Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders

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Abstract | The translocator protein (18 kDa) (TSPO) is localized primarily in the outer mitochondrial membrane of steroid-synthesizing cells, including those in the central and peripheral nervous system. One of its main functions is the transport of the substrate cholesterol into mitochondria, a prerequisite for steroid synthesis. TSPO expression may constitute a biomarker of brain inflammation and reactive gliosis that could be monitored by using TSPO ligands as neuroimaging agents. Moreover, initial clinical trials have indicated that TSPO ligands might be valuable in the treatment of neurological and psychiatric disorders. This Review focuses on the biology and pathophysiology of TSPO and the potential of currently available TSPO ligands for the diagnosis and treatment of neurological and psychiatric disorders.

Cholesterol
A 27-carbon steroid present in cells and bodily fluids. It is a basic component of membranes and a precursor of steroid hormones, bile acids and vitamins.

Microglia
A type of glial cell that is the resident macrophage in the brain and spinal cord, and the primary mediator of the immune system of the central nervous system.

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The translocator protein (18 kDa) (TSPO) is a five transmembrane domain protein that is localized primarily in the outer mitochondrial membrane[1,2] and is expressed predominantly in steroid-synthesizing tissues, including the brain[3–7]. TSPO is involved in the translocation of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting step in the synthesis of steroids and neurosteroids[1,2] and is one of the most well-characterized functions of this protein.

TSPO is currently under investigation as a biomarker of brain inflammation and reactive gliosis that are associated with various neuropathologies. For this reason, various TSPO ligands have been developed as neuroimaging agents; for example, for positron emission tomography (PET) studies[8,9]. Furthermore, TSPO ligands have been shown to have substantial *in vivo* efficacy in animal models of neurodegeneration[10], and in anxiety models in animals[11] and humans[12]. These studies highlight their potential use for neuroprotection, limiting neuroinflammation, promoting regeneration and for treating dysfunctions of the nervous system. However, many questions remain regarding the use of TSPO ligands as diagnostic tools to assess activation of microglia, their efficacy in treating neurological or psychiatric disorders, and their possible side-effect profiles compared with existing treatments. Here, we review the biology and pathophysiology of this promising molecular target in the central and peripheral nervous system and discuss the properties of the available TSPO ligands, with the aim of providing a basis for future discussions on the development of TSPO ligands for the diagnosis and treatment of neurological and psychiatric disorders.

TSPO

Structure, organization and distribution. TSPO is a well-conserved ubiquitous protein that is encoded by nuclear DNA and localized primarily in the outer mitochondrial membrane[1,13]. TSPO has previously been known as the peripheral-type benzodiazepine receptor and as the mitochondrial benzodiazepine receptor; the name TSPO was adopted in 2006 in view of new insights into its structure and molecular functions[1]. Hydropathy profile analysis of the 169-amino-acid TSPO sequence suggested a putative five transmembrane helix structure that has since been experimentally confirmed[14]. The presence of specific mitochondrial proteins that interact with TSPO has suggested that TSPO forms a complex composed of proteins residing in both the outer and inner mitochondrial membrane, such as the voltage-dependent anion channel (VDAC) and the adenine nucleotide transporter (ANT)[15–17]. These findings suggest that TSPO is a component of the outer/inner

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membrane mitochondrial contact sites and thereby facilitates the passage of lipophilic molecules across the aqueous intermembrane space (FIG. 1).

Consistent with this hypothesis, TSPO is predominantly localized at these contact sites when formed[18]. The ability of TSPO to form homopolymers (mainly dimers and trimers) seems to increase with mitochondrial activity, such as during cell proliferation and activated steroid synthesis[5,6]. The tissue-specific and cell-specific protein and lipid compositions of the mitochondrial membranes may affect and even determine cell-specific TSPO function. Moreover, cytosolic proteins have been shown to interact with TSPO[19], which suggests that TSPO serves as a mitochondrial anchor that transduces intracellular signals to mitochondria[20].

Recently, a paralogous protein, TSPO2, was identified. TSPO2 is predominantly expressed in haematopoietic tissue and is involved in cholesterol redistribution during erythrocyte maturation[21]. This paralogue arose from an ancient gene duplication event before the divergence of the bird and mammal classes. It cannot bind drug ligands, but retains the ability to bind cholesterol. In contrast to TSPO1, it is localized at the endoplasmic reticulum and nuclear membranes.

Function and physiological role. Functional inactivation of TSPO induces an early embryonic lethal phenotype in mice[22]. Together with the observation that TSPO is well conserved throughout evolution[21], this finding highlights the importance of TSPO for tissue development and function.

Although TSPO is expressed in many organs, the highest levels are found in tissues containing steroid-synthesizing cells, such as adrenal, gonad and brain cells[1,6]. In the central nervous system (CNS), TSPO is usually expressed in microglia[23,24] and in reactive astrocytes[25,26]. However, TSPO expression has also been detected in some neuronal cell types, such as neurons of the mammalian olfactory bulb[27,28], in neuroblastoma SHEP and glioblastoma SNB79 cell lines[29], in primary cell cultures of mammalian cortical astrocytes and neurons, in cerebellar granule cells, in BV-2 microglial cell lines[3,4] and in rat dorsal root ganglia sensory neurons[30].

As a major component of the outer mitochondrial membrane, TSPO mediates various mitochondrial functions, including cholesterol transport and steroid hormone synthesis, mitochondrial respiration, mitochondrial permeability transition (MPT) pore opening, apoptosis, and cell proliferation[6,23,24,31–33]. Notably, the role of TSPO in most of these functions was discovered using TSPO ligands, but has only been directly demonstrated in a few of these functions. For example, knocking out TSPO by homologous recombination[34] or knocking down TSPO using TSPO antisense vectors[35,36], antisense oligonucleotides[37] or silencing RNAs[38–40] demonstrated the crucial role of TSPO in steroidogenesis, apoptosis and cell proliferation. It is likely that some of these functions may also be related to a general cellular mechanism involving a TSPO-mediated generation of reactive oxygen species, which could control both mitochondrial steroidogenesis and apoptosis pathways[5,17,40,41].

In steroidogenic cells, TSPO mediates the translocation of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting step in the synthesis of steroid hormones and neurosteroids[1,2,6]. TSPO ligands were initially found to stimulate steroid formation in steroidogenic cells and in isolated mitochondria, and to induce cholesterol translocation from the outer to the inner mitochondrial membrane[6]. Targeted disruption of the *TSPO* gene in Leydig cells arrests cholesterol transport into mitochondria and steroid formation, whereas transfection of TSPO-disrupted cells with a TSPO cDNA rescues steroidogenesis. Further *in vivo* studies demonstrated a correlation between TSPO levels and steroidogenesis[6]. To determine the specific role of TSPO in steroidogenesis, three-dimensional models of human and mouse TSPO were developed, which indicated that TSPO may function as a channel, accommodating a cholesterol molecule in the space delineated by the five transmembrane helices[18,42] (FIG. 1). Subsequent site-directed mutagenesis and *in vitro* expression studies identified a region of the cytosolic carboxyl terminus of the protein containing a cholesterol recognition amino acid consensus (CRAC) domain[43,44] (FIG. 1). *In vitro* reconstitution experiments, confirmed by nuclear magnetic resonance spectroscopy, showed that TSPO binds cholesterol at the CRAC domain with an affinity in the low nanomolar range[45–47]. These data suggest that the C-terminus of TSPO, exposed to the cytosol, plays an important role in cholesterol uptake from a cytosolic donor and import into the inner mitochondrial membrane.

In a recent study, the three-dimensional structure of bacterial TSPO was determined[48]. It confirmed previous results that monomeric TSPO comprises five transmembrane alpha helices and forms homodimers, and indicated that the arrangement of the transmembrane domains of TSPO facilitates substrate translocation.

Although the regulation of steroid hormone biosynthesis by peripheral endocrine organs in response to pituitary hormones leads to the generation of large amounts of steroids that are needed to supply the entire body, only small amounts of neurosteroids that regulate and modulate local neuronal function are made in the brain. Currently, TSPO is the only known effector in neurosteroid production.

Reactive astrocytes
In response to injury and degenerative conditions, astrocytes become hypertrophic and extend processes, accompanied by increased expression of surface molecules, neurotrophic factors, hormones and cytokines. They can exert both beneficial and detrimental effects on neuronal survival and axon regeneration.

Mitochondrial permeability transition
(MPT). The increase in the permeability of the mitochondrial membrane to solutes with molecular mass ≤1,500 daltons. It is caused by the opening of the high-conductance permeability transition pore, inducing mitochondrial depolarization, uncoupling of oxidative phosphorylation and swelling, leading to ATP depletion and cell death.

Because TSPO can bind cholesterol and import it into mitochondria, TSPO may also play a part in mitochondrial membrane biogenesis. Mitochondrial proliferation occurs during cell proliferation and/or repair. Therefore, the marked and prolonged increase in TSPO expression in neural cells after injury or in nervous system disorders, as discussed further below, suggests that it may be involved in the response mechanisms of these cells to degenerative processes and other damaging stimuli.

