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Translocator protein 18 kDa (TSPO): Molecular sensor of brain injury and repair

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Abstract

For over 15 years, the peripheral benzodiazepine receptor (PBR), recently named translocator protein 18 kDa (TSPO) has been studied as a biomarker of reactive gliosis and inflammation associated with a variety of neuropathological conditions. Early studies documented that in the brain parenchyma, TSPO is exclusively localized in glial cells. Under normal physiological conditions, TSPO levels are low in the brain neuropil but they markedly increase at sites of brain injury and inflammation making it uniquely suited for assessing active gliosis. This research has generated significant efforts from multiple research groups throughout the world to apply TSPO as a marker of "active" brain pathology using *in vivo* imaging modalities such as Positron Emission Tomography (PET) in experimental animals and humans. Further, in the last few years, there has been an increased interest in understanding the molecular and cellular function(s) of TSPO in glial cells. The latest evidence suggests that TSPO may not only serve as a biomarker of active brain disease but also the use of TSPO-specific ligands may have therapeutic implications in brain injury and repair. This review presents an overview of the history and function of TSPO focusing on studies related to its use as a sensor of active brain disease in experimental animals and in human studies.

Keywords: Peripheral benzodiazepine receptor; Translocator protein 18 kDa; TSPO; Brain injury; Neurodegeneration; Biomarker; PET

Abbreviations: ACBP, acyl coenzyme-A binding protein; AD, Alzheimer's disease; AGP, α1-acid glycoprotein; AIF, apoptosis inducing factor; ANC, adenine nucleotide carrier; Apaf-1, apoptosis activating factor 1; BBB, blood brain barrier; Bmax, maximal number of binding sites; CBR, central benzodiazepine receptors; CNS, central nervous system; CRAC, cholesterol recognition/interaction amino acid consensus; DA, dopamine; DAA1106, *N*-(2,5-dimethoxybenzyl)-N-(5-fluoro-2-phenoxyphenyl) acetamide; DHEA, dehydroepiandrosterone; DPA173, *N*,*N*-diethyl-2-[2-(4-methoxyphenyl)-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-acetamide; ENP, eicosaneuropeptide; FGIN-1-27, *N*,*N*-dihexyl-2-(4-fluorophenly) indole-3-acetamide; GABA, gamma-aminobutyric acid; GFAP, glial fibrillary acidic protein; IL, interleukin; Kd, dissociation constant; kDa, kilodaltons; KO, knockout; Liss-Con-PK-11195, lissamine conjugated form of PK11195; Ln-PK-11195, lanthanide chelated PK-11195; MPT, mitochondrial permeability transition; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MS, multiple sclerosis; NBD, 7-nitrobenz-2-oxa-1,3-diazol-4-yl; NO, nitric oxide; ODN, octadecaneuropeptide; P-450scc, side chain cleavage cytochrome P-450 enzyme; PAF, platelet activating factor; PAP7, peripheral benzodiazepine receptor and protein kinase A associated protein 7; PBR, peripheral benzodiazepine receptor; PD, Parkinson's disease; PET, positron emission tomography; PK 11195, 1-(2-chlorophenyl)-*N*-methyl-(1-methypropyl)-3-isoquinoline carboxamide; PKA, protein kinase A; PMN, polymorphonuclear neutrophil; PRAX-1, peripheral benzodiazepine receptor associated protein-1; PREG, pregnenolone; PROG, progesterone; RO5-4864, 7-chloro-5-(4-chlorophenyl)-1-methyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one; ROS, reactive oxygen species; SPECT, single photon emission computer tomography; SRTM, simplified reference tissue model; StAR, steroidogenic acute regulatory protein; TMT, trimethyltin; TNF, tumor necrosis factor; TSPO, translocator protein (18 kDa); TTN, triakontatetraneuropeptide; VDAC, voltage-dependent anion channel.

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Contents

1. Reactive gliosis as a biomarker of brain injury

Reactive gliosis comprises the activation of microglia and astrocytes and is a hallmark response of the CNS to injury (O'Callaghan, 1991; Norton et al., 1992; O'Callaghan, 1993; Raivich et al., 1999; Streit et al., 1999; Streit, 2000; McGraw et al., 2001; Norenberg, 2004; Sriram & O'Callaghan, 2004; Streit, 2004; Ladeby et al., 2005; O'Callaghan & Sriram, 2005; Streit et al., 2005). Reactive gliosis has a graded morphological response that is directly associated with the degree of damage in all forms of brain pathology (Raivich et al., 1999).

Reactive gliosis based on morphological examination is a microscopic finding in brain tissue sections and can only be obtained either from invasive biopsy or postmortem autopsy. Therefore, the development and validation of an *in vivo* biomarker of reactive gliosis is a major advance in the detection of active CNS disease, to monitor disease progression and to assess the effectiveness of therapeutic interventions. To this end, the peripheral benzodiazepine receptor (PBR) or translocator protein (18 kDa)(TSPO), a new nomenclature for PBR (Papadopoulos et al., 2006), is located exclusively in glial cells in the brain parenchyma and has been used as a sensitive biomarker of reactive gliosis and inflammation associated with a variety of brain insults including chemical-induced neurotoxicity (Guilarte et al., 1995; Kuhlmann & Guilarte, 1997, 1999, 2000; Guilarte et al., 2003; Chen et al., 2004; Chen & Guilarte, 2006), ischemic stroke (Stephenson et al., 1995; Pappata et al., 2000; Gerhard et al., 2000, 2005), physical trauma (Miyazawa et al., 1995; Raghavendra Rao et al., 2000), CNS degenerative diseases (Cagnin et al., 2001a; Gerhard et al., 2003; Versijpt et al., 2003; Cagnin et al., 2004; Henkel et al., 2004; Ouchi et al., 2005; Gerhard et al., 2006a, 2006b; Chen et al., in press) and CNS inflammatory disease (Vowinckel et al., 1997; Banati et al., 2000; Debruyne et al., 2003; Mankowski et al., 2003; Hammoud et al., 2005) to name a few (see Tables 1 and 2). Importantly, increased TSPO levels following brain injury are specific to primary or secondary areas of injury expressing activated glial cells, and TSPO can be visualized and quantified using *in vitro* and *in vivo* imaging techniques (Kuhlmann & Guilarte, 1997, 1999, 2000; Banati et al., 2000; Pappata et al., 2000; Cagnin et al., 2001a, 2001b; Mankowski et al., 2003; Versijpt et al., 2003; Chen et al., 2004; Gerhard et al., 2005). Therefore, this approach offers great potential for *in vivo* imaging of a wide variety of neuropathological conditions.

2. The peripheral benzodiazepine receptor/translocator protein 18 kDA—what is it?

Benzodiazepines are one of the most commonly prescribed drugs that have anxiolytic, anticonvulsant, muscle-relaxant, and hypnotic properties. Some of these therapeutic effects are mediated via specific benzodiazepine receptors located in the CNS. The central benzodiazepine receptor (CBR) is coupled to the γ-aminobutyric acid $(GABA)_A$ receptor and modulates GABA-regulated opening of Cl^- channels and inhibition of neuronal activity (Tallman et al., 1978, 1980). In addition to CBR, another type of benzodiazepine receptor was identified using rat kidneys as control tissue during CBR binding studies using [^{3}H]-diazepam (Braestrup & Squires, 1977; Schoemaker et al., 1981). This "peripheral" [^{3}H]-diazepam binding site was shown to be abundantly distributed in peripheral tissues and was defined as peripheral-type benzodiazepine binding site (PBBS) or peripheral benzodiazepine receptor (PBR). Subsequently, studies demonstrated the presence of the PBR in glial and in ependymal cells of the brain (Richards & Mohler, 1984; Gavish

