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## **Role of Sigma-1 Receptors in Neurodegenerative Diseases**

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## Critical review

## Role of sigma-1 receptors in neurodegenerative diseases



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## ABSTRACT

Neurodegenerative diseases with distinct genetic etiologies and pathological phenotypes appear to share common mechanisms of neuronal cellular dysfunction, including excitotoxicity, calcium dysregulation, oxidative damage, ER stress and mitochondrial dysfunction. Glial cells, including microglia and astrocytes, play an increasingly recognized role in both the promotion and prevention of neurodegeneration. Sigma receptors, particularly the sigma-1 receptor subtype, which are expressed in both neurons and glia of multiple regions within the central nervous system, are a unique class of intracellular proteins that can modulate many biological mechanisms associated with neurodegeneration. These receptors therefore represent compelling putative targets for pharmacologically treating neurodegenerative disorders. In this review, we provide an overview of the biological mechanisms frequently associated with neurodegeneration, and discuss how sigma-1 receptors may alter these mechanisms to preserve or restore neuronal function. In addition, we speculate on their therapeutic potential in the treatment of various neurodegenerative disorders.

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## 1. Introduction

Neurodegeneration is characterized by the progressive loss of neuronal integrity, in both structure and function. Alzheimer's disease and Parkinson's disease are the most common neurodegenerative disorders worldwide, affecting approximately 10% of individuals over the age of 60. Current therapies for these and other neurodegenerative conditions focus on symptomatic treatment, and there remains an urgent need to identify and develop effective therapeutics to protect and restore neuronal integrity. To achieve this goal, a better understanding of cellular targets and processes involved in neurodegeneration and regeneration is needed.

Among the putative therapeutic targets being studied, sigma receptors have gained attention for their involvement in modulating cell survival and function. Originally misclassified as a subtype of opioid receptor in the 1970s, sigma receptors are now recognized as a unique class of intracellular proteins, distinct from G protein-coupled and ionotropic receptors (1). They are capable of modulating a variety of cellular processes relevant to neurodegeneration (1,2). The two established subtypes, sigma-1 and sigma-2, are both highly expressed in the central nervous system (CNS), and can be distinguished by their distinct pharmacological profiles and molecular characteristics (1).

Over the past decade, significant advances have been made in our understanding of the sigma-1 receptor subtype in both pathological and physiological processes. The contribution of the sigma-2 subtype, however, remains less well understood due to the paucity of available experimental tools to study its functions. This review focuses on the role of sigma-1 receptors in neurodegeneration,

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beginning with a brief overview of sigma-1 receptor biology, followed by a summary of the common mechanisms of neurodegeneration and how sigma-1 receptor ligands may modulate these mechanisms to elicit neuroprotective and/or restorative effects. Finally, we discuss the potential application of sigma-1 receptor modulation to specific therapeutic interventions.

## 2. Sigma-1 receptor structure and functions

The sigma-1 receptor is a small (28 kDa), highly conserved, transmembrane protein located in the endoplasmic reticulum (ER) membrane. It is specifically enriched in the ER subregion contacting mitochondria, called the mitochondrial-associated membrane (MAM). Localization studies also report the sigma-1 receptor at or in i) neuronal nuclear, mitochondrial, and plasma membranes, ii) multiple other CNS cell types (astrocytes, microglia and oligodendrocytes), and iii) CNS-associated immune and endocrine tissues (1). The varied sites at which sigma-1 receptors are present suggest multiple pathways by which these receptors may influence physiological and pathological processes.

The sigma-1 receptor can migrate between different organellar membranes in response to ligand binding (3,4). As chaperone proteins, sigma-1 receptors do not have their own intrinsic signaling machinery. Instead, upon ligand activation, they appear to operate primarily via translocation and protein-protein interactions to modulate the activity of various ion channels and signaling molecules, including inositol phosphates, protein kinases, and calcium channels (3). The characteristics of sigma-1 interactions in each pathway are still being determined.

Because sigma-1 receptors exhibit no homology to other mammalian proteins, genetic manipulation has been instrumental in investigating their functions in experimental systems. These studies allow the results of pharmacological manipulation, which is more amenable for potential therapeutic intervention, to be interpreted as either agonistic or antagonistic. By convention, “antagonists” are those compounds that recapitulate the gene knockdown phenotype; they generally have no effects on their own, but attenuate the effects of sigma-1 stimulation. Sigma-1 receptor antagonists that are commonly cited in the literature, including this review, include: BD1047 (N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino) ethylamine), BD1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine), and NE-100 (4-methoxy-3-(2-phenylethoxy)-N,N-dipropylbenzeneethanamine). In contrast, sigma-1 “agonists” are those compounds that recapitulate the phenotypes of receptor overexpression, either autonomously or additive to the effects of other compounds. Common selective sigma-1 receptor agonists include: (+)-pentazocine, (+)-SKF10,047, PRE084 (2-morpholin-4-ylethyl 1-phenylcyclohexane-1-carboxylate), and SA4503 (1-[2-(3,4-dimethoxyphenyl)ethyl]-4-(3-phenylpropyl)piperazine). Many currently marketed drugs (e.g., haloperidol, donepezil, and fluvoxamine) interact with sigma-1 receptors, but are not selective for them. The involvement of the sigma-1 subtype in a given system therefore requires careful analysis and verification using selective genetic and pharmacological tools.

## 3. Common mechanisms of neurodegeneration

### 3.1. Excitotoxicity and calcium overload

Glutamate is the major excitatory neurotransmitter in the CNS, and its interaction with specific membrane receptors is responsible for many neurologic functions, including learning and memory. Sustained release of glutamate, however, causes persistent (and only partially desensitizable) activation of N-methyl-D-aspartate (NMDA) receptors, leading to neuronal excitotoxicity. In addition to

transporting sodium, NMDA receptors also transport calcium. Persistent activation therefore increases intracellular calcium levels, followed by stochastic failure of calcium homeostasis and necrotic cell death (5). This toxicity does not result from superoxide free radical production, as initially proposed (6), but rather from activation of the mitochondrial permeability transition pore opening triggered by membrane potential-dependent uptake of calcium into the mitochondrial matrix (7,8). The identity of the pore itself has recently been proposed to be the Fo portion of the FoF1 adenosine triphosphate (ATP) synthase (9). Excitotoxicity and excess intracellular calcium contribute to neurodegeneration in many acute CNS diseases, including stroke and traumatic brain injury, and are also implicated in chronic diseases, including amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and Parkinson's disease (5,10,11).

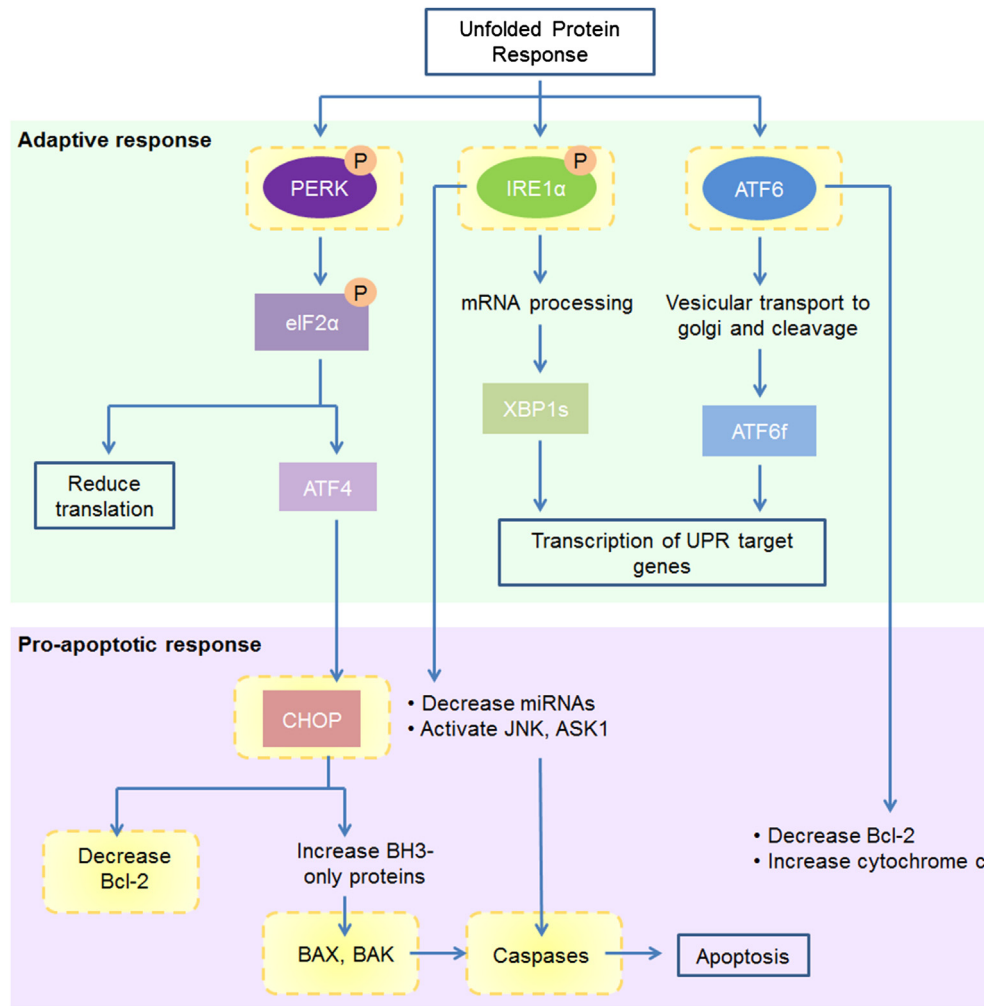
### 3.2. Oxidative and nitrosative stress

