Neuropathological imaging: *in vivo* detection of glial activation as a measure of disease and adaptive change in the brain

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Glial cells form a structural and functional network with complex cell–cell communication pathways that enable fast and slow signalling amongst themselves as well as with neurons. They exert regulatory influence on normal synaptic transmission and alter it in disease. It is becoming increasingly clear that an understanding of brain function in disease conditions requires a better account of the highly plastic, disease-associated changes in glial physiology *in vivo*. Particularly, microglia, the brain’s ubiquitous but normally inconspicuous immune effector cell, are prominently involved in many brain diseases. They respond rapidly and in a territorially highly confined way to subtle, acute and chronic pathological stimuli. Detection of microglial activation provides diagnostically useful formal parameters of disease, such as the accurate spatial localisation, disease progression and the secondary neurodegenerative or adaptive changes remote from the primary site of disease. The latter has potential relevance for the understanding of disease-induced brain plasticity. Systematic attempts are now undertaken, using positron emission tomography and a ligand with relative selectivity for activated microglia, to develop generic imaging tools for a cellular *in vivo* neuropathology.

For today’s neuropathologist, it must be hard to believe that microglia were once considered an endangered species. Yet, a few years ago, it was suggested that the existence of the microglia is in doubt and that their name should be abandoned. What can be regarded a gross scientific error by today’s standards has a long and complicated history. Discovered independently by Nissl and Robertson, microglia were first studied in detail by del Rio-Hortega. He also deserves the credit for establishing valuable knowledge on the role of microglia in CNS pathology. However, in the following years, and mainly due to a lack of cell type-specific markers, controversy arose around microglial embryonic development and their ‘nature’ as well as their cellular ‘identity’. Thus, in the mid-1980s, microglia were ‘rediscovered’ with the advent of immunocytochemistry and lectin markers. Meanwhile, the esoteric debate that surrounded microglia for decades had
given way to research activity involving a broad circle of scientists. As a result, more than 1000 papers have been published on microglia over the last few years. The biology and function of microglia is central to many issues in modern neuropathology. Microglia and brain macrophages have been recognized to play crucial roles in important diseases such as viral infections, autoimmunity and neurodegenerative disorders. HIV encephalitis, multiple sclerosis and Alzheimer’s disease are examples where understanding the role of microglia promises to hold essential information concerning disease pathogenesis. In addition, it is becoming increasingly clear that certain molecules expressed by microglia have the potential of serving as diagnostic ‘sensors’ in day-to-day neuropathological practice. These markers point to subtle tissue pathology that may otherwise go undetected.

Graeber and Kreutzberg (1994)¹

The brain contains an estimated 100 billion neurons and approximately 10–50 times as many glial cells. Approximately 1 out of 10 glial cells is a microglia, a cell-type of mononuclear-phagocyte lineage that is distributed throughout the entire brain in non-overlapping territories. Microglia constitute the brain’s autochthonous source of tissue macrophages and thus are part of its intrinsic immune system. Through various signalling pathways, many of which are not yet known or fully understood, these normally dormant cells can respond to even the most subtle neuronal injury by changing their highly ramified morphology to resemble gradually, in shape and function, typical macrophages. This activation process is associated with an increase the number of microglia through mitotic proliferation and the de novo expression of numerous proteins and cell-surface molecules, many of which are important for cell–cell communication. A survey of the scientific literature on microglia listed in Medline since 1966 shows that by the time this article has gone to press, the number of references containing information on microglia will exceed 6000 of which at least 5000 alone have been published after 1994. Much of the literature reflects an application of known macrophage biology to the special case of microglia in various brain diseases. However, increasingly sensitive techniques for the detection of these cells demonstrate that microglial responses take place very early in inflammatory as well as neurodegenerative brain disease and often occur in histologically normal-appearing tissue. The latter, in particular, extends the traditional macrophage conception of microglia as merely cell debris removing phagocytes. The conventional definition of inflammation, a process that involves the recruitment of cells of the peripheral immune system with specific cell–cell interactions, too, cannot be readily applied to the process of microglial activation. Likewise, a wealth of new data about microglial cell biology shows that their activities can no longer be categorized in a dichotomous way as either ‘neurotoxic’ or ‘neuroprotective’. Readers interested in detailed
accounts of the complex and manifold functions of microglia in disease and experimental models are referred to a number of introductory and exhaustive reviews\(^2\)–\(^{12}\).