TSPO expression in neuropsychiatric disorders

Radiolabelled TSPO ligands are used as neuroimaging agents and are becoming important diagnostic tools (TABLE 1). TSPO seems to be a sensitive biomarker of brain damage and neurodegeneration, particularly of inflammation and reactive gliosis[8] (FIG. 2a; TABLE 1). Specifically, its levels of expression are low in the normal brain but are locally upregulated in damaged regions, thereby allowing sensitive and accurate localization of

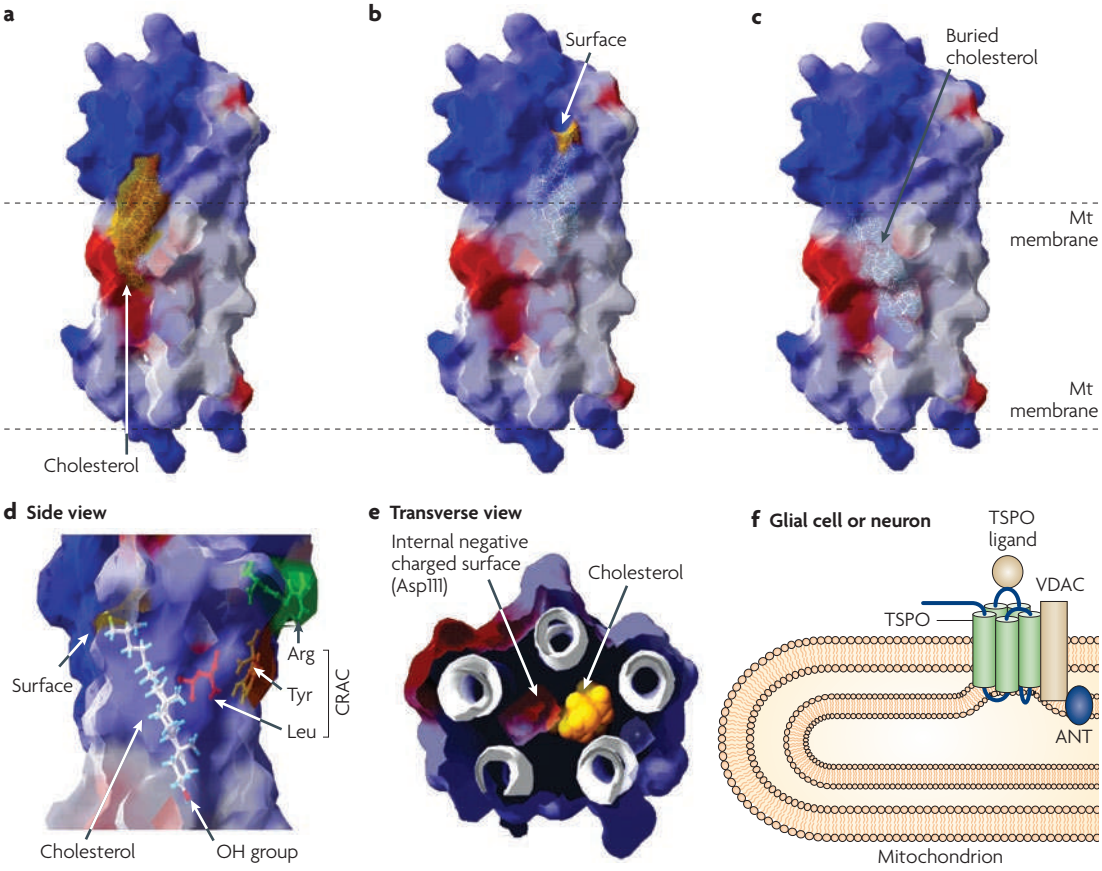

Figure 1 | **Structure of TSPO, docking with cholesterol and mitochondrial localization.** Monomeric translocator protein (18 kDa) (TSPO) forms a channel-like structure from its five transmembrane alpha helices. Cholesterol from various intracellular sources binds to the cholesterol recognition amino acid consensus (CRAC) domain at the carboxyl terminus of TSPO, and moves through the channel owing to hydrophobic forces or to another unknown mechanism. **a** | Electrostatic potential of the mouse TSPO three-dimensional model and molecular docking with cholesterol by shape fitting to optimize the contact surface between the ligand and the protein. The molecular docking was carried out using Hex 4.5 software[189]. **b**,**c** | Two conformational models (selected based on their rankings in minimized energy generated by Hex 4.5 software) indicate that cholesterol enters the TSPO channel from the top (cytosolic) surface (**b**) and is then pulled down by an internal negatively charged patch. It is likely that the polar β-hydroxyl group of cholesterol, which has positive and negative poles, binds to the acidic side-chain of Asp111 (see part **e**). A similar process has been shown for other cholesterol-transporting proteins[190]. This suggests that cholesterol initially binds either at an electrostatic (**a**) or hydrophobic (**b**) region, and then moves to the other side of the mitochondrial (Mt) membrane, where it is involved in more hydrophobic interactions (**c**). The red colour shows negatively charged regions, the blue colour shows positively charged areas and the white colour shows the hydrophobic surface. The surface of the buried and/or exposed cholesterol is mapped as brown. **d** | A high-resolution view of the internalized cholesterol with the CRAC domain consisting of Leu/Tyr/Arg residues on the side view of part **b**. The hydroxyl group of the cholesterol is indicated as a ball and stick model. **e** | Transverse view of the three-dimensional model of TSPO in part **d**, showing that the internal negatively charged patch (Asp111) may assist the trafficking of single cholesterol molecules through the TSPO molecular channel. The white circles represent the five alpha helices of TSPO. **f** | Mitochondrial localization of TSPO in the outer mitochondrial membrane, where it associates with voltage-dependent anion channel (VDAC) and the adenine nucleotide transporter (ANT) of the inner mitochondrial membrane. Homology models of mouse TPSO (TSPO1) were deduced through the SWISS-MODEL services under project (optimize) mode using the crystal structures of apolipophorin-III from *Manduca sexta* (RCSB Protein Data Bank ID code: 1EQ1) as a template[191–193].

Shape fitting
Shape fitting used in protein docking methods is based on the concept that if a ligand molecule has a similar shape or volume to the binding pocket in a protein, it should overlay well, and any volume mismatch would be a measure of dissimilarity. The fit between the ligand and the binding pocket is based on the matching of both three-dimensional shape and chemical functionalities.

lesions and active disease processes. The binding of labelled TSPO ligands can be visualized and quantified by *in vivo* imaging techniques such as PET and single photon emission computed tomography (SPECT). Moreover, following experimental exposure to neurotoxins, substantial elevations in TSPO are evident before overt pathological and structural changes[7]. However, different binding affinity patterns for distinct TSPO PET ligands have been identified in healthy human volunteers, which suggests that apparent reductions in TSPO ligand binding should not be simply interpreted as a reduction in TSPO density[49].

Peripheral nervous system lesions. In response to injury, TSPO expression is strongly upregulated in the peripheral nervous system in Schwann cells, macrophages and neurons[30,50,51] (FIG. 2b; TABLE 1). Following peripheral nerve injury, TSPO expression returns to resting levels only when nerve regeneration is completed, which suggests that TSPO has a key role in nerve repair processes[51]. Gene expression profiling studies in rats have identified a pronounced upregulation of *TSPO* in dorsal root ganglia after axotomy or nerve ligation, which is a model of neuropathic pain[52–54]. However, increased TSPO expression in peripheral neuropathies has not yet been demonstrated experimentally. In contrast to the CNS, in which TSPO is currently used as a biomarker of neurological disorders, TSPO has not been used as a diagnostic tool in the peripheral nervous system.

Brain damage. Initially, TSPO expression in the brain was considered to be specific for activated microglia and infiltrating macrophages, thereby representing an inflammation biomarker[55] (FIG. 2a; TABLE 1). However, it is now well established that reactive astrocytes also express TSPO, although with a different spatiotemporal profile[25,56]. Moreover, TSPO has also been found in certain CNS neurons[3,4]. The upregulation of TSPO in microglia and astrocytes in response to lesions is directly associated with the degree of damage[7,57]. For this reason, TSPO imaging has become a valuable tool for assessing brain lesions with considerable pathophysiological heterogeneity, such as those observed in stroke patients[58,59]. Experimental studies suggest that TSPO ligands also serve as markers for the state and progression of traumatic brain injury[60], in which mitochondria are primary targets. Importantly, the timing of TSPO expression tracks the glial cell activation that occurs not only due to injury but also during regeneration, and thus may qualify as a molecular sensor of active repair processes[61].

Neurodegenerative diseases. The cellular distribution of TSPO has recently been mapped in the human brain under normal and pathological conditions[62]. Increased TSPO ligand binding has also been investigated as a molecular *in vivo* sensor of neuronal damage and inflammation in patients with neurodegenerative diseases that are characterized by neuronal loss in discrete areas of the CNS. These include Alzheimer's disease[63–65], frontotemporal dementia[66], multiple sclerosis[67,68], Huntington's disease[69], amyotrophic lateral sclerosis[70] and Parkinson's disease[71,72] (TABLE 1). In all these diseases, TSPO is strongly upregulated at the sites of degenerative changes, but sometimes also in more remote sites (TABLE 1).

Interestingly, a recent study using different transgenic mouse models of Alzheimer's disease and various experimental models of neurodegeneration showed that predominant expression of TSPO in microglia was associated with substantial neuronal loss, whereas predominant expression of TSPO in astrocytes was associated with reduced neuronal damage[73]. Moreover, studies using animal models of demyelinating diseases such as multiple sclerosis — for example, experimental allergic encephalomyelitis and toxin-induced demyelination of nerve fibres followed by remyelination — revealed that TSPO levels remain elevated during recovery from disease and myelin repair, which suggests a possible role for TSPO in regenerative processes[57,74].

Brain tumours. TSPO is overexpressed in various cancers, including brain tumours. It may therefore be used as a target for imaging brain tumours, serving as a biomarker for clinical diagnosis and follow-up[75,76]. Although TSPO is a well-documented imaging target in neurological disorders, its importance for neuro-oncology remains less explored[77]. Efforts are underway to develop new, potent TSPO ligands for imaging brain tumours[78]. However, in view of the high levels of mitochondrial activity in rapidly proliferating tumour cells, it is unclear whether TSPO expression is related more to the cause or to the prognosis of brain tumours.

Psychiatric disorders. Despite the importance of neurosteroids as modulators of depression and anxiety[79–81], few studies have investigated the expression of TSPO in psychiatric disorders. These studies investigated either the expression of *TSPO* mRNA[82,83] in peripheral mononuclear cells or the binding characteristics of the synthetic isoquinoline carboxamide TSPO ligand 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide (PK-11195) on platelet membranes[84–92] (FIG. 3; TABLE 1).

TSPO expression on peripheral mononuclear cells and platelets was reduced in anxious subjects[82,87], although whether this also applies to TSPO expression in distinct brain areas remains to be investigated. Reduced TSPO expression has also been reported in generalized anxiety disorder[83], social anxiety disorder[86], post-traumatic stress disorder[85], and panic disorder in the presence of adult separation anxiety disorder[88].

