding, it has been suggested that in CNS injury, the peripheral benzodiazepine binding sites may influence neuronal functioning and possibly participate directly in regeneration by regulating the synthesis of neurosteroids (Lacor et al., 1999; di Michele et al., 2000). All this cautions against the simplifying equation of microglial activation with tissue destruction (Banati and Graeber, 1994).

An important question for which clinical in vivo observations are still outstanding is the length of time necessary for the process of microglial activation to subside completely. Some neuropathological evidence exists that even years after a single toxic event, such as in MPTP-induced parkinsonism (Langston et al., 1999), activated microglia are present in selective areas, indicating continuing or secondary neurodegenerative processes. Indications are that after repeated injury, e.g., of. lumbar spinal roots, glial responses, including activated microglial responses, become more persistent (Hunt et al., 2001). A continued presence of microglial activation has also been described after peripheral inflammatory nerve lesion where it appears to be accompanied by prolonged changes in nociception (Yeo et al., 2001). Thus, the time course of microglial activation is expected to reflect any persistence of pathological stimuli or continuation of secondary disease-maintaining processes. However, most experimental lesion models focus on acute pathological events, and few produce chronically active disease. It is unclear, therefore, how far the data derived from these experimental models can be extrapolated to human brain diseases, many of which show chronic progression and sometimes appear to have started from a single pathological event. It is here that molecular in vivo imaging has the potential to provide new insights into the cellular phenotype, particularly at early disease stages, where tissue for neuropathological examination is rarely available. Methodological issues related to quantifiability, sensitivity, and scarcity of available glial cell markers now call for the development of new and improved specific markers. The observations presented justify the hope that molecular in vivo imaging can indeed be extended to an in vivo neuropathology and provide the necessary phenotyping of human brain diseases in parallel with their genomic and transcriptomic characterisation.

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