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History of *Tspo* deletion and induction *in vivo*: Phenotypic outcomes under physiological and pathological situations

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ABSTRACT

The mitochondrial translocator protein (TSPO) is an outer mitochondrial protein membrane with high affinity for cholesterol. It is expressed in most tissues but is more particularly enriched in steroidogenic tissues. TSPO is involved in various biological mechanisms and TSPO regulation has been related to several diseases. However, despite a considerable number of published studies interested in TSPO over the past forty years, the precise function of the protein remains obscure. Most of the functions attributed to TSPO have been identified using pharmacological ligands of this protein, leading to much debate about the accuracy of these findings. In addition, research on the physiological role of TSPO has been hampered by the lack of *in vivo* deletion models. Studies to perform genetic deletion of *Tspo* in animal models have long been unsuccessful, which led to the conclusions that the deletion was deleterious and the gene essential to life. During the last decades, thanks to the significant technical advances allowing genome modification, several models of animal genetically modified for TSPO have been developed. These models have modified our view regarding TSPO and profoundly improved our fundamental knowledge on this protein. However, to date, they did not allow to elucidate the precise molecular function of TSPO and numerous questions persist concerning the physiological role of TSPO and its future as a therapeutic target. This article chronologically reviews the development of deletion and induction models of TSPO.

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Abbreviations: Amhr2, anti-Mullerian hormone receptor type II; Nr5a1, steroidogenic factor 1; Cyp11a1, cytochrome 11A1; StAR, steroidogenic acute regulatory protein.

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1. Introduction

Since its discovery by Braestrup & Squires [1] in the rat kidney, the function of the 18 kDa peripheral benzodiazepine receptor, which was subsequently named mitochondrial translocator protein (TSPO; [2]) is still not clearly elucidated. TSPO is highly expressed in steroidogenic tissues and has a very high affinity for cholesterol [3]. Anholt et al. [4] and Besman et al. [5] were the first to suggest that this protein could play a role in the stimulation of steroidogenesis. Then, studies demonstrated that TSPO is involved in the mitochondrial cholesterol transfer through the transduceosome (hormone-induced multiprotein complex, [6]), the first step in steroidogenesis and in the minor path of bile acid biosynthesis. The first remains the main function described for this protein but also the most debated. TSPO has been involved in many other biological processes such as inflammation, calcium handling, apoptosis, oxydative stress, etc. [2,7]. TSPO has also been studied in erythropoiesis as heme and its precursors are natural ligands of TSPO [8,9]. However, most of the functions attributed to TSPO have been identified using specific ligands of this protein, leading to much debate about the accuracy of these findings [2], and those functions are now challenged by the development of *in vivo* models of *Tspo* gene deletion. Despite the involvement of TSPO in various biological mechanisms, its exact role remains to be clarified [10]. However, its ability to bind endogenous ligands of different structures such as cholesterol, acylcoenzyme A binding protein/diazepam binding inhibitor, and protoporphyrin IX seems to be clues for its function. More particularly, its high affinity for cholesterol, which can not exist free in a membrane or the cytosol, suggests a function on cholesterol compartmentalization for needs the cell and mitochondria may have.

Clinical studies showed that the expression of TSPO varies in response to different pathologies, *i.e.*, decreasing in neuropsychological disorders (depression and anxiety) and increasing in neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's and multiple sclerosis). As a result, TSPO ligands were widely studied in these pathologies [11]. Furthermore, TSPO is overexpressed in neuroinflammation, and positron emission tomography using radiolabeled ligands of TSPO is a current clinical method to assess this parameter [12]. TSPO ligands were also shown to produce beneficial effects in *in vivo* models of various diseases ranging from Alzheimer disease to cardiac ischemia-reperfusion [13,14]. However, research on the physiological role of TSPO has long been hampered by the lack of *in vivo* deletion models as studies to perform genetic deletion of *Tspo* in animal models have been unsuccessful. The animals died at the embryonic stage, suggesting that TSPO is essential for basic functions necessary for embryonic development [15]. However, in the last decades, important advances have been made in the techniques allowing genome modification, and animal deletion models for *Tspo*, either tissue-specific or global, have been developed (Fig. 1). This review provides a chronological summary of the development of these models, the results they have yielded, and the debate on the physiological role of TSPO that they have generated. In this review, only animal models are presented; experimental models of *Tspo* deletions in bacteria, yeast and cells gave birth to important data on the impact of mitochondrial functions, from respiration and structure to steroid formation, but they are not discussed in this review.

2. Non-murine models of *Tspo* deletion

The first successful deletion of *Tspo* was obtained in a study analyzing the role of TSPO in zebrafish development. TSPO specific morpholinos antisense oligonucleotides that target TSPO mRNA were injected to zebrafish embryos. These embryos displayed a high mortality linked to a specific erythropoietic cell depletion, associated with a significant decrease in hemoglobin. This lack of blood cells is independent of *Tspo* cholesterol binding sites [16]. Soon after, Lin et al. [17] succeeded to inactivate the *Tspo* gene in drosophila with two different methods to delete *Tspo*: they inserted a P-element in the 3′ regulatory region of *Tspo* gene and they injected a transgene, transcribed into a dsRNA that targets the mRNA. These genetic deletions of *Tspo* did not affect the development or morphology of drosophila and flies from both sexes were protected from apoptosis, but only lifespan was extended in males who were resistant to oxidative stress. However, during aging, mitochondrial function of *Tspo*-deleted flies was altered with a decrease in mitochondrial respiration linked to a reduction of the activity of respiratory chain complexes and an increase in mitochondrial oxidative stress [17]. The same group used these fly models to study the mechanisms of susceptibility to alcohol use disorders [18]. The authors hypothesized that TSPO might be involved in alcohol addiction because of its involvement in mitochondrial processes (bioenergetics, reactive oxygen species production and apoptosis), its use for neuro-inflammatory diagnosis and its ability to bind benzodiazepines. They demonstrated that the deletion of *Tspo* in drosophila induced an increase in sedation and tolerance to ethanol, mediated in particular by an increased production of reactive oxygen species. This study also revealed a significant difference in TSPO expression between males and females [18]. Similar drosophila models of *Tspo* deletion were also used to look at the role of TSPO and VDAC (voltage-dependent anion channel) in the innate immune response and wound healing. *Tspo* deletion in these flies impaired their response to infection and wound. The study suggests that mitochondrial TSPO-VDAC complex could cooperate with Parkin (an E3 ubiquitin ligase whose mutation can result in mitochondrial dysfunction and fly survival after infection) to mediate responses against infection and wound [19]. All the results obtained with the models presented in this section are summarized in Table 1.

