

This is the author's accepted manuscript of the following article:

Gatliff, J. and Campanella, M. (2016) 'TSPO: kaleidoscopic 18-kDa amid biochemical pharmacology, control and targeting of mitochondria', *Biochemical Journal*, 473(2), 107-121.

The final publication is available at the journal website via the following link:

<http://dx.doi.org/10.1042/BJ20150899>.

The full details of the published version of the article are as follows:

TITLE: TSPO: kaleidoscopic 18-kDa amid biochemical pharmacology, control and targeting of mitochondria

AUTHORS: Jemma Gatliff; Michelangelo Campanella

JOURNAL TITLE: Biochemical Journal

VOLUME/EDITION: 473/2

PUBLICATION DATE: 5 January 2016

PUBLISHER: Portland Press

DOI: 10.1042/BJ20150899

TSPO: kaleidoscopic 18kDa amid biochemical pharmacology, control and targeting of mitochondria

Jemma Gatliff¹ and Michelangelo Campanella^{1, 2}

¹Department of Comparative Biomedical Sciences, The Royal Veterinary College, University of London and ²University College London Consortium for Mitochondrial Research, Royal College Street NW1 0TU, London, United Kingdom

To whom correspondence should be addressed:

Michelangelo Campanella

Royal College Street, NW1 0TU, London, United Kingdom

Email: mcampanella@rvc.ac.uk

Abstract

The 18 kDa Translocator Protein (TSPO) localizes in the outer mitochondrial membrane of the cells and is readily up-regulated under various pathological conditions such as cancer, inflammation, mechanical lesions, and neurological diseases. Capable to bind with high affinity synthetic and endogenous ligands, its core biochemical function resides in the translocation of cholesterol into the mitochondria influencing the subsequent steps of (neuro-) steroid synthesis and systemic endocrine regulation.

Over the years however TSPO has also been linked to core cellular processes such as apoptosis and autophagy. It interacts and forms complexes with other mitochondrial proteins such as the voltage-dependent anion channel (VDAC) via which signaling and regulatory transduction of these core cellular events may be influenced. Despite nearly 40 years of study, the precise functional role of TSPO beyond cholesterol trafficking remains elusive even though the recent breakthroughs on its high-resolution crystal structure and contribution to quality control signaling of mitochondria.

All this along with a captivating pharmacological profile naturally provides novel opportunities for investigating and understanding the significance of this highly conserved protein and the envisaged therapeutics development as here presented and discussed.

Summary statement

An alternative binding site for the benzodiazepines was discovered in the periphery of the central nervous system at the end of the seventies. Since then, the Peripheral Benzodiazepines Receptor (PBR), as it was initially named before the current nomenclature of Translocator Protein (TSPO), has exponentially gained interest from various disciplines of experimental and applied biomedicine. Inspired by the series of discoveries recorded in the past twelve months we here aim to review the most compelling aspects of TSPO science in order to inform on the biochemical and molecular pharmacology, homeostatic relevance and therapeutic potential of this mitochondrial stress-response pathway.

Keywords: TSPO, PBR, Steroids, Metabolism, Autophagy, Biochemistry and Mitochondrial Pharmacology

Abbreviations: Translocator Protein (TSPO); Peripheral Benzodiazepine Receptor (PBR); Voltage-Dependent Anion Channel (VDAC);

Introduction

The cross-disciplinary nature of TSPO has been recently remarked by prominent discoveries, which have pointed out this as an underlying regulatory pathway in cell homeostasis and signaling to fine-tune mitochondrial function and its quality control to acute and chronic stress conditions. This has

indeed unveiled a fundamental relevance in the molecular and biochemical physiopathology of mitochondria and their interplay with the intracellular environment.

TSPO was discovered in the 1977(1) and initially named as the peripheral benzodiazepine receptor (PBR) for the ability to bind benzodiazepine drugs outside of the central nervous system. It is an abundant, evolutionary conserved, protein found in every organs but with particularly high constitutive expression in steroidogenic tissue, including adrenal glands, gonads, placenta and activated brain microglia (2-4).

The pattern of TSPO expression and the early discovery that TSPO was an high-affinity binding protein of cholesterol resident in the outer mitochondrial membrane, established an exciting link between cholesterol transport and biosynthesis of steroids, including the neuronal kind of these (2, 5, 6). A large body of evidence has gradually defined TSPO as an essential component of cholesterol transport across the mitochondrial membrane and a rate-limiting step of steroid hormone production (7-10). This feature, in addition to the putative roles in protein import, porphyrins binding and transport, resulted in a new nomenclature as Translocator Protein. Remarkably, this was introduced almost 30 years after its discovery and adopted to better describe TSPO function as cholesterol-translocator protein replacing the original name (11, 12).

TSPO is widely expressed throughout the body yet its density appears tissue-dependent. Steroidogenic tissues possess the majority of the protein whereas renal and myocardial tissues have reduced levels, and lower still, the brain and liver (13-15). Notably, distribution is not homogenous within a given organ. For instance, in the adrenal glands, the medulla is almost lacking in TSPO, while far greater levels of the protein are expressed in the cortex (16). It is localized to the outer mitochondrial membrane (2, 17, 18), and for this we call it a mitochondrial protein however it has been reported in the nucleus (19) and on the plasma membrane (20). Hitherto neither of these two alternative localizations has been properly validated.

TSPO expression rises readily in various cell types following exposure to pro-pathological stressors or physicochemical insults (10, 21, 22). Such a dynamic increase of expression is particularly evident in microglia cells of the brain (23), which has led TSPO to be exploited for diagnostic purposes as a biomarker of active disease or disease-related tissue remodeling through the generation of TSPO radioligands (12, 21).

The recurring association with cell and tissue pathology likewise its known druggability have made TSPO an attractive therapeutic target. Various are indeed the chemical entities with affinity for TSPO that have been reported with some of which entering clinical trials as either diagnostics or therapeutics (12).

Animal models of disease that were reported to benefit from treatments with TSPO ligands include those for Alzheimer's Disease (AD), Multiple Sclerosis (MS), anxiety disorders, neuropathic pain, peripheral nerve injury, diabetes, rheumatoid arthritis, cancer and cardiac ischemia (21, 24-30). Among the therapeutic interventions exploiting TSPO the most promising one, hitherto, is based on

the prediction that neurosteroids could be beneficial in the treatment of inflammation and selective TSPO ligands would consequently hold a therapeutic potential by promoting the function of TSPO in steroidogenesis (21, 24, 31).

A role for TSPO in the formation of the mitochondrial membrane permeability transition pore (MPTP) has been for long proposed, providing a rationale for the evidences linking TSPO with apoptosis, Ca^{2+} signaling and redox stress homeostasis. However, recent studies on gene-edited mice (knocked out for TSPO) have ruled this out and showed that MPTP is formed regardless of the presence of TSPO (32-36). Nevertheless, a regulatory role for TSPO and that of its drug ligands cannot be excluded.

Several are the endogenous ligands for TSPO such as diazepam binding inhibitors (DBIs), porphyrins and cholesterol. There is substantial literature to date that describes cholesterol binding but much less about the physiological relevance of DBIs and porphyrins. DBIs exhibit micromolar affinity with both TSPO and the central benzodiazepine receptor, and are widely distributed in glial cells of the CNS and in steroidogenic peripheral tissues (37). They may be cleaved into other biologically active fragments and are shown to stimulate steroidogenesis via interactions with TSPO (38). Porphyrins, tetrapyrrolic pigments important in heme biosynthesis pathways, may be scavenged by TSPO (39, 40). A number of interacting proteins (e.g. ACDB3, ACBD1, PRAX1, VDAC, 14-3-3, phospholipase A2) have been identified, which are involved in steroidogenesis and other TSPO-related functions including apoptosis and autophagy. Notably, the molecular link between TSPO and the Voltage Dependent Anion Channel (VDAC), a core element of the mitochondrial outer membrane, is restated by most of the recent literature, which highlights novel functional significance for this protein (41).

In this review we will outline the current knowledge and the implications of the most recent findings in the understanding of TSPO as core molecular determinant of mitochondrial biochemistry and pharmacology.

TSPO and a bi-directional transcriptional signaling network

The *Tspo* gene is composed of four exons, with a large intron that separates the first two. Multiple transcription start sites have been identified in the *Tspo* promoter in different species (42). The promoter lacks a TATA box or CCAAT elements but contains proximal GC-rich motifs and five tandem binding sites for specificity protein 1/ specificity protein 3 (Sp1/Sp3) factors. The promoter region also harbors putative binding sites that are conserved between mouse and human TSPO for a number of additional transcription factors including activator protein 1 (AP1), v-ets erythroblastosis virus E26 oncogene homolog (Ets) transcription factors, Myc and STAT members (42-46). It is likely that tissue-specific regulatory elements exist as in steroidogenic MA-10 and Y1 cells 585 base pairs of the promoter was required to maintain full activity compared to an extended 805 bp region in non-steroidogenic NIH-3T3 cells (42).

Tspo is predominantly under the control of a PKC ϵ -dependent signal transduction pathway, which is constitutively active in steroidogenic and inducible in non-steroidogenic cells (46, 47). PKC ϵ activates

the Raf-MEK-ERK MAPK pathway and triggers *Tspo* transcription through cooperation with c-Jun, Ets, AP1 and STAT3 transcription factors(46-48). Basal *Tspo* transcription requires Ets and Sp1/Sp3 transcription factors (48), which are located in promoter regions 805-515 and 123-1 (47). Evidence has demonstrated that TSPO is directly related to changes in ROS generation and there are multiple ROS-sensitive elements that drive the expression of the *Tspo* gene, such as AP1, ERK1/2 and PKC ϵ (46, 48). For example, increased ROS production in astrocytes results in nuclear accumulation of the Sp1 transcription factor, which increases TSPO mRNA (49). Upregulation of TSPO has also been observed after treatment with other ROS-producing compounds including TNF α (50, 51), phorbol-12-myristate 13-acetate (PMA) (46, 52) and the mitogen-activated protein kinase kinase (MEK) inhibitor, 2-(2'-amino-3'-methoxyphenyl)-oxanophthalen-4-one (PD98059) (48).

We have shown that overexpression of TSPO is associated with increased ROS production, which we have reported as a causative factor in TSPO-mediated mitophagy inhibition(41). The idea that overexpression of TSPO creates an oxidative cellular environment is very important as the expression of the *Tspo* is driven by downstream effectors of ROS (48). Under oxidative conditions, an increase in TSPO levels may be physiologically important to provide mitochondrial and cellular protection against initial ROS damage; should this be sustained, a positive feedback cycle in which the gene is re-expressed and mitophagy continuously impaired may manifest by leading to cumulative mitochondrial damage, which would impact cell and tissue health over time (53).

There are anyway reports showing, on the contrary, that sustained oxidative stress decreases TSPO expression contributing cell death (22, 48) although the transcriptional or protein degradation pathways in such conditions are ill-defined.

Numerous hormones including estradiol and aldosterone also regulate TSPO (48, 54, 55); the presence of hormones appears to be necessary for maintaining constitutive TSPO expression, since decrease in TSPO is observed in steroidogenic tissues following surgical removal of the pituitary gland (6) and the adrenal gland (54). Several studies have described that exposure to steroid hormones modifies TSPO levels, determined through TSPO binding studies (56-58). mRNA and protein levels do not always correlate, however, and the protein is reported to undergo conformational changes that result in increased ligand binding (55, 59). Hormones do cause a change in TSPO binding affinity to typical ligands and induce structural alterations including post-translation modifications such as cyclic adenosine monophosphate (cAMP, cyclic AMP, or 3'-5'-cyclic adenosine monophosphate)- dependent PKA-mediated phosphorylation (48, 60).

It is well established that hormones activate the cAMP pathway, being an important element in steroidogenesis contributing to lipid synthesis, cholesterol trafficking and protein phosphorylation. cAMP is a second messenger important in many biological processes including growth and differentiation, and is used for intracellular signal transduction in many different organisms.

Activation of the cAMP pathway leads to a redistribution of Acyl-coenzyme A binding domain containing 3 (ACBD3) from the Golgi to mitochondria, where it interacts with TSPO and recruits PKA

(61, 62). This has relevance in the activation of steroidogenesis and has been described in estradiol-induced neuroprogesterone synthesis in the hypothalamic astrocytes (63).

Prolonged exposure to hormones including estradiol can lead to a decrease in TSPO levels, signifying a feedback mechanism (48, 55). A negative feedback loop has been described to regulate *Tspo* transcription following the identification of microtranscriptional regulation by cAMP dependent natural antisense transcripts (NAT). Antisense transcription was initially considered as transcriptional noise, however, it is increasingly becoming recognized as an important regulator of gene expression and may form self-regulatory circuits that allow genes to regulate their own expression [for review see (64)]. At the *Tspo* locus a short interspersed element (SINE) of the B2 family was identified that was able to drive the expression of long transcripts. Its extension overlapped exon 3 of the *Tspo* gene and formed a NAT specific for *Tspo* that was dependent on cAMP (65).

Alternatively, peroxisome proliferator-activated receptor alpha (PPAR α) (66) has been linked to decreases in TSPO expression (48, 67-69). PPAR α interferes with ROS-activated NF κ B, STATs and AP-1 transcription signaling pathways (70), factors with binding sites in the *Tspo* promoter (47, 48). It has been also suggested that PPAR α reduces the binding of AP-1 to its PKC ϵ -associated site on the *Tspo* promoter resulting in a decrease in TSPO mRNA (48) while previous studies have shown a positive correlation between PPARs and increased TSPO expression (52). Interestingly, activation of PPAR α may also occur via a cAMP-PKA dependent mechanism creating a further compatible regulatory mechanism as activation of PPAR α does bear anti-inflammatory effect (71-73), which may be logically linked to a decrease in TSPO expression (**Figure 1**).