Depression has not been associated with reduced TSPO expression[91]. However, co-morbid adult separation anxiety[84,92] or suicidality[90] was associated with reduced TSPO expression in patients with depression or bipolar disorder. In addition, a genetic case–control association study[93] has shown that patients with depression and adult separation anxiety disorder had a high frequency of the Ala147Thr polymorphism allelic variant of TSPO, which may affect the synthesis of the neurosteroid pregnenolone[94].

In schizophrenia, reduced TSPO expression has been associated with anxiety, distress and aggression[89]. Moreover, a genetic polymorphism in exon 4 of *TSPO*

Table 1 | **Changes in TSPO expression patterns in the nervous system**

Experimental studies	Changes in TSPO expression	Species
Central nervous system		
Hippocampus, neurotoxin-induced lesion	↑ in hippocampal microglia and astrocytes	Rat[25]
Striatum, ethanol neurotoxicity	↑ in striatal microglia and astrocytes	Rat[26]
Alzheimer's disease	Astrocyte-dominant TSPO expression in amyloid precursor mutants; microglia-dominant TSPO expression in TAU protein mutants	Mouse[73]
Experimental focal traumatic brain injury	↑ in activated microglia	Rat[60]
Transient focal cerebral ischaemia	↑ in activated microglia and astrocytes surrounding infarction	Rat[154]
Toxin (cuprizone)-induced demyelination	↑ in areas of demyelination (astrocytes and microglia)	Mouse[57]
	↑ in corpus callosum (astrocytes and microglia)	Mouse[153]
Experimental autoimmune encephalomyelitis	↑ at sites of neuroinflammation (astrocytes and microglia)	Mouse[67]
	↑ in spinal cord	Rat[74]
Demyelinating jimpy and shiverer mutants	↑ in reactive astrocytes	Mouse[155]
Peripheral nervous system		
Sciatic nerve transection	↑ in DRG sensory neurons	Rat[30]
	↑ in DRG	Rat[52]
	↑ in DRG	Rat[54]
Sciatic nerve transection or freeze injury	↑ in the sciatic nerve distal to injury site	Rat[51]
Spinal nerve ligation	↑ in DRG	Rat[53]
Neurological disorders		
Ischaemic stroke	↑ in primary lesion and remote areas	Human[58]
Alzheimer's disease	↑ in activated microglia of cortical regions with amyloid load	Human[63]
	↑ in multiple brain regions at relatively early disease stages	Human[64]
Alzheimer's disease, ischaemic stroke, multiple sclerosis	↑ in microglia, macrophages and hypertrophic astrocytes	Human[62]
Frontotemporal dementia	↑ in frontotemporal brain regions	Human[66]
Huntington's disease	↑ in striatum correlating with disease severity	Human[69]
Amyotrophic lateral sclerosis	↑ in multiple brain regions	Human[70]
Parkinson's disease	↑ in nigrostriatal pathway correlating with dopaminergic terminal loss	Human[71]
	↑ in the primary lesion and remote brain regions	Human[72]
Multiple sclerosis	↑ at sites of active lesions	Human[67]
	↑ in white matter lesions correlating with brain atrophy and disability	Human[68]
Psychiatric disorders		
Anxious patients	↓ in lymphocytes	Human[82]
Generalized anxiety disorder	↓ in lymphocytes	Human[83]
Social anxiety disorder (social phobia)	↓ in platelets	Human[86]
Post-traumatic stress disorder	↓ in platelets	Human[85]
Panic and adult separation anxiety disorder	↓ in platelets	Human[88]
Panic disorder	Contribution of *TSPO* gene polymorphism	Human[95]
Major depression with adult separation anxiety disorder	↓ in platelets	Human[84]
Bipolar disorder with adult separation anxiety disorder	↓ in platelets	Human[92]
Suicidal adolescent population	↓ in platelets	Human[90]
Untreated depressed patients	No change in platelets	Human[91]
Schizophrenia*	↓ in platelets	Human[89]
Normal persons under stressful conditions	↑ in platelets correlated with anxiety scores	Human[87]

DRG, dorsal root ganglion; TSPO, translocator protein (18 kDa). *Decreased platelet TSPO was associated with aggression, hostility and anxiety[89]. Moreover, positron emission tomography labelling TSPO has shown a positive correlation with positive symptoms[96].

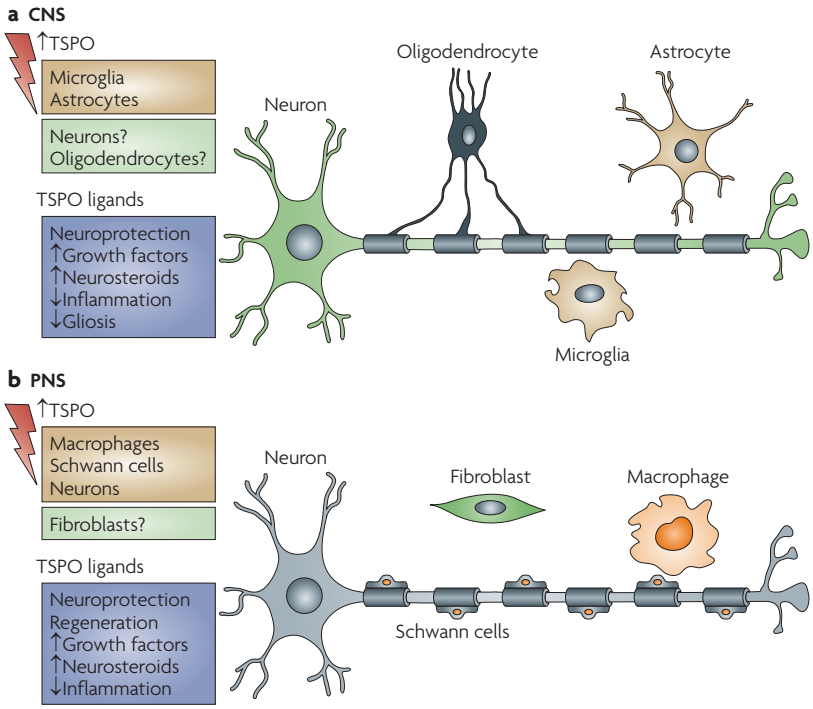

Figure 2 | **TSPO expression in the central and peripheral nervous system, and effects of TSPO ligands. a** | In the central nervous system (CNS), levels of translocator protein (18 kDa) (TSPO) are low under normal conditions. However, in response to injury or degenerative diseases (red lightning symbols), TSPO expression is markedly increased in reactive microglia and in astrocytes. TSPO expression may serve as a sensitive marker of inflammation in the brain, and specific radiolabelled TSPO positron emission tomography ligands have been developed for *in vivo* imaging. TSPO is also present in certain types of cell-cultured neurons, but whether specific populations of brain neurons express relevant levels of TSPO *in vivo* remains to be clarified. TSPO expression in oligodendrocytes (the myelinating glial cells of the CNS) has not been observed. In the CNS, TSPO ligands are neuroprotective, and they modulate inflammatory responses and gliosis. Whether they also promote myelin repair needs to be investigated. As microglia and astrocytes are the main targets of TSPO ligands, their beneficial effects on other neural cells are probably indirect, and may be mediated by cytokines, hormones and neurotrophic factors. **b** | In the peripheral nervous system (PNS), TSPO expression is strongly upregulated in response to traumatic injury in macrophages, either resident or derived from the circulation, and in Schwann cells (the myelinating glial cells of the PNS). After peripheral nerve lesion, TSPO expression is also induced in sensory neurons of dorsal root ganglia and in spinal motor neurons. It is not known how peripheral neuropathies affect TSPO expression. In the PNS, TSPO ligands promote axonal regeneration and modulate neuroinflammation in experimental models of injury and neuropathies.

seems to increase the susceptibility to panic disorder[95]. Recently, a PET study revealed a positive correlation between the binding of the TSPO ligand [^{11}C]DAA1106 and the positive symptoms and duration of illness in patients with schizophrenia, suggesting that glial reaction might be involved in the pathophysiology of positive symptoms[96].

TSPO ligands

Endogenous ligands of TSPO. Cholesterol and porphyrins are important endogenous ligands of TSPO, and show nanomolar and micromolar affinities for TSPO, respectively[43,97] (FIG. 3; see Supplementary information S1 (table)). Further endogenous ligands of TSPO include the endozepines, which are a family of neuropeptides originally isolated from rat brain extracts on the basis of their ability to displace benzodiazepines from their binding site at the $GABA_A$ (γ-aminobutyric acid type A) receptor[98].

Whereas cholesterol binds to the cytosolic C-terminus of TSPO containing a conserved CRAC domain[43,44], all other drug ligands bind to a region within the amino terminus[43,99,100]. Nevertheless, multiple binding sites for benzodiazepines have also been reported[101]. Moreover, partner proteins such as VDAC are required for maximal benzodiazepine binding[15,16]. Given that endozepines were identified by their ability to displace benzodiazepines from TSPO, it is likely that endozepines and porphyrins also bind at the N-terminal binding sites.

Endozepines are derived by endogenous proteolytic processing from a common polypeptide precursor, the diazepam-binding inhibitor (DBI), which is encoded by a single gene that is widely expressed in the nervous system[102]. The major biologically active peptide fragments are the octadecaneuropeptide DBI33–50 (ODN) and the triakontatetraneuropeptide DBI17–50 (TTN)[103], which stimulate mitochondrial steroid synthesis[104]. DBI has been shown to bind long-chain (C_{12}–C_{22}) acyl coenzyme A (acyl-CoA) esters with high affinity, and so is also known as acyl-CoA-binding protein[105]. Recently, DBI was classified as a member of the acyl-CoA-binding domain-containing proteins (ACBD) and renamed ACBD1 (REF. 106). Interestingly, in independent studies, a protein named PAP7 was found to bind TSPO[107] and to be part of a signal transduction protein complex at the outer mitochondrial membrane that mediates cholesterol import into mitochondria in steroidogenic cells[19]. PAP7 contains an acyl-CoA-binding domain, belongs to the same family of proteins as DBI, and is now called ACBD3 (REF. 106).

