PERSPECTIVE

Translocator protein (18kDa) TSPO: a new diagnostic or therapeutic target for stress-related disorders?

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Efficient treatment of stress-related disorders, such as depression, is still a major challenge. The onset of antidepressant drug action is generally quite slow, while the anxiolytic action of benzodiazepines is considerably faster. However, their long-term use is impaired by tolerance development, abuse liability and cognitive impairment. Benzodiazepines act as positive allosteric modulators of ɣ-aminobutyric acid type A ($GABA_A$) receptors. 3α-reduced neurosteroids such as allopregnanolone also are positive allosteric $GABA_A$ receptor modulators, however, through a site different from that targeted by benzodiazepines. Recently, the administration of neurosteroids such as brexanolone or zuranolone has been shown to rapidly ameliorate symptoms in post-partum depression or major depressive disorder. An attractive alternative to the administration of exogenous neurosteroids is promoting endogenous neurosteroidogenesis via the translocator protein 18k Da (TSPO). TSPO is a transmembrane protein located primarily in mitochondria, which mediates numerous biological functions, e.g., steroidogenesis and mitochondrial bioenergetics. TSPO ligands have been used in positron emission tomography (PET) studies as putative markers of microglia activation and neuroinflammation in stress-related disorders. Moreover, TSPO ligands have been shown to modulate neuroplasticity and to elicit antidepressant and anxiolytic therapeutic effects in animals and humans. As such, TSPO may open new avenues for understanding the pathophysiology of stress-related disorders and for the development of novel treatment options.

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INTRODUCTION

Efficient treatment of stress-related disorders, such as depression, is still a major challenge. A disadvantage of most current pharmacological and non-pharmacological treatment options is their rather slow onset of action, which usually takes several weeks until the occurrence of a clinically meaningful therapeutic effect [1]. Within the plethora of available antidepressants and anxiolytics, only benzodiazepines are fast acting anxiolytics already after acute treatment and have found their place even as emergency medication for many conditions [2, 3]. However, their long-term use is hampered by side effects, such as tolerance development and abuse liability [2, 3]. Although add-on treatment with benzodiazepines to antidepressants has been shown to be superior to antidepressant treatment alone in the early treatment phase, this effect is not maintained throughout treatment and has to be balanced against the above mentioned side effects [4, 5]. As such, other treatment options, which exert rapid antidepressant and/or anxiolytic effects lacking the side effects of benzodiazepines are needed for the treatment of stress-related disorders.

Benzodiapines act as positive allosteric modulators of $GABA_A$ receptors through a benzodiazepine binding site, which is determined by differential composition of α subunits [6], thereby enhancing GABA-gated chloride currents. 3α-reduced neurosteroids, such as allopregnanolone, have been identified as another class of positive allosteric modulators of $GABA_A$ receptors [7]. Neurosteroid binding sites have been proposed to be located at interfaces of β subunits [8]. However, the characterization of the molecular pharmacology underlying the modulation of $GABA_A$ receptors by neurosteroids appears to be even more difficult than that of benzodiazepines. Nevertheless, a considerable body of literature suggests that endogenous GABAergic neurosteroids including allopregnaolone are sensitive to stress, and may modulate anxiety- and stress-related behavior [9]. Intriguingly, also antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), may increase allopregnanolone levels through interference with neurosteroidogenic enzymes, e.g., the 3α-hydroxysteroid oxidoreductase [10, 11]. This mechanism may contribute to the well-known anxiolytic effects of SSRIs. First clinical studies in stress-related disorders, e.g., depression and panic disorder, showed an imbalance of GABAergic neurosteroids assessed both in plasma and CSF in patients suffering from depression and anxiety disorders. Moreover, an effect of treatment with antidepressants on neurosteroid composition has been revealed [11–15].

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The neurosteroid field has recently attracted considerable interest regarding stress-related disorders, e.g., postpartum depression or major depression, due to industrial efforts to promote neurosteroid compounds as therapeutic treatment options. A first successful development is brexanolone, which is an intravenous formulation of allopregnanolone [16] and has recently been approved by the FDA for the treatment of postpartum depression. Actually, brexanolone is the only approved neurosteroid compound for treatment of a psychiatric condition with a fast onset of action within a few days [16]. On the one hand, it is no major surprise that a neurosteroid compound may exert beneficial effects in a psychiatric condition, which is accompanied by a dramatic drop of endogenous progesterone levels and related metabolites, such as allopregnanolone, following parturition. However, the further development of zuranolone, a modified allopregnanolone molecule with high bioavailability after oral administration, challenged this assumption in that it has been shown to be effective for treating major depressive disorder within 14 days of treatment [17, 18]. Intriguingly, neither tolerance development nor abuse liability have been reported so far, which, however, needs to be confirmed after more prolonged or repeated administration. It may even be an option that neurosteroids, such as zuranolone, may work as an interval therapy by treating on demand only for 14 days. This would constitute an enormous paradigm shift in the pharmacological treatment of stress-related disorders, which usually require acute and maintenance therapy for at least several months.