What emerges from the existing literature is that TSPO represents an important element in facilitating cellular adaptation to various pathological and pro-pathological stimuli by integrating hormone and REDOX sensitive pathways.

Detailed identification of the transcriptional regulatory pathways and feedback loops could prove fundamental to our understanding of this protein's function and responsiveness to stress conditions.

TSPO and its phylogenetic structure

TSPO has tryptophan-rich regions that are highly conserved between prokaryotes and eukaryotes (**Figure 2**). The structure of TSPO was first predicted based on its secondary structure and modelled using molecular dynamics simulation to give a two-dimensional membrane topological model comprising an intramitochondrial short amino-terminal region and five amphipathic α -helices linked by hydrophilic loops leading to an extramitochondrial carboxyl-terminal tail (74).

An early 3D structure of TSPO, built upon the 7 transmembrane rhodopsin structure, which was almost the only membrane protein structure available at the time, was modelled as only long enough to span one phospholipid layer rather than crossing the entire bilayer membrane (74). Later TSPO topological classification in the mitochondrial membranes of yeast supported a five-transmembrane structure but with extended α -helices that are in fact able to traverse the entire membrane bilayer (75). However, CD spectrum results from mouse TSPO (MmTSPO) demonstrated the presence of a mainly helical secondary structure while NMR data confirmed the presence of at least five helical transmembrane segments (76).

Bacterial homologues have also been used to provide detailed insights into the structure of TSPO: TSPO from *Rhodobacter sphaeroides* (RsTSPO), one of the closest ancestors of mitochondria, not only has a primary structure similar to that of human TSPO but is also reported to have functional similarities (77). Cryo-EM and image analysis studies of RsTSPO revealed a dimeric, five transmembrane domain architecture for TSPO at 10 Å resolution (78).

This structural information indicated monomeric TSPO could function as a channel and could therefore translocate cholesterol across the outer mitochondrial membrane, in line with its well-defined role in steroidogenesis (21, 79). Recently, a high-resolution structure of mammalian TSPO reconstituted in detergent micelles in complex with the ligand PK 11195 has been obtained, further supporting a five transmembrane alpha helical conformation (80).

Another powerful study has described crystal structures for *Bacillus cereus* TSPO (BcTSPO) at 1.7 Å resolution in complex with PK 11195. These authors demonstrated oxidative catalytic activity between TSPO and PpIX (protoporphyrin IX), a reaction also observed in TSPO from *Xenopus tropicalis* and *Homo sapiens* that leads to PpIX degradation. This has been attributed to ROS-mediated generation of tryptophan radicals at residues W53 (hs numbering; transmembrane domain 2) and W143 (hs numbering; transmembrane domain 5), which are both shown to participate in the stabilization and cleavage of PpIX. Two other conserved tryptophan residues are located in Loop 1 (W32 and W40; hs numbering) and in the cholesterol recognition amino acid consensus (CRAC) cytoplasmic domain located at the C-terminus (W155; hs numbering), which are considered to be important in other TSPO-ligand and –protein interfaces (77, 81-83). The identification of potential catalytic regions in TSPO is particularly interesting and it would be worth understanding how such biochemical activity influences TSPO molecular interactions and downstream processes under conditions of REDOX stress since

different experimental conditions are already known to affect K_d values for PpIX binding; e.g. 0.3 μM (81) versus 8.6 μM (78).

Quite notably, TSPO has been characterized in monomeric, dimeric and multimeric states although the functional significance of its polymerization requires further clarification. Analysis of RsTSPO revealed a dimer (78, 81) while studies on MmTSPO portray a monomeric protein in complex with PK 11195 (80) although there is a possibility of oligomeric states (84). BcTSPO on the other hand has been extracted as monomeric, dimeric and as higher oligomeric species with a dimeric model in complex with PK 11195 (40). Interestingly, cellular REDOX status has been previously linked with TSPO oligomeric status s in response to ROS increases TSPO preferentially forms covalent polymers (85).

TSPO can function both as a monomer and polymer. In its monomeric form TSPO binds PK 11195 and cholesterol with nanomolar affinity (79) although the translocation of cholesterol is preferentially mediated through polymeric forms or in complex with other protein binding partners (86).

A TSPO genetic polymorphism in humans Ala(147) \rightarrow Thr(147) is associated with psychiatric disorders (87-89) and reduced pregnenolone production (90).

The polymorphism resides one helical turn before the CRAC cholesterol binding motif, and a reduction in cholesterol binding has been identified with a four- to fivefold lower affinity than wild-type (91, 92). Complications in the application of certain TSPO PET ligands, including [11C]-PBR28, [18F]-PBR111 and [(18)F]-FEPPA for imaging brain inflammation have been linked to this polymorphism (88, 93-96). However, structural analyses have shown that A147T TSPO is able to retain the same structural profile as the wild-type protein, and binds the first generation TSPO ligand, PK 11195, with comparable affinity (97). Contradictory to this study, which used mTSPO (97), RsTSPO A139T exhibited a lower binding affinity to PK 11195 as well as PpIX (92) and this is supported by crystal structures of the A139T mutant at 1.8 and 2.4 Å resolution in RsTSPO, which show conformational changes that alter the structural environment in both the CRAC domain and possible ligand binding domains. These conflicting reports however can be ascribed to the structural differences between mammalian and bacterial species. Nevertheless, they still raise the question as to how functional activity data obtained in bacterial systems can be applied to mammalian TSPO and therefore if this has been preserved intact in the phylogenesis. Additional studies conducted in mammalian TSPO systems will therefore be of essence particularly with regards to the apparent catalytic activity exhibited by TSPO in the degradation of PpIX as already pointed out (40).

Anyhow the tremendous effort by colleagues who endeavored, in series, to generate the actual structure of the protein is of invaluable importance not only to inform TSPO biochemistry but also to pave novel avenues of research to dissect and exploit the nature of TSPO-ligands binding as well as the design of novel chemicals.

***Tspo*^{-/-} knockout mice: new insights of an old function**

The most studied role assigned to TSPO is in the transport of cholesterol into mitochondria for hormone biosynthesis. Hormone-induced steroidogenesis in all tissues begins with the conversion of the precursor cholesterol to pregnenolone, a reaction catalysed by the enzyme P450 cholesterol side chain cleavage enzyme (CYP11A1), located in the IMM, facing the matrix [reviewed in (98)]. The rate-limiting step is the transport of cholesterol from the cellular stores across the mitochondrial membranes. In order for this step to be achieved, cholesterol must accumulate at the outer mitochondrial membrane (OMM) and then be transferred to the IMM.

The steroidogenic acute regulatory protein (StAR) is a hormone induced mitochondria-targeted protein that has been shown to initiate cholesterol transfer into mitochondria (99-101). It binds cholesterol (102) and is active at the OMM where it is proposed to interact with TSPO to form a complex that facilitates mitochondrial cholesterol import (62, 101, 103, 104). Other components of the complex include ACBD3 (Acyl-coenzyme A binding domain containing 3) and the regulatory subunit R1 α of PKA (62, 83, 105). TSPO can bind cholesterol through its CRAC domain (106), and mutagenesis in this region interferes with cholesterol binding and transfer of cholesterol into the IMM, preventing steroidogenesis (107). TSPO gene silencing blocks cholesterol transport into the mitochondria, also reducing steroid production, while reintroduction of TSPO is able to restore steroidogenic capacity (83). Parallel studies conducted in a bacterial system expressing MmTSPO demonstrated time- and temperature-dependent uptake of radiolabeled cholesterol. A number of non-steroidal ligands that interact with the CRAC motif were recently identified through molecular modeling and in silico screening of chemical libraries and were shown to potently inhibit steroidogenesis (108). Taken together there is a large body of evidence supporting a function for TSPO in cholesterol transport; although it has also been suggested that it stores cholesterol until later requirement since cholesterol loaded membranes release cholesterol in response to PK 11195 (79, 109).

Recently, however, a number of studies from independent groups have reported that TSPO global knock out mice are viable and that loss of the protein has no effect on basal steroid hormone biosynthesis (32, 35, 110, 111). These studies not only question the role TSPO holds in steroidogenesis but contrast early attempts to generate *Tspo*^{-/-} knockout mice, which resulted in embryonic lethality (5). However, the viability of mice has been shown to vary depending on methodology: Nr5a1-driven conditional knock-outs were born at a normal Mendelian ratio while Amhr2-Cre driven conditional knock-outs were born at a ratio of 4.4% (111). Global *Tspo* knockout phenotypes can range from lethal, when whole gene deletion is performed, to no phenotype, as in the case of cre-loxP technique, thus presenting the possibility that methodological differences may be the cause of such discrepancy (112). Phenotypic characterization carried out on currently available global and conditional knock-out strains is summarized in **Table 1**. However, it is important to consider that compensatory mechanisms may play an important part in ensuring ongoing steroidogenesis in a TSPO null setting, and these may be different in the various strains, which may be attributed to methodology as highlighted already. In both global and conditional *Tspo* knock-out studies, several

genes involved in steroidogenic processes were found to be upregulated including *Cyp21a2* (110), *Abca2* (110), and *Scarb1* (111) in the adrenal glands and *Lhcgr* (111) in the testes (**Table 2**). The study by Fan et al describes how tissue-specific deletion of *Tspo* in gonadal tissues had little effect on gonadal steroidogenesis although ACTH stimulation of corticosterone production in *Tspo* depleted adrenals was severely impaired in both male and female animals (111). Interestingly, in *Tspo*^{-/-} mice, there is an accumulation of lipid droplets in the adrenal glands, implying decreased lipid metabolism, while instead lipid stores were depleted in testes and ovaries, implying excessive substrate consumption (111). These observations provide strong evidence that there is an important role for TSPO in hormone-mediated steroidogenesis and lipid and cholesterol metabolism.

TSPO is also attributed to immune regulation, with elevated expression observed in microglia and macrophages. Recently it was highlighted that TSPO is involved in cholesterol trafficking in macrophages, since its overexpression leads to increased transcription of proteins involved in cholesterol efflux, ACBA1 (ATP-binding cassette A1), PPAR α (peroxisome-proliferator-activated receptor α) and LXR α (liver X receptor α), and corresponds with increased efflux of cholesterol to acceptors (113). This pathway is activated upon a moderate cholesterol load stress and it is proposed as a protective mechanism to reduce macrophage cholesterol mass (113). Other genes involved in immune regulation are similarly altered in *Tspo*^{-/-} mice (**see Table 2**) further validating a role for TSPO in the regulation of these processes. *Tmem178* is proposed as a negative regulator of macrophage activation and Ca²⁺ signaling at the ER level (114), and is reduced in *Tspo*^{-/-} mice. Conversely, there are increased levels of *Zbtb7b*, which is associated with T-cell maturation (115) and regulates the development of Natural Killer T (NKT) cells (116) and ties in the Tu et al study (110) with the recent work by Banati et al (32), who observed an increase in NKT cells present during haematological analysis in female *Tspo*^{-/-} mice. TSPO has previously been reported as having antiretroviral activity by inhibiting Env protein expression (117). In support of this study, decreased levels of *Trim12a*, coding for protein within the anti-retroviral TRIM family, and *Pydc4*, coding for an AIM2-like receptor that activates STING-dependent interferon (IFN) production as part of the antiviral response (118), were observed in *Tspo*^{-/-} mice (110). The reduced levels suggest that TSPO is involved in an upstream transcriptional signaling cascade that influences the antiviral response.

Another interesting observation in *Tspo*^{-/-} mice is a disparity in body weight that occurs only in females, with an increase in weight in the knock-out strain (34), which the authors attribute to a hormone-dependent metabolic pathway. Previously the TSPO ligand, PK 11195, was shown to alter the expression of metabolic genes in white adipose tissue (22) and liver (29) of male mice although no study has examined the application of ligands in female mice yet to assess possible sexual disparity in ligand efficacy. Notably PPAR α , regulated by hormones and cAMP, holds a key role in integrating the mammalian clock and energy metabolism (119) and notably influences *Tspo* transcriptional regulation (48). Since gender based differences have been observed previously in metabolic patterns of circadian rhythms (120), this may be an explanation for the gender-based differences in body

weight seen in *Tspo*^{-/-} mice. This is particularly interesting as it could reveal a therapeutic potential for TSPO ligands in the development of gender-based personalized medicine for human diseases relating to metabolic disorders, which are often linked to circadian disruption (121).

The overarching conclusions that are consistent between all the knock-out studies indicate that due to the relative lack of phenotype in *Tspo*^{-/-} mice, TSPO is likely to be most involved in pathological and stress-related conditions. Future follow on studies could therefore focus to assessing susceptibility in standard stress models of diseases where TSPO has already been linked to, for example, LPS-induced inflammation, ischemia-reperfusion injury, radiation/chemical-induced cancers, overfeeding or bacterial/viral infection. Nonetheless, age-related studies should also be recommended based on the brain-imaging evidences lately produced (96).