3. Murine models of *Tspo* tissue-specific deletion

Tissue-specific deletion of *Tspo* models have been generated in different organs to study the functions attributed to TSPO. *Tspo* deletion in steroidogenic tissues (testes, adrenal or brain) was used to study the role of TSPO in steroidogenesis, heart and liver deletion for the implication of TSPO in mitochondrial stress, and brain deletion to address TSPO involvement in neuro-inflammation pathologies notably. The different models presented in this section are chronologically presented and all the results and conclusions they generated are summarized in Table 2.

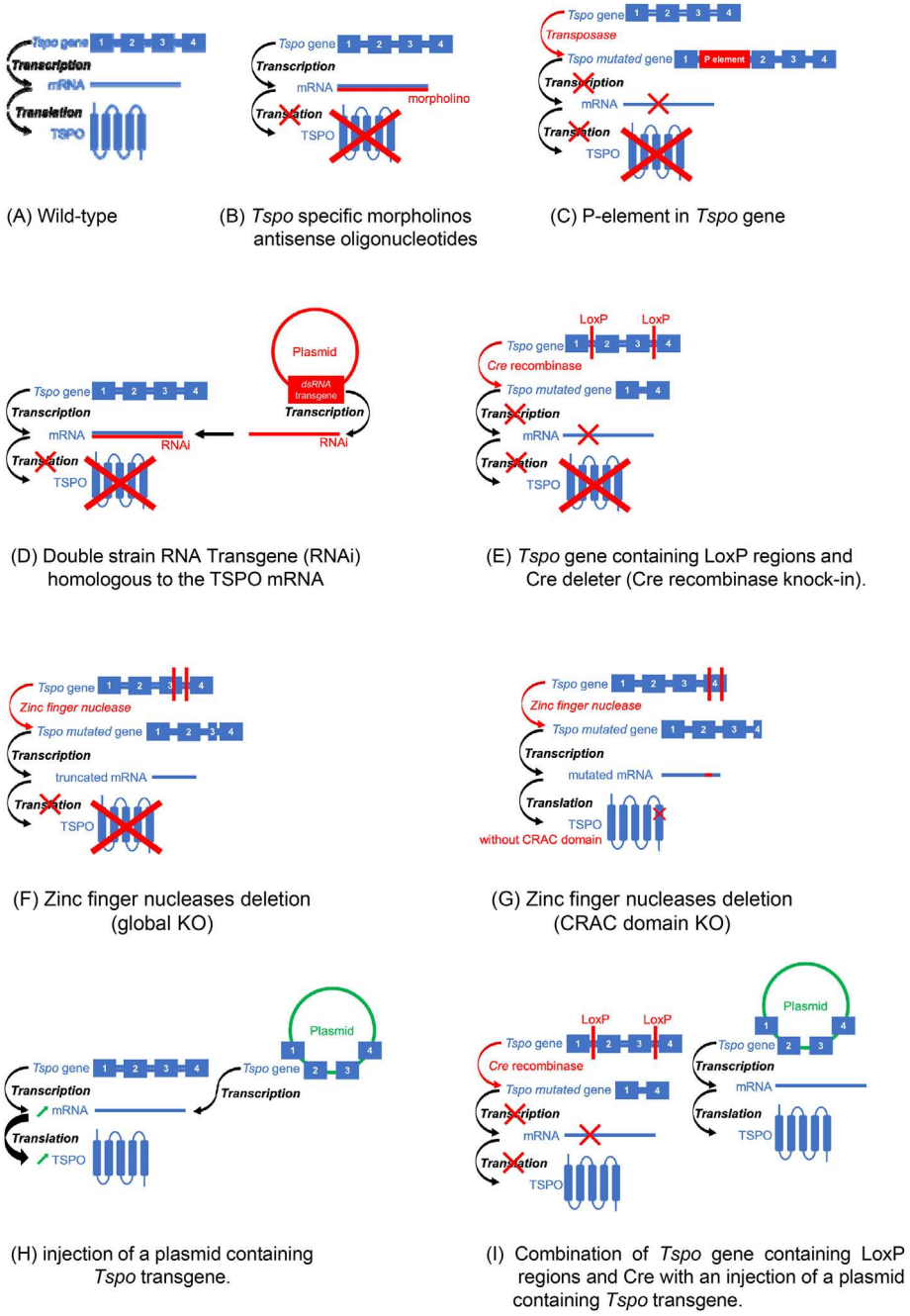

Fig. 1. Schematic representation of Tspo gene editing methods exposed in this review.
A Wild-type: *Tspo* gene is transcripted in *Tspo* mRNA. mRNA is then translated into the TSPO protein.
B Tspo specific morpholinos antisense oligonucleotides (Inducible global knock-down): a *Tspo*-specific morpholino binds to the *Tspo* mRNA and blocks the translation.
C P-element in *Tspo* gene (Constitutive global knock-out): P-element produces Transposase which exises P-element to another location within the chromosome. Here, the P-element is transposed within the *Tspo* gene enabling the transcription.
D Double strain RNA transgene (RNAi) homologous to the TSPO mRNA (Inducible global knock-down): the transgene is transcripted into a RNAi that binds specifically to the *Tspo* mRNA and blocks the translation. dsRNA: double strain ribonucleic acid; RNAi: ribonucleic acid interference.
E *Tspo* gene containing *LoxP* regions and *Cre deleter* (Cre recombinase knock-in): *Tspo* gene contains LoxP region that guides Cre recombinase deletion. In these models, the gene promoter regulating Cre recombinase expression defines the type of deletion: an ubiquitous promoter induces a constitutive global knock-out, a tissue-specific promoter induces a constitutive tissue-specific knock-out. The promoter can also be regulated by exogenous compound: if the promoter is inducible by tamoxifen injection, Cre recombinase expression and therefore *Tspo* knock-out is tamoxifen-inducible.
F Zinc finger nucleases deletion (Constitutive global knock-out). Zinc finger nucleases induce a deletion between exon 3 and intron 3 of *Tspo* gene. The mRNA transcripted is truncated and not translated into TSPO protein.
G Zinc finger nucleases deletion (Constitutive global CRAC domain knock-out): Zinc finger nucleases induce a deletion in the CRAC motif within exon 4 of the *Tspo* gene. The TSPO protein translated does not contain the CRAC motif.
H Lentivirus with plasmid containing *Tspo* gene (Inducible tissue-specific knock-in): The plasmid injected contains a *Tspo* transgene that expresses TSPO. This expression is localized in the injection localization that defines the tissue-specificity of the *Tspo* knock-in.
I Combination of *Tspo* gene containing LoxP regions and Cre with an injection of a plasmid containing *Tspo* transgene. This method combines a constitutive global knock-out described in method (E) and the inducible tissue-specific knock-in described in method (H). This method results in the expression of *Tspo* only in the injected tissue.

Table 1
Animal models of global *Tspo* deletion.