These findings suggest that acyl-CoA-binding proteins or acyl-CoA regulate mitochondrial TSPO function. Moreover, the fatty acid regulation of mitochondrial cholesterol transport may be mediated by TSPO, as arachidonic acid modulates TSPO ligand binding[108] and long-chain fatty acids contribute to steroid formation[109].

Endozepines are synthesized by Schwann cells in the peripheral nervous system. In response to injury, the local production of endozepines is upregulated concurrently with increased TSPO expression[51]. In the CNS, the *DBI* gene is primarily expressed in glial cells. Interestingly, the synthesis of endozepines by astrocytes is stimulated by amyloid-β peptide[110], a peptide that is thought to have a key pathogenic role in Alzheimer's disease. Moreover, elevated levels of endozepines have been measured in the cerebrospinal fluid of patients with Alzheimer's disease[111]. Taken together, these observations suggest that TSPO is involved in autocrine and paracrine signalling responses of glial cells to disease and injury, which underlines the great therapeutic potential for synthetic TSPO ligands.

Synthetic ligands of TSPO. Classical synthetic ligands of TSPO include PK-11195 and the benzodiazepine 7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one (Ro5-4864). PK-11195 binds exclusively

Porphyrins
Heterocyclic compounds formed of four pyrrole rings linked by unsaturated carbons to form a large ring. They can chelate metals such as iron and magnesium, and are crucial constituents of haemoglobin, chlorophyll and cytochromes.

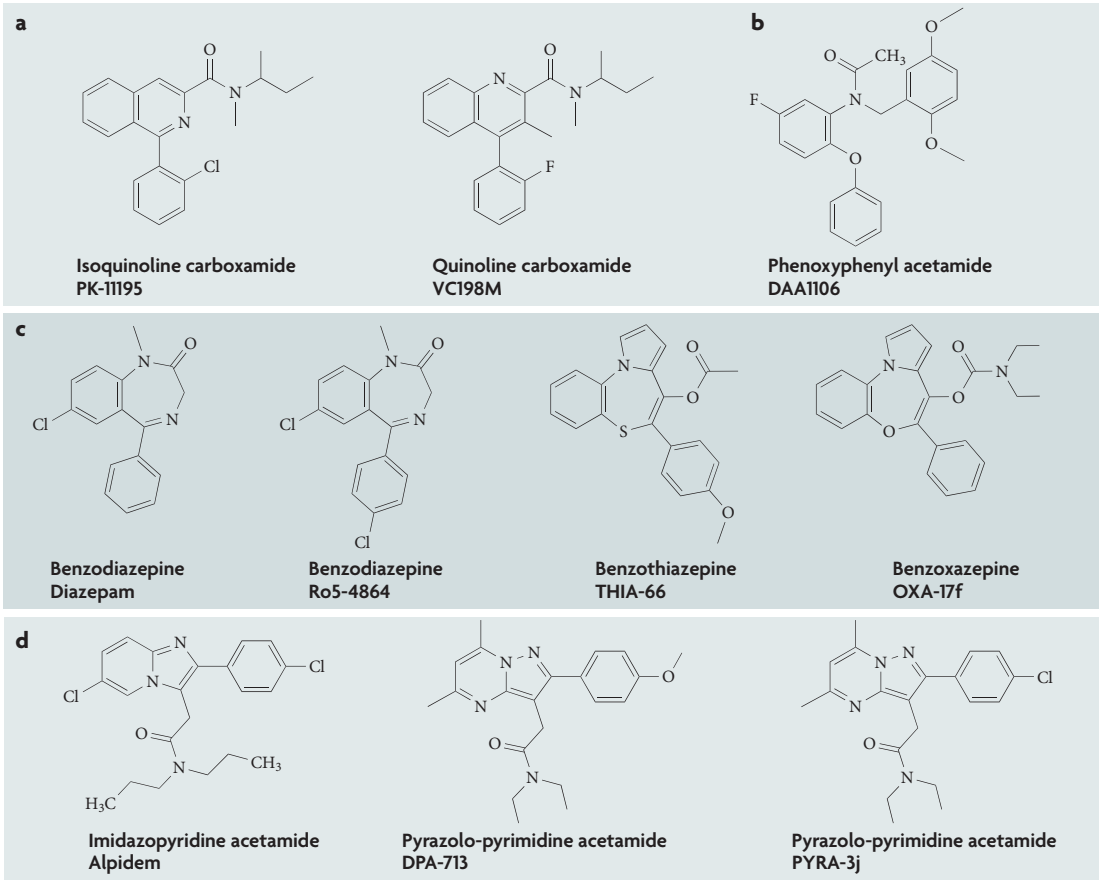

Figure 3 | **Classes, names and structures of representative TSPO ligands.** Representative translocator protein (18 kDa) (TSPO) ligands from key classes are presented with class, name and structure. IUPAC names and further derivatives for each class are shown in Supplementary information S1 (table). The isoquinoline carboxamide PK-11195 (**a**) and the benzodiazepine Ro5-4864 (**c**) selectively bind to TSPO with nanomolar affinity and have been extensively used for its identification and investigation of its characteristics and function[55]. PK-11195 is considered a prototypical ligand, and has been used as the basis to generate new TSPO ligands. Labelled quinoline carboxamides (**a**) are also used as radioligands for TSPO imaging[194]. The phenoxyphenyl acetamide derivative DAA1106 (**b**) has become an attractive ligand for TSPO imaging because of its subnanomolar binding affinity and selectivity[96,127]. Some classical benzodiazepines, such as diazepam (**c**), target central benzodiazepine receptor (CBR) sites associated with $GABA_A$ (γ-aminobutyric acid type A) receptors and TSPO, whereas benzothiazepines and benzoxazepines (**c**) are selective TSPO ligands. Other classes of organic compounds, such as imidazopyridine and pyrazolo-pyrimidine acetamides (**d**) are used as TSPO ligands for *in vivo* imaging[127].

to TSPO, whereas the benzodiazepines (Ro5-4864 and AHN-086) require other mitochondrial protein components for full binding capacity. Isoquinolines became important diagnostic ligands for characterizing the expression and function of TSPO in various tissues and cells, and their discovery was crucial for the further isolation and characterization of the TSPO protein.

Over the past two decades, various additional TSPO ligands have been developed, which can be subdivided into distinct chemical classes (FIGS 3,4; see Supplementary information S1 (table)). These include the imidazopyridines such as alpidem — which also binds to central $GABA_A$/benzodiazepine receptors — and related molecules CLINDE, CLINME, CB-34, DPA, indole derivatives FGIN-1-27 and SSR180575, pyrrolobenzoxazepines, phenoxyphenyl acetamide derivatives DAA1106 and PBR28, and many others (FIGS. 3,4; see Supplementary information S1 (table)).

Most of these ligands were developed primarily as neuroimaging agents and as diagnostic tools for brain inflammation associated with various neuropathological conditions[8] (TABLE 1). However, as discussed in the following section, some TSPO ligands may also have therapeutic potential in neuroprotection, neuroregeneration and anxiety (TABLE 2). For example, the selective phenylpurine TSPO ligand XBD173 (AC-5216/emapunil) exerts rapid anxiolytic effects not only in animal models but also in human volunteers[12]. This molecule may thus provide a lead structure for the development of new drugs for the treatment of anxiety disorders as well as other TSPO-related indications.

Less selective molecules may also offer therapeutic possibilities (TABLE 2). For example, the benzoxazine etifoxine, which stimulates neurosteroid formation[112], shows considerable affinity for TSPO, but also binds to $GABA_A$ receptors[113,114]. Its anxiolytic effects may

Acyl coenzyme A
(Acyl-CoA). A temporary product formed when coenzyme A attaches to the end of a long-chain fatty acid, which is a step in fatty acid oxidation.

Autocrine and paracrine signalling
During autocrine signalling, a cell secretes a protein and/or a chemical messenger that binds to receptors on the same cell. This differs from paracrine signalling, which targets adjacent cells.

