The sigma-1 receptor: roles in neuronal plasticity and disease

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Sigma-1 receptors (Sig-1Rs) have been implicated in many neurological and psychiatric conditions. Sig-1Rs are intracellular chaperones that reside specifically at the endoplasmic reticulum (ER)–mitochondrion interface, referred to as the mitochondrion-associated ER membrane (MAM). Here, Sig-1Rs regulate ER–mitochondrion Ca2+ signaling. In this review, we discuss the current understanding of Sig-1R functions. Based on this, we suggest that the key cellular mechanisms linking Sig-1Rs to neurological disorders involve the translocation of Sig-1Rs from the MAM to other parts of the cell, whereby Sig-1Rs bind and modulate the activities of various ion channels, receptors, or kinases. Thus, Sig-1Rs and their associated ligands may represent new avenues for treating aspects of neurological and psychiatric diseases.

Introduction

The Sig-1R is an ER-resident protein that has been implicated in many diseases, ranging from cocaine or alcohol addiction to the most recently reported familial adult or juvenile amyotrophic lateral sclerosis (ALS) [1–3]. The amino acid sequence of the Sig-1R does not resemble that of any other mammalian proteins. So far, no other members have been found in this class of protein except for a short variant of the Sig-1R that has been recently reported [4]. The so-called ‘sigma-2 receptor’ (Sig-2R) was identified by binding assays in which certain ligands showed slightly different affinities from those at the Sig-1R. However, the Sig-2R has not yet been cloned. The Sig-1R contains two transmembrane regions (Box 1).

Sig-1Rs reside at the specialized ER membrane directly apposing mitochondria, the so-called ‘MAM’ [5,6]. At the MAM, Sig-1Rs have been demonstrated to regulate dendritic spine formation and dendrite arborization [7]. Interestingly, the localization of Sig-1Rs is dynamic in nature. Specially, Sig-1Rs have been shown to translocate from the MAM to other areas of the cell [8,9] where they can interact with a plethora of membrane targets, including voltage-gated ion channels (VGICs), glutamate and GABA ionotropic receptors, the dopamine (DA) D1 receptor (D1R), muscarinic and nicotinic acetylcholine receptors, neurotrophic tyrosine kinase receptor type 2 (TrkB), and intracellular targets, such as kinases (e.g., Src kinase) and inositol triphosphate (IP3) receptors [9,10]. For brevity, this review focuses on the interaction between Sig-1Rs and ion channels and receptors known to be relevant in neuronal excitability or synaptic strength, and analyzes how these interactions reveal the role of Sig-1Rs in neuronal functions and dysfunctions.

Mechanistic considerations

Information transmission within the brain involves complex and subtle variations in neuronal activity. In particular, electrical signals in the brain are constantly modulated and are heavily influenced by excitatory (glutamate) and inhibitory (GABA) inputs. These, in turn, are translated into excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs, respectively), which eventually give rise to action potentials (APs). An AP travels from the somato-dendritic compartment along an axon to its presynaptic terminal, where it triggers the release of neurotransmitters. These are molecules that transmit information chemically by binding to their specific postsynaptic receptors on adjacent neurons. Over the past two decades, Sig-Rs (Sig-1R when possible) have been shown to affect each stage of this process, in both the central nervous system (CNS) and peripheral nervous system (PNS) [3,11]. A persistent change in any of these events affects information coding and impacts underlying cognitive or sensory processes, which may result in adaptive or maladaptive neuronal functions [12,13]. VGICs are critical for shaping APs [14]. The voltage-gated sodium (Na+), calcium (Ca2+), and potassium (K+) channels are divided in many subtypes, with each family being composed of subfamilies (for a comprehensive review of these channels, see [14,15]).

The following sections focus on the principal ion channels that shape global neuronal excitability, that is, VGICs (e.g., Na+, Ca2+, and K+) for intrinsic excitability and ligand-gated ion channels (LGICs) (i.e., glutamate and GABA type A receptors (GABAAR) for synaptic excitability.)

Keywords: neuronal excitability; voltage-gated ion channels; glutamate receptors; GABA receptors; mitochondrion-associated ER membrane; binding immunoglobulin protein

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762
Box 1. Sig-Rs: topology and ligand binding sites

The Sig-1R contains two transmembrane domains with a short N terminus and a long C terminus facing the ER lumen (Figure I). In Figure I, the long C terminus of Sig-1R is shown as attaching to the ER membrane. However, a study has suggested that the hydrophobic region at the C terminus associates with either one or both of the transmembrane domains [69]. The chaperone activity of the Sig-1R occurs at its long C terminus from amino acid 116 to amino acid 223. Sig-1Rs exist not only in the brain, but also in the peripheral organs, including lung, kidney, liver, pancreas, spleen, adrenal gland [20], and heart [70]. The function of Sig-1Rs in those organs has not been systematically explored except in the heart, where Sig-1Rs are known to play an important role in cardioprotection [70].