Species	*Tspo* gene editing types	Methods	Deletion outcomes	Refs
Zebrafish	Inducible global KD	TSPO specific morpholinos antisense oligonucleotides injected in embryo (Fig. 1B)	Enhanced mortality and erythropoietic cell depletion.	[16]
Mouse	Constitutive global KO	Crossing mice expressing *Tspo* gene containing *LoxP* regions and "*Cre* deleter mice" (*Cre* recombinase knock-in) (Fig. 1E)	Normal phenotype (reproduction and development), normal blood composition, less ATP production in microglia.	[32]
Fly	Constitutive global KO (strain). Inducible global KD (transgene).	*Tspo* deficient strain obtained by inserting a P-element in the gene (Fig. 1C). Transgene injection to induce expression of dsRNA (RNAi) homologous to the TSPO mRNA regulated by the Gal4/UAS system (Fig. 1D)	Normal development, extended male fly lifespan, inhibition of Aβ42-induced neurodegeneration, decreased caspase activation, resistance to oxidative stress.	[17]
Mouse	Constitutive global KO	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the *Ddx4* promoter (Fig. 1E)	Normal steroidogenesis.	[33]
Fly	Constitutive global KO (strain). Inducible global KD (transgene).	Previously described in Ref. [17] (Fig. 1C and D)	Normal development, defective responses to infection and wound.	[19]
Fly	Constitutive global KO (strain). Inducible global KD (transgene).	Previously described in Ref. [17] (Fig. 1C and D)	Normal development, increased sensitivity to ethanol sedation.	[18]
Mouse	Constitutive global KO	Previously described in Ref. [32] (Fig. 1E)	Confirmation of binding specificity of TSPO ligands.	[34]
Mouse	Constitutive global KO	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the *protamine* promoter (Fig. 1E)	Normal phenotype (viability at birth, reproduction and organs) and normal gene expression profile of lung tissues.	[39]
Mouse	Constitutive global KO	Previously described in Ref. [33] (Fig. 1E)	Normal erythropoiesis, heme biosynthesis and Protoporphyrin IX biosynthesis.	[35]
Rat	Constitutive global KO	Injection of rat embryos with zinc finger nucleases: deletion between exon 3 and intron 3 of *Tspo* gene (Fig. 1F)	Normal phenotype, impaired steroido-genesis	[36]
Rat	Constitutive global CRAC domain KO	Injection of rat embryos with zinc finger nucleases: deletion in the CRAC motif within exon 4 (Fig. 1G)	Normal phenotype, impaired steroido-genesis.	[36]
Mouse	Constitutive global KO	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the *Camk2* promoter (Fig. 1E)	Subtle steroidogenic abnormalities exacerbated with aging.	[37]
Mouse	Constitutive global KO	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the *Best1* promoter (Fig. 1E)	Normal retinal morphology and function, no effect on photoreceptor degeneration or microglia activation in experimental retinal degeneration models.	[43]
Mouse	Constitutive global KO (first considered as a constitutive testes-specific KO)	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the *Amhr2* promoter. Previously described in Ref. [22] (Fig. 1E)	Abnormal early embryonic development.	[24]
Mouse	Constitutive global KO	Previously described in Ref. [39] (Fig. 1E)	Confirmation of effects specificity of TSPO ligands.	[42]
Mouse	Constitutive global KO	Previously described in Ref. [39] (Fig. 1E)	Inhibition of microglial activation through regulation of mitochondrial metabolism.	[40]
Mouse	Constitutive global KO	Previously described in Ref. [33] (Fig. 1E)	Normal retinal morphology, elevated levels of lipids and microglial activation in retina.	[44]
Mouse	Constitutive global KO	Previously described in Ref. [37] (Fig. 1E)	Reduced microglia activation and neuroinflammation in an septic encephalopathy experimental model.	[45]
Mouse	Constitutive global KO (first considered as a constitutive testes-specific KO)	Previously described in Ref. [22] (Fig. 1E)	Highlighting a role for TSPO in the evolution of nonalcoholic fatty liver disease.	[48]
Rat	Constitutive global KO	Previously described in Ref. [34] (Fig. 1F)	Highlighting a role for TSPO in the evolution of nonalcoholic fatty liver disease.	[48]
Mouse	Constitutive global KO	*Tspo* gene containing *LoxP* regions and *Cre* deleter (*Cre* recombinase knock-in) (Fig. 1E)	No changes in food intake, body mass or energy homeostasis in an altered diet experimental model.	[47]
Mouse	Constitutive global KO	Previously described in Ref. [39] (Fig. 1E)	Impaired microglial phagocytosis, enhanced neuroinflammation and cerebral accumulation of Aβ in an Alzheimer's disease experimental model.	[41]
Mouse	Constitutive global KO	Previously described in Ref. [33] (Fig. 1E)	Higher inflammation and epithelial alternation and worse outcome in an ulcerative colitis experimental model	[50]
Mouse	Constitutive global KO	Previously described in Ref. [39] (Fig. 1E)	Higher inflammation and pyroptisis in an inflammatory bowel disease experimental model.	[51]
Mouse	Constitutive global KO	Previously described in Ref. [37] (Fig. 1E)	Higher mortality and worse anxiety/depression outcome in an sepsis experimental model.	[46]
Mouse	Constitutive global knock-out	Previously described in Ref. [32] (Fig. 1E)	Normal steroidome.	[38]

Aβ: amyloid beta peptides; Aβ42: amyloid beta peptide 42; ATP: Adenosine triphosphate; Best1: Bestrophin 1; Camk2: Calmodulin-dependent protein kinase II a; CRAC: Cholesterol recognition/interaction amino acid consensus; Ddx4: ATP-dependent RNA helicase DDX4; dsRNA: Double-stranded ribonucleic acid; Gal4/UAS: yeast transcription activator protein Gal4/Upstream activation sequence; KO: Knock-out; KD: Knock-down; mRNA: Messenger ribonucleic acid; RNAi: Ribonucleic acid interference; TSPO: Mitochondrial 18 kDa translocator protein.

3.1. Steroidogenic organs

The first murine model of *Tspo* deletion was a conditional knockout mouse with a deletion in testicular Leydig and Sertoli cells [20]. These mice were obtained with the *Cre/LoxP* system, which was used to induce a deletion between the exons 2 and 3 of the TSPO gene by placing the *Cre* recombinase under the control of the anti-Mullerian hormone receptor type II (Amhr2) promoter. The testicular deletion of *Tspo* did not impair testicular development. Testicular morphology was normal as well as the gene expression of proteins involved in steroidogenesis (*i.e.*, steroidogenic acute regulatory protein (StAR), cytochrome 11A1 (Cyp11a1) and 3-beta-hydroxysteroid dehydrogenase). Tspo deletion did not affect spermatogenesis and testosterone production. In the same

Table 2
Animal models of conditional *Tspo* deletion.

