Positron Emission Tomography Imaging of Neuroinflammation

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Summary: In the diseased brain, upon activation microglia express binding sites for synthetic ligands designed to recognize the 18-kDa translocator protein TP-18, which is part of the so-called *peripheral benzodiazepine receptor* complex. PK11195 [1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide], the prototype synthetic ligand, has been widely used for the functional characterization of TP-18. Its cellular source in activated microglia has been established using high-resolution, single-cell autoradiography with the R-enantiomer [^{3}H](*R*)-PK11195. Radiolabeled [^{11}C](*R*)-PK11195 has been used to image active brain disease with positron emission tomography. Consistent with experimental and postmortem observations of a characteristically distributed pattern of microglia activation in areas of focal pathology, as well as in anterograde and retrograde projection areas, the *in vivo* regional [^{11}C](*R*)-PK11195 signal is found in active focal lesions and over time also along the affected neural tracts and their respective cortical and subcortical projection areas. Thus, a profile of active disease emerges that matches some of the typical distribution patterns known from structural neuroimaging techniques, but additionally shows involvement of brain regions linked through neural pathways. In the context of cell-based *in vivo* neuropathology, the image data are thus best interpreted in the context of the emerging cellular understanding of brain disease or damage, rather than the definitions of clinical diagnosis. One important observation, borne out by experiment, is the long latency with which activated microglia or increased PK11195 retention appear to gradually emerge and remain in distal areas secondarily affected by disease, supporting speculations that the presence of activated microglia is an important corollary of brain plasticity. **Key Words:** Peripheral benzodiazepine receptor, PBR, PK11195, microglia, neuroinflammation, PET, brain.

MICROGLIA AND NEUROINFLAMMATION

Microglia are mesoderm-derived brain macrophages and represent the resident immunocompetent cells of the CNS. In conditions of an intact blood–brain barrier when blood-borne cells are largely absent, microglia together with perivascular cells are the first line of the immune defense system of the brain. In response to neuronal injury or even subtle perturbations of the CNS environment, resident microglia react with a set of stereotypic changes in their physiological state. The transition of microglia into an activated state includes a change in their morphology, migration toward the site of neuronal damage, proliferation until they quadruplicate in number, and cell surface expression and release of a widespread variety of proinflammatory molecules. If cell death occurs, activated microglia mature into full-blown macrophages.[1] This responsiveness of microglia to a wide variety of pathological stimuli has led to their description as a "sensor for pathological events in the CNS."[1]

Recently, it has been demonstrated by two-photon microscopy that so-called resting microglia found in healthy tissue continuously change their morphology, sending out extensions from their ramified processes in an apparent attempt to sample and assess the microenvironment and make contact with other cells and blood vessels. Even in the resting state, therefore, microglia are thus far from being dormant or functionally at rest.[2,3]

Rapid local activation of microglia can occur without the lymphocytic infiltrations characteristic of classical inflammatory brain disease, such as in multiple sclerosis (MS). This observation has led to the concept of *neuroinflammation* in a variety of primarily noninflammatory neurological conditions, including neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD),[4] metabolic encephalopathy such as hepatic encephalopathy,[5] and processes involving neuronal remodeling and plasticity after brain or

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peripheral nerve injury.[6,7] The general concept of microglial activation has been extensively reviewed with respect to its potential relevance for cytotoxicity,[8,9] plasticity,[7] and inflammation and site-directed homing of inflammatory cells to areas containing activated microglia.[10] More comprehensive data looking at the full complement of activation-induced genes in microglia have now also become available.[11,12]

Here, we outline the rational for studying *in vivo* activated microglia by using brain positron emission tomography (PET). The *in vivo* detection of activated microglia may serve as a surrogate marker of neuronal damage, CNS disease activity, and, more speculatively, brain plasticity.[13]

PET IMAGING OF THE PERIPHERAL BENZODIAZEPINE RECEPTOR

Cellular source and function

The *peripheral benzodiazepine receptor* (PBR) is a hetero-oligomeric complex, that appears to be largely, though not exclusively, localized in the outer membrane of mitochondria.[14,15] However, there is also disproportionately high binding to nonmitochondrial fractions of brain extract and mitochondria-free cells.[14,16] The name derives from the binding of certain benzodiazepines, such as diazepam, to this receptor, which is distinct from the central benzodiazepine receptor associated $GABA_A$-regulated channels. Other names have also been used such as *mitochondrial benzodiazepine receptor* and *peripheral benzodiazepine binding site*.

In the CNS, the PBR is exclusively found on non-neuronal cells, primarily activated microglia or, in the case of a disrupted blood–brain barrier, on invading cells of mononuclear-phagocyte lineage.[17–19] Early studies of the functional role of the PBR were performed in astrocytes cell cultures, which express high levels of PBR *in vitro*.[20] As will be discussed shortly, however, these *in vitro* observations could not be fully extended to the *in vivo* state.[18–20] Differences in the functional behavior of cells between the *in vivo* and *in vitro* state are not unusual. For example, although astrocytes *in vivo* show hypertrophy in response to brain disease, they generally do not proliferate, whereas *in vitro* they have a high proliferation rate. In contrast, microglia proliferate both *in vitro* and *in vivo*.

Given that the PBR is closely linked to cell cycle control,[22,23] this obvious difference in the proliferative behavior of astrocytes *in vitro* cautions against extrapolating to conclude that astrocytic PBR expression will also occur *in vivo*. The PBR is not expressed significantly in normal brain parenchyma, except for low-level expression in certain areas, such as those constitutively without blood–brain barrier (i.e., the choroid plexus and the ependymal cells lining the ventricles).

The gene for the PBR is remarkably well preserved across species, implying an important housekeeping function. A role in the cerebral steroid biosynthesis and regulation of immunologic responses has been reported.[24] More recently, the term *translocator protein (18 kDa)* has been proposed.[25] The proposed name refers to the main molecular functions of the 18-kDa protein, namely, the binding and transport into the mitochondria of cholesterol, proteins, and porphyrin, with regulation of the steroid synthesis being the most extensively studied function.

There remains considerable lack of clarity as to whether other observed effects of purported PBR ligands (such as increasing cell survival by modulating free radical production, regulating cell-death, and promoting cell proliferation) are solely due to the 18-kDa protein. Some of these cellular effects have been seen using high doses of synthetic PBR ligands, which cause other nonspecific modulatory effects, such as the inhibition of ATP kinases.[26] Also, there is still insufficient information with regard to the existence of PBR subunits or functionally and structurally related binding sites, which would help to explain the reported differences between different PBR ligands in their cellular distribution and function.