Table 1
Reactive gliosis and TSPO expression in experimental animal models

Animal models	Reactive gliosis and TSPO expression	Reference
6-OHDA induced Parkinsonism	Striatum	Cicchetti et al., 2002
Cuprizone-induced demyelination	Corpus callosum, fiber bundles in striatum, and cerebellar deep nuclei	Chen et al., 2004
Cuprizone-induced demyelination and remyelination	Corpus callosum	Chen and Guilarte, 2006
Demoic acid neurotoxicity	Hippocampus, subiculum, dentate gyrus, amygdala, striatum	Kuhlmann and Guilarte, 1997
Experimental autoimmune encephalomyelitis	Multiple sclerosis plaques	Vowinckel et al., 1997
Experimental autoimmune encephalomyelitis	Multiple sclerosis plaques	Banati et al., 2000
Facial nerve axotomy	Facial nucleus	Banati et al., 1997
Facial nerve axotomy	Facial nucleus	Gehlert et al., 1997
Ischemia	Peripheral of infarct zone	Benavides et al., 1990
Ischemia	Forebrain	Demerle-Pallardy et al., 1991
Ischemia	Cerebral cortex	Myers et al., 1991
Kainic acid injection	Olfactory/limbic	Altar and Baudry, 1990
Methamphetamine	Striatum, thalamus, hippocampus, dorsal raphe/central gray	Guilarte et al., 2003
MPTP induced Parkinsonism	Striatum and substantia nigra	Kuhlmann and Guilarte, 1999
MPTP induced Parkinsonism	Cerebral cortex and subcortical regions including caudate/putamen	Chen et al., in press
Sciatic nerve degeneration and regeneration	Sciatic nerve	Lacor et al., 1999
Simian immunodeficiency virus encephalitis	Frontal cortical white matter	Mankowski et al., 2003
Simian immunodeficiency virus encephalitis	Frontal cortical white/gray matter, basal ganglion, hippocampus	Venneti et al., 2004
Stab wounds	Injury site	Miyazawa et al., 1995
Transient ischemia	Forebrain	Stephenson et al., 1995
Transient ischemia	Forebrain	Rao et al., 2001
Transient ischemia	Infarct core and peripheral zone	Rojas et al., 2007
Traumatic brain injury	Thalamus	Raghavendra Rao et al., 2000
Trimethyltin	Hippocampus, olfactory cortex, amygdaloid nucleus, subiculum, and entorhinal cortex	Guilarte et al., 1995
Trimethyltin	Hippocampus	Kuhlmann and Guilarte, 2000

et al., 1999). It was also determined that this "peripheral" site that binds diazepam was pharmacologically, anatomically, structurally, and physiologically distinct from the CBR (Woods & Williams, 1996; Gavish et al., 1999). Despite the fact that for many years, this nomemclature was used, it was misleading and a group of scientists studying the PBR decided to rename the protein to best represent its function and reached a consensus on a new nomenclature: translocator protein (18 kDa) (TSPO) (Papadopoulos et al., 2006).

2.1. Molecular properties of TSPO

TSPO is an 18 kDa protein consisting of 169 amino-acids (Casellas et al., 2002). It is highly hydrophobic and rich in tryptophan (Casellas et al., 2002). The cDNA encoding TSPO has been cloned from various species such as rodents, bovines and humans (Sprengel et al., 1989; Parola et al., 1991; Riond et al.,

Table 2
In vivo TSPO expression in human neurological disorder

Human neurological disorder	TSPO expression	Reference
AIDS dementia	Thalamus, putamen, frontal, temporal, and occipital cortex	Hammoud et al., 2005
Alzheimer's dementia	Entorhinal, temporoparietal, and cingulate cortex	Cagnin et al., 2001a
Alzheimer's dementia	Frontal and mesotemporal cortex	Versijpt et al., 2003
Amyotrophic lateral sclerosis	Motor cortex, prefrontal cortex, pons, thalamus	Turner et al., 2004
Cerebral vasculitis	Occipital, temporoparietal cortex	Goerres et al., 2001
Corticobasal degeneration	Caudate/putamen, substantia nigra, pons, pre- postcentral gyrus, and frontal cortex	Gerhard et al., 2004
Corticobasal degeneration	Basal ganglia, temporal and parietal cortex	Henkel et al., 2004
Frontal temporal dementia	Frontal temporal cortex	Cagnin et al., 2004
Herpes encephalitis	Primary and secondary projected neuron	Cagnin et al., 2001b
Huntington's disease	Putamen, frontal cortex	Messmer and Reynolds, 1998
Huntington's disease	Caudate/putamen, cortical regions including prefrontal cortex and anterior cingulate	Pavese et al., 2006
Huntington's disease carrier (presymptomatic)	Caudate/putamen, cortical regions	Tai et al., 2007
Idiopathic Parkinson's disease	Midbrain	Ouchi et al., 2005
Idiopathic Parkinson's disease	Pons, basal ganglia, frontal and temporal cortex	Gerhard et al., 2006a, 2006b
Ischemic stroke	Cerebral cortex	Ramsay et al., 1992
Ischemic stroke	Cerebral cortex	Gerhard et al., 2000
Ischemic stroke	Primary lesion and remote pathological changes following Wallerian degeneration	Gerhard et al., 2005
Ischemic stroke	Thalamus	Pappata et al., 2000
Ischemic stroke	Peri-infact zone	Price et al., 2006
Multiple sclerosis	Multiple sclerosis plaques, cerebral central gray	Banati et al., 2000
Multiple sclerosis	Normal-appearing white matter	Debruyne et al., 2003
Multiple sclerosis	Normal-appearing white matter	Versijpt et al., 2005
Multiple system atrophy	Prefrontal cortex, putamen, pallidum, pons, and substantia nigra	Gerhard et al., 2003
Progressive supranuclear palsy	Basal ganglia, midbrain, frontal cortex, and cerebellum	Gerhard et al., 2006a, 2006b
Rasmussen's encephalitis	Affected hemisphere	Banati et al., 1999

1991; Chang et al., 1992; Garnier et al., 1994) and there is an 80% sequence homology amongst these species (Casellas et al., 2002). The location of the TSPO gene is in the q13.3 region of the long arm of human chromosome 22 (Riond et al., 1991).

TSPO can form a multimeric complex with the 32 kDa voltage-dependent anion channel (VDAC) also called mitochondrial porin and the 30 kDa adenine nucleotide carrier (ANC) (McEnery et al., 1992) in the outer mitochondrial membrane (Anholt et al., 1986; Bribes et al., 2004). Topographic studies of TSPO reveal that the 18 kDa TSPO subunit is organized in clusters of 4–6 molecules associated with one VDAC subunit (Papadopoulos et al., 1990, 1994, 1997). Studies using three-dimensional modeling reveal TSPO as a structure with five α-helices spanning one phospholipid layer of the mitochondrial membrane (Bernassau et al., 1993).

There are two other TSPO associated proteins, PBR and protein kinase A associated protein 7 (PAP7) and steroidogenic acute regulatory protein (StAR) localized in steroidogenic tissue and participating in steroid synthesis (Lacapere & Papadopoulos, 2003). StAR can regulate the transport of cholesterol by binding cholesterol in the cytoplasm and transferring it to TSPO on the outer membrane of mitochondria (Lacapere & Papadopoulos, 2003). Phosphorylation of StAR by PKA is facilitated by interaction with PAP7. Furthermore, PAP7 contains an acyl-CoA motif and has sequence identity to diazepine binding inhibitor (DBI), suggesting a common site of interaction between PAP7, DBI and TSPO (Lacapere & Papadopoulos, 2003). A schematic of TSPO and associated proteins is presented in Fig. 1.

2.2. TSPO pharmacology

The TSPO binding site has distinct pharmacological properties from the CBR, although diazepam binds to both classes of receptors with high affinity. Certain benzodiazepines, such as 7-chloro-5-(4-chlorophenyl)-1-methyl-1,3-dihydrobenzo[e][1,4] diazepin-2-one (Ro5-4864), interact weakly with the CBR but have nanomolar affinity for TSPO (Schoemaker et al., 1981; Benavides et al., 1983a, 1983b). In contrast, clonazepam binds with high affinity to CBR but binds with extremely low affinity to TSPO. On the other hand, 1-(2-chlorophenyl)-*N*-methyl-(1-methypropyl)-3-isoquinoline carboxamide (PK11195), an isoquinoline carboxamide derivative, is the first non-benzodiazepine ligand found to bind TSPO with nanomolar affinity (Le Fur et al., 1983a, 1983b, 1983c). While the binding affinity of Ro5-4864 for TSPO varies across species (Bolger et al., 1985), PK11195 displays high affinity in all species (Casellas et al., 2002). This suggests that their binding domains are overlapping but not identical (Farges et al., 1993). Thermodynamic analysis indicates that the [^{3}H]-PK11195 binding is entropy driven, whereas the [^{3}H]-RO5-4864 binding is enthalpy driven (Le Fur et al., 1983c). Therefore PK-11195 might be an antagonist of TSPO, and RO5-4864 might be an agonist or a partial agonist (Le Fur et al., 1983c).

Further studies demonstrated that TSPO binds with high affinity to other classes of organic compounds, such as phenoxyphenyl-acetamides, pyrazolopyrimidine, indolacetamide, and imidiazopyridines, which could prospectively act as potential selective TSPO ligands (James et al., 2006 and Section 4.7).