An attractive alternative to administering exogenous neurosteroids may constitute the enhancement of endogenous neurosteroidogenesis. The mitochondrial translocator protein 18 kDa (TSPO) has been identified as a key target for promoting endogenous steroid synthesis and is expressed in steroidogenic tissues including the brain [19, 20]. In fact, preclinical and translational work in animals and humans has shown that TSPO ligands, such as XBD173, may enhance GABAergic neurotransmission via neurosteroidogenesis and may exert anxiolytic effects in animals and humans lacking the inherent side effects of benzodiazepines [2]. Moreover, studies have suggested a role of TSPO for brain inflammation and bioenergetics, which shed further light on this interesting molecule regarding its relevance for pathophysiology and treatment of stress-related disorders [21]. Here, we will delineate complex physiology and pharmacology of TSPO in relation to neurosteroidogenesis and bioenergetics. Moreover, we will discuss, how TSPO is related to neuroplasticity modulated by stress and discuss its implications in animal studies of stress-related disorders. Finally, we will highlight recent developments regarding TSPO PET and MRI imaging and clinical studies applying TSPO ligands.

THE ROLE OF MITOCHONDRIA AND TSPO IN STRESS-RELATED DISORDERS

Given the potential of neurosteroids as modulators of stress and stress-related disorders, it is important to look also at molecular and subcellular determinants of steroid synthesis in steroidogenic tissues including the brain. At a subcellular level, neurosteroid synthesis, just like the synthesis of all steroids in the body, has its origin in the mitochondria, which moves these organelles into focus for stress-related disorders. Mitochondria are of central importance for the provision of energy in form of ATP generated by the oxidative phosphorylation system (OXPHOS) [22, 23]. The transport of electrons and translocation of protons are crucial for this process, thereby generating a proton gradient and an electromotive force, which results in a mitochondrial membrane potential and also drives the generation of reactive oxygen species (ROS) [24]. Depending on their concentrations, ROS exert important signaling properties. Moreover, extended oxidative stress induces detrimental effects on lipids, proteins and nucleic acids, thereby leading to mitophagy or even apoptosis [25, 26]. Mitochondria are not only the powerhouse of a cell, but also important signal integrators. They are in crosstalk with a wide variety of environmental, metabolic, and neuroendocrine stress mediators, such as glucocorticoids, estrogen, and others [27–29]. Moreover, they can generate signals of adaptation to physiological and environmental challenges, e.g., psychological stress [30]. Importantly, mitochondria modify cellular bioenergetics, produce biochemical signals, such as Krebs cycle metabolic intermediates, regulate cellular Ca^{2+} homeostasis, release mitochondrial DNA (mtDNA) and peptides, and modify the epigenome. In the context of stress regulation, mitochondria initiate and regulate the synthesis of a plethora of steroid hormones, including glucocorticoids and sex hormones.

A major determinant of steroid synthesis is TSPO, a 169 amino acid comprising protein of the outer mitochondrial membrane (OMM) [19]. TSPO is a highly interacting protein, associated with other proteins residing in the OMM such as voltage-dependent anion channel (VDAC), but also with cytosolic proteins, e.g., the steroidogenic acute regulatory protein (STAR) and proteins of the inner mitochondrial membrane (IMM), such as the adenine nucleotide transporter (ANT) [31, 32]. TSPO is expressed in many tissue types, especially in steroidogenic tissues [20, 33]. TSPO has been shown to be involved in the translocation of cholesterol from the cytosol to the mitochondrial matrix, where it is metabolized to the first steroid pregnenolone by activation of P450scc (Cyp11A1) [34] (Fig. 1). Pregnenolone, in turn, diffuses into the endoplasmatic reticulum and the cytoplasm, and is converted to other steroid hormones and neurosteroids involving multiple metabolic pathways [35]. Although the definite role and function of TSPO in the context of steroidogenesis has recently been challenged by TSPO knockout (KO) models [36, 37], there is numerous evidence that TSPO expression and administration of endogenous or synthetic TSPO ligands stimulate steroid synthesis, but also affect mitochondrial and cellular functional parameters, such as bioenergetics, Ca^{2+} homeostasis, cell proliferation and differentiation, anion and porphyrin transport, heme synthesis, inflammatory response, and apoptosis [33, 38, 39]. Moreover, TSPO is an important modulator of stress-related cellular functions, e.g., generation of ROS and programmed cell death [40]. Given the crucial role of cellular functionality, it is obvious that mitochondrial dysregulation is likely involved in the etiology and pathophysiology of neurodegenerative diseases (Parkinson´s disease, amyotrophic lateral sclerosis), but also in a variety of psychiatric and stress-related disorders (autism spectrum disorder, bipolar disorder, major depression) [41–43]. Thus, in the context of this multimodal interactive network, the neurosteroidogenic capacity of mitochondria and their regulation by TSPO might open new therapeutic avenues for the treatment of stress-related disorders.