PK11195 binding to PBR: from *in vitro* ligand to *in vivo* PET tracer

PK11195, or 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide, is a specific ligand for the PBR. It has been widely used for the functional characterization of the PBR and identification of its cellular source in brain tissue. High-resolution microautoradiography with the R-enantiomer [^{3}H](*R*)-PK11195, combined with immunohistochemical cell identification in MS and experimental autoimmune encephalomyelitis tissue, has shown that increased binding of [^{3}H](*R*)-PK11195 is found on infiltrating blood-borne cells and activated microglia but not on reactive astrocytes.[21,27] Indeed, numerous experimental models have shown that autoradiographically detected PK11195 binding correlates better with the temporospatial profile of microglial activation than with that of reactive astrocytes.[18,19,28,29]

Discrepancies with other reported cellular localization data using a polyclonal antibody[30] might hint to differences between the sites or PBR subunits recognized by the antibody and those to which (*R*)-PK11195 binds on tissue sections and *in vivo*. Cellular localization data from a model of chemically induced demyelination using cuprizone reported by the same group, indicate that in certain lesion-types, astrocytes, too, bind PK11195.[31] However, the high-resolution (photoemulsion) autoradiographic binding pattern in the demyelinated areas shows a different, less dense and obvious localization of the silver grains to astrocytic processes compared with the high background signal and the high density seen on activated microglia.[21]

Labeled with carbon-11, PK11195 is a suitable ligand for PET studies. There is *in vivo* evidence from PET with the ligand [^{11}C](*R*)-PK11195 in favor of a largely microglial source of PK11195 binding. A [^{11}C](*R*)-PK11195 PET study performed in stable epilepsy patients with hippocampal sclerosis, a condition characterized by marked astrogliosis, showed no increases of [^{11}C](*R*)-PK11195 binding, supporting the view that the *in vivo* [^{11}C](*R*)-PK11195 signal is preferentially if not exclusively due to the presence of activated microglia.[32] Likewise, there is little binding of (*R*)-PK11195 in surgically removed tissue of hippocampal sclerosis. Where any binding has been seen, it appears to be on activated microglia lying amid the astrogliotic scar tissue.[8]

Thus, most *in vivo* data suggest that activated microglia (in the absence of blood-borne inflammatory cells) are the dominant source of PK11195 binding in areas of active pathology. Given the much lower sensitivity with which ligand binding can be detected *in vivo*, and the inherent threshold in the calculation of the values of specific binding, the signal measured by [^{11}C](*R*)-PK11195 PET is unlikely to reflect any significant contribution by astrocytes.

Advances in kinetic modeling of PET images with PK11195

Parametric images of regional [^{11}C](*R*)-PK11195 binding at the voxel level have been obtained using a simplified reference tissue model[33,34] and cluster analysis.[35–38] Parametric maps of [^{11}C](*R*)-PK11195 binding potential were generated using a basis function implementation of a simplified reference tissue model[34] (FIG. 1).

The choice of the simplified reference tissue model reflects the empirical observation that in the case of PK11195, the use of a plasma input function is less sensitive than the calculation of parametric images using a reference tissue-derived input function. The simplified reference tissue model is expected to show a dependency on regional blood flow and underestimation of the true signal when the reference region selected is not a pure reference: that is, the a priori assumption that the region of interest (ROI) is devoid of specific binding is violated. Therefore, the often-used cerebellar reference region is not, unlike for many other ligands, well suited for the definition of the tissue input function of PK11195, because it contains significant constitutively expressed PBR and numerous relatively large blood vessels within the folia.

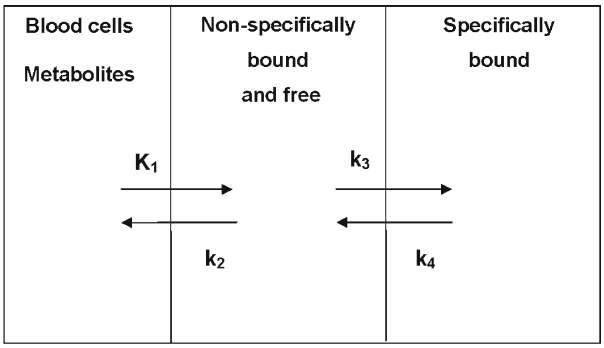

FIG. 1. Compartmental model for [^{11}C](*R*)-PK11195 assumes that there are two tissue compartments and four rate constants. The two tissue compartments represent the concentration of free (and possibly nonspecific bound fraction) and the concentration of specific bound fraction of the tracer to the peripheral benzodiazepine receptor (PBR) in brain tissue. The k_1 and k_2 represent first-order rate constants for the transport of ligand from plasma to tissue and vice versa; k_3 and k_4 represent rate constants between the free and specifically bound compartments in brain tissue. Binding potential (BP) is the measure of specific binding of the tracer in the target region (BP = k_3/k_4).

The possible contamination with a constitutive binding signal in a purely anatomically defined reference region is also relevant in conditions where global changes in binding may occur. Cluster analysis offers an alternative data-led approach for the extraction of the normal ligand kinetic that can serve as the reference input function. Cluster analysis allows the automatic segmentation of the dynamic raw data into a number of clusters of concentration time–activity curves (TACs) on the basis of the shape of the TAC. The process then associates each voxel of the image with one of the cluster curves according to the likelihood with which the TAC of the voxel belongs to one of the clusters.

In normal brain, the majority (~90%) of the voxels are segregated into two clusters, one representing the TAC mainly from the skull and scalp, and one representing the TAC of voxels mostly located in the cerebral cortex (FIG. 2). The latter was identified as the kinetic of the ligand in normal brain tissue. The suitability of the TAC extracted by cluster analysis as the individual normal ligand kinetic was confirmed by testing for dissimilarity with a normal population input kinetic (χ^2 test < 0.95) (FIG. 2). A normalized mean TAC (population input kinetic) was calculated from the normal ligand kinetics as previously identified by cluster analysis and cortical gray ROI in a group of normal subjects. A highly significant correlation was obtained between calculated values using the cluster analysis approach and those calculated using the reference input kinetic generated from a cerebellar ROI in a sample of images obtained from normal controls and patients affected by herpes encephalitis, a disease that spares the cerebellum.[39]

The robustness of cluster analysis for the extraction of a reference tissue region for the quantification of [^{11}C](*R*)-PK11195 PET studies has recently been confirmed by Turkheimer et al.[40] The authors also described that an improvement in the signal to noise ratio can be achieved by a clustering algorithm that selects reference voxels avoiding regions with nonspecific binding and specific binding located in the blood vessel walls.