2.3. Endogenous TSPO ligands

A wide variety of endogenous molecules that bind to TSPO have been identified. One putative endogenous ligand is the diazepam binding inhibitor (DBI) or endozepine (Guidotti et al., 1983). As its name suggests, DBI was originally described based on its ability to inhibit the binding of [^{3}H]-diazepam to CBR (Guidotti et al., 1983). DBIs are widely distributed in the CNS (predominantly in glial cells) and in peripheral organs, especially steroidogenic cells (Bovolin et al., 1990; Alho et al., 1991; Malagon et al., 1993; Alho et al., 1994; Lihrmann et al., 1994). DBIs have similar micromolar (μM) affinities for both TSPO and CBR. DBI is a 10-kDa peptide of 86 amino-acids which can be further cleaved into several biologically active fragments including octadecaneuropeptide (ODN or DBI_{33-50}), eicosaneuropeptide (ENP or DBI_{26-50},) and triakontatetraneuropeptide (TTN or DBI_{17-50},) (Ferrero et al., 1986; Slobodyansky et al., 1989). TTN has similar affinity for TSPO as DBI but is more selective, whereas the ODN is less potent (Ferrero et al., 1986; Slobodyansky et al., 1989).

DBIs can stimulate steroidogenesis by interacting with TSPO (Besman et al., 1989). It has been found that cultured rat astrocytes contain and release DBI-related peptides especially TTN (Lamacz et al., 1996; Patte et al., 1999). TTN can stimulate neurosteroid synthesis in C6 glioma cells by acting on TSPO (Papadopoulos et al., 1991). Later studies confirm the identical sequences between DBI and the acyl coenzyme-A binding protein (ACBP), suggesting the potential role of DBIs in fatty acid metabolism (Knudsen, 1991; Knudsen et al., 1993).

Cholesterol is also considered an endogenous ligand for TSPO with nanomolar affinity (Lacapere et al., 2001). Cholesterol can bind to the cholesterol recognition/interaction amino acid consensus (CRAC) sequence in the carboxyl terminus of TSPO for transport to the mitochondrial inner-membrane for subsequent steroidogenesis (Li et al., 2001).

There are other potential endogenous ligands such as the porphyrins (protoporphyrin IX, mesoporphyrin IX, deuteroporphyrin IX, hemin), which exhibit a very high (nM) affinity for TSPO but not for CBR (Snyder et al., 1987; Verma et al., 1987). Porphyrins are tetrapyrrolic pigments formed in the biosynthesis pathway of heme, mitochondrial cytochrome, hemoglobin, and other heme proteins (Verma & Snyder, 1989). The concept that porphyrins are endogenous ligands fits with the mitochondrial location of TSPO since the initial and final steps in porphyrin biosynthesis occur within the mitochondria (Verma & Snyder, 1989). This also implicates one of the potential physiological functions of TSPO related to heme biosynthesis (Verma & Snyder, 1989).

2.4. Subcellular location and tissue distribution of TSPO

The subcellular localization of TSPO has been demonstrated primarily in the outer mitochondrial membrane by use of the selective ligand [^{3}H]-PK11195 binding studies in rat adrenal glands (Anholt et al., 1986), as well as in rat testis, lung, kidney, heart, liver, and skeletal muscle (Antkiewicz-Michaluk et al., 1988). Later studies using TSPO immunohistochemistry with

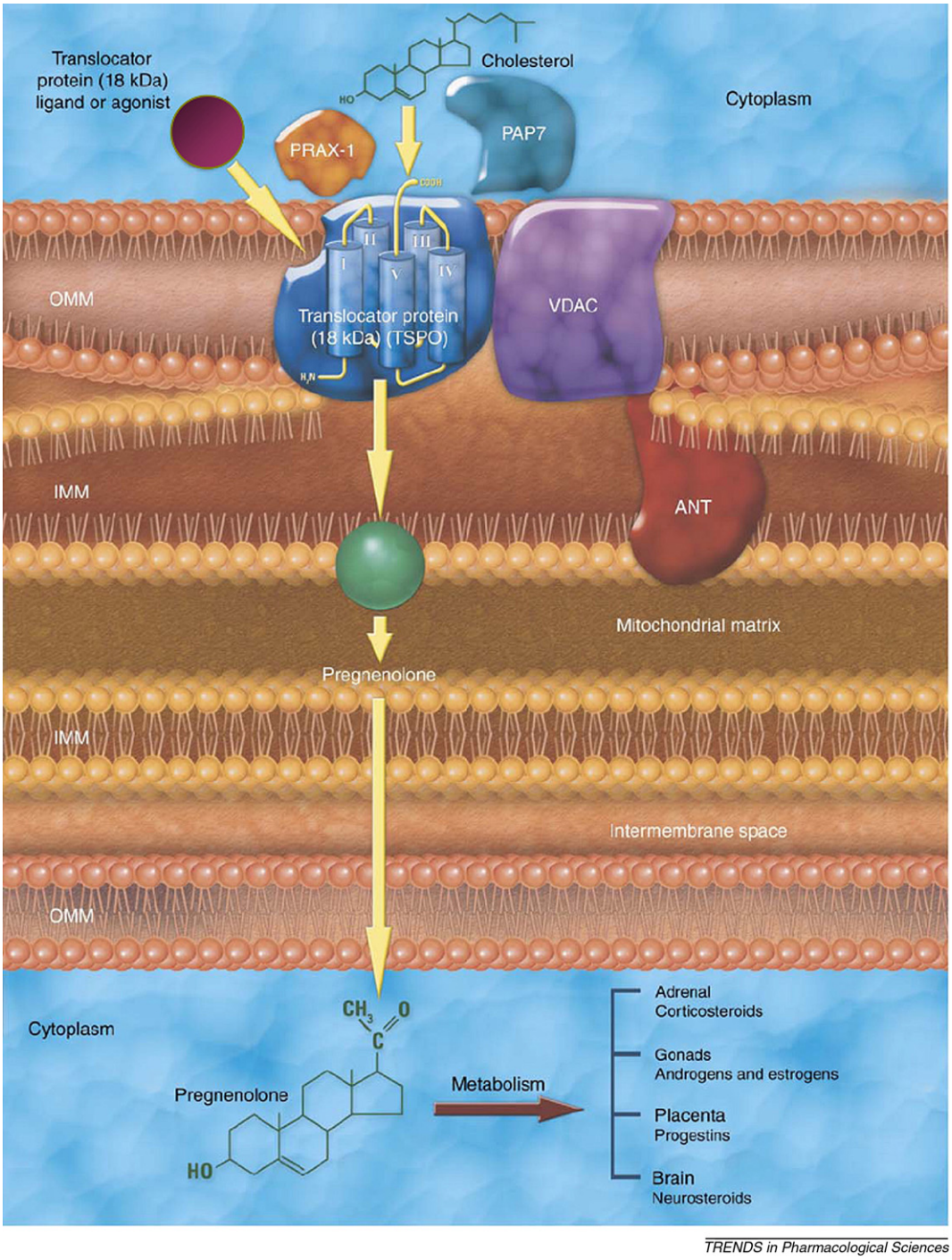

Fig. 1. Schematic of TSPO and associated proteins. Published with permission from Elsevier Limited. Taken from Papadopoulos et al., *TIPS* 27: 402–409, 2006.

confocal microscopy (Garnier et al., 1994) and electron microscopy (Bribes et al., 2004) confirmed the outer mitochondrial membrane localization of TSPO. Although TSPOs are primarily located in the mitochondria, one study demonstrates TSPOs in red blood cells, which are devoid of mitochondria (Olson et al., 1988). This result indicates that TSPOs might also be located in a non-mitochondrial fraction (Olson et al., 1988). Other studies also suggest that small numbers of TSPO may be localized to the plasma membrane of certain peripheral organs, such as liver (O'Beirne et al., 1990; Woods et al., 1996). And there are studies demonstrating that TSPOs are located in the nucleus and perinuclear area in malignant human breast cancer cell lines (Hardwick et al., 1999), glial cells in the CNS (Kuhlmann & Guilarte, 2000), human glioma cell lines (Brown et al., 2000b) and hepatic tumor cells (Corsi et al., 2005).

Radioligand binding assays and autoradiography using either of the selective TSPO tritium labeled ligands Ro5-4864 or PK-11195 have been used to detect the anatomical distribution of TSPO in the body. Glandular and secretory tissues such as the pineal gland, adrenal glands, salivary glands,

olfactory epithelium and gonads are particularly abundant in TSPO with renal and myocardial tissue showing intermediate levels (Gavish et al., 1999). In contrast, the liver and brain express relatively low levels of TSPO (Gavish et al., 1999). The tissue distribution of TSPO is not homogeneous within a given organ. In the adrenal glands, the medulla is devoid of TSPO, whereas density in the adrenal cortex is very high (Anholt et al., 1986). In rat liver, the mitochondrial-form of TSPO is located in hepatocytes and the non-mitochondrial-form (plasma) of TSPO is located in biliary epithelial cells (Woods et al., 1996). TSPO is also expressed in circulating blood cells with the highest concentrations in monocytes and polymorphonuclear neutrophils (PMN) (Canat et al., 1993).