Species	*Tspo* gene editing types	Methods	Deletion outcomes	Refs
Mouse	Constitutive testes-specific KO	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the *Amhr2* promoter (Fig. 1E).	Normal development and steroidogenesis.	[20]
Mouse[1]	Constitutive liver-specific KO	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the *albumin* promoter (Fig. 1E)	Normal phenotype (longevity, morphology, mitochondrial functions), no effect on mitochondrial permeability transition pore opening, off targets effects for TSPO ligands.	[27]
Mouse	Constitutive cardiac-specific KO	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the *alpha-myosine* promoter (Fig. 1E)	No effects on mitochondrial permeability transition pore opening and ischemia-reperfusion injury *in vitro*.	[27]
Mouse[2]	Constitutive testes-specific KO (then considered Constitutive global KO)	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the *Amhr2* promoter (Fig. 1E)	High embryonic lethality.	[22]
Mouse	Constitutive testes-specific KO	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the *Nr5a1* promoter (Fig. 1E)	Impaired hormone-induced corticosterone production, adrenal glands lipid droplets accumulation indicative of blockade of cholesterol utilization for steroidogenesis, lipid stock in gonads reduction.	[22]
Mouse	Constitutive neuro-specific (neurones) KO	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the GFAP promoter (Fig. 1E)	Normal development and neuronal activity, protection in an experimental multiple sclerosis model.	[31]
Mouse	Tamoxifen-inducible cardiac-specific KO	*Tspo* gene containing *LoxP* regions and *Cre* recombinase under the control of the tamoxifen-inducible *alpha-myosine* promoter (Fig. 1E)	Protection against heart failure *in vivo* in an experimental model of aortic pressure overload (cardiac morphology, function and mitochondria).	[30]
Mouse	Constitutive testes-specific KO	Previously described in Ref. [22] (Fig. 1E)	Prediabetes phenotype.	[23]
Mouse	Constitutive neuro-specific (hypothalamus) KO	*Tspo* gene containing *LoxP* regions from Ref. [27] and *Cre* recombinase under the control of the *Rax* promoter (Fig. 1E)	Food intake reduction and energy expenditure elevation.	[28]
Mouse	Constitutive neuro-specific (microglia) KO	*Tspo* gene containing *LoxP* regions from Ref. [27] and *Cre* recombinase under the control of the *interleukin-8* promoter (Fig. 1E)	Prevention of phagocytes reactivity in a laser- induced model of neovascular age-related macular degeneration.	[29]

Amhr2: Anti-mullerian hormone receptor type 2; GFAP: Glial Fibrillary Acidic Protein KO: knock-out; Nr5a1: Steroidogenic factor 1, Rax: Retina and anterior neural fold homeobox, Tspo: Mitochondrial 18 kDa translocator protein. [1]The mice strain with the *Tspo* gene containing *LoxP* regions is now commercialized by Jackson Laboratories (JAX:024976). [2]The mice strain with the *Tspo* gene containing *LoxP* regions is now commercialized by the Mutant Mouse Resource and Research Center (MMRRC_067168-UCD).

way, estradiol and progesterone levels were unchanged in female *Tspo*-deleted mice. The breeding of these animals was not affected.

The authors had ensured that the deletion of *Tspo* was not compensated by the expression of *Tspo2*. The authors concluded that TSPO is not essential to steroidogenesis [20].

This work was immediately criticized for its biases, limitations and shortcuts. Indeed, it concluded that TSPO had no role in steroidogenesis, even though the study was limited to Leydig and Sertoli cells. Moreover, mitochondrial cholesterol transport does not result from a single-protein event. It seems to be due to a complex that facilitates cholesterol translocation, the transduceosome, consisting of StAR and mitochondrial membrane proteins including TSPO, VDAC, the ATPase family AAA domain-containing protein 3 (ATAD3) and the Cyp11A1.

It is therefore difficult to argue that TSPO is not involved in steroidogenesis especially since the results of several major studies in the field, conducted in different models including primary cells, cell lines or isolated mitochondria, confirm its involvement in mitochondrial cholesterol transport and in steroidogenesis [21].

These *Tspo* deletion experiments [20] were replicated in another study, but a low percentage of *Tspo*-deleted animals was obtained (4% versus the expected 25%; [22]). The authors hypothesized that at an early embryonic stage, *Amhr2* would be expressed and, thus, *Tspo* would be deleted, in an ectopic and not in a specific way in steroidogenic tissues. This would result in embryo death. They did not observe a reduction in the expression of *Tspo* at the testicular level but a reduction was observed in the ovary. This suggests that sex-dependent factors were influencing recombinase activity. They therefore decided to develop a similar deletion model but with the *Cre* recombinase under the control of the promoter of another gene than *Amhr2*: *Nr5a1* (steroidogenic factor 1). The litters obtained followed Mendel's laws for sex and genotype distribution. In this model, *Nr5a1* induced *Tspo* deletion in gonadal and stereoidogenic tissues, but not in brain. *Tspo*-deleted mice had blood levels of steroid hormones (testosterone, progesterone, corticosterone) and transduceosome protein expression (VDAC, ATAD3, Star and the Cyp11A1) similar to wild-type mice. Corticosterone production induced by hormonal stimulation of the *Tspo*-deleted mice was significantly reduced, in contrast to testosterone which was not affected by *Tspo* deletion. An accumulation of lipid droplets was observed in the adrenal glands of the deleted mice. This was associated with a reduction of the lipid stock in the testes and ovaries. These observations indicate that the lipid metabolism of these animals would thus be altered [22].

In this model, heterozygous crosses generated homozygous deleted pups of small size, with poorer growth than other pups from the same litter. In adulthood, HDL and triglycerides blood levels of the deleted mice were comparable to wild-type mice. However, *Tspo*-deleted mice exhibited a prediabetic phenotype with hyperglycemia, hypercholesterolemia, increased hemoglobin A1c glycation, and increased insulin secretion [23]. Furthering their research on embryogenesis of deleted mice, this team demonstrated that $Tspo^{+/-}$ and $Tspo^{-/-}$ embryos exhibited growth retardation compared to $Tspo^{+/+}$ embryos and concluded that TSPO is necessary for early embryonic development [24].

According to Ref. [24], these models of *Tspo* deletion using *Cre* under the control of the *Amhr2* promoter [20,22] are models of global deletion and not steroidogenic tissue-specific deletions. Indeed, *Amhr2* is expressed at an early stage of embryogenesis and would be responsible for a global deletion of *Tspo*. In this *Amhr2* model of *Tspo* deletion, Fan et al. [24] showed an incomplete deletion in the testes, an alteration of lipid homeostasis and a decrease in testosterone levels. This is consistent with results obtained in intersticial Leydig cells. Cells isolated from *Tspo*-deleted mice failed to respond to saturating concentrations of human chorionic gonadotropin, whereas cells isolated from wild-type

animals showed a three-fold stimulation of testosterone production in response to human chorionic gonadotropin. Whether these changes are related to steroid precursor levels is unknown. This contrasts with their *Nr5a1* model, which allows a specific and complete deletion with no effect on testosterone. Similarly, the *Amhr2* model resulted in altered expression of certain proteins involved in cholesterol binding and transport (lower expression of Star and higher expression of Cyp11A1), which would reflect compensatory mechanisms in the absence of TSPO.

