

## Review Article

## S1P-S1PRs Alliance in Neuropathic Pain Processing

Jin-Chao Hou, and Xiang-Ming Fang

## ABSTRACT

**Aim of review:** Neuropathic pain (NPP), a common clinical condition, is characterized by allodynia and hyperalgesia. In the setting of NPP, components in the peripheral and central transmission pathway of pain exhibit tremendous plasticity, facilitating pain signal conduction and giving rise to hypersensitivity. Clarifying the signal path of nociceptive processing and discovering the key molecules involved in NPP may allow improvement of its treatment.

**Method:** This article reviewed the sphingosine-1-phosphate (S1P) and sphingosine-1-phosphate receptors (S1PRs) alliance that initiates and maintains peripheral and central sensitization in NPP, underlying the fundamental contribution and multiple mechanisms of this alliance in synaptic plasticity.

**Recent findings:** In the peripheral nervous system, S1P-S1PRs alliance contributes to the inflammatory chemical milieu formation and ion channel function, which increase the ectopic action potentials of sensory neurons. In addition, S1P-S1PRs alliance affects thermal hyperalgesia through enhancing inward tetrodotoxin-resistant  $I_{Na^+}$  and decreasing total outward  $I_K^+$  on the nociceptors. In the central nervous system, S1P-S1PRs alliance facilitates the neuroinflammatory response and glutamate accumulation at the "first pain synapse", which leads to persistent central hypersensitization. Furthermore, S1P-S1PRs alliance could disequilibrate the extracellular glutamate via reducing the activities of glutamine synthetase (GS) and glutamate transporters (GTs), which are the key players in glutamate metabolism.

**Summary:** Targeting S1P-S1PRs alliance may be an "Achilles' heel" of the "Trojan horse" model of NPP.

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**Citation:** Jin-Chao Hou, Xiang-Ming Fang. S1P-S1PRs alliance in neuropathic pain processing. *J Anesth Perioper Med* 2015; 2: 87-95.

Neuropathic pain (NPP) is defined as "pain arising as a direct consequence of a lesion or disease affecting the somatosensory system" (1). It is characterized by spontaneous ongoing or shooting pain and evoked amplified pain responses after noxious or non-noxious stimulation (2). As a chronic condition, NPP affects millions of people worldwide and its incidence rate is as high as 10% in community-dwelling adults (3). Complications, such as depression and anxiety, are common and suffering (4). Despite increased focuses on NPP from investigators all over the

world, there is still no consensus on the pathogenesis of NPP, which hampers its treatments.

The majority of research suggested that the tremendous plasticity in both the peripheral and central nervous system (PNS/CNS) are involved in NPP processing (5, 6). The persistence of plasticity may result in peripheral and central sensitization, and enhance pain signaling and produce hypersensitivity (5, 7). Although numerous cytokines, chemokines and neurotransmitters have been implicated in this pathophysiological process, accumulated data have im-

licated that sphingosine-1-phosphate and sphingosine-1-phosphate receptors (S1P-S1PRs) alliance may play pivotal roles in initiating and maintaining peripheral and central sensitization, further leading to NPP.

S1P is a bioactive lipid molecule that acts as both an extracellular signaling molecule and an intracellular second messenger (8). The cell-extrinsic S1P executes key functions through S1PRs in the immune, nervous and cardiovascular systems (9, 10). The potential role of S1P-S1PRs alliance in NPP processing is already revealed by the observations that S1P participates in peripheral sensitization and hyperalgesia by directly increasing the excitability of rat sensory neurons at the insult (11-13), at least in part via activation of S1P receptor subtypes 1 (S1P1) (14). Besides its direct role in excitability modulation, S1P is an important mediator of neuro-immune response recently shown *in vitro* to increase the expression levels of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$  and inducible nitric oxide synthase (iNOS) in activated microglia, which are critical signaling molecules in the development of central sensitization (15). This "central sensitization" causes allodynia, hyperalgesia and causalgia. It has been realized that the activated S1P1 has been the potential contributor of the formation of those inflammatory mediators, since conditional nociceptor-specific deletion of S1P1 in mice has remarkably ameliorated S1P or inflammation-induced hypersensitivity (16). In addition, FTY720, a functional antagonist for S1P1, also reduces nociceptive behaviors during NPP through a similar role to gabapentin (a commonly used drug for relieving NPP) (17). These observations prompt us to consider that S1PRs expressed in the nervous system play important roles in NPP processing and S1P-S1PRs alliance may serve as an initiating factor for an explosion of perturbations that culminate in the generation of persistent NPP. We focus here on S1P-S1PRs alliance's neural-specific roles in the peripheral and secondary sensory neurons in the spinal cord, and disclose how these neural activities subsequently initiate and maintain the peripheral and central sensitization, further leading to NPP.

### Metabolism and Distribution of S1P in the Nervous System

S1P is derived from phosphorylation of sphingosine, a backbone component of all sphingolipids, in a reaction catalysed by two isoforms of sphingosine kinase, SphK1 and SphK2. SphKs have distinct and overlapping functions (10). So far, numerous pro-inflammatory cytokines and growth factors could induce rapid activation and translocation of SphK1 from the cytosol to the membrane via mechanisms involving protein phosphorylation, protein-lipid binding, protein-protein interaction and calcium/calmodulin regulation, promoting the formation of S1P (18-23). S1P, in turn, can be partly exported from the cell by an adenosine triphosphate (ATP)-binding cassette (ABC) transporter to activate S1PRs in auto-crine and/or paracrine manners (23-26). This "inside-out signaling" process of S1P is important and typical for many of the S1P-regulated immune cells and neurons (10). Diverse functions consequently exhibited by these cells and neurons are depend on the repertoire of S1PRs expression (27). In contrast, S1P synthesized by SphK2 has a putative nuclear localization signal. In the CNS, cerebellar granule cells and astrocytes have been shown to be the dominant source of S1P, implicating the possible roles of S1P in the CNS (19, 28).