2.5. Physiological functions of TSPO

Many physiological functions have been attributed to TSPO, including cell growth and proliferation (Wang et al., 1984; Carmel et al., 1999; Lee et al., 2004), steroidogenesis (Kelly-Hershkovitz et al., 1998; Papadopoulos et al., 1990, 1991; Papadopoulos, 1998; Lacapere & Papadopoulos, 2003), bile acid synthesis (Woods & Williams, 1996; Woods et al., 1996; Lacapere & Papadopoulos, 2003), calcium flow (Azarashvili et al., 2005; Hong et al., 2006), chemotaxis and cellular immunity (Ruff et al., 1985; Lenfant et al., 1986), heme biosynthesis (Verma & Snyder, 1989), and mitochondrial respiration and apoptosis (Hirsch et al., 1989, 1998; Casellas et al., 2002). The fact that TSPO knockout (KO) mice die at an early embryonic stage (Papadopoulos et al., 1997) strongly suggests that TSPO is involved in basic house-keeping functions and is essential for embryonic development.

Amongst the potential physiological functions of TSPO, steroidogenesis is the best characterized. TSPO is abundantly expressed in steroidogenetic tissues where TSPO mediates cholesterol transport into mitochondria (Papadopoulos et al., 1997). TSPO ligands have been shown to stimulate steroidogenesis in adrenal, placental, testicular, ovarian and glial systems (Papadopoulos et al., 1997). TSPO can bind cholesterol and facilitate the transport of cholesterol from the outer to the inner mitochondrial membrane, the rate-determining step of steroidogenesis (Papadopoulos et al., 1997). The side chain cleavage cytochrome P-450 enzyme (P-450scc or CYP11A1), located in the inner mitochondrial membrane, can convert cholesterol to pregnenolone (PREG), a steroid precursor, and initiate steroidogenesis such as neurosteroid production in glial cells (Papadopoulos & Brown, 1995; Papadopoulos, 1998; Brown et al., 2000a; Brown & Papadopoulos, 2001). More recently, studies have also shown that in astrocytes high concentrations of TSPO specific ligands are able to induce changes in intracellular cholesterol trafficking that are not necessarily related to steroid biosynthesis (Falchi et al., 2007). Therefore, it is highly likely that other cholesterol-related functions for TSPO and/or its ligands are yet to be discovered.

Because of its location on the outer membrane of mitochondria and its association with VDAC, TSPO is suggested to be involved in a multimeric protein complex known as the mitochondrial permeability transition (MPT) pore (Casellas et al., 2002; Kinnally et al., 1993). The MPT pore performs like Ca^{2+}, H^{+} and redox-gated channels with multiple levels of conductance and low selectivity (Kroemer & Reed, 2000). The MPT pore is maintained by the mitochondrial inner membrane potential and the matrix pH, which are regulated by several mitochondrial proteins such as the apoptosis-inhibitory oncoprotein Bcl-2 (Hirsch et al., 1998; Kroemer & Reed, 2000; Casellas et al., 2002). Opening of the MPT pore leads to colloid osmotic swelling of the mitochondrial matrix, defective oxidative phosphorylation, cessation of ATP synthesis and the generation of free radicals (Kroemer & Reed, 2000). The collapse of the mitochondrial inner membrane potential is a critical initiating event for the apoptotic cascades, which include the increase or swelling of the mitochondrial matrix, disruption of the mitochondrial membrane, and the release of cytochrome c and apoptosis inducing factor (AIF) (Kroemer & Reed, 2000; Casellas et al., 2002). AIF can induce nuclear chromatin condensation and DNA fragmentation (Kroemer & Reed, 2000). Cytochrome c can interact with apoptosis activating factor 1(Apaf-1) and pro-caspase 9, cause the activation of caspase 9, which subsequently activates caspase 3 and a series of enzymes causing programmed cell death (Kroemer & Reed, 2000). Myxoma poxvirus M11L is an anti-apoptotic protein localized in the mitochondria and can regulate the MPT pore complex by direct modulation of TSPO, therefore preventing the loss of mitochondrial inner membrane potential in response to induction of apoptosis (Everett et al., 2002). This provides direct evidence that TSPO is involved in the modulation of apoptosis (Everett et al., 2002; Veenman et al., 2007).

3. TSPO: a molecular sensor of active brain disease

Under normal physiological conditions TSPO levels in the CNS are very low and limited to glial cells (astrocytes and microglia). A dramatic increase in TSPO levels occurs in glial cells in response to brain injury or inflammation (Guilarte et al., 1995; Kuhlmann & Guilarte, 1997, 1999, 2000; Guilarte et al., 2003; Chen et al., 2004; Chen & Guilarte, 2006). Because of the availability of high affinity and selective ligands such as PK-11195, which can be labeled with various radioisotopes (^{3}H, ^{11}C, ^{123}I and ^{125}I), the distribution of TSPO can be visualized and measured using *in vitro* receptor autoradiography and binding assays as well as *in vivo* imaging techniques, such as PET or SPECT. The fact that TSPO levels are low in the brain parenchyma and regionally increase in the injured brain makes TSPO an ideal and sensitive marker to detect small changes in the region of injury in comparison to the low levels in normal regions. Time-dependent and region-specific increases of TSPO levels have been measured in areas with neuronal loss or axonal injury following exposure to neurotoxicants (Benavides et al., 1987; Altar & Baudry, 1990; Guilarte et al., 1995; Kuhlmann & Guilarte, 1997, 1999, 2000; Guilarte et al., 2003) (see Table 1). For example, Fig. 2 demonstrates pseudocolor images of [^{3}H]-PK11195 binding to TSPO in rodent brain. Panels A–C represent the temporal TSPO response to the degenerative effects of a single injection of the neurotoxicant TMT, a toxicant whose primary target is the limbic system. In these images, the