The latter article has been criticized and the conclusions drawn were contradicted [25]. The authors recalled that more than a hundred publications rely on the *Cre/LoxP* system under the control of the Amhr2 promoter to generate steroidogenic tissue-specific deletions. They point out that it is crucial to use only *Cre*-carrying males and not females as this can lead to global and non-tissue-specific deletions. They hypothesized that Fan et al. [22] could have failed to follow this recommendation, which would explain the global deletion obtained in their study. The authors concluded that TSPO is not involved in *de novo* steroidogenesis, supported by a review of the different deletion models of *Tspo* [25]. However, a recent study revealed that the *Amhr2* promoter is not as specific as originally thought and can lead to global genetic modifications in the mouse [26]. This can contribute, at least partially, to the variability of the results. To date, there is no consensus on the role of TSPO in steroidogenesis (testosterone, corticosterone synthesis), when investigating testis specific *Tspo* deletion mouse model.

3.2. Heart & liver

Two *Tspo* deleted mouse models were also developed to study its role in the induction of the mitochondrial permeability transition pore in the liver and in the heart [27]. The deletion between exons 2 and 3 of the *Tspo* gene was obtained using the Cre/LoxP system with Cre recombinase under the control of the albumin promoter for the liver deletion model and alpha-myosin promoter for the heart. Hepatic deletion of *Tspo* did not lead to modification of the hypothetical TSPO partner proteins constituting the mitochondrial permeability transition pore (i.e., adenine nucleotide translocase, VDAC, cyclophilin D, mitochondrial phosphate transporter, and the ATPase beta subunit). Similarly, the deleted mice did not exhibit any particular phenotype, in terms of longevity and hepatic morphology. *Tspo* deletion did not cause morphological nor functional alterations in mitochondria (respiration, membrane potential, calcium retention capacity, induction of mitochondrial permeability transition pore). Moreover, *Tspo* deletion did not alter the extent of reperfusion injury, in an isolated heart hypoxia-reoxygenation model. The authors concluded that TSPO is not required for the mitochondrial permeability transition pore opening. Surprisingly, the different TSPO ligands tested in this study (PK-11195, 4′-chlorodiazepam, and protoporphyrin IX) decreased calcium retention capacity similarly in mitochondria from wild-type and deleted mice. It should be noted that the authors had previously ensured that mitochondria from deleted mice did not bind [3H]PK-11195. These results called into question the involvement of TSPO in the specific effect produced by these ligands and the conclusions obtained with their use. Indeed, TSPO has been identified as an actor in many physio-pathological processes (proliferation, oncology, inflammation, neurodegeneration, etc.) thanks to the effects of its ligands in these processes. This study tended to demonstrate that the effects of TSPO ligands were due to their interaction with other targets [27]. Other teams have used this mouse model to generate other tissue-specific deletion models. In hypothalamic glial cells, *Tspo* inactivation increased the use of fat by AMPK-induced lipophagy, which overcomed energy surplus and improved the obese phenotype [28]. In the retina, specific deletion of *Tspo* revealed that TSPO is a key regulator of NADoxidase1-dependent neurotoxic ROS production in the retina [29].

Another cardiac and conditional *Tspo* deletion model was developed to address the cardioprotective effect of TSPO with an *in vivo* model of heart failure [30]. Two LoxP regions were added before exon 2 and after exon 3 of the *Tspo* gene and the *Cre* recombinase gene was placed under the control of the tamoxifen-inducible myosin promoter. The experimental heart failure was induced by subjecting the mice to a transverse aortic constriction. This model of aortic pressure overload increased cardiac *Tspo* expression in wild-type mice. *Tspo*-deleted mice were protected from the effects of transverse aortic constriction with limited progression to heart failure. Indeed, these mice had a significantly preserved ejection fraction and reduced dilatation, hypertrophy, and remodeling compared with wild-type mice. Wild-type mice showed impaired mitochondrial calcium uptake, preserved by *Tspo* deletion without modification of the mitochondrial permeability transition pore, either in basal or in heart failure conditions. Transverse aortic constriction caused a decrease in ATP production in wild-type mice, while it was maintained in *Tspo*-deleted mice. Reactive oxygen species production was reduced in *Tspo*-deleted mice, and mitochondrial respiratory chain complex I activity, mitochondrial membrane potential, and mitophagy were preserved. In this study, there was no difference between wild type and *Tspo*-deleted mice under basal conditions for the majority of the parameters studied. It is the application of a stress (transverse aortic constriction) that revealed the involvement of TSPO in these different mechanisms [30].

3.3. Brain

In parallel with research in steroidogenesis and cardioprotection, deletion experiments have been conducted to study TSPO at the brain level. The first neurospecific *Tspo* deletion was achieved by placing the *Cre* recombinase under the control of the Glial Fibrillary Acidic Protein promoter, and did not cause any developmental alteration or loss of neuronal activity in the mice. In an experimental autoimmune encephalomyelitis model of multiple sclerosis, *Tspo* knockout mice were protected compared to wild-type mice, making TSPO a therapeutic target for the treatment of this pathology [31].

Wolf et al. [29] developed another model of brain deletion of *Tspo* from the strain generated by Šileikytė et al. [27]. With the *Cre* recombinase under the control of the Cx3cr1 (interleukin-8 receptor) promoter, the deletion was microglia-specific. The deleted mice did not show an altered microglia phenotype, with branching and networking comparable to wild-type mice and the mitochondrial function of retinal microglial cells (ATP production, mitochondrial membrane potential) was not impaired. However, *Tspo* deletion significantly reduced inflammation related to vascular damage in age-related macular degeneration, with a decrease in the phagocytic capacity of monocytes and in neo-angiogenesis. The authors also demonstrated that TSPO was responsible for microglial production of reactive oxygen species via its effect on cellular calcium and NADPH oxidase 1 [29].

The *Tspo*-deleted mouse strain developed by Šileikytė et al. [27] was also used to generate a hypothalamic-specific *Tspo* deletion model [28] in an obesity study. The deleted mice consumed less food and expended more energy than wild-type mice when fed a high-fat diet. *Tspo* deletion induced lipophagy, releasing fatty acids contained in lipid droplets. The authors conclude that TSPO allows lipid sensing at the hypothalamic level, thus participating in the regulation of energy homeostasis [28].