The second Sig-R subtype, Sig-2R, was identified by binding studies that showed that certain common ligands exhibited different affinities at this binding site from those exhibited at Sig-1Rs. Thus, the site was termed 'Sig-2R'. Although the Sig-2R has yet to be cloned, a complex of progesterone receptor membrane component 1 was purported to contain the Sig-2R in a photoaffinity labeling study [71]. Sig-2Rs also exist in the CNS.

There are several selective ligands for Sig-1R and Sig-2R [72], as exemplified in the following list (ordered by their relative affinities).

| Sig-1R: haloperidol > (+)pentazocine > DTG > progesterone > fluvoxamine > dextromethorphan > cocaine > N,N-dimethyltryptamine, Sig-2R: DTG > (-)pentazocine > haloperidol > progesterone > dextromethorphan. |

VGICs
Voltage-gated Ca\(^{2+}\) channels
Calcium is probably the ion that controls most neuronal functions, both directly and indirectly. For example, calcium channels control the flux of calcium from extracellular to intracellular compartments, and this may regulate neurotransmitter release at the synaptic level. Calcium can also act as a second messenger to trigger specific intracellular signaling pathways. Overall, whereas Na\(^{+}\) and K\(^{+}\) channels are involved in processes requiring fast transduction signal, calcium plays a role in both fast synaptic transmission and slow changes in neuronal function through its action on intracellular signaling pathways [16,17].

Through a variety of ways, Sig-Rs strongly modulate the intracellular calcium concentration in both neuronal and non-neuronal cells (reviewed in [3,9,11]). However, it is not clear whether this calcium regulation is mediated by Sig-1R, Sig-2R, or both. By contrast, most of these effects seem to be mediated through indirect pathways.

To date, few studies have demonstrated that the direct action of Sig-1Rs on Ca\(^{2+}\) channels can occur in the nervous system (Table 1 and Figure 1a). For example, in cultured retinal ganglion cells, the Sig-1R agonist (+)-SKF10047 directly inhibited Ca\(^{2+}\) currents, an effect that was prevented by the Sig-1R antagonist BD 1047. Direct association between the Sig-1R and L-type Ca\(^{2+}\) channel was supported by co-immunoprecipitation [18].

In another study, activation of Sig-1Rs by the endogenous neuroactive steroid pregnenolone sulfate (PREGS) facilitated an NMDA receptor (NMDAR)-independent form of long-term potentiation (LTP) in acute slices from the CA1 region of the hippocampus [19]. However, the Sig-1R antagonist BD 1047 prevented PREGS facilitation of L-type Ca\(^{2+}\) channel-dependent LTP and mimicked the effect of nimodipine, a specific L-type Ca\(^{2+}\) channel blocker [19]. Although it is unclear whether Sig-1Rs act directly via L-type Ca\(^{2+}\) channels in this model to induce this form of LTP, data suggest that current flow is facilitated, which is in contrast with Sig-1R-induced inhibition of L-type Ca\(^{2+}\) channel observed in retinal ganglion cells [18] (Figure 2a).

For brevity, this section focuses on Ca\(^{2+}\) currents mediated by voltage-gated Ca\(^{2+}\) channels; however, through physical interactions, the Sig-1R can also regulate Ca\(^{2+}\) influx through non-voltage-gated Ca\(^{2+}\)-permeable channels. This includes regulation of IP3 receptors (reviewed in [3,9,11]), resulting in proper ER to mitochondria Ca\(^{2+}\) signaling and thought to regulate mitochondrial bioenergetics [20] (reviewed in [9,11]), and plasma membrane acid-sensing ion channel 1as (ASIC1as) [21,22], which