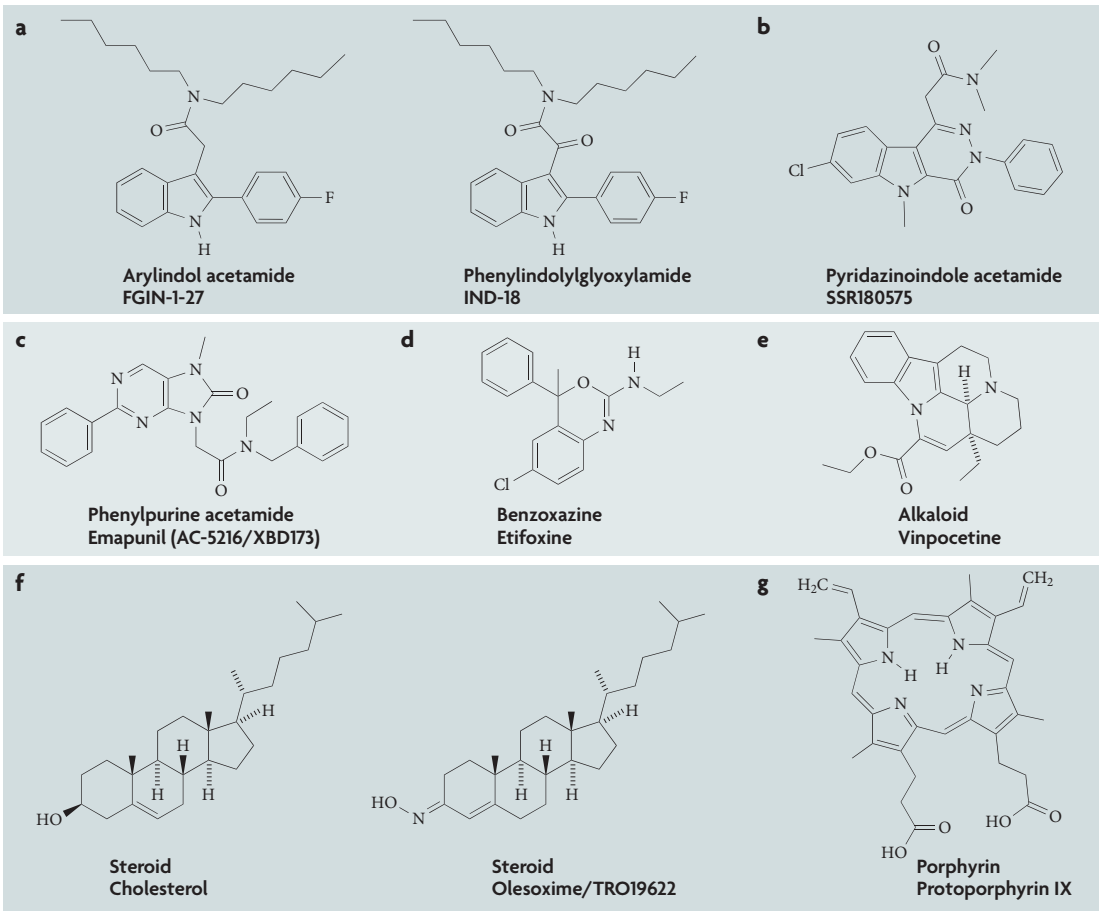

Figure 4 | **Classes, names and structures of representative TSPO ligands.** Representative translocator protein (18 kDa) (TSPO) ligands from key classes are presented with class, name and structure. IUPAC names and further derivatives for each class are shown in Supplementary information S1 (table). The indol acetamides (**a**) include highly specific TSPO ligands such as IND-18 (REF. 195) and FGIN-1-27 (REF. 196). The pyridazinoindole acetamide SSR180575 (**b**) shows high affinity and selectivity for TSPO with neuroprotective and neuroregenerative properties[120]. The selective phenylpurine TSPO ligand emapunil (AC-5216/XBD173) (**c**) exerts rapid anxiolytic effects in animals and humans[12], and may provide a lead structure for the development of TSPO ligands as drugs. The benzoxazine etifoxine (**d**) binds to TSPO and $GABA_A$ (γ-aminobutyric acid type A) receptors. Whereas its anxiolytic effects involve $GABA_A$ receptors and TSPO, its neuroregenerative effects mainly involve TSPO[10,112]. The vinca alkaloid vinpocetine (**e**) has neuroprotective properties[197], and binds to TSPO[198] in addition to other receptors, including adrenergic receptors. Cholesterol (**f**) and porphyrins (**g**) are endogenous TSPO ligands. They bind with nanomolar affinities to TSPO, which transports them across the outer mitochondrial membrane from the cytoplasm[44,97,199,200]. Olesoxime (TRO19622) (**f**), a cholesterol derivative with neuroprotective properties, can displace the binding of cholesterol to TSPO and also interact with voltage-dependent anion channel, another protein of the outer mitochondrial membrane that is associated with TSPO[124].

therefore involve direct targeting of both TSPO and $GABA_A$ receptors[115]. Importantly, alpidem, another TSPO ligand that binds to $GABA_A$ receptors[116], was approved for anxiety in France in 1991 but was withdrawn in 1994 after observations of liver dysfunction with severe or even lethal consequences[117–119]. However, these side effects might also be related to its binding to $GABA_A$ receptors[116], which may occasionally be also expressed in liver tissue — for example, in hepatocellular carcinomas.

TSPO ligands as neurotherapeutics

Considerable beneficial effects of TSPO ligands on neuronal viability, regenerative processes and neuroinflammatory responses have been documented in various experimental lesion and disease models (TABLE 2). Recent clinical trials further underline the therapeutic potential of TSPO ligands for numerous indications.

Peripheral nervous system lesions. Neuroprotective effects of TSPO ligands have been observed in experimental models of peripheral neuropathies[120–122]. Recent experimental data suggest that TSPO is a promising target for the management of neuropathic pain, as has been shown in preclinical models of diabetic and chemotherapy-induced neuropathies[121,123]. In addition, after injury of the facial nerve in young rats, treatment with the selective TSPO ligand SSR180575 promoted functional recovery[120]. Administration of olesoxime also rescued motor neurons from axotomy-induced cell death in neonatal rats and

promoted sciatic nerve regeneration following crush injury[124]. Olesoxime interacts with TSPO at the cholesterol-binding site rather than at the PK-11195-binding site, as well as with VDAC, but its precise mechanism of therapeutic action remains unknown.

A Phase II study of SSR180575 investigating the rate of regeneration of epidermal nerve fibres in patients with diabetic peripheral neuropathy has been conducted by Sanofi–Aventis (ClinicalTrials.gov identifier: NCT00502515). There are also ongoing clinical trials with olesoxime in the treatment of chemotherapy-induced peripheral neuropathy (Phase II) and as an adjunct to riluzole in the treatment of amyotrophic lateral sclerosis (Phase II and III) sponsored by Trophos (ClinicalTrials.gov identifier: NCT00868166).

The potency of TSPO ligands for promoting axonal regeneration was recently demonstrated in the rat sciatic nerve after freeze-injury or nerve transection. Treatment with etifoxine resulted in acceleration in axonal regrowth. Although etifoxine also binds the $GABA_A$ receptor, these effects seemed to be solely dependent on TSPO, as they could be mimicked by selective ligands of TSPO but not of $GABA_A$ receptors[10]. Most importantly, axonal regeneration was associated with a marked improvement in both the rate and quality of functional recovery[10].

Brain damage. Studies concerning the potential of TSPO ligands in the CNS have focused primarily on its neuroprotective and anti-inflammatory actions in experimental models of excitotoxic and traumatic brain injury[61] (TABLE 2). In rats, the intravenous intracerebroventricular or intracerebral injection of kainic acid constitutes a widely used model of excitotoxic brain injury, resulting in a substantial loss of neurons and astrogliosis within the hippocampus. Administration of the classical TSPO benzodiazepine ligand Ro5-4864 before kainic acid prevented hippocampal neuronal death and reactive gliosis[125]. Pretreatment of rats with Ro5-4864 also increased the number of surviving neurons and preserved neuronal networks after contusion injury of the cerebral cortex[126].

Whereas TSPO imaging has become an important diagnostic tool for ischaemic stroke, there is only indirect evidence that TSPO ligands may also be useful for reducing cerebral infarction[127,128]. In particular, TSPO ligands might be useful for preventing secondary pathophysiological consequences and neuronal loss after traumatic, excitotoxic or ischaemic brain damage. Moreover, TSPO ligands have been shown to efficiently protect other organs, such as the heart and kidney, against ischaemic damage[129,130].

Investigations of the contribution of TSPO to CNS neuroprotection have been limited mostly to the classical ligands Ro5-4864 and PK-11195. However, their usefulness is limited *in vivo* owing to their low solubility, low brain uptake and inconsistent effects. Moreover, PK-11195 displays agonistic or antagonistic effects depending on the cell type and tissue environment and/or on the presence of an endogenous ligand[131]. Thus, it would be interesting to investigate the neuroprotective effects of new TSPO ligands that have already shown efficacy in experimental models of injury or neurodegeneration. Although regeneration is considerably more limited in the CNS than in peripheral nerves, recent experimental findings indicate that TSPO may also be involved in neuroplastic changes and play a role in regenerative processes within the brain[7,73].

Neurodegenerative diseases. To date, few investigations have examined the potential beneficial effects of TSPO ligands in experimental models of neurodegenerative and demyelinating diseases within the CNS. However, recent observations suggest that the induction of TSPO expression may be involved in the response of nervous tissues to degenerative processes, and that TSPO ligands could have therapeutic potential. For example, in a transgenic mouse model of familial amyotrophic lateral sclerosis, treatment with the TSPO ligand olesoxime improved motor performance and delayed the onset of the disease phenotype[124]. Moreover, studies in various models of neurodegeneration and Alzheimer's disease indicated that upregulated TSPO levels in astrocytes are associated with neurotrophic support[73].

Neuroinflammation. TSPO is also an attractive drug target for controlling neuroinflammation. After peripheral nerve injury, activated macrophages derived from resident populations or recruited from the circulation have a key role in the anterograde degeneration of nerve fibres, known as "Wallerian degeneration", which is followed by the regrowth of the lesioned axons[132]. However, prolonged and robust inflammation may cause severe damage to neurons and nerve fibres. Thus, modulation of the inflammatory response is essential in this context, and there is strong evidence for the involvement of TSPO. For example, administration of the TSPO ligand etifoxine modulated macrophage activation and blunted the production of inflammatory cytokines after peripheral nerve injury[10]. This anti-inflammatory effect of etifoxine is likely to involve TSPO because the selective TSPO ligands PK-11195 and Ro5-4864 have also been shown to inhibit inflammatory responses in a pain model induced by carrageenan injection into the rat hind paw[133].

In the CNS, the activation of microglia is closely associated with the expression of TSPO[134] (FIG. 2a). Microglia provide a first defence against damage and disease[135] and contribute to an environment that supports neuronal viability and regeneration, and myelin sheath formation[136–138]. However, chronic activation of microglia may become deleterious for neuronal cells and constitute an important factor in neurodegenerative processes[139]. In animal models of excitotoxic brain damage, Ro5-4864 and PK-11195 reduced the level of microglial activation and the production of pro-inflammatory cytokines[125,140]. Interestingly, TSPO ligands attenuate inflammatory responses in the brain even in the absence of neuronal death, as observed in rats after intracerebroventricular infusion of the bacterial endotoxin lipopolysaccharide[141].

Brain tumours. Mitochondria are key cellular organelles that mediate intrinsic pathways of cell death by apoptosis. Targeting TSPO is therefore of interest owing to the

Astrogliosis
The presence of reactive astrocytes.

