

# Neuro-oxysterols and neuro-sterols as ligands to nuclear receptors, GPCRs, ligand-gated ion channels and other protein receptors

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The brain is the most cholesterol rich organ in the body containing about 25% of the body's free cholesterol. Cholesterol cannot pass the blood–brain barrier and be imported or exported; instead, it is synthesised *in situ* and metabolised to oxysterols, oxidised forms of cholesterol, which can pass the blood–brain barrier. 24S-Hydroxycholesterol is the dominant oxysterol in the brain after parturition, but during development, a myriad of other oxysterols are produced, which persist as minor oxysterols after birth. During both development and in later life, sterols and oxysterols interact with a variety of different receptors, including nuclear receptors, membrane bound GPCRs, the oxysterol/sterol sensing proteins INSIG and SCAP, and the ligand-gated ion channel NMDA receptors found in nerve cells. In this review, we summarise the different oxysterols and sterols found in the CNS whose biological activity is transmitted via these different classes of protein receptors.

## KEYWORDS

24S-hydroxycholesterol, 24S,25-epoxycholesterol, brain, cholesterol, G protein-coupled receptor, nuclear receptor, oxysterol

## 1 | INTRODUCTION

It has been known for decades that  $C_{18}$ – $C_{21}$  steroids are ligands to nuclear receptors and since the 1990s that oxysterols are ligands to “orphan,” now “adopted,” nuclear receptors (Evans & Mangelsdorf, 2014). Importantly, nuclear receptors are expressed in the brain where oxysterols are also abundant (Warner & Gustafsson, 2015). These receptors work via regulating gene and hence protein expression. **N-methyl-D-aspartate receptors** (NMDARs) are expressed in nerve cells and work on a much shorter time scale. They are ligand-gated ion channels activated by the neurotransmitter glutamate, critical to the regulation of excitatory synaptic function. NMDARs are

modulated by excitatory neurosteroids and by the neuro-oxysterol **24S-hydroxycholesterol** (24S-HC, Figure 1, see Table S1 for systematic and common names and their abbreviations) (Paul et al., 2013).

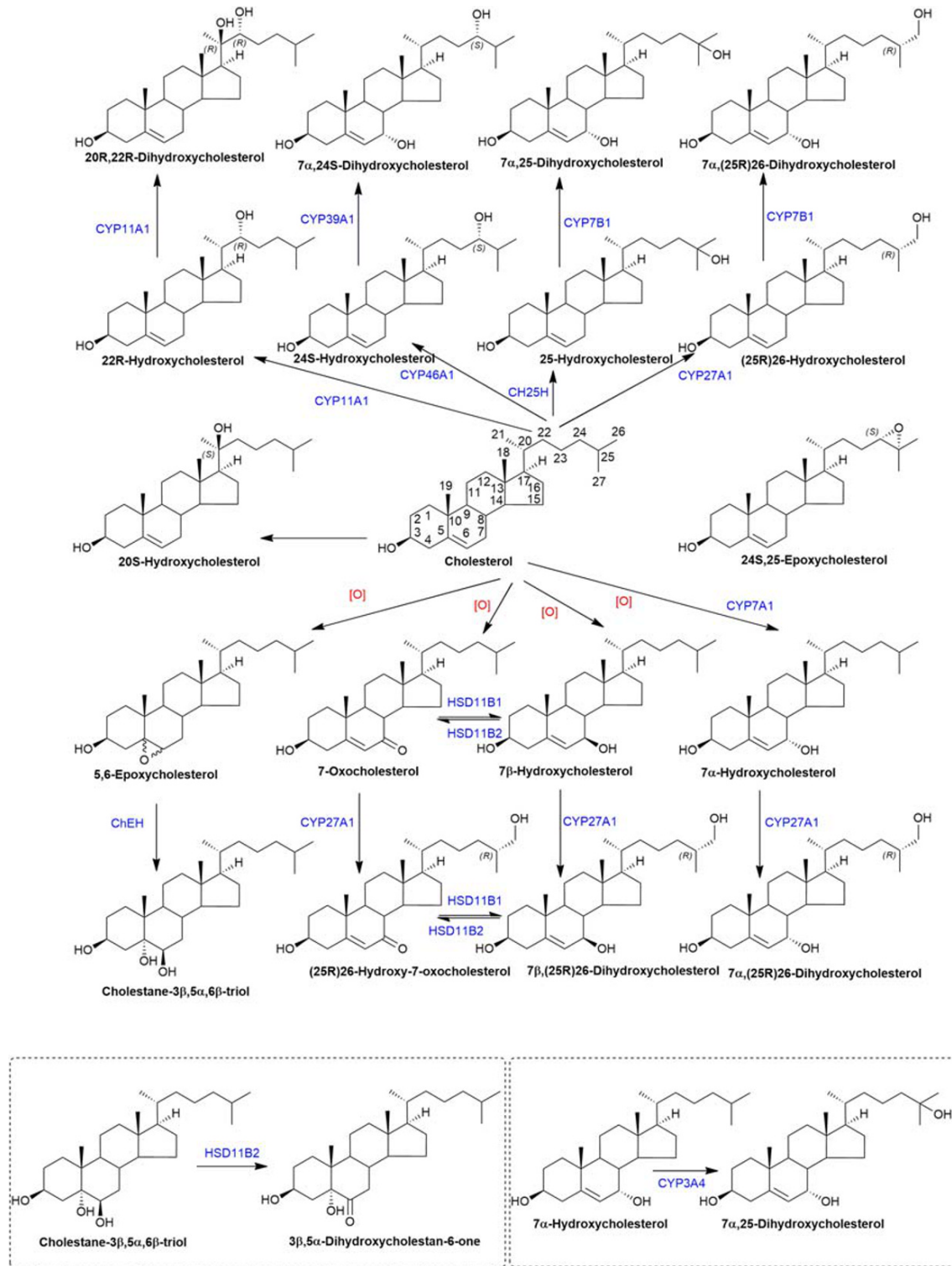
For simplicity, in this article, we use the term oxysterol to cover oxidised forms of cholesterol and its precursors (Schroepfer, 2000) and use the term sterol to specifically embrace cholesterol and its cyclic precursors. By extension, neuro-oxysterols and neuro-sterols are the respective terms to define oxysterols and sterols found in the CNS. The sterol definition is at variance with the formal definition of sterols by lipid maps to include all molecules based on the cyclopentanoperhydrophenanthrene skeleton and ring-opened versions thereof (Fahy et al., 2005).

**G protein-coupled receptors (GPCRs)** are cell membrane receptors that are activated by molecules outside the cell and activate signal transduction pathways within the cell. Cell membranes are rich in

Much of this article was written during the Coronavirus (COVID-19) outbreak. The article is dedicated to all healthcare workers throughout the world.

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**FIGURE 1** Biosynthesis of simple oxysterols. Enzymes are marked in blue. Non-enzymatic oxidation marked in red. Oxysterol names are linked to their abbreviations and biological activities in Table S1

cholesterol and membrane cholesterol plays an important role in GPCR structure and function (Sengupta & Chattopadhyay, 2015). Several cholesterol interaction sites have been identified in GPCRs whose occupancy may modulate GPCR activity. **Smoothered** (SMO), a member of the Frizzled-class of GPCRs and a critical component of the hedgehog (Hh) signalling pathway, has a cholesterol binding site

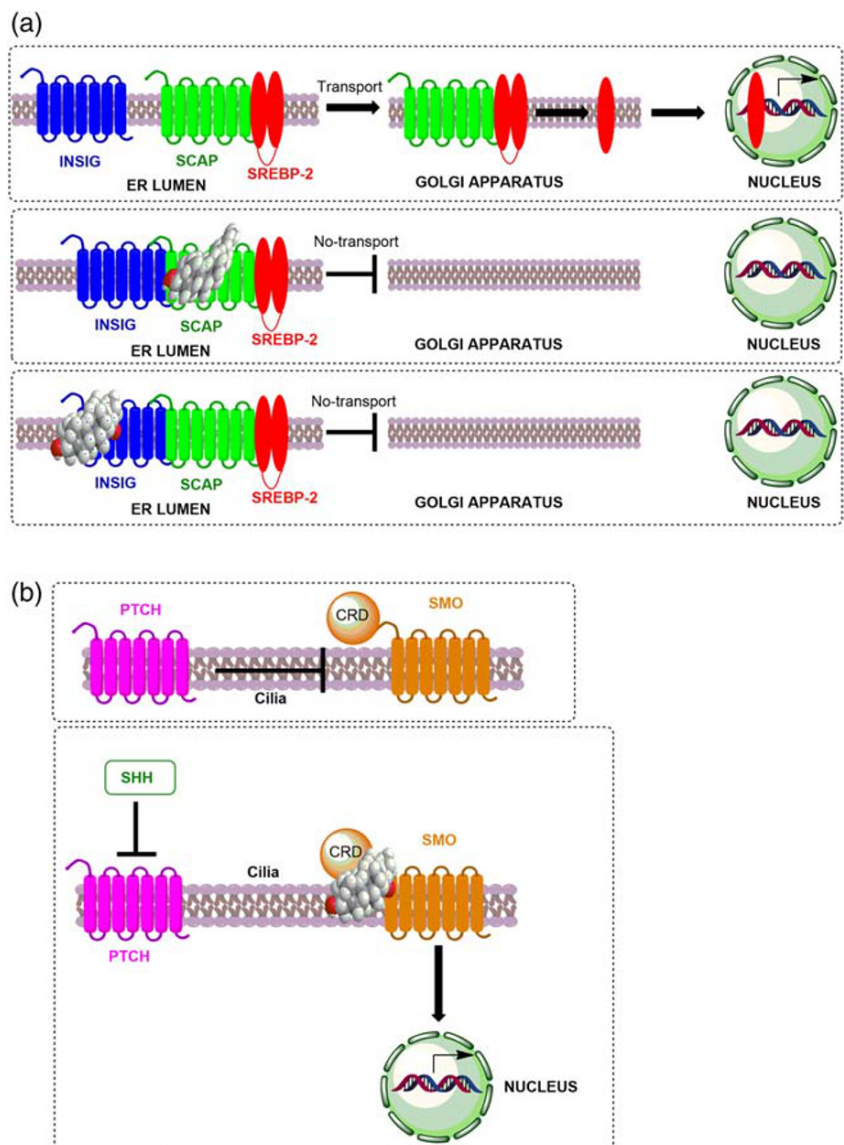
within its extracellular cysteine rich domain, mutational modification of which impairs the ability of SMO to transmit hedgehog signals (Byrne et al., 2016). The hedgehog pathway is critical for tissue patterning during development and abnormal function is associated with birth defects and cancer (Kong, Siebold, & Rohatgi, 2019). Defective hedgehog signalling is implicated in Smith–Lemli–Opitz syndrome,

which presents not only with dysmorphology but also learning and behavioural problems, highlighting the importance of hedgehog signalling in the brain (Cooper et al., 2003; Kelley et al., 1996). Smith-Lemli-Opitz syndrome results from deficiency in 7-dehydrocholesterol reductase (DHCR7), an enzyme near the end of the cholesterol biosynthesis pathway. Numerous oxysterols have also been found to activate the hedgehog signalling pathway by binding to the cysteine rich domain of SMO (Nedelcu, Liu, Xu, Jao, & Salic, 2013; Qi et al., 2019; Raleigh et al., 2018), many of these are found in the brain and are hence neuro-oxysterols (Yutuc et al., 2020). **GPR183**, also known as Epstein Barr Virus-Induced G protein-coupled receptor 2 or EBI2, is involved in the trafficking of immune cells towards their EBI2 ligands, that is, oxysterols with a dihydroxycholesterol (diHC) structure, where one additional hydroxy group is at C-7 $\alpha$  and the second on the side-chain (Hannedouche et al., 2011; Liu et al., 2011). As T-cell trafficking plays a major role in the neurodegenerative autoimmune disease multiple sclerosis and in its animal model, experimental autoimmune encephalitis (EAE), GPR183 and neuro-oxysterols have been studied in this regard

(Duc, Vigne, & Pot, 2019). **7 $\alpha$ ,25-Dihydroxycholesterol** (7 $\alpha$ ,25-diHC) and **7 $\alpha$ ,(25R)26-dihydroxycholesterol** (7 $\alpha$ ,(25R)26-diHC, also known as 7 $\alpha$ ,27-dihydroxycholesterol, note, if stereochemistry at C-25 is not indicated it is assumed to be 25R) are both GPR183 agonists which are present in the brain (Griffiths, Crick, et al., 2019) and 7 $\alpha$ ,25-diHC has been found to be increased in spinal cord during experimental autoimmune encephalitis development (Wanke et al., 2017).

Most, if not all, cells in vertebrates express the enzymatic machinery to synthesise cholesterol. The cholesterol biosynthesis pathway, also known as the mevalonate pathway (see [http://www.lipidmaps.org/pathways/pathway\\_lipids\\_list.php](http://www.lipidmaps.org/pathways/pathway_lipids_list.php) for exact details), is regulated by the master transcription factor SREBP-2 (sterol regulatory element-binding protein 2) which also regulates the expression of the LDL receptor (Horton, Goldstein, & Brown, 2002). When cholesterol levels are low, SREBP-2 is transported by SCAP (SREBP cleavage activating protein) from the endoplasmic reticulum to the Golgi apparatus where it is processed to its active form, which translocates to the nucleus and activates target gene transcription, hence up-regulating

**FIGURE 2** Cartoon representations of the regulation of SREBP-2 processing and of hedgehog (Hh) signalling by cholesterol and oxysterols. (a) Upper panel:- SCAP transports SREBP-2 from the endoplasmic reticulum to the Golgi where it is cleaved to its active form which translocates to the nucleus and activates transcription of genes coding for enzymes of the cholesterol biosynthesis pathway and of the LDL receptor. Middle panel:- When cholesterol levels are high, cholesterol binds to SCAP which binds to the endoplasmic reticulum resident protein INSIG, tethering the SCAP-SREBP-2 complex in the endoplasmic reticulum and preventing transport to the Golgi. Lower panel: Oxysterols may fine-tune the regulation of SREBP-2 processing by binding to INSIG which results in INSIG binding to the SCAP-SREBP-2 complex and preventing transport of SREBP-2 to the Golgi for further processing. (b) Upper panel: PTCH is the receptor for Hh ligands (e.g. sonic hedgehog, SHH). In the absence of Hh ligands, PTCH inhibits SMO and prevents signalling across the membrane. Lower panel: Hh ligands (e.g. SHH) bind to PTCH and relieve the inhibition of SMO by PTCH allowing Hh signalling. The exact mechanism by which PTCH inhibits SMO is unclear, but it is suggested that PTCH acts as a sterol-pump, pumping cholesterol and/or oxysterols out of the membrane preventing them binding to and activating SMO



cholesterol biosynthesis and import via LDL receptors and restoring cholesterol levels in the cell (Figure 2) (Goldstein, DeBose-Boyd, & Brown, 2006). When cholesterol levels are elevated, cholesterol in the endoplasmic reticulum binds to SCAP, which becomes tethered to the resident endoplasmic reticulum protein INSIG (insulin induced gene) and prevents transport of SREBP-2 to the Golgi apparatus for activation (Goldstein et al., 2006; Sun, Seemann, Goldstein, & Brown, 2007). In this way, cholesterol regulates its own biosynthesis and import via the LDL receptor. Oxysterols also inhibit cholesterol biosynthesis, but in this case by binding to INSIG and tethering the SCAP-SREBP-2 complex in the endoplasmic reticulum (Figure 2) (Radhakrishnan, Ikeda, Kwon, Brown, & Goldstein, 2007). Although the turnover of cholesterol in the adult brain is slow (0.4% per day in mouse, 0.03% in human), the rate of synthesis is much higher during development (Dietschy & Turley, 2004). In the fetal mouse, the blood-brain barrier (BBB) is formed at E10-E11 and after E11-E12, the brain is the source of essentially all new sterols (Tint, Yu, Shang, Xu, & Patel, 2006). Importantly, cholesterol 24-hydroxylase (**cytochrome P450 46A1, CYP46A1**) expression is low until E18, conserving cholesterol and restricting the biosynthesis of the neuro-oxysterol 24S-HC (Tint et al., 2006). CYP46A1 is the dominant cholesterol hydroxylase in the brain and hydroxylation of cholesterol to 24S-HC is responsible for 50–60% of all cholesterol metabolism in adult brain (Russell, Halford, Ramirez, Shah, & Kotti, 2009).