This review’s prime aim is not only to present a rational for imaging microglia \textit{in vivo} but also to emphasise the pathophysiological relevance of neuron-glial-microglial interactions in the disturbance and recovery of brain function. More specific details of the currently tested ligand have been reviewed elsewhere\(^{11,12}\).

The formal pathology of microglia

Since glial reactions (like a number of other neuropathological phenomena) are mostly ‘non-specific’ in the sense that they are not exclusively associated with either one or the other nosological entity, this review is not ‘disease-oriented’, but focuses on the formal aspects of microglial activation in acute and chronic brain disease.

The use of microglial responses as markers of disease location, progression, chronicity and recovery has its biological basis in the tight coupling between neuronal activity and the functional state of microglia. There are a number of theories on how the neuron–microglial coupling is achieved. One is that it is mediated by fluctuations in the local ion \textit{milieu}, such as the local increase in potassium in the wake of a neuronal injury\(^{13}\). Depending on their functional state, microglia lack the outwardly rectifying potassium channel and, therefore, are particularly sensitive to increased extracellular potassium concentrations\(^{14,15}\). Likewise, microglia have purinergic receptors and enzymes involved in the turnover of ATP\(^{16,17}\), a common co-transmitter. Importantly, the efficacy of these signalling pathways seems to depend on the type and degree of the underlying pathology and thus the amount of glial activation\(^{18–20}\). The complexity of neuron–microglial communication is further increased through the participation of other glial cell types, such as astrocytes whose Ca\(^{2+}\) waves evoke microglial responses\(^{21}\). The interaction between neurons and microglia is bi-directional. Microglia secrete trophic factors, such as growth factors, which promote neurite formation or exert indirect influence \textit{via} other glial cells, notably astrocytes. Activated microglia appear to support sprouting \textit{via} the insulin-like growth factor-1\(^{22}\). Similarly, neuronal sprouting of serotonergic and dopaminergic fibres co-incides with the presence of activated microglia expressing glial cell line-derived and brain-derived neurotrophic factor mRNA\(^{23}\). Perineuronal microglia are involved in the removal of synapses (‘synaptic stripping’) from the somata and dendrites of lesioned neurons and could thus be a subtle cellular correlate of clinically observed disconnection syndromes\(^{5}\).
Unlike astrocytes, that form a syncytium allowing large-scale synchronization of their functional state, microglia normally do not form cell–cell contacts between them (Fig. 1). Signals between microglia except under conditions of gross brain damage with massive blood–brain barrier disruption do not appear to travel far. Consequently, their activation can initially be confined to individual cells remaining highly localized to the source of the activating stimulus (e.g. an injured neuron or neuronal pathway). Hence, the distribution pattern of microglial activation closely reflects the distributed network structure of the brain’s neural tract system. For example, a lesion of the

Fig. 1 (A) Dormant microglia form a dense network of cellular surveillance that operates throughout the entire CNS. (B) Upon activation, microglia change their highly ramified morphology (1, 2), proliferate, express numerous biomolecules and gradually transform into tissue macrophages. Activated perineuronal microglia participate in the removal of synapses from the surface of injured neurons (‘synaptic stripping’) (3). (C) Microglial activation follows the injured neuronal pathway in antero- and retrograde direction. (D) The transition from the resting to the activated state is accompanied by an expression of the PK11195 binding site, which is near absent in resting microglia. Labelled with carbon-11, PK11195 can be used to image this process of activation by PET as a measure of active disease in the brain.
facial nerve results in a retrograde neuronal reaction with activation of perineuronal microglia around the injured motoneurons of the facial nucleus (Fig. 1C and Plate III A–B, see end of file p.132). Similarly, an injury to the sciatic nerve is accompanied not only by an activation of microglia in the spinal cord but also in the remote projection area of the gracile nucleus that receives the ipsilaterally ascending nerve fibres of the affected sciatic nerve. This simple principle of retro- and anterograde microglial activation can be applied to any focal brain lesion that affects regional pathways connecting to remote projection areas. An interesting question is whether microglial activation may occur even beyond the primarily affected neural pathways (i.e. in trans-synaptic regions). Trans-synaptic ‘knock-on’ effects have indeed been reported for lesions in the visual system and in experimental lesion models of Huntington’s disease. Functionally important, late trans-synaptic 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.