Oxygen (O<sub>2</sub>) is critical to meet the energetic demands of biological tissues through the production of ATP by oxidative phosphorylation. However, aberrant O<sub>2</sub> reduction produces radical species that can cause extensive damage to cellular components, cells, and tissues. This phenomenon of “oxidative stress” is defined by a broad range of phenotypes, including the accumulation of oxidized molecules and the disruption of normal cellular processes and viability. Oxidative stress is typically considered to be the state in which these phenotypes are measurable at higher levels than in a “normal” state. Neurons may be particularly vulnerable to oxidative stress due to their terminally differentiated state, complex morphology, and dependence on surrounding glia for metabolic substrates and glutathione (12). Reactive oxygen species (ROS) are generated by multiple conditions and sources, including sustained neurotransmission (e.g., of glutamate, dopamine, or serotonin), mitochondrial dysfunction, and production by glial cells. Depending on the species and location of the ROS, oxidative damage can affect nucleic acids, proteins and lipids. The best evidence that ROS may be an underlying cause of neurodegeneration is the strong association between the detection of increased ROS production and the increased oxidative damage observed in CNS disorders such as Parkinson's disease, Alzheimer's disease and ALS (12,13). Oxidative stress can also impair mitochondrial function, leading to a depletion of ATP and decreased antioxidant capacity (13). Along with ROS, reactive nitrogen species (RNS) can also be generated under pathological conditions in the CNS.

### 3.3. Endoplasmic reticulum (ER) stress

The ER plays an important role in protein synthesis and folding as well as cellular homeostasis. Different perturbations, such as calcium dysregulation and oxidative stress, can alter ER function and lead to the accumulation of unfolded or misfolded proteins within the ER lumen. This triggers a stress response by the ER known as the unfolded protein response (UPR) to restore protein folding homeostasis (Fig. 1). Three major signaling pathways mediate the UPR: protein kinase RNA-like ER kinase (PERK), inositol-requiring enzyme 1 alpha (IRE1 $\alpha$ ), and activating transcription factor 6 (ATF6).

The downstream activities of all three pathways have been implicated in protective or adaptive responses to the protein accumulation as well as in the promotion of apoptosis. Adaptive responses include a reduction in global protein translation by PERK to decrease the protein load to the ER, and an upregulation of proteins involved in the UPR by IRE1 $\alpha$  and ATF6 to increase ER folding capacity and ER-associated protein degradation (ERAD). Conversely, PERK activation can also lead to apoptosis. Some



**Fig. 1.** Schematic diagram of the unfolded protein response (UPR) and modulation by sigma-1 receptors (adapted from (14)). Accumulation of misfolded or unfolded proteins in the endoplasmic reticulum (ER) activates the UPR to restore protein folding homeostasis and promote cell survival (adaptive response). Prolonged exposure to ER stress overcomes the adaptive response of the UPR and induces apoptosis (pro-apoptotic response). See text for details and further information (Section 3.3). To promote cell survival, sigma-1 receptors have been reported to modulate the activity and/or levels of the three major ER stress proteins (PERK, IRE1 $\alpha$ , and ATF6), decrease CHOP, BAX and caspases and increase Bcl-2, as discussed in the text (Section 4.3 and Section 5) and represented here by yellow boxes.

proteins, most notably ATF4, can bypass the PERK-mediated translational repression. ATF4 then promotes the expression of various apoptotic activators, including C/EBP-homologous protein (CHOP). Sustained activation of PERK thus leads to the CHOP upregulation, which in turn inhibits the expression of anti-apoptotic Bcl-2 while upregulating expression of the pro-apoptotic BH3-only proteins. This cascade of events can result in the activation of Bak- and Bax-dependent apoptosis (14). IRE1 $\alpha$  can also induce the activation of c-JUN amino-terminal kinase (JNK) and apoptosis signal-regulating kinase 1 (ASK1) and degrade microRNAs that inhibit caspase expression, contributing to caspase-activated apoptotic cell death (14). Additionally, ATF6 can decrease Bcl-2 levels and increase cytochrome C release, adding to the activation of the apoptotic cascade (15).

Whether ER stress elicits a UPR-adaptive or pro-apoptotic response depends on the accumulation of misfolded proteins and the timing of the stress exposure (14). Under acute stress and moderate misfolded protein accumulation, the UPR is activated to clear accumulations through the ERAD machinery linked to the ubiquitin proteasome system (UPS) or through autophagy, restoring cellular homeostasis (14). Although the threshold for apoptosis is unclear, it is reasonable to assume that prolonged or severe protein accumulation could cause the ER to trigger cell death

rather than cell maintenance programs. Consistent with this, constitutive activity of the ER stress response has been linked to neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease (14,16). Therapeutic strategies predicted to inhibit neurodegeneration might enhance UPR signaling responses that attenuate ER stress inducers, while inhibiting those portions of UPR signaling that promote apoptosis.

### 3.4. Mitochondrial dysfunction

Mitochondria play multiple critical roles in neuron maintenance. In addition to supplying ATP and providing metabolic and biosynthetic substrates, mitochondria also regulate calcium homeostasis and the initiation of apoptosis. Aberrant mitochondrial function is associated with multiple neurodegenerative diseases (13,17).

As described above, high intracellular calcium as a result of sustained glutamate receptor activity is a proposed trigger for excitotoxic cell death. This process is dependent on polarized mitochondria; cells treated with mitochondrial inhibitors are not susceptible to the calcium deregulation that mediate excitotoxicity (18), though the artificial conditions that confer this protection would result in ATP insufficiency that in vivo would not be feasible.

A more recent mechanistic model of excitotoxic cell death proposes a key role for spare respiratory capacity, or the ability of the cell to increase respiration beyond its basal rate to meet increased ATP demand. In the context of sustained glutamate signaling, the influx of sodium along with calcium through the NMDA receptor require increased activity of the sodium-potassium ATPase to restore resting ion concentrations (18), increasing ATP demand and requiring ATP production to increase. Indeed, pharmacological inhibition of respiratory capacity increases the stochastic failure of cell viability under these conditions, suggesting ATP insufficiency upstream of calcium dysregulation as the initial trigger of the excitotoxic cascade.

Alterations in mitochondrial structure and dynamics are also strongly associated with neurodegenerative diseases. Mitochondrial fission and fusion are part of normal organellar maintenance, allowing mitochondrial transport to distal regions of the cell. Fission and fusion are particularly significant in axons, in which mitochondria may have to travel long distances. These processes also allow the sequestration of damaged mitochondrial material for engulfment by autophagosomes (19). Multiple diseases are associated with defects of either fusion or fission, though causality is not always clear. In addition, genetic or RNA-level disruption of proteins that mediate fission and fusion can recapitulate multiple disease phenotypes.

Recent work has identified the ER as a crucial component of the mitochondrial fission machinery, where dynamin-related protein 1 (Drp1) is recruited to ER-mitochondria contact sites and mediates fission (20). Homozygous knockout of Drp1 is lethal (21), while fragmented mitochondria and elevated or modified Drp1 (i.e., increased fission activity) are associated with Alzheimer's disease, Parkinson's disease, and Huntington's disease (17). The close apposition of mitochondria to a particular subset of the ER, the MAM, is important for multiple other aspects of normal mitochondrial and cellular function. Proper interaction between mitochondria and the MAM maintains lipid synthesis and trafficking, calcium homeostasis, and regulation of mitochondrial-dependent apoptosis. Mitochondria-MAM dysregulation has been proposed as the underlying cause of Alzheimer's disease (22), and may contribute to neuronal loss in other disease contexts (23).