TSPO, CELLULAR REPRESENTATION, NEUROPLASTICITY, AND COGNITION

Although most cell types in the CNS express TSPO to various degrees [44, 45], the evidence available to date points to microglia as the effectors of TSPO-mediated alterations in synaptic plasticity and cognition. However, also neurons are steroidogenic cells in the brain, and more recently, single-cell transcriptomics and immunolabeling with TSPO-KO validated antibodies confirmed neuronal TSPO expression in the rodent and human brain [46–48]. Neuronal activation results in an upregulation of hippocampal and cortical neuronal TSPO expression in the hippocampus and cortex. The functional significance of neuronal TSPO has recently been revealed, as the anxiogenic phenotype induced by global TSPO knockout could partially be rescued by selective neuronal TSPO expression [49]. Moreover, cell-specific neuronal TSPO overexpression also reduced depression-related behavior, an effect found to be dependent on steroidogenesis [49]. Such regionally

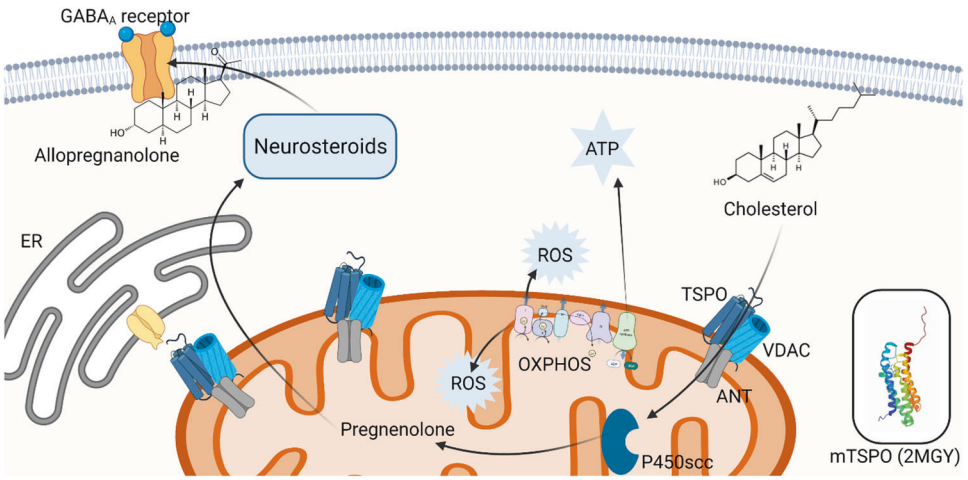

Fig. 1 Schematic presentation of various cellular functions of TSPO. TSPO is expressed in the outer mitochondrial membrane (OMM) and associated with other proteins such as VDAC and the adenine nucleotide transporter (ANT) forming a super-complex. TSPO is involved in the translocation of cholesterol from the cytosol to the mitochondrial matrix, where it is converted to pregnenolone by the activity of P450scc. After diffusion out of the mitochondria, pregnenolone is metabolized to neurosteroids, e.g., allopregnanolone, in the cytosol and endoplasmic reticulum (ER). Allopregnanolone is a positive modulator at the GABA$_A$ receptor. In addition, TSPO influences the generation of ATP and reactive oxygen species (ROS) via the oxidative phosphorylation system (OXPHOS). The inset shows the structure of the monomeric mouse TSPO (2MGY). Created with BioRender.com.

synthesized neurosteroids in neurons may have rapid and efficient access to the target site and can trigger pharmacological effects even at low concentrations. Intriguingly, reduced hippocampal volumes have been noted in various studies in depressed patients [50], which is in line the well-known overdrive of hypothalamic-pituitary-adrenal (HPA) axis activity in depression. Nevertheless, in addition to neurons, numerous other cells contribute to TSPO expression in the brain both under normal and pathological conditions, such as choroid plexus, endothelial cells, immune cells, astrocytes, and microglia [51, 52]. A recent milestone in TSPO research has been the discovery of the link between neuronal activity and intracerebral TSPO expression based on the hypothesis that TSPO expression may mirror the increased demands in energy production under conditions of increased neuronal activity [48]. While confocal laser scanning microscopy identified TSPO in both neuronal and non-neuronal cells (astrocytes, microglia, vascular endothelial cells) of cortical and subcortical brain regions, pharmacological or chemogenetic stimulation of neuronal activity stimulated TSPO expression in neurons, but not glia cells. A similar upregulation of neuronal TSPO occurred after exposure to an acute stressor.