Other receptors for oxysterols include cytoplasmic oxysterol-binding protein (OSBP) and a family of proteins showing sequence homology to OSBP called OSBP-related (ORP) or OSBP-like (OSBPL) (Olkonen & Hynynen, 2009). OSBP regulates lipid transport between the endoplasmic reticulum and Golgi and also can act as a sterol-dependent scaffold for protein phosphatases that dephosphorylate ERK (Olkonen & Hynynen, 2009).

## 2 | NUCLEAR RECEPTORS

### 2.1 | Liver X receptors

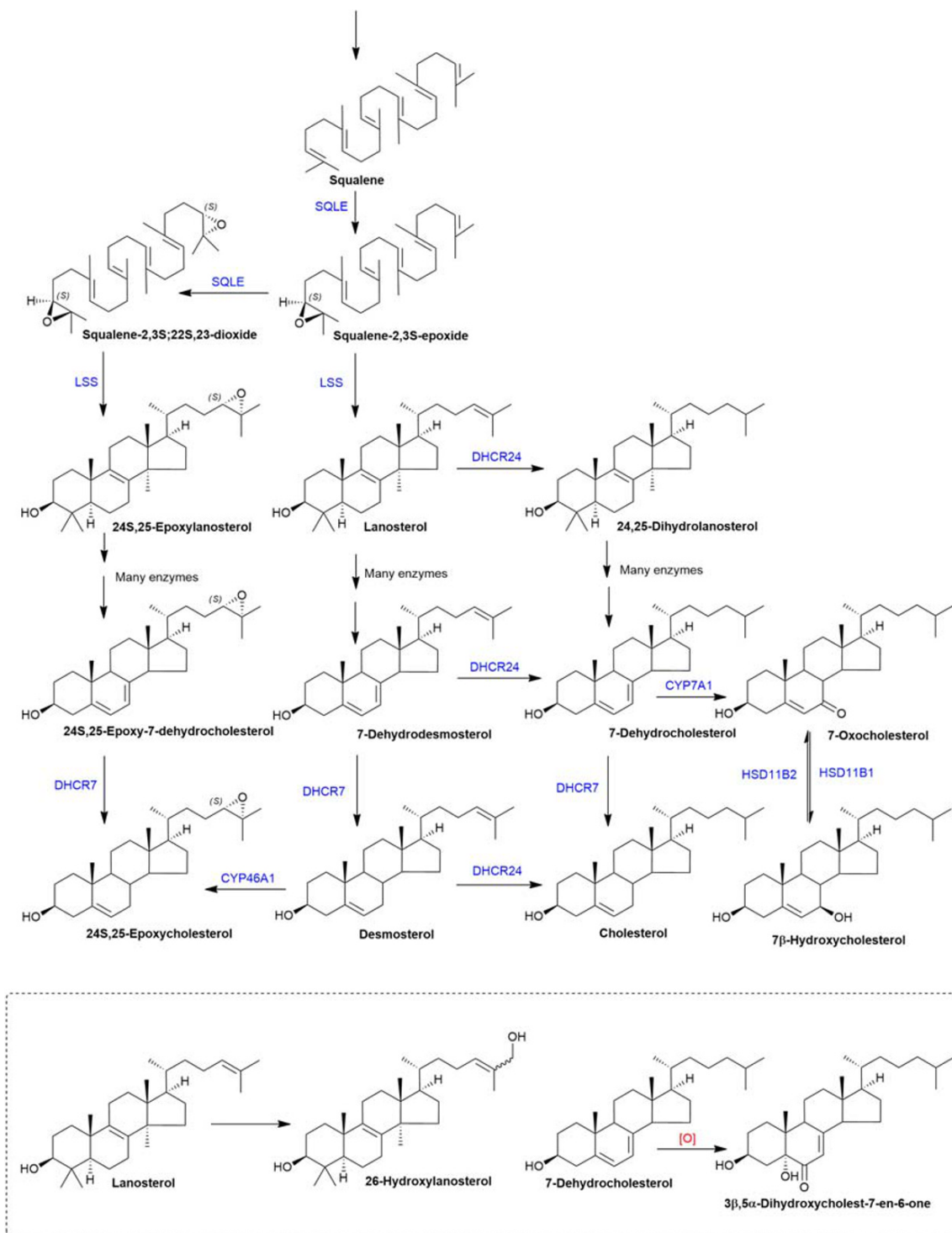
Perhaps the most well-studied activity of oxysterols is as ligands to nuclear receptors (Evans & Mangelsdorf, 2014; Warner & Gustafsson, 2015). Oxysterols are ligands to the liver X receptors  $\alpha$  (**LXR $\alpha$** , NR1H3) and  $\beta$  (**LXR $\beta$** , NR1H2) (Janowski, Willy, Devi, Falck, & Mangelsdorf, 1996; Lehmann et al., 1997), the latter of which is highly expressed in the brain (Wang et al., 2002). The most potent oxysterols are ones in which the side-chain of cholesterol has been modified by hydroxylation to give monohydroxycholesterols (HC), epoxidation to give **24S,25-epoxycholesterol** (24S,25-EC) (Janowski et al., 1996; Lehmann et al., 1997) or carboxylation to give a carboxylic acid (Song & Liao, 2000; Theofilopoulos et al., 2014), although other oxysterols also activate these receptors (Segala et al., 2017).

Following birth, the dominant neuro-oxysterol in the brain is 24S-HC (Bjorkhem, 2007; Dietschy & Turley, 2004). This is a ligand to both the liver X receptors. Surprisingly, the *cholesterol 24-hydroxylase*

knockout (*Cyp46a1<sup>-/-</sup>*) mouse has a mild phenotype but does show defective motor learning and in vitro studies indicate impaired hippocampal LTP (Kotti, Head, McKenna, & Russell, 2008; Kotti, Ramirez, Pfeiffer, Huber, & Russell, 2006; Lund et al., 2003). Thanks to the tight regulation of cellular cholesterol levels by cholesterol itself (Goldstein et al., 2006), the cholesterol levels in the brain of the *Cyp46a1<sup>-/-</sup>* mouse are not distorted (Lund et al., 2003; Meljon, Wang, & Griffiths, 2014). However, Kotti et al. found that a reduced rate of production of cholesterol precursors, specifically geranylgeraniol, as a consequence of an absence of CYP46A1, was the explanation for impaired hippocampal LTP (Kotti et al., 2006; Kotti et al., 2008).

Before birth, 24S,25-EC is the major neuro-oxysterol (Theofilopoulos et al., 2013; Wang et al., 2009) and can be formed by at least two pathways. In the cholesterol biosynthesis pathway, **squalene epoxidase (SQLE)** introduces a 2,3-epoxy group into squalene, the resultant squalene-2,3S-epoxide is then cyclised to **lanosterol** by the enzyme lanosterol synthase (LSS, Figure 3). If levels of squalene-2,3S-epoxide are elevated squalene epoxidase can introduce a second epoxy group to give squalene-2,3S;22S,23-dioxide, which is metabolised in parallel to squalene-2,3S-epoxide by the same enzymes (except 24-dehydrocholesterol reductase, DHCR24) to give 24S,25-EC rather than cholesterol (Gill, Chow, & Brown, 2008; Nelson, Steckbeck, & Spencer, 1981). The second pathway involves oxidation of **desmosterol** to give 24S,25-EC. In the brain the likely catalyst is CYP46A1 expressed in neurons (Goyal, Xiao, Porter, Xu, & Guengerich, 2014). However, the reaction can also proceed in fibroblasts raising the possibility of the involvement of an alternative catalyst or CYP46A1 expression in these cells (Saucier, Kandutsch, Gayen, Nelson, & Spencer, 1990).

Theofilopoulos et al. (2013) have shown the importance of 24S,25-EC in the neurogenesis of midbrain dopaminergic neurons through activation of the liver X receptors. They showed that midbrain progenitor cells derived from *Lxr* double knockout (*Lxr $\alpha$ <sup>-/-</sup>Lxr $\beta$ <sup>-/-</sup>*) mouse embryos have reduced neurogenic capacity (Sacchetti et al., 2009), while exogenous 24S,25-EC promoted dopaminergic neurogenesis in midbrain progenitor cells derived from wild type embryos (Theofilopoulos et al., 2013). 24S,25-EC also promoted the differentiation of embryonic stem cells to dopaminergic neurons, suggesting that 24S,25-EC, or perhaps more chemically stable liver X receptor ligands, may contribute to the development of cell replacement and regenerative therapies for Parkinson's disease, a disease characterised by the loss of dopaminergic neurons (Theofilopoulos et al., 2013). In the *CYP46A1* transgenic mouse (*CYP46A1tg*) overexpressing human CYP46A1, the concentration of 24S,25-EC is elevated in the developing ventral midbrain (Theofilopoulos et al., 2019). Theofilopoulos et al. exploited this mouse to show an increase in midbrain dopaminergic neurons in vitro and in vivo. Importantly, 24S-HC which is also elevated in *CYP46A1tg* mouse developing midbrain does not affect in vitro neurogenesis of midbrain dopaminergic neurons (Theofilopoulos et al., 2013; Theofilopoulos et al., 2019). Intracerebroventricular injection of 24S,25-EC to WT mouse brain embryos in utero increased the number of midbrain dopaminergic neurons in vivo, adding further weight to the hypothesis that the



**FIGURE 3** Biosynthesis of 24S,25-EC and other oxysterols from cholesterol precursors. Enzymes are marked in blue. Non-enzymatic oxidation marked in red. Oxysterol names are linked to their abbreviations and biological activities in Table S1

neuro-oxysterol 24S,25-EC promotes dopaminergic neurogenesis (Theofilopoulos et al., 2019). This led to the suggestion that increasing the levels of 24S,25-EC in vivo may be a useful strategy to combat the loss of midbrain dopaminergic neurons in Parkinson's disease. Interestingly, the concept of adeno-associated virus (AAV) gene transfer of *CYP46A1* for the treatment of Alzheimer's disease (Burlot et al., 2015), Huntington's disease (Boussicault et al., 2016; Kacher

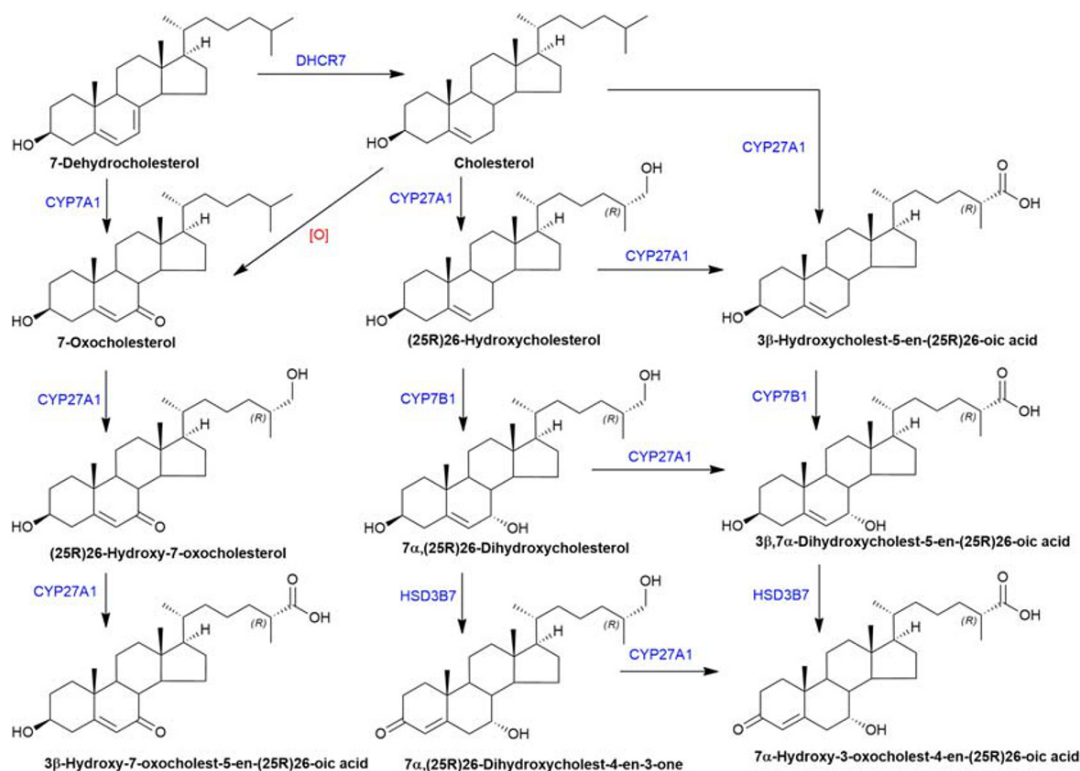
et al., 2019) and spinocerebellar ataxias (Nobrega et al., 2019) has been tested in mouse models of these diseases with success, with the aim of enhancing cholesterol metabolism via oxidation to 24S-HC. Such administration of *CYP46A1* no doubt enhances 24S,25-EC biosynthesis as well, although this was not measured in these studies and could potentially be used to also treat Parkinson's disease. In the study by Kacher et al. using the zQ175 mouse model of Huntington's

disease, CYP46A1 gene transfer to striatum was found to rectify defects in cholesterol metabolism and alleviate the Huntington's disease phenotype (Kacher et al., 2019). The expression of the liver X receptor target gene *ApoE* was up-regulated and the transport of cholesterol from astrocytes to neurons partially explained the improved phenotype (Kacher et al., 2019). It should be noted that the anti-HIV drug efavirenz activates CYP46A1 and could provide an alternative route to enhancing 24S-HC and 24S,25-EC formation in the brain (Mast et al., 2017).

Studies on the *Lxr $\alpha$ <sup>-/-</sup>Lxr $\beta$ <sup>-/-</sup>* adult mouse reveal progressive accumulation of lipids in the brain and loss of spinal cord motor neurons and ventral midbrain dopaminergic neurons, indicating further that these receptors are important in the brain in both the developing and adult mouse (Wang et al., 2002). Surprisingly, studies of the CYP46A1tg adult mouse found little change in the mRNA of liver X receptor target genes in the brain (Shafaati et al., 2011), despite elevation in 24S-HC and 24S,25-EC levels (Shafaati et al., 2011; Theofilopoulos et al., 2019). This may be a consequence of making global measurements, where changes in specific brain regions are lost by averaging signal levels in bulk tissue.