So far, *Tspo* deletion in glial cells or specific brain regions did allow to obtain a clear picture of the role of TSPO in

neuroinflammation and oxidative stress.

4. Murine models of global *Tspo* deletion

The different methodological approaches to obtain the total *Tspo*-deletion models as well as the results of the different studies performed with these models are summarized in Table 1.

4.1. Global Tspo deletion and steroidogenesis

In 2014, two independent studies generated the first models of global deletion for *Tspo* in mice [32,33]. The first mouse model called Guwiyang Wurra ("fire mouse" in Dharawal, an Australian aboriginal language) was obtained by crossing mice carrying LoxP site between exons 2 and 3 of the *Tspo* gene and *Cre* knock-in mice that constitutively express the Cre recombinase called "Cre deleter mice" [32]. Global *Tspo* deletion did not altered the reproduction of these mice, nor in their development, and overall, their health and behaviors were not affected. Similarly, mitochondrial cholesterol catabolism by CYP11A1, serum pregnenolone (precursor of steroid hormone biosynthesis, derived from cholesterol) and heme metabolism, were comparable in *Tspo* knockout and wild-type mice. Unlike the *Tspo* knockdown zebrafish [16], the blood composition of *Tspo* knockout mice was not altered. The authors, however, found a decrease in mitochondrial activity associated with a reduction in ATP production in primary microglia cells from these mice [32]. A subsequent study using this model confirmed the binding specificity of some TSPO ligands [34], which had been questioned previously [27]. Indeed, whole animal *in vivo* imaging of *Tspo* knockout mice with PBR111 and membrane binding using PK11195 in testicular and kidney tissue showed no specific ligand binding.

The second model of global *Tspo* deletion in mice was also achieved using the *Cre/LoxP* system, but under the control of the *Ddx4* (ATP-dependent RNA helicase DDX4) promoter [33]. Like the "fire mice", these mice showed a normal phenotype and were fertile. Indeed, steroid organ morphology (gonads, adrenal glands), plasma levels of steroid hormones (glucocorticoids, mineralocorticoids, androgens, estrogens, and progestogens), mitochondrial structure, and fertility were comparable in wild type and *Tspo* knockdown mice. *Tspo* deletion did not alter the gene expression of other proteins involved in steroidogenesis (StAR, Cyp11a1, hydroxy-delta-5-steroid dehydrogenase (Hsd3b1), VDAC, adenine nucleotide translocase, Hexokinase 2, Acyl-CoA-binding domain-containing 3 protein (ACBD3)) and these mice did not show any increase in *Tspo2* expression, similarly to the mice carrying the testicular deletion of *Tspo* [20]. However, the expression of other genes was modified. These genes were mainly involved in immunomodulation and lipid transport. The authors concluded that TSPO is not required for steroidogenesis and hypothesized that the effects of TSPO ligands were due to a conformational change of the protein inducing either a release of cholesterol, thus available for steroidogenesis, or a modification of the membrane conformation allowing interactions between steroidogenic proteins. Therefore, the role of TSPO could be mainly linked to biological stress situations rather than physiological mechanisms [33]. This mouse model was also used to study the role of TSPO in heme biosynthesis. *Tspo* deficiency did not affect erythropoiesis and tissue heme and protoporphyrin IX levels. The authors conclude that TSPO is not essential for heme biosynthesis [35].

Since these first two global deletion models did not lead to definitive conclusions regarding the role of TSPO in steroidogenesis, Papadopoulos's group addressed this issue and generated two new models in rats: a *Tspo* deletion and a *Tspo* mutation at the Cholesterol Recognition/Interaction Amino Acid Consensus (CRAC) motif of TSPO [36]. Both of these genetic modifications were performed by injecting rat embryos with zinc finger nucleases targeting the *Tspo* gene. Rats deleted for *Tspo* were obtained by inducing a deletion between exon 3 and intron 3 of the gene. Rats deleted for the CRAC domain were obtained by truncating the gene at exon 4, which results in the expression of a truncated TSPO protein without a CRAC binding domain. Obtaining these rats was difficult since out of 474 transfected embryos, only two deleted rats were born with a global deletion of TSPO and only one rat out of 130 embryos with a CRAC domain mutation. Rats mutated for *Tspo* (global or CRAC domain deletion) showed no particular phenotype. However, an accumulation of lipid droplets (source of cholesterol used for steroidogenesis) was observed in the steroidogenic tissues of both rat models. Testosterone levels in *Tspo*-deleted rats were significantly lower than in wild type rats and *Tspo*-deleted rats did not respond, or only partially, to the stimulation of corticosterone synthesis by corticotropin. This study concluded that steroidogenesis is impaired in the absence of TSPO when it is stimulated by corticotropin but not under basal condition [36].

Approximately similar results were obtained by Barron et al. [37] who developed their own model of *Tspo* global deletion in mice, with the *Cre/LoxP* system under the control of Camk2a (calmodulin-dependent protein kinase II a) promoter. They observed a decrease in total steroidogenesis with specific reduction in the plasma levels of progesterone, corticosterone and aged-dependent deficiency androgenic steroids. This study concluded that TSPO is not required for basal steroidogenesis but that its deletion would affect this process, especially during aging [37].

Recently, the brain, adrenal glands, testes and plasma steroidomes of the "fire mouse" generated by Banati et al. [32] were characterized under basal conditions. Liere et al. [38] demonstrated that all steroid hormones (progestogens, mineraloglucocorticosteroids and androgens) and their precursor (pregnenolone) were present in both wild type and *Tspo*-deleted mice, with similar concentrations. However, in *Tspo*-deleted mice, they found significant lower 5α-reduced metabolites of steroid hormones concentrations in adrenal glands and in plasma but without an alteration of VDAC-1, CYP11A1 and 5α-reductase expression. The authors concluded that *Tspo* deletion does not impair *de novo* steroidogenesis, suggesting other regulatory functions for TSPO [38].

For now, the different models of *Tspo* deletion, whether specific or global, do not allow to conclude on the potential role of TSPO in steroidogenesis. However, they indicate that *Tspo* is not necessary for the *de novo* synthesis of pregnenolone under basal condition but that *Tspo* deletion would affect steroid levels following hormone stimulation.