### 3.5. Reactive gliosis

Neural tissue insults arise from various sources and involve nearly all cell types contained within the CNS. One of the most ubiquitous responses to CNS insults including neurodegenerative disorders is reactive gliosis (24). Although present in a wide array of neurodegenerative disorders, its contribution to neurodegeneration and the progression of neurodegenerative disorders is still poorly understood.

Reactive astrogliosis is classified as the "activation" of astrocytes within the CNS. This activation leads to the increased expression of various genes, including glial fibrillary acidic protein (GFAP), a component of astrocytic filaments and commonly utilized marker of reactive astrogliosis. The upregulation of GFAP is a downstream result of STAT3 (signal transducer and activator of transcription 3) phosphorylation and activation, an effect demonstrated in multiple models of neurodegenerative disorders (25). Neural damage that results in astrogliosis and the subsequent upregulation of GFAP causes astrocytes to proliferate, migrate, and in cases of severe neural damage, form glial scars (25,26). Glial scar formation is hypothesized to protect surrounding neuronal tissue from further damage as a result of excess inflammation; however the formation of glial scars also can impede repair processes and thereby can inhibit the ability of neuronal tracts to regenerate (25). Reactive astrocytes can also produce several factors (cytokines, chemokines,

and neurotrophic factors) to further improve or aggravate brain damage (25).

Microglia are a separate and distinct type of glial cell compared to astrocytes. They are the macrophage-derived resident immune cells of the CNS. Under normal physiological conditions, microglia are responsible for synaptic pruning, ultimately affecting neuronal connectivity and signaling (27). The ability of microglia to monitor the surrounding microenvironment and react to both changes in neuronal signaling as well as CNS insults underlies their importance in neurodegeneration (28). As a result of disruptions in CNS homeostasis, microglia are activated in a manner similar to macrophages in the periphery (28). Although multiple microglial phenotypes are believed to occur in response to CNS insult, they are typically classified as M1 and/or M2 responses, similar to peripheral macrophages (28). M1 microglial responses are pro-inflammatory in nature, and can cause further damage to the CNS through the release of ROS/RNS and pro-inflammatory cytokines such as interleukin (IL)-1 $\beta$ , while M2 microglial responses are believed to mediate repair, including remyelination, in response to various CNS insults (28).

Further elucidation of precisely how the physiological changes in astrocytes and microglia affect neurodegeneration may provide a framework for development of therapeutic strategies that target endogenous regenerative processes. For example, interventions aimed at limiting the inflammatory M1 response while enhancing the reparative M2 responses may slow or even reverse the neurodegeneration that is characteristic of injury and disease progression.

## 4. Neuroprotective actions by sigma ligands

### 4.1. Modulation of calcium homeostasis and glutamate activity

One major mechanism by which sigma-1 receptor ligands may confer neuroprotection is through the regulation of intracellular calcium homeostasis. The sigma-1 agonist (+)-pentazocine, for example, can induce a mono- or biphasic transient calcium response in place of sustained flux in primary rat cortical neurons exposed to toxic concentrations of glutamate. This shift is indicative of neuroprotection, possibly through modulation of receptor and voltage-gated calcium channel activity (29). Additionally, sigma-1 agonists can attenuate intracellular calcium elevations in response to an *in vitro* model of ischemia induced by sodium azide and glucose deprivation (30). Notably, sigma-1 receptors can respond to perturbations in ER calcium concentrations by promoting calcium entry into mitochondria through stabilization of type 3 inositol triphosphate (IP<sub>3</sub>) receptors (IP<sub>3</sub>R3) at the MAM (4).

Activation of sigma-1 receptors has also been shown to attenuate the release of glutamate following ischemia (31), and under certain conditions inhibit NMDA receptors (32), which, among the glutamate receptor subtypes, appear to be the principal mediators of excitotoxic damage (10). The exact mechanisms by which sigma-1 receptors modulate the activity of NMDA receptors is unclear, but may involve direct interaction with specific subunits of the NMDA receptor (33) or indirect effects of other ion channel modulation (34).

### 4.2. Attenuation of reactive species production

Activation of sigma-1 receptors may also mitigate ROS accumulation, possibly through modulation of ROS-neutralizing proteins. Sigma-1 receptor knockout or knockdown can increase oxidative damage (35,36). Liver and lung tissue homogenates as well as primary hepatocytes extracted from sigma-1 knockout mice showed higher levels of superoxide as measured by an increased

fluorescence of 2',7'-dichlorofluorescein (DCF) compared to wild-type mice (36). Metabolomic screening and 2D gel electrophoresis of liver homogenates of the two mouse groups indicated significant differences in the levels of metabolites and proteins associated with free radical production and clearance (36). Moreover, knockdown of sigma-1 receptors in Chinese hamster ovary (CHO) cells caused increased DCF fluorescence intensity compared to control cells (35). On the other hand, expression of sigma-1 receptors in monkey kidney fibroblast (COS-7) and mouse monocyte macrophage (RAW 264.7) cells led to decreased DCF fluorescence intensity (36). Addition of (+)-pentazocine to COS-7 cells further attenuated the fluorescence signal, while the sigma antagonist haloperidol blocked the expression phenotype, conferring higher ROS formation (36). The apparent sigma-1-dependent decrease in ROS levels corresponded with the upregulation of antioxidant response element (ARE) genes including NAD(P): quinone oxidoreductase 1 (NQO1) and superoxide dismutase 1 (SOD1) (36).

Sigma-1 agonists may also ameliorate nitrosative stress. The sigma receptor agonist PPBP (4-phenyl-1-(4-phenylbutyl)piperidine) attenuated nitric oxide (NO) production as well as nitrosative damage to proteins and nucleic acids (37,38). The decrease in NO generation may be linked to the ability of sigma-1 receptor activation to decrease nitric oxide synthase (NOS) activity (38–40).

#### 4.3. Modulation of ER and mitochondrial function

Sigma-1 receptor localization within the ER and at mitochondrial membranes suggests a role in interorganellar communication and regulation, as well as separate influences in each structure (4). As chaperones, sigma-1 receptors can modulate the UPR. In its dormant state, the sigma-1 receptor forms a complex at the MAM with the ER chaperone and signaling regulator BiP/Grp78 (4). During ER stress or via ligand stimulation, sigma-1 receptors dissociate from BiP/Grp78 and can modulate the activity of other proteins, including PERK, IRE1 $\alpha$ , and ATF6 (4,41). In addition, ER stress is associated with the upregulation of sigma-1 expression (4). Overexpression of sigma-1 receptors has been shown to decrease the activation of PERK and ATF6 and increase cell survival, whereas knockdown of sigma-1 receptors destabilizes the conformation of IRE1 and decreases cell survival following administration of the ER stressor thapsigargin *in vitro* (4,41). The precise mechanism(s) by which sigma-1 receptors may alter the activities or amounts of these stress response proteins is unclear, but may involve direct protein-protein interactions (41) and transcriptional regulation (42,43). Future investigations must address the downstream effects of sigma-1 receptor-mediated modulation on these stress response proteins, and the timing of sigma-1 receptor regulation in response to ER stress in order to better understand the conditions and timing under which pharmacological modulation of sigma-1 may be of the greatest benefit.

Sigma-1 receptors may also alter mitochondrial function, as sigma receptor ligands have been shown to ameliorate bioenergetic deterioration in a variety of cells. For example, the sigma-1 receptor ligand BHDP (N-benzyl-N-(2-hydroxy-3,4-dimethoxybenzyl)-piperazine) protected rat liver cells from ischemic stress, preserving mitochondrial respiration and ATP synthesis compared to the control group (44). The cytoprotective effects of BHDP are likely mediated through agonist activity at sigma-1 receptors, as similar results were observed with the sigma-1 agonist SA4503 in cardiomyocytes treated with angiotensin II to induce hypertrophy; SA4503 protected cardiomyocytes from impaired ATP production (45). Co-administration of the sigma-1 antagonist NE-100 blocked this effect, confirming specificity of sigma-1 receptor involvement (45). Finally, in cultured astrocytes, BHDP overcame hypoxia-induced impairment of ATP production (46).