Cholestenic acids are also ligands to liver X receptors, these include 3 $\beta$ -hydroxycholest-5-en-(25R)26-oic (3 $\beta$ -HCA), 3 $\beta$ -hydroxy-7-oxocholest-5-en-(25R)26-oic (3 $\beta$ H,7O-CA) and 3 $\beta$ ,7 $\alpha$ -dihydroxycholest-5-en-(25R)26-oic (3 $\beta$ ,7 $\alpha$ -diHCA) acids. 7 $\alpha$ -Hydroxy-3-oxocholest-4-en-(25R)26-oic acid (7 $\alpha$ H,3O-CA), the down-stream metabolite of 3 $\beta$ ,7 $\alpha$ -diHCA, lacks a 3 $\beta$ -hydroxy group and is not an liver X receptor ligand (Figure 4) (Song & Liao, 2000; Theofilopoulos

et al., 2014). All of these acids are identified in human CSF, with the exception of 3 $\beta$ H,7O-CA which has been found in human plasma and bovine retinal pigment epithelium (Griffiths, Yutuc, et al., 2019; Heo et al., 2011; Theofilopoulos et al., 2014). The two 7 $\alpha$ -hydroxy acids have been identified in mouse brain making them neuro-oxysterol-acids (Yutuc et al., 2020). 3 $\beta$ -HCA, 3 $\beta$ ,7 $\alpha$ -diHCA and 7 $\alpha$ H,3O-CA have also been identified in the human brain. Remarkably, 3 $\beta$ -HCA is neurotoxic towards motor neurons, while 3 $\beta$ ,7 $\alpha$ -diHCA is protective and 3 $\beta$ H,7O-CA promotes maturation of precursor cells into motor neurons, with each activity mediated by liver X receptors (Theofilopoulos et al., 2014). Theofilopoulos et al. suggested that the loss of motor function in two diseases resulting from inborn errors of metabolism, cerebrotendinous xanthomatosis (deficiency in CYP27A1) and hereditary spastic paresis type 5 (deficiency in CYP7B1) is a consequence of a reduced production of 3 $\beta$ ,7 $\alpha$ -diHCA in cerebrotendinous xanthomatosis, with the additional over production of neurotoxic 3 $\beta$ -HCA in spastic paresis type 5 providing a double-hit mechanism in the latter disease (see Figure 4 for CYP catalysed reactions) (Theofilopoulos et al., 2014). Interestingly, 3 $\beta$ ,7 $\alpha$ -diHCA is found to be most abundant in mouse cerebellum (Yutuc et al., 2020) but is absent in cerebellum from cerebrotendinous xanthomatosis patients. Cerebellar ataxia, impaired co-ordination of voluntary movements due to underdevelopment of the cerebellum, is a common characteristic of cerebrotendinous xanthomatosis and may occur in spastic paresis type 5 also (Bjorkhem, 2013; Clayton, 2011), linking cholestenic acids and liver X receptors to brain development. Besides the



**FIGURE 4** Biosynthesis of cholestenic acids. Enzymes are marked in blue. Non-enzymatic oxidation marked in red. Oxysterol names are linked to their abbreviations and biological activities in Table S1

cholestenic acids, **(25R)26-hydroxycholesterol** ((25R)26-HC, also called by the non-systematic name 27-hydroxycholesterol, 27-HC, note if stereochemistry at C-25 is not defined it is assumed to be 25R), a weak liver X receptor agonist (Fu et al., 2001), also accumulates in CSF of spastic paresis type 5 patients (Schols et al., 2017; Theofilopoulos et al., 2014) and Hauser et al. have suggested that neurotoxic effects of (25R)26-HC are major contributors to the spastic paresis type 5 phenotype (Hauser et al., 2019). It is noteworthy that neither *Cyp27a1*<sup>-/-</sup> or *Cyp7b1*<sup>-/-</sup> mice shows a motor neuron phenotype. A possible explanation for these differences between human and mouse is the prevalence of alternative pathways to produce neuroprotective 3 $\beta$ ,7 $\alpha$ -diHCA in mouse (Griffiths, Crick, et al., 2019; Meljon et al., 2019) or alternatively the presence of lower levels of neurotoxic neuro-oxysterols in mouse than in human (Hauser et al., 2019). Gene therapy has been suggested as a potential treatment for spastic paresis type 5 (Hauser et al., 2019) and also cerebrotendinous xanthomatosis. Interestingly, CYP27A1 the deficient enzyme in cerebrotendinous xanthomatosis, is a mitochondrial enzyme and mitochondrial dysfunction has been linked to several neurodegenerative diseases (Fivenson et al., 2017).

24S-HC through its activation of liver X receptors has been suggested to be protective against glioblastoma, the most common primary malignant brain tumour in adults (Han et al., 2020). Efavirenz, an antiretroviral medication that crosses the BBB and activates CYP46A1 (Mast et al., 2017), was shown to inhibit glioblastoma growth, the effect being explained by enhanced synthesis of 24S-HC, activation of liver X receptor and inhibition of the cholesterol synthesis pathway by inhibition of SREBP processing (Han et al., 2020). The results reported by Han et al. are interesting in a number of regards (Han et al., 2020):- (a) Despite the name, the cellular origin of glioblastoma is unknown, but if glia are the origin of the cancer the action of 24S-HC must be via a paracrine mechanism as CYP46A1 is expressed in neurons not glia (Lund, Guileyardo, & Russell, 1999); (b) the SREBP studied by Han et al. was SREBP-1, not SREBP-2 the dominating transcription factor regulating cholesterol synthesis (Horton et al., 2002). 24S-HC was found to reduce nuclear SREBP-1, which is expected (Wang, Muneton, Sjoval, Jovanovic, & Griffiths, 2008), but SREBP-1 primarily activates fatty acid not cholesterol synthesis (Horton et al., 2002), hence the link to reduced cholesterol synthesis and uptake via LDL receptor is less clear. Nevertheless, the beneficial effects of 24S-HC suggest CYP46A1 as a potential therapeutic target.

## 2.2 | Oestrogen receptors

The oestrogen receptors  $\alpha$  (ER $\alpha$ , NR3A1) and  $\beta$  (ER $\beta$ , NR3A2) are both present in rodent brain, although in different locations (Warner & Gustafsson, 2015) as are their classical steroid hormone agonists (Li & Gibbs, 2019) and oxysterol modulators (Griffiths, Crick, et al., 2019; Meljon et al., 2019). ER $\beta$  has a role in the migration of cortical neurons in the developing brain, where it is detectable at E12.5 (Wang, Andersson, Warner, & Gustafsson, 2003) and in adult in the maintenance of 5-hydroxytryptamine containing neurons in the dorsal raphé

nucleus, involved in fear, anxiety and depression (Suzuki et al., 2013). ER $\alpha$  is expressed in spinal cord motor neurons where it has a role in protecting neurons against cytokine toxicity (Das et al., 2011). Both ER $\alpha$  and ER $\beta$  are protective against experimental autoimmune encephalitis, a mouse model of multiple sclerosis (Spence et al., 2013). The protective effect of ER $\alpha$  is through astrocytes but that of ER $\beta$  through microglia (Wu et al., 2013). Microglia are the macrophages of the brain and when activated can damage healthy neurons in the region of insult. ER $\beta$  selective agonists dampen the activation of microglia and reduce the proinflammatory potential of invading T-cells (Warner & Gustafsson, 2015; Wu, Tan, et al., 2013).

Besides the ER ligands based on the estradiol skeleton, oxysterols can also act as ligands to both ER $\alpha$  and ER $\beta$  (DuSell, Umetani, Shaul, Mangelsdorf, & McDonnell, 2008; Umetani et al., 2007). Umetani et al. (2007) and DuSell et al. (2008) both identified 27-HC, presumably (25R)26-HC, as a selective oestrogen receptor modulator (SERM). SERMs are ER ligands whose relative agonist/antagonist activities vary in a cell- and promotor-dependent manner (Wardell, Nelson, & McDonnell, 2014). Selectivity is based on the ability of ligands to induce alterations in the ER structure leading to differential recruitment of co-activators and co-repressors. (25R)26-HC induces conformational changes in both ER $\alpha$  and ER $\beta$ . Interestingly, (25R)26-HC is a competitive antagonist of ER action in the vasculature (Umetani et al., 2007) but has ER agonist activity in breast cancer cells (DuSell et al., 2008). Although most studies of (25R)26-HC and ER have been made in the context of breast cancer (Nelson et al., 2013; Wu et al., 2013), the possibility exists that (25R)26-HC may act as a SERM in the CNS.

In mouse and human brain levels of (25R)26-HC are low ( $\sim 1$  ng $\cdot$ mg<sup>-1</sup>) (Griffiths, Crick, et al., 2019; Heverin et al., 2004; Meljon et al., 2019) but are elevated in the *Cyp7b1*<sup>-/-</sup> mouse ( $\sim 5$  ng $\cdot$ ml<sup>-1</sup>) (Meljon et al., 2019) and presumably in sufferers of spastic paresis type 5 where CYP7B1 is deficient and one route of (25R)26-HC metabolism blocked (Figure 4). A raised content of (25R)26-HC in spastic paresis type 5 brain has not been considered in the context of (25R)26-HC as a SERM, but the role of ER $\alpha$  in the protection of spinal cord motor neurons may be relevant with (25R)26-HC acting as a potential ER $\alpha$  antagonist in spastic paresis type 5 where motor neurons are lost. **25-Hydroxycholesterol** (25-HC) is also a SERM (Simigdala et al., 2016) and is also elevated in *Cyp7b1*<sup>-/-</sup> mouse brain (Meljon et al., 2019).

## 2.3 | Glucocorticoid receptor

The **glucocorticoid receptor** (GR, NR3C1) is expressed in the brain in both rodent and human, in neurons, glia and vascular epithelial cells (de Kloet, Meijer, de Nicola, de Rijk, & Joels, 2018; Tanaka et al., 1997). The GR is most widely expressed in stress-related centres, yet within these regions, there is differential expression over time. Besides cortisol and corticosterone, the steroid hormone ligands towards GR, this receptor is also activated by 3 $\beta$ ,5 $\alpha$ -dihydroxycholestan-6-one (3 $\beta$ ,5 $\alpha$ -diHC-6O, also called 6-oxocholestan-3 $\beta$ ,5 $\alpha$ -diol, OCDO, see inset Figure 1), a recently discovered oxysterol derived from cholestane-

3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol through enzymatic oxidation by hydroxysteroid dehydrogenase 11B2 (HSD11B2) (Voisin et al., 2017), an enzyme expressed in the brain (Holmes et al., 2006). Cholestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol itself is derived from 5,6-epoxycholesterol (5,6-EC) by the enzyme cholesterol-5,6-epoxide hydrolase (ChEH), an enzyme derived from two subunits DHCR7 and D8D7I (3 $\beta$ -hydroxysterol-delta-8,7-isomerase also called emopamil-binding protein); however, the enzymatic origin of 5,6-EC has yet to be defined (de Medina, Paillassé, Segala, Poirot, & Silvente-Poirot, 2010). In the context of neuro-oxysterols 3 $\beta$ ,5 $\alpha$ -diHC-6O has been found to be exported from human brain indicating its biosynthesis in this organ (Iuliano et al., 2015).

## 2.4 | Retinoic acid receptor-related orphan receptors

There are three retinoic acid receptor-related orphan receptors (RORs): **ROR $\alpha$**  (NR1F1), **ROR $\beta$**  (NR1F2) and **ROR $\gamma$**  (NR1F3). ROR $\gamma$  exists as two isoforms ROR $\gamma$ 1, or simply ROR $\gamma$  and ROR $\gamma$ 2 also called ROR $\gamma$ t. ROR $\gamma$ t has a shorter N-terminus compared to ROR $\gamma$  but with otherwise identical domains. ROR $\gamma$ t is highly expressed in thymus and is an essential transcription factor for Th17 cell development. Th17 cells are pro-inflammatory T-helper cells (CD4<sup>+</sup>) that express IL-17. Th17 cells are mediators of experimental autoimmune encephalitis, the mouse model of multiple sclerosis (Duc et al., 2019). ROR $\gamma$  is expressed in multiple organs but not normally in the CNS. However, ROR $\gamma$ t-immunoreactive cells have been found in the meninges, the three membranes that envelop the brain and spinal cord, of multiple sclerosis patients, presumably as infiltrating immune cells (Serafini et al., 2016). ROR $\alpha$  and ROR $\beta$  are both expressed in the brain. ROR $\alpha$  is abundant in cerebellum and thalamus and plays a key role in development, particularly in the regulation of the maturation and survival of Purkinje cells (Jetten & Joo, 2006). ROR $\beta$  is highly expressed in the brain and also the retina (Jetten & Joo, 2006). Oxysterols have been found to bind to the ligand binding domains of both ROR $\alpha$  and ROR $\gamma$  (including ROR $\gamma$ t), but not as yet ROR $\beta$  (Duc et al., 2019).

In 2010, a number of papers were published identifying sterols and oxysterols as ligands to ROR $\alpha$  and ROR $\gamma$  (Jin et al., 2010; Wang, Kumar, Crumbley, Griffin, & Burris, 2010; Wang, Kumar, Crumbley, et al., 2010); these included the archetypical neuro-oxysterol 24S-HC, 24S,25-EC, **20S-hydroxycholesterol** (20S-HC), **22R-hydroxycholesterol** (22R-HC), 25-HC, 7-oxocholesterol (7-OC), **7 $\alpha$ -hydroxycholesterol** (7 $\alpha$ -HC) and **7 $\beta$ -hydroxycholesterol** (7 $\beta$ -HC), all of which have been identified in mouse or human brain (Figure 1) (Yutuc et al., 2020). It is noteworthy that the side-chain oxysterols 20S-HC, 22R-HC 24S-HC, 24S,25-EC and 25-HC are also ligands to the LXRs (Janowski et al., 1996; Lehmann et al., 1997) and with the exception of 20S-HC, which has not been tested, to INSIG (Radhakrishnan et al., 2007). Cholesterol sulphate, the dominating neuro-sterol sulphate in rat brain (Liu, Sjoval, & Griffiths, 2003), has also been shown to bind to the ligand binding domain (LBD) of ROR $\alpha$  (Kallen, Schlaeppli, Bitsch, Delhon, & Fournier, 2004), while desmosterol, one of the most abundant neurosterols and also an LXR ligand (Yang et al., 2006), has been shown to be a

potent ROR $\gamma$  agonist (Hu et al., 2015). ROR $\alpha$  is an unusual nuclear receptor in that it is constitutively active in the absence of ligand and despite binding to the LBD of ROR $\alpha$  cholesterol sulphate does not appear to affect the transcriptional activity of ROR $\alpha$  or ROR $\gamma$  (Wang, Kumar, Solt, et al., 2010). In contrast, Wang et al. reported 7 $\alpha$ -HC, 7 $\beta$ -HC and also 7-OC to be ligands to ROR $\alpha$  and ROR $\gamma$  and to suppress the transcriptional activity of these receptors acting as inverse agonists (Wang, Kumar, Crumbley, et al., 2010). Although CYP7A1, the enzyme required to synthesise 7 $\alpha$ -HC and 7-OC, is not expressed in the brain, 7 $\alpha$ -HC, 7-OC and 7 $\beta$ -HC can cross the BBB and be imported from the periphery (Griffiths, Crick, et al., 2019) or alternatively be formed via non-enzymatic reactions initiated by ROS (Griffiths & Wang, 2020; Murphy & Johnson, 2008). Interestingly, **HSD11B1** (11- $\beta$ HSD1) is expressed in the brain and will convert 7-OC to 7 $\beta$ -HC (Cobice et al., 2013; Mitic et al., 2013) (Figure 1).