Microglia are well known to modulate synapses during brain development, both in normal brain function and disease [53, 54]. The best studied mechanism by which microglia modulate synapses is synaptic pruning [55], where specific signaling molecules at the surface of synapses, such as complement component proteins [56] or phosphatidylserine [57] stimulate microglia to remove synapses. In mice, TSPO ligands, such as XBD173 or diazepam activate the same mechanism, leading to increased synaptic C1q deposition, removal of excitatory synapses and microglial phagocytosis of synaptic proteins [58] (Fig. 2). Interestingly, increased neurosteroid synthesis does not seem to play a role for this TSPO-mediated synapse loss, as systemic allopregnanolone administration caused an increase in the number of excitatory synapses [58]. These recent findings of impaired cognitive function due to a loss of hippocampal and cortical excitatory synapses, which is reversible after drug discontinuation [58–60], may help to explain why certain benzodiazepines such as diazepam cause cognitive impairment in humans, which is in line with clinical findings that long-term benzodiazepine use may impair cognition [61–64]. Furthermore, benzodiazepines may also increase the risk for neurodegenerative disease [65–67], although recent studies could not establish a causal link between benzodiazepine use and neurodegeneration [68, 69]. However, these findings are now explained by the studies mentioned above [58–60] in that diazepam may impair cognitive function via TSPO in susceptible individuals. Given the prominent role of microglia in neurodegenerative disease [70, 71], the role of TSPO in the pathogenesis of neurodegenerative diseases and whether TSPO ligands may have beneficial or even detrimental effects in these diseases warrants further research.

TSPO AND TSPO LIGANDS IN ANIMAL MODELS OF STRESS AND DEPRESSION

Meanwhile, the consequences of manipulation of the brain TSPO system, either in glial cells, neurons or in both, have intensely been studied in relevant rodent models of anxiety, posttraumatic stress disorder (PTSD), and depression. Animal models of distinct psychopathologies are indispensable to study the involvement of brain TSPO and its underlying mechanisms in the context of socio-emotional dysfunctions. Most rodent models employed used repeated or chronic exposure to either non-social stress paradigms, such as unpredictable chronic mild stress (UCMS), unpredictable exposure to foot shocks, or social stress paradigms, such as chronic social defeat and chronic subordinate colony housing. Moreover, distinct pharmacological treatments with anxiogenic or depression-inducing properties were used to model symptoms of psychiatric disorders. The acute anxiolytic properties of the TSPO ligands have been described as early as 1972 [72], and have been multifold confirmed, for example, in stress-sensitive and stress-resistant mouse lines, i.e., C57BL/6J and BALB/cByJ mice, respectively. Here, the TSPO ligand etifoxine reversed the behavioral effects of restraint stress exposure selectively seen in the stress-sensitive line often used as a model of high anxiety and depression [73]. Similarly, the TSPO ligand XBD173 exerted acute anxiolytic effects, which were prevented by the TSPO antagonist PK11195. XBD173 also counteracted pharmacologically induced panic attacks both in rodents and humans without sedative effects [2].

In line, in mice exposed to UCMS, daily oral application of the TSPO ligand ZBD - an analog of XBD173 - over two weeks attenuated the chronic stress-induced increase in anxiety [74]. The