TSPO and its molecular partnership with VDAC1

TSPO is included in a complex with the 32kDa voltage-dependent anion channel (VDAC) (17), of which there are three different isoforms. The relative ratio of these proteins is tissue and condition dependent (122). Early studies indicate that 4-6 molecules of TSPO associate with one molecule of VDAC to form a single mitochondrial pore (5, 123) but the precise biochemical properties governing the interaction remain unclear and require further investigation, which may be facilitated with the recent high-resolution characterization of its 3D structure (80).

There is a growing body of work that supports a functional interplay between TSPO and VDAC particularly in dictating the efficiency of mitochondrial metabolism and quality control.

The long standing role of TSPO in steroidogenesis appears to be linked to its interactions with VDAC (124, 125), and relative levels of each protein have comparative expression profiles in steroidogenic tissues (126). STAR, TSPO and VDAC all contain binding motifs for 14-3-3 ϵ , a newly identified negative regulator of cAMP-mediated induction of steroidogenesis (124, 127). VDAC is a primary target of 14-3-3 ϵ , with which forms a protein-protein scaffold that influences downstream TSPO interactions leading to reduced cholesterol import into mitochondria (124). In fish testis, *tspo* and *vdac* mRNA levels are both correlated with reproductive stage and gonadosomatic index (GSI) while in females, gonadal *tspo* and *vdac* expression are negatively correlated with GSI and levels of plasma testosterone and 17 β -estradiol (126).

As mentioned above, TSPO has a regulatory metabolic role. For example the ligands, FGIN-1-27 and PPIX are both shown to reduce glycolytic activity and cellular ATP levels (128, 129) while PK 11195 increases ATP levels (130). TSPO overexpression is associated with reduced Ca²⁺-dependent activation of mitochondrial respiration (41), which has been linked to VDAC activity while the effect of PK 11195 on quinolinic acid-induced glucose metabolic disturbance is reported to involve VDAC (131).

TSPO is strongly associated with inflammation, and studies have demonstrated the importance of the TSPO-VDAC interplay in this response too. In vascular endothelial cells, TNF α and other

inflammatory cytokines induce mitochondrial ROS production and expression of vascular cell adhesion molecule-1 (VCAM-1) although TSPO overexpression interferes with this sequence of events (132). In contrast to the apparent parallel expression profiles in steroidogenic tissues, TSPO expression negatively correlates with VDAC1 expression in endothelial cells, an observation that the authors attribute to the reduced mitochondrial ROS and lower levels of VCAM-1 in overexpressing TSPO conditions (132).

The mitochondrial TSPO-VDAC complex is further involved downstream of PARK2-mediated activity (41, 133). We determined that increased TSPO expression in mouse embryonic fibroblast cells leads to a reduction in PARK2-mediated mitochondrial ubiquitination during FCCP-induced mitophagy (41). Mechanistically, VDAC1 was required for this TSPO-dependent activity and further attributed to a ROS-signaling pathway since mitophagy could be restored in over-expressing TSPO cells in the presence of the antioxidant, MnTBAP (Manganese (III) tetrakis (4-benzoic acid)porphyrin chloride) (41).

A further study has confirmed a role for TSPO-VDAC in regulating PARK2 function, showing that TSPO-VDAC is involved downstream of the PARK2-mediated immune response in *Drosophila* since PARK2 overexpression failed to rescue lethality caused by septic injury in TSPO-silenced larvae (133).

This evidence suggests that the interplay between VDAC and TSPO is important in all the major functions attributed to TSPO including steroidogenesis, hormone biosynthesis, immune/inflammatory regulation and energy metabolism, processes for which mitochondria are critically involved. The removal of different VDAC isoforms can yield mice without mitochondrial dysfunction or evident phenotype, which is also the case in certain strains of TSPO KO mice (please see section above). This may appear particularly confounding, as each of these proteins can comprise up to 5% of total outer mitochondrial membrane protein levels in certain tissues. However, some functional redundancy has been described between the various VDAC isoforms, which may explain the apparent lack of phenotype. Furthermore, in the case of the VDAC1^{-/-} strains, there was considerable variation in phenotype, which was notably dependent upon the methodology used to generate the line. VDAC1^{-/-} mice obtained from a mixed genetic background (C57BL6/129SvEv) were born at a lower than expected mendelian ratio whilst VDAC1^{-/-} mice, bred onto the C57B16 background, showed near complete lethality (134).

TSPO in cellular and tissues pathology

Cancer

TSPO is overexpressed in a variety of cancers including those affecting the brain (135). TSPO expression is elevated in human prostatic intraepithelial neoplasia, primary prostate cancer and metastases when evaluated against normal prostate tissue and benign prostatic hyperplasia (136). A

positive correlation with disease progression was observed too and immunohistochemical studies in oral cancer have yielded similar results. Interestingly, the five-year survival probability dropped from 65% in patients with TSPO negative tumors to just 7% in patients with high tumoral TSPO content (137). Results, from colorectal carcinomas in human patients, indicated that TSPO was overexpressed in 67% of tumors in comparison to corresponding normal mucosa in which expression was found to be significantly higher. And in this study, TSPO did not differ for expression between intermediate versus high-grade tumors or in lymph node-positive versus negative patients (138). Overexpression of TSPO has also been observed in breast cancer cell lines (139) and in clinical studies (140). In the former, estrogen receptor-negative breast cancer cell lines had significantly higher TSPO expression compared to ER-positive lines and the levels of TSPO were positively correlated with the proliferation marker, Ki-67 (139). This observation was mirrored in the clinical study in which TSPO expression was significantly increased in tumoral versus normal breast cells and TSPO was again correlated with Ki-67 levels. TSPO was anyway never associated with reduced prognosis in the whole patient sample, but in lymph-node negative group of patients, elevated TSPO was linked to a shorter disease-free survival period, indicating that TSPO could be used to identify a higher risk population in this category (140). TSPO is reported overexpressed in oesophageal cancer cells compared to normal oesophageal epithelium (141), as well as in human endometrial carcinoma, where significantly more mitochondrial TSPO was observed in contrast to normal endometrium (142). The link between TSPO and cancer is therefore consolidated even though the underlying pathogenic mechanism driving a primary oncogenic profile is still lacking.

Inflammatory Brain conditions

High levels of TSPO are recurrently associated with glial cell activation and inflammation (143, 144). Chronic inflammation is an early feature of many neurological conditions and other age-related conditions including atherosclerosis [reviewed in (145)]. Uptake of TSPO ligands in the brain is generally low in normal healthy adults, but overall uptake is increased with age (146). An increase in TSPO expression has been observed during the onset of disease in amyotrophic lateral sclerosis (ALS) patients (147). Similarly, brains of subjects with mild cognitive impairment (MCI), who are at an increased risk of developing Alzheimer's disease (AD), or subjects with mild and early forms of AD, exhibit higher ligand binding compared to age-matched controls (148, 149) although other studies contradict this observation (150). Widespread glial activation is likewise associated with the pathological process that occurs in Parkinson's disease (PD) and TSPO ligands have shown increased binding profiles in rat models mimicking the early stages the disease (151). Clinical studies show that compared to controls, PD patients have increased levels of TSPO ligand binding in the regions of the brain (152), although the degree of binding did not alter over a two-year period and was not associated with clinical severity demonstrating that TSPO overexpression and inflammation are likely early diagnostic factors (153). In stroke patients, TSPO expression and glial activation is

increased in the peri-infarct zone for several weeks following insult (154) while bio-imaging has revealed elevated TSPO expression in inflamed atherosclerotic plaques of the vasculature (155, 156). The precise molecular mechanisms characterizing a functional role for TSPO in these diseases are unclear leaving a general understanding that the upregulation of TSPO is an adaptive consequence rather than a causative factor on which the mitochondrial dependent rewiring of cell signaling and metabolism of the cells, is affected. It is anyway known that factors playing part in the regulation of TSPO expression such as ROS, Interleukin-1 (IL-1) and TNF α , are clearly involved in this process suggesting that a molecular pro-inflammatory role is plausible (18, 157).

TSPO is also implicated in peripheral inflammatory diseases for example the Inflammatory Bowel Disease (IBD). In this it has been demonstrated an increase in TSPO expression in the colon likewise in human intestinal biopsies from IBD patients (18). TSPO ligands have been indeed developed or adapted along the years to illustrate areas of inflammation through *in vivo* imaging analysis, making this one of the most active fields for biomedical applications targeting the protein. Interestingly, almost unexplored is whether these same tools may be effective as therapeutic means against the diseases in which they are adopted. In this regard, some preliminary material seems to support this notion (21, 24, 158) but more is to be done.

The pharmacology of a natural mitochondrial target

The consolidated profile as a multi-drugs binding protein has made TSPO a topic of natural interest for pharmacologists. Numerous are indeed the publications reporting the use of TSPO targeting chemicals to dissect and/or correct biological processes associated with the protein. The archetype synthetic TSPO ligands, prevalently used are the 4'-chloro-derivative of diazepam, Ro 5-4864, and the isoquinoline carboxamide, PK11195 which respectively bind TSPO with nanomolar affinity even though this is greater for PK11195 ($K_D < 20\text{nM}$) (159). Several endogenous ligands for the protein have been also identified among the porphyrins (cholesterol, hemin, protoporphyrin IX, mesoporphyrin IX, deuteroporphyrin IX) (160, 161).

In spite of the extensive use, the most recent evidences suggest that these may not be so reliable since Ro 5-4864 varies its efficacy across species whilst PK11195, exerts cellular effects independent of TSPO (162-165) soon as it is over-dosed (μM rather than nM).

This has consequently stimulated the search for alternative compounds based on different chemical structures. Another class of compounds generated and still binding TSPO with nM affinity was identified in the 2 aryl-3-indoleacetamides (FGIN-1) (166), which retains good cross-species efficacy. Furthermore this drug does hold anxiolytic-like effects (167) likewise the XBD173 (AC-5216; Emapunil). The mechanisms by which these two chemicals operate are univocal by inducing a boost of the production of neurosteroids. Once they are bound to TSPO they would in turn potentiate the GABAA receptor-based neurotransmission leading to a beneficial effect linked with the regulatory cascade depending on this receptor.

However, a series of highly specific synthetic ligands for TSPO have been successfully developed in the form of radiotracers for PET bio-imaging of inflammation, for example the 7-chloro-*N,N*,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide (SSR180575) (168) and 2-(5, 7-diethyl-2-(4-(2-fluoroethoxy)phenyl)pyrazolo[1,5-*a*]pyrimidin-3-yl)-*N,N*-diethylacetamide (VUHS1008) (169) and the 12a ([¹⁸F]GE-180). Thus, the exploitation of TSPO upregulation for the *in vivo* diagnostic imaging of patients is probably the primary and mostly corroborated clinical value for TSPO and holds the potential to grow and extend across multiple fields by refinement in the chemistry of TSPO-binding ligands in order to overcome imaging complications. In spite of this the pharmacological regulation of TSPO does remain debated.

Very recently Selvaraj and Stocco have, very timely and elegantly, stimulated attention towards the word 'ligand' as loosely used in the TSPO literature in reference to all substances that bind TSPO at various distinct sites (170). This is quite appropriate, as findings on ligand-binding response have proved inconsistent along the years, ranging from steroid hormone production to cell proliferation, signaling and apoptosis. The word ligand thus describes a small-molecular substance that binds to a target biomolecule (or complex) without the need to elicit or modulate a specific biological response as required instead by an agonistic or antagonistic which directly associated with the function of the target. In the case of TSPO such function is -for now- represented solely by the translocation of cholesterol and consequent synthesis of steroids.

However, gene-edited animal models for TSPO do not reassemble, challenged with TSPO-binding chemicals, the pharmacological effects recorded *in vitro* experiments, implying these chemicals may have alternative biochemical/pharmacological relevance beyond TSPO or the TSPO dependent translocation of cholesterol may not represent *per se* the perfect assay to sample their activity. Although basal steroids could be still formed without TSPO being expressed a regulatory role for the protein cannot be excluded and thus TSPO ligands, which do mediate a robust effect on steroids as well as neurosteroids formation, may also be affected.

The manufacture of steroids might indeed entail concurrent limiting steps depending on alternative molecular elements and therefore likely to be too elaborated to allow fast discrimination of TSPO pharmacology. The effort should be therefore to outline alternative fast-response assays, which could insight, not only the binding capacity but also the agnostic/antagonistic profiles of the compounds.

In this the recent advancements in comprehending the structure and the functional role of TSPO in respect of cell signaling mitochondrial cell biology may represent the solution. Even without a direct etiological link, this quest for TSPO-binding therapies has been extensive in recent years and applied to various diseases prompted by the pressing need for drug discovery pipelines, being the upregulation of TSPO constant in pathological lesions.

Thus, TSPO ligands have still proven efficacious in ameliorating disease pathology in numerous preclinical models and most remarkably in models bearing phenotypes in the central nervous system

(24, 171-173). Recently, Ro5-4864 was shown to amend Alzheimer's disease associated phenotypes *in vivo* (25) by mediating positive effects in steroid hormones production (109) (**Table 3**).

Human clinical trials have been performed for Emapunil to treat anxiety disorders with promising outcomes reported in an induced model of panic disorder (31). However, the high TSPO binding variability across human subjects -recently observed- does render unlikely a drug development via this mechanism of targeting (operating by inducing a boost of the production of neurosteroids as recorded following challenge with TSPO ligands) which remains, anyhow the most promising one (174). On the other hand, the early assessment of mutations in TSPO (e.g. A147T SNP), via advanced imaging protocols, could lead to personalized therapies and consequent tangible benefit for patients. What is known since long time is that TSPO-binding drugs can cause death of cancer cells and therefore considered potential anticancer therapies. This was prevalently associated with the putative role of TSPO in activating the Mitochondrial Permeability Transition Pore (MPTP) that was also hypothesized to be involved in the protection mediated by the TSPO ligands as heart protectors and tools to reduce infarct size after ischemia-reperfusion (175, 176). The recent advancements in the clarification of the MPTP molecular entity rule TSPO in its formation and regulation and therefore in the consequent protein-associated pharmacological mechanisms thus calling for these to be clarified if therapeutic uses are to be exploited.