Although the desirable absolute quantification of PK11195 binding has not been achieved, the potential errors introduced by the cluster analytical approach have been found to be systematic across healthy and diseased

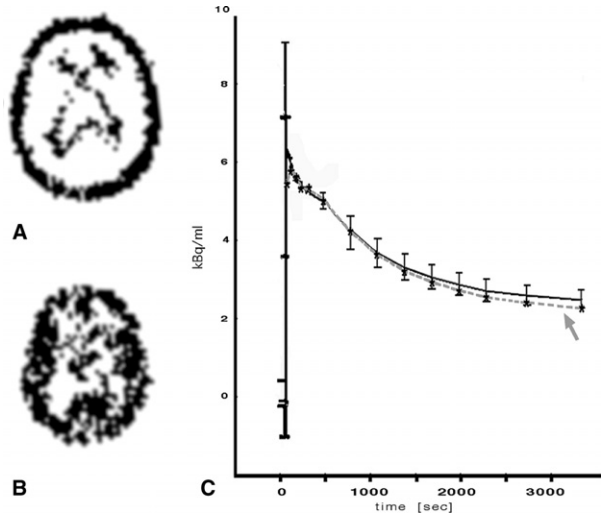

FIG. 2. Binary mask images derived from cluster analysis of a [^{11}C](*R*)-PK11195 scan of a normal subject. The two images show the distribution of voxels characterized by the two main clusters corresponding to (A) scalp and white matter, (B) the cerebral cortex, and (C) individual TACs. The individual time–activity curve (TAC) extracted by cluster analysis in a single subject (gray line and arrow) is tested against the normal population kinetic (black line). Individual TACs not significantly dissimilar from the normal population kinetic were used as reference input function.

subjects and thus do not affect the statistical significance of differences found between comparator groups. A trend toward a small underestimation of the true signal and thus reduced sensitivity is currently the most obvious shortcoming. It is likely that a genuine increase in sensitivity will come from the development of new PBR ligands with better pharmacokinetic properties rather than from improved tracer modeling.

IMAGING NEUROINFLAMMATION IN INFLAMMATORY BRAIN DISEASES

Multiple sclerosis

In MS, quantitative [^{11}C](*R*)-PK11195 PET demonstrated increased PBR expressed by activated microglia–macrophages in areas of focal pathology identified by T1- and T2-weighted MRI, and also in MRI normal-appearing cerebral regions, including gray matter.[27] The overlap between areas with MRI-defined pathology and with increased [^{11}C](*R*)-PK11195 binding reached a peak in gadolinium-enhancing lesions, in which 30% of the volume had a significant increase of [^{11}C](*R*)-PK11195 binding. For patients studied during an acute relapse the overlap increased in both T2-weighted hyperintense areas and also the T1 black holes with respect to MS patients not in relapse.

One patient with secondary progressive MS showed high [^{11}C](*R*)-PK11195 binding colocalizing with the hypointensities of the T1-weighted MRI, reaching 40% overlap. The [^{11}C](*R*)-PK11195 overlap with the T1-weighted MRI hypointensities positively correlated with the total Expanded Disability Status Scale (EDSS) score. Increased [^{11}C](*R*)-PK11195 binding was detected in anatomical structures with a normal appearance MRI and in gray matter, along neuronal fiber tracts.

In patients with optic neuritis, a white matter tract pathology associated with anterograde glial reactions in the projection areas of the optic nerves, increased [^{11}C](*R*)-PK11195 binding was found in the lateral geniculate bodies. Given that MRI did not reveal disruption of the blood–brain barrier or obvious structural abnormalities in the lateral geniculate bodies, the increased [^{11}C](*R*)-PK11195 binding is indicative of a subtle microglial activation secondary to the degeneration of the optic tract.

In vitro experiments using [^{3}H]PK11195 microautoradiography after peripheral axotomy demonstrated that activated [^{3}H]PK11195-binding microglial cells are also found remote from the primary lesion in regions that are connected via retrograde and anterograde neuronal projections.[21] Axotomy of the facial nerve results in an activation of microglial cells in the facial nucleus as a response to the retrograde neuronal reaction. Likewise, axotomy of the sciatic nerve induces an anterograde microglial activation in the ipsilateral nucleus gracilis, the nucleus receiving the ipsilaterally projecting fibers ascending from the dorsal root ganglion.

Rasmussen's encephalitis

Rasmussen's encephalitis is a rare progressive inflammatory brain disease clinically presenting as epilepsia partialis continua and frequently associated with hemiplegia, hemianopsia and intellectual deterioration.[41] Neuropathologically, the disease is characterized by multifocal inflammation, neuronophagia, activated microglia, microglial nodules, and brain atrophy generally confined to one cerebral hemisphere. A second pathology, such as cortical dysplasia, vascular malformations, and astrocytomas, has been found in ~10% of patients with Rasmussen's encephalitis. It has been suggested that Rasmussen's encephalitis may have an autoimmune origin; however, the autoimmune mechanisms and the precise role played by antibody-mediated mechanisms, T-cell immunity, and viral antigens remain unclear.

Although the peculiar anatomic restriction of the pathologic process to a single hemisphere is a consistent finding, Rasmussen and others have noted that a "chronic encephalitis" can frequently be seen in the absence of invading blood-borne cells.[42] In two patients with Rasmussen's encephalitis a significant increase of [^{11}C](*R*)-PK11195 binding has been detected in the affected cerebral hemisphere compared with the unaffected contralateral hemisphere.[32] Furthermore, the distribution pattern of increased [^{11}C](*R*)-PK11195 binding corresponded to the pattern of brain atrophy detected by

MRI[32] and to other brain abnormalities, such as subependymal gray matter heterotopia, that often coexists in this condition (unpublished data). The extent to which this microglial activation suggests a primary neuroinflammatory disease process or is secondary to neuronal damage caused by the high seizure frequency in these patients, remains to be established.