### Table 1. Summary of direct effects of Sig-1R activation on VGICs

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<thead>
<tr>
<th>Effects on VGICs</th>
<th>Experimental system</th>
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<tr>
<td>Ca(^{2+}) channels*</td>
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<tr>
<td>L-type</td>
<td>Retinal ganglion cells (cell culture)</td>
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<tr>
<td>L-type</td>
<td>CA1 field of hippocampus (brain slices)</td>
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<td>Na(^{+}) channels</td>
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<td>h(_{\text{Na}}) (Nav1.5)</td>
<td>Cardiac myocytes and cell lines (cell culture)</td>
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<td>h(_{\text{Na}})</td>
<td>Intracardiac ganglion neurons (cell culture)</td>
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<td>K(^{+}) channels</td>
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<td>h(_{\text{K}A}(\text{Kv1.4}))</td>
<td>Neurohypophysial terminals (pituitary gland slices)</td>
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<tr>
<td>h(_{\text{K}D})</td>
<td>Parasympathetic intracardiac neurons (cell culture)</td>
<td>[30]</td>
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<tr>
<td>h(_{\text{K}A}(\text{Kv1.4}))</td>
<td>Xenopus oocytes</td>
<td>[31]</td>
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<td>Kv1.3</td>
<td>Xenopus oocytes</td>
<td>[33]</td>
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<td>h(_{\text{K}C})</td>
<td>CA1 field of hippocampus (brain slices)</td>
<td>[46]</td>
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<tr>
<td>h(_{\text{ERG}})</td>
<td>Xenopus oocytes</td>
<td>[32]</td>
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*Abbreviations: h\(_{\text{Na}}\), A-type K\(^{+}\) current; h\(_{\text{ERG}}\), large-conductance Ca\(^{2+}\)-activated K\(^{+}\) current; h\(_{\text{K}D}\), Human ether-a-go-go K\(^{+}\) current; h\(_{\text{K}C}\), delayed outwardly rectifying K\(^{+}\) current; h\(_{\text{K}A}\), voltage-gated Na\(^{+}\) current; h\(_{\text{K}C}\), small conductance Ca\(^{2+}\)-activated K\(^{+}\) current.
**Figure 1.** Examples of direct modulation of Ca\(^{2+}\), Na\(^{+}\), and K\(^{+}\) voltage-gated channels by sigma-1 receptors (Sig-1Rs). (a) (i) The Sig-1R agonist, (+)-SKF10047, inhibited voltage-gated Ca\(^{2+}\) currents in a concentration-dependent manner [18]. Whole-cell Ca\(^{2+}\) currents were recorded using patch clamp on cultured retinal ganglion cells (2 days in vitro) prepared from adult rats. Currents were evoked with a depolarization voltage step from -90 to 0 mV for 55 ms. (ii) The inhibitory effect of (+)-SKF10047 was prevented by the Sig-1R antagonist BD 1047. Treatment with BD 1047 alone had little effect on whole-cell Ca\(^{2+}\) currents. (b) (i) In rat intracardiac ganglion neurons, the Sig-1R antagonist BD 1063 blocked the effects of Sig-R pan-selective agonist 1,3-di(2-toly)guanidine (DTG) on peak Na\(^{+}\) currents [26]. Na\(^{+}\) currents were evoked by voltage steps from -90 to -10 mV in a single neuron in the absence (i.e., control; blue line) and presence of 30 µM DTG (red line), in the presence of 100 nM BD 1063 alone (orange line) and following co-application of both drugs (green line). (ii) DTG-induced inhibition of Na\(^{+}\) currents resulted in delayed action potential (AP) latency and decreased firing rate. APs generated by 400 ms depolarizing current ramps (0–200 nA) from a neuron in the absence (blue line) and presence of DTG (30 µM) (red line) are also shown. The inset shows the first APs generated by the ramps on an expanded time scale. Arrows in the inset indicate the start point of the injected current ramp and points at which latency times were measured. Broken lines represent 0 mV, and the unbroken line above the voltage traces represents the current ramp protocol used. (c) Sig-1Rs bidirectionally modulate K\(^{+}\) currents, which occurs through direct protein–protein interactions and results in either the modulation of K\(^{+}\) channel function [31,33] or the regulation of subunit trafficking [32]. (i) Sig-1R expression stimulated human ether-a-go-go K\(^{+}\) currents (hERG) in Xenopus oocytes [32]. Traces represent families of tail currents recorded in non-injected (NI), hERG cRNA-injected (hERG; 25 pg/oocyte), and hERG + Sig1R cRNA-injected (hERG + Sig1R; 25 pg and 5 ng/oocyte, respectively) oocytes (representative experiment). Tail currents were recorded following pre-pulses from -70 to 40 mV. (ii) Sig-1R coexpression accelerated Kv1.3 inactivation kinetics [33]. Current decay of Kv1.3 alone and coexpressed with the Sig-1R were fitted with a double exponential function (broken lines). A double exponential fit to the current decay showed a voltage-independent fast (A1*exp(-t/τau1)) and a voltage-dependent slow component (A2*exp (-τt/τau2)) of the inactivation time constants. Both time constants were smaller in the co-injected oocytes (open circles) than in those injected with Kv1.3 cRNA only (closed circles). The fast component demonstrated significant difference at all voltages (*P<0.05). Adapted, with permission, from [18] (a), [26] (b), [32] (cii), and [33] (cii).
results in ASIC1a-mediated Ca\(^{2+}\) current inhibition and, consequently, intracellular Ca\(^{2+}\) accumulation [22].