24S-HC has also been found to be an inverse agonist of ROR $\alpha$  and ROR $\gamma$  suppressing the constitutive activity of these receptors (Wang, Kumar, Crumbley, et al., 2010). Interestingly, 24S,25-EC selectively suppresses the activity of ROR $\gamma$  (Wang, Kumar, Crumbley, et al., 2010). Conversely, 20S-HC, 22R-HC and 25-HC have been shown to be agonists towards ROR $\gamma$ , promoting the recruitment of co-activators (Jin et al., 2010). The crystal structure of the LBD of ROR $\gamma$  with 20S-HC, 22R-HC, or 25-HC bound showed the AF-2 helix in a conformation that is permissive for interactions with co-activator proteins. Importantly, mutations that disrupt the binding of these hydroxycholesterols abolish ROR $\gamma$  transcriptional activity, suggesting a critical role for hydroxycholesterols in activating ROR $\gamma$  (Jin et al., 2010). With respect to brain, it is interesting to note that CYP46A1, the enzyme that generates 24S-HC, is expressed in Purkinje cells (Lund et al., 1999) and that ROR $\alpha$  plays a role in the regulation of the maturation and survival of these cells (Jetten & Joo, 2006). The cerebellum of ROR $\alpha$ -deficient mice contains significantly fewer Purkinje cells and exhibits a loss of cerebellar granule cells (Jetten & Joo, 2006). It is tempting to speculate that 24S-HC plays a role in modulating maturation and survival of Purkinje cells via its inverse agonist activity towards ROR $\alpha$ , however defects in the Purkinje cell layer have not been reported in the *Cyp46a1*<sup>-/-</sup> or *CYP46A1tg* mice (Kotti et al., 2006; Shafaati et al., 2011), although *Cyp46a1*<sup>-/-</sup> mice do display defects in motor learning (Kotti et al., 2006). Interestingly, *Cyp7b1* has been reported as a target gene of ROR $\alpha$  (Wada et al., 2008). CYP7B1 was first reported in the brain and functions as an oxysterol 7 $\alpha$ -hydroxylase to side-chain oxysterols including 25-HC and (25R)26-HC (Rose et al., 2001), but not 24S-HC, where CYP39A1 is the 7 $\alpha$ -hydroxylase (Li-Hawkins, Lund, Bronson, & Russell, 2000). Recent evidence suggests CYP39A1 is also regulated by ROR $\alpha$  (Matsuoka et al., 2020).

There is good evidence for the production of 7 $\alpha$ ,(25R)26-diHC in the brain from either imported or in situ synthesised (25R)26-HC (Heverin et al., 2005; Iuliano et al., 2015; Yutuc et al., 2020). Based on the publications of Jin et al. which indicates that (25R)26-HC activates ROR $\gamma$  and Wang et al. that 7 $\alpha$ -HC suppresses the activity of ROR $\gamma$ , it is difficult to predict whether 7 $\alpha$ ,(25R)26-diHC should be an agonist or inverse agonist towards ROR $\gamma$  (Jin et al., 2010; Wang, Kumar, Solt,



et al., 2010). Soroosh et al. have answered this conundrum and shown that  $7\alpha,(25R)26$ -diHC and also  **$7\beta,26$ -dihydroxycholesterol** ( $7\beta,26$ -diHC also called  $7\beta,27$ -diHC), presumably  $7\beta,(25R)26$ -diHC, are ROR $\gamma$ t agonists, reversing the inhibitory effects of the ROR $\gamma$ t antagonist, ursolic acid, in cell-based reporter assays (Soroosh et al., 2014). In primary cells, both oxysterols were found to enhance the differentiation of IL-17-producing cells in a ROR $\gamma$ t-dependent manner and Th17 cells were found to produce both oxysterols (Soroosh et al., 2014). Importantly, the pro-inflammatory cytokine IL17-producing CD4<sup>+</sup> Th17 cells play a key pathogenic role in multiple sclerosis and as stated above, ROR $\gamma$ t-immunoreactive cells have been found in the meninges of multiple sclerosis patients, presumably from infiltrating immune cells (Serafini et al., 2016). While reaction mechanisms for the formation of  $7\alpha,(25R)26$ -diHC are well established, mostly reactions catalysed by CYP27A1 and CYP7B1 or by CYP7A1 and CYP27A1 (Griffiths & Wang, 2020), the formation of  $7\beta,(25R)26$ -diHC is less clear cut. However, it has been identified in plasma from patients suffering from Niemann Pick (NP) disease where  $7\beta$ -HC and 7-OC are abundant (Griffiths, Yutuc, et al., 2019) and Smith–Lemli–Opitz syndrome patients where 7-dehydrocholesterol (7-DHC) is abundant, so mechanisms are available for its formation (Shinkyō et al., 2011), at least extra-cerebrally (Figures 1 and 3).

### 3 | G-PROTEIN COUPLED RECEPTORS

#### 3.1 | GPR183

GPR183, or EBI2, is a member of the rhodopsin-like subfamily of class A transmembrane spanning GPCRs (Daugvilaite, Arfelt, Benned-Jensen, Sailer, & Rosenkilde, 2014). It is expressed on the surface of immune cells including T-lymphocytes, monocytes, dendritic cells, astrocytes and innate lymphoid cells (Duc et al., 2019). GPR183 is believed to act as a chemoattractant receptor participating in the migration of immune cells up a gradient towards  $7\alpha,25$ -diHC or  $7\alpha,(25R)26$ -diHC (Hannedouche et al., 2011; Liu et al., 2011). Importantly, inflamed white matter of multiple sclerosis patients shows a high expression of GPR183 compared to non-inflamed white matter (Clottu et al., 2017) and  $7\alpha,25$ -diHC has been found to be increased in spinal cord during development of experimental autoimmune encephalitis, a mouse model of multiple sclerosis (Wanke et al., 2017), linking GPR183, its neuro-oxysterol ligand, immune cell migration and multiple sclerosis. Importantly, GPR183 is expressed by Th17 cells during inflammation and infiltrating cells in multiple sclerosis lesions express GPR183 (Wanke et al., 2017). Furthermore, expression of cholesterol 25-hydroxylase (CH25H) and CYP7B1, two enzymes involved in  $7\alpha,25$ -diHC biosynthesis (Figure 1), was found to change during the course of experimental autoimmune encephalitis with strong up-regulation in spinal cord leading to increased concentration of  $7\alpha,25$ -diHC (Wanke et al., 2017). Remarkably, microglia express CH25H early in experimental autoimmune encephalitis, while CYP7B1 is expressed by infiltrating monocytes and lymphocytes (Wanke et al., 2017). These data lead to the hypothesis that GPR183 and

$7\alpha,25$ -diHC play a role in the migration of inflammatory CD4<sup>+</sup> T cells into the CNS.  $7\alpha,(25R)26$ -diHC is also a GPR183 ligand and it is perhaps no coincidence that this molecule is also an agonist towards ROR $\gamma$ t.

#### 3.2 | Smoothed

SMO belongs to the Class F (Frizzled, FZD) of GPCRs and of the 11 members of this class 10 are FZD paralogues, the other is SMO (Kozielewicz et al., 2020). SMO is a seven-pass transmembrane (7TM) protein, essential to the hedgehog signalling pathway critical in normal animal development, for example, activation of the hedgehog pathway is required for the differentiation of neural progenitor cells into motor neuron progenitors, as well as pathological malignancies, including medulloblastoma, the most common paediatric brain tumour.

The hedgehog signalling pathway is regulated in primary cilia where the transporter protein Patched 1 (PTCH1) and SMO are co-located (Rohatgi, Milenkovic, Corcoran, & Scott, 2009). PTCH1 is the receptor for hedgehog ligands, for example, sonic hedgehog (SHH), and in the absence of ligand inhibits SMO and the transduction of hedgehog signals across the plasma membrane (Figure 2) (Kong et al., 2019). When hedgehog ligands bind to PTCH1 inhibition is released and SMO transmits the hedgehog signal across the membrane. PTCH1 is structurally related to the sterol transporting protein **NPC1** (Lange & Steck, 1998). It has 12 transmembrane domains and two sterol/oxysterol binding cavities and inactivation of PTCH1 by hedgehog ligands is suggested to allow sterols/oxysterols to accumulate in the cilia sufficiently to activate SMO (Deshpande et al., 2019). The identity of the activating sterol/oxysterol is a point of considerable debate.

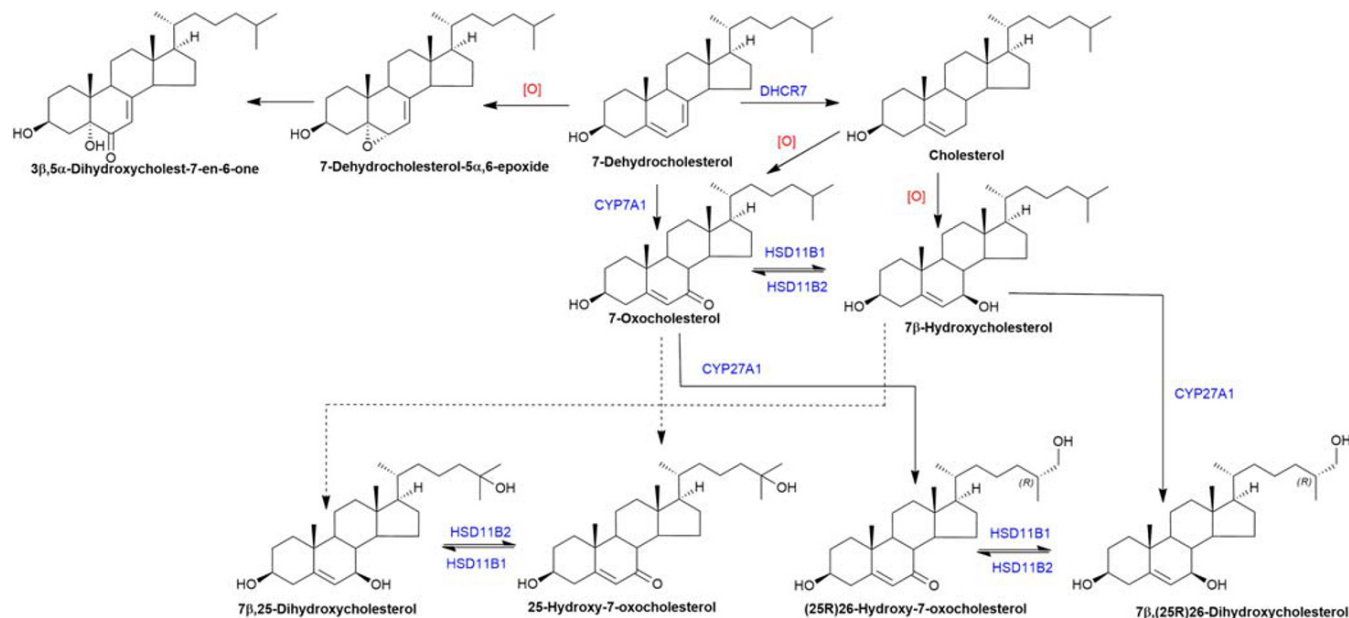
Current theories suggest at least two sterol/oxysterol binding sites in the SMO protein. Based on the crystal structure of mouse SMO stabilised in an active state, Deshpande et al. suggest one binding site within the 7TM pocket of SMO and a second deep within the extracellular cysteine rich domain, with a third pocket for the sterol-like antagonist cyclopamine (Deshpande et al., 2019). Deshpande et al. argued that both sterol-binding pockets are likely occupied by cholesterol but acknowledged that oxysterols may be alternative occupants of these pockets (Deshpande et al., 2019). Other crystal structures based on inactive SMO failed to identify a 7TM sterol/oxysterol and emphasised cholesterol binding to the cysteine rich domain as the critical event controlled by PTCH1 (Byrne et al., 2016; Huang et al., 2016). Deshpande et al. and Kinnebrew et al. have both proposed biophysical models to explain how abundant cholesterol can behave as a signalling molecule (Deshpande et al., 2019; Kinnebrew et al., 2019); there is a precedent for this in the regulation of cholesterol synthesis (and uptake) via the SCAP/SREBP-2 pathway (Goldstein et al., 2006). Deshpande et al. proposed a hydrophobic tunnel between TM5 and TM6 of active SMO that opens on the inner leaflet of the membrane bilayer; this may enable sterols/oxysterols in this leaflet to activate SMO without energetically costly membrane de-solvation (Deshpande et al., 2019). Activation of SMO via the

7TM-binding sterol/oxysterol may lead to a displacement of TM<sub>6</sub>, which is further stabilised by sterol/oxysterol binding to the cysteine rich domain (Deshpande et al., 2019). On the other hand, Kinnebrew et al. introduced the concept of “accessible cholesterol” in membrane and cilia in their model (Kinnebrew et al., 2019). They defined three pools of cholesterol: a fixed pool essential to maintain membrane integrity, a sphingomyelin sequestered pool of low accessibility and an available pool to interact with proteins and be transported to the endoplasmic reticulum (Kinnebrew et al., 2019). The idea of multiple pools of cholesterol was discussed earlier by Radhakrishnan et al. and in respect to brain by Dietschy and Turley (2004) (Radhakrishnan, Anderson, & McConnell, 2000). According to Kinnebrew et al.'s model, the pool of accessible cholesterol in cilia, the subcellular compartment where PTCH1 and SMO are located together, is particularly low. PTCH1 functions in this compartment to selectively transport accessible cholesterol from the cilia to intracellular or extracellular receptors, precluding its binding to the cysteine rich domain and TM-binding site of SMO (Kinnebrew et al., 2019). Inactivation of PTCH1 will lead to an increase in accessible cholesterol in both leaflets of the ciliary membrane leading to SMO activation through cholesterol binding to both sterol binding sites (Kinnebrew et al., 2019). While Deshpande et al. acknowledged that oxysterols may be alternative ligands to these binding sites (Deshpande et al., 2019), Kinnebrew et al. argued against this idea based on a CRISPR screen targeting lipid-related genes exploiting the NIH/3T3 cell line (Kinnebrew et al., 2019). While they identified many genes of the cholesterol biosynthesis pathway as positive regulators of the hedgehog pathway, they failed to find oxysterol synthesising genes to positively regulate hedgehog signalling (Kinnebrew et al., 2019). However, in the absence of excess cholesterol, it is uncertain whether these cells generate oxysterols under the conditions employed. Interestingly, genes encoding enzymes for sphingomyelin biosynthesis suppressed hedgehog signalling, promoting the concept of a sphingomyelin sequestered inaccessible pool of cholesterol (Kinnebrew et al., 2019).

Despite the study of Kinnebrew et al. (2019) and the crystal structures showing cholesterol bound to SMO (Byrne et al., 2016; Deshpande et al., 2019; Huang et al., 2016), there is also good evidence that oxysterols activate the hedgehog signalling pathway, perhaps in a fine-tuning mode akin to their regulation of cholesterol biosynthesis via the INSIG/SCAP/SREBP-2 pathway (Gill et al., 2008). Synthetic oxysterols known to bind to SMO and activate hedgehog signalling include 20S-HC, 24S-HC, 25-HC, 24S,25-EC, 24-oxocholesterol (24-OC), 7 $\beta$ ,(25R)26-diHC, 25-hydroxy-7-oxocholesterol (25H,7O-C), (25R)26-hydroxy-7-oxocholesterol ((25R)26H,7O-C) but not 7 $\alpha$ -HC, 7-OC nor 19-HC (Corcoran & Scott, 2006; Dwyer et al., 2007; Kim, Meliton, Amantea, Hahn, & Parhami, 2007; Myers et al., 2013; Nachtergaele et al., 2012; Nachtergaele et al., 2013; Nedelcu et al., 2013; Qi et al., 2019; Raleigh et al., 2018). Conversely, 3 $\beta$ ,5 $\alpha$ -dihydroxycholesterol-7-en-6-one (DHCEO, Figure 3), an oxysterol derived from 7-DHC and identified in the brain of a mouse model of Smith–Lemli–Opitz syndrome where 7-DHC is abundant (Xu et al., 2012), binds to SMO and blocks hedgehog signalling (Sever et al., 2016). Of the above oxysterols, 24S,25-EC

is abundant in embryonic mouse brain, particularly the ventral mid-brain, while 24S-HC and 25-HC are also present during development but at concentrations about one order of magnitude lower than 24S,25-EC (Theofilopoulos et al., 2013; Wang et al., 2009). In the new-born mouse, 24S,25-EC is still the most abundant oxysterol (Meljon et al., 2012), but in the adult mouse, 24S-HC is by far the most abundant oxysterol and 20S-HC is also present but at a low level (Meljon et al., 2012; Yutuc et al., 2020). Smith–Lemli–Opitz syndrome phenocopies dysregulated hedgehog signalling (Cooper et al., 2003), however, at least in the new-born mouse the pattern of SMO-activating oxysterols in the brain is similar to the WT (Meljon, Watson, Wang, Shackleton, & Griffiths, 2013). Of the other oxysterols suggested to activate the hedgehog pathway through binding to SMO, we have identified 24-OC in the brain, although we used a LC-MS method incorporating a derivatisation step in which, as a side-reaction, the 24,25-epoxy function isomerises to the 24-oxo function. So we cannot be sure if the observed 24-OC is present in the brain as a natural molecule or is an “artificial” isomer of endogenous 24S,25-EC, while 7 $\beta$ ,(25R)26-diHC, (25R)26H,7O-C and 25H,7O-C are present in Smith–Lemli–Opitz syndrome plasma but essentially absent from control plasma (Meljon et al., 2012; Meljon et al., 2013).