This further highlights how structure and conformational adaptation of the protein are essential to better dissect the pharmacology of this pathway and in this way ease the comprehension and validation of current and future chemicals.

Concluding remarks

The aspects of TSPO above discussed report of a multifaceted mitochondrial pathway characterized by interdisciplinary interest. The evidences gathered along the years have enriched the landscape of TSPO science favoring its current exploitation by diagnostic and therapeutic protocols.

Being brought up to general attention, as an off-target binding site of the benzodiazepines TSPO is now a corroborated regulatory element in the mitochondrial and cellular pharmacology of lipids.

In the attempt to interpret the series of information homogenously it seems that the accumulation of TSPO, reconciled during inflammation and therefore targeted by advanced protocols of *in vivo* imaging, prevents the removal of mitochondria by the autophagy-operated quality control mechanisms leaving the organelles to be primed by the cells for steroids synthesis and undermining other core functions that are indeed compromised by the accumulation of TSPO.

The concerted cooperation among disciplines, a structured cooperation by experts and the advancements in the technology and experimental protocols will continue to improve and modernize TSPO science incrementing its significance in mammalian pathophysiology to obtain an informed understanding of this regulator and target of the mitochondrial function.

Declaration

Authors disclose no conflicting interests related to the matter of this publication,

Acknowledgements

The research activities on TSPO lead by MC are supported by: BBSRC, MRC, PPCT, The Umberto Veronesi Foundation, MarieCurie Actions and LAM-Bighi Grant to the all of which we are sincerely grateful.

References

1. Braestrup C. Specific benzodiazepine receptors in rat brain characterized by high-affinity [3H]diazepam binding. *Proc Natl Acad Sci USA*. 1977;74(9):3805-9.
2. Anholt RR, Pedersen PL, De Souza EB, Snyder SH. The peripheral-type benzodiazepine receptor. Localization to the mitochondrial outer membrane. *J Biol Chem*. 1986;261(2):576-83. Epub 1986/01/15.
3. Antkiewicz-Michaluk L, Guidotti A, Krueger KE. Molecular characterization and mitochondrial density of a recognition site for peripheral-type benzodiazepine ligands. *Mol Pharmacol*. 1988;34(3):272-8.
4. Benavides J, Guilloux F, Rufat P, Uzan A, Renault C, Dubroeuq MC, et al. In vivo labelling in several rat tissues of 'peripheral type' benzodiazepine binding sites. *European journal of pharmacology*. 1984;99(1):1-7.
5. Papadopoulos V, Amri H, Boujrad N, Cascio C, Culty M, Garnier M, et al. Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis. *Steroids*. 1997;62(1):21-8. Epub 1997/01/01.
6. Anholt RR, De Souza EB, Kuhar MJ, Snyder SH. Depletion of peripheral-type benzodiazepine receptors after hypophysectomy in rat adrenal gland and testis. *European journal of pharmacology*. 1985;110(1):41-6.
7. Papadopoulos V, Guarneri P, Kreuger KE, Guidotti A, Costa E. Pregnenolone biosynthesis in C6-2B glioma cell mitochondria: regulation by a mitochondrial diazepam binding inhibitor receptor. *Proc Natl Acad Sci U S A*. 1992;89(11):5113-7.
8. Papadopoulos V, Berkovich A, Krueger KE, Costa E, Guidotti A. Diazepam binding inhibitor and its processing products stimulate mitochondrial steroid biosynthesis via an interaction with mitochondrial benzodiazepine receptors. *Endocrinology*. 1991;129(3):1481-8.
9. Li H, Papadopoulos V. Peripheral-type benzodiazepine receptor function in cholesterol transport. Identification of a putative cholesterol recognition/interaction amino acid sequence and consensus pattern. *Endocrinology*. 1998;139(12):4991-7. Epub 1998/12/01.
10. Gavish, M., I. Bachman, R. Shoukrun, Y. Katz, L. Veenman, G. Weisinger, and A. Weizman. Enigma of the peripheral benzodiazepine receptor. *Pharmacological reviews*. 1999;51(4):629-50.
11. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapere JJ, Lindemann P, et al. Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci*. 2006;27(8):402-9. Epub 2006/07/11.
12. Liu GJ, Middleton RJ, Hatty CR, Kam WW, Chan R, Pham T, et al. The 18 kDa translocator protein, microglia and neuroinflammation. *Brain pathology*. 2014;24(6):631-53.
13. De Souza EB, Anholt RR, Murphy KM, Snyder SH, Kuhar MJ. Peripheral-type benzodiazepine receptors in endocrine organs: autoradiographic localization in rat pituitary, adrenal, and testis. *Endocrinology*. 1985;116(2):567-73. Epub 1985/02/01.
14. Anholt RR, De Souza EB, Oster-Granite ML, Snyder SH. Peripheral-type benzodiazepine receptors: autoradiographic localization in whole-body sections of neonatal rats. *The Journal of pharmacology and experimental therapeutics*. 1985;233(2):517-26.

15. Gehlert DR, Yamamura HI, Wamsley JK. Autoradiographic localization of "peripheral-type" benzodiazepine binding sites in the rat brain, heart and kidney. *Naunyn-Schmiedeberg's archives of pharmacology*. 1985;328(4):454-60.
16. Anholt R. The Peripheral-type Benzodiazepine Receptor - Localization to the Mitochondrial Outer Membrane. *The Journal of Biological Chemistry*. 1986;261(15):576-83.
17. McEnery MW, Snowman AM, Trifiletti RR, Snyder SH. Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier. *Proc Natl Acad Sci U S A*. 1992;89(8):3170-4. Epub 1992/04/15.
18. Ostuni MA, Issop L, Peranzi G, Walker F, Fasseu M, Elbim C, et al. Overexpression of translocator protein in inflammatory bowel disease: potential diagnostic and treatment value. *Inflamm Bowel Dis*. 2010;16(9):1476-87. Epub 2010/03/12.
19. Hardwick M, Fertikh D, Culty M, Li H, Vidic B, Papadopoulos V. Peripheral-type benzodiazepine receptor (PBR) in human breast cancer: correlation of breast cancer cell aggressive phenotype with PBR expression, nuclear localization, and PBR-mediated cell proliferation and nuclear transport of cholesterol. *Cancer Res*. 1999;59(4):831-42. Epub 1999/02/24.
20. Olson JM, Ciliax BJ, Mancini WR, Young AB. Presence of peripheral-type benzodiazepine binding sites on human erythrocyte membranes. *European journal of pharmacology*. 1988;152(1-2):47-53. Epub 1988/07/26.
21. Rupprecht, R., V. Papadopoulos, G. Rammes, T.C. Baghai, J. Fan, N. Akula, G. Groyer, D. Adams, and M. Schumacher. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat Rev Drug Discov*. 2010;9(12):971-88. Epub 2010/12/02.
22. Thompson MM, Manning HC, Ellacott KL. Translocator protein 18 kDa (TSPO) is regulated in white and brown adipose tissue by obesity. *PloS one*. 2013;8(11):e79980. Epub 2013/11/22.
23. Banati RB, Myers R, Kreutzberg GW. PK ('peripheral benzodiazepine')--binding sites in the CNS indicate early and discrete brain lesions: microautoradiographic detection of [3H]PK11195 binding to activated microglia. *Journal of neurocytology*. 1997;26(2):77-82.
24. Daugherty DJ, Selvaraj V, Chechneva OV, Liu XB, Pleasure DE, Deng W. A TSPO ligand is protective in a mouse model of multiple sclerosis. *EMBO Mol Med*. 2013;5(6):891-903. Epub 2013/05/18.
25. Barron, A.M., L.M. Garcia-Segura, D. Caruso, A. Jayaraman, J.W. Lee, R.C. Melcangi, and C.J. Pike. Ligand for translocator protein reverses pathology in a mouse model of Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2013;33(20):8891-7.
26. Kondo, D., H. Saegusa, R. Yabe, I. Takasaki, T. Kurihara, S. Zong, and T. Tanabe. Peripheral-type benzodiazepine receptor antagonist is effective in relieving neuropathic pain in mice. *Journal of pharmacological sciences*. 2009;110(1):55-63.
27. Decaudin D, Castedo M, Nemati F, Beurdeley-Thomas A, De Pinieux G, Caron A, et al. Peripheral benzodiazepine receptor ligands reverse apoptosis resistance of cancer cells in vitro and in vivo. *Cancer Res*. 2002;62(5):1388-93.
28. Bribes E, Bourrie B, Esclançon M, Galiegue S, Vidal H, Casellas P. Involvement of the peripheral benzodiazepine receptor in the development of rheumatoid arthritis in Mrl/lpr mice. *European journal of pharmacology*. 2002;452(1):111-22.
29. Gut, P., B. Baeza-Raja, O. Andersson, L. Hasenkamp, J. Hsiao, D. Hesselson, K. Akassoglou, E. Verdin, M.D. Hirschey, and D.Y. Stainier. Whole-organism screening for gluconeogenesis identifies activators of fasting metabolism. *Nature chemical biology*. 2013;9(2):97-104. Epub 2012/12/04.
30. Obame FN, Zini R, Souktani R, Berdeaux A, Morin D. Peripheral benzodiazepine receptor-induced myocardial protection is mediated by inhibition of mitochondrial membrane permeabilization. *The Journal of pharmacology and experimental therapeutics*. 2007;323(1):336-45. Epub 2007/07/21.
31. Rupprecht, R., G. Rammes, D. Eser, T.C. Baghai, C. Schule, C. Nothdurfter, T. Troxler, C. Gentsch, H.O. Kalkman, F. Chaperon, et al. Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. *Science*. 2009;325(5939):490-3.
32. Banati RB, Middleton RJ, Chan R, Hatty CR, Kam WW, Quin C, et al. Positron emission tomography and functional characterization of a complete PBR/TSPO knockout. *Nature communications*. 2014;5:5452.

33. Sileikyte J, Blachly-Dyson E, Sewell R, Carpi A, Menabo R, Di Lisa F, et al. Regulation of the Mitochondrial Permeability Transition Pore by the Outer Membrane does not Involve the Peripheral Benzodiazepine Receptor (TSPO). *J Biol Chem*. 2014. Epub 2014/04/03.
34. Tu LN, Morohaku K, Manna PR, Pelton SH, Butler WR, Stocco DM, et al. Peripheral benzodiazepine receptor/translocator protein global knock-out mice are viable with no effects on steroid hormone biosynthesis. *J Biol Chem*. 2014;289(40):27444-54.
35. Morohaku K, Pelton SH, Daugherty DJ, Butler WR, Deng W, Selvaraj V. Translocator protein/peripheral benzodiazepine receptor is not required for steroid hormone biosynthesis. *Endocrinology*. 2014;155(1):89-97. Epub 2013/11/01.
36. Tu LN, Zhao AH, Stocco DM, Selvaraj V. PK11195 effect on steroidogenesis is not mediated through the translocator protein (TSPO). *Endocrinology*. 2015;156(3):1033-9.
37. Alho H, Harjuntausta T, Schultz R, Pelto-Huikko M, Bovolín P. Immunohistochemistry of diazepam binding inhibitor (DBI) in the central nervous system and peripheral organs: its possible role as an endogenous regulator of different types of benzodiazepine receptors. *Neuropharmacology*. 1991;30(12B):1381-6.
38. Besman MJ, Yanagibashi K, Lee TD, Kawamura M, Hall PF, Shively JE. Identification of des-(Gly-Ile)-endozepine as an effector of corticotropin-dependent adrenal steroidogenesis: stimulation of cholesterol delivery is mediated by the peripheral benzodiazepine receptor. *Proc Natl Acad Sci U S A*. 1989;86(13):4897-901.
39. Vanhee C, Zapotoczny G, Masquelier D, Ghislain M, Batoko H. The Arabidopsis multistress regulator TSPO is a heme binding membrane protein and a potential scavenger of porphyrins via an autophagy-dependent degradation mechanism. *The Plant cell*. 2011;23(2):785-805.
40. Guo Y, Kalathur RC, Liu Q, Kloss B, Bruni R, Ginter C, et al. Protein structure. Structure and activity of tryptophan-rich TSPO proteins. *Science (New York, NY)*. 2015;347(6221):551-5.
41. Gatiloff J, East D, Crosby J, Abeti R, Harvey R, Craigen W, et al. TSPO interacts with VDAC1 and triggers a ROS-mediated inhibition of mitochondrial quality control. *Autophagy*. 2014:0.
42. Giatzakis C, Papadopoulos V. Differential utilization of the promoter of peripheral-type benzodiazepine receptor by steroidogenic versus nonsteroidogenic cell lines and the role of Sp1 and Sp3 in the regulation of basal activity. *Endocrinology*. 2004;145(3):1113-23. Epub 2003/11/25.
43. Batarseh A, Barlow KD, Martinez-Arguelles DB, Papadopoulos V. Functional characterization of the human translocator protein (18kDa) gene promoter in human breast cancer cell lines. *Biochim Biophys Acta*. 2012;1819(1):38-56. Epub 2011/10/01.
44. Oberto A, Longone P, Krueger KE. Identification of three transcriptional regulatory elements in the rat mitochondrial benzodiazepine receptor-encoding gene. *Gene*. 1995;167(1-2):255-60. Epub 1995/12/29.
45. Giatzakis C, Batarseh A, Dettin L, Papadopoulos V. The role of Ets transcription factors in the basal transcription of the translocator protein (18 kDa). *Biochemistry*. 2007;46(16):4763-74. Epub 2007/04/04.
46. Batarseh A, Giatzakis C, Papadopoulos V. Phorbol-12-myristate 13-acetate acting through protein kinase Cepsilon induces translocator protein (18-kDa) TSPO gene expression. *Biochemistry*. 2008;47(48):12886-99. Epub 2008/11/04.
47. Batarseh A, Li J, Papadopoulos V. Protein kinase C epsilon regulation of translocator protein (18 kDa) Tspo gene expression is mediated through a MAPK pathway targeting STAT3 and c-Jun transcription factors. *Biochemistry*. 2010;49(23):4766-78. Epub 2010/05/18.
48. Batarseh A, Papadopoulos V. Regulation of translocator protein 18 kDa (TSPO) expression in health and disease states. *Mol Cell Endocrinol*. 2010;327(1-2):1-12. Epub 2010/07/06.
49. Kruczek, C., B. Gorg, V. Keitel, E. Pirev, K.D. Kroncke, F. Schliess, and D. Haussinger. Hypoosmotic swelling affects zinc homeostasis in cultured rat astrocytes. *Glia*. 2009;57(1):79-92. Epub 2008/08/19.
50. Rey C, Mauduit C, Naureils O, Benahmed M, Louisot P, Gasnier F. Up-regulation of mitochondrial peripheral benzodiazepine receptor expression by tumor necrosis factor alpha in testicular leydig cells. Possible involvement in cell survival. *Biochemical pharmacology*. 2000;60(11):1639-46. Epub 2000/11/15.