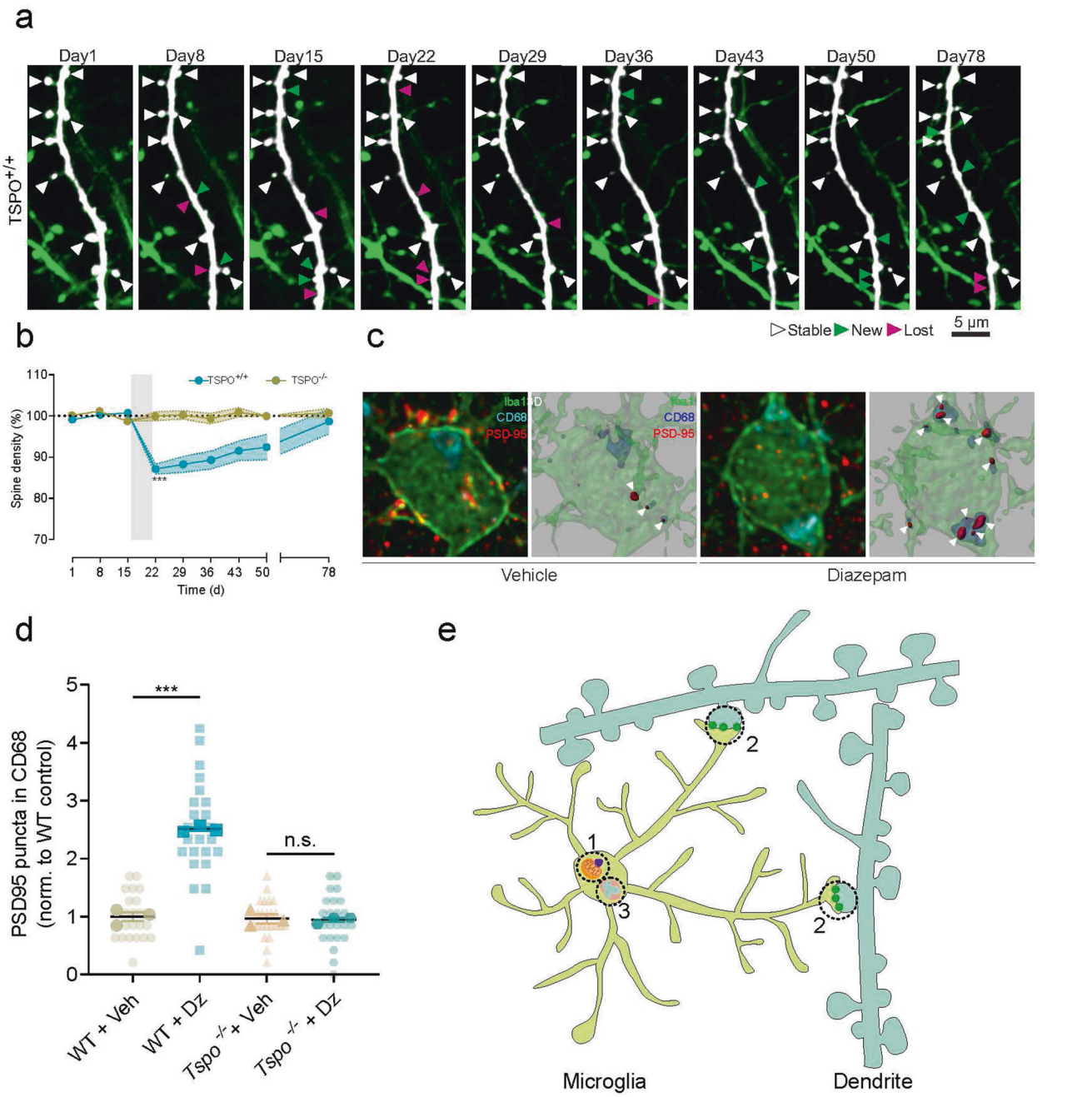

Fig. 2 Diazepam may lead to synapse loss by increased microglial synaptic pruning via TSPO. a In vivo two-photon micrographs of apical dendritic tufts of layer 5 pyramidal neurons in the somatosensory cortex of TSPO wildtype (TSPO+/+) mice. Between day 15 and day 22, 5 mg/kg diazepam were administered daily by oral gavage. Arrowheads mark dendritic spines, which are the morphological correlate of excitatory postsynapses. **b** Relative densities of dendritic spines in TSPO wildtype and TSPO knockout (TSPO-/-) mice before, during (gray bar) and after diazepam administration. **c** Confocal images and 3D reconstruction of synaptic material (PSD95, red) in lysosomes (CD68, cyan) in microglia (Iba1, green) in the somatosensory cortex after one week of vehicle or diazepam treatment. **d** Quantification of confocal stains of synaptic material inside lysosomes of microglia in vehicle or diazepam (dz) treated wildtype or TSPO knockout mice. **e** Graphical summary of the putative mechanism of TSPO- mediated synapse loss. (1) TSPO ligands bind to TSPO on mitochondria in microglia. (2) Increased synaptic deposition of C1q and increased contacts of microglia processes with dendritic spines. (3) Phagocytosis of synaptic material, which can be observed in microglial lysosomes. Panels a-d are reproduced with permission from Shi et al., 2022 [58].

same treatment also diminished the stress-induced passive stress coping in the forced swim and tail suspension tests. Interestingly, both effects were prevented by the TSPO antagonist PK11195. However, these data should be interpreted with caution, since proper treatment controls are not shown in this study. Nevertheless, they further revealed that the behavioural effects were due to the capacity of the ligand to regulate the balance between GABAergic and glutamatergic transmission in the basolateral amygdala and hippocampus [74].

TSPO ligands may be of particular interest for the treatment of mood disorders associated with sexual steroid dysbalances, such as postpartum depression. In a rat model of postpartum depression induced by ovarian steroid hormone withdrawal, treatment with the TSPO ligands XBD173 or YL-IPA08 over 2 or

3 weeks prevented or reversed the reduced levels of TSPO and neurosteroids in the hippocampus, basolateral amygdala, and PFC, and the increased anxiety- and depression-related behaviors [75, 76]. Interestingly, the TSPO ligand had a shorter onset compared with treatment with the positive control drug sertraline [75].