Similar to the situation with cholesterol, there appear to be multiple binding sites for oxysterols on SMO. Oxysterols can bind to the same cysteine rich domain pocket as cholesterol (Byrne et al., 2016) and also within a 7TM pocket (Qi et al., 2019; Raleigh et al., 2018). 24S,25-EC appears to bind to and activate SMO via both pockets, while 20S-HC, 7 $\beta$ ,(25R)26-diHC, (25R)26H,7O-C and 25H,7O-C act exclusively through the cysteine rich domain. As discussed above, PTCH1 acts to repress SMO by removing agonist ligands from the plasma membrane in proximity to SMO and exploiting this concept Qi et al. were able to extract 24S,25-EC and also 24-OC, 24S-HC and 25-HC from purified PTCH1 (Qi et al., 2019). PTCH1 and SMO function together in cilia and Raleigh et al. found 24S,25-EC, 24-OC and 7 $\beta$ ,(25R)26-diHC to be enriched in cilia purified from embryonic sea urchin (Raleigh et al., 2018). 7 $\beta$ ,(25R)26-diHC and (25R)26H,7O-C are metabolically linked by HSD11B enzymes (Figure 5) (Beck, Inderbinen, et al., 2019; Schweizer, Zurcher, Balazs, Dick, & Odermatt, 2004) and both HSD11B1 and HSD11B2 are expressed in the brain (Holmes & Seckl, 2006). Interestingly, HSD11B2 is expressed during brain development (Holmes et al., 2006) and will catalyse the oxidation of the 7 $\beta$ -hydroxy group to a 7-oxo, while HSD11B1 catalyses the reverse reduction (Beck, Inderbinen, et al., 2019; Beck, Kanagaratnam, et al., 2019; Schweizer et al., 2004). Importantly, *Hsd11b2* is enriched in mouse models of medulloblastoma and *HSD11B2* is enriched in hedgehog-pathway associated human medulloblastoma (Raleigh et al., 2018). Raleigh et al. proposed a mechanism involving HSD11B2 and CYP27A1 by which hedgehog agonists 7 $\beta$ ,(25R)26-diHC and (25R)26H,7O-C could be formed from 7 $\beta$ -HC and, remarkably, pharmacological inhibition of HSD11B2 reduced hedgehog signalling and tumour growth in mouse medulloblastoma (Raleigh et al., 2018). The contribution of HSD11B2 to oncogenic hedgehog signalling suggests that oxysterols produced by this enzyme are required for high-level pathway activity. Our interpretation of these data is that while



**FIGURE 5** Biosynthesis of SMO ligands from 7-DHC. Enzymes are marked in blue. Non-enzymatic oxidation marked in red. CYP27A1 is the likely catalyst for reactions with broken arrows. Oxysterol names are linked to their abbreviations and biological activities in Table S1

(25R)26H,7O-C and 7β,(25R)26-diHC are both agonists to SMO, (25R)26H,7O-C must be more potent. Whether this is a consequence of differences in SMO binding or simply accessibility of ligand to receptor is unclear. While 20S-HC, 24S-HC, 25-HC and 24S,25-EC have been identified in the brain and are bona fide neuro-oxysterols, neither 7β,(25R)26-diHC nor (25R)26H,7O-C have been identified in the brain or medulloblastoma; however, their precursors 7-OC and 7β-HC have (Griffiths, Crick, et al., 2019; Meljon et al., 2019). Importantly, 7-OC and 7β-HC can traverse the blood-brain barrier and provide an extracerebral source of precursors for (25R)26-hydroxylation by CYP27A1 (Iuliano et al., 2015).

#### 4 | NMDA RECEPTORS

NMDA receptors are glutamate-gated ion-channels critical in the regulation of excitatory synaptic function. They are involved in experience-dependent synaptic plasticity and implicated in the cognitive defects of schizophrenia and some forms of autism (Paul et al., 2013). 24S-HC is a positive allosteric modulator (PAM) of NMDA receptor interacting with the receptors at a site distinct from other allosteric modulators, for example, the positive allosteric modulator pregnenolone sulphate. Even at sub-micromolar concentrations, 24S-HC potentiates NMDA receptor-mediated excitatory post-synaptic currents (EPSC) and enhances long term potentiation (LTP) (Paul et al., 2013). While the presence of [pregnenolone sulphate](#) in the brain is debatable (Liu et al., 2003), 24S-HC is present in rodent and human brain at concentrations of 10–20 ng·mg<sup>-1</sup> (25–50 μM). Importantly, 24S-HC does not alter membrane currents on its own in the absence of NMDA, indicating that the effect is independent of

nuclear receptors, for example, LXRs. The synthetic 24-HC analogue, 24,24-dimethylchol-5-en-3β,24-diol (SGE-201), is also a positive allosteric modulator, somewhat more potent than the parent molecule and it likewise potentiates LTP and, remarkably, reverses behavioural deficits (e.g. spatial working memory) induced by NMDA receptor channel blockers (Paul et al., 2013). Importantly, the *Cyp46a1*<sup>-/-</sup> mouse shows a deficiency in hippocampal LTP and a deficit in spatial, associative and motor learning (Kotti et al., 2006). This was explained by Kotti et al. to be a consequence of reduced flux of metabolites through the cholesterol biosynthetic pathway and through the mevalonate pathway towards geranylgeranyl diphosphate (Kotti et al., 2006). Administration of geranylgeraniol to hippocampal slices restored LTP to wild type levels indicating that it is a deficit in this molecule, or its diphosphate, which is responsible for the impaired LTP in the *Cyp46a1*<sup>-/-</sup> mouse (Kotti et al., 2006; Kotti et al., 2008). In light of the data of Paul et al. indicating that 24S-HC is a NMDA receptor positive allosteric modulator (Paul et al., 2013), it is not unreasonable to speculate that a loss of this molecule in the *Cyp46a1*<sup>-/-</sup> mouse may also be responsible for some of the deficiency in hippocampal LTP. It is likely that 24S-HC modulates NMDA receptors in an autocrine or paracrine manner at a distinct site from other positive allosteric modulators (Paul et al., 2013). Interestingly, the *CYP46A1*tg mouse shows improved spatial memory and increased hippocampal NMDAR activity (Maioli et al., 2013). Surprisingly, the other side-chain oxysterols 22R-HC and 20S-HC are not modulators of NMDA receptors, although 25-HC is a weak positive modulator (Linsenbardt et al., 2014; Paul et al., 2013). In fact, 25-HC is an antagonist of the positive allosteric modulator activity of 24S-HC to NMDA receptors but remarkably through a different site (Linsenbardt et al., 2014).

## 5 | INSIG, SCAP AND HMG-COA REDUCTASE

Sterols and oxysterols regulate cholesterol synthesis by binding to SCAP or INSIG and via SREBP-2 modulate the expression of the enzymes of the cholesterol biosynthesis pathway (Figure 2) (Radhakrishnan et al., 2007; Sun et al., 2007). At times of cholesterol excess, cholesterol in the endoplasmic reticulum binds to SCAP, which is already in complex with SREBPs and causes a conformational change in SCAP which results in binding of the SCAP-SREBP complex to the endoplasmic reticulum-anchored protein INSIG. The consequence of this event is retention of SREBP-2 in the endoplasmic reticulum and prevention of its transport by SCAP to the Golgi for processing to its active form as a transcription factor for the enzymes of the cholesterol biosynthesis pathway and for the LDL receptor (Goldstein et al., 2006; Sun et al., 2007). Oxysterols also have a role in regulating cholesterol biosynthesis (Kandutsch, Chen, & Heiniger, 1978) but in most cases by providing “fine tuning” of this regulation (Gill et al., 2008). The brain is an organ with high oxysterol concentrations (24S-HC 10–20 ng·mg<sup>-1</sup> cf. cholesterol 10–20 µg·mg<sup>-1</sup>) and it may be in the CNS that the involvement of oxysterols in regulation of cholesterol biosynthesis is most significant (Saeed et al., 2014). Another case when oxysterols may be especially important in regulating cholesterol biosynthesis is in response to infection, both bacterial and virus (Bauman et al., 2009; Blanc et al., 2013). Rather than bind to SCAP, side-chain oxysterols, that is, 22R-HC, 24S-HC, 25-HC, (25R)26-HC and 24S,25-EC, bind to INSIG and tether the SCAP-SREBP-2 complex in the endoplasmic reticulum (Radhakrishnan et al., 2007). Elegant experiments performed by Radhakrishnan et al. exploiting [<sup>3</sup>H]25-HC and site-directed mutagenesis confirmed that side-chain oxysterols bind to INSIG via a mechanism involving transmembrane helices 3 and 4, which are also involved in INSIG binding to SCAP (Radhakrishnan et al., 2007).

In the context of neuro-oxysterols and infection, *Ch25h*<sup>-/-</sup> mice show exacerbated experimental autoimmune encephalitis, which was explained by Reboldi et al. to result from enhanced processing of SREBP-2, a consequence of a diminished negative feedback inhibition, normally provided by 25-HC (Reboldi et al., 2014). Interestingly, Crick et al. reported that plasma from multiple sclerosis patients show reduced levels of 25-HC, indicating an impaired capacity of immune cells from these patients to synthesise 25-HC (Crick et al., 2017). Based on Reboldi et al.'s work (Reboldi et al., 2014), Crick et al. suggested that a reduced production of 25-HC in multiple sclerosis patients may result in reduced activation of LXRs and enhanced activity of the cholesterol biosynthesis pathway leading to overproduction of *IL-1β*, both of which lead to enhanced activity of macrophages recruited to the CNS during multiple sclerosis relapse (Crick et al., 2017).

Radhakrishnan et al. reported that 19-HC, 4,4-dimethylcholesterol, and lanosterol did not bind to either INSIG or SCAP and did not inhibit the processing of SREBP-2 (Radhakrishnan et al., 2007). Conversely, lanosterol, the first sterol in the cholesterol biosynthesis pathway (Figure 3), stimulates the formation of an INSIG-HMG-CoA reductase (HMGCR) complex (Song, Javitt, & DeBose-Boyd, 2005) leading to the ubiquitination and degradation

of HMGCR, the rate-limiting enzyme of the cholesterol biosynthesis pathway. Cholesterol has no effect on INSIG-HMGCR formation; however 25-HC and other side-chain oxysterols, for example, (25R)26-HC, 25H,7O-C and 26-hydroxylanosterol (26-HL, also called 27-hydroxylanosterol) can induce the formation of the INSIG-HMGCR complex and reductase ubiquitination (Song et al., 2005). In an early and largely forgotten study, Axelson et al. showed that (25R)26-HC, (25R)26-hydroxycholest-4-en-3-one ((25R)26-HCO), 7 $\alpha$ ,(25R)26-dihydroxycholest-4-en-3-one (7 $\alpha$ , (25R)26-diHCO), 7 $\alpha$ ,12 $\alpha$ ,26-trihydroxycholest-4-en-3-one (7 $\alpha$ ,12 $\alpha$ ,26-triHCO) were potent suppressors of HMGCR (Axelson, Larsson, Zhang, Shoda, & Sjovall, 1995). The first three of these compounds have been found in the brain (Griffiths, Crick, et al., 2019; Meljon et al., 2019). However, Axelson et al.'s findings were before the involvement of INSIG in HMGCR degradation was uncovered and a mechanism of HMGCR suppression by oxysterols was not described by these authors (Axelson et al., 1995). Radhakrishnan et al. suggested that it is the oxysterol bound form of INSIG that can form a complex with HMGCR, just as it can with SCAP, while lanosterol acts by binding to HMGCR, much like cholesterol binds to SCAP (Radhakrishnan et al., 2007). Lanosterol is a 4,4-dimethyl sterol and a very recent study has confirmed its ability to stimulate HMGCR degradation and its inability to inhibit SREBP-2 cleavage (Chen et al., 2019). Surprisingly, other 4,4-dimethyl sterols in the cholesterol biosynthesis pathway promoted both HMGCR degradation and inhibited SREBP-2 cleavage (Chen et al., 2019).

## 6 | ANALYSIS OF NEURO-OXYSTEROLS AND NEURO-STEROLS

Much of our understanding of the biochemistry of neuro-sterols and neuro-oxysterols depends on their reliable detection and quantification in brain tissue, CSF, plasma and in cells of the nervous systems. Both GC-MS and LC-MS methods have been extensively applied. Gold standard GC-MS methods are based on the classic paper by Dzeletovic et al. describing the analysis of oxysterols in plasma (Dzeletovic, Breuer, Lund, & Diczfalusy, 1995; Heverin et al., 2004). There are many LC-MS methods, with and without the use of derivatisation. A major study using LC-tandem MS (MS/MS) was performed by Russell and colleagues in Dallas in which they analysed over 3,000 serum samples (Stiles et al., 2014). Their analytical method relied on multiple reaction monitoring (MRM), exploiting a tandem quadrupole to achieve maximum sensitivity, with the use of multiple isotope labelled standards for quantification (McDonald, Smith, Stiles, & Russell, 2012). To achieve high sensitivity, others have exploited derivatisation in combination with LC-MS/MS (Honda et al., 2009; Roberg-Larsen et al., 2014; Sidhu et al., 2015; Xu et al., 2011).

Our preference is to use the Girard P (GP) derivatisation reagent in combination with enzymatic oxidation in a methodology called “enzyme-assisted derivatisation for sterol analysis” EADSA (Crick et al., 2015; Griffiths et al., 2013). The method is applicable to any sterol/oxysterol with an oxo group or with a hydroxy group amenable to enzymatic oxidation to a carbonyl. The most commonly used

enzyme is cholesterol oxidase which converts 3 $\beta$ -hydroxy-5-ene sterols to 3-oxo-4-enes and 3 $\beta$ -hydroxy-5 $\alpha$ -hydrogen structures to 3-ones (Karu et al., 2007). The oxo group is then reacted with the GP hydrazine reagent to give a GP hydrazone which effectively tags the sterol/oxysterol with a positive charge (see Figure S1). The consequence of charge-tagging is a major improvement in LC-MS sensitivity. Besides enhancing signal, the charge-tag directs fragmentation in MS/MS or in MS with multistage-fragmentation (MS<sup>n</sup>), improving confidence for identification and allowing the identification of unexpected sterols/oxysterols (Abdel-Khalik et al., 2018; Griffiths, Crick, et al., 2019). We find the use of MS<sup>3</sup> in ion-trap mass spectrometers particularly valuable for structural elucidation, where in a first step the GP-derivative [M]<sup>+</sup> ion is selected (MS<sup>1</sup>), in a second step the [M]<sup>+</sup> ion is fragmented (MS<sup>2</sup>), the major fragmentation route is the loss of pyridine (Py) from the derivative to give a [M-Py]<sup>+</sup> ion, which is then fragmented in the MS<sup>3</sup> step to reveal structurally informative fragment-ions (see Figures S1 and S2). To augment MS<sup>3</sup>, we like to combine fragmentation information with exact mass information available on Fourier transform hybrid and tribrid mass spectrometers. We have extensively utilised this method to identify and quantify neuro-sterols and neuro-oxysterols in the brain and in CSF and to monitor the import and export of these molecules into and out of brain (Iuliano et al., 2015). We have now extended this method to on-surface analysis of tissue slices to localise neuro-oxysterols and neuro-sterols in mouse brain (Yutuc et al., 2020).