Table 2 | **Effects of TSPO ligands in the central and peripheral nervous systems**

Experimental studies	TSPO ligands	Effects of TSPO ligands	Refs
Cell cultures of neural cells			
Dorsal root ganglia	PK-11195, Ro5-4864	Neurite outgrowth	50
Trophic-support-deprived spinal motor neurons	Olesoxime (TRO19622)	Increased survival	124
Neuronal PC12 cells	Etifoxine	Increased neurite outgrowth	10
Cerebellar granule neurons	PK-11195, Ro5-4864, diazepam	Promotes colchicine-induced apoptosis	3
Astrocytes, microglia and neurons	PK-11195, Ro5-4864, protoporphyrin IX	Increased free-radical production	4
Glioma cells	DBI, TTN, flunitrazepam	Increased pregnenolone synthesis	104,162
Brain mitochondria			
Isolated mitochondria	PK-11195, Ro5-4864	Inhibition of MPT pore	157
	TSPO-specific antibody, protoporphyrin IX	Opening of MPT pore	156
Rodent brains			
Intact rats	XBD173	↑ brain allopregnanolone levels	12
	SSR180575	↑ brain and sciatic nerve pregnenolone levels	120
Rats deprived of their steroidogenic endocrine glands	Etifoxine	↑ brain neurosteroid levels	112
	Imidazopyridine acetamides (CB-34, CB-50, CB-54)	↑ brain neurosteroid levels	149
	FGIN-1-27, 4′-chlorodiazepam, PK-11195	↑ brain neurosteroid levels	164
	FGIN-1-27, PK-11195	↑ brain neurosteroid levels	165
	FGIN-1-27, 4-MA	↑ brain neurosteroid levels	163
Central nervous system injury models			
Facial nerve axotomy in neonatal rats	SSR180575	Increased motor neuron survival	120
	Olesoxime (TRO19622)	Increased motor neuron survival	124
Freeze lesion of the facial nerve in immature rats	SSR180575	Improved functional recovery	120
Excitatory amino acid toxicity in hippocampus	Ro5-4864, PK-11195	Neuroprotection and decrease in reactive astrocytes and microglia	125
	PK-11195	Neuroprotection and decrease in reactive astrocytes and microglia	125,140
Contusion injury of cerebral cortex	Ro5-4864, PK-11195	Improved neuron survival and preservation of neurofilament networks	126
Transient cerebral ischaemia	Diazepam	Only indirect evidence for neuroprotective effect	128
Transgenic mouse model of amyotrophic lateral sclerosis	Olesoxime (TRO19622)	Improved motor neuron survival, delayed clinical symptoms	124
Different models of neuron degeneration in the brain	TSPO imaging	TSPO upregulation in astrocytes	73
Lipopolysaccharide-induced neuroinflammation	Ro5-4864, PK-11195	Reduced microglial activation in the absence of neurodegeneration	141

potential to influence the proliferative activity of tumour cells. Indeed, knock down of TSPO reduced cancer cell mortality and exerted anti-apoptotic effects, probably by affecting the MPT pore[17,33]. Moreover, TSPO overexpression increases the motility, transmigration and proliferation properties of rat C6 glioma cells[142].

PK-11195, Ro5-4864 (which have an affinity for TSPO in the nanomolar range) and FGIN-1-27 cause apoptosis of various cancer cell types, including neuroblastomas and glioblastomas, and they can potentiate the effects of chemotherapeutic drugs at micromolar concentrations[33]. However, the high concentrations needed and the fact that the pro-apoptotic actions of these ligands can still be observed after TSPO knockdown or in cells not expressing the TSPO protein indicate that TSPO may not primarily be involved in these effects[143,144]. Indeed, at nanomolar concentrations, PK-11195 and Ro5-4864 even protect cells against apoptosis[145,146]. The detailed mechanisms by which high concentrations of PK-11195, Ro5-4864 and FGIN-1-27 promote cell death are not yet completely understood. Moreover, the potential cytotoxicity of other TSPO ligands at higher concentrations needs to be investigated.

Table 2 (cont.) | **Effects of TSPO ligands in the central and peripheral nervous systems**

Experimental studies	TSPO ligands	Effects of TSPO ligands	Refs
Peripheral nervous system injury models			
Sciatic nerve crush injury	Ro5-4864, PK-11195	Initiation of axonal regrowth	50
	Olesoxime (TRO19622)	Improved nerve regeneration	124
Freeze lesion of the sciatic nerve	Etifoxine	Accelerated axonal regeneration, improved functional recovery	10
		Reduced macrophage activation and cytokine production	
Acrylamide-induced neuropathy	SSR180575	Improved functional recovery	120
Streptozotocin-induced diabetic neuropathy	Olesoxime (TRO19622)	↓ neuropathic pain	121
	Ro5-4864	↓ severity of diabetic neuropathy symptoms	122
Chemotherapy (vincristine)-induced peripheral neuropathy	Olesoxime (TRO19622)	↓ neuropathic pain symptoms	121
	Etifoxine	↓ neuropathic pain symptoms	123
Carrageenan injection into hind paw (neuroinflammatory pain)	Ro5-4864, PK-11195	↓ oedema and inflammatory cytokines	133
	PK-11195	↑ TSPO-mediated allopregnanolone synthesis in spinal dorsal horns	166
	PK-11195	↑ TSPO-mediated allopregnanolone synthesis in spinal dorsal horns, effect on decay of IPSCs	167
Experimental psychopharmacological models			
Water-lick conflict test	Etifoxine	Anti-anxiety effects	112
	Imidazopyridine acetamides (CB-34, CB-50, CB-54)	Anti-anxiety effects	149
Various anxiety and depression models	XBD173	Anti-anxiety and antidepressant-like effects	147,151
Light/dark exploration test and elevated plus-maze test	DAA1106, DAA1097	Anti-anxiety effects	148,150
Social exploration test and elevated plus-maze test	XBD173	Anti-anxiety effects	12
Lactate- or CCK4-induced panic in rodent paradigms	XBD173	Anti-panic effects	12
Neuropsychiatric disorders			
Adjustment disorders with anxiety	Etifoxine	Anti-anxiety effects	115
CCK4-induced panic in healthy male volunteers	XBD173	Anti-panic effects	12

CCK4, cholecystokinin tetrapeptide; DBI, diazepam-binding inhibitor; IPSCs, inhibitory postsynaptic currents; MPT pore, mitochondrial permeability transition pore; TSPO, translocator protein (18 kDa); TTN, triakontatetraneuropeptide.

Psychiatric disorders. Various TSPO ligands have been shown to exert acute anxiolytic/anticonflict activity in rodents[112,147–150] (TABLE 2). Initial studies with etifoxine have provided the first evidence for a clinical anxiolytic effect of TSPO ligands, which showed comparable efficacy to the benzodiazepine lorazepam in patients suffering from adjustment disorders with anxiety[115]. However, etifoxine is also a weak, direct $GABA_A$ receptor modulator[114], and so the extent to which the effects observed are related to TSPO binding is not yet clear.

In a recent translational study, the new selective and high-affinity TSPO ligand XBD173 enhanced GABAergic neurotransmission in brain slices via the induction of neurosteroidogenesis[12,151] and counteracted pharmacologically induced panic attacks in rodents in the absence of sedation[12]. Most importantly, XBD173 displayed antipanic and anxiolytic efficacy in humans using an experimental anxiety paradigm involving challenge with cholecystokinin tetrapeptide (CCK4)[12]. Whereas the benzodiazepine alprazolam caused sedation and withdrawal symptoms after only 7 days of treatment, these were absent in the XBD173-treated subjects[12]. Moreover, repeated administration of XBD173 induced neither tolerance to its anxiolytic-like effects nor withdrawal symptoms in rodents[12,151]. Thus, TSPO may represent a promising target for the development of fast-acting anxiolytics with a more favourable side-effect profile than benzodiazepines[12]. A Phase II study on the efficacy, safety and tolerability of XBD173 has been conducted by Novartis in patients with generalized anxiety disorder (ClinicalTrials.gov identifier: NCT00108836).

In general, it will be important to determine whether medium-term to long-term treatment with new selective TSPO ligands is devoid of liver-related side effects, which have been observed with alpidem[117–119].

Mechanisms and cellular targets

Drug development targeting TSPO could be aided by a greater understanding of the mechanisms underlying the neuroprotective and regenerative effects of TSPO ligands. These involve the regulation of mitochondrial activity, functions related to cell viability and steroid biosynthesis. Increasing evidence suggests that intramitochondrial transport of cholesterol and acute regulation of steroid synthesis, the most well-characterized functions of TSPO, have a key role in this context.

Cell-specific actions of TSPO ligands. In response to injury, inflammation and disease, TSPO expression is induced primarily in microglia and astrocytes in the CNS, and in macrophages and/or Schwann cells in the peripheral nervous system (FIG. 2). Thus, glial and inflammatory cells probably mediate the beneficial effects of TSPO ligands on neurons, either directly or indirectly, via secreted neurosteroids, other metabolites or proteins. However, neurons can also express TSPO under certain circumstances, such as inflammatory states, which is consistent with direct neuronal actions of TSPO ligands[3,4,10,50]. Moreover, the discovery of a TSPO paralogous protein, TSPO2 (REF. 21), and the presence of a distinct TSPO protein in Jurkat cells[152], suggest that a neuron-specific TPSO might exist or that the neuron-specific microenvironment defines certain properties and function of TSPO in these cells.

The fine regulation of neuroinflammatory responses plays a key role in neuroprotection, and TSPO ligands have been shown to reduce the activation of microglia and macrophages, and the production of inflammatory cytokines. However, the signalling mechanisms involved in the modulation of neuroinflammation by TSPO remain unexplored. Notably, TSPO also regulates the viability and functions of immune cells, including lymphocytes and macrophages, and virtually all cell types of the immune system express TSPO[33].

TSPO upregulation in response to neurotoxic insults is delayed in astrocytes compared with microglia. However, the increase in TSPO expression in astrocytes is long-lasting and may be crucial for the beneficial effects of TSPO ligands on neuronal survival and regeneration[25,153,154]. Moreover, in experimental models of myelin-related disorders, TSPO expression in astrocytes has been associated with recovery from demyelination and enhanced neurosteroid formation[57,155]. Further studies should address the mechanisms underlying the neurotrophic support of TSPO, which may involve the stimulation of growth factor and neurosteroid synthesis by glial cells[4,73].