IMAGING NEUROINFLAMMATION IN DEGENERATIVE DISEASES

There have been consistent observations of activated microglia in primarily noninflammatory neurodegenerative diseases, such (among others) AD and PD.[43] It is experimentally well established that neuronal injury per se, in the absence of any other contributing pathology (such as damage to blood vessels), evokes a rapid, highly localized activation of microglia around the somata of injured neurons and in anatomical projection areas.[1]

Movement disorders

Microglial activation has been demonstrated *in vivo* using [^{11}C](*R*)-PK11195 PET in the brains of patients with idiopathic PD [44,45] and atypical parkinsonism, such as multiple system atrophy,[46] progressive supranuclear palsy,[47] and corticobasalganglionic degeneration.[48] In these conditions, the distribution of [^{11}C](*R*)-PK11195 binding mirrors the spatial pattern of known distinct neuropathologic hallmarks. These studies also revealed marked within-group variation, with a certain degree of overlap between the cases of idiopathic PD and atypical parkinsonism. Although imaging of activated microglia with [^{11}C](*R*)-PK11195 PET does not serve to override an a priori clinical classification, it does provide a generic measurement of disease location and progression that may help to understand the heterogeneity in the clinical presentation of patients with Parkinsonian or other neurodegenerative disorders.

Two recent reports focused on assessing microglial activation in patients with PD with a combined PET study using [^{11}C](*R*)-PK11195 and a tracer binding to presynaptic dopamine transporter ([^{11}C]CFT)[44] or [^{18}F]fluorodopa,[45] for the study of dopaminergic nigrostriatal projections. Ouchi et al.[44] found an increase of [^{11}C](*R*)-PK11195 binding in the midbrain region (encompassing substantia nigra and other structures in this region) in 10 early-stage, drug-naïve PD patients. Furthermore, the level of [^{11}C](*R*)-PK11195 binding in the midbrain correlated inversely with the loss of dopaminergic nigrostriatal projection in the putamen and positively with the degree of motor disability. They failed to detect any significant increase of [^{11}C](*R*)-PK11195 binding in the striatum.

In contrast, Gerhard et al.[45] found a more widespread spatial pattern of microglial activation in PD patients both at early and late stages of the disease. In 18 non-demented PD patients, a significant increase of [^{11}C](*R*)-PK11195 binding was found in the striatum, pallidum, thalamus, and pons, as well as in cortical regions such as frontal lobe, cingulate cortex, temporal lobe, and hippocampus. No close correlation between the level of regional [^{11}C](*R*)-PK11195 binding and either clinical parameters (disease duration and severity) or putaminal [^{18}F]fluorodopa signal was found. In addition, 8 PD patients underwent a second [^{11}C](*R*)-PK11195 scan after an interval time of 18–28 months from the first scan. No significant changes of [^{11}C](*R*)-PK11195 binding from baseline to follow-up were found despite further disease progression, measured clinical rating motor scales, and confirmed additional reductions of the putaminal [^{18}F]fluorodopa signal.

The discrepancy between the two studies[44,45] could be due at least in part to methodologic differences, such as the resolution of PET scanner, the kinetic modeling applied to the parametric images, and the type of image analysis performed. The study of Ouchi et al.[44] did not explore and interrogate the parametric [^{11}C](*R*)-PK11195 images for increases of signal in regions outside the midbrain and basal ganglia. Gerhard et al.[45] performed both an ROI analysis, sampling several cortical regions, and a statistical parametric mapping analysis to localize significant mean differences of ligand binding potential at a voxel level. Statistical parametric mapping allows exploratory voxel by voxel group comparison throughout the entire brain volume without requiring a priori hypothesis. The authors in essence confirmed *in vivo* what is known from neuropathologic studies;[49] namely, that there is widespread microglial activation in the substantia nigra and basal ganglia, but also in cortical association regions (i.e., hippocampus, transentorhinal cortex, cingulated cortex), the same regions that have a high burden of Lewy body pathology in the early stages of the disease.[50]

It is important to note that PD is emerging as a multisystem disease with involvement of regions outside the nigrostriatal connections and that a widely distributed tissue pathology is already present in the early clinical phase of the disease. Furthermore, it is important to be aware of differences in the respective patient population when comparing the results of the aforementioned studies. It is possible that differences in the disease severity and, particularly, in the presence and duration of drug therapy may play a role in the different level of [^{11}C](*R*)-PK11195 binding potential detected, for example, in the striatum.

Two additional considerations arise from the results of [^{11}C](*R*)-PK11195 PET studies in PD. First, the mean level of [^{11}C](*R*)-PK11195 binding, reflecting the degree of microglial activation, is not a function of disease severity. For example, follow-up [^{11}C](*R*)-PK11195 PET after 18–28 months did not detect changes of [^{11}C](*R*)-

PK11195 binding from the baseline, despite evidence of clinical and functional disease progression. Although it could be that this interval time is too short to determine differences in microglial activation, nonetheless it is more likely that the result reflects the time course of neuroinflammation, with a peak in the preclinical or early stages of PD that reaches a plateau with ongoing activation of microglial cells in the following stages.

In experimental studies, it has been shown that [^{3}H]PK11195 binding by activated microglia increases rapidly after neuronal injury, but then does not increase any further when transformation of microglia into macrophages occurs, following neuronal cell death.[21] Second, increased microglial activation in the striatum may reflect loss of nigral dopaminergic projections, subtle molecular neuronal changes due to drug therapy effects, and possibly excitotoxicity from hyperactivity of the subthalamic nucleus, rather than direct pathologic involvement.

In a postmortem study, activated microglia have been found in brains of patients with Huntington disease (HD), particularly in regions known to be targeted, such as the striatum, globus pallidus, and frontal cortex.[51] *In vivo* evidence of increased [^{11}C](*R*)-PK11195 binding in the striatum and cortical regions in symptomatic HD patients compared with healthy controls has been recently published.[52] In that study, [^{11}C](*R*)-PK11195 binding correlated with clinical severity of HD rated with Unified HD Rating Scale motor scores, and inversely with the level of striatal dopamine D2 receptor loss as assessed by [^{11}C]raclopride PET. The authors also reported that increased striatal [^{11}C](*R*)-PK11195 binding was present in a few presymptomatic HD gene carriers.[53] However, a minority of the presymptomatic HD gene carriers studied did not exhibit abnormal striatal [^{11}C](*R*)-PK11195 or [^{11}C]-raclopride binding. It may be that some of these carriers were further away from neuronal dysfunction and disease onset. Nevertheless, the results indicate that microglial activation is an early process in HD pathogenesis and may be present in the HD brain before the onset of symptoms.