Sigma-1 receptors may confer these protective effects through modulation of mitochondrial calcium uptake at the MAM (4). Under conditions of ER stress, sigma-1 receptors have been shown in CHO cells to dissociate from BiP/Grp78 and interact directly with IP<sub>3</sub>R3, which selectively mediates calcium uptake by mitochondria (4). The sigma-1 receptor-IP<sub>3</sub>R3 interaction stabilizes IP<sub>3</sub>R3, which could in turn promote both mitochondrial calcium uptake and, ultimately, cell survival (4). Supporting this, Shioda and colleagues identified a truncated splice variant of the sigma-1 receptor (short form sigma-1 or sigma-1S) in the mouse hippocampus that localizes to the MAM and complexes with non-truncated sigma-1 receptors, but does not complex with IP<sub>3</sub>R (47). In mouse neuroblastoma C3100 (Neuro-2a) cells, exogenous overexpression of non-truncated sigma-1 receptors enhanced ATP- or IP<sub>3</sub>-induced mitochondrial calcium uptake whereas overexpression of sigma-1S decreased mitochondrial calcium uptake compared to control cells (47). Following tunicamycin-induced ER stress, the exogenous overexpression of non-truncated sigma-1 receptors protected IP<sub>3</sub>R proteins from degradation and enhanced ATP production, promoting cell survival (47). Conversely, overexpression of sigma-1S enhanced IP<sub>3</sub>R degradation and decreased mitochondrial calcium uptake, resulting in increased apoptosis (47). These findings suggest that sigma-1S destabilizes IP<sub>3</sub>R and diminishes IP<sub>3</sub>R-driven mitochondrial calcium uptake through loss of sigma-1 IP<sub>3</sub>R3 interaction, resulting in impaired ATP production and increased apoptosis (47). More work is needed to determine how truncated sigma-1 receptors interfere with normal receptor function to affect mitochondrial stability. This question is of high clinical relevance as aberrant forms of sigma-1 receptors have been found to occur in neurodegenerative conditions such as ALS (48,49).

Along with their effects on mitochondrial ATP production and calcium mobilization, sigma-1 receptors may also influence the expression of anti- and pro-apoptotic signals that target the mitochondria. Sigma-1 receptor activity positively regulates Bcl-2 expression, possibly through nuclear factor kappa B (NF- $\kappa$ B) and/or extracellular signal-regulated kinase (ERK) pathways (42,50). In CHO cells, the overexpression of sigma-1 receptors increases Bcl-2 mRNA transcript and protein levels, while knockdown decreases Bcl-2 mRNA and protein and potentiates hydrogen peroxide-induced apoptosis (50). Decreased Bcl-2 levels are also seen in retinal neurons from sigma-1 receptor null mice (42). Additionally, pharmacological activation of sigma receptors with the sigma agonists PPBP and afobazole protected cells against Bcl-2 decreases and apoptosis induced by O<sub>2</sub> or glucose deprivation, glutamate, or amyloid beta (A $\beta$ ) in primary cortical neurons (51,52). Since Bcl-2 has also been shown to interact with IP<sub>3</sub>R and enhance their activity (53), this positive regulation of Bcl-2 level may be another mechanism by which sigma-1 activity increases IP<sub>3</sub>R-mediated mitochondrial calcium uptake and ATP production, in addition to the sigma-1 receptor-IP<sub>3</sub>R interaction described above. Activation of sigma-1 receptors may also decrease expression of Bax and apoptosis-associated caspases, further promoting cell survival (52,54).

#### 4.4. Modulation of glial activity

Several recent studies have shown the ability of sigma ligands to ameliorate reactive astrogliosis. For example, the sigma agonist 1,3-di-(2-tolyl)guanidine (DTG) attenuated the increase in GFAP expression that occurs in a rodent stroke model of middle cerebral artery occlusion (MCAO) (55). Similar effects have been shown in a mouse model of ALS, where treatment with the selective sigma-1 agonist PRE084 decreased GFAP immunoreactivity (56). Cellular studies using cultured astrocytes appear to corroborate these findings, as changes in sigma-1 receptor expression and ligands targeting sigma receptors have been found to modulate the activity

of these cells (57,58). Sigma receptors may also modulate the Janus kinase 2 (JAK2)/STAT3 signaling pathway to inhibit astrocyte activation (59).

In addition to mitigating reactive astrogliosis, sigma ligands have been shown to modulate microglial activity in animal models of Parkinson's disease and ALS (11,56,60). Sigma ligands may affect M1 and/or M2 microglial responses, though most studies to date have focused more on the amelioration of the M1 type. In primary cultures of microglia, the sigma agonists DTG and afobazole suppressed microglial activation and migration as well as the release of inflammatory cytokines in response to microglial activators such as ATP, uridine triphosphate, lipopolysaccharide (LPS), and monocyte chemoattractant protein-1 (MCP-1) (61,62). Conversely, in animals with motor neuron (MN) disease, treatment with the selective sigma-1 agonist PRE084 increased the number of cells positive for the pan-macrophage marker cluster of differentiation 68 (CD68) and of CD206-positive cells, which are associated with neuronal repair (56). Sigma ligands also improved microglial cell survival during and 24 hours after ischemia (61) as well as after toxic exposure with A $\beta$  (63). These data suggest that sigma receptors may elicit neuroprotective and/or neurorestorative effects by maintaining the proper balance of inflammatory and reparative microglial responses. Additional studies are needed to determine the effects mediated by specific sigma receptor subtypes on glial function, as DTG and afobazole bind to both sigma-1 and sigma-2 receptors. Further elucidation of the mechanisms by which sigma-1 receptors modulate glial activity is also warranted.

## 5. Potential therapeutic opportunities

### 5.1. Stroke

A major contributor to cerebral damage following stroke is glutamate-mediated excitotoxicity (10). Sigma-1-preferring and mixed sigma receptor agonists have been shown to decrease glutamate release and block intracellular calcium overload following ischemia in vitro (30,31). Moreover, given their ability to alter NMDA receptor expression and activity (11,32,34,64), sigma-1 receptor agonists could be an appealing strategy for treating stroke.

Numerous studies have shown the acute benefits of sigma agonists in multiple animal models of stroke (Table 1). Of note, in rat models of stroke, decreased infarct volume as well as enhanced neuronal survival have been observed with sigma agonist treatment 24 hours after onset of ischemia (55,65).

Other means by which sigma agonists appear to decrease cerebral damage include attenuation of radical species production and inhibition of neuro-inflammation. Administration of the sigma agonist PPBP has been shown to decrease NO production in a rat model of transient MCAO (40) as well as decrease nitrosative and oxidative stress in a piglet model of neonatal hypoxic-ischemia (38). The decrease in NO production is likely mediated through modulation of NOS activity (38–40). Treatment with sigma agonists following stroke has also been shown to attenuate reactive gliosis (55) as well as increase levels of anti-inflammatory cytokines and reduce pro-inflammatory ones (66).

In addition to neuroprotection following stroke, sigma-1 receptor activation can facilitate neuronal re-growth and functional recovery (38,58,65,66). For example, chronic treatment with SA4503 starting two days after transient MCAO in rats conferred significantly better recovery of sensorimotor function compared with the vehicle group, without affecting infarct size (58). SA4503 also upregulated neurabin and neurexin-1 expression in membrane rafts in peri-infarct regions (58). As neurabin is a protein involved in the formation of neurite outgrowth and neurexin 1 is associated with presynaptic differentiation, this suggests that activation of sigma-1 receptors might stimulate neural regrowth (58). The initiation of treatment two days after stroke in this (58) and another study (65) is the most delayed time point to show beneficial effects of using a sigma ligand.

With success in the preclinical realm, a phase II trial exploring the safety and efficacy of the sigma-1 agonist cutamesine (SA4503) in patients with ischemic stroke has been conducted (67). Sixty subjects were randomized between 48 and 72 hours after stroke to receive cutamesine (1 or 3 mg/d, oral administration) or placebo for 28 days (67). Safety and efficacy were assessed at baseline, at end of treatment (day 28) and at end of follow-up (day 56). Treatment with placebo or cutamesine at both dosages caused no significant difference in the incidence of adverse events, suggesting cutamesine is

**Table 1**  
Summary of protective effects following acute to subacute administration of sigma-1 preferring and mixed sigma agonists in a variety of in vivo stroke models. MCAO, middle cerebral artery occlusion. NOS, nitric oxide synthase. ROS, reactive oxygen species. RNS, reactive nitrogen species.