Various somatic diseases show comorbidity with anxiety disorders and/or depression, e.g., diabetes mellitus [77, 78] and inflammatory bowel syndrome (IBS) [79, 80]. Intriguingly, in a clinically relevant rat model of diabetes mellitus type 2 induced by high fat diet and streptozotocin, administration of the TSPO ligand AC-5216 (XBD173) reversed the diabetes-induced anxiogenic and depression-like phenotype, as seen by reduced anxiety levels in the open field, increased sucrose intake in the sucrose preference test, shortened latency to approach food in the novelty-suppressed feeding test, and increased active stress coping in the forced swim test, respectively. Most of these effects were prevented by the TSPO antagonist PK11195 [81]. Interestingly, opposite effects of the antagonistic TSPO ligand ONO-2952 have been reported [82].

Important genetic-based evidence that TSPO is causally involved in regulating anxiety- and depression-related behaviors and steroidogenesis in the brain has come from TSPO knockout and neuronal TSPO transgenic mice models [49, 83]. TSPO deletion has not only been associated with a marked depletion in brain neuroactive steroid levels, including allopregnanolone, but also with an anxiogenic phenotype in mice [49]. This is in line with observations that a perturbation in brain levels of allopregnanolone, a positive allosteric modulator of $GABA_A$ receptors, have been associated with stress-related disorders including anxiety [14, 84].

Moreover, the anxiolytic effect of various TSPO ligands, such as AC-5216 (XBD173), could not be observed after TSPO knockdown and, thus, indeed requires the TSPO protein [85]. Also, in the forced swim test, the antidepressant effect of AC-5216 was only seen in wildtype mice. The essential role of TSPO in mediating the effects of AC-5216 was further confirmed in a chronic unpredictable stress paradigm performed over 6 weeks. Two to four days of AC-5216 treatment produced fast-onset antidepressant-like effects in the novelty-suppressed feeding and sucrose preference tests, as well as memory-enhancing effects in the novel object recognition test, but again, only in wildtype, but not TSPO KO mice [85]. In addition, after five days of treatment, the levels of blood corticosterone, prefrontal cortex allopregnanolone, and mTOR signaling-related proteins (mBDNF, p-mTOR, PSD-95, synapsin-1, GluR1) were restored only in stressed wildtype mice as soon as 5 days of AC-5216 treatment [85].

In addition to profound anxiolytic and anti-depressive effects of TSPO and TSPO ligands, beneficial treatment effects have also been revealed in animal models of PTSD. For example, in the single prolonged stress model [86], i.e., single exposure of rats to restraint (2 h), forced swim (20 min), and ether anesthesia, daily oral administration of the TSPO and central benzodiazepine receptor ligand midazolam over two weeks reversed the PTSD-like behavioral deficits seen in treatment controls [87]. Thus, midazolam ameliorated anxiety-like behavior in the open field and on the elevated plus-maze and freezing in the contextual fear conditioning. Similarly, after exposure to inescapable electric foot-shocks, a mouse model of PTSD, daily application of the TSPO ligand YL-IPA08 over two weeks prevented the enhanced anxiety and contextual fear, and restored levels of allopregnanolone in the prefrontal cortex and serum [88]. Both behavioral and allopregnanolone effects were blocked by a single i.p. injection of the TSPO antagonist PK11195 administered 30 min before testing. In confirmation of a role of TSPO in foot shock-induced PTSD-like symptoms, viral overexpression of TSPO in the hippocampal dentate gyrus prevented the increase in contextual freezing and in anxiety-related behavior seen in treatment controls [89]. The hippocampal overexpression of TSPO was accompanied by increased local allopregnanolone levels and neurogenesis, which may partly underlie the observed behavioral effects. In both studies, the respective antagonist prevented these effects indicating that the actions of TSPO ligands involve, at least partly, TSPO, $GABA_A$ receptors, and neurosteroidogenesis.

TSPO EXPRESSION, NEUROIMAGING, AND THERAPEUTIC EFFECTS OF TSPO LIGANDS IN STRESS-RELATED DISORDERS

In healthy volunteers, an acute virtual reality Trier social stress test elicited a prominent cortisol response, which was accompanied by a decrease in TSPO expression in platelets [90]. A variety of studies have investigated the expression of TSPO in stress-related disorders [91]. These studies investigated either the expression of TSPO mRNA in peripheral mononuclear cells, the binding characteristics of the TSPO ligand PK11195 to platelet membranes, or protein expression in thrombocytes [92–94]. In view of various reports of reduced TSPO expression in peripheral mononuclear cells and platelets of anxious subjects in stress-related disorders [92, 95–99], the question of a causal relationship between brain TSPO expression and behavior and putative therapeutic effects of TSPO ligands turned into focus. Moreover, a genetic association study in patients with depression and adult separation anxiety disorder revealed a higher frequency of a genetic variant of TSPO, likely affecting neurosteroid synthesis [100].