Dementia

The process of neurodegeneration in AD is associated with local glial responses. Activated microglia have been found clustered at the site of senile plaques and associated with fibrillar amyloid peptide deposition and dystrophic neuritis, where they play a role in the phagocytosis of amyloid fibrils.[54] Some studies have implied an active role of microglia in the mediation of amyloid toxicity and subsequent secondary tissue damage via release of cytokines and cytotoxic molecules.[55] This observation had led to the hypothesis that a chronic, self-perpetuating neuroinflammatory stimulation of microglia in AD pathology is an important pathogenetic mechanism that may contribute to disease progression.[55]

Cagnin et al.[56] showed that it was possible to detect and quantify *in vivo* microglial activation in the brain of patients with mild to moderate AD using PET with the radioligand [^{11}C](*R*)-PK11195. The spatial distribution of [^{11}C](*R*)-PK11195 binding potential matched the known pattern of pathologic changes typical of AD—inferior and medium temporal cortex, amygdala, parietal lobule, and posterior cingulate cortex—and mirrored the regional distribution of cerebral hypometabolism detected with [^{18}F]fluorodeoxyglucose (^{18}FDG) PET in individual patients scanned with the two combined PET tracers (FIG. 3). The spatial pattern and extent of microglial activation in the brain of AD patients was predictive of which cerebral regions would develop more pronounced brain atrophy in the following years as assessed by longitudinal MRI scans.

This suggests that activated microglial cells clustered in areas of ongoing neurodegenerative changes and that the measure of microglial activation using [^{11}C](*R*)-PK11195 PET may be used as a surrogate marker of disease activity. [^{11}C](*R*)-PK11195 binding was also increased in the brain of a patient with mild cognitive impairment suggesting that microglial activation, like other pathological processes, such as brain amyloid deposition, is an early event starting years before the clinical manifestation becomes apparent.

Immunization against amyloid β_{1-42} (Aβ) in mice overexpressing amyloid precursor protein has been shown to reverse the amyloid burden in elderly animals

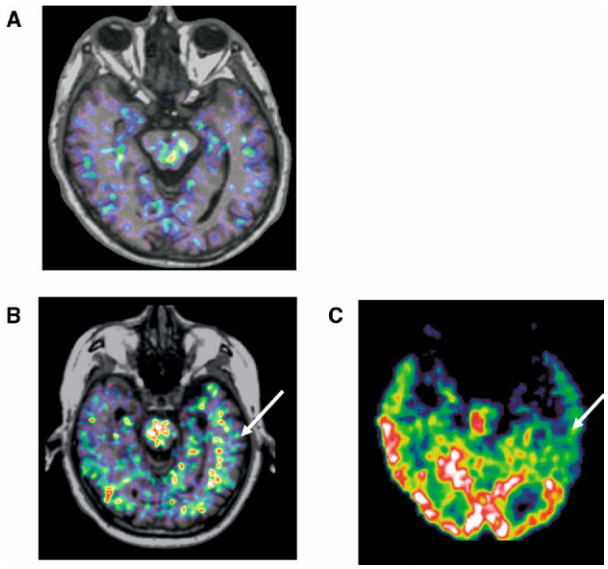

FIG. 3. Transverse sections of [^{11}C](*R*)-PK11195 binding potential images coregistered to the individual MRI scans in (A) a healthy elderly subject and (B) a patient with Alzheimer's disease (AD). Only constitutive binding in the pons was detected in the control subject, whereas increased [^{11}C](*R*)-PK11195 binding potential was found in the lateral and mesial temporal lobe (arrows) of the AD patient, matching the spatial distribution of the cerebral hypometabolism as shown by (C) its own ^{18}FDG PET scan.

and to prevent amyloid deposition in young ones,[57] possibly through the increased clearance by activated microglia and an efflux of amyloid from the brain into the blood stream. The mechanism of amyloid clearance was attributed to an optimized microglial phagocytosis via Fc receptor-mediated opsonization or phagocytosis. Initial human immunization experiments with an antibody against amyloid have led to variable, in some cases fatal, outcomes for the development of encephalitis. Trials with Aβ vaccinations are ongoing, and PET imaging of activated microglia has potential to contribute to the monitoring of the effectiveness or collateral effects of such neurotherapies.

It is important to remember that microglial activation is not driven by a specific neurodegenerative pathological process, such as the deposition of amyloid fibrils, but instead is triggered by ongoing tissue pathology and neuronal injury irrespective of the primary cause. It is thus not surprising that increased [^{11}C](*R*)-PK11195 binding is also found in non-amyloid-related degenerative dementia, such as frontotemporal lobar degeneration, a disease characterized by intracellular tau-positive or ubiquitin-positive inclusions and neuronal degeneration.[58] In frontotemporal lobar degeneration, increased [^{11}C](*R*)-PK11195 binding is prominent in frontotemporal regions and basal ganglia, a regional distribution different from that previously reported for AD patients, and overlaps with the distribution of the ensuing brain atrophy.

IMAGING NEUROINFLAMMATION IN STROKE

Experimental PK11195 binding studies have shown expression by blood-derived and microglia-derived brain macrophages in the infarct core and periphery as early as 3 days, reaching maximum levels after ~7 days, in various stroke models.[18,29] A longitudinal [^{11}C](*R*)-PK11195 PET study in patients after ischemic stroke[59] demonstrated that [^{11}C](*R*)-PK11195 binding was present after the first week in the ischemic border zone, with an early detection beyond 72 hours,[60] and later spread beyond this region into the thalamus and neocortex. The later involvement in regions connected by degenerating white matter tracts to the ischemic core, such as the ipsilateral thalamus via the damaged corticothalamic projections,[61] was due to microglial activation occurring along the neuronal pathways expected to develop Wallerian degeneration and may have a role in long-term plasticity and recovery.[7]

IMAGING NEUROINFLAMMATION IN NEURONAL REMODELING AND NEUROPLASTICITY

Post-herpetic encephalitis

Secondary neuroinflammatory reactions along fiber tracts have been observed after a lesion disconnecting projecting areas, such as the ipsilateral thalamus after a cortical stroke, or the geniculate body after optic neuritis in MS. Such distributed and projected neuroinflammation along neuronal networks can also occur throughout an entire neuronal circuitry, as has been seen subacutely in patients with herpes simplex encephalitis.

Cagnin et al.[39] studied clinically stable patients with unilateral or asymmetric herpes simplex encephalitis between 5 and 8 months after disease onset and conducted serial follow-up [^{11}C](*R*)-PK11195 PET and volumetric MRI over a period of 2 years. Increased [^{11}C](*R*)-PK11195 signals in the cerebral regions most damaged overlapped with those areas that subsequently developed the most marked atrophic changes. Additionally, microglial activation was found widely distributed beyond the recognizable structural damage. In fact, [^{11}C](*R*)-PK11195 binding was increased also in regions contralateral to the affected site, and in areas spared from structural damage but connected to the lesioned areas through white matter tracts.