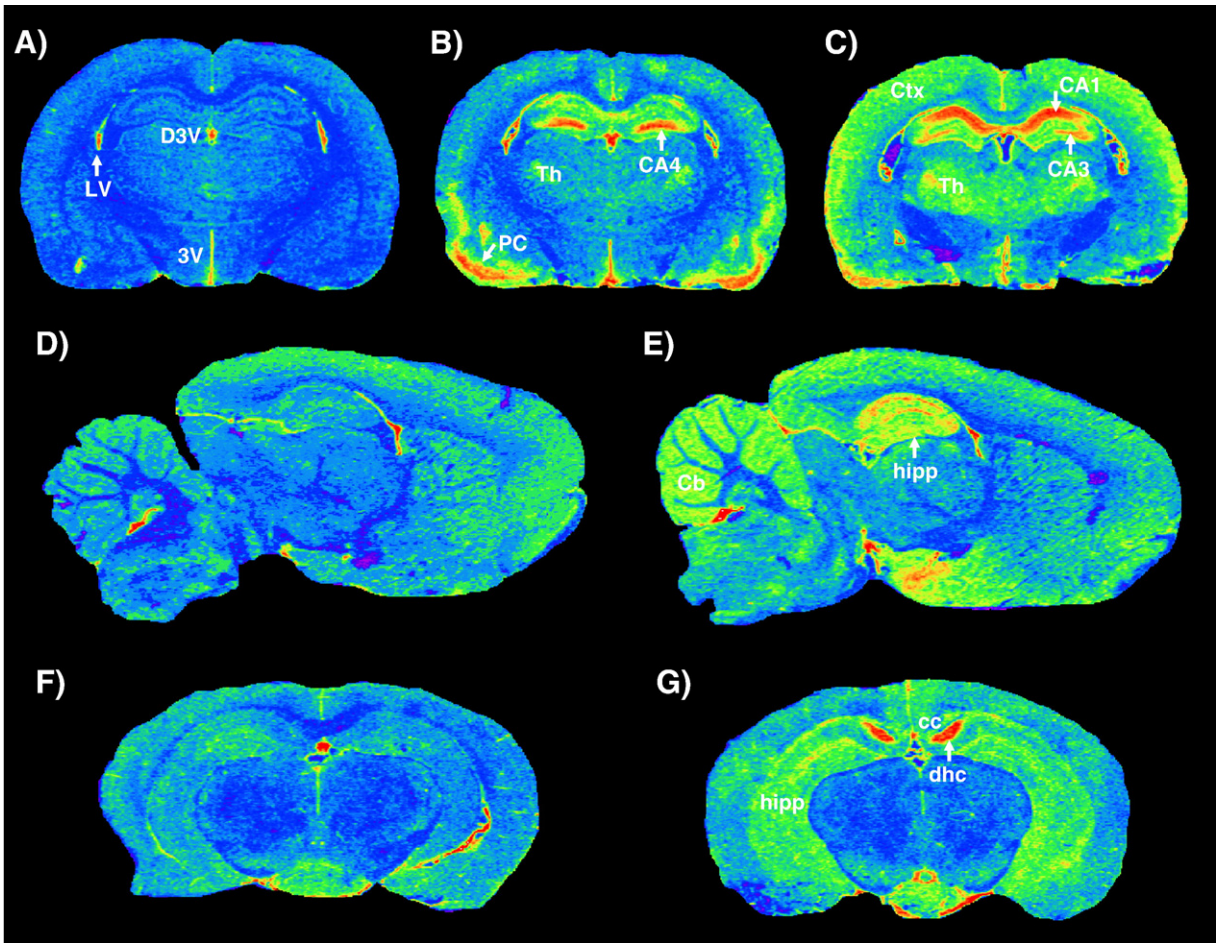

Fig. 2. Pseudocolor images of [^{3}H]-R-PK11195 binding to TSPO in rodent brain. Color in images represents levels of [^{3}H]-R-PK11195 binding with blue representing low levels, green–yellow representative of intermediate levels, and red high levels of binding. Panels **A**, **D**, **F** are pseudocolor images from control animals and **B**, **C**, **E**, **G** are from animals treated with different neurotoxicants. Panels **B** and **C** represent levels of TSPO following a single injection (8 mg/kg) of the neurotoxicant trimethyltin. Panel **B** represents levels of TSPO at 14 days after TMT administration and panel **C** after 6 weeks following TMT administration. There is a dramatic increase in hippocampal regions and piriform cortex at 14 days with a marked increase in different hippocampal regions at 6 weeks. See progressive increase in the thalamus. Panel **E** is representative of an animal that had a seizure after domoic acid administration (3 mg/kg). Compare image in **E** to control in **D**. Panel **G** represents a mouse that had been administered cuprizone in the diet for 4 weeks. Increased levels of TSPO are noted in the dorsal hippocampal commisure and in the hippocampus proper, two brain regions that are known to develop demyelination in this model. D3V = dorsal third ventricle; 3V = third ventricle; LV = lateral ventricle; Th = thalamus; PC = piriform cortex; CA4 = CA4 region of the hippocampus; CA3 = CA3 region of the hippocampus; CA1 = CA1 region of the hippocampus; Ctx = cerebral cortex; Cb = cerebellum; hipp = hippocampus; cc = corpus callosum; dhc = dorsal hippocampal commisure.

blue color areas in panel A (control) represent low levels of binding with the notable exception being the lining of the ventricles and choroid plexus that express high levels of binding or red color. In panel B, at 14 days after TMT, there is extensive upregulation of TSPO (red and yellow areas) in the piriform cortex and in the hippocampus, in particular the CA4 region of the dentate gyrus. However, at 6 weeks (panel C), the pattern of TSPO is different with the CA4 and CA1 regions of the hippocampus being more prominent. Also see the progressive increase in TSPO binding in the thalamus (A–C). These changes in TSPO levels following TMT are consistent and correlate with the known neuropathology of this agent in rodent brain (Guilarte et al., 1995). Panels D and E are representative of a control and an animal following seizure elicited by domoic acid, respectively. Note the prominent increase of TSPO in the hippocampus of the domoic acid treated animal. Finally, panels F and G are representative of a control (F) and an animal in which cuprizone, a demyelinating agent was included in the diet for 4 weeks (G). There is increased TSPO in the dorsal hippocampal commisure and in the corpus callosum, brain regions known to exhibit demyelination in this model. These images are just a few examples of the power of using quantitative autoradiography to assess brain injury in rodent models.

Besides its use to assess the temporal and spatial pattern of glial cell activation in neurotoxicant-induced injury, TSPO has been used as a biomarker of brain injury and inflammation in neurodegenerative diseases (Messmer & Reynolds, 1998; Cagnin et al., 2001a; Gerhard et al., 2003; Cagnin et al., 2004; Gerhard et al., 2004; Ouchi et al., 2005; Gerhard et al., 2006a, 2006b; Pavese et al., 2006), in ischemia or stroke (Demerle-Pallardy et al., 1991; Myers et al., 1991; Ramsay et al., 1992; Stephenson et al., 1995; Pappata et al., 2000; Gerhard et al., 2005; Price et al., 2006), in inflammatory diseases such as multiple sclerosis and experimental autoimmune encephalitis

(Vowinckel et al., 1997; Banati et al., 2000; Debruyne et al., 2002, 2003; Mattner et al., 2005; Versijpt et al., 2005), virus induced encephalitis (Cagnin et al., 2001b; Mankowski et al., 2003; Venneti et al., 2004; Hammoud et al., 2005) and in physical trauma (Miyazawa et al., 1995; Raghavendra Rao et al., 2000; Rao et al., 2001). Therefore, the use of TSPO as a biomarker of brain injury using *ex vivo* brain tissue is well validated (see Table 1).

The nature of the increase in TSPO measured in these models of brain injury has been studied using radioligand binding studies. Saturation isotherms and Scatchard analysis of [^{3}H]-PK11195 binding to TSPO demonstrate that this service and the increase in binding is due to an increase in the maximal number of [^{3}H]-PK11195 binding sites (Bmax) with no change in the affinity of TSPO to this radioligand (Kd) (See Fig. 3)(Guilarte et al., 1995; Kuhlmann & Guilarte, 1997, 1999; Banati et al., 2000; Chen et al., 2004).

Despite many studies showing an increase of TSPO as a result of brain injury the mechanisms and physiological implications of how and why TSPO responds to brain injury are still not known. This is an area of research that requires a greater amount of attention (See section below).

4. TSPO expression in glial cell types following brain injury

4.1. Temporal pattern of glial cells responses during brain injury and repair

The pattern of glial activation to neuropathological events appears to be a programmed response of the CNS to injury. Microglia respond and become activated within a short amount of time following perturbation of their environment by injury or inflammation (Davalos et al., 2005; Nimmerjahn et al., 2005). The microglia response peaks sometimes after the injury, depending upon the nature of the injury, and it decays with a temporal expression dependent upon the degree and chronicity

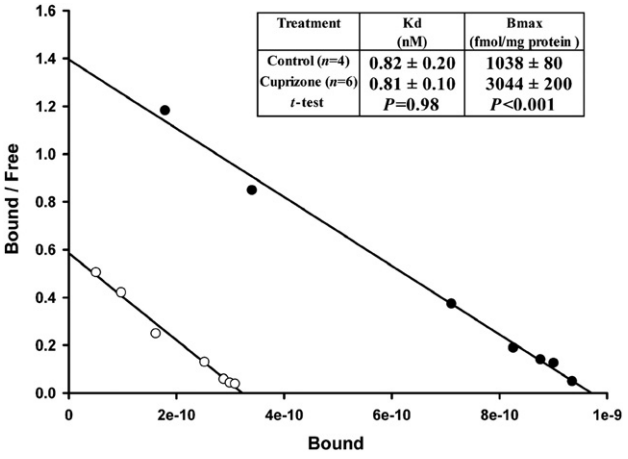

Fig. 3. Scatchard analysis of [^{3}H]-*R*-PK11195 binding to TSPO in the cerebral cortex of animals exposed to cuprizone for 4 weeks. The data clearly show that the effect of cuprizone treatment on [^{3}H]-*R*-PK11195 specific binding is in the maximal number of binding sites (Bmax) and not in the affinity (Kd). Published with permission from Oxford University Press. Taken from Chen et al., Brain 127: 1379–1392, 2004.

of the injury. On the other hand, astrocytes become activated sometime after microglia but they appear to have a more protracted period of activation than microglia (McCann et al., 1996; Liberatore et al., 1999; Kuhlmann & Guilarte, 2000; Chen et al., 2004; Guilarte, 2004). This pattern of microglia and astrocyte activation appears to be true not only following brain injury but also following recovery from injury (Chen & Guilarte, 2006). For example, our laboratory has studied the temporal response of microglia and astrocyte in brain injury models of neurotoxicant exposure (Kuhlmann & Guilarte, 2000; Chen et al., 2004). We observed an early and transient microglial response to injury that eventually decayed to normal levels. Importantly, despite significant reductions in microglia activation there was a late and protracted activation of astrocytes. A similar effect was observed following recovery from a treatment that induces demyelination (Chen & Guilarte, 2006). That is, mice that were administered cuprizone in the diet, a chemical that induces demyelination, can recover if the cuprizone is removed from the diet (Matsushima & Morell, 2001). In this model of demyelination/remyelination we also observed a very rapid decrease in the number of activated microglia with a more protracted decay of the astrocytic response in the corpus callosum during the remyelination or recovery phase (Chen & Guilarte, 2006). In these models of neurotoxicant-induced injury and recovery, TSPO tracks activation of both microglia and astrocytes. Further, these findings were the first to show that TSPO tracks glial cell activation not only as a result of injury but also during recovery from injury.