Animal	Model	Sigma ligand	Time of treatment	Major outcome	Reference
Mouse	Transient MCAO	(+)-Pentazocine	5 min before reperfusion and continued for 24 h	• Reduces infarct size through inhibition of inducible NOS	(39)
Rat	Transient MCAO	PPBP	Infusion following MCAO and continued during 22 h of reperfusion	• Reduces infarct volume in cerebral cortex and striatum	(40)
	Embolic MCAO	PRE084	Injections 3 and 24 h post-MCAO	• Decreases NO production in ischemic and non-ischemic striatum	(66)
	Focal MCAO	Fluvoxamine	6 h before and immediately after ischemic onset, or immediately after ischemia onset and 2 h later	• Reduces infarct volume, neurological deficits, and pro-inflammatory cytokines	(103)
	Permanent MCAO	DTG	Injections at 24, 48, and 72 h post-MCAO	• Decreases stroke volume, and improves sensorimotor dysfunction	(55)
Piglet	Neonatal hypoxic-ischemia	Afobazole	Daily injections until 96 h post-MCAO starting at 6–48 h post-MCAO	• Neuroprotective effects were blocked with the selective sigma-1 receptor antagonist NE-100	(65)
		PPBP	Infusion from 5 min to 6 h post-resuscitation	• Decreases infarct size, improves survival, and enhances grip strength	(38)
Gerbil	Bilateral carotid artery occlusion	(+)-SKF10,047	Infusion from 5 min to 6 h post-resuscitation	• Reduces striatal neuronal damage and ROS/RNS stress	(105)
Cat	Transient Focal Ischemia	PPBP	Infusion 75 min after initiation of ischemia and continued during 4 h of reperfusion	• Modulates neuronal NOS/postsynaptic density-95 coupling	(106)
				• Neuroprotection of hippocampal neurons	(105)
				• Decreases infarct size, improves survival, and enhances grip strength	(65)
				• Reduces striatal neuronal damage and ROS/RNS stress	(38)
				• Modulates neuronal NOS/postsynaptic density-95 coupling	(105)
				• Neuroprotection of hippocampal neurons	(105)
				• Decreases infarct size, improves survival, and enhances grip strength	(65)
				• Reduces striatal neuronal damage and ROS/RNS stress	(38)
				• Modulates neuronal NOS/postsynaptic density-95 coupling	(105)
				• Neuroprotection of hippocampal neurons	(105)
				• Decreases infarct volume and improves somatosensory recovery	(106)



safe and well tolerated (67). No significant effect was observed on the primary efficacy measure (change in National Institutes of Health Stroke Scale, or NIHSS) or modified Rankin Scale and Barthel Index scores (67). Greater improvement ( $P < 0.05$ ) in NIHSS scores among moderately and severely affected patients (baseline NIHSS  $\geq 7$  and  $\geq 10$ , respectively), however, were seen in post-hoc analysis of the 3 mg/d cutamesine group compared to placebo (67). Additional studies may focus on the moderate to severe patient subgroups, higher dosages, and/or longer treatment durations. The potential to treat with cutamesine or other sigma agonists at extended times following the initial injury warrants further investigation, as the only available treatment approved for use in humans is the administration of thrombolytics, which is limited to 6 hours post-stroke due to the risk for hemorrhagic transformation (10).

### 5.2. Parkinson's disease

Sigma-1 receptors have been shown through positron emission tomography to be downregulated in the brains of early stage Parkinson's disease patients (68). Recently, the sigma-1 agonist PRE084 was shown to elicit both histological and behavioral improvements in an animal model of Parkinson's disease (60). Mice with intrastriatal 6-hydroxydopamine lesions were treated daily with PRE084 for 5 weeks, starting on the same day as the lesion induction (60). At the dose of 0.3 mg/kg/day, PRE084 gradually and significantly improved spontaneous forelimb use, along with a modest recovery of dopamine levels and increased dopaminergic fiber densities compared to saline-treated animals (60). PRE084 also upregulated multiple neurotrophic factors, including brain derived neurotrophic factor (BDNF) and glial cell derived neurotrophic factor (GDNF), as well as activated the trophic factor mediators ERK 1 and 2 (ERK1/2) and protein kinase B (Akt) (60). These findings suggest that a restoration of synaptic connectivity may contribute to functional recovery in Parkinson's disease (60). Of note, neuro-inflammation is a significant contributor in the pathophysiology of Parkinson's disease, and treatment with PRE084 also attenuated M1 microglial responses induced by 6-hydroxydopamine lesions (60).

Along with alleviating neuro-inflammation, sigma-1 receptors may also attenuate dopamine-induced toxicity in Parkinson's disease (35). Endogenous dopamine can undergo both enzymatic and auto-oxidation, generating ROS and causing degenerative damage to dopaminergic neurons. Mori and colleagues showed that exposing CHO cells to dopamine at a physiologically relevant concentration (10  $\mu$ M) increased intracellular ROS in wildtype cells and potentiated the elevated basal levels of ROS in sigma-1 receptor knockdown cells (35). Dopamine, however, caused apoptosis only in the latter cells (35). Moreover, the apoptosis seen by the dopamine/sigma-1 knockdown combination was blocked by Bcl-2 overexpression (35). Since dopamine also potentiated NF- $\kappa$ B activation and Bcl-2 protein downregulation in sigma-1 receptor knockdown cells (35), these results suggest that the sigma-1 receptor-NF- $\kappa$ B-Bcl-2 pathway plays a crucial role against dopamine-induced apoptosis (35). Dopamine at 10  $\mu$ M was also shown to increase sigma-1 receptor expression through oxidative stress-related mechanisms (35). These in vitro data suggest that sigma-1 receptors are one of the endogenous substrates that counteract the dopamine cytotoxicity that would otherwise cause apoptosis. Future work in dopaminergic neurons is needed to validate the sigma-1 receptor response to the damaging effects caused by dopamine and protection against developing Parkinson's disease.

### 5.3. Alzheimer's disease

Decreased labeling of sigma-1 receptors are observed in the brains of patients living with Alzheimer's disease (69) and in

postmortem tissue samples (70,71). However, only Jansen and colleagues excluded patients who had taken sigma-1 receptor-binding drugs, which may confound these results, and should be a consideration for future studies. Because sigma-1 receptor levels do not change considerably in normal aging (72), future studies should also address the etiology of this decrease and its relationship to Alzheimer's pathology.

A genetic polymorphism of the sigma-1 receptor is associated with lower levels of the sigma-1 receptor protein (73, 74), but evidence is inconclusive regarding sigma-1 polymorphisms as risk factors for sporadic Alzheimer's disease (75,76). Certain combinations of sigma-1 receptor and apolipoprotein E (apoE) genotypes may synergistically increase the risk of Alzheimer's disease (73), but it may be that the influence apoE polymorphisms outweigh sigma-1 receptor genotype in determining sporadic Alzheimer's disease risk (77).

With its wide range of activities, induction or activation of sigma-1 receptors could improve clinical symptoms of Alzheimer's disease and protect against associated neuropathologic changes. Indeed, a variety of sigma agonists protect against  $A\beta_{25-35}$ -induced toxicity in cultured neurons (52,78) and prevent memory deficits when  $A\beta_{25-35}$  is injected intracerebroventricularly in mice (79–81). The importance of sigma-1 receptors in conferring a therapeutic benefit is supported by the ability of sigma-1 receptor antagonists to block the anti-amnesic effects of the agonists (79–81).

The mechanisms of sigma-1 receptor-mediated neuroprotective and anti-amnesic effects are not fully understood, but may include regulation of calcium (52), modulation of Bcl-2 and caspase levels (52,79), and attenuation of oxidative stress (79,80). Sigma-1 receptor activation may also limit the propagation of downstream pathological cascades, because the mixed sigma-1 receptor and muscarinic agonist ANAVEX 2-73 prevented tau hyperphosphorylation and  $A\beta_{1-42}$  production in  $A\beta_{25-35}$ -treated mice by altering glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) activity (82). Additionally, sigma agonists may have anti-inflammatory effects; afobazole, for instance, reduced microglial activation while concomitantly decreasing Bax and caspase-3 levels and increasing survival in cultured cells exposed to  $A\beta_{25-35}$  (63).

While preclinical evidence suggests that sigma-1 receptor agonists may be useful in treating Alzheimer's disease, no selective sigma-1 agonist is currently available for clinical use. Two currently approved drugs, donepezil and memantine, both act on sigma-1 receptors in addition to their other primary pharmacological targets, but whether any of their therapeutic effects are mediated by sigma-1 receptor activity has not been determined.