51. Trincavelli ML, Marselli L, Falleni A, Gremigni V, Ragge E, Dotta F, et al. Upregulation of mitochondrial peripheral benzodiazepine receptor expression by cytokine-induced damage of human pancreatic islets. *Journal of cellular biochemistry*. 2002;84(3):636-44. Epub 2002/01/29.
52. Campioli E, Batarseh A, Li J, Papadopoulos V. The endocrine disruptor mono-(2-ethylhexyl) phthalate affects the differentiation of human liposarcoma cells (SW 872). *PloS one*. 2011;6(12):e28750. Epub 2011/12/30.
53. Gatliff J, Campanella M. TSPO is a REDOX regulator of cell mitophagy. *Biochemical Society Transactions*. 2015;43(4):543-52.
54. Basile AS, Ostrowski NL, Skolnick P. Aldosterone-reversible decrease in the density of renal peripheral benzodiazepine receptors in the rat after adrenalectomy. *The Journal of pharmacology and experimental therapeutics*. 1987;240(3):1006-13. Epub 1987/03/01.
55. Mazurika, C., L. Veenman, R. Weizman, M. Bidder, S. Leschiner, I. Golani, I. Spanier, G. Weisinger, and M. Gavish. Estradiol modulates uterine 18 kDa translocator protein gene expression in uterus and kidney of rats. *Mol Cell Endocrinol*. 2009;307(1-2):43-9. Epub 2009/06/16.
56. Bitran D, Carlson D, Leschiner S, Gavish M. Ovarian steroids and stress produce changes in peripheral benzodiazepine receptor density. *European journal of pharmacology*. 1998;361(2-3):235-42.
57. Bar-Ami S, Amiri Z, Fares F, Gavish M. Modulation of peripheral benzodiazepine receptors in female rat genital organs by various gonadal steroids. *Life sciences*. 1994;54(25):1965-75.
58. Gavish M, Okun F, Weizman A, Youdim MB. Modulation of peripheral benzodiazepine binding sites following chronic estradiol treatment. *European journal of pharmacology*. 1986;127(1-2):147-51.
59. Boujrad N, Gaillard JL, Garnier M, Papadopoulos V. Acute action of choriogonadotropin on Leydig tumor cells: induction of a higher affinity benzodiazepine-binding site related to steroid biosynthesis. *Endocrinology*. 1994;135(4):1576-83.
60. Whalin ME, Boujrad N, Papadopoulos V, Krueger KE. Studies on the phosphorylation of the 18 kDa mitochondrial benzodiazepine receptor protein. *Journal of receptor research*. 1994;14(3-4):217-28.
61. Fan J, Liu J, Culty M, Papadopoulos V. Acyl-coenzyme A binding domain containing 3 (ACBD3; PAP7; GCP60): an emerging signaling molecule. *Progress in lipid research*. 2010;49(3):218-34.
62. Liu J, Rone MB, Papadopoulos V. Protein-protein interactions mediate mitochondrial cholesterol transport and steroid biosynthesis. *J Biol Chem*. 2006;281(50):38879-93. Epub 2006/10/20.
63. Chen C, Kuo J, Wong A, Micevych P. Estradiol modulates translocator protein (TSPO) and steroid acute regulatory protein (StAR) via protein kinase A (PKA) signaling in hypothalamic astrocytes. *Endocrinology*. 2014;155(8):2976-85.
64. Pelechano V, Steinmetz LM. Gene regulation by antisense transcription. *Nature reviews Genetics*. 2013;14(12):880-93. Epub 2013/11/13.
65. Fan J, Papadopoulos V. Transcriptional regulation of translocator protein (Tspo) via a SINE B2-mediated natural antisense transcript in MA-10 Leydig cells. *Biol Reprod*. 2012;86(5):147, 1-15. Epub 2012/03/02.
66. Vega RB, Huss JM, Kelly DP. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Molecular and cellular biology*. 2000;20(5):1868-76. Epub 2000/02/12.
67. Hardwick M, Rone J, Han Z, Haddad B, Papadopoulos V. Peripheral-type benzodiazepine receptor levels correlate with the ability of human breast cancer MDA-MB-231 cell line to grow in SCID mice. *Int J Cancer*. 2001;94(3):322-7. Epub 2001/12/18.
68. Gazouli M, Yao ZX, Boujrad N, Corton JC, Culty M, Papadopoulos V. Effect of peroxisome proliferators on Leydig cell peripheral-type benzodiazepine receptor gene expression, hormone-stimulated cholesterol transport, and steroidogenesis: role of the peroxisome proliferator-activator receptor alpha. *Endocrinology*. 2002;143(7):2571-83. Epub 2002/06/20.
69. Boujrad N, Vidic B, Gazouli M, Culty M, Papadopoulos V. The peroxisome proliferator perfluorodecanoic acid inhibits the peripheral-type benzodiazepine receptor (PBR) expression and hormone-stimulated mitochondrial cholesterol transport and steroid formation in Leydig cells. *Endocrinology*. 2000;141(9):3137-48. Epub 2000/08/31.

70. Delerive P, De Bosscher K, Besnard S, Vanden Berghe W, Peters JM, Gonzalez FJ, et al. Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. *J Biol Chem.* 1999;274(45):32048-54. Epub 1999/11/05.
71. Poynter ME, Daynes RA. Peroxisome proliferator-activated receptor alpha activation modulates cellular redox status, represses nuclear factor-kappaB signaling, and reduces inflammatory cytokine production in aging. *J Biol Chem.* 1998;273(49):32833-41. Epub 1998/11/26.
72. Yang, S., C. Chen, H. Wang, X. Rao, F. Wang, Q. Duan, F. Chen, G. Long, W. Gong, M.H. Zou, and D.W. Wang. Protective effects of Acyl-coA thioesterase 1 on diabetic heart via PPARalpha/PGC1alpha signaling. *PloS one.* 2012;7(11):e50376. Epub 2012/12/12.
73. Gervois P, Mansouri RM. PPARAlpha as a therapeutic target in inflammation-associated diseases. *Expert opinion on therapeutic targets.* 2012;16(11):1113-25. Epub 2012/08/29.
74. Bernassau JM, Reversat JL, Ferrara P, Caput D, Lefur G. A 3D model of the peripheral benzodiazepine receptor and its implication in intra mitochondrial cholesterol transport. *J Mol Graph.* 1993;11(4):236-44. 5. Epub 1993/12/01.
75. Joseph-Liauzun E, Delmas P, Shire D, Ferrara P. Topological analysis of the peripheral benzodiazepine receptor in yeast mitochondrial membranes supports a five-transmembrane structure. *J Biol Chem.* 1998;273(4):2146-52. Epub 1998/01/27.
76. Murail, S., J.C. Robert, Y.M. Coic, J.M. Neumann, M.A. Ostuni, Z.X. Yao, V. Papadopoulos, N. Jamin, and J.J. Lacapere. Secondary and tertiary structures of the transmembrane domains of the translocator protein TSPO determined by NMR. Stabilization of the TSPO tertiary fold upon ligand binding. *Biochim Biophys Acta.* 2008;1778(6):1375-81. Epub 2008/04/19.
77. Yeliseev AA, Kaplan S. TspO of rhodobacter sphaeroides. A structural and functional model for the mammalian peripheral benzodiazepine receptor. *J Biol Chem.* 2000;275(8):5657-67. Epub 2000/02/22.
78. Korkhov VM. Three-dimensional structure of TspO by electron cryomicroscopy of helical crystals. *Structure.* 2010;18(6):677.
79. Lacapere JJ, Delavoie F, Li H, Peranzi G, Maccario J, Papadopoulos V, et al. Structural and functional study of reconstituted peripheral benzodiazepine receptor. *Biochemical and biophysical research communications.* 2001;284(2):536-41.
80. Jaremko L, Jaremko M, Giller K, Becker S, Zweckstetter M. Structure of the mitochondrial translocator protein in complex with a diagnostic ligand. *Science (New York, NY).* 2014;343(6177):1363-6. Epub 2014/03/22.
81. Li F, Xia Y, Meiler J, Ferguson-Miller S. Characterization and modeling of the oligomeric state and ligand binding behavior of purified translocator protein 18 kDa from Rhodobacter sphaeroides. *Biochemistry.* 2013;52(34):5884-99.
82. Scarf AM, Auman KM, Kassiou M. Is there any correlation between binding and functional effects at the translocator protein (TSPO) (18 kDa)? *Current molecular medicine.* 2012;12(4):387-97.
83. Li H, Yao Z, Degenhardt B, Teper G, Papadopoulos V. Cholesterol binding at the cholesterol recognition/ interaction amino acid consensus (CRAC) of the peripheral-type benzodiazepine receptor and inhibition of steroidogenesis by an HIV TAT-CRAC peptide. *Proc Natl Acad Sci U S A.* 2001;98(3):1267-72. Epub 2001/02/07.
84. Teboul D, Beaufils S, Taveau JC, Iatmanen-Harbi S, Renault A, Venien-Bryan C, et al. Mouse TSPO in a lipid environment interacting with a functionalized monolayer. *Biochim Biophys Acta.* 2012;1818(11):2791-800.
85. Delavoie F, Li H, Hardwick M, Robert JC, Giatzakis C, Peranzi G, et al. In vivo and in vitro peripheral-type benzodiazepine receptor polymerization: functional significance in drug ligand and cholesterol binding. *Biochemistry.* 2003;42(15):4506-19.
86. Lacapere JJ, Papadopoulos V. Peripheral-type benzodiazepine receptor: structure and function of a cholesterol-binding protein in steroid and bile acid biosynthesis. *Steroids.* 2003;68(7-8):569-85. Epub 2003/09/06.
87. Costa B, Pini S, Martini C, Abelli M, Gabelloni P, Landi S, et al. Ala147Thr substitution in translocator protein is associated with adult separation anxiety in patients with depression. *Psychiatric genetics.* 2009;19(2):110-1.