Taken together, although it is clear that the Sig-1R modulates Ca\(^{2+}\) channels, the modulation can be direct or indirect, and facilitatory or inhibitory, depending on the physiological context. This highlights the complexity and diversity of Sig-1R actions and suggests that the Sig-1R exerts opposite effects depending on the brain regions or neuronal types.

**Voltage-gated Na\(^{+}\) channels**

It is only over the past few years that the Sig-1R has been found to inhibit Na\(^{+}\) channels both directly and indirectly, an effect that has been observed in different types of cell and cellular preparations. One of the first studies was performed on prefrontal cortex slice preparations [23] and showed that Sig-1R activation by the neurosteroid dehydroepiandrosterone sulphate (DHEAS) inhibited a persistent Na\(^{+}\) current, an effect that seemed to occur via the activation of the Gi protein and protein kinase C (PKC). However, over the past 2 years, several studies have revealed that the Sig-1R can also modulate the Na\(^{+}\) current through direct interaction [24–26] (Table 1). Although unequivocal evidence is still needed, Sig-1R activation in mouse cardiac myocytes and fibroblast-like cell lines [e.g., COS-7 and human embryonic kidney cells (HEK 293)] by SKF-10047, (+)-pentazocine [25] or N,N-dimethyltryptamine (DMT) [24] (a newly discovered Sig-1R endogenous ligand), inhibited Nav1.5-mediated currents without alterations in channel kinetics or shifts in voltage dependence [25]. This effect was attenuated in cells from Sig-1R knockout mice [25]. Further analysis showed that Sig-1Rs inhibited Nav1.5-mediated currents without requiring ATP or GTP [25]. This suggests that their inhibitory action is not dependent on G proteins and protein kinases, and is likely through the direct association of Sig-1Rs with Nav1.5 channels. Further supporting DMT as an endogenous ligand that can efficiently and quickly activate Sig-1Rs, a recent study performed in mouse motoneurons (MN) revealed subsurface cisternae colocalization of the Sig-1R and indole-N-methyltransferase (INMT), an enzyme that synthesizes DMT [27]. So far, using cultured intracardiac ganglion neurons, only one study has revealed a similar effect of Sig-1R activation on Na\(^{+}\) currents in the nervous system [26] (Figure 1b). The inhibitory effect of the Sig-R agonists 1,3-di(2-toly)guanidine (DTG) and (+)-pentazocine on Na\(^{+}\) currents (an effect blocked by the specific Sig-1R antagonist BD 1063) resulted in delayed AP latency, decreased firing rate, and a shift in
steadystate inactivation of Na+ channels to more negative potentials [26]. Thus, Sig-1R activation can decrease intrinsic excitability not only by decreasing the Na+ current, but also by decreasing Na+ channel availability when needed. Taken together, and in contrast to the bidirectional action of Sig-1R on Ca2+ currents, the effect of Sig-1R on Na+ currents to date has been shown to be inhibitory (Figure 2a).

Voltage-gated K+ channels

Sig-1R inhibition of various K+ channels in both non-neuronal and neuronal cells can involve direct or indirect interaction with channels (reviewed in [3,9]). Similar to the effect of Sig-1R on Na+ channels [26], Sig-1R activation shifts the steady-state inactivation curve toward more negative potentials, which could decrease K+ channel availability upon excitation and delay neuronal repolarization. To exacerbate this effect, the Sig-1R can also delay K+ channels recovery from inactivation. In contrast to its effect on Na+ channels, Sig-1R activation changes the channel kinetics by accelerating current decay, which would lead to increased cell excitability overall.