### 5.4. Retinal degeneration

Glaucoma, diabetic retinopathy, age-related macular degeneration, and retinitis pigmentosa, despite their differing etiologies, are all characterized by the progressive loss of retinal neurons that lead to eventual blindness. Retinal degeneration can also occur secondary to other neurodegenerative conditions such as stroke and Alzheimer's disease. Sigma-1 receptors have been detected in a variety of cell types in the eye using RT-PCR and immunoblotting, with supporting immunohistochemical data from retinal ganglion cells (RGCs), inner segments of photoreceptors, and retinal pigment epithelium (RPE) cells (83,84). Similar to the other neurodegenerative conditions reviewed here, common mechanistic defects in retinal degeneration include increased inflammation, oxidative stress, and activation of apoptotic pathways (85). Glial cells also play an important role (85), and are amenable to intervention by sigma-1 receptor agonists.

Numerous studies have demonstrated that sigma-1 receptor agonists can mitigate apoptosis of RGCs in a variety of clinically

relevant in vitro and in vivo models. The selective sigma-1 receptor agonist (+)-pentazocine has been most commonly used for these studies, where it attenuates excitotoxic cell death induced by glutamate or homocysteine (86,87) as well as apoptosis resulting from oxidative stress (43) in primary RGCs and RGC-5 cells. In addition to (+)-pentazocine, other sigma-1 receptor agonists such as (+)-SKF10,047 and SA4503 can also confer retinal cell protection (54). Consistent with the specific involvement of sigma-1 receptors in these processes, sigma-1 antagonists such as NE-100 and BD1047 attenuated the protective effects of the agonists (54,88). The mechanisms by which agonists such as (+)-pentazocine can attenuate apoptosis in these in vitro models appear to be through modulation of a number of molecular and pathway targets, including intracellular calcium, Bax levels, caspase-3 and caspase-9 cleavage, FasL and TRAIL expression, and ER stress response proteins (PERK, ATF4, ATF6, IRE1, CHOP) (43,54). Additionally, sigma-1 phosphorylation has been shown to increase following xanthine:xanthine oxidase (X:XO)-induced ROS production, and is attenuated by (+)-pentazocine binding (43). This indicates that phosphorylation may diminish sigma-1 activity (43), and further studies are needed to understand the effect of phosphorylation in altering sigma-1 receptor activity.

The potential therapeutic relevance of the in vitro observations described above is supported by in vivo models of diabetic retinal degeneration. (+)-Pentazocine can attenuate retinal cell apoptosis in a streptozotocin (STZ)-induced diabetic mouse model as well as in spontaneous diabetic *Ins2<sup>Akita/+</sup>* mice (89). In the latter model, intraperitoneal (i.p.) administration of (+)-pentazocine (0.5 mg/kg) twice a week for up to 22 weeks beginning at diabetes onset preserved retinal architecture, reduced apoptotic cell death, and maintained radial organization of glia processes in Müller cells and the number of cells in the ganglion cell layer (89). In addition, oxidative damage to protein and lipid targets was suppressed by (+)-pentazocine administration, as shown by decreased elevations of nitrotyrosine and decreased 4-hydroxynonenal levels (89). Blood glucose levels remained high during (+)-pentazocine treatment in these mice, suggesting that the oxidative damage measured in this model was secondary to hyperglycemia. Increased expression of ER stress response genes (PERK, ATF6, IRE1, ATF4, CHOP) was also suppressed by (+)-pentazocine in the *Ins2<sup>Akita/+</sup>* mice (43). A full list of genes that were altered by (+)-pentazocine in these mice is published, and includes genes whose protein products are involved in apoptosis, axon guidance, calcium ion binding, and cell differentiation (43).

Sigma-1 receptor agonists can also mitigate retinal damage resulting from ischemia-reperfusion injury. In these studies, the sigma-1 receptor agonist PRE084 or the sigma-1-active neurosteroid dehydroepiandrosterone sulfate (DHEA-S) were administered intraperitoneally to rats just prior to ischemia and also immediately after reperfusion (90). Pretreatment with the sigma-1 receptor antagonist BD1047 prevented the protective effects of these sigma-1 agonists (90). Similar agonist-antagonist effects have also been reported in a rat model of A $\beta$  retinal toxicity (91). In this study, intravitreal delivery of PRE084 before A $\beta$  injection decreased retinal damage, Bax level elevation, and phosphorylated JNK, all of which were attenuated by BD1047 co-administration (91).

Evidence from sigma-1 receptor knockout mice supports the relevance of this subtype to retinal degeneration pathways. In sigma-1 knockout mice, retinal development appears normal, with measurable deficits observed only with advanced age, suggesting that the functional consequences of this protein manifest primarily under conditions of stress or accumulated damage (83,92). Consistent with this idea, a recent study showed that RGC death is accelerated in sigma-1 receptor knockout mice compared to wildtype following optic nerve crush, a model system for triggering apoptotic

responses similar to those seen in glaucoma (83). More extensive characterization has also been performed in sigma-1 receptor knockout mice with STZ-induced diabetes. Similar to the ocular crush model, STZ treatment accelerated retinal damage in sigma-1 receptor knockout mice; diabetic sigma-1 knockout mice showed fewer RGCs and more caspase-3 positive cells compared to non-diabetic wildtype mice, while sigma-1 knockout alone had no effects (92). Additionally, relative to the other groups tested (non-diabetic knockout, non-diabetic wildtype and diabetic wildtype), diabetic sigma-1 receptor knockout mice showed increased intraocular pressure and deficits in scotopic threshold responses, which are the most sensitive electroretinogram (ERG) responses observable with dim stimuli in the dark-adapted state and reflect RGC health (92). When primary RGCs from wildtype and sigma-1 knockout mice were cultured under X:XO-induced oxidative stress, (+)-pentazocine could not prevent oxidative stress-induced cell death in the sigma-1 knockout group, but conferred protection against cell death in the wildtype group, confirming that the protective effects of (+)-pentazocine were sigma-1 dependent (92). Together, these findings suggest that sigma-1 receptors contribute to tissue maintenance and resistance to stressors or homeostatic imbalance.

In addition to effects on retinal neurons, a recent study also evaluated the effects of (+)-pentazocine on retinal microglia isolated from rat pups. Inflammation was induced using LPS under conditions that did not affect cell viability or sigma-1 receptor expression, but did alter the cell morphology (93). (+)-Pentazocine was able to mitigate LPS-induced formation of NO and intracellular ROS as well as release of pro-inflammatory cytokines, including tumor necrosis factor alpha, IL-10, and MCP-1 (93). (+)-Pentazocine also inhibited LPS-induced ERK and JNK phosphorylation, suggesting it may confer these protective effects through modulation of the mitogen-activated protein kinase (MAPK) signaling pathway, which has been implicated in controlling the expression of these immune-related factors in microglia (93). The sigma-1 receptor antagonist BD1063 prevented the protective effects conferred by (+)-pentazocine (93), confirming the sigma-1 receptor specificity of these effects. These results suggest that the protective effects of sigma-1 receptor agonists in the retina may involve modulation of glial responses in addition to effects on neurons.

One limitation of the studies conducted thus far is that they have all focused on histology and analysis of a limited number of biochemical markers. Functional assessments, such as ERG measurements, were conducted in only one study (92). Moreover, the most relevant in vivo interventions were begun at disease onset and it is unclear whether protective or restorative effects are possible if the interventions had been delayed to a later point in the disease progression.

## 5.5. ALS

ALS is characterized by the progressive loss of MNs in the spinal cord, brainstem and motor cortex causing paralysis and ultimately death. Sigma-1 receptors are enriched in MNs (94), and mutations in the sigma-1 receptor gene have been found in patients with frontotemporal lobar degeneration (FTLD)-ALS and a juvenile form of ALS (48,49). More recently, a significant reduction in the overall levels of the sigma-1 receptor protein has been reported in the lumbar spinal cord of ALS patients (95). In addition, the alpha MNs of ALS patients showed abnormal accumulation of sigma-1 receptors in ER structures and enlarged C terminals (specialized regions of synaptic input within MNs in the spinal cord) (95). These findings suggest that aberrant modifications of sigma-1 receptors may contribute to the pathogenesis of ALS. Attempts to target sigma-1 receptors to treat ALS have yielded promising results using both in vitro and in vivo models.