88. Kreisl, W.C., K.J. Jenko, C.S. Hines, C.H. Lyoo, W. Corona, C.L. Morse, S.S. Zoghbi, T. Hyde, J.E. Kleinman, V.W. Pike, F.J. McMahon, R.B. Innis, and P.E.T.R.P.T. Biomarkers Consortium. A genetic polymorphism for translocator protein 18 kDa affects both in vitro and in vivo radioligand binding in human brain to this putative biomarker of neuroinflammation. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2013;33(1):53-8.
89. Colasanti A, Owen DR, Grozeva D, Rabiner EA, Matthews PM, Craddock N, et al. Bipolar Disorder is associated with the rs6971 polymorphism in the gene encoding 18 kDa Translocator Protein (TSPO). *Psychoneuroendocrinology*. 2013;38(11):2826-9.
90. Costa B, Pini S, Gabelloni P, Da Pozzo E, Abelli M, Lari L, et al. The spontaneous Ala147Thr amino acid substitution within the translocator protein influences pregnenolone production in lymphomonocytes of healthy individuals. *Endocrinology*. 2009;150(12):5438-45.
91. Li F, Liu J, Valls L, Hiser C, Ferguson-Miller S. Identification of a key cholesterol binding enhancement motif in translocator protein 18 kDa. *Biochemistry*. 2015;54(7):1441-3.
92. Li F, Liu J, Zheng Y, Garavito RM, Ferguson-Miller S. Protein structure. Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism. *Science (New York, NY)*. 2015;347(6221):555-8.
93. Owen DR, Yeo AJ, Gunn RN, Song K, Wadsworth G, Lewis A, et al. An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2012;32(1):1-5.
94. Mizrahi, R., P.M. Rusjan, J. Kennedy, B. Pollock, B. Mulsant, I. Suridjan, V. De Luca, A.A. Wilson, and S. Houle. Translocator protein (18 kDa) polymorphism (rs6971) explains in-vivo brain binding affinity of the PET radioligand [(18F)-FEPPA. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2012;32(6):968-72.
95. Yoder KK, Nho K, Risacher SL, Kim S, Shen L, Saykin AJ. Influence of TSPO genotype on 11C-PBR28 standardized uptake values. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*. 2013;54(8):1320-2.
96. Guo, Q., A. Colasanti, D.R. Owen, M. Onega, A. Kamalakaran, I. Bennacef, P.M. Matthews, E.A. Rabiner, F.E. Turkheimer, and R.N. Gunn. Quantification of the specific translocator protein signal of 18F-PBR111 in healthy humans: a genetic polymorphism effect on in vivo binding. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*. 2013;54(11):1915-23.
97. Jaremko M, Jaremko L, Giller K, Becker S, Zweckstetter M. Structural integrity of the A147T polymorph of mammalian TSPO. *Chembiochem : a European journal of chemical biology*. 2015.
98. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocrine reviews*. 2011;32(1):81-151. Epub 2010/11/06.
99. Epstein LF, Orme-Johnson NR. Regulation of steroid hormone biosynthesis. Identification of precursors of a phosphoprotein targeted to the mitochondrion in stimulated rat adrenal cortex cells. *J Biol Chem*. 1991;266(29):19739-45. Epub 1991/10/15.
100. Clark BJ, Wells J, King SR, Stocco DM. The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). *J Biol Chem*. 1994;269(45):28314-22. Epub 1994/11/11.
101. Bose HS, Lingappa VR, Miller WL. Rapid regulation of steroidogenesis by mitochondrial protein import. *Nature*. 2002;417(6884):87-91. Epub 2002/05/03.
102. Petrescu AD, Gallegos AM, Okamura Y, Strauss JF, 3rd, Schroeder F. Steroidogenic acute regulatory protein binds cholesterol and modulates mitochondrial membrane sterol domain dynamics. *J Biol Chem*. 2001;276(40):36970-82.
103. Hauet, T., J. Liu, H. Li, M. Gazouli, M. Culty, and V. Papadopoulos. Peripheral-type benzodiazepine receptor-mediated action of steroidogenic acute regulatory protein on cholesterol entry into leydig cell mitochondria. *Mol Endocrinol*. 2005;19(2):540-54. Epub 2004/10/23.
104. Papadopoulos V. Peripheral-type benzodiazepine/diazepam binding inhibitor receptor: biological role in steroidogenic cell function. *Endocrine reviews*. 1993;14(2):222-40. Epub 1993/04/01.

105. Hauet T, Liu J, Li H, Gazouli M, Culty M, Papadopoulos V. PBR, StAR, and PKA: partners in cholesterol transport in steroidogenic cells. *Endocrine research*. 2002;28(4):395-401.
106. Jamin, N., J.M. Neumann, M.A. Ostuni, T.K. Vu, Z.X. Yao, S. Murail, J.C. Robert, C. Giatzakis, V. Papadopoulos, and J.J. Lacapere. Characterization of the cholesterol recognition amino acid consensus sequence of the peripheral-type benzodiazepine receptor. *Mol Endocrinol*. 2005;19(3):588-94. Epub 2004/11/06.
107. Midzak A, Akula N, Lecanu L, Papadopoulos V. Novel androstenetriol interacts with the mitochondrial translocator protein and controls steroidogenesis. *J Biol Chem*. 2011;286(11):9875-87. Epub 2011/01/07.
108. Midzak AS, Akula N, Rone MB, Papadopoulos V. Computational modeling and biological validation of novel non-steroidal ligands for the cholesterol recognition/interaction amino acid consensus (CRAC) motif of the mitochondrial translocator protein (TSPO). *Pharmacological research : the official journal of the Italian Pharmacological Society*. 2015.
109. Rone MB, Fan J, Papadopoulos V. Cholesterol transport in steroid biosynthesis: role of protein-protein interactions and implications in disease states. *Biochim Biophys Acta*. 2009;1791(7):646-58. Epub 2009/03/17.
110. Tu, L.N., K. Morohaku, P.R. Manna, S.H. Pelton, W.R. Butler, D.M. Stocco, and V. Selvaraj. Peripheral Benzodiazepine Receptor/Translocator Protein Global Knockout Mice are Viable with no Effects on Steroid Hormone Biosynthesis. *J Biol Chem*. 2014. Epub 2014/06/18.
111. Fan J, Campioli E, Midzak A, Culty M, Papadopoulos V. Conditional steroidogenic cell-targeted deletion of TSPO unveils a crucial role in viability and hormone-dependent steroid formation. *Proc Natl Acad Sci U S A*. 2015.
112. Papadopoulos V, Aghazadeh Y, Fan J, Campioli E, Zirkin B, Midzak A. Translocator protein-mediated pharmacology of cholesterol transport and steroidogenesis. *Mol Cell Endocrinol*. 2015;408:90-8.
113. Taylor JM, Allen AM, Graham A. Targeting mitochondrial 18 kDa translocator protein (TSPO) regulates macrophage cholesterol efflux and lipid phenotype. *Clinical science*. 2014;127(10):603-13.
114. Decker C, Faccio R, editors. Tmem178 Is A Negative Regulator Of Bone Homeostasis And Osteoclast Activation In Mice And Humans Via A Novel Negative Feedback Loop Targeting Endoplasmic Reticulum Ca²⁺ Mobilization. Orthopaedic Research Society (ORS) Annual Meeting; 2015; Las Vegas, NV, USA.
115. Wang L, Wildt KF, Castro E, Xiong Y, Feigenbaum L, Tessarollo L, et al. The zinc finger transcription factor Zbtb7b represses CD8-lineage gene expression in peripheral CD4⁺ T cells. *Immunity*. 2008;29(6):876-87.
116. Enders A, Stankovic S, Teh C, Uldrich AP, Yabas M, Juelich T, et al. ZBTB7B (Th-POK) regulates the development of IL-17-producing CD1d-restricted mouse NKT cells. *Journal of immunology*. 2012;189(11):5240-9.
117. Zhou T, Dang Y, Zheng YH. The mitochondrial translocator protein, TSPO, inhibits HIV-1 envelope glycoprotein biosynthesis via the endoplasmic reticulum-associated protein degradation pathway. *Journal of virology*. 2014;88(6):3474-84.
118. Brunette RL, Young JM, Whitley DG, Brodsky IE, Malik HS, Stetson DB. Extensive evolutionary and functional diversity among mammalian AIM2-like receptors. *The Journal of experimental medicine*. 2012;209(11):1969-83.
119. Chen L, Yang G. PPARs Integrate the Mammalian Clock and Energy Metabolism. *PPAR research*. 2014;2014:653017.
120. Yang X, Zhang YK, Esterly N, Klaassen CD, Wan YJ. Gender disparity of hepatic lipid homeostasis regulated by the circadian clock. *Journal of biochemistry*. 2009;145(5):609-23.
121. Maury E, Ramsey KM, Bass J. Circadian rhythms and metabolic syndrome: from experimental genetics to human disease. *Circulation research*. 2010;106(3):447-62.
122. Golani I, Weizman A, Leschiner S, Spanier I, Eckstein N, Limor R, et al. Hormonal regulation of peripheral benzodiazepine receptor binding properties is mediated by subunit interaction. *Biochemistry*. 2001;40(34):10213-22. Epub 2001/08/22.
123. Papadopoulos V, Boujrad N, Ikonovic MD, Ferrara P, Vidic B. Topography of the Leydig cell mitochondrial peripheral-type benzodiazepine receptor. *Mol Cell Endocrinol*. 1994;104(1):R5-9. Epub 1994/08/01.

124. Aghazadeh Y, Martinez-Arguelles DB, Fan J, Culty M, Papadopoulos V. Induction of androgen formation in the male by a TAT-VDAC1 fusion peptide blocking 14-3-3varepsilon protein adaptor and mitochondrial VDAC1 interactions. *Molecular therapy : the journal of the American Society of Gene Therapy*. 2014;22(10):1779-91.
125. Campanella M. Peptide targeting of mitochondria elicits testosterone formation. *Molecular therapy : the journal of the American Society of Gene Therapy*. 2014;22(10):1727-9.
126. Doperalski NJ, Martyniuk CJ, Prucha MS, Kroll KJ, Denslow ND, Barber DS. Cloning and expression of the translocator protein (18 kDa), voltage-dependent anion channel, and diazepam binding inhibitor in the gonad of largemouth bass (*Micropterus salmoides*) across the reproductive cycle. *General and comparative endocrinology*. 2011;173(1):86-95.
127. Aghazadeh Y, Rone MB, Blonder J, Ye X, Veenstra TD, Hales DB, et al. Hormone-induced 14-3-3gamma adaptor protein regulates steroidogenic acute regulatory protein activity and steroid biosynthesis in MA-10 Leydig cells. *J Biol Chem*. 2012;287(19):15380-94.
128. Rosenberg N, Rosenberg O, Weizman A, Veenman L, Gavish M. In vitro effect of FGIN-1-27, a ligand to 18 kDa mitochondrial translocator protein, in human osteoblast-like cells. *Journal of bioenergetics and biomembranes*. 2014;46(3):197-204.
129. Rosenberg N, Rosenberg O, Weizman A, Veenman L, Gavish M. In vitro catabolic effect of protoporphyrin IX in human osteoblast-like cells: possible role of the 18 kDa mitochondrial translocator protein. *Journal of bioenergetics and biomembranes*. 2013;45(4):333-41.
130. Rosenberg, N., O. Rosenberg, A. Weizman, S. Leschiner, Y. Sakoury, F. Fares, M. Soudry, G. Weisinger, L. Veenman, and M. Gavish. In vitro mitochondrial effects of PK 11195, a synthetic translocator protein 18 kDa (TSPO) ligand, in human osteoblast-like cells. *Journal of bioenergetics and biomembranes*. 2011;43(6):739-46.
131. Amitani M, Ohashi A, Hatazawa J, Gee A, Inoue O. Effect of PK11195 on attenuating the enhancement of glucose utilization induced by quinolinic acid infusion in the rat brain. *Synapse*. 2008;62(4):253-8.
132. Joo, H.K., Y.R. Lee, S.Y. Lim, E.J. Lee, S. Choi, E.J. Cho, M.S. Park, S. Ryoo, J.B. Park, and B.H. Jeon. Peripheral benzodiazepine receptor regulates vascular endothelial activations via suppression of the voltage-dependent anion channel-1. *FEBS Lett*. 2012;586(9):1349-55. Epub 2012/05/24.
133. Cho JH, Park JH, Chung CG, Shim HJ, Jeon KH, Yu SW, et al. Parkin-mediated responses against infection and wound involve TSPO-VDAC complex in *Drosophila*. *Biochemical and biophysical research communications*. 2015;463(1-2):1-6.
134. Raghavan A, Sheiko T, Graham BH, Craigen WJ. Voltage-dependant anion channels: novel insights into isoform function through genetic models. *Biochim Biophys Acta*. 2012;1818(6):1477-85.
135. Miettinen, H., J. Kononen, H. Haapasalo, P. Helen, P. Sallinen, T. Harjuntausta, H. Helin, and H. Alho. Expression of peripheral-type benzodiazepine receptor and diazepam binding inhibitor in human astrocytomas: relationship to cell proliferation. *Cancer Res*. 1995;55(12):2691-5. Epub 1995/06/15.
136. Fafalios A, Akhavan A, Parwani AV, Bies RR, McHugh KJ, Pflug BR. Translocator protein blockade reduces prostate tumor growth. *Clin Cancer Res*. 2009;15(19):6177-84. Epub 2009/10/01.
137. Nagler, R., O. Ben-Izhak, D. Savulescu, E. Krayzler, S. Akrish, S. Leschiner, I. Otradnov, S. Zeno, L. Veenman, and M. Gavish. Oral cancer, cigarette smoke and mitochondrial 18kDa translocator protein (TSPO) - In vitro, in vivo, salivary analysis. *Biochim Biophys Acta*. 2010;1802(5):454-61. Epub 2010/01/21.
138. Konigsrainer, I., U.F. Vogel, S. Beckert, K. Sotlar, S. Coerper, A. Braun, N. Lember, W. Steurer, A. Konigsrainer, and S. Kupka. Increased translocator protein (TSPO) mRNA levels in colon but not in rectum carcinoma. *Eur Surg Res*. 2007;39(6):359-63. Epub 2007/07/27.
139. Beinlich A, Strohmeier R, Kaufmann M, Kuhl H. Relation of cell proliferation to expression of peripheral benzodiazepine receptors in human breast cancer cell lines. *Biochemical pharmacology*. 2000;60(3):397-402. Epub 2000/06/17.
140. Galiegue S, Casellas P, Kramar A, Tinel N, Simony-Lafontaine J. Immunohistochemical assessment of the peripheral benzodiazepine receptor in breast cancer and its relationship with survival. *Clin Cancer Res*. 2004;10(6):2058-64. Epub 2004/03/26.