At present, there is a lack of knowledge on the functional significance of increased TSPO levels in glial cells following neuronal injury or in recovery and whether the enhanced TSPO expression in microglia serves similar functions as in astrocytes. For example, microglia constantly survey their environment and have the ability to respond to brain injury within minutes by directing their ramifications to the site(s) of damage (Nimmerjahn et al., 2005). They proliferate and migrate to the sites of brain injury (Streit et al., 1988; Kreutzberg, 1996; Streit et al., 1999; Streit, 2000), characteristics that are not possessed by astrocytes (Norton et al., 1992; Norenberg, 2004). Previous studies indicate that TSPO ligands can influence both the rate of DNA synthesis and the chemotactic potential of breast cancer cell lines (Hardwick et al., 1999), gliomas (Miccoli et al., 1999) and hepatic tumor cell lines (Corsi et al., 2005). Further, TSPO ligands have been shown to modulate chemotaxis and phagocytosis in peripheral monocytes and neutrophils (Ruff et al., 1985; Cosentino et al., 2000; Marino et al., 2001). Since microglia are the monocytes/macrophages of the brain (Streit et al., 1988; Kreutzberg, 1996; Streit et al., 1999; Streit, 2000), it is possible that injury-induced upregulation of TSPO in microglia may be associated with their proliferative, migratory and phagocytic capacity, characteristics that are essential for the microglial response to injury. Another potential role of increased TSPO expression in microglia may be related to the secretion of inflammatory cytokines. Choi et al. (2002) have shown that the TSPO antagonist PK11195 can inhibit lipopolysaccharide-induced increases in cyclooxygenase-2 and tumor necrosis factor-α levels in cultured human microglia

(Choi et al., 2002). Further, PK11195 can reduce the expression of pro-inflammatory cytokines and neuronal death in the quinolinic acid-injected rat striatum (Ryu et al., 2005).

Astrocytes also increase TSPO level following injury possibly to increase neurosteroid synthesis at the sites of damage. Studies have shown that TSPO activation in astrocytes promotes the synthesis of pregnenolone and progesterone (Le Goascogne et al., 2000), two neurosteroids that possess neurotrophic and neuroprotective activity (Le Goascogne et al., 2000; Schumacher et al., 2000; Veiga et al., 2005). Relevant to a potential difference in the function of the TSPO in microglia and astrocytes, it has been noted that astrocytes but not microglia are capable of synthesizing neurosteroids in culture (Cascio et al., 2000).

4.2. Examination of the TSPO response in microglia and astrocytes in rodent models of brain injury

Since TSPO is present in both microglia and astrocytes, it is important to understand the proportion of the overall TSPO increase after brain injury that is contributed by each of these glial cell types. The cellular localization of TSPO in the injured brain was first studied in rodent models of ischemia. Benavides et al. (1990) reported that TSPO expression in brain cells appeared to have both astrocytic and macrophage-like morphology. However, other studies did not confirm the astrocytic component of the TSPO response in ischemia (Myers et al., 1991; Stephenson et al., 1995). Elevated TSPO levels were consistently correlated with macrophages and microglia rather than astrocytes in the regions of maximal neuronal damage (Myers et al., 1991; Stephenson et al., 1995). Because blood brain barrier (BBB) disruption is a consequence of the ischemic models, it is likely that the entry of peripheral macrophages and other inflammatory elements contribute to the high TSPO levels (Canat et al., 1993).

Later studies using axotomy models that did not result in disturbance of BBB integrity provided evidence of an association between elevated TSPO levels and microglial markers, with no consistent association with astrocytic markers (Banati et al., 1997; Gehlert et al., 1997; Banati et al., 2000). [^{3}H]-PK11195 emulsion microautoradiography combined with immunohistochemistry provided evidence of colocalization of TSPO and activated microglia but not astrocytes (Banati et al., 1997, 2000). However, these studies did not examine the temporal patterns of expression such as the late time course after the injury. This is an important consideration since it has been shown that the temporal response is very distinct for both microglia and astrocytes. Therefore, if studies only examine the cellular sources of the TSPO response shortly after injury, then a microglia localization and hence a greater association of TSPO with microglial markers is to be expected.

The ability to examine the temporal TSPO response and the glial cell types responsible for the increase in TSPO levels following brain injury was the result of developing methods to assess radioligand binding to TSPO using microautoradiography techniques and immunohistochemical methods in the same cell types (Kuhlmann & Guilarte, 2000; Chen et al., 2004, Chen & Guilarte, 2006). Using this approach, we assessed the TSPO response in rat brains after a single injection of the neurotoxicant trimethyltin, a chemical that targets limbic structures of the brain. This study provided unequivocal evidence of cellular TSPO localization in both activated microglia and astrocytes and it was confirmed by double-labeling fluorescence (Kuhlmann & Guilarte, 2000). It is also noted that in the same study, TSPO expression is abundant not only in the cytoplasm but also in nuclear and perinuclear region in both microglia and astrocytes (Kuhlmann & Guilarte, 2000). The nuclear and perinuclear localization may be associated with the capacity of microglia to differentiate, proliferate, and migrate following a brain insult (Streit et al., 1988; Kreutzberg, 1996). More recent studies from our laboratory using a cuprizone model of demyelination and remyelination also showed that both microglia and astrocytes contributed to the elevation of TSPO levels in areas of demyelination (Chen et al., 2004) and during recovery from demyelinaton (Chen & Guilarte, 2006).

The examination of the glial cell types responsible for the increased density of TSPO following brain injury is now being performed more frequently by several laboratories. For example, recent studies using a transient focal cerebral ischemia rodent model show that the TSPO signal arising from the site of injury derived from TSPO in the infarcted core is primarily associated with microglia. Further, the TSPO signal arising from the rim surrounding the core is primarily from astrocytes (Rojas et al., 2007). Maeda and colleagues (2007) have examined this same question in a model of intrastriatal injection of ethanol. They showed that the astrocytic and microglial expression of TSPO changed longitudinally following injury and revealed increased TSPO levels in both glial cell types. In this particular model, the microglia activation was more persistent than astrocytes, a response that is different from other models of brain injury (Maeda et al., 2007). Nevertheless, these studies indicate that when careful examination of glial cell types is performed in a longitudinal design, brain injury produces increased TSPO levels in both microglia and astrocytes. In many of the studies in which the TSPO response to brain injury is solely attributed to microglia, either only one time point following injury was examined, the contribution from astrocytes was not examined, or double labeling of glial specific immunohistochemical markers with radioligand microautoradiography was not performed. For example, in a recent study examining the TSPO temporal response in an axotomy model of the perforant path to produce a hippocampal lesion, it was concluded that increased TSPO levels in the hippocampus were associated with microglia activation as defined by CD11 immunohistochemistry (Pedersen et al., 2006). However, they did not perform immunohistochemistry with an astrocytic marker such as glial fibrillary acidic protein (GFAP). Therefore, the contribution of astrocytes to the TSPO signal was not studied. Nevertheless, close examination of their data indicates that while at 2 and 5 days following hippocampal lesion there was increased TSPO binding and significant microglia activation, at the latest time point examined (10 days) CD11 immunohistochemistry was normal but [^{3}H]-PK11195 binding to TSPO was still significantly elevated from controls. This suggests that it is likely that increased TSPO levels at the latest

time point (10 days) are not contributed by microglia since microglia were of a normal phenotype. Rather, it is likely that at this time point a significant portion of the TSPO signal was associated with astrocytes. However, this was not investigated.

Another study using rat models of intrastriatal injection of lipopolysaccharide (LPS) or 6-hydroxydopamine described a close association of the TSPO response to microglia (Venneti et al., 2007). However, if one examines the correlation of GFAP (an astrocyte marker) and CD68 (a microglia marker) with the binding of the TSPO ligand [^{3}H]-DAA1106 it is clear that the association of [^{3}H]-DAA1106 Bmax and CD68 is mainly driven by two out of the seven data points (see Fig. 2-J in Venneti et al., 2007). Further, an increase in [^{3}H]-DAA1106 Bmax from approximately 250–500 fmol/mg was present only when GFAP levels (but not CD68 levels) were elevated based on immunohistochemistry indicating a clear astrocytic response in the absence of microglia. Therefore, the contribution of microglia and astrocytes to the TSPO signal in active brain disease remains a point of contention in the literature.

4.3. Sensitivity of the TSPO response to brain injury

Our laboratory has been interested in determining the degree of brain injury necessary in order to elicit an increase in TSPO levels following exposure to a variety of neurotoxicants. The result indicate that the TSPO response to injury is directly associated with the degree of damage. That is, there is a robust increase in TSPO levels as results of frank neuronal cell loss (Guilarte et al., 1995; Kuhlmann & Guilarte, 1997, 2000) and demyelination (Chen et al., 2004; Chen & Guilarte, 2006) with smaller increases measured due to loss of neuronal terminals (Kuhlmann & Guilarte, 1999; Guilarte et al., 2003; Chen et al., in press). These studies also showed that TSPO is a much more sensitive indicator in detecting brain damage than histological techniques since significant elevations in TSPO levels are measured prior to pathological changes (Chen et al., 2004; Kuhlmann & Guilarte, 1997). Further, increased levels of TSPO are also measured in secondary areas of brain injury resulting from the primary lesion providing a more extensive assessment of damage associated with neural networks (Kuhlmann & Guilarte, 1999; Banati et al., 2000; Cagnin et al., 2001b; Turner et al., 2004). As it will be described below, an important advantage of the TSPO as a biomarker of brain injury is that it can be visualized and quantified using not only in vitro methods such as quantitative autoradiography but also using *in vivo* imaging techniques such as PET. Therefore, this approach offers great potential for the *in vivo* imaging of a wide variety of human neurological diseases.