## 7 | THERAPEUTIC MANAGEMENT OF CNS DISEASE

Throughout this review, we have attempted to draw attention to potential therapies to neuro-sterol and neuro-oxysterol related CNS disorders and here we proved a summary of these therapeutic possibilities.

Treatment is available for cerebrotendinous xanthomatosis in the form of chenodeoxycholic acid (Bjorkhem, 2013; Clayton, 2011); however, while proving effective in stabilising systemic manifestations of the disease, it fails to prevent worsening of the neurological outcome and ultimately death from the disease (Bjorkhem, 2013). A curative approach to cerebrotendinous xanthomatosis is more likely to require complementation of 26-hydroxylated metabolites deficient in this disease by, for example, gene transfer of *CYP27A1*. Similar to cerebrotendinous xanthomatosis, oxysterol 7 $\alpha$ -hydroxylase (*CYP7B1*) deficiency has been treated with chenodeoxycholic acid and this has proved successful in respect to improving liver function (Dai et al., 2014). However, whether this treatment improves neurological function in the spastic paraplegia form of this disease, that is, spastic paresis type 5, is unclear (Marelli et al., 2018). Hauser et al. have suggested *CYP7B1* gene therapy as a potential treatment for spastic paresis type 5 (Hauser et al., 2019). Gene therapy in the form of AAV-*CYP46A1* shows promising results in mouse models for the treatment of Alzheimer's disease, Huntington's disease and spinocerebellar ataxias (Boussicault et al., 2016; Burlot et al., 2015; Kacher et al., 2019; Nobrega et al., 2019) and perhaps also Parkinson's disease (Theofilopoulos et al., 2019). An alternative way of enhancing

*CYP46A1* activity is through the anti-HIV drug Efavirenz which activates *CYP46A1* and is able to cross the BBB from the circulation (Mast et al., 2017). 24S,25-EC one of the products of *CYP46A1* catalysis has been suggested to have value in cell replacement therapy to treat Parkinson's disease based on its role in promoting neurogenesis of dopaminergic neurons via activation of liver X receptors (Theofilopoulos et al., 2013). Another liver X receptor ligand, 3 $\beta$ ,7 $\alpha$ -diHC may have value in the treatment of motor neuron disease based on its protective action towards motor neurons (Theofilopoulos et al., 2014).

## 8 | CONCLUSIONS

The dominating neuro-oxysterol and neuro-sterol in the brain are 24S-HC and cholesterol, respectively. During embryonic development and in the new-born, 24S,25-EC is more abundant than 24S-HC in the brain. Besides the dominating neuro-oxysterols, there are numerous other oxysterols present in the brain but at lower concentrations. This is also true for the neuro-sterols where comparatively low levels of cholesterol precursors are evident. It is, however, the most abundant species that appear to be most biochemically active. For example, both 24S-HC and 24S,25-EC are ligands to LXRs, 24S-HC is an inverse agonist to ROR $\alpha$  and ROR $\gamma$ , while 24S,25-EC is an inverse agonist to ROR $\gamma$  only (see Table S1). Both 24S-HC and 24S,25-EC are reported as agonists to SMO and to activate hedgehog signalling, while 24S-HC is a positive allosteric modulator of the NMDA receptors, but 24S,25-EC has not been tested. In addition, both 24S-HC and 24S,25-EC will bind to INSIG and modulate SREBP-2 regulated cholesterol biosynthesis. Why this redundancy? The answer may be provided by the *Cyp46a1*<sup>-/-</sup> mouse, which does not synthesise 24S-HC via its usual pathway and 24S-HC is only present in trace amounts in its brain. However, other than some learning difficulties the *Cyp46a1*<sup>-/-</sup> mouse has a mild phenotype. Even in the absence of *CYP46A1* in this mouse, 24S,25-EC is still the most abundant oxysterol in the brain, although at a reduced level compared to the wild type and may take on the roles normally performed by the more abundant 24S-HC. Significantly, the *Cyp46a1*<sup>-/-</sup> mouse shows both impaired learning and hippocampal LTP in vitro and while 24S-HC acts as a positive allosteric modulator of the NMDA receptors and induces LTP, 24S,25-EC has not been reported to do so. So perhaps, the roles of 24S-HC being so important, biology has devised a back-up system using 24S,25-EC. Besides these two-dominating neuro-oxysterols, lower abundance relatives may perform specific roles in defined cell populations, at different times and in response to different stimuli.

### 8.1 | Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the

common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Mathie et al. 2019; Alexander, Christopoulos et al., 2019).

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## CONFLICT OF INTEREST

W.J.G. and Y.W. are listed as inventors on the patent "Kit and method for quantitative detection of steroids" US9851368B2. W.J.G., E.Y. and Y.W. are shareholders in CholesteniX Ltd.

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## REFERENCES

- Abdel-Khalik, J., Crick, P. J., Yutuc, E., DeBarber, A. E., Duell, P. B., Steiner, R. D., ... Griffiths, W. J. (2018). Identification of 7 $\alpha$ ,24-dihydroxy-3-oxocholest-4-en-26-oic and 7 $\alpha$ ,25-dihydroxy-3-oxocholest-4-en-26-oic acids in human cerebrospinal fluid and plasma. *Biochimie*, 153, 86–98. <https://doi.org/10.1016/j.biochi.2018.06.020>
- Axelsson, M., Larsson, O., Zhang, J., Shoda, J., & Sjoval, J. (1995). Structural specificity in the suppression of HMG-CoA reductase in human fibroblasts by intermediates in bile acid biosynthesis. *Journal of Lipid Research*, 36, 290–298.
- Alexander, S. P. H., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., & CGTP Collaborators (2019). The Concise Guide to PHARMACOLOGY 2019/20: G protein-coupled receptors. *British Journal of Pharmacology*, 176(S1), S21–S141.
- Alexander, S. P. H., Mathie, A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., & CGTP Collaborators. (2019). The Concise Guide to PHARMACOLOGY 2019/20: Ion channels. *British Journal of Pharmacology*, 176(S1), S142–228.
- Bauman, D. R., Bitmansour, A. D., McDonald, J. G., Thompson, B. M., Liang, G., & Russell, D. W. (2009). 25-Hydroxycholesterol secreted by macrophages in response to toll-like receptor activation suppresses immunoglobulin A production. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 16764–16769.
- Beck, K. R., Inderbinen, S. G., Kanagaratnam, S., Kratschmar, D. V., Jetten, A. M., Yamaguchi, H., & Odermatt, A. (2019). 11 $\beta$ -hydroxysteroid dehydrogenases control access of 7 $\beta$ ,27-dihydroxycholesterol to retinoid-related orphan receptor  $\gamma$ . *Journal of Lipid Research*, 60(9), 1535–1546.
- Beck, K. R., Kanagaratnam, S., Kratschmar, D. V., Birk, J., Yamaguchi, H., Sailer, A. W., ... Odermatt, A. (2019). Enzymatic interconversion of the oxysterols 7 $\beta$ ,25-dihydroxycholesterol and 7-keto,25-hydroxycholesterol by 11 $\beta$ -hydroxysteroid dehydrogenase type 1 and 2. *The Journal of Steroid Biochemistry and Molecular Biology*, 190, 19–28.
- Bjorkhem, I. (2007). Rediscovery of cerebrosterol. *Lipids*, 42, 5–14.
- Bjorkhem, I. (2013). Cerebrotendinous xanthomatosis. *Current Opinion in Lipidology*, 24, 283–287.
- Blanc, M., Hsieh, W. Y., Robertson, K. A., Kropp, K. A., Forster, T., Shui, G., ... Meljon, A. (2013). The transcription factor STAT-1 couples macrophage synthesis of 25-hydroxycholesterol to the interferon antiviral response. *Immunity*, 38, 106–118.
- Boussicault, L., Alves, S., Lamaziere, A., Planques, A., Heck, N., Moumne, L., ... Galvan, L. (2016). CYP46A1, the rate-limiting enzyme for cholesterol degradation, is neuroprotective in Huntington's disease. *Brain*, 139, 953–970. <https://doi.org/10.1093/brain/awv384>
- Burlot, M. A., Braudeau, J., Michaelsen-Preusse, K., Potier, B., Ayciriex, S., Varin, J., ... Fernandez-Gomez, F. J. (2015). Cholesterol 24-hydroxylase defect is implicated in memory impairments associated with Alzheimer-like tau pathology. *Human Molecular Genetics*, 24, 5965–5976.
- Byrne, E. F., Sircar, R., Miller, P. S., Hedger, G., Luchetti, G., Nachtergaele, S., ... Sansom, M. S. (2016). Structural basis of smooth-endoregulation by its extracellular domains. *Nature*, 535, 517–522.
- Chen, L., Ma, M. Y., Sun, M., Jiang, L. Y., Zhao, X. T., Fang, X. X., ... Song, B. L. (2019). Endogenous sterol intermediates of the mevalonate pathway regulate HMGCR degradation and SREBP-2 processing. *Journal of Lipid Research*, 60, 1765–1775.
- Clayton, P. T. (2011). Disorders of bile acid synthesis. *Journal of Inherited Metabolic Disease*, 34, 593–604.
- Clottu, A. S., Mathias, A., Sailer, A. W., Schluep, M., Seebach, J. D., Du Pasquier, R., & Pot, C. (2017). EBI2 expression and function: Robust in memory lymphocytes and increased by natalizumab in multiple sclerosis. *Cell Reports*, 18, 213–224.
- Cobice, D. F., Mackay, C. L., Goodwin, R. J., McBride, A., Langridge-Smith, P. R., Webster, S. P., ... Andrew, R. (2013). Mass spectrometry imaging for dissecting steroid intracrinology within target tissues. *Analytical Chemistry*, 85, 11576–11584.
- Cooper, M. K., Wassif, C. A., Krakowiak, P. A., Taipale, J., Gong, R., Kelley, R. I., ... Beachy, P. A. (2003). A defective response to hedgehog signaling in disorders of cholesterol biosynthesis. *Nature Genetics*, 33, 508–513.
- Corcoran, R. B., & Scott, M. P. (2006). Oxysterols stimulate sonic hedgehog signal transduction and proliferation of medulloblastoma cells. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 8408–8413.
- Crick, P. J., Griffiths, W. J., Zhang, J., Beibel, M., Abdel-Khalik, J., Kuhle, J., ... Wang, Y. (2017). Reduced plasma levels of 25-hydroxycholesterol and increased cerebrospinal fluid levels of bile acid precursors in multiple sclerosis patients. *Molecular Neurobiology*, 54, 8009–8020.
- Crick, P. J., William Bentley, T., Abdel-Khalik, J., Matthews, I., Clayton, P. T., Morris, A. A., ... Wang, Y. (2015). Quantitative charge-tags for sterol and oxysterol analysis. *Clinical Chemistry*, 61, 400–411.
- Dai, D., Mills, P. B., Footitt, E., Gissen, P., McClean, P., Stahlschmidt, J., ... Mizuuchi, T. (2014). Liver disease in infancy caused by oxysterol 7 $\alpha$ -hydroxylase deficiency: Successful treatment with chenodeoxycholic acid. *Journal of Inherited Metabolic Disease*, 37, 851–861.
- Das, A., Smith, J. A., Gibson, C., Varma, A. K., Ray, S. K., & Banik, N. L. (2011). Estrogen receptor agonists and estrogen attenuate TNF- $\alpha$ -induced apoptosis in VSC4.1 motoneurons. *The Journal of Endocrinology*, 208, 171–182.
- Daugvilaite, V., Arfelt, K. N., Benned-Jensen, T., Sailer, A. W., & Rosenkilde, M. M. (2014). Oxysterol-EBI2 signaling in immune regulation and viral infection. *European Journal of Immunology*, 44, 1904–1912.
- de Kloet, E. R., Meijer, O. C., de Nicola, A. F., de Rijk, R. H., & Joels, M. (2018). Importance of the brain corticosteroid receptor balance in metaplasticity, cognitive performance and neuro-inflammation. *Frontiers in Neuroendocrinology*, 49, 124–145.
- de Medina, P., Paillasse, M. R., Segala, G., Poirot, M., & Silvente-Poirot, S. (2010). Identification and pharmacological characterization of