The time course of microglial activation is, depending on the method of detection, between minutes to days. The first activation signals, such a transient changes in extracellular ion concentration are likely to occur within milliseconds after a lesion. In acute experimental lesion models, the peak of microglial activation has been found to be between 2–3 days after the insult. With the persistence of the pathological stimulus, microglial activation continues. There are few data on chronic microglial activation as there are currently no generally excepted animal models that would truly replicate the chronicity of disease that is associated with most human neurodegenerative disorders. In humans, even years after a single toxic event, such as in MPTP-induced Parkinsonism, activated microglia may be present in selective areas indicating continuing or secondary neurodegenerative processes.

Applied to a focally destructive condition, a number of early and late microglial effects can be expected (Fig. 2).

1 The separation of the peripheral immune system from the brain normally maintained by the intact blood-brain barrier is disturbed locally with influx of blood-borne cells. Under this condition, invading cells of mononuclear-phagocyte lineage cannot be readily distinguished from locally activated microglia that have transformed into macrophages. The latter is true for any focal lesions, such as stroke lesion or plaques in multiple sclerosis.

2 As a consequence of damage to neuronal pathways, secondary microglial activation occurs in remote projection areas. Due to the lack of in vivo markers, little is known about how these remote effects relate to the overall functional impact of a focal lesion and to the eventual outcome during recovery. In any case, the presence of activated microglia in areas...
not primarily damaged introduces an additional compartment constituted by numerous receptors and other biomolecules that are expressed de novo and potentially impact on neuronal functioning.

One further consequence of microglial activation in areas of intact blood-brain barrier (so far studied in peripheral nerve lesion models\textsuperscript{37,38}) is that the tissue can become ‘immune alert’ and attract cells of the peripheral immune system (Fig. 2D). This observation is important as it points to the possibility that, primarily, neuronal pathology in the absence of overt blood–brain barrier damage can elicit secondary immune responses that themselves may contribute to further secondary disease progression.

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Fig. 2 (A) The blood–brain barrier (BBB) separates the peripheral immune system from the healthy brain. (B) In pathology with disturbance of the BBB, cells of the peripheral immune system invade the area of the focal lesion. The strict compartmental separation of CNS peripheral immune system is lifted and it is difficult to distinguish the response of the brain’s intrinsic immune effector cell, microglia, from invading blood-borne macrophages. (C) Primarily neuronal pathology, such as after peripheral nerve lesion, does not cause obvious BBB damage but evokes a glial inflammation, i.e. a microglial response locally and in projection areas, that does not involve the peripheral immune system. (D) The presence of activated microglia can confer regional ‘immune alertness’ with the secondary, site-directed recruitment of, for example, circulating T-lymphocytes. The neuronally triggered glial inflammation evolves in toward a delayed inflammatory response.
Imaging activated microglia in vivo

The isoquinoline PK11195 binds to the peripheral benzodiazepine binding site (PBBS) originally so named because it partially displaces certain benzodiazepines, such as diazepam. It is, however, a misnomer since the PK11195 binding site is not related to the central benzodiazepine receptor associated with γ-aminobutyric acid (GABA)-regulated channels.