4.4. In vivo assessment of TSPO in animal models of brain injury

While TSPO-PET has been used in several human neurodegenerative disorders (see Section 4.5 below), there is a lack of studies showing its utility in small animals such as rodents. Cicchetti and colleagues (2002) investigated the microglial response to degeneration of dopaminergic neurons *in vivo* using an in-house built PET scanner (PCR-I with resolution 4.5 mm) in a rat model of Parkinson's disease (PD) by unilateral intrastriatal administration of 6-hydroxydopamine (6-OHDA). Increased [^{11}C]-PK11195 binding ratio in the striatum (67%) and substantia nigra (45%) was demonstrated after 3 weeks of 6-OHDA treatment using the cerebellum as reference region (Cicchetti et al., 2002). Our laboratory began to use small animal PET imaging to demonstrate *in vivo* changes in TSPO levels in the same animals longitudinally during demyelination and remyelination in a cuprizone-induced demyelination murine model (Chen & Guilarte, 2006). The use of TSPO PET imaging in the living mouse brain provides a significant advance in monitoring brain injury during demyelination and remyelination in the same animal. This approach may be useful in studying the function of the TSPO in animal models of chemical-induced neurotoxicity or in models of human neurodegenerative disorders. The use of small-animal imaging provides a novel approach to examine the effects of neurotoxicants on the brain in a longitudinal fashion since the same animal can be imaged repeatedly over time. Further, the use of small-animal imaging significantly reduces the number of animals needed since the same animal can be used at multiple time points. As the improvement of dedicated small animal PET with better resolution (less than 1 mm), investigators can confidently evaluate the biochemical changes in the rodent models *in vivo* and further utilize these models for the development of new pharmaceuticals for diagnosis and therapy.

4.5. In vivo assessment of TSPO in human neurological diseases

TSPO has been widely investigated as an *in vivo* marker of human neurological diseases using [^{11}C]-(R)-PK11195 PET (see Table 2). These include patients with ischemic stroke (Pappata et al., 2000; Gerhard et al., 2005), multiple sclerosis (Vowinckel et al., 1997; Banati et al., 2000; Versijpt et al., 2005), cerebral vasculitis (Goerres et al., 2001), Rasmussen's and herpes encephalitis (Banati et al., 1999; Cagnin et al., 2001a, 2001b), HIV encephalitis (Hammoud et al., 2005), Alzheimer's dementia (Cagnin et al., 2001a; Versijpt et al., 2003), frontotemporal lobe dementia (Cagnin et al., 2004), amyotrophic lateral sclerosis (Turner et al., 2004), corticobasal degeneration (Gerhard et al., 2004; Henkel et al., 2004), multiple system atrophy (Gerhard et al., 2003), Parkinson's disease (PD) (Ouchi et al., 2005; Gerhard et al., 2006a), and Huntington's disease (HD)(Pavese et al., 2006; Tai et al., 2007). Although most of the human studies demonstrated statistically significant increase of [^{11}C]-(R)-PK11195 binding in either the primary or secondary areas of injury expressing activated glial cells, the degree of increased [^{11}C]-(R)-PK11195 binding varied amongst the different neurological diseases. The degree of the TSPO response measured in PET is closely related to the nature of the insult and the subsequent reactive gliosis. For example, Ouchi and colleagues (2005) have found no significant changes in [^{11}C]-(R)-PK11195 binding to TSPO in the striatum of patients in the early stages of PD, a time in which significant degeneration has already occurred (Ouchi et al., 2005). These authors did find a small but significant increase in TSPO levels in the substantia nigra (SN). On the other hand, Gerhard and colleagues (2006a) demonstrated a small but significant increase

in [^{11}C]-(R)-PK11195 binding to TSPO in striatum and extrastriatal brain regions including the pallidum, thalamus, pons and cerebral cortex but not in the SN of a group of early and advanced PD patients (Gerhard et al., 2006a). It is possible that the glial response elicited by dopaminergic terminal degeneration in the striatum in PD patients may not be sufficiently large to be detected with the sensitivity and resolution of current PET scanners. This notion is consistent with the finding that higher levels of TSPO are measured in the striatum of HD patients (Pavese et al., 2006), a neurodegenerative condition with frank neuronal loss intrinsic to the striatum. The glial TSPO response to the loss of neuronal cell bodies is likely to be more robust and more readily detectible than the response generated from the loss of neuronal terminals as occurs in PD.

4.6. Limitation of in vivo TSPO detection and the development of new classes of radioligands

Most of the work on the validation of TSPO as marker of reactive gliosis has been based on the selective ligand (PK11195) binding to TSPO using either *in vitro* receptor autoradiography or receptor binding assays. For these types of studies, the experimental conditions are controlled and are maximized to obtain the best signal to noise ratio (i.e., total vs non-specific binding). Thus, increases in radioligand binding to TSPO in the injured brain could be easily identified and visualized compared to the very low levels of TSPO in non-disease areas of the brain. The results from *in vitro* receptor binding reflects the available receptors/or binding sites in tissues. However, when one applies the TSPO ligands in living experimental animals or humans using PET imaging, one is dealing with a complex environment composed of different kinetic compartments including distribution (blood circulation, permeability through blood brain barrier or plasma membrane of glial cells), metabolism, and excretion. In addition, most of the injected TSPO ligand will bind to TSPO that is abundantly expressed in peripheral steroidogenic organs and only a small portion of the tracer is able to reach the brain. Further, the TSPO-PET imaging is a combination of specific binding, non-specific binding, free [^{11}C]-(*R*)-PK11195 and its metabolites in brain regions, and [^{11}C]-(*R*)-PK11195 and its metabolites in the blood pool of the brain. Because of the extremely lipophilic nature of PK11195, there is a significant amount of non-specific binding that contributes to the background levels in TSPO-PET imaging. In addition, the non-specific binding of TSPO ligand in the brain can not be completely determined by pharmacological blocking *in vivo* (Petit-Taboue et al., 1991; Venneti et al., 2006).

Previous studies have shown an increase of [^{11}C]-PK11195 uptake in the brain after pre-administration of PK11195 or administration of unlabeled PK11195 afterward (Petit-Taboue et al., 1991). The explanation for this phenomenon is that PK11195 blocked TSPO in the peripheral tissues and caused increased [^{11}C]-PK11195 concentration in the blood and more [^{11}C]-PK11195 entered the brain (Petit-Taboue et al., 1991; Venneti et al., 2006). Compartmental or other non-compartmental mathematical modeling with arterial blood input function can help to extrapolate the meaningful biological binding parameters or representative parametric images from dynamic PET images. However, the [^{11}C]-(*R*)-PK11195 binding is subject to change depending on the model chosen and there is currently no ideal model available to fit the [^{11}C]-(*R*)-PK11195 PET time activity curves and to provide sensitive detection to the limited increase of TSPO levels from reactive gliosis in neurodegenerative diseases. Therefore, further investigation for the improvement of the TSPO-PET is necessary. The development of new ralioligands for TSPO with lower lipophilicity and higher affinity may solve the problem of high non-specific binding and provide better TSPO-PET imaging for quantification (see Section 4.8. below).

4.7. Quantification methodology and new ligands for in vivo TSPO PET imaging

Imaging the TSPO levels with [^{11}C]-(*R*)-PK11195 PET is currently the best characterized and most widely used radioligand for studies in humans. The most important feature of TSPO as a useful marker of brain injury is the low level of TSPO expression in normal brain. This advantage on the other hand provides some challenges for the modeling of this tracer (Turkheimer et al., 2007). Because of the low TSPO level in the normal brain, the signal from [^{11}C]-(*R*)-PK11195 in blood vessels and its non-specific binding in tissue becomes predominant (Turkheimer et al., 2007). In addition, as mentioned previously, the abundance of TSPO in peripheral organs also affects the availability of [^{11}C]-(*R*)-PK11195 for binding in the brain (Turkheimer et al., 2007). Because of the ubiquity of glial cells in the CNS, an ideal reference tissue for mathematical modeling of [^{11}C]-(*R*)-PK11195 PET is lacking (Turkheimer et al., 2007).