To date, only a few studies have suggested a physical interaction between the Sig-1R and K+ channels (28–30), reviewed in [3; Table 1], although the evidence is indirect. However, unequivocal evidence for a direct physical interaction between Sig-1Rs and K+ channels is accumulating [31–33] (Figure 1a). A seminal study [31], combining electrophysiological recordings and co-immunoprecipitation data from posterior pituitary and Xenopus oocytes, showed that the Sig-1R inhibits K+ currents formed by Kv1.4 through a direct protein–protein interaction. Interestingly, depending on the absence or presence of ligand, the Sig-1R differentially inhibited the Kv1.4-mediated current, which suggests that the Sig-1R acts as a ligand-regulated auxiliary K+ channel subunit. Given that Kv1.4 is an important contributor to transient A-type K+ currents (Ih), regulation of Kv1.4 currents may have profound effects on dendritic excitability and, therefore, on intrinsic and synaptic plasticity [34]. Two recent studies have provided additional evidence for K+ current regulation involving a direct interaction mechanism [32,33]. The first showed that, in HEK cells, the Sig-1R co-immunoprecipitates with human ether-à-gogo K+ channel (hERG) [32] (Figure 1c), a voltage-dependent K+ channel that regulates cardiac repolarization [35,36]. Interestingly and in contrast to previous studies, when expressed in Xenopus oocytes, the interaction between Sig-1Rs and hERG channels resulted in increased hERG-mediated K+ currents, a mechanism that seemed to occur through regulation of the maturation and stability of channel subunits [32]. The second study, reminiscent of previous findings [31], also showed a ligand-independent modulation of K+ channel function [33] (Figure 1c). Specifically, coexpression in Xenopus oocytes of Sig-1Rs and Kv1.3 channels, a slowly-inactivating outward voltage-gated K+ channel that is predominantly expressed in T lymphocytes [37] and in cerebellum [38], resulted in Kv1.3-mediated current inhibition by accelerating channel inactivation [33].

In summary, the Sig-1R bidirectionally modulates K+ currents, an effect that can occur through direct protein–protein interaction and results in either the modulation of K+ channel function [31,33] or the regulation of subunit trafficking [32] (Figure 2a). These findings suggest that the Sig-1R acts as an auxiliary subunit for voltage-gated K+ channels.

Ligand-gated channels: glutamate and GABA ionotropic receptors

The capability of Sig-R ligands to modulate excitatory transmission in the brain is now well established. Although specific antagonists were not yet available, by combining Sig-R agonists and antagonists with electrophysiological recordings, the first studies showed that the Sig-1R has the potential to modulate NMDAR transmission bidirectionally. This phenomenon has been shown in both the CNS and PNS, including the CA3 field of rat dorsal hippocampus [39–41], cultured neuronal cells from fetal rat telencephalon [42], pyramidal cells of medial prefrontal cortex [43], and spinal cord [44,45]. However, modulation of AMPA receptor (AMPAR) transmission by the Sig-1R seems modest [43]. More specifically, Sig-1R agonists (e.g., (+)-pentazocine, DTG, and SR 31742A) induce a biphasic modulatory action on NMDAR transmission, wherein a low dose is excitatory and a high dose inhibitory, resulting in bidirectional modulation on evoked excitatory transmission [43].

The effect of Sig-R activation on AMPAR-mediated currents has also been studied and has generated mixed results. In contrast to the lack of effect of Sig-1R activation on AMPAR current amplitude [43], a study performed on cultured pyramidal neurons from the CA1 field of hippocampus showed that PREGS increased the frequency of AMPAR-mediated miniature excitatory post synaptic currents (EPSCs), an effect mimicked by Sig-1R agonists [(+)pentazocine and DHEAS] and blocked by Sig-1R antagonists (haloperidol and BD 1063). However, the effect on AMPAR appears to be relatively modest compared with that on NMDARs [43]. Thus, we speculate that changes in excitatory transmission due to the Sig-1R originate primarily from its modulation of NMDAR-mediated currents. Although the cellular basis of this has been studied, the mechanistic picture is far from clear. To date, some data suggest that it is either through a NMDAR phosphorylation mechanism [44,45] and/or indirectly through blockade of the small conductance Ca2+-activated K+ (SK) channel [46]. Indeed, (+)-pentazocine and apamin, a specific Sig-1R agonist and SK channel blocker, respectively, similarly blocked the SK channel, which in turn potentiated NMDAR currents and LTP [46]. Furthermore, the Sig-1R can also regulate AMPAR and NMDAR mRNA and protein expression levels [47]. For example, repeated exposure to E-5842, a putative atypical antipsychotic and preferential Sig-1R ligand, differentially regulated levels of AMPAR and NMDAR subunits (i.e., GluA2 and GluN2A) in a regionally specific manner [47].

Little is known about the relation between the Sig-1R and GABAAR-mediated transmission. However, the few studies published on this topic have all shown a decrease in GABAAR-mediated currents via a Sig-1R-dependent presynaptic mechanism [48,49].