The binding site for PK11195 is particularly abundant in peripheral organs and haematogenous cells, but barely present in the normal CNS. The PBBS co-precipitates with the outer membrane of mitochondria, hence its other name, mitochondrial benzodiazepine receptor. PK11195-binding is also present in non-mitochondrial fractions of brain extracts, and mitochondria-free erythrocytes while immunocytochemical staining hints to the possible presence PBBS in cell nuclei. Amongst others, the PBBS plays an important role in steroid synthesis and regulates immunological responses in mononuclear phagocytes. The putative functions of the PBBS, that have not yet support a coherent theory of its biological role, have recently been reviewed by Gavish et al.\(^28\).

In vitro, astrocytes are found to have high binding of PK11195. In experimental lesion models and human diseases, however, focally increased PK11195 binding appears mostly due to binding to infiltrating haematogenous cells or locally activated microglia. In vivo studies of conditions without blood–brain barrier damage demonstrate that the distribution pattern of increased PK11195 binding matches closest the distribution of activated microglia rather than that of reactive astrocytes (see recent review by Banati et al.\(^11\)). These findings are supported by high-resolution micro-autoradiography with \(^{3}H\)-(R)-PK11195 combined with immunohistochemical cell identification performed on the same tissue section in inflammatory disease, such as multiple sclerosis and experimental allergic encephalomyelitis. They suggest that whilst reactive astrocytes and microglia regularly occur side by side, increased binding of \(^{3}H\)-(R)-PK11195 is found either on infiltrating blood-borne cells or activated microglia. The latter appear to become the dominant source of binding in areas without any obvious histopathology and remote from the primary pathological focus\(^29\). Though PK11195 is not truly cell-specific, activated but not resting microglia appear to be the dominant source of PK11195 binding in the brain in vivo. With respect to above micro-autoradiographic double-labelling data, it is potentially important 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, rather than the commonly used racemate. As there are currently no entirely conclusive data available that show that the site of (R)-PK11195 binding is identical with the epitope recognised by polyclonal antibody staining against the PBBS, it may be advisable not to view altered
(R)-PK11195 binding as synonymous with changed PBBS expression. Recent data suggest that an interaction between the subunits of the PK11195 binding site rather than transcriptional, post-transcriptional or translational mechanisms may cause changes in cellular binding.

While the exact function of the PBBS has remained elusive, a potentially useful clinical application exists for its specific ligand, PK11195, based on three observations: (i) normal brain shows only minimal binding of PK11195; (ii) in CNS pathology, in vivo PK11195 binding is predominantly found on activated microglia; and (iii) [11C]-PK11195 can be used as a ligand for positron emission tomography (PET). [11C](R)-PK11195 PET has been used to image active brain pathology in stroke, multiple sclerosis, herpes encephalitis, vasculitis and Alzheimer’s disease. The lack of significantly increased [11C](R)-PK11195 binding in astrocyte-rich tissue, such as in patients with hippocampal sclerosis, supports the view that microglial (R)-PK11195 binding appears to be the relatively largest contributor to the PET signal measured in vivo. Importantly, these patients had a 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 lesions identified as hypo-intense areas in the MRI and known to be surrounded by reactive astrogliosis do not show an increased [11C](R)-PK11195 PET signal.

As predicted from experimental data, one observation common to all these conditions is the presence of increased [11C](R)-PK11195 PET signals remote from the primary lesion in areas that may appear structural normal. For example, in stroke patients, increased [11C](R)-PK11195 binding is regularly found in the ipsilateral thalamus, indicating the presence of activated microglia in the degenerating projection areas remote from the primary lesion in the cortex. Similarly, patients recovering from unilateral herpes encephalitis, show a distribution pattern of activated glial cells emerging over a period of months, which gradually propagates through the entire affected limbic system well beyond the initial lesion focus. The increased [11C](R)-PK11195 binding in these patients follows projecting axonal pathways, such as the large association bundles interconnecting mesocortical areas, subicular allocortices and subcortical amygdaloid nuclei.