In a SOD1<sup>G93A</sup> mouse model of ALS, the sigma-1 agonist PRE084 was shown to slow the progression of ALS symptoms and prolong life-span (11). Daily administration of PRE084 (0.25 mg/kg, i.p.) from 8 to 16 weeks of age improved spinal MN function, demonstrated by the preservation of neuromuscular connections and MNs in the spinal cord, maintenance of muscle action potential amplitudes, and improvement of locomotor performance (11). Delayed treatment with PRE084 from 12 weeks of age, which marks the beginning of the classical symptomatic phase in SOD1<sup>G93A</sup> mice and mimics the time point at which patients might start treatment, also improved motor function and MN survival compared to untreated mice (11). Additionally, sigma-1 receptor agonists may be effective in cases of ALS not linked to SOD1 mutations, as chronic treatment with PRE084 (0.25 mg/kg, i.p. three times a week for 8 weeks) has been shown to improve MN survival and locomotor performances in the wobbler mouse, a model of spontaneous MN degeneration (56). In contrast, gene ablation of sigma-1 receptors caused an earlier appearance of motor decline signs and decreased longevity in the SOD1<sup>G93A</sup> mouse model (94).

Although the pathophysiology of ALS remains largely unknown, pathological hallmarks include calcium dysregulation and excitotoxicity, neuro-inflammation, accumulation of misfolded proteins, and degradation of MNs (11), most of which have been shown to be amenable to modulation via sigma-1 receptors. For example, the MN of mice lacking sigma-1 receptors showed increased firing frequency in a SOD1<sup>G93A</sup> mouse model of ALS, suggesting that sigma-1 receptors may act as a brake on MN excitability (94) and decrease excitotoxic cell death. Moreover, knockdown of sigma-1 receptors in MN-like (NSC34) cells destabilized membrane lipid rafts and disturbed ER structural integrity, leading to improper calcium handling at the MAM (95) and implicating sigma-1 receptors in normal regulation of intracellular calcium homeostasis. The altered ER integrity and calcium dysregulation may contribute to a significant induction of the UPR, compromising ubiquitin-proteasome function by increasing the misfolded protein load, and thereby further exacerbating misfolded protein accumulation, all of which were also observed in the sigma-1 receptor knockdown NSC34 cells (95). In addition, sigma-1 receptor accumulation was observed in lumbar alpha MNs of ALS patients and SOD1<sup>G93A</sup> mice, cultured fibroblasts from ALS-8 patients with the P56S-VABP mutation, and in NSC34 cells transfected with the P56S-VABP mutation (95). These accumulations co-localized with VAPB (vesicle-associated membrane protein-associated protein B) in the fibroblasts and NSC34 cells with the P56S-VABP mutation (95). VAPB is another ER protein, in which the P56S point mutation causes severe misfolding of the peptide and leads to formation of cytoplasmic inclusion bodies and familial ALS (95). Since sigma-1 receptors can regulate ERAD (96), this sigma-1 accumulation and co-localization with aberrant protein aggregates may reflect a compensatory, albeit inadequate, effort by sigma-1 receptors to clear misfolded proteins in ALS. Importantly, activation of sigma-1 receptors by PRE084 in P56S-VABP NSC34 cells ameliorated mutant VAPB aggregation and increased the degradation of soluble mutant VAPB without affecting the normal level of the wildtype proteins (95). Taken together, these results suggest targeting sigma-1 receptors with agonists can help ameliorate protein aggregation and inhibit disease progression by enhancing their innate chaperone activity.

With regard to neuro-inflammation, chronic treatment with PRE084 has been shown to reduce immunoreactivity to the microglial marker Iba1 (ionized calcium binding adapter molecular 1) in the lumbar spinal cord of SOD1<sup>G93A</sup> mice (11). Interestingly, in a more recent study using the wobbler mouse model, chronic treatment with PRE084 caused an increase in CD11b expression, another marker for resting and active microglial cells, in the cervical spinal cord (56). Further characterization of the microglial

phenotype involved in this mouse model revealed an increase in the number of cells positive for the pan-macrophage marker CD68 and cells positive for the M2 marker CD206 (56). Moreover, while chronic treatment with PRE084 reduced GFAP immunoreactivity in the wobbler mouse (56), it did not have any effect in the SOD1<sup>G93A</sup> mouse (11). These data suggest that, depending on the existing pathological state, activation of sigma-1 receptors may modulate different inflammatory and reparative glial phenotypes in favor of a pro-regenerative response.

### 5.6. Huntington's disease

Huntington's disease is a genetic disorder characterized by progressive motor and cognitive deficits resulting from selective neuronal loss, particularly in the striatum and cerebral cortex. The disease is caused by an autosomal dominant mutation in the huntingtin gene in which a repetitive CAG region that is normally less than 27 repeats long expands through replication error to 35 or more. CAG encodes the amino acid glutamine, hence the characterization of Huntington's disease as a "polyglutamine" (polyQ) disease. Though polyQ expansion occurs in all cells of an affected patient, the above regions of the CNS are particularly sensitive to the effects of the mutation in ways that remain poorly understood. Known pathological phenotypes include increased oxidative damage, increased activation of astrocytes and microglia in addition to neuronal damage and loss, deficits in intracellular signaling pathways associated with ER stress, and impaired protein folding and trafficking (97,98).

Although no reports of huntingtin interaction with sigma-1 receptors have been reported *in vivo*, a recent study in a neuronal cell line (PC6.3) showed decreased sigma-1 receptor levels in the presence of mutant huntingtin proteins expression (99). In this cell line, decreases in sigma-1 protein expression were observed in cells expressing N-terminal huntingtin fragment proteins with 120 polyQ repeats (120Q-huntingtin) as well as cells expressing the full-length huntingtin protein with 75 repeats (75QFL). In contrast, sigma-1 receptor expression did not differ from controls in cells expressing the 18Q-huntingtin N-terminal fragment protein or a wildtype 17Q-huntingtin (17QFL).

Administration of the sigma-1 agonist PRE084 restored the deficits in sigma-1 receptor protein levels caused by the mutant huntingtin expression in the PC6.3 neuronal Huntington's disease model; there were no changes in sigma-1 mRNA levels, suggesting modulation of post-transcriptional processes to restore expression (99). PRE084 also promoted cell viability, and decreased caspase-3 cleavage and the resulting poly ADP ribose polymerase (PARP) cleavage in mutant huntingtin-expressing cells (99). In addition, it decreased caspase-12 (an ER-resident caspase) cleavage, presumably attenuating ER stress through the activation of sigma-1 receptors (99). Oxidative stress responses may also be attenuated by PRE084, which decreased losses of SOD1, SOD2, thioredoxin 2, Bcl-XL expression and attenuated mutant huntingtin-induced increases in ROS levels (99). This study also demonstrated that PRE084 and overexpression of sigma-1 receptors in control cells enhances calpastatin expression (99), and that PRE084 can restore the down-regulated levels of calpastatin and NF- $\kappa$ B-p65 and prevent the decrease in NF- $\kappa$ B signaling in mutant huntingtin expressing cells (99). Calpastatin is an endogenous inhibitor of calpains, which are proteases that can cleave NF- $\kappa$ B-p65 and reduce the activity of NF- $\kappa$ B (100). Together these data suggest PRE084 elicits neuroprotection by modulating the calpastatin/calpain system, which positively affects NF- $\kappa$ B signaling to upregulate various cellular antioxidants and decrease ROS levels. Future studies are needed to determine whether similar interactions and alterations occur *in vivo*, particularly in clinical populations, as they may be amenable to therapeutic interventions with sigma-1 receptor agonists.

## 6. Future considerations

Sigma receptor ligands confer protective effects against many pathological insults leading to neurodegeneration *in vitro* and *in vivo*. They also confer neuroprotection in different animal models of neurodegenerative disorders. While the majority of the studies discussed here have used sigma-1 receptor-preferring ligands, in some cases such as stroke and A $\beta$ -induced neurotoxicity, agonists with high sigma-1 and sigma-2 binding affinities (particularly DTG and afobazole) have also proven beneficial. The role of the sigma-2 subtype remains understudied and poorly understood due to the paucity of experimental tools; future investigation in this area are needed.

Several recent studies also demonstrate that abnormal modification of sigma-1 receptors may contribute to the pathogenesis of neurodegenerative diseases (Table 2). For example, mutations in the sigma-1 receptor gene are reported in ALS (48,49), and sigma-1 receptors accumulate in abnormal intracellular protein deposits in various neurodegenerative diseases (95,101). Since sigma-1 receptors have chaperone and regulatory roles (4,41,96), this accumulation may reflect a failed adaptive response to clear the inclusions during the course of the various diseases. Moreover, this accumulation may contribute to disease progression by limiting the number of soluble sigma-1 receptors, which can in turn predispose cells to further ER stress and subsequent apoptosis. It is thus likely that sigma-1 receptor dysfunction participates somewhere downstream rather than upstream of the pathologic process, when neurodegeneration has begun but may or may not have manifested in clinical symptoms. Pharmacological activation of sigma-1 receptors has been shown to help induce the clearance of specific protein aggregates and alleviate ER stress exerted by the aggregates (95). Further work is needed to characterize the potential for using sigma-1 receptor ligands to slow the progression of neurodegeneration and/or reverse an existing pathology. Clarifying the cellular mechanisms and molecular targets of sigma-1 receptors in the CNS will also be critical to understanding the physiological roles and pathological alterations of sigma-1 receptors.