4.2. Global Tspo deletion and neuropathologies

Tspo global deletion models have also been developed in other research fields than steroidogenesis, in particular to study the role of this protein in different neurological pathologies. Thus, a study using the *Cre/LoxP* system under the control of protamine promoter [39] to generate a global deletion of *Tspo* in mice concluded that the deletion did not induce impairment of reproduction and phenotype and did not alter the development of major organs. RNAseq analysis of these mice also revealed that deletion of *Tspo* did not induce modification in gene expression of TSPO partners, including StAR, VDAC, and Cyp11a1 [39]. In this model, *Tspo* deletion was associated with an inhibition of microglial activation and mitochondrial metabolism in microglia cells was impaired, with a significant reduction in mitochondrial membrane potential, oxidative phosphorylation, and glycolysis [40]. In an experimental Alzheimer's disease model, these mice show impaired microglial phagocytosis,

strong neuroinflammation and thus, brain accumulation of beta amyloid peptide. Since numerous studies have shown overexpression of TSPO in Alzheimer's disease, the authors concluded that TSPO would play a neuroprotective role in this pathology [41]. The model generated by Wang et al. [39] was also used to verify that the specificity of antidepressant effects of a TSPO ligand (AC-5216 also known as XBD-173 or Emapunil). This study demonstrated that, in chronically stressed mice, TSPO mediated anxiolytic- and antidepressant-like effects, through BDNF-mTOR pathway activation and increase in the total dendritic length and branching points of pyramidal neurons [42].

4.3. Global Tspo deletion and retinal degeneration

Another model of global Cre/LoxP deletion of *Tspo* under the control of the *Best1* (bestrophin 1) promoter was generated in mice, as part of a study on retinal degeneration, a disease in which TSPO is overexpressed. Mice deleted for *Tspo* showed a normal retinal phenotype, with morphology and function similar to wild-type mice. Likewise, *Tspo* deletion had no effect on susceptibility to retinal degeneration. The authors hypothesized that TSPO requires its partners for some of its functions and that the interaction of TSPO with its ligands may be necessary to modulate retinal degeneration [43]. Another study using the mouse model developed by Tu et al. [33] investigated this pathology and confirmed the absence of alteration in the retinal phenotype [44]. However, changes in lipids (cholesterol, triglycerides, and phospholipids) and cholesterol efflux were observed in *Tspo*-deleted mice retinal cells. These effects were accompanied by pro-inflammatory cytokine production and microglia activation. The authors conclude that TSPO could be a potential target in the development of therapeutic strategies for age-related macular degeneration [44].

4.4. Global Tspo deletion and infection

As Cho et al. [19] suggested a role for TSPO in the responses against infection and wound, studies were conducted on mouse models to address this hypothesis, especially in pathologies where TSPO is overexpressed. Therefore, the mouse model developed by Barron et al. [37] was used in a study on septic encephalopathy. This pathology was reproduced *in vivo* by injection of a bacterial toxin, inducing neuroinflammation with microglia activation and cytokine release associated with behavioral inhibition. All of these effects were significantly reduced in the *Tspo*-deleted mice. The authors conclude that TSPO would play a major role in septic encephalopathy, the mechanisms of which remain to be elucidated [45]. Another study addressed the role of TSPO in bacterial infection response as psychiatric and cognitive impairments are often observed in patients after sepsis. Kikutani et al. [46] subjected *Tspo*-deleted mice generated by Barron et al. [37] to a cecal ligation and puncture surgery, an experimental sepsis model. Interestingly, *Tspo* deletion led to higher mortality and prolonged weight loss and exacerbated anxiety-like and depressive-like behavior with cognitive impairment after surgery [46].

4.5. Global Tspo deletion and metabolism

Morrissey et al. [47] generated a new model of global *Tspo* deletion using a method similar to the one used by Banati et al. [32] for their “fire mouse”. This model challenges the results obtained by Fan et al. [23] and Kim et al. [28] with tissue-specific deletion models. Indeed, they observed no modification of body mass or food intake in *Tspo*-deleted mice. An altered diet (prolonged fasting or high fat) did not affect energy homeostasis in *Tspo*-deleted mice and does not induce a prediabetic phenotype. Overall, they found that *Tspo* deletion did not impaired the physiological response to deviations in systemic energy availability at the whole organism level [47].

4.6. Global Tspo deletion and inflammatory disorders

Recently, Papadopoulos' group examined the role of TSPO in non-alcoholic fatty liver disease [48] using the *Tspo*-deleted models they developed in mice [22] and rats [36]. This study was undertaken because the expression of TSPO increases when the pathology evolves from a simple steatosis to a non-alcoholic steatohepatitis and because the radiolabeling of TSPO is a potential diagnostic tool for non-alcoholic steatohepatitis as for neuroinflammation. This study shows that *Tspo*-deletion induces adaptive cellular antioxidant mechanisms and decreases acetyl-CoA acetyltransferase 2 expression. This favors free cholesterol accumulation, which causes the loss of adaptive mechanisms and thus endoplasmic reticulum stress. In the experimental model of non-alcoholic steatohepatitis, *Tspo*-deletion is accompanied by a decrease in the cytochromes Cyp7A1 and Cyp27A1. The authors conclude that TSPO regulates steatosis and bile acid synthesis in non-alcoholic fatty liver disease. This study highlights the complexity of studying TSPO, which role evolves during this pathology: its deletion promotes the progression of simple steatosis but improves fibrosis in nonalcoholic steatohepatitis [48].

TSPO is overexpressed in ulcerative colitis, an inflammatory bowel disease, and could be an interesting target for therapeutic development [49]. Jimenez et al. [50] subjected *Tspo*-deleted mice developed by Tu et al. (2014) to an ulcerative colitis experimental model and demonstrated that *Tspo*-deletion worsened the disease, with higher inflammation and epithelial integrity loss. These effects were associated with lower proliferation capacity and elevated mitochondrial fatty acid oxidation. This study concluded that TSPO limits inflammation in ulcerative colitis and should be further studied to elucidate the underlying mechanisms [50]. Those results were confirmed by another study addressing the pathophysiological role of TSPO in inflammatory bowel disease. Indeed, *Tspo*-deleted mice, generated by Wang et al. [39], developed more severe colitis with higher inflammation and pyroptosis than wild-type mice. They concluded that rapid TSPO upregulation in response to inflammatory injury protects from pyroptosis [51].

5. Murine models of *Tspo* tissue-specific induction

To date, two *in vivo* models of *Tspo* induction, using two different techniques, have been published and both were used to study TSPO in the brain. Zhang et al. [52] induced *Tspo* overexpression by injecting, into the hippocampal dentate gyrus, a lentivirus with a plasmid containing the *Tspo* gene. This brain overexpression of *Tspo* induced a specific increase in progesterone and its derivative allopregnanolone in the CA1 hippocampal region and protected from neuroinflammations induced by different experimental models of cognitive deficits [52,53]. Two other studies confirmed the neurosteroidogenesis enhancement effect of *Tspo* overexpression and demonstrated its anxiolytic and antidepressant effects [54,55]. The second model of *Tspo* induction was generated from a mouse model of global *Tspo* deletion previously described [37], coupled with the injection of a transgene to express *Tspo* only in neurons. Global *Tspo* deletion induced an accentuation of anxiety behaviors and a reduction in cerebral steroidogenesis without modifying depressive behaviors. Neuronal *Tspo* overexpression decreased depressive behaviors, a neurosteroidogenesis-dependent effect, and partially reversed anxiety-like behavior in *Tspo*-deleted mice. The authors concluded that TSPO is required for cerebral steroidogenesis and would thus be involved in anxious and depressive behaviors [56],

which is consistent with conclusions drawn with the first *Tspo* induction model [52–55]. The main results obtained with the models presented in this section are summarized in Table 3. This table clearly shows that conditional *Tspo* induction in animal results in beneficial outcomes, including neuroprotection, anxiolytic-and anti-depression-like effects, some of which are mediated by specific increased production of allopregnanolone.