There is evidence that peripheral denervation with long-lasting abnormal stimuli may also evoke a trans-synaptic glial response beyond the first-order projection areas of the injured neural pathway. For example, increased [11C](R)-PK11195 binding has been seen in the normal-appearing, contralateral thalamus of patients who have lost an upper limb 2–23 years ago and suffering to various degrees from painful phantom sensations. This increase in [11C](R)-PK11195 binding in the thalamus, but not somatosensory cortex, indicates the presence of activated microglia, possibly as a consequence of subtle transneuronal changes. Formal histological confirmation is, however, still outstanding.
The important implication is that glial activation can occur trans-synaptically and may be driven purely by altered neuronal activity. If confirmed, this would support the view that subtle, activity-dependent, microstructural changes in the thalamus are at least partially responsible for lasting re-arrangements of the cortical representational maps\textsuperscript{26} and that there is active glial participation in brain plasticity. While in this specific context microglial activation does not suggest tissue destruction, recent studies of Alzheimer’s disease patients demonstrated a correlation between the regional $[^{11}C]-(R)$-PK11195 binding pattern (e.g. in the temporal lobe) and the subsequent anatomical pattern of atrophy as shown by MR-difference imaging\textsuperscript{32,34} (Plate IIIG–I, see end of file p.132). The extent to which microglial activation in patients with Alzheimer’s disease is more than a concomitant marker of disease progression but indeed an active inducer of neuronal damage requires more comprehensive knowledge about the particular disease-state related balance of detrimental and beneficial microglial activities.

**Conclusions**

Neuropathological *in vivo* imaging, particularly of glial cells as prominent constituents of tissue pathology, can be used to study a wide variety of brain diseases, such as stroke, multiple sclerosis, autoimmune encephalitis, vasculitis, Parkinson’s disease, atypical Parkinsonism and others. However, it is important at this descriptive level that the aim is to find generic disease markers in their own right rather than support the effort of keeping clinically defined syndromes apart. The purpose of this review was to describe the basic microglial response pattern in disease generally and to raise the awareness that brain function in health and disease is the joint result of neuronal and glial activity. The more comprehensive information about all disease-associated cellular changes, including microglial activation, that is necessary to understand their exact pathophysiological relevance, is now being furnished by the application of genomic and proteonomic array techniques to well define neuropathological specimen. For a useful clinical application, the main requirement is the development of improved or new ligands suitable for an ‘*in vivo* neuropathology’, appropriate biomathematical modelling and signal quantification, aspects that were not covered in this outline of the basic concept.

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Plate III  (A) Transient facial nerve paralysis (Bell's palsy) leads to a retrograde neuronal reaction with concomitant glial responses in the lesioned facial nucleus. (B) This patient with Bell's palsy shows a regional increase in the [11C]-(R)-PK11195 PET signal in the area of the ipsilateral facial nucleus. The PET image has been co-registered to the patient's MRI. (C) In another patient with facial nerve palsy, MRI reveals a small gadolinium-enhancing lesion (+ gd) in the vicinity of (but not in) the facial nucleus. Under the condition of an open BBB, the associated increase in the [11C]-(R)-PK11195 PET signal cannot be solely attributed to the local activation of microglia. (Note: extra-cerebral [11C]-(R)-PK11195 binding, such as in the nasal mucosa, has been masked. Some areas, such as the area postrema or around the central canal, where the BBB is constitutively absent, show low-grade microglial activation. Thus significant [11C]-(R)-PK11195 signals, particularly in the brain stem, can be seen that are not indicative of a disease.) (D–F) Regionally increased [11C]-(R)-PK11195 PET signal in the superior longitudinal fascicle (SLF) in the left frontal operculum correlating with the clinical presentation of a transient speech dyspraxia in this multiple sclerosis patient. (G–I) 75-year-old patient with dementia (mini mental state examination: 20) showing increased [11C]-(R)-PK11195 PET binding in the cortex, particularly in the temporal lobe. The prominent left temporal [11C]-(R)-PK11195 PET signal matches the area of relatively greater loss of volume as measured by MRI subtraction imaging.