In the sections above, we provided a brief overview of the many effects of the Sig-1R on aspects of neuronal transmission, including generation and conduction of APs, release of neurotransmitters from presynaptic terminals, and
postsynaptic receptors function. The challenge now is to understand how neuronal activity is affected by the combination of various effects of Sig-Rs on such a diverse variety of targets. This complexity has been the main issue limiting understanding of how Sig-1R function affects CNS activity. However, in the next section, we discuss possible explanations for this variety of outcomes.

**How does Sig-1R activity affect overall neuronal excitability?**

A substantial amount of information on the effects of Sig-1R activation has been reported. However, little is known about how these effects modulate intrinsic and synaptic excitability and, thus, how they affect overall neuronal excitability. Because of the various effects of the Sig-1R on individual channels, this task might be difficult to resolve. For example, inhibition of Na+ currents by the Sig-1R should decrease AP firing, whereas inhibition of K+ currents should, by contrast, increase AP firing. To add a supplementary level of complexity, depending on the action site, the Sig-1R can facilitate or inhibit voltage-gated Ca2+ channels. Thus, how would a combination of such opposite effects on VGICs affect intrinsic excitability? It is difficult to infer the changes in firing capacity from changes in individual (or even several) currents [50] and thus, a direct measurement of firing properties is necessary to determine Sig-1R-induced changes in intrinsic excitability. To date, the few studies that measured the effect of Sig-1R activation on basal firing have provided mixed results, showing both excitation and inhibition [23,26,30,51].

Why does Sig-1R activation lead to various consequences on neuronal excitability? Although difficult to assess, experimental conditions are likely to affect functional outcomes of Sig-1R activation. For example, *in vitro* and *in vivo* preparations exhibit different levels of neuronal network integrity, which is absent in cultured neurons, semi-preserved in brain slices, and intact in *in vivo* studies. More importantly, one might consider intra-, membrane and extracellular environments as biological factors that may dictate how a neuron will respond to the Sig-1R stimulation. Specifically:

(i) The Sig-1R is differentially expressed throughout the brain [52–55]. We speculate that a neuronal type that highly expresses Sig-1R will respond differently to Sig-1R activation compared with a neuron that exhibits a low level of expression. Along the same line, a dose-curve analysis revealed that modulation of NMDAR currents by some Sig-R ligands followed a bell-shaped curve [43,56]. Does this indicate that the levels of activation and/or expression of Sig-1Rs influence target functions, or does it reflect the existence of several subtypes of Sig-R and/or simply the off-target effects of ligands when used at high doses.

(ii) The availability of the targets. Subtypes of Na+, Ca2+, and K+ channels are heterogeneously and not proportionately distributed throughout the nervous system. Combined with variability in their subcellular distribution [57,58], these factors are likely to influence the resulting effect of Sig-1R activation on neuronal excitability.

(iii) Along the same lines as the previous point, the intracellular milieu and molecular substrates available at the membrane may also play important roles. Indeed, it appears that protein kinases [e.g., extracellular signal-regulated kinase (ERK)] and Sig-1Rs can mutually regulate one another [59,60]. Protein kinases are strong modulators of VGICs [61]. Therefore, variations in the levels and types of protein kinase, an effect that is directly related to levels of neuronal activity, may lead to differential effects of Sig-1R activation on neuronal excitability. This also likely provides a powerful way for Sig-1R to fine tune neuronal excitability. This is a critical point because one of the characteristics that define neuronal types is the level of tonic activity, which suggests that modulatory effects mediated through the Sig-1R will also be influenced by neuronal and/or neural system basal activity.

(iv) Finally, a factor that is often underestimated is age. Studies of whether Sig-1R levels vary with age have provided mixed results [62,63]; however, the levels of neuroactive steroids (important endogenous ligands for the Sig-1R) decrease with age [10]. As mentioned earlier, the action of neurosteroids on Sig-1Rs can affect both VGICs and LGICs, which indicates that endogenous age-related variations in neurosteroids levels could also contribute to physiological regulation of neuronal activity. This raises concerns when investigating the effects of Sig-1R stimulation on neuronal excitability using preparations from animals of different ages. In other words, exogenous experimental conditions may interact with the endogenous level of neuroactive steroids and lead to different functional outcomes. For example, administration of the same concentration of neuroactive steroids to preparations from animals of different ages may lead to different results when neuronal excitability is investigated, a situation that may also lead to different behavioral effects in the case of *in vivo* administration.

Thus, the net effect of Sig-1R activation on neuronal activity may depend not only on the level of Sig-1R expression, but also on several other factors, including availability, anatomical and subcellular localization of VGICs, and the intracellular milieu, a factor that is influenced by the level of basal neuronal activity and age.