Meanwhile, various positron emission tomography (PET) studies reported increased TSPO expression in depression [21, 101] and obsessive-compulsive disorder [102]. More recently, using [^{18}F] FEPPA as a tracer, a therapeutic study indicated that regional TSPO total distribution volume (TSPO V_T) can, for example, even predict the clinical response to treatment with the COX2 inhibitor celecoxib in major depression [103], thereby highlighting the potential of TSPO ligands as personalized medicine approaches both in diagnostics and for selecting treatment procedures in relation to their outcome. Moreover, in patients suffering from PTSD, it has been shown that higher C-reactive protein (CRP) levels are associated with lower prefrontal-limbic TSPO availability and PTSD severity [104]. Moreover, in neurodegenerative disorders, such as Alzheimer´s disease, but also depression with cognitive impairment, upregulation of TSPO labeling has been reported in PET studies [101, 105]. However, it should be noted that PET scans may provide valuable information on discrete signs of neuroinflammation, e.g., increased glial or neuronal activity, but are not suitable for making differential diagnosis of stress-related or other psychiatric disorders. Moreover, TSPO expression should not unequivocally be considered as a marker of neuroinflammation, since also neuronal activation may increase TSPO levels [48]. Nevertheless, these findings are in line with the hypothesis that TSPO ligands may exert antidepressant effects through their anti-inflammatory properties. Furthermore, gene variants, such as the rs6971 TSPO polymorphism, which affects ligand binding and cholesterol uptake, should be considered in clinical studies, when assessing TSPO binding or function. For example, both bipolar disorder and diurnal cortisol rhythm in bipolar disorder have been linked to this TSPO polymorphism [106, 107].

Regarding putative therapeutic effects, in a translational study, the selective TSPO ligand XBD173 enhanced GABAergic neurotransmission in brain slices via the induction of neurosteroidogenesis and effectively reduced the number of pharmacologically induced panic attacks in rodents and humans in the absence of sedation [2]. However, XBD173 has not been further developed for clinical use after completion of a phase II trial in generalized anxiety disorder. Currently the only clinically available TSPO ligand is etifoxine, which has been approved in France. Etifoxine has a dual mode of action, as it targets TSPO but also directly α_2 and α_3 containing $GABA_A$ receptors [108]. Initial clinical studies with etifoxine have provided the first evidence for a clinical anxiolytic

effect of etifoxine, which showed comparable efficacy to the benzodiazepine lorazepam in patients suffering from adjustment disorders with anxiety [109]. The anxiolytic effects of etifoxine comparable to clonazepam have recently been confirmed in a randomized controlled double-blind clinical trial in patients with anxiety disorder [110]. Although no clinical studies are available in depressed patients to date, TSPO ligands may also be promising agents for the treatment of depression. This assumption is based on animal studies, which suggest that TSPO mediates fast-onset antidepressant-like effects of cognate ligands [85], the antidepressant potential of exogenous neurosteroids, e.g., brexanolone or zuranolone [16, 18], and the discrete signs of neuroinflammation together with hypercortisolemia seen in depression. A particular advantage of this approach might be the shorter onset of action within 14 days compared to conventional antidepressants, as it has been noted for zuranolone and esketamine [18, 111]. Currently, etifoxine and its derivatives such as GRX-917 [112] are of interest in this context, as we are not aware of other TSPO ligands under clinical development for stress-related disorders. Further advances regarding the application of TSPO ligands in stress-related disorders may arise from sophisticated neuroimaging approaches. Strategies for future imaging research may include a combination of TSPO-PET and fMRI to investigate how altered TSPO expression relates to patterns of dysfunction in cognitive domains such as memory, attention or the processing of emotions affected by stress-related disorders. Furthermore, it is important to consider the dynamic nature of pathophysiology on different time scales (Fig. 3). For example, combining TSPO-PET with fMRI measures of functional connectivity dynamics, a proxy of metabolic activity and cognitive flexibility [113, 114] has the potential to unravel the metabolic underpinnings of how the brain adapts to psychological challenges and how such adaptive processes may be altered in stress-related disorders. Another dynamic aspect is the investigation of neuroimaging biomarkers of early onset of therapeutic responses that may predate and predict clinically relevant effects of therapeutic interventions (Fig. 3). The combination of multiple sources of data from TSPO-PET, from fMRI and clinical scales that rely on spatial as well as temporal information may help creating innovative prediction models for psychiatric disorders. However, it should be noted that putative therapeutic effects of TSPO ligands are not restricted to stress-related disorders in view of preclinical studies suggesting neuroprotective effects of etifoxine or XBD173 in mouse models of Alzheimer´s disease [115, 116] or multiple sclerosis [117, 118].