Mitochondrial activity and apoptosis. TSPO is involved in MPT pore opening. Treatment of rat brain mitochondria with a TSPO-specific antibody delayed dissipation of the mitochondrial membrane potential, diminished mitochondrial Ca^{2+} efflux and inhibited the release of pro-apoptotic factors, which is consistent with an inhibition of MPT pore opening[156]. However, PK-11195, Ro5-4864 and protoporphyrin IX stimulated free-radical production in neural cells; this TSPO-dependent increase in free radicals seemed to require MPT pore opening, as it could be prevented by cyclosporin A, which blocks the pore[4].

However, beneficial effects of TSPO ligands with respect to MPT pore opening have also been reported. Both Ro5-4864 and PK-11195 inhibited mitochondrial permeability and cytochrome *c* release in cell cultures of neurons exposed to an excitotoxic insult[157]. This is consistent with the observation that TSPO activation by 4-chlorodiazepam increased the resistance of mitochondria to Ca^{2+}-induced MPT pore opening in cardiac tissue[158]. Moreover, TSPO ligands may be effective in blocking injury-induced MPT pore changes, cytochrome *c* release and apoptosis in neurons[61]. So, whether TSPO ligands increase or decrease mitochondrial permeability seems to depend on the respective microenvironment of the cells or tissues under investigation.

Mitochondria-dependent neurosteroid synthesis. The transport of cholesterol from the outer to inner mitochondrial membrane by TSPO[20] is crucial for the induction of steroid synthesis, which involves an array of steroid hormones and neurosteroids[159–161]. These include oestradiol, testosterone, pregnenolone, pregnenolone sulphate, progesterone, allopregnanolone, allotetrahydrodeoxycorticosterone (3α,5α-THDOC), dehydroepiandrosterone and dehydroepiandrosterone sulphate (FIG. 5).

Initially, endogenous and synthetic TSPO ligands were shown to stimulate the synthesis of pregnenolone from endogenous cholesterol in glioma cells[104,162]. Subsequently, a series of *in vivo* studies demonstrated that the administration of TSPO ligands efficiently increased neurosteroidogenesis in rat brain[112,149,163–165]. In the dorsal horns of the rat spinal cord, TSPO is involved in the local increase in allopregnanolone synthesis following peripheral nerve inflammation. Because allopregnanolone is a potent positive modulator of $GABA_A$ receptors, its TSPO-dependent upregulation potentiates $GABA_A$ receptor-mediated inhibitory synaptic transmission, thereby exerting a marked analgesic effect[166,167].

Cell-specific neurosteroid signalling. The TSPO-mediated translocation of cholesterol from the outer to the inner mitochondrial membrane is the rate-limiting step in the synthesis of pregnenolone, which is the precursor of all other neurosteroids[1,2,6]. TSPO ligands differentially affect neurosteroidogenesis depending on their affinity and intrinsic activity.

Neurosteroids are potent modulators of almost all types of synaptic transmission. They can alter the release of multiple neurotransmitters or the activity of neurotransmitter receptors[168] and thus may act as inhibitors or enhancers of neuronal excitability[168,169]. Neurosteroid biosynthesis is region-specific and neuron-specific and depends not only on the relative TSPO abundance but also on the expression of the respective neurosteroidogenic enzyme machinery (FIG. 6). For example, 5α-reductase and 3α-hydroxysteroid dehydrogenase, which sequentially catalyse the synthesis of the positive

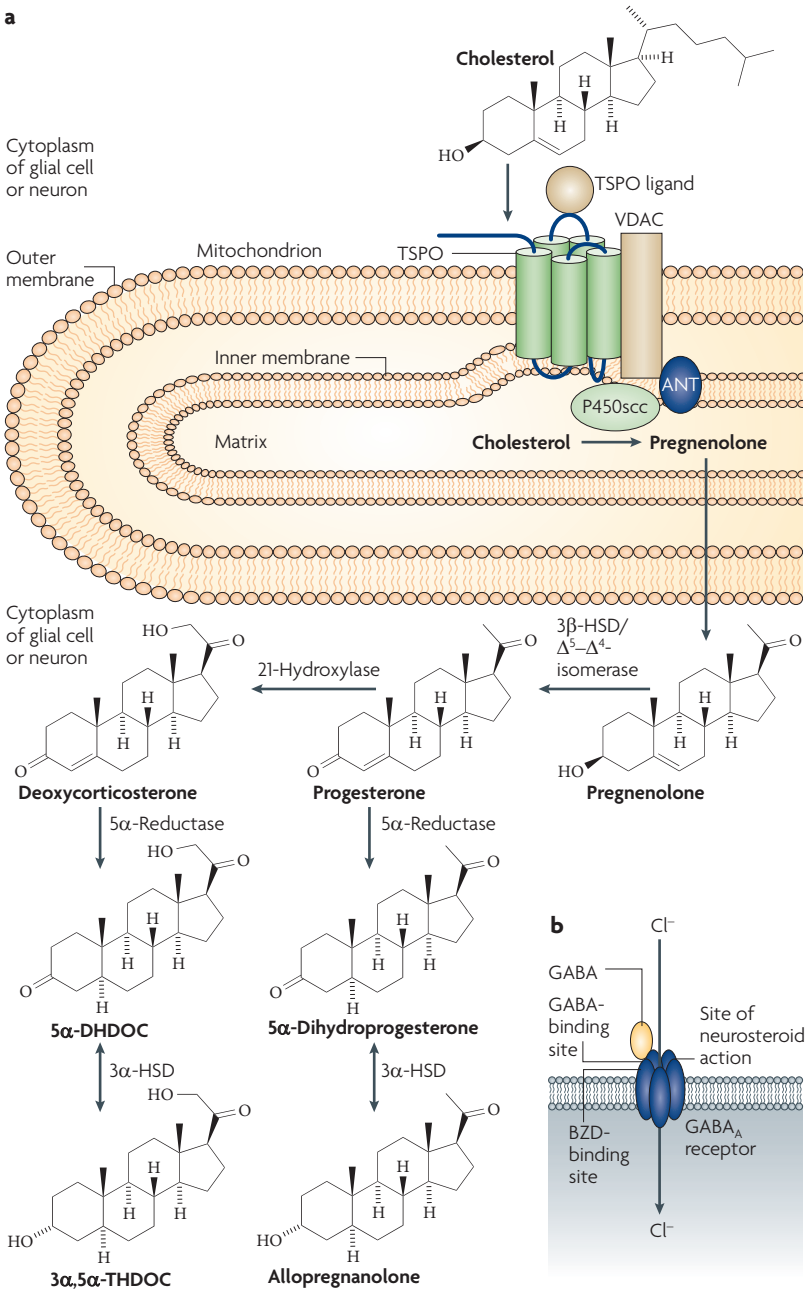

Figure 5 | **Neurosteroidogenesis and neurosteroid signalling induced by TSPO ligands. a** | Binding of a translocator protein (18 kDa) (TSPO) ligand favours the transport of cholesterol to the inner mitochondrial membrane. The cholesterol side-chain-cleaving cytochrome P450 enzyme (P450scc; encoded by *CYP11A1*), which is located at the inner mitochondrial membrane, converts cholesterol to pregnenolone, which is a neurosteroid[1,2,80,160]. After diffusion into the cytoplasm, pregnenolone is converted into progesterone by the microsomal 3β-hydroxysteroid dehydrogenase (3β-HSD)/Δ^5-Δ^4 isomerase[80,160]. Progesterone is metabolized to deoxycorticosterone by 21-hydroxylase (encoded by *CYP21B*). Progesterone and deoxycorticosterone are reduced by 5α-reductase to the 5α-pregnane steroids 5α-dihydroprogesterone and 5α-dihydrocorticosterone (5α-DHDOC) in the cytoplasm. Here, they are further reduced by the 3α-hydroxysteroid dehydrogenase (3α-HSD) to the neurosteroids allopregnanolone and allotetrahydrodeoxycorticosterone (3α,5α-tetrahydrodeoxycorticosterone; 3α,5α-THDOC)[80,160,201]. **b** | Presumably by diffusion through the cell membrane, these 3α-reduced neurosteroids act in an autocrine and paracrine manner and are potent positive allosteric modulators of synaptic and extrasynaptic $GABA_A$ (γ-aminobutyric acid type A) receptors[202]. They modulate $GABA_A$ receptor function through a binding site different from that for benzodiazepines (BDZs)[203]. The effect of neurosteroids on $GABA_A$ receptors is dependent on receptor subunit composition.

allosteric $GABA_A$ receptor modulators allopregnanolone and allotetrahydrodeoxycorticosterone, colocalize in cultured type 1 and type 2 rat astrocytes and oligodendrocytes[170–172], but not in S100β- or glial fibrillary acidic protein-positive astrocytes within the mouse brain. Here, they are present in principal output neurons (glutamatergic pyramidal, GABAergic reticulothalamic, striatal and Purkinje neurons)[173], but almost absent in telencephalic or hippocampal GABAergic interneurons[173]. The region-specific synthesis of neurosteroids in turn is responsible for the modulation of neurotransmitter function in distinct brain areas involved in sensory, motor, cognitive and emotional functions.

Neurosteroids synthesized in cortical glutamatergic principal neurons may act at $GABA_A$ receptors through autocrine (that is, at postsynaptic receptors expressed on the same neuron) and/or paracrine (that is, at receptors located at distal cortical neurons) mechanisms (FIG. 6). Although *in vitro* studies suggest that neurons express TSPO[3,4,10,25,56], this has not been confirmed *in vivo*. Whether GABAergic interneurons express TSPO is still to be determined. Thus, the more likely mechanism seems to be the paracrine release of neurosteroids from glial cells and microglia[174].