141. Sutter, A.P., K. Maaser, M. Hopfner, B. Barthel, P. Grabowski, S. Faiss, P. Carayon, M. Zeitz, and H. Scherubl. Specific ligands of the peripheral benzodiazepine receptor induce apoptosis and cell cycle arrest in human esophageal cancer cells. *Int J Cancer*. 2002;102(4):318-27. Epub 2002/10/29.
142. Batra S, Iosif CS. Peripheral benzodiazepine receptor in human endometrium and endometrial carcinoma. *Anticancer Res*. 2000;20(1A):463-6. Epub 2000/04/19.
143. Lavisse S, Guillermier M, Herard AS, Petit F, Delahaye M, Van Camp N, et al. Reactive astrocytes overexpress TSPO and are detected by TSPO positron emission tomography imaging. *J Neurosci*. 2012;32(32):10809-18. Epub 2012/08/10.
144. Owen DR, Matthews PM. Imaging brain microglial activation using positron emission tomography and translocator protein-specific radioligands. *International review of neurobiology*. 2011;101:19-39. Epub 2011/11/05.
145. Libby P, Tabas I, Fredman G, Fisher EA. Inflammation and its resolution as determinants of acute coronary syndromes. *Circulation research*. 2014;114(12):1867-79. Epub 2014/06/07.
146. Kumar A, Muzik O, Shandal V, Chugani D, Chakraborty P, Chugani HT. Evaluation of age-related changes in translocator protein (TSPO) in human brain using (11)C-[R]-PK11195 PET. *J Neuroinflammation*. 2012;9:232. Epub 2012/10/06.
147. Corcia, P., C. Tauber, J. Vercoullie, N. Arlicot, C. Prunier, J. Praline, G. Nicolas, Y. Venel, C. Hommet, J.L. Baulieu, J.P. Cottier, C. Roussel, M. Kassiou, D. Guilloteau, and M.J. Ribeiro. Molecular imaging of microglial activation in amyotrophic lateral sclerosis. *PloS one*. 2012;7(12):e52941. Epub 2013/01/10.
148. Yasuno, F., J. Kosaka, M. Ota, M. Higuchi, H. Ito, Y. Fujimura, S. Nozaki, S. Takahashi, K. Mizukami, T. Asada, and T. Suhara Increased binding of peripheral benzodiazepine receptor in mild cognitive impairment-dementia converters measured by positron emission tomography with [(1)(1)C]DAA1106. *Psychiatry research*. 2012;203(1):67-74. Epub 2012/08/16.
149. Cagnin, A., D.J. Brooks, A.M. Kennedy, R.N. Gunn, R. Myers, F.E. Turkheimer, T. Jones, and R.B. Banati. In-vivo measurement of activated microglia in dementia. *Lancet*. 2001;358(9280):461-7. Epub 2001/08/22.
150. Varrone, A., P. Mattsson, A. Forsberg, A. Takano, S. Nag, B. Gulyas, J. Borg, R. Boellaard, N. Al-Tawil, M. Eriksdotter, T. Zimmermann, M. Schultze-Mosgau, A. Thiele, A. Hoffmann, A.A. Lammertsma, and C. Halldin. In vivo imaging of the 18-kDa translocator protein (TSPO) with [18F]FEDAA1106 and PET does not show increased binding in Alzheimer's disease patients. *European journal of nuclear medicine and molecular imaging*. 2013;40(6):921-31. Epub 2013/02/26.
151. Maia, S., N. Arlicot, E. Vierron, S. Bodard, J. Vergote, D. Guilloteau, and S. Chalon. Longitudinal and parallel monitoring of neuroinflammation and neurodegeneration in a 6-hydroxydopamine rat model of Parkinson's disease. *Synapse*. 2012;66(7):573-83. Epub 2012/02/18.
152. Ouchi, Y., E. Yoshikawa, Y. Sekine, M. Futatsubashi, T. Kanno, T. Ogusu, and T. Torizuka. Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann Neurol*. 2005;57(2):168-75. Epub 2005/01/26.
153. Gerhard, A., N. Pavese, G. Hotton, F. Turkheimer, M. Es, A. Hammers, K. Eggert, W. Oertel, R.B. Banati, and D.J. Brooks. In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol Dis*. 2006;21(2):404-12. Epub 2005/09/27.
154. Gulyas, B., M. Toth, M. Schain, A. Airaksinen, A. Vas, K. Kostulas, P. Lindstrom, J. Hillert, and C. Halldin. Evolution of microglial activation in ischaemic core and peri-infarct regions after stroke: a PET study with the TSPO molecular imaging biomarker [((11)C]vinpocetine. *Journal of the neurological sciences*. 2012;320(1-2):110-7. Epub 2012/07/31.
155. Cuhlmann, S., W. Gsell, K. Van der Heiden, J. Habib, J.L. Tremoleda, M. Khalil, F. Turkheimer, M.J. Meens, B.R. Kwak, J. Bird, A.P. Davenport, J. Clark, D. Haskard, R. Krams, H. Jones, and P.C. Evans. In Vivo Mapping of Vascular Inflammation Using the Translocator Protein Tracer 18F-FEDAA1106. *Molecular imaging*. 2014;13(0):1-10. Epub 2014/05/16.
156. Bird, J.L., D. Izquierdo-Garcia, J.R. Davies, J.H. Rudd, K.C. Probst, N. Figg, J.C. Clark, P.L. Weissberg, A.P. Davenport, and E.A. Warburton. Evaluation of translocator protein quantification as a tool for characterising macrophage burden in human carotid atherosclerosis. *Atherosclerosis*. 2010;210(2):388-91. Epub 2010/01/09.

157. Karlstetter, M., C. Nothdurfter, A. Aslanidis, K. Moeller, F. Horn, R. Scholz, H. Neumann, B.H. Weber, R. Rupprecht, and T. Langmann. Translocator protein (18 kDa) (TSPO) is expressed in reactive retinal microglia and modulates microglial inflammation and phagocytosis. *J Neuroinflammation*. 2014;11:3. Epub 2014/01/09.
158. Zhao YY, Yu JZ, Li QY, Ma CG, Lu CZ, Xiao BG. TSPO-specific ligand vinpocetine exerts a neuroprotective effect by suppressing microglial inflammation. *Neuron glia biology*. 2011;7(2-4):187-97. Epub 2012/08/10.
159. Le Fur, G., M.L. Perrier, N. Vaucher, F. Imbault, A. Flamier, J. Benavides, A. Uzan, C. Renault, M.C. Dubroeuq, and C. Gueremy. Peripheral benzodiazepine binding sites: effect of PK 11195, 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide. I. In vitro studies. *Life sciences*. 1983;32(16):1839-47. Epub 1983/04/18.
160. Verma A, Nye JS, Snyder SH. Porphyrins are endogenous ligands for the mitochondrial (peripheral-type) benzodiazepine receptor. *Proc Natl Acad Sci U S A*. 1987;84(8):2256-60. Epub 1987/04/01.
161. Pastorino JG, Simbula G, Gilfor E, Hoek JB, Farber JL. Protoporphyrin IX, an endogenous ligand of the peripheral benzodiazepine receptor, potentiates induction of the mitochondrial permeability transition and the killing of cultured hepatocytes by rotenone. *J Biol Chem*. 1994;269(49):31041-6. Epub 1994/12/09.
162. Gonzalez-Polo, R.A., G. Carvalho, T. Braun, D. Decaudin, C. Fabre, N. Larochette, J.L. Perfettini, M. Djavaheri-Mergny, I. Youlyouz-Marfak, P. Codogno, M. Raphael, J. Feuillard, and G. Kroemer. PK11195 potently sensitizes to apoptosis induction independently from the peripheral benzodiazepine receptor. *Oncogene*. 2005;24(51):7503-13. Epub 2005/08/11.
163. Gastaldello A, Callaghan H, Gami P, Campanella M. Ca²⁺-dependent autophagy is enhanced by the pharmacological agent PK11195: Pharmacological tools in autophagy. *Autophagy*. 2010;6(5). Epub 2010/05/01.
164. Le Fur, G., F. Guilloux, P. Rufat, J. Benavides, A. Uzan, C. Renault, M.C. Dubroeuq, and C. Gueremy. Peripheral benzodiazepine binding sites: effect of PK 11195, 1-(2-chlorophenyl)-N-methyl-(1-methylpropyl)-3 isoquinolinecarboxamide. II. In vivo studies. *Life Sci*. 1983;32(16):1849-56. Epub 1983/04/18.
165. Casellas P, Galiegue S, Basile AS. Peripheral benzodiazepine receptors and mitochondrial function. *Neurochem Int*. 2002;40(6):475-86. Epub 2002/02/19.
166. Romeo, E., J. Auta, A.P. Kozikowski, D. Ma, V. Papadopoulos, G. Puia, E. Costa, and A. Guidotti. 2-Aryl-3-indoleacetamides (FGIN-1): a new class of potent and specific ligands for the mitochondrial DBI receptor (MDR). *The Journal of pharmacology and experimental therapeutics*. 1992;262(3):971-8. Epub 1992/09/01.
167. Guillon J, Boulouard M, Lelong V, Dallemagne P, Rault S, Jarry C. Synthesis and preliminary behavioural evaluation in mice of new 3-aryl-3-pyrrol-1-ylpropanamides, analogues of FGIN-1-27 and FGIN-1-43. *The Journal of pharmacy and pharmacology*. 2001;53(11):1561-8. Epub 2001/12/06.
168. Chauveau, F., H. Boutin, N. Van Camp, C. Thominaux, P. Hantraye, L. Rivron, F. Marguet, M.N. Castel, T. Rooney, J. Benavides, F. Dolle, and B. Tavitian. In vivo imaging of neuroinflammation in the rodent brain with [¹¹C]SSR180575, a novel indoleacetamide radioligand of the translocator protein (18 kDa). *European journal of nuclear medicine and molecular imaging*. 2011;38(3):509-14. Epub 2010/10/12.
169. Tang D, Nickels ML, Tantawy MN, Buck JR, Manning HC. Preclinical Imaging Evaluation of Novel TSPO-PET Ligand 2-(5,7-Diethyl-2-(4-(2-[¹⁸F]fluoroethoxy)phenyl)pyrazolo[1,5-a]pyrimidin-3-yl)-N,N-diethylacetamide ([¹⁸F]VU0152801) in Glioma. *Molecular imaging and biology* : MIB : the official publication of the Academy of Molecular Imaging. 2014. Epub 2014/05/23.
170. Selvaraj V, Stocco DM. The changing landscape in translocator protein (TSPO) function. *Trends Endocrinol Metab*. 2015;26(7):341-8.
171. Li, X.B., H.L. Guo, T.Y. Shi, L. Yang, M. Wang, K. Zhang, Y.Y. Guo, Y.M. Wu, S.B. Liu, and M.G. Zhao. Neuroprotective effects of a novel translocator protein (18 kDa) ligand, ZBD-2, against focal cerebral ischemia in vivo and NMDA-induced neurotoxicity in vitro. *Clinical and experimental pharmacology & physiology*. 2015.

172. Arbo BD, Benetti F, Garcia-Segura LM, Ribeiro MF. Therapeutic actions of translocator protein (18kDa) ligands in experimental models of psychiatric disorders and neurodegenerative diseases. *The Journal of steroid biochemistry and molecular biology*. 2015;154:68-74.
173. Owen DR, Lewis AJ, Reynolds R, Rupprecht R, Eser D, Wilkins MR, et al. Variation in binding affinity of the novel anxiolytic XBD173 for the 18 kDa translocator protein in human brain. *Synapse (New York, NY)*. 2011;65(3):257-9.
174. Xiao J, Liang D, Zhang H, Liu Y, Li F, Chen YH. 4'-Chlorodiazepam, a translocator protein (18 kDa) antagonist, improves cardiac functional recovery during postischemia reperfusion in rats. *Exp Biol Med (Maywood)*. 2010;235(4):478-86. Epub 2010/04/22.
175. Schaller, S., S. Paradis, G.A. Ngoh, R. Assaly, B. Buisson, C. Drouot, M.A. Ostuni, J.J. Lacapere, F. Bassissi, T. Bordet, A. Berdeaux, S.P. Jones, D. Morin, and R.M. Pruss. TRO40303, a new cardioprotective compound, inhibits mitochondrial permeability transition. *The Journal of pharmacology and experimental therapeutics*. 2010;333(3):696-706.