In one patient, for example, increased [^{11}C](*R*)-PK11195 binding was present in structurally normal-appearing regions all belonging to the right limbic circuitry and along the superior longitudinal fasciculus and right angular gyrus. That latter observation appeared particularly relevant, because this patient presented with a peculiar cognitive deficit characterized by a decreased ability to recognize facial emotions, a task in which right angular gyrus seemed critically involved. These findings illustrate that focal damage can lead to widespread microglial activation along an entire neural network (limbic structures and associated areas) and that the pattern of glial activation thus closely reflects neuronal function or dysfunction.

Persistent microglial activation can be detected many months after successful antiviral therapy for herpes encephalitis, and its regional distribution predicts which areas may subsequently undergo atrophic changes. It is important to understand the significance of the persistent neuroinflammatory response that occurs in regions directly connected with the primary lesion site and are not accompanied by further clinical impairment such as cognitive or behavioral deterioration in post-herpetic encephalitis.

A persistent inflammatory tissue response does not necessarily imply continued viral activity. Viral products tend to disappear after the first 3 weeks, and often viral genome can not be detected consistently.[62,63] This might suggest that late increases of [^{11}C](*R*)-PK11195 signals reflect primarily a delayed neuroinflammatory response to Wallerian-type degeneration of axons and synaptic terminals of the initially damaged neurons. Microglial activation may be involved in long-lasting modifications of the architecture of neuronal network after an acute insult by contributing to neuroplasticity and remodeling of distributed neuronal network. There are suggestions that activated microglia may also support rather neces-

sarily hinder neuronal survival by releasing growth factors and taking part in remodeling of synapses, thus actively contributing to brain plasticity.[9,13]

Traumatic peripheral nerve injury

Late trans-synaptic microstructural changes are found in the thalamus of limb-amputated primates, where they underlie at least in part the cortical plasticity induced by the lesion.[64] Using [^{11}C](*R*)-PK11195 PET, long-term trans-synaptic glial responses have been shown in the human thalamus after peripheral nerve injury.[6] In all seven patients with peripheral nerve or spinal root lesions, increased binding of [^{11}C](*R*)-PK11195 was found in the thalamus contralateral to the side of injury, up to two decades after the lesion. In contrast, one patient with cervical spinal cord injury showed a bilateral and symmetrical increase of [^{11}C](*R*)-PK11195 binding in both thalami. These findings were not associated with thalamic atrophy or alterations of MRI signal.

The presence of trans-synaptic glial responses of a remote neuronal injury may contribute to the removal of synapses from deafferented neurons, a process called *synaptic stripping*, thus mediating cortical plasticity. Such persistence of activated microglia may suggest that even in the chronic stages of the post-traumatic recovery process, structural plasticity continues to occur.

NEW PBR LIGANDS FOR PET STUDIES OF MICROGLIAL ACTIVATION

The isoquinoline carboxamide [^{11}C](*R*)-PK11195 is at present the most widely used pharmacologic probe for studying the function and expression of PBR. Recently, several other compounds have been evaluated: [^{11}C]vinpocetine, [^{11}C]VC195, [^{11}C]DAA1106, [^{18}F]FMDAA1106, and [^{18}F]FEDAA1106.[65]

Maeda et al.,[66] Zhang et al., [67,68] and Fujimara et al.[69] have extensively described [^{11}C]DAA1106 and fluorinated derivatives in *ex vivo* binding experiments on rat brain sections and biodistribution studies in mice. PET imaging has also been performed in normal monkeys or healthy patients, but so far no published data from *in vivo* experiments using DAA1106 and derivatives in disease states have been published. Guylas et al.[70,71] have investigated [^{11}C]vinpocetine using PET, but only in healthy subjects or monkeys, and pharmacologic data indicate that vinpocetine may bind to binding sites other than the PBR. Belloli et al.[72] have evaluated carbon-11 labeled VC193M, VC195, and VC198M in a rat model of striatal damage (quinolinic acid), and their *in vivo* distribution by microdissection of the brain. In these experiments, similar results were obtained when using either PK11195 or VC195. More recently the pyrazolopyrimidines have emerged as exciting new molecular probes for the PBR, with DPA-713 representing one of the lead members;[73] however, further evaluation in humans is still required to determine their clinical utility.

SUMMARY

PBR ligands, such as [^{11}C](*R*)-PK11195 that bind with relative cellular selectivity to activated microglia can be used to

1) detect *in vivo* neuroinflammatory changes occurring in a variety of brain diseases and at different disease stages;
2) monitor the progression of neuroinflammation as a generic *in vivo* marker of disease activity; and
3) detect cellular responses in areas remote from the primary lesion site that may be involved in axonal Wallerian degeneration and neuronal remodeling.

The use of [^{11}C](*R*)-PK11195 PET is a systematic attempt at measuring the emerging phenomenology tissue pathology itself—as opposed to measuring, for example, the loss of neuronal function or structure. Although methodologic issues, such as sensitivity or absolute quantification of the specific ligand, remain, [^{11}C](*R*)-PK11195 PET applications have served as proof of principle for the clinical utility of imaging glial cells *in vivo*, with a corresponding potential for establishing cell biology-based *in vivo* neuropathology.

REFERENCES

1. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. TINS 1996;19:312–318.
2. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005;308:1314–1318.
3. Davalos D, Grutzendler J, Jang G, et al. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 2005;8:752–758.
4. Graeber MB. Glial inflammation in neurodegenerative diseases [abstract]. Immunology 2001;101(Suppl 1):52.
5. Cagnin A, Taylor-Robinson SD, Forton DM, Banati RB. In vivo imaging of cerebral "peripheral benzodiazepine binding sites" in patients with hepatic encephalopathy. Gut 2006;55:547–553.
6. Banati RB, Cagnin A, Brooks DJ, et al. Long-term trans-synaptic glial responses in the human thalamus after peripheral nerve injury. Neuroreport 2001;12:3439–3442.
7. Banati RB. Brain plasticity and microglia: is transsynaptic glial activation in the thalamus after limb denervation linked to cortical plasticity and central sensitisation? J Physiol Paris 2002;96:289–299.
8. Banati RB, Gehrmann J, Schubert P, Kreutzberg GW. Cytotoxicity of microglia. Glia 1993;7:111–118.
9. Banati RB, Graeber MB. Surveillance, intervention and cytotoxicity: is there a protective role of microglia? Dev Neurosci 1994;16:114–127.
10. Raivich G, Banati R. Brain microglia and blood-derived macrophages: molecular profiles and functional roles in multiple sclerosis and animal models of autoimmune demyelinating disease. Brain Res Brain Res Rev 2004;46:261–281.
11. Duke DC, Moran LB, Turkheimer F, Banati RB, Graeber MB. Microglia in culture: what genes do they express? Dev Neurosci 2004;26:30–37.