Quantification of [^{11}C]-(*R*)-PK11195 PET studies has so far been approached either by normalization of the uptake to a reference region or by application of the simplified reference tissue model (SRTM) (Lammertsma & Hume, 1996) with a "reference" region devoid of TSPO derived from cluster analysis (Banati et al., 2000; Pappata et al., 2000; Turkheimer et al., 2000). In brief, Banati and colleagues transformed dynamic TSPO-PET imaging into several clusters of voxels with indistinguishable kinetic behavior and selected one as the "reference" region, which has the fastest clearance and most similarity to the cluster derived from the average of normal controls (Banati et al., 2000; Turkheimer et al., 2000). This approach has some important limitations (Venneti et al., 2006). Recently, full kinetic characterization of [^{11}C]-(*R*)-PK11195 with measurement of arterial input function has been reported with the application of appropriate 2-tissue compartments, 4-rate-constants model (Kropholler et al., 2005). Further work has shown that blood input modeling provides binding potentials ($BP = k_3/k_4$) that correlate significantly with those estimated with the simplified reference region model (Kropholler et al., 2005). Recent work from Turkheimer et al. (2007) provides an alternative approach for the selection of a reference region devoid of TSPO under supervision to prevent the selection of a reference region outside the brain (Turkheimer et al., 2007). The use of a tissue input function may provide practical advantages with no need for extensive arterial blood sampling in patients

with brain injury. However, most of the TSPO-PET studies conducted in humans have not been validated by postmortem receptor autoradiography (Venneti et al., 2006).

Another methodological issue in regards to the use of the [^{11}C]-(*R*)-PK11195 in previous studies is the highly variable kinetic behavior of [^{11}C]-(*R*)-PK11195 in plasma (Lockhart et al., 2003). Lockhard et al. (2003) measured the binding of racemic [^{3}H]PK11195 to whole human blood and found a low level of binding to blood cells but extensive binding to plasma protein, with strong binding to α1-acid glycoprotein (AGP) and much weaker interaction with albumin. AGP is an acute phase protein, and its levels vary during infection and pathological inflammatory diseases such as multiple sclerosis (Lockhart et al., 2003). This could significantly alter the free plasma concentrations of the ligand and contribute to its variable kinetic behavior. Furthermore, local synthesis of AGP at the site of brain injury may potentially contribute to the [^{11}C]-(*R*)-PK11195 binding observed in neuroinflammatory diseases (Lockhart et al., 2003). Therefore, the measurement of AGP levels in blood samples or brain tissues is important to future [^{11}C]-(*R*)-PK11195 PET studies.

In the development of new TSPO ligands, several studies have demonstrated that TSPO binds with high affinity to other classes of organic compounds, such as phenoxyphenyl-acetamides, pyrazolopyrimidine, indolacetamide, and imidiazopyridines, which could act as TSPO ligands *in vivo* (James et al., 2006). The phenoxyphenyl-acetamide derivative, *N*-(2,5-dimethoxybenzyl)-N-(5-fluoro-2-phenoxyphenyl) acetamide (DAA1106), has subnanomolar affinity (Ki: 0.043 nM) for TSPO compared to PK11195 (Ki: 0.77 nM) when competing with [^{3}H]-DAA1106 (Chaki et al., 1999). [^{11}C]-DAA1106 binding (% injected dose) is four times higher compared to the binding of [^{11}C]-PK11195 in the monkey occipital cortex. Specific binding is estimated as 80% of total binding. Thus, [^{11}C]-DAA1106 might be a good ligand for *in vivo* imaging of TSPO (Maeda et al., 2004). The most recent work with [^{11}C]-DAA1106 in an animal model of inflammation indicates that this radioligand has higher affinity and greater retention in the brain than [^{11}C]-PK11195 (Venneti et al., 2007).

Another acetamide derivative [^{11}C]-CLINME (2-[6-chloro-2-(4-iodophenyl)-imidazo[1,2-*a*] pyridin-3-yl]-*N*-ethyl-*N*-methyl-acetamide) has recently been shown to perform better than [^{11}C]-PK11195 in terms of specific to nonspecific ratio *in vivo* using small animal PET in a rat model with intra-striatal injection of AMPA (α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid)(Boutin et al., 2007b).

The pyrazolopyrimidine, *N*, *N*-diethyl-2-[2-(4-methoxyphenyl)-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidin-3-yl]-acetamide (DPA-713), has also been reported to be a potent ligand for TSPO, displaying higher affinity than PK11195. [^{11}C]-DPA173 was shown to bind TSPO selectively in the baboon brain with higher brain uptake and relatively slow wash out compared to [^{11}C]-(*R*)-PK11195 (James et al., 2005). The most recent work with [^{11}C]-DPA173 also demonstrated that this ligand has better signal-to noise ratio than [^{11}C]-PK11195 *in vivo* (Boutin et al., 2007a). With molecular imaging being increasingly used in medical diagnostics, potential ligands for TSPO will continue to be developed to enhance studies of disease processes. Further improvements in radioligands, methodology for imaging analysis, and instrumentation for imaging acquisition are required in order to provide greater sensitivity and accuracy.

4.8. TSPO ligands as potential therapeutic agents for brain injury and inflammation

Emerging evidence suggests that the administration of TSPO selective ligands may be useful in the treatment of inflammatory conditions (Torres et al., 2000) as well as attenuation of seizures and brain injury (Ferzaz et al., 2002; Veenman et al., 2002; Ryu et al., 2005; Veiga et al., 2005). The exact mechanisms by which TSPO specific ligands confer protection are not precisely known. However, associations between TSPO activation and stimulation of neurosteroid synthesis have been noted (Lacapere & Papadopoulos, 2003). Cascio et al. (2000) have shown a correlation between TSPO expression, steroid synthesis, myelination and oligodendrocyte differentiation. Thus, TSPO activation may assist in the recovery from injury that produces demyelination. Consistent with this hypothesis, Lacor et al. (1999) have shown that TSPO levels and DBI (the putative endogenous ligand of TSPO) are increased after peripheral nerve injury. Following regeneration, TSPO and DBI levels decreased to normal levels; in the absence of regeneration, TSPO and DBI remained elevated. In the same studies, they also showed that TSPO activation by an exogenous ligand Ro5-4864 increased pregnenolone levels in the injured tissue. These findings strongly suggest that TSPO plays an important role in regeneration and neurosteroid synthesis and may be a trophic factor in recovery from brain injury.

4.9. New probes for studying the cellular and sub-cellular localization of TSPO in injured brain tissue

The ability to selectively detect and measure the cellular and sub-cellular distribution of TSPO in cultured cells and *in vivo* prompted the development of TSPO ligands labeled with fluorescence and MR probes. TSPO ligands with fluorescent probes will become popular for the *in vitro* detection of TSPO in living cells. To this aim, Kozikowski et al. (1997) reported the first fluorescent high-affinity TSPO ligand 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)-labeled FGIN-1-27 (*N*,*N*-dihexyl-2-(4-fluorophenly)-indole-3-acetamide) and provided a tool allowing the direct imaging by fluorescence microscopy of the 18 kDa TSPO proteins in living cells (Kozikowski et al., 1997). However, NBD is not the ideal fluorescent dye due to its low sensitivity and limited signal to noise ratio related to its potential autofluorescence and scatter in tissues and cells (Manning et al., 2006).

Another TSPO ligand, a lanthanide chelated PK-11195 (Ln-PK-11195) with the potential to provide both an optical and MR signature depending on the metal ion that is chelated (i.e. Eu^{3+} for optical and Gd^{3+} for MR), was developed by Manning et al. (2004). A fluorescence dye lissamine conjugated form of PK11195 (Liss-Con-PK-11195), which has a relatively high molar extinction coefficient, offers improved sensitivity for microscopy imaging and high-throughput screening detection

in multiwell plates (Manning et al., 2006). Liss-Con-PK11195 specifically binds to TSPO and displays attractive optical properties for live cell fluorescence microscopic imaging and high-throughput plate reader assays (Manning et al., 2006). However, the permeability of Gd-PK11195 through the BBB is questionable and this may limit the use of MRI with Gd-PK11195 in the CNS.

5. Concluding remarks

In vivo imaging of TSPO as a biomarker of reactive gliosis has gained a great deal of attention in the last decade. This is based on the fact that imaging and quantitation of TSPO levels provides an excellent approach for the detection of active brain disease, to study the progression of neurodegeneration, and to monitor the effects of therapeutic strategies. Current efforts to improve the *in vivo* characteristics of TSPO specific radioligands, the development of improved mathematical models for image analysis, and the development of PET instrumentation with greater resolution and sampling acquisition are required in order to make TSPO imaging useful in the clinical and research settings. Lastly, it is critically important to understand the function and how TSPO expression is regulated in the different glial cell types in order to gain a better understanding of this rather unique protein and its expression in health and disease.

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