The evidence presented here predominately supports the hypothesis that sigma-1 receptor activation confers specific neuro-protective and/or neurorestorative effects. Conversely, disruption of the sigma-1 receptor gene is associated with deleterious effects. In some *in vitro* and *in vivo* models of neurodegeneration, however, the putative sigma-1 receptor antagonist haloperidol has protective effects, including attenuation of glutamate released during *in vitro* ischemia (anoxia without glucose) (31), protection against glutamate-induced cell death in a mouse hippocampal cell line (HT-22) (102), and reduction in infarct volume in a rat model of transient MCAO (102). The protective potency of haloperidol positively correlates with its affinity to sigma-1 receptors when compared to other butyrophenone compounds (102); however, haloperidol has nanomolar affinity to other targets, including dopamine, serotonin, and alpha adrenergic receptors, making it difficult to attribute sigma-1 receptor antagonism as the primary drug mechanism in these models. Moreover, very few other studies have found beneficial effects using more selective sigma-1 receptor antagonists, further weakening the hypothesis that sigma-1 receptor antagonism is a primary mechanism contributing to the neuroprotective effects of haloperidol. Hence, though select compounds with sigma-1 antagonism activity may be beneficial in certain conditions, the bulk of the collected data to date indicate that sigma-1 agonist-based therapeutics are more likely to protect against neurodegeneration than antagonists.

While reported beneficial effects of selective sigma-1 ligands in the treatment of neurodegeneration are numerous in preclinical studies, the U.S. Food and Drug Administration (FDA) has not approved any selective sigma-1 receptor ligands for use in humans. In addition, in the single clinical study that tested a selective sigma-1 ligand for treatment of neurodegeneration, the sigma-1 agonist SA4503 failed to elicit significant functional recovery in the subject population as a whole compared to the placebo group after ischemic stroke (67). It remains unclear why sigma-1 receptor ligands have been ineffective against neurodegenerative diseases in the clinic thus far. Future studies may need to refine the patient inclusion criteria and treatment regimen in order to optimize potential therapeutic effects, particularly with regard to severity of

**Table 2**  
Summary of abnormal modifications of sigma-1 receptors in human samples with neurodegenerative disorders. PET, positron emission tomography. FTLD, frontotemporal lobar degeneration. ER, endoplasmic reticulum. VAPB, vesicle-associated membrane protein.

Disease	Sample	Major findings	Reference
Parkinson's disease (PD)	Brain of live patients	<ul style="list-style-type: none"> <li>[<sup>11</sup>C]SA4503 PET showed reduced density of sigma-1 receptors on the more affected than the less affected side of the anterior putamen</li> <li>No significant difference in sigma-1 receptor density between PD patients and controls</li> </ul>	(68)
Alzheimer's disease (AD)	Brain of live patients	<ul style="list-style-type: none"> <li>[<sup>11</sup>C]SA4503 PET showed reduced density of sigma-1 receptors in the frontal, temporal, and occipital lobes, cerebellum and thalamus of early AD patients</li> </ul>	(69)
	Hippocampal brain tissue	<ul style="list-style-type: none"> <li>Reduced [<sup>3</sup>H]DTG binding in CA1 stratum pyramidal region in hippocampi of AD patients that correlates with loss of pyramidal cells in the same region</li> </ul>	(71)
Amyotrophic lateral sclerosis (ALS)	Cortical brain tissue	<ul style="list-style-type: none"> <li>Decreased sigma-1 receptor levels in cortical brain tissue of AD patients</li> </ul>	(70)
	Brains tissue; lymphocytes	<ul style="list-style-type: none"> <li>Mutations in the 3'-untranslated region of the sigma-1 receptor (<i>SIGMAR1</i>) gene found in three ALS-FTLD/FTLD families</li> <li>Brains of c.672*51G &gt; T carriers showed unique pathology of cytoplasmic inclusions of transactivating response element DNA binding protein (TDP-43) or fused in sarcoma protein (FUS)</li> <li>Postulated that mutations altered the transcript stability and <i>SIGMAR1</i> gene expression which leads to a pathogenic alteration of TDP-43 and FUS</li> </ul>	(49)
	Lymphocytes	<ul style="list-style-type: none"> <li>Missense mutation in exon 2 of the <i>SIGMAR1</i> gene encoding sigma-1 receptors (c.304G &gt; C) of patients with juvenile ALS affects highly conserved amino acid located in the transmembrane domain of the encoded protein</li> <li>In motor neuron-like (NSC-34) cells, transfection with the mutated <i>SIGMAR1</i> caused aberrant subcellular distribution of the protein and reduced resistance to ER stress-induced apoptosis</li> </ul>	(48)
	Lumbar spinal cord; skin fibroblasts with P56S-VAPB mutation (ALS8)	<ul style="list-style-type: none"> <li>Reduced levels of sigma-1 receptor proteins in the lumbar spinal cord</li> <li>Abnormal sigma-1 receptor accumulations in enlarged C-terminals and ER structures in alpha motor neurons</li> <li>Accumulations co-localized with the 20S component of the proteasome in alpha motor neurons and patient's fibroblasts</li> <li>Accumulations also co-localized with mutant VAPB aggregations in the fibroblasts</li> </ul>	(95)

the disease as well as dose and duration of treatment. Moreover, despite affecting multiple mechanisms and neural cell types that contribute to neurodegeneration, sigma-1 receptor activation as a stand-alone treatment may not be sufficient in a pathophysiological context. To combat the complex and multi-dimensional nature of neurodegenerative diseases, a multi-treatment approach would likely be most beneficial. Many currently marketed psychotropic medications have significant affinity for sigma-1 receptors. Whether or not the therapeutic effects of these medications reflect actions through sigma-1 receptors in humans remains unclear, but preclinical studies have shown that compounds such as fluvoxamine, DHEA-S and donepezil elicit neuroprotective effects in part through activation of sigma-1 receptors, as their effects were attenuated with sigma-1 antagonists (80,90,103). Therefore, the repurposing or development of sigma-1 receptor active drugs, selective or not, and usage of selective sigma-1 ligands as an adjunct treatment, require further investigation as viable therapeutic approaches for treating neurodegenerative diseases.

One highly valuable feature of sigma-1 ligands is their favorable safety profiles, particularly in humans. This may be due to the modulatory action of sigma-1 receptor agonists, which appear to function selectively only under pathological conditions while sparing normal physiological activity, thus limiting adverse side effects. Consistent with this notion, the selective sigma-1 receptor agonist (+)-pentazocine prolonged the association of sigma-1 receptors with IP<sub>3</sub>R3 under ER stress but had no effect under normal conditions (4). In addition, the selective sigma-1 receptor agonist PRE084 improved motor function and restored dopaminergic fibers in a rodent model of Parkinson's disease but had no effects in the sham-lesioned cohort (60). In the stroke study (67) and the few other clinical trials that have tested selective sigma ligands for various CNS-related disorders (104), few major side effects have been reported. The varied efficacy of sigma-1 ligands for their targeted indications and favorable safety profile suggests that further investigation involving clinical populations will be vital for evaluating the neuroprotective potential of sigma-1 receptor ligands for neurodegenerative conditions.

## 7. Conclusion

Neurodegenerative disorders as diverse as stroke, Parkinson's disease, Alzheimer's disease, retinal degeneration, ALS, and Huntington's disease may share common cellular and molecular pathological mechanisms including excitotoxicity, calcium dysregulation, oxidative/nitrosative stress, ER stress and mitochondrial dysfunction. Dysfunction of glial cells may also contribute to neurodegeneration. Sigma-1 receptor ligands have been shown to modulate multiple aspects of these neurodegenerative processes, affecting neurons as well as glia. Although further work is needed to clarify the cellular actions of sigma-1 receptors in order to provide greater insight into their potential therapeutic roles in the broad spectrum of neurodegenerative diseases, findings to date suggest that sigma-1 receptors are promising targets for developing new drugs to understand and to treat neurodegeneration.

## Conflicts of interest

There are no conflicts to disclose.

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