Legends to Figures

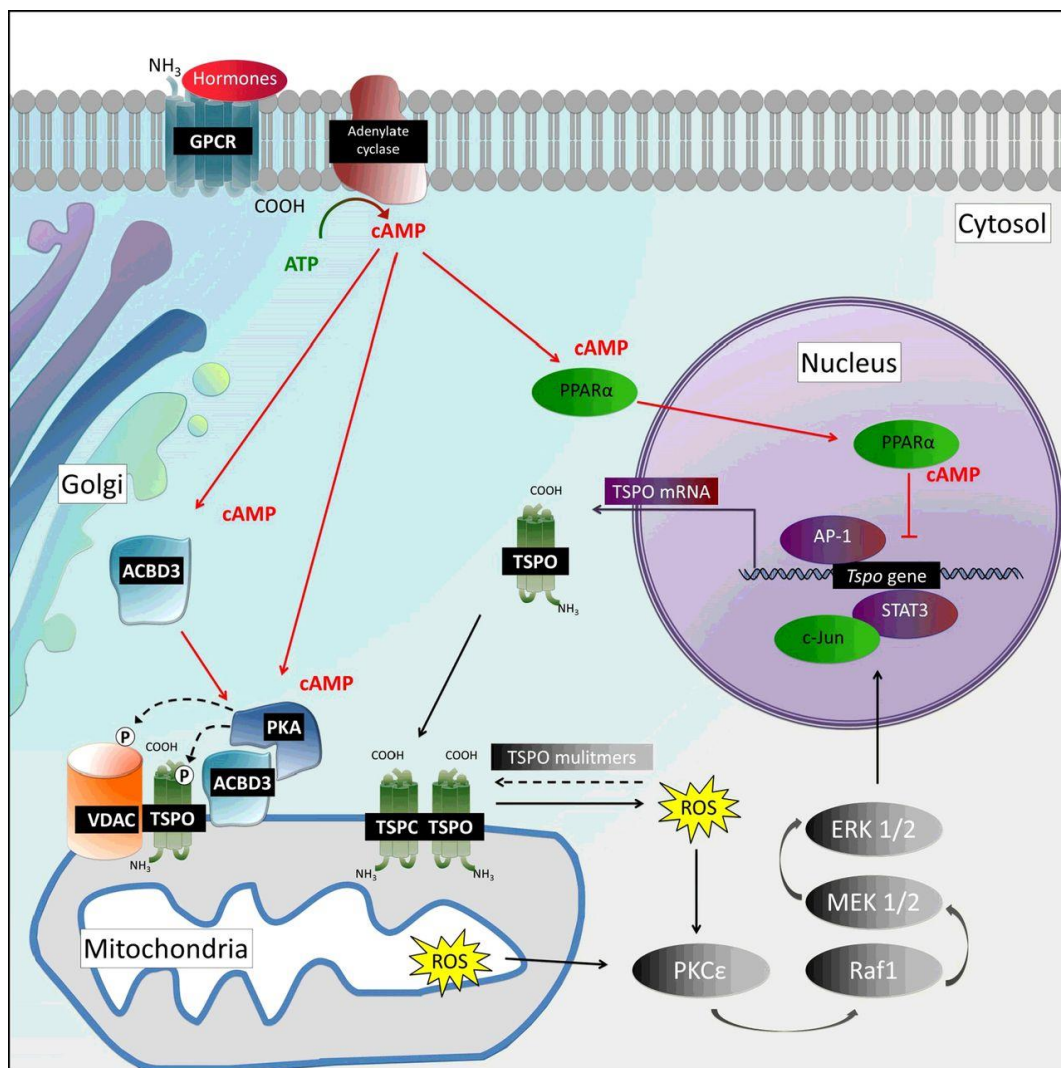


Figure 1: TSPO transcriptional regulation- crosstalk between hormone and REDOX sensitive signal transduction pathways

Hormonal stimuli that bind to G-protein coupled receptors (GPCR) in the plasma membrane results in cAMP synthesis from ATP via adenylate cyclases. cAMP is a signaling molecule that triggers the release of ACBD3 from the Golgi, which translocates to mitochondria, where it recruits PKA through its interaction with TSPO. PKA has been demonstrated to phosphorylate TSPO and VDAC and its kinase activity is dependent upon local concentrations of cAMP. cAMP also activates PPARα, which interferes with *Tspo* transcription factors including AP-1. TSPO is also sensitive to changes in REDOX status. Cytoplasmic or mitochondrial sources of ROS may activate the PKCε-Raf1-MEK1/2-ERK1/2 signaling cascade, which promotes *Tspo* transcription via the activity of c-Jun and STAT-3 transcription factors. An increase in TSPO may potentiate this response by maintaining an oxidative environment. ROS have also been demonstrated as promoting the formation of TSPO multimers. VDAC – voltage dependent anion channel; TSPO – translocator protein; ACBD3 – Acyl-coenzyme A binding domain containing 3; PKA – cAMP dependent protein kinase A; cAMP - cyclic adenosine monophosphate; ROS – reactive oxygen species; PPARα - peroxisome proliferator-activated receptor alpha; STAT3 – signal transducer and activator of transcription 3; AP-1 – activator protein 1

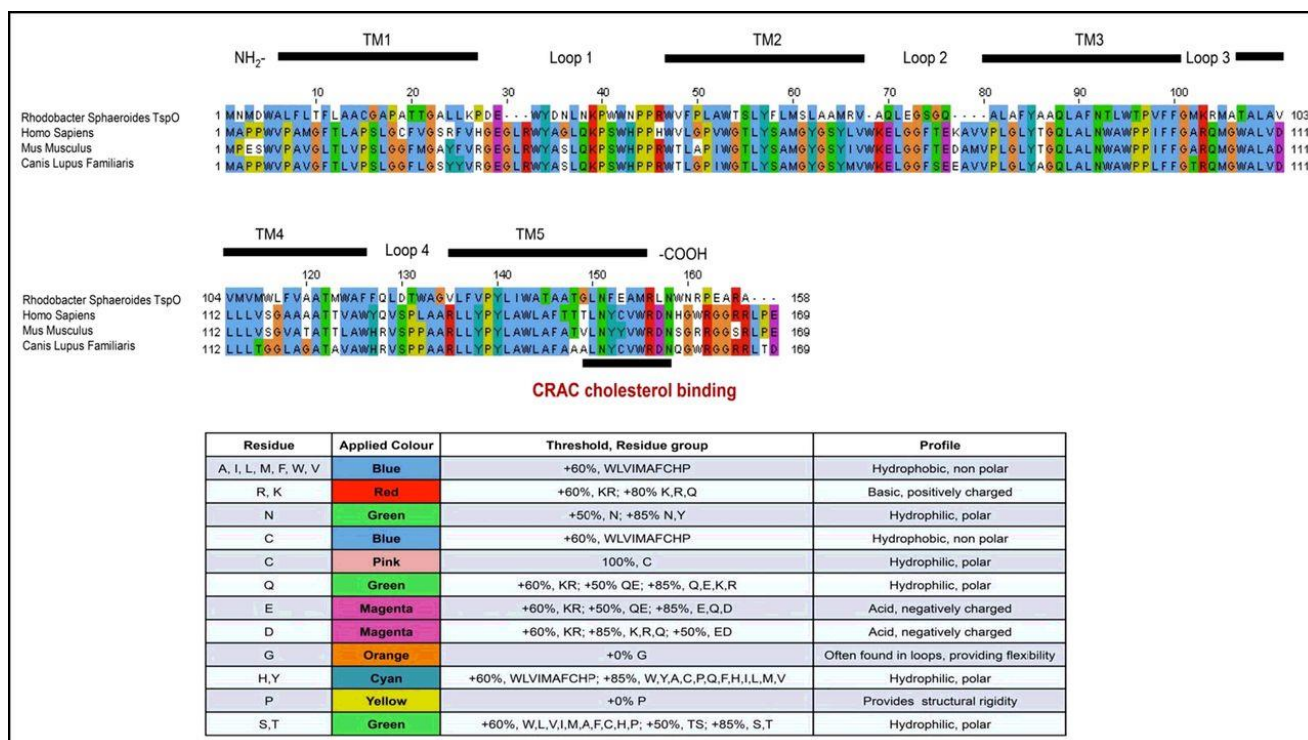


Figure 2: TSPO domain architecture and cross-species sequence alignment

Sequence alignment of TSPO using ClustalX software, showing conserved regions including the cholesterol-binding domain (CRAC sequence) in rhodobacter sphaeroides TspO, Homo sapiens, mus musculus and canis lupus familiaris. Transmembrane (TM) domains are denoted by black lines (TM1: 6-26 a.a; TM2: 47-67 a.a; TM3: 80-100 a.a; TM4: 106-126 a.a; TM5: 135-155 a.a.). Loops 1 (6-26) and 3 are cytosolic; loops 2 and 4 are facing the mitochondrial intermembrane space. The longer cytosolic loop 1 is implicated in dimerization, possible VDAC interaction, and the binding of ligands. b) The default colour scheme used for sequence alignments generated in ClustalX, the graphical interface for the ClustalW multiple sequence alignment programs. Residues in the alignment are assigned a colour according to the amino acid profile. The table shown gives the criteria as clauses +X%, xx, y; where X is the minimum percentage presence of any of the xx or y residues accompanied by a functional profile. White regions are unconserved.

Table 1: Phenotypic characterization of global and conditional *Tspo*^{-/-} mice

	Global C57BL/6- <i>Tspo</i> ^{tm1GuMu(GuwiyangWurra)}	Global (Tu et al)	<i>Nr5a1</i> -Cre Driven <i>Tspo</i> Conditional Knockout
Mitochondria phenotype	reduced microglial mitochondrial metabolic activity	Mitochondrial volume in primary fibroblasts unchanged; Mitochondrial morphology unchanged in testis and ovary	
Body weight	No differences observed	Increased body weight in female mice (~1g, 1-5 weeks of age)	
Lipid metabolism		No effect on lipid deposits in adrenals	Depletion of lipid storage in testis; Increased accumulation of lipids in adrenals
Hormone-induced steroidogenesis		hCG-induced plasma testosterone unchanged Increased levels of plasma estradiol	Impaired ACTH stimulation of corticosterone production hCG-induced plasma testosterone unchanged Increased epinephrine production
Steroidogenesis	No change in basal steroidogenesis	No change in basal steroidogenesis	No change in basal steroidogenesis
Immune response	Microglial activation following neuronal injury normal; Increased levels of NKT cells in female mice		

Table 2: Compensatory gene expression in *Tspo*^{-/-} mice: focus on the immune response and hormone regulation

TSPO Function	Gene	Tissue	Protein product function	Expression
Immune Response	<i>Tmem178</i>	Adrenal	Negative regulator of macrophage activation ¹⁰⁹	↓ ¹⁰⁶
	<i>Zbtb7b</i>	Adrenal	T-cell maturation ^{110, 111}	↑ ¹⁰⁶
	<i>Trim12a</i>	Adrenal	Anti-viral response	↓ ¹⁰⁶
	<i>Pydc4</i>	Adrenal	Anti-viral response ¹¹³	↓ ¹⁰⁶
Hormone Regulation	<i>Cyp21a2</i>	Adrenal	P450 enzyme required for adrenal steroidogenesis ¹¹⁷	↑ ¹⁰⁶
	<i>Abca2</i>	Adrenal	Regulates cholesterol efflux to ApoE3 ¹¹⁸	↑ ¹⁰⁶
	<i>Scarb1</i>	Adrenal	Mediates uptake of HDL-derived cholesterol and cholesteryl ester ¹¹⁹	↑ ¹⁰⁷
	<i>Lhcgr</i>	Testes	Hormone receptor, required for reproduction	↑ ¹⁰⁷

Table 3: in vivo assessment of TSPO ligands in animal models of disease

Ligand	Chemical Class	Disease-relevant study	Outcome	Ref
ZBD-2	Phenylpurine acetamide	Mouse: Middle cerebral artery occlusion	Neuroprotection	168
		Mouse: Hindpaw injection of Freund's adjuvant (CFA)	Altered excitatory and inhibitory transmission in the basolateral amygdala (BLA), reduced anxiolytic effects	174
Midazolam	Benzodiazepine	Rat: Single prolonged stress model	Reversed PTSD-associated freezing and anxiety-like behaviour via neurosteroidogenesis	175
YL-IPA08	Pyridazinoindole acetamide	Mouse: Inescapable electric foot shock induced model of PTSD	Reduced anxiety and fear, mediated by allopregnanolone synthesis; antagonized by PK 11195	176
		Mouse: Novelty suppressed feeding test, Vogel drinking conflict test, elevated plus-maze test, forced swimming test; tail suspension test	Anxiolytic and antidepressant effects	177
PK 11195	Isoquinoline carboxamide	Rat: Model of cortical contusion with hyperbaric oxygen therapy	Reversed protective effects of hyperbaric hyperoxia in brain injury	178
Etifoxine	Benzoxazine	Mouse: Experimental autoimmune encephalomyelitis (EAE) model	Reduced inflammatory pathology in spinal cord, promoted oligodendroglial regeneration; promoted recovery when administered before and after development of symptoms	24
Ro-5-4864	Benzodiazepine	Rat: 30 min coronary occlusion/ 15 min reperfusion	Reduced infarct size and improved mitochondrial function post injury, reduced oxidative stress and oxysterol formation by lowering cholesterol accumulation	179
		Mouse: Lesion to facial nerve in adults	Treated mice showed improved recovery of whisker function and whisker pad reinnervation	180
		Rat: Single injection of streptozotocin in sciatic nerve to simulate diabetes	Stimulate levels of pregnenolone, progesterone and testosterone in sciatic nerves, restored skin innervation density, improved Na ⁺ , K ⁺ -ATPase activit.	181
MPIGA	Phenylindolylgly oxylamide	Rat: Elevated maze test	Anxiolytic effects	182
FGIN-1-27	Arylindol acetamide	Mouse: Subcutaneous xenograft of HT29 cells in right thigh	40 % reduction in the growth rate of grafted tumors	183
		Rat: L5 spinal nerve ligation	Reduced mechanical allodynia and thermal hyperalgesia; same effects observed with Ro5-4864	184
SSR 180575	Pyridazinoindole acetamide	Mouse: MRL/lpr model	TSPO ligands reduced pulmonary inflammation and alveolitis onset; similar effects seen with PK 11195 and Ro5-4864	185
		Mouse: MRL/lpr model	Delayed onset of arthritis symptoms	28
Emapunil	Phenylpurine acetamide	Rat: Social exploration test; rat elevated plus maze test	Anxiolytic activity	31
		Mouse: Vogel-type conflict test; light/dark box, social interaction tests	Anti-anxiety effects	31
		Rat: Forced swimming test	Anti-anxiety effects	186