**Cellular neurobiology of Sig-1Rs**

At the MAM, Sig-1Rs reside in the ceramide-enriched microdomains where they appear to bind ceramide [54]. Sig-1Rs at the MAM have also been shown to bind to binding immunoglobulin protein (BiP), another ER chaperone protein that normally prevents the Sig-1R from translocation [8,20]. The exact relation among the binding of the Sig-1R, BiP, and ceramide is unknown at present. As mentioned earlier, Sig-1Rs can regulate dendrite arborization and dendritic spine formation in hippocampal neurons [7]. Whether Sig-1Rs have other functions at the MAM remains to be clarified. However, accumulating evidence indicates that, upon stimulation by Sig-1R agonists such as cocaine, Sig-1Rs can dissociate from BiP and translocate to...
the nucleus, the plasma membrane, and even the extracellular space [8,9] (Figure 2).

**Sig-1Rs in CNS diseases**

Sig-1Rs have been implicated in many CNS disorders, including amnesia, depression, stroke, Alzheimer’s disease, age-related cognitive impairments, neuropathic pain, and cocaine and alcohol addiction (Table 2). Here, we review only neurological studies that clearly implicate the translocation of Sig-1Rs in these disorders. For example, a recent study indicated that the anti-ischemic action of Sig-1Rs in stroke involves the increased translocation of Sig-1Rs to the lipid raft of neurons [64]. Given that lipid rafts are cholesterol-enriched specialized regions of the plasma membrane that serve as platforms for functional proteins [65], this anti-ischemic finding suggests that Sig-1Rs recruit synaptic proteins required for brain repair to the lipid rafts to stimulate brain plasticity (Figure 2b). As another example, the translocation of Sig-1Rs from the ER into the nucleus has been found to correlate with the development of frontotemporal lobar degeneration-motor neuron disease [2].

It is known that cocaine causes the translocation of Sig-1Rs from the ER to other parts of the cell [8]. In primary

### Table 2. Neurological and psychiatric disorders associated with changes in the expression and/or function of Sig-1Rs: clinical and preclinical findings

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Sig-1R expression</th>
<th>Rodent studies</th>
<th>Ligand with therapeutic potential</th>
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<td><strong>Drug addiction</strong></td>
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<td><strong>Psychiatric disorders</strong></td>
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<td>Schizophrenia</td>
<td>Binding site (occipital, front, temporal, cingulate cortices, cerebellum)</td>
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<td>Positive symptoms: antagonist (panamesine, eliprodil, rimcazole, BMY14802, DuP734) [86–90] Negative symptoms and/or cognitive deficits: agonist (fluvoxamine) [86–90]</td>
</tr>
<tr>
<td>Depression</td>
<td></td>
<td></td>
<td>Agonist [86,91–93]</td>
</tr>
<tr>
<td>Anxiety disorders</td>
<td></td>
<td></td>
<td>Agonist/agonist-like ligands (opipramol [94], afobazole [95])</td>
</tr>
<tr>
<td><strong>Neurodegenerative disorders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke (including neurosurgery)</td>
<td>binding sites (frontal, occipital lobes, cerebellum, thalamus, hippocampus)</td>
<td></td>
<td>Agonist (cutamesine [64], BMY14503, DM4 [96])</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>(polymorphism: TT-241-240TT)</td>
<td></td>
<td>Agonist</td>
</tr>
<tr>
<td>Juvenile ALS</td>
<td>mutation: c.304G&gt;C</td>
<td></td>
<td>Agonist?</td>
</tr>
<tr>
<td>Pseudobulbar effect (e.g., ALS, MS)</td>
<td></td>
<td></td>
<td>Agonist (carbetapentane, DM)</td>
</tr>
<tr>
<td>Frontotemporal lobar degeneration-motor neuron disease</td>
<td></td>
<td></td>
<td>Agonist?</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-associated neurocognitive disorder</td>
<td></td>
<td></td>
<td>Agonist (DM)</td>
</tr>
<tr>
<td>Pain</td>
<td></td>
<td></td>
<td>Agonist (DM) or antagonist</td>
</tr>
</tbody>
</table>

*Abbreviations: ALS, amyotrophic lateral sclerosis; DHEA, dehydroepiandrosterone; DM, dextromethorphan; MS, multiple sclerosis; NAc, nucleus accumbens; PRE-084, 2-(4-morpholino) ethyl 1-phenylcyclohexane-1-carboxylate.

*Post-mortem study.

*Conflicting results.