- cholesterol-5,6-epoxide hydrolase as a target for tamoxifen and AEBS ligands. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 13520–13525.
- Deshpande, I., Liang, J., Hedeem, D., Roberts, K. J., Zhang, Y., Ha, B., ... Myers, B. R. (2019). Smoothed stimulation by membrane sterols drives hedgehog pathway activity. *Nature*, 571, 284–288.
- Dietschy, J. M., & Turley, S. D. (2004). Thematic review series: Brain lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *Journal of Lipid Research*, 45, 1375–1397.
- Duc, D., Vigne, S., & Pot, C. (2019). Oxysterols in autoimmunity. *International Journal of Molecular Sciences*, 20, 4522. <https://doi.org/10.3390/ijms20184522>
- DuSelle, C. D., Umetani, M., Shaul, P. W., Mangelsdorf, D. J., & McDonnell, D. P. (2008). 27-hydroxycholesterol is an endogenous selective estrogen receptor modulator. *Molecular Endocrinology*, 22, 65–77.
- Dwyer, J. R., Sever, N., Carlson, M., Nelson, S. F., Beachy, P. A., & Parhami, F. (2007). Oxysterols are novel activators of the hedgehog signaling pathway in pluripotent mesenchymal cells. *The Journal of Biological Chemistry*, 282, 8959–8968.
- Dzeletovic, S., Breuer, O., Lund, E., & Diczfalusy, U. (1995). Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry. *Analytical Biochemistry*, 225, 73–80.
- Evans, R. M., & Mangelsdorf, D. J. (2014). Nuclear receptors, RXR, and the Big Bang. *Cell*, 157, 255–266.
- Fahy, E., Subramaniam, S., Brown, H. A., Glass, C. K., Merrill, A. H. Jr., Murphy, R. C., ... Shimizu, T. (2005). A comprehensive classification system for lipids. *Journal of Lipid Research*, 46, 839–861.
- Fivenson, E. M., Lautrup, S., Sun, N., Scheibye-Knudsen, M., Stevnsner, T., Nilsen, H., ... Fang, E. F. (2017). Mitophagy in neurodegeneration and aging. *Neurochemistry International*, 109, 202–209.
- Fu, X., Menke, J. G., Chen, Y., Zhou, G., MacNaul, K. L., Wright, S. D., ... Lund, E. G. (2001). 27-hydroxycholesterol is an endogenous ligand for liver X receptor in cholesterol-loaded cells. *The Journal of Biological Chemistry*, 276, 38378–38387.
- Gill, S., Chow, R., & Brown, A. J. (2008). Sterol regulators of cholesterol homeostasis and beyond: The oxysterol hypothesis revisited and revised. *Progress in Lipid Research*, 47, 391–404.
- Goldstein, J. L., DeBose-Boyd, R. A., & Brown, M. S. (2006). Protein sensors for membrane sterols. *Cell*, 124, 35–46.
- Goyal, S., Xiao, Y., Porter, N. A., Xu, L., & Guengerich, F. P. (2014). Oxidation of 7-dehydrocholesterol and desmosterol by human cytochrome P450 46A1. *Journal of Lipid Research*, 55, 1933–1943.
- Griffiths, W. J., Crick, P. J., Meljon, A., Theofilopoulos, S., Abdel-Khalik, J., Yutuc, E., ... Wang, Y. (2019). Additional pathways of sterol metabolism: Evidence from analysis of Cyp27a1<sup>-/-</sup> mouse brain and plasma. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, 1864, 191–211.
- Griffiths, W. J., Crick, P. J., Wang, Y., Ogundare, M., Tuschl, K., Morris, A. A., ... Wang, Y. (2013). Analytical strategies for characterization of oxysterol lipidomes: Liver X receptor ligands in plasma. *Free Radical Biology & Medicine*, 59, 69–84.
- Griffiths, W. J., & Wang, Y. (2020). Oxysterols as lipid mediators: Their biosynthetic genes, enzymes and metabolites. *Prostaglandins & Other Lipid Mediators*, 147, 106381. <https://doi.org/10.1016/j.prostaglandins.2019.106381>
- Griffiths, W. J., Yutuc, E., Abdel-Khalik, J., Crick, P. J., Hearn, T., Dickson, A., ... Jones, S. A. (2019). Metabolism of non-enzymatically derived oxysterols: Clues from sterol metabolic disorders. *Free Radical Biology & Medicine*, 144, 124–133.
- Han, M., Wang, S., Yang, N., Wang, X., Zhao, W., Saed, H. S., ... Miletic, H. (2020). Therapeutic implications of altered cholesterol homeostasis mediated by loss of CYP46A1 in human glioblastoma. *EMBO Molecular Medicine*, 12, e10924.
- Hannedouche, S., Zhang, J., Yi, T., Shen, W., Nguyen, D., Pereira, J. P., ... Knochenmuss, R. (2011). Oxysterols direct immune cell migration via EB12. *Nature*, 475, 524–527.
- Harding, S. D., Sharman, J. L., Faccenda, E., Southan, C., Pawson, A., Ireland, S., & NC-IUPHAR. (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Research*, 46(D1), D1091–D1106. <https://doi.org/10.1093/nar/gkx1121>
- Hauser, S., Poenisch, M., Schelling, Y., Hoflinger, P., Schuster, S., Teegler, A., ... Chevessier-Tünnesen, F. (2019). mRNA as a novel treatment strategy for hereditary spastic paraplegia type 5. *Mol Ther Methods Clin Dev*, 15, 359–370.
- Heo, G. Y., Bederman, I., Mast, N., Liao, W. L., Turko, I. V., & Pikuleva, I. A. (2011). Conversion of 7-ketocholesterol to oxysterol metabolites by recombinant CYP27A1 and retinal pigment epithelial cells. *Journal of Lipid Research*, 52, 1117–1127.
- Heverin, M., Bogdanovic, N., Lutjohann, D., Bayer, T., Pikuleva, I., Bretillon, L., ... Björkhem, I. (2004). Changes in the levels of cerebral and extracerebral sterols in the brain of patients with Alzheimer's disease. *Journal of Lipid Research*, 45, 186–193.
- Heverin, M., Meaney, S., Lutjohann, D., Diczfalusy, U., Wahren, J., & Björkhem, I. (2005). Crossing the barrier: Net flux of 27-hydroxycholesterol into the human brain. *Journal of Lipid Research*, 46, 1047–1052.
- Holmes, M. C., Sangra, M., French, K. L., Whittle, I. R., Paterson, J., Mullins, J. J., & Seckl, J. R. (2006). 11 $\beta$ -Hydroxysteroid dehydrogenase type 2 protects the neonatal cerebellum from deleterious effects of glucocorticoids. *Neuroscience*, 137, 865–873.
- Holmes, M. C., & Seckl, J. R. (2006). The role of 11 $\beta$ -hydroxysteroid dehydrogenases in the brain. *Molecular and Cellular Endocrinology*, 248, 9–14.
- Honda, A., Yamashita, K., Hara, T., Ikegami, T., Miyazaki, T., Shirai, M., ... Matsuzaki, Y. (2009). Highly sensitive quantification of key regulatory oxysterols in biological samples by LC-ESI-MS/MS. *Journal of Lipid Research*, 50, 350–357.
- Horton, J. D., Goldstein, J. L., & Brown, M. S. (2002). SREBPs: Activators of the complete program of cholesterol and fatty acid synthesis in the liver. *The Journal of Clinical Investigation*, 109, 1125–1131.
- Hu, X., Wang, Y., Hao, L. Y., Liu, X., Lesch, C. A., Sanchez, B. M., ... Toogood, P. L. (2015). Sterol metabolism controls T(H)17 differentiation by generating endogenous ROR $\gamma$  agonists. *Nature Chemical Biology*, 11, 141–147.
- Huang, P., Nedelcu, D., Watanabe, M., Jao, C., Kim, Y., Liu, J., & Salic, A. (2016). Cellular cholesterol directly activates smoothed in hedgehog signaling. *Cell*, 166(1176–1187), e1114.
- Iuliano, L., Crick, P. J., Zerbini, C., Tritapepe, L., Abdel-Khalik, J., Poirot, M., ... Griffiths, W. J. (2015). Cholesterol metabolites exported from human brain. *Steroids*, 99, 189–193.
- Janowski, B. A., Willy, P. J., Devi, T. R., Falck, J. R., & Mangelsdorf, D. J. (1996). An oxysterol signalling pathway mediated by the nuclear receptor LXR $\alpha$ . *Nature*, 383, 728–731.
- Jetten, A. M., & Joo, J. H. (2006). Retinoid-related orphan receptors (RORs): Roles in cellular differentiation and development. *Advances in Developmental Biology*, 16, 313–355.
- Jin, L., Martynowski, D., Zheng, S., Wada, T., Xie, W., & Li, Y. (2010). Structural basis for hydroxycholesterols as natural ligands of orphan nuclear receptor ROR $\gamma$ . *Molecular Endocrinology*, 24, 923–929.
- Kacher, R., Lamaziere, A., Heck, N., Kappes, V., Mounier, C., Despres, G., ... Messent, V. (2019). CYP46A1 gene therapy deciphers the role of brain cholesterol metabolism in Huntington's disease. *Brain*, 142, 2432–2450.
- Kallen, J., Schlaeppi, J. M., Bitsch, F., Delhon, I., & Fournier, B. (2004). Crystal structure of the human ROR $\alpha$  ligand binding domain in complex with cholesterol sulfate at 2.2 Å. *The Journal of Biological Chemistry*, 279, 14033–14038.

- Kandutsch, A. A., Chen, H. W., & Heiniger, H. J. (1978). Biological activity of some oxygenated sterols. *Science*, *201*, 498–501.
- Karu, K., Hornshaw, M., Woffendin, G., Bodin, K., Hamberg, M., Alvelius, G., ... Griffiths, W. J. (2007). Liquid chromatography-mass spectrometry utilizing multi-stage fragmentation for the identification of oxysterols. *Journal of Lipid Research*, *48*, 976–987.
- Kelley, R. L., Roessler, E., Hennekam, R. C., Feldman, G. L., Kosaki, K., Jones, M. C., ... Muenke, M. (1996). Holoprosencephaly in RSH/Smith-Lemli-Opitz syndrome: Does abnormal cholesterol metabolism affect the function of sonic hedgehog? *American Journal of Medical Genetics*, *66*, 478–484.
- Kim, W. K., Meliton, V., Amantea, C. M., Hahn, T. J., & Parhami, F. (2007). 20(S)-hydroxycholesterol inhibits PPAR $\gamma$  expression and adipogenic differentiation of bone marrow stromal cells through a hedgehog-dependent mechanism. *Journal of Bone and Mineral Research*, *22*, 1711–1719.
- Kinnebrew, M., Iverson, E. J., Patel, B. B., Pusapati, G. V., Kong, J. H., Johnson, K. A., ... Siebold, C. (2019). Cholesterol accessibility at the ciliary membrane controls hedgehog signaling. *eLife*, *8*, e50051.
- Kong, J. H., Siebold, C., & Rohatgi, R. (2019). Biochemical mechanisms of vertebrate hedgehog signaling. *Development*, *146*, dev166892. <https://doi.org/10.1242/dev.166892>
- Kotti, T., Head, D. D., McKenna, C. E., & Russell, D. W. (2008). Biphasic requirement for geranylgeraniol in hippocampal long-term potentiation. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 11394–11399.
- Kotti, T. J., Ramirez, D. M., Pfeiffer, B. E., Huber, K. M., & Russell, D. W. (2006). Brain cholesterol turnover required for geranylgeraniol production and learning in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 3869–3874.
- Kozielewicz, P., Turku, A., Bowin, C. F., Petersen, J., Valnohova, J., Canizal, M. C. A., ... Schulte, G. (2020). Structural insight into small molecule action on Frizzleds. *Nature Communications*, *11*, 414.
- Lange, Y., & Steck, T. L. (1998). Four cholesterol-sensing proteins. *Current Opinion in Structural Biology*, *8*, 435–439.
- Lehmann, J. M., Kliewer, S. A., Moore, L. B., Smith-Oliver, T. A., Oliver, B. B., Su, J. L., ... Willson, T. M. (1997). Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *The Journal of Biological Chemistry*, *272*, 3137–3140.
- Li-Hawkins, J., Lund, E. G., Bronson, A. D., & Russell, D. W. (2000). Expression cloning of an oxysterol 7 $\alpha$ -hydroxylase selective for 24-hydroxycholesterol. *The Journal of Biological Chemistry*, *275*, 16543–16549.
- Li, J., & Gibbs, R. B. (2019). Detection of estradiol in rat brain tissues: Contribution of local versus systemic production. *Psychoneuroendocrinology*, *102*, 84–94.
- Linsenbardt, A. J., Taylor, A., Emmett, C. M., Doherty, J. J., Krishnan, K., Covey, D. F., ... Mennerick, S. (2014). Different oxysterols have opposing actions at N-methyl-D-aspartate receptors. *Neuropharmacology*, *85*, 232–242.
- Liu, C., Yang, X. V., Wu, J., Kuei, C., Mani, N. S., Zhang, L., ... Karlsson, L. (2011). Oxysterols direct B-cell migration through EBI2. *Nature*, *475*, 519–523.
- Liu, S., Sjoval, J., & Griffiths, W. J. (2003). Neurosteroids in rat brain: extraction, isolation, and analysis by nanoscale liquid chromatography-electrospray mass spectrometry. *Analytical Chemistry*, *75*, 5835–5846.
- Lund, E. G., Guileyardo, J. M., & Russell, D. W. (1999). cDNA cloning of cholesterol 24-hydroxylase, a mediator of cholesterol homeostasis in the brain. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 7238–7243.
- Lund, E. G., Xie, C., Kotti, T., Turley, S. D., Dietschy, J. M., & Russell, D. W. (2003). Knockout of the cholesterol 24-hydroxylase gene in mice reveals a brain-specific mechanism of cholesterol turnover. *The Journal of Biological Chemistry*, *278*, 22980–22988.
- Maioli, S., Bavner, A., Ali, Z., Heverin, M., Ismail, M. A., Puerta, E., ... Björkhem, I. (2013). Is it possible to improve memory function by upregulation of the cholesterol 24S-hydroxylase (CYP46A1) in the brain? *PLoS ONE*, *8*, e68534.
- Marelli, C., Lamari, F., Rainteau, D., Lafourcade, A., Banneau, G., Humbert, L., ... Ollagnon, E. (2018). Plasma oxysterols: Biomarkers for diagnosis and treatment in spastic paraplegia type 5. *Brain*, *141*, 72–84.
- Mast, N., Saadane, A., Valencia-Olvera, A., Constans, J., Maxfield, E., Arakawa, H., ... Pikuleva, I. A. (2017). Cholesterol-metabolizing enzyme cytochrome P450 46A1 as a pharmacologic target for Alzheimer's disease. *Neuropharmacology*, *123*, 465–476.
- Matsuoka, H., Katayama, M., Ohishi, A., Miya, K., Tokunaga, R., Kobayashi, S., ... Michihara, A. (2020). Orphan nuclear receptor ROR $\alpha$  regulates enzymatic metabolism of cerebral 24S-hydroxycholesterol through CYP39A1 intronic response element activation. *International Journal of Molecular Sciences*, *21*, 3309.
- McDonald, J. G., Smith, D. D., Stiles, A. R., & Russell, D. W. (2012). A comprehensive method for extraction and quantitative analysis of sterols and secosteroids from human plasma. *Journal of Lipid Research*, *53*, 1399–1409.
- Meljon, A., Crick, P. J., Yutuc, E., Yau, J. L., Seckl, J. R., Theofilopoulos, S., ... Griffiths, W. J. (2019). Mining for oxysterols in Cyp7b1(–/–) mouse brain and plasma: Relevance to spastic paraplegia type 5. *Biomolecules*, *9*, 149.
- Meljon, A., Theofilopoulos, S., Shackleton, C. H., Watson, G. L., Javitt, N. B., Knolker, H. J., ... Griffiths, W. J. (2012). Analysis of bioactive oxysterols in newborn mouse brain by LC/MS. *Journal of Lipid Research*, *53*, 2469–2483.
- Meljon, A., Wang, Y., & Griffiths, W. J. (2014). Oxysterols in the brain of the cholesterol 24-hydroxylase knockout mouse. *Biochemical and Biophysical Research Communications*, *446*, 768–774.
- Meljon, A., Watson, G. L., Wang, Y., Shackleton, C. H., & Griffiths, W. J. (2013). Analysis by liquid chromatography-mass spectrometry of sterols and oxysterols in brain of the newborn Dhcr7(Delta3–5/T93M) mouse: A model of Smith-Lemli-Opitz syndrome. *Biochemical Pharmacology*, *86*, 43–55.
- Mitic, T., Shave, S., Semjonous, N., McNae, I., Cobice, D. F., Lavery, G. G., ... Andrew, R. (2013). 11 $\beta$ -Hydroxysteroid dehydrogenase type 1 contributes to the balance between 7-keto- and 7-hydroxy-oxysterols in vivo. *Biochemical Pharmacology*, *86*, 146–153.
- Murphy, R. C., & Johnson, K. M. (2008). Cholesterol, reactive oxygen species, and the formation of biologically active mediators. *The Journal of Biological Chemistry*, *283*, 15521–15525.
- Myers, B. R., Sever, N., Chong, Y. C., Kim, J., Belani, J. D., Rychnovsky, S., ... Beachy, P. A. (2013). Hedgehog pathway modulation by multiple lipid binding sites on the smoothed effector of signal response. *Developmental Cell*, *26*, 346–357.
- Nachtergaele, S., Mydock, L. K., Krishnan, K., Rammohan, J., Schlesinger, P. H., Covey, D. F., & Rohatgi, R. (2012). Oxysterols are allosteric activators of the oncoprotein smoothed. *Nature Chemical Biology*, *8*, 211–220.
- Nachtergaele, S., Whalen, D. M., Mydock, L. K., Zhao, Z., Malinauskas, T., Krishnan, K., ... Rohatgi, R. (2013). Structure and function of the smoothed extracellular domain in vertebrate hedgehog signaling. *eLife*, *2*, e01340.
- Nedelcu, D., Liu, J., Xu, Y., Jao, C., & Salic, A. (2013). Oxysterol binding to the extracellular domain of smoothed in hedgehog signaling. *Nature Chemical Biology*, *9*, 557–564.
- Nelson, E. R., Wardell, S. E., Jasper, J. S., Park, S., Suchindran, S., Howe, M. K., ... Umetani, M. (2013). 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science*, *342*, 1094–1098.