12. Moran LB, Duke DC, Turkheimer FE, Banati RB, Graeber MB. Towards a transcriptome definition of microglial cells. Neurogenetics 2004;5:95–108.
13. Banati RB. Visualising microglial activation in vivo. Glia 2002; 40:206–217.
14. Hertz L. Binding characteristics of the receptor and coupling to transport proteins. In: Giessen-Crouse E, editor. Peripheral benzodiazepine receptors. London: Academic Press, 1993:27–51.
15. Anholt RR, Pedersen PL, De Souza EB, Snyder SH. The peripheral-type benzodiazepine receptor: localization to the mitochondrial outer membrane. J Biol Chem 1986;261:576–583.
16. Olson JM, McNeel W, Young AB, Mancini WR. Localization of the peripheral-type benzodiazepine binding site to mitochondria of human glioma cells. J Neurooncol 1992;13:35–42.
17. Benavides J, Cornu P, Dennis T, et al. Imaging of human brain lesions with an omega-3 site radioligand. Ann Neurol 1988;24: 708–712.
18. Myers R, Manjil LG, Cullen BM, Price GW, Frackowiak RSJ, Cremer JE. Macrophage and astrocyte populations in relation to [^{3}H]PK 11195 binding in rat brain cortex following a local ischaemic lesion. J Cereb Blood Flow Metab 1991;11:314–332.
19. Conway EL, Gundlach AL, Craven JA. Temporal changes in glial fibrillary acidic protein messenger RNA and [^{3}H]PK11195 binding in relation to imidazoline-I2-receptor and alpha 2-adrenoceptor binding in the hippocampus following transient global forebrain ischaemia in the rat. Neuroscience 1998;82:805–817.
20. Itzhak Y, Baker L, Norenberg MD. Characterization of the peripheral-type benzodiazepine receptors in cultured astrocytes: evidence for multiplicity. Glia 1993;9:211–218.
21. Banati RB, Myers R, Kreutzberg GW. PK ('peripheral benzodiazepine')-binding sites in the CNS indicate early and discrete brain lesions: microautoradiographic detection of [3H]PK11195 binding to activated microglia. J Neurocytol 1997;26:77–82.
22. Veenman L, Levin E, Weisinger G, et al. Peripheral-type benzodiazepine receptor density and in vitro tumorigenicity of glioma cell lines. Biochem Pharmacol 2004;68:689–698.
23. Lee DH, Kang SK, Lee RH, et al. Effects of peripheral benzodiazepine receptor ligands on proliferation and differentiation of human mesenchymal stem cells. J Cell Physiol 2004;198:91–99.
24. Gavish M, Bachman I, Shoukrun R, et al. Enigma of the peripheral benzodiazepine receptor. Pharmacol Rev 1999;51:629–650.
25. Papadopoulos V, Baraldi M, Guilarte TR, et al. Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. Trends Pharmacol Sci 2006;27:402–409.
26. Cleary J, Johnson KM, Opipari AW Jr, Glick GD. Inhibition of mitochondrial F_1F_0-ATPase by ligands of the peripheral benzodiazepine receptor. Bioorg Med Chem Lett 2007;17:1667–1670.
27. Banati RB, Newcombe J, Gunn RN, et al. The peripheral benzodiazepine binding site in the brain in multiple sclerosis: quantitative in vivo-imaging of microglia as a measure of disease activity. Brain 2000;123:2321–2337.
28. Stephenson DT, Schober DA, Smalstig EB, Mincy RC, Gehlert DR, Clemens JA. Peripheral benzodiazepine receptors are colocalized with activated microglia following transient global forebrain ischemia in the rat. J Neurosci 1995;15:5263–5274.
29. Rao VL, 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;16;102–114.
30. Kuhlmann AC, Guilarte TR. Cellular and subcellular localization of peripheral benzodiazepine receptors after trimethyltin neurotoxicity. J Neurochem 2000;4:1694–1704.
31. Chen MK, Baidoo K, Verina T, Guilarte TR. Peripheral benzodiazepine receptor imaging in CNS demyelination: functional implications of anatomical and cellular localization. Brain 2004;127: 1379–1392.
32. Banati RB, Goerres GW, Myers R, Gunn RN, Turkheimer FE, Kreutzberg GW, et al. [^{11}C](*R*)-PK11195 PET-imaging of activated microglia in vivo in Rasmussen's encephalitis. Neurology 1999;53:2199–2203.
33. Lammertsma AA, Hume SP. Simplified reference tissue model for PET receptor studies. Neuroimage 1996;4:153–158.
34. Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. Neuroimage 1997;6:279–287.
35. Ashburner J, Haslam J, Taylor C, Cunningham V, Jones T. A cluster analysis approach for the characterization of dynamic PET data. In: Myers R, Cunningham V, Bailey D, Jones T, editors. Quantification of brain function using PET. San Diego: Academic Press, 1996:301–306.
36. Acton PD, Pilowsky LS, Costa DC, Ell PJ. Multivariate cluster analysis of dynamic iodine-123 iodobenzamide SPET dopamine D2 receptor images in schizophrenia. Eur J Nucl Med 1997;2:111–118.
37. Gunn RN, Lammertsma AA, Cunningham VJ. Parametric imaging of ligand–receptor interactions using a reference tissue model and cluster analysis. In: Carson R, Daule M, Witherspoon P, Herscovitch P, editors. Quantitative functional brain imaging with positron emission tomography. San Diego: Academic Press, 1998: 401–406.
38. Myers R, Gunn RN, Cunningham V, Banati RB, Jones T. Cluster analysis and the reference tissue model in the analysis of clinical [^{11}C](*R*)-PK11195 PET [Abstract]. J Cereb Blood Flow Metab 1999;19(Suppl):S789.
39. Cagnin A, Myers R, Gunn RN, et al. In vivo visualization of activated glia by [^{11}C](*R*)-PK11195 PET following herpes encephalitis reveals projected neuronal damage beyond the primary focal lesion. Brain 2001;124:2014–2027.
40. Turkheimer FE, Edison P, Pavese N, et al. Reference and target region modeling of [^{11}C](*R*)-PK11195 brain studies. J Nucl Med 2007;48:158–167.
41. Rasmussen T, Andermann F. Rasmussen's syndrome: symptomatology of the syndrome of chronic encephalitis and seizures: 35-year experience with 51 cases. In: Lüders H, editor. Epilepsy surgery, 1st ed. New York: Raven Press, 1991:173–182.
42. Oguni H, Andermann F, Rasmussen TB. The syndrome of chronic encephalitis and epilepsy: a study based on the MNI series of 48 cases. Adv Neurol 1992;57:419–433.
43. McGeer PL, McGeer EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Rev 1995;21:195–218.
44. Ouchi Y, Yoshikawa E, Sekine Y, et al. Microglial activation and dopamine terminal loss in early Parkinson's disease. Ann Neurol 2005;57;168–175.
45. Gerhard A, Pavese N, Hotton G, et al. In vivo imaging of microglial activation with [^{11}C](*R*)-PK11195 PET in idiopathic Parkinson's disease. Neurobiol Dis 2006;21;404–412.
46. Gerhard A, Banati RB, Goerres GB, et al. [^{11}C](*R*)-PK11195 PET imaging of microglial activation in multiple system atrophy. Neurology 2003;61:686–689.
47. Gerhard A, Trender-Gerhard I, Turkheimer F, Quinn NP, Bhatia KP, Brooks DJ. In vivo imaging of microglial activation with [^{11}C](*R*)-PK11195 PET in progressive supranuclear palsy. Mov Disord 2006;21:89–93.
48. Gerhard A, Watts J, Trender-Gerhard I, et al. In vivo imaging of microglial activation with [^{11}C](*R*)-PK11195 PET in corticobasal degeneration. Mov Disord 2004;19:1221–1226.
49. Imamura K, Hishikawa N, Sawada M, et al. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. Acta Neuropathol (Berl) 2003;106:518–526.
50. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197–211.
51. Sapp E, Kegel KB, Aronin N, et al. Early and progressive accumulation of reactive microglia in the Huntington disease brain. J Neuropathol Exp Neurol 2001;60:161–172.
52. Pavese N, Gerhard A, Tai YF, et al. Microglial activation correlates with severity in Huntington disease: a clinical and PET study Neurology 2006;66:1638–1643.
53. Tai YF, Pavese N, Gerhard A, et al. Microglial activation in presymptomatic Huntington's disease gene carriers. Brain 2007 Mar 30 [Epub ahead of print].
54. Veerhuis R, Hoozemans JJM, Cagnin A, Eikelemboom P, Banati RB. The activation of microglia as an early sign of disease pro-