*The efficacy of fluvoxamine against psychotic depression needs further verification.

*This mutation did not affect the level of Sig-1Rs but relocated Sig-1Rs to detergent-resistant membranes of lower density. Inasmuch as Sig-1R agonists are well known to be neuroprotective, it is speculated that the agonist serves as a therapeutic agent.

*Whether this clinical effect of DM involves Sig-1Rs is not yet determined.

*Some studies suggested that Sig-1R antagonists do not have antipsychotic action.
human brain microvascular endothelial cells, it was shown that cocaine ‘hijacks’ Sig-1Rs from the ER to the plasma membrane lipid rafts and causes the transmigration of microglia across the blood–brain barrier (BBB). This results in the enhancement of neuroinflammation associated with HIV infection and the exacerbation of HIV-associated neurological dementia [66,67].

Cocaine binding to Sig-1Rs may have a bearing on the development of cocaine addiction. For example, cocaine causes a dissociation of the Sig-1R from its binding partner at the ER, BiP, and thus allows Sig-1Rs to translocate and interact with other functional proteins that may play a role in cocaine addiction [9,20]. For example, in rat brains and in cell lines, cocaine was reported to trigger translocation of Sig-1Rs from the ER to the plasma membrane to interact with D1Rs, which are well known to be important for cocaine addiction [68]. Thus, inasmuch as Sig-1Rs are intracellular ER proteins, cocaine may exert its effects both intra- and extracellularly (Figure 2). The extracellular action of cocaine leads to the increase of DA at the synapse by the blockade of dopamine transporters.

Concluding remarks
In summary, the Sig-1R, through various means and diverse targets, is capable of affecting each stage of neuronal transmission. This may explain why the Sig-1R is associated with many brain functions and neurological disorders. A clear, region-specific understanding of how Sig-1Rs can regulate neuronal activity through the modulation of VGICs and glutamate and/or GABA transmission will provide information not only on how Sig-1Rs participate in shaping neuronal activity, but also on how its disruption can lead to symptoms observed in brain disorders. In this regard, the clarification of many outstanding questions on the fundamental properties of Sig-1Rs will help advance understanding of the role of Sig-1R dysfunction in these disorders (Box 2).

Acknowledgments
This work is supported by the Intramural Research Program of NIDA, NIH/DHHS.

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Box 2. Outstanding questions

- How can a two-transmembrane protein, such as Sig-1R, move quickly upon stimulation by agonists? For example, Sig-1Rs are seen in the extracellular space and the nucleus just 10–15 min after cocaine stimulation [8]. It takes a tremendous amount of energy to move proteins along the biological membrane, let alone a two-transmembrane protein. How can the energy barrier be so easily overcome?
- Chaperone proteins are known to be able to accommodate many client proteins and, thus, have a great degree of tolerance in binding clients of different structures [106]. What is the motif or domain of the Sig-1R that enables it to play such a role?
- Why does Sig-1R interact with so many different proteins? Sig-1Rs are also known to bind many different classes of compound, including neurosteroids [20], antidepressants [86], benzomor- phans [3], and cocaine [20]. Why can the binding pocket of Sig-1R accept so many different classes of ligand? What is the biological implication of this?
- One peculiar property of Sig-1Rs is that they can translocate or efflux into the outer space of cells when, for example, stimulated by cocaine [8]. So far, this has only been reported in cell culture systems, and it is still unclear whether this phenomenon is relevant in vivo. Although other chaperone proteins exist outside of the cell [106], most of them do not have transmembrane regions. Thus, how do Sig-1Rs cross the plasma membrane? Is it through exocytosis? Furthermore, how can a two-transmembrane protein, such as Sig-1R, exist outside of the cell? Is it in the form of an exosome? What is the Sig-1R doing in the extracellular space? Is it working as a chaperone protecting the conformation of the motifs of client proteins in the extracellular space? Can Sig-1R interact not only with the intracellular portion of its client proteins, but also with the extracellular domain of the same protein? Can Sig-1Rs serve as retrograde chaperones, that is, are they released from postsynaptic sites to chaperone presynaptic proteins across the synaptic cleft?
- Inasmuch as cocaine can also cause the translocation of Sig-1Rs from the ER into the nucleus [8], do Sig-1Rs mediate aspects of cocaine-induced gene regulation? Further experiments are needed to confirm whether this is the case.
- Because the Sig-1R is a chaperone protein, important questions that need to be addressed are: What is/are the atomic structural changes that occur in Sig-1R when selective agonists or antagonists bind to it? What are the conformers that allow Sig-R1 interaction(s) with its various protein partners?
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