OUTLOOK

The TSPO molecule with its pleiotropic actions is a fascinating example for promoting theranostic approaches in stress-related disorders, because TSPO labeling may serve as a neuroimaging maker, e.g., for microglia activity or inflammatory processes, and, on the other hand, TSPO ligands may exert putative therapeutic effects. Particularly, patients showing discrete signs of neuroinflammation and/or imbalance of neurosteroids might profit from treatment with TSPO ligands. For example, targeted activation of TSPO to promote steroidogenesis through the development of ligands with higher brain permeability or brain/neuron-targeted gene therapy may represent an effective approach for the treatment of anxiety and depression-related symptoms [49]. The induction of endogenous neurosteroidogenesis may constitute an attractive alternative pathway to administering exogenous neurosteroid molecules. However, TSPO mediates numerous functions beyond steroidogenesis, e.g., modulation of mitochondrial bioenergetics and calcium homeostasis, which may also contribute to the pleiotropic actions of TSPO ligands. As such, TSPO ligands differ from exogenous neurosteroids in view of their broader pharmacological profile. Finally, also off-target effects should be considered, when discussing putative clinical effects of TSPO ligands. These multiple actions of TSPO cannot be dissected under clinical conditions and may contribute to reported side effects of the TSPO ligand etifoxine, e.g., liver disturbances or skin reactions. Nevertheless, this fascinating molecule has the potential to put

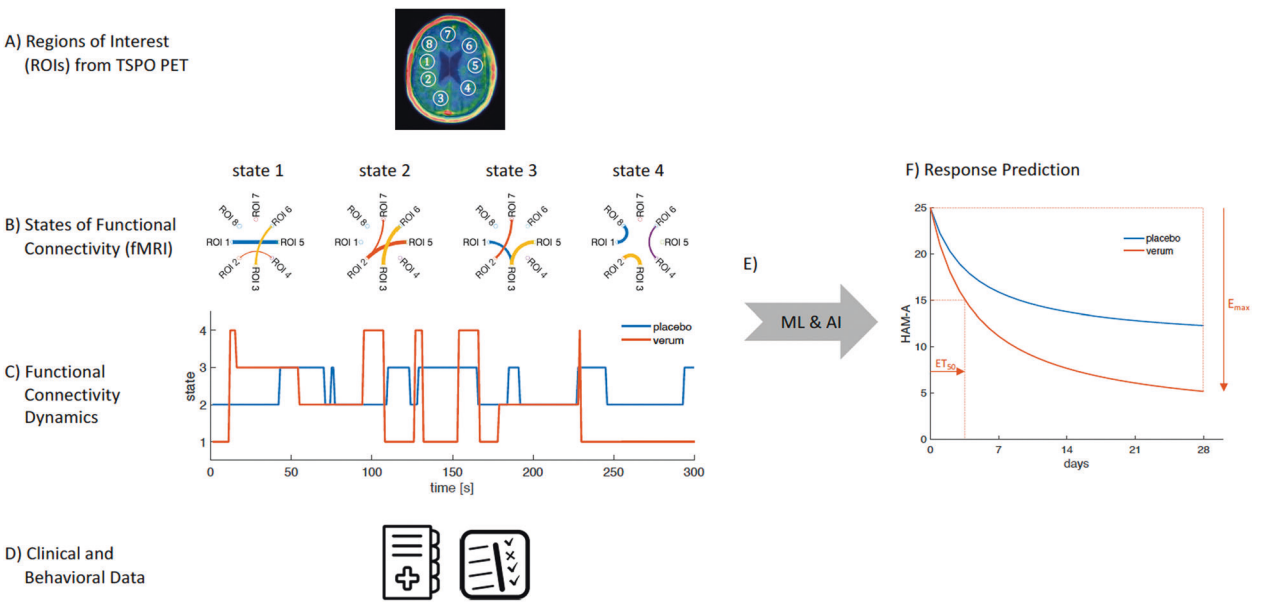

Fig. 3 Neuroimaging-derived parameters using machine learning (ML) and artificial intelligence (AI) models may predict the speed and efficiency of a therapeutic intervention with TSPO ligands. a Receptor density maps from TSPO-PET yield regions of interest (ROIs) which allow extracting time-courses from resting-state fMRI data of the same patient. **b** Clustering of pairwise correlations of windowed fMRI time-courses may produce a stable set of functional connectivity states. **c** Each time point from the rs-fMRI scan can now be assigned to one of these connectivity states, which yields state trajectories in time for patients treated with either placebo or verum. **d** Clinical ratings as well as data from standardized tests help to identify neuro-behavioral subtypes. Parameters from **a**–**d** can be used in machine learning applications **e** such as deep neural networks or support vector regression to **f** predict speed (ET_{50}) and efficiency (E_{max}) of the response to a therapeutic intervention with a TSPO ligand.

forward our understanding of the pathophysiology of stress-related disorders and to open the avenue for novel treatment options.

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AUTHOR CONTRIBUTIONS

RR drafted and revised the whole manuscript, CW contributed to the chapter on mitochondria, MS to the neurosteroid part, MD and JH contributed findings on neuroplasticity, IDN to the chapter on animal models, and JS and NA to the clinical neuroimaging part. All authors carefully read and revised the manuscript.

COMPETING INTERESTS

RR has received consultancy honoraria from SAGE and GABA Therapeutics.

ADDITIONAL INFORMATION

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