gression in Alzheimer's disease. In: Kettenmann H, Ransom BR, editors. Neuroglia, 2nd ed. New York: Oxford University Press, 2004:1027–1043.
55. Akiyama H, Barger S, Barnum S, et al. Inflammation and Alzheimer's disease. Neurobiol Aging 2000;21:383–421.
56. Cagnin A, Brooks DJ, Kennedy AM, et al. In-vivo measurement of activated microglia in dementia. Lancet 2001;358:461–467 Erratum in: Lancet 2001;358:766].
57. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999;400:173–177.
58. Cagnin A, Rossor M, Sampson EL, MacKinnon T, Banati RB. In vivo detection of microglia activation in frontotemporal dementia. Ann Neurol 2004;56:894–897.
59. Gerhard A, Schwarz J, Myers R, Wise R, Banati RB. Evolution of microglial activation in patients after ischemic stroke: a [^{11}C](*R*)-PK11195 PET study. Neuroimage 2005;24:591–595.
60. Price CS, Wang D, Menon DK, et al. Intrinsic activated microglia map to the peri-infarct zone in the subacute phase of ischemic stroke. Stroke 2006;37:1749–1753.
61. Pappata S, Levasseur M, Gunn RN, et al. Thalamic microglial activation in ischemic stroke detected in vivo by PET and [^{11}C]PK11195. Neurology 2000;55:1052–1054.
62. Sobel RA, Collins AB, Colvin RB, Bhan AK. The in situ cellular autoimmune response in acute herpes simplex encephalitis. Am J Pathol 1986;125:332–338.
63. Esiri MM, Drummond CW, Morris CS. Macrophages and microglia in HSV-1 infected mouse brain. J Neuroimmunol 1995;62: 201–205.
64. Jones EG. Cortical and subcortical contributions to activity-dependent plasticity in primate somatosensory cortex. Annu Rev Neurosci 2000;23:1–37.
65. Kassiou M, Meikle SR, Banati RB. Ligands for peripheral benzodiazepine binding sites in glial cells. Brain Res Brain Res Rev 2005;48:207–210.
66. Maeda J, Suhara T, Zhang MR, et al. Novel peripheral benzodiazepine receptor ligand [^{11}C]DAA1106 for PET: an imaging tool for glial cells in the brain. Synapse 2004;52:283–291.
67. Zhang MR, Kida T, Noguchi J, et al. [^{11}C]DAA1106: radiosynthesis and in vivo binding to peripheral benzodiazepine receptors in mouse brain. Nucl Med Biol 2003;30:513–519.
68. Zhang MR, Maeda J, Ogawa M, et al. Development of a new radioligand, *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-[18F]fluoroethyl-5-methoxybenzyl) acetamide, for PET imaging of peripheral benzodiazepine receptor in primate brain. J Med Chem 2004;22:2228–2235.
69. Fujimura Y, Ikoma Y, Yasuno F, et al. Quantitative analyses of 18 F-FEDAA1106 binding to peripheral benzodiazepine receptors in living human brain. J Nucl Med 2006;47:43–50.
70. Gulyas B, Halldin C, Sandell J, et al. PET studies on the brain uptake and regional distribution of [^{11}C]vinpocetine in human subjects. Acta Neurol Scand 2002;106:325–332.
71. Gulyas B, Halldin C, Vas A, et al. [^{11}C]Vinpocetine: a prospective peripheral benzodiazepine receptor ligand for primate PET studies. J Neurol Sci 2005;15:219–223.
72. Belloli S, Moresco RM, Matarrese M, et al. Evaluation of three quinoline-carboxamide derivatives as potential radioligands for the in vivo PET imaging of neurodegeneration. Neurochem Int 2004; 44:433–440.
73. James ML, Fulton RR, Henderson DJ, et al. Synthesis and in vivo evaluation of a novel peripheral benzodiazepine receptor PET radioligand. Bioorg Med Chem 2005;13:6188–6194.