Moreover, the neurosteroid sensitivity of a given neuron is also determined by the subunit composition of its synaptic and extrasynaptic $GABA_A$ receptors[169]. Extrasynaptic $GABA_A$ receptors contain $\alpha_{1,4,5,6}\beta_{2,3}\delta/\varepsilon$ subunits in dentate gyrus granular cells of the hippocampus, in the ventrobasal nucleus of the thalamus and in cingulate gyrus granular cells[175–184], and $\alpha_5\beta\gamma_2/\delta$ subunits in the CA1 region of the hippocampus[178]. GABA is released from GABAergic interneurons and targets presynaptic (α_2-containing[178,185]), postsynaptic ($\alpha_{1,2,3,6}\beta_{2,3}\gamma_2$-containing[178,186]) and extrasynaptic receptors at glutamatergic principal output neurons (FIG. 6).

In the dorsal horns of the spinal cord, for example, extrasynaptic $GABA_A$ receptors mediating persistent tonic inhibition of neuronal activity have been identified as major targets for the analgesic actions of allopregnanolone[187]. The formation of neurosteroids induced by TSPO ligands might therefore result in a brain region-specific enhancement of GABAergic neuronal inhibition. Indeed, the TSPO agonist CB34 increased the amplitude and decay of $GABA_A$ receptor-mediated inhibitory postsynaptic currents recorded from CA1 pyramidal neurons in isolated rat hippocampal slices in a 5α-reductase-dependent manner[188]. In hypothalamic neurons, etifoxine enhanced the tonic inhibition mediated by extrasynaptic $GABA_A$ receptors, an effect that was partly blocked by the 5α-reductase inhibitor finasteride[114]. Consistent with this observation, etifoxine caused an elevation of plasma and brain levels of pregnenolone, progesterone, 5α-dihydroprogesterone and allopregnanolone. The increase in neurosteroid levels was independent from peripheral sources, indicating a brain-specific release of neurosteroids. These data suggest that activation of brain neurosteroidogenesis partially contributes to the anxiolytic-like effects of etifoxine[112]. Moreover, the reduction of chemotherapy-induced neuropathic pain by etifoxine is mediated by allopregnanolone[123].

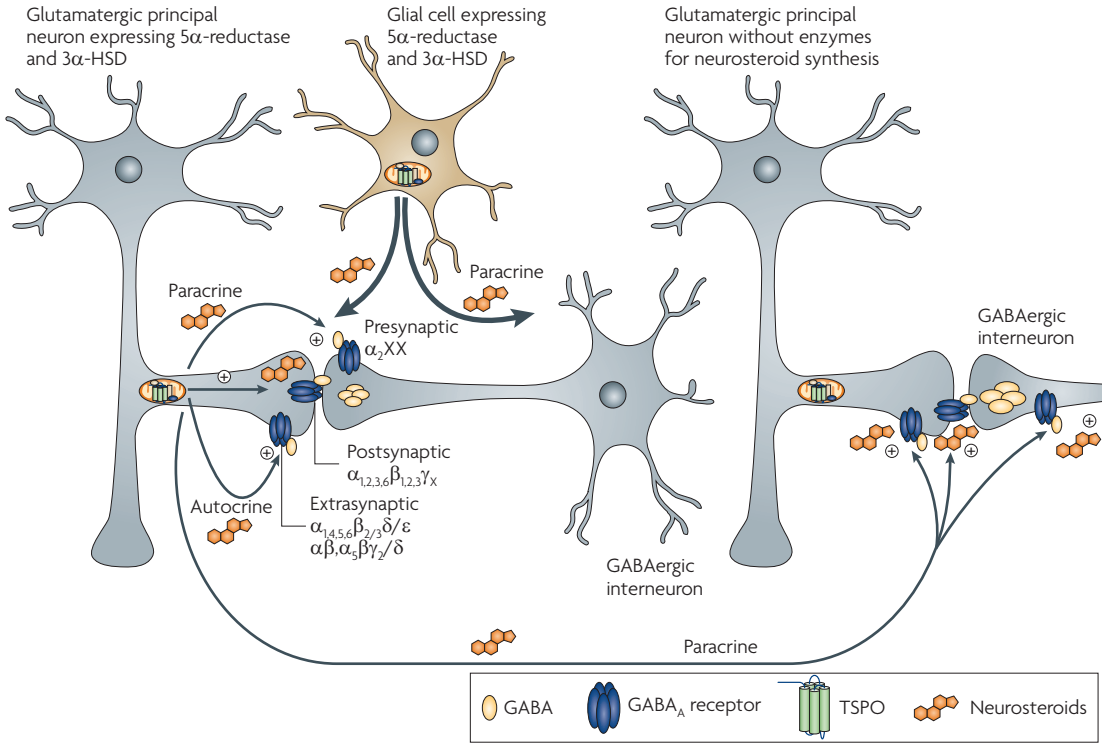

Figure 6 | **Neuronal networks targeted by TSPO ligand-induced neurosteroid signalling.** The synthesis of neurosteroids results in the potentiation or inhibition of neurotransmitter receptor function. 3α-pregnane-reduced neurosteroids generally act as efficient positive modulators of $GABA_A$ (γ-aminobutyric acid type A) receptors (although this may differ between neurons and $GABA_A$ receptor subtypes), whereas certain other neurosteroids are generally negative modulators[204]. An example of the formation of neurosteroids by translocator protein (18 kDa) (TSPO) in glial cells and glutamatergic principal output neurons and the positive allosteric modulation of different types of $GABA_A$ receptors is shown. Neuronal $GABA_A$ receptors are predominantly modulated by neurosteroids derived from glial cells and microglia (a paracrine mechanism). In addition, neurosteroids released from principal neurons may modulate the different subtypes of $GABA_A$ receptors located at the same neuron (an autocrine mechanism) or $GABA_A$ receptor subtypes located at distal neurons (a paracrine mechanism). The known subunit configuration of different $GABA_A$ receptor subtypes is indicated. X depicts unknown subunits. Extrasynaptic $GABA_A$ receptors contain $\alpha_{1,4,5,6}\beta_{2,3}\delta/\varepsilon$ subunits in dentate gyrus granular cells of the hippocampus, in the ventrobasal nucleus of the thalamus and in cingulate gyrus granular cells[175–184]. Extrasynaptic $GABA_A$ receptors contain $\alpha_5\beta\gamma_2/\delta$ subunits in the CA1 region of the hippocampus[178]. GABA is released from GABAergic interneurons and targets presynaptic (α_2-containing[178,185]), postsynaptic ($\alpha_{1,2,3,6}\beta_{2,3}\gamma_2$-containing[178,186]) and extrasynaptic receptors at glutamatergic principal output neurons. Depending on the respective receptor subunit composition, the neurosteroids allopregnanolone and allotetrahydrodeoxycorticosterone (3α,5α-tetrahydrodeoxycorticosterone; 3α,5α-THDOC) differentially increase or decrease the net chloride current through $GABA_A$ receptors[202,205,206]. For example, GABA-evoked responses that are mediated by $\alpha_1\beta_1\gamma_2$ or $\alpha_3\beta_1\gamma_2$ receptors are enhanced by low concentrations of allopregnanolone, whereas equivalent receptors that incorporate the α_2-, α_4-, α_5- or α_6-subunits require threefold to tenfold higher concentrations for potentiation[207]. Receptors that contain the γ_1-subunit are less sensitive to allopregnanolone than equivalent receptors that express either γ_2- or γ_3-subunits[206,207]. Moreover, 3α,5α-THDOC potently enhanced GABA-evoked currents through $\alpha_1\beta_3\delta$-containing receptors, in contrast to $\alpha_1\beta_3\gamma_{2L}$-containing receptors[205]. 3α-HSD, 3α-hydroxysteroid dehydrogenase.

The selective TSPO ligand XBD173 also potentiated the amplitude and duration of GABA-mediated inhibitory postsynaptic currents in mouse medial prefrontal cortical neurons, which was prevented by finasteride[12]. In contrast to diazepam, XBD173 did not act directly on postsynaptic $GABA_A$ receptors expressed in WSS-1 cells[12]. These data provide further evidence that neurosteroidogenesis is involved in the differential effects of TSPO ligands on GABAergic neurotransmission.

Conclusion and future directions

Because TSPO mediates a broad range of biological functions both in peripheral tissues and in the CNS, TSPO ligands may be used as diagnostic tools for monitoring physiological and pathophysiological processes in the CNS and peripheral nervous system. Moreover, specific TSPO ligands are also under development for the treatment of various neurological and psychiatric disorders and may therefore constitute an as yet unexploited class of compounds related to the pathophysiology of these disorders. Possible indications may include peripheral neuropathies, neurodegenerative or traumatic processes within the CNS, and psychiatric disorders, especially anxiety disorders. It is intriguing that TSPO ligands target both the underlying pathophysiology and clinical symptoms of the respective treatment indications. For example, their neuroregenerative properties may constitute an advantage for the treatment of peripheral neuropathies

compared with symptomatic treatment alone. For amyotrophic lateral sclerosis, no effective treatment is available. For anxiety disorders, TSPO ligands offer the possibility for developing fast-acting anxiolytics that lack sedation and withdrawal symptoms.

Although results from the first clinical trials are promising, several issues must be addressed in future research. First, what is the medium-term and long-term efficacy of TSPO ligands in distinct neuropsychiatric indications? And, given the high expression of TSPO in peripheral tissues, what is the side-effect profile of these ligands after prolonged administration? In this context, initial experiences with etifoxine, which was approved in France for the treatment of anxiety disorders in 1982, are promising[115]. The fact that Phase II and Phase III trials with different TSPO ligands have been initiated by various companies also seems encouraging. Nevertheless, given the issue of potential liver-related side effects, differential dosing regimens — for example, adopting the approach used in cytostatic cancer therapy involving treatment for several weeks followed by a drug-free interval — should also be considered. Do TSPO ligands really offer an improved benefit–risk profile relative to existing treatment options for their proposed indications, as suggested by initial short-term human studies[12]? Will TSPO ligands find their place primarily as diagnostic tools, novel treatment molecules or both? These questions can only be answered by systematic clinical studies involving prolonged administration and safety monitoring. Given the broad range of putative applications of TSPO ligands in neurological and psychiatric disorders, further studies of this kind are eagerly awaited.

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Competing interests statement

The authors declare competing financial interests: see web version for details.

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