- Nelson, J. A., Steckbeck, S. R., & Spencer, T. A. (1981). Biosynthesis of 24,25-epoxycholesterol from squalene 2,3;22,23-dioxide. *The Journal of Biological Chemistry*, 256, 1067–1068.
- Nobrega, C., Mendonca, L., Marcelo, A., Lamaziere, A., Tome, S., Despres, G., ... de Almeida, L. P. (2019). Restoring brain cholesterol turnover improves autophagy and has therapeutic potential in mouse models of spinocerebellar ataxia. *Acta Neuropathologica*, 138, 837–858.
- Olkkonen, V. M., & Hynynen, R. (2009). Interactions of oxysterols with membranes and proteins. *Molecular Aspects of Medicine*, 30, 123–133.
- Paul, S. M., Doherty, J. J., Robichaud, A. J., Belfort, G. M., Chow, B. Y., Hammond, R. S., ... Mennerick, S. J. (2013). The major brain cholesterol metabolite 24(S)-hydroxycholesterol is a potent allosteric modulator of N-methyl-D-aspartate receptors. *The Journal of Neuroscience*, 33, 17290–17300.
- Qi, X., Liu, H., Thompson, B., McDonald, J., Zhang, C., & Li, X. (2019). Cryo-EM structure of oxysterol-bound human smoothed coupled to a heterotrimeric Gi. *Nature*, 571, 279–283.
- Radhakrishnan, A., Anderson, T. G., & McConnell, H. M. (2000). Condensed complexes, rafts, and the chemical activity of cholesterol in membranes. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 12422–12427.
- Radhakrishnan, A., Ikeda, Y., Kwon, H. J., Brown, M. S., & Goldstein, J. L. (2007). Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: Oxysterols block transport by binding to INSIG. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 6511–6518.
- Raleigh, D. R., Sever, N., Choksi, P. K., Sigg, M. A., Hines, K. M., Thompson, B. M., ... Krup, A. L. (2018). Cilia-associated oxysterols activate smoothed. *Molecular Cell*, 72(316–327), e315.
- Reboldi, A., Dang, E. V., McDonald, J. G., Liang, G., Russell, D. W., & Cyster, J. G. (2014). Inflammation. 25-Hydroxycholesterol suppresses interleukin-1-driven inflammation downstream of type I interferon. *Science*, 345, 679–684.
- Roberg-Larsen, H., Lund, K., Vehus, T., Solberg, N., Vesterdal, C., Misaghian, D., ... Lundanes, E. (2014). Highly automated nano-LC/MS-based approach for thousand cell-scale quantification of side chain-hydroxylated oxysterols. *Journal of Lipid Research*, 55, 1531–1536.
- Rohatgi, R., Milenkovic, L., Corcoran, R. B., & Scott, M. P. (2009). Hedgehog signal transduction by Smoothed: Pharmacologic evidence for a 2-step activation process. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 3196–3201.
- Rose, K., Allan, A., Gaudie, S., Stapleton, G., Dobbie, L., Dott, K., ... Gustafsson, J. Å. (2001). Neurosteroid hydroxylase CYP7B: Vivid reporter activity in dentate gyrus of gene-targeted mice and abolition of a widespread pathway of steroid and oxysterol hydroxylation. *The Journal of Biological Chemistry*, 276, 23937–23944.
- Russell, D. W., Halford, R. W., Ramirez, D. M., Shah, R., & Kotti, T. (2009). Cholesterol 24-hydroxylase: An enzyme of cholesterol turnover in the brain. *Annual Review of Biochemistry*, 78, 1017–1040.
- Sacchetti, P., Sousa, K. M., Hall, A. C., Liste, I., Steffensen, K. R., Theofilopoulos, S., ... Gustafsson, J. Å. (2009). Liver X receptors and oxysterols promote ventral midbrain neurogenesis in vivo and in human embryonic stem cells. *Cell Stem Cell*, 5, 409–419.
- Saeed, A. A., Genove, G., Li, T., Lutjohann, D., Olin, M., Mast, N., ... Betsholtz, C. (2014). Effects of a disrupted blood-brain barrier on cholesterol homeostasis in the brain. *The Journal of Biological Chemistry*, 289, 23712–23722.
- Saucier, S. E., Kandutsch, A. A., Gayen, A. K., Nelson, J. A., & Spencer, T. A. (1990). Oxygenation of desmosterol and cholesterol in cell cultures. *Journal of Lipid Research*, 31, 2179–2185.
- Schols, L., Rattay, T. W., Martus, P., Meisner, C., Baets, J., Fischer, I., ... Scarlato, M. (2017). Hereditary spastic paraplegia type 5: Natural history, biomarkers and a randomized controlled trial. *Brain*, 140, 3112–3127.
- Schroepfer, G. J. Jr. (2000). Oxysterols: Modulators of cholesterol metabolism and other processes. *Physiological Reviews*, 80, 361–554.
- Schweizer, R. A., Zurcher, M., Balazs, Z., Dick, B., & Odermatt, A. (2004). Rapid hepatic metabolism of 7-ketocholesterol by 11 $\beta$ -hydroxysteroid dehydrogenase type 1: Species-specific differences between the rat, human, and hamster enzyme. *The Journal of Biological Chemistry*, 279, 18415–18424.
- Segala, G., David, M., de Medina, P., Poirot, M. C., Serhan, N., Vergez, F., ... Caron, N. (2017). Dendrogenin A drives LXR to trigger lethal autophagy in cancers. *Nature Communications*, 8, 1903.
- Sengupta, D., & Chattopadhyay, A. (2015). Molecular dynamics simulations of GPCR-cholesterol interaction: An emerging paradigm. *Biochimica et Biophysica Acta*, 1848, 1775–1782.
- Serafini, B., Rosicarelli, B., Veroni, C., Zhou, L., Reali, C., & Aloisi, F. (2016). ROR $\gamma$ t expression and lymphoid neogenesis in the brain of patients with secondary progressive multiple sclerosis. *Journal of Neuropathology and Experimental Neurology*, 75, 877–888.
- Sever, N., Mann, R. K., Xu, L., Snell, W. J., Hernandez-Lara, C. I., Porter, N. A., & Beachy, P. A. (2016). Endogenous B-ring oxysterols inhibit the hedgehog component smoothed in a manner distinct from cyclopamine or side-chain oxysterols. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 5904–5909.
- Shafaati, M., Olin, M., Bavner, A., Pettersson, H., Rozell, B., Meaney, S., ... Björkhem, I. (2011). Enhanced production of 24S-hydroxycholesterol is not sufficient to drive liver X receptor target genes in vivo. *Journal of Internal Medicine*, 270, 377–387.
- Shinkyo, R., Xu, L., Tallman, K. A., Cheng, Q., Porter, N. A., & Guengerich, F. P. (2011). Conversion of 7-dehydrocholesterol to 7-ketocholesterol is catalyzed by human cytochrome P450 7A1 and occurs by direct oxidation without an epoxide intermediate. *The Journal of Biological Chemistry*, 286, 33021–33028.
- Sidhu, R., Jiang, H., Farhat, N. Y., Carrillo-Carrasco, N., Woolery, M., Ottinger, E., ... Jiang, X. (2015). A validated LC-MS/MS assay for quantification of 24(S)-hydroxycholesterol in plasma and cerebrospinal fluid. *Journal of Lipid Research*, 56, 1222–1233.
- Simigdala, N., Gao, Q., Pancholi, S., Roberg-Larsen, H., Zvelebil, M., Ribas, R., ... Martin, L. A. (2016). Cholesterol biosynthesis pathway as a novel mechanism of resistance to estrogen deprivation in estrogen receptor-positive breast cancer. *Breast Cancer Research*, 18, 58.
- Song, B. L., Javitt, N. B., & DeBose-Boyd, R. A. (2005). INSIG-mediated degradation of HMG CoA reductase stimulated by lanosterol, an intermediate in the synthesis of cholesterol. *Cell Metabolism*, 1, 179–189.
- Song, C., & Liao, S. (2000). Cholestenoid acid is a naturally occurring ligand for liver X receptor  $\alpha$ . *Endocrinology*, 141, 4180–4184.
- Soroosh, P., Wu, J., Xue, X., Song, J., Sutton, S. W., Sablad, M., ... Luna, R. (2014). Oxysterols are agonist ligands of ROR $\gamma$ t and drive Th17 cell differentiation. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 12163–12168.
- Spence, R. D., Wisdom, A. J., Cao, Y., Hill, H. M., Mongerson, C. R., Stapornkul, B., ... Voskuhl, R. R. (2013). Estrogen mediates neuroprotection and anti-inflammatory effects during EAE through ER $\alpha$  signaling on astrocytes but not through ER $\beta$  signaling on astrocytes or neurons. *The Journal of Neuroscience*, 33, 10924–10933.
- Stiles, A. R., Kozlitina, J., Thompson, B. M., McDonald, J. G., King, K. S., & Russell, D. W. (2014). Genetic, anatomic, and clinical determinants of human serum sterol and vitamin D levels. *Proceedings of the National Academy of Sciences of the United States of America*, 111, E4006–E4014.
- Sun, L. P., Seemann, J., Goldstein, J. L., & Brown, M. S. (2007). Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: INSIG renders sorting signal in SCAP inaccessible to COPII proteins. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 6519–6526.
- Suzuki, H., Barros, R. P., Sugiyama, N., Krishnan, V., Yaden, B. C., Kim, H. J., ... Gustafsson, J. Å. (2013). Involvement of estrogen receptor  $\beta$  in

- maintenance of serotonergic neurons of the dorsal raphe. *Molecular Psychiatry*, 18, 674–680.
- Tanaka, J., Fujita, H., Matsuda, S., Toku, K., Sakanaka, M., & Maeda, N. (1997). Glucocorticoid- and mineralocorticoid receptors in microglial cells: The two receptors mediate differential effects of corticosteroids. *Glia*, 20, 23–37.
- Theofilopoulos, S., Abreu de Oliveira, W. A., Yang, S., Yutuc, E., Saeed, A., Abdel-Khalik, J., ... Griffiths, W. J. (2019). 24(S),25-Epoxycholesterol and cholesterol 24S-hydroxylase (CYP46A1) overexpression promote midbrain dopaminergic neurogenesis in vivo. *The Journal of Biological Chemistry*, 294, 4169–4176.
- Theofilopoulos, S., Griffiths, W. J., Crick, P. J., Yang, S., Meljon, A., Ogundare, M., ... Morris, A. A. (2014). Cholestenic acids regulate motor neuron survival via liver X receptors. *The Journal of Clinical Investigation*, 124, 4829–4842.
- Theofilopoulos, S., Wang, Y., Kitambi, S. S., Sacchetti, P., Sousa, K. M., Bodin, K., ... Karu, K. (2013). Brain endogenous liver X receptor ligands selectively promote midbrain neurogenesis. *Nature Chemical Biology*, 9, 126–133.
- Tint, G. S., Yu, H., Shang, Q., Xu, G., & Patel, S. B. (2006). The use of the DHCR7 knockout mouse to accurately determine the origin of fetal sterols. *Journal of Lipid Research*, 47, 1535–1541.
- Umetani, M., Domoto, H., Gormley, A. K., Yuhanna, I. S., Cummins, C. L., Javitt, N. B., ... Mangelsdorf, D. J. (2007). 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. *Nature Medicine*, 13, 1185–1192.
- Voisin, M., de Medina, P., Mallinger, A., Dalenc, F., Huc-Claustre, E., Leignadier, J., ... Noguer, E. (2017). Identification of a tumor-promoter cholesterol metabolite in human breast cancers acting through the glucocorticoid receptor. *Proceedings of the National Academy of Sciences of the United States of America*, 114, E9346–E9355.
- Wada, T., Kang, H. S., Angers, M., Gong, H., Bhatia, S., Khadem, S., ... Xie, W. (2008). Identification of oxysterol 7 $\alpha$ -hydroxylase (Cyp7b1) as a novel retinoid-related orphan receptor  $\alpha$  (ROR $\alpha$ ) (NR1F1) target gene and a functional cross-talk between ROR $\alpha$  and liver X receptor (NR1H3). *Molecular Pharmacology*, 73, 891–899.
- Wang, L., Andersson, S., Warner, M., & Gustafsson, J. A. (2003). Estrogen receptor (ER) $\beta$  knockout mice reveal a role for ER $\beta$  in migration of cortical neurons in the developing brain. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 703–708.
- Wang, L., Schuster, G. U., Hultenby, K., Zhang, Q., Andersson, S., & Gustafsson, J. A. (2002). Liver X receptors in the central nervous system: From lipid homeostasis to neuronal degeneration. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 13878–13883.
- Wang, Y., Kumar, N., Crumbley, C., Griffin, P. R., & Burris, T. P. (2010). A second class of nuclear receptors for oxysterols: Regulation of ROR $\alpha$  and ROR $\gamma$  activity by 24S-hydroxycholesterol (cerebrosterol). *Biochimica et Biophysica Acta*, 1801, 917–923.
- Wang, Y., Kumar, N., Solt, L. A., Richardson, T. I., Helvering, L. M., Crumbley, C., ... Chalmers, M. J. (2010). Modulation of retinoic acid receptor-related orphan receptor  $\alpha$  and  $\gamma$  activity by 7-oxygenated sterol ligands. *The Journal of Biological Chemistry*, 285, 5013–5025.
- Wang, Y., Muneton, S., Sjoval, J., Jovanovic, J. N., & Griffiths, W. J. (2008). The effect of 24S-hydroxycholesterol on cholesterol homeostasis in neurons: Quantitative changes to the cortical neuron proteome. *Journal of Proteome Research*, 7, 1606–1614.
- Wang, Y., Sousa, K. M., Bodin, K., Theofilopoulos, S., Sacchetti, P., Hornshaw, M., ... Griffiths, W. J. (2009). Targeted lipidomic analysis of oxysterols in the embryonic central nervous system. *Molecular BioSystems*, 5, 529–541.
- Wanke, F., Moos, S., Croxford, A. L., Heinen, A. P., Graf, S., Kalt, B., ... Yogev, N. (2017). EBI2 is highly expressed in multiple sclerosis lesions and promotes early CNS migration of encephalitogenic CD4 T cells. *Cell Reports*, 18, 1270–1284.
- Wardell, S. E., Nelson, E. R., & McDonnell, D. P. (2014). From empirical to mechanism-based discovery of clinically useful selective estrogen receptor modulators (SERMs). *Steroids*, 90, 30–38.
- Warner, M., & Gustafsson, J. A. (2015). Estrogen receptor  $\beta$  and liver X receptor  $\beta$ : Biology and therapeutic potential in CNS diseases. *Molecular Psychiatry*, 20, 18–22.
- Wu, Q., Ishikawa, T., Sirianni, R., Tang, H., McDonald, J. G., Yuhanna, I. S., ... Umetani, M. (2013). 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. *Cell Reports*, 5, 637–645.
- Wu, W. F., Tan, X. J., Dai, Y. B., Krishnan, V., Warner, M., & Gustafsson, J. A. (2013). Targeting estrogen receptor  $\beta$  in microglia and T cells to treat experimental autoimmune encephalomyelitis. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 3543–3548.
- Xu, L., Korade, Z., Rosado, D. A. Jr., Liu, W., Lamberson, C. R., & Porter, N. A. (2011). An oxysterol biomarker for 7-dehydrocholesterol oxidation in cell/mouse models for Smith-Lemli-Opitz syndrome. *Journal of Lipid Research*, 52, 1222–1233.
- Xu, L., Mirnics, K., Bowman, A. B., Liu, W., Da, J., Porter, N. A., & Korade, Z. (2012). DHCEO accumulation is a critical mediator of pathophysiology in a Smith-Lemli-Opitz syndrome model. *Neurobiology of Disease*, 45, 923–929.
- Yang, C., McDonald, J. G., Patel, A., Zhang, Y., Umetani, M., Xu, F., ... Hobbs, H. H. (2006). Sterol intermediates from cholesterol biosynthetic pathway as liver X receptor ligands. *The Journal of Biological Chemistry*, 281, 27816–27826.
- Yutuc, E., Angelini, R., Baumert, M., Mast, N., Pikuleva, I., Newton, J., ... Griffiths, W. J. (2020). Localization of sterols and oxysterols in mouse brain reveals distinct spatial cholesterol metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, 117, 5749–5760.

## SUPPORTING INFORMATION

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