6. Conclusion

For a long time, the deletion of the *Tspo* gene in rodents was considered lethal at an embryonic stage, thus conferring a vital character to TSPO [15]. However, recent improvements in techniques to induce gene deletions allowed to obtain viable and normally developing *Tspo*-deleted animals. The absence of a drastic phenotype altered by the deletion of *Tspo* is not synonymous with the inanity of this protein. Indeed, gene deletion, even when the genes are essential, can result in normal phenotypes and reveal impairment only under stress conditions [57]. For example, the deletion of FoxO1 does not cause any particular phenotype in male and young mice (classically used in studies) but this deletion causes hyperglycemia and reduction in the mass of pancreatic beta cells in multiparous females and older males [58].

In the same way, as pointed out by Papadopoulos et al. [59], other proteins critical for mitochondrial functions, such as VDAC and the adenine nucleotide translocator 1, show no altered phenotype despite their apparent importance. This suggests that functional redundancies may exist for some mitochondrial proteins because of their importance in cell physiology [59].

Moreover, although ancient and highly conserved genes are generally considered “essential” for fundamental physiological mechanisms, these genes are mainly associated with pathologies and would be more frequently involved in stress response mechanisms [60]. TSPO, which is highly conserved, might not have a physiological function *per se* but may be involved in certain pathological or cellular stress adaptation processes. Indeed, the majority of studies carried out under “basal” conditions have not shown any major effect of *Tspo*-deletion. On the other hand, the presence of a stress (as observed in aging, neurodegenerative diseases, heart failure and high inflammation such as colitis) causes significant differences in *Tspo*-deleted animals.

It will be interesting to investigate whether the effect of a stressor, such as adrenocorticotrophic hormone for the adrenal, or activator of cells, such as luteinizing hormone for the testis, would be affected by the lack of TSPO. It should be noted that a recent study identified a first link between TSPO and the human stress response [36]. In humans, the rs6971 polymorphism on the TSPO gene has been associated with anxiety-related disorders [61,62]. This polymorphism lead to an amino acid substitution in the fifth transmembrane loop of TSPO, which is where the cholesterol-binding domain of the protein is located. The presence of rs6971 polymorphism in humans alters adrenocorticotropic hormone-induced plasma corticosteroid concentrations in the same way as shown in rats TSPO deletion mutations [36].

The variability of models (animal species and strains, breeding patterns, genetic modification techniques, etc.) could be at the origin of the disparity of results obtained following *Tspo*-deletion. For example, *Cre recombinase* expression is under the control of different promoters and they could be activated at different stages of embryonic development: *Tspo*-deletion would therefore be more or less early depending on the model. Papadopoulos et al. [59] advanced another hypothesis based on the physical proximity of the *Tspo* gene with two other genes: *Ttll12* (Tubulin tyrosine ligase-like family member 12) which is involved in histone methylation and microtubule assembly and *Mcat* (Mitochondrial malonyl-CoA:ACP acyltransferase) which is involved in mitochondrial fatty acid biosynthesis. Depending on the deletion technique, these genes could potentially be altered and participate in the observed phenotype differences. CRISPR/Cas9 genome editing development [63] and its successful use to create a mouse model of gene deletion ten years ago [64] offers new possibilities to generate models to study TSPO *in vivo* in the future.

In conclusion, this review shows that, in a short time, *Tspo*-deletion and induction studies have profoundly improved our fundamental knowledge on this protein. However, the disparity of the phenotypes obtained and the contradictory results of the different studies have fueled a debate on the pathophysiological functions of TSPO, questioned the specificity of the effects obtained with its ligands and the interactions with its partner proteins (especially within the transduceosome and the mitochondrial permeability transition pore). Despite these advances, the physiological role of *Tspo* remains an open question.

One of the main difficulties in the study of TSPO lies in the absence of an *endpoint*, a parameter to be measured that reflects its activity since this is not clearly established. Research should focus on identifying signaling pathways involving TSPO, with defined inducers and effectors. Numerous studies question the role of TSPO in stress situations: is it a deregulation of TSPO that causes the dysfunctions or is it the dysfunctions that cause a deregulation of TSPO expression? Moreover, the role of TSPO seems to be able to evolve during pathologies, making its study even more complicated. However, this does not call into question the numerous *in vitro, in vivo* and clinical studies that reveal the involvement of TSPO under chronical stress conditions or upon administration of its ligands. TSPO therefore remains an interesting target to study in many pathologies. Since its discovery and the first attempts to

Table 3
Animal models of conditional *Tspo* induction.

Species	*Tspo* gene editing types	Methods	Induction outcomes	Refs
Mouse	Inducible neuro-specific (hippocampal dentate gyrus) KI.	Injection of lentivirus with plasmid containing *Tspo* gene in the hippocampal dentate gyrus (Fig. 1H)	Neuroprotection and enhanced neurosteroidogenesis in a systemic inflammation model.	[52]
Mouse	Inducible neuro-specific (hippocampal dentate gyrus) KI.	Previously described in Ref. [52] (Fig. 1E)	Protection against cognitive impaired in a systemic inflammation model.	[53]
Mouse	Inducible neuro-specific (hippocampal dentate gyrus) KI	Previously described in Ref. [52] (Fig. 1E)	Increased allopregnenolone and anxiolytic and antidepressant-like behavioral effects.	[54]
Mouse	Inducible neuro-specific (hippocampal dentate gyrus) KI.	Previously described in Ref. [52] (Fig. 1E)	Increased allopregnenolone and anxiolytic and antidepressant-like behavioral effects in a PTSD experimental model.	[55]
Mouse	Constitutive neuro-specific (neurons) KI coupled with a constitutive global KO	Injection of neuro-specific *Tspo* transgene and breeding with global *Tspo*-deleted mice from Ref. [37] (Fig. 1I, mix from methods 1E and 1H)	Increased allopregnenolone and anxiolytic and antidepressant-like behavioral effects.	[56]

KI: knock-in; KO: knock-out; Tspo: Mitochondrial 18 kDa translocator protein, PTSD: post-traumatic stress disorder.

develop animal models of deletion more than twenty years ago, the function of TSPO remains an enigma, as evidenced by the plethora of studies published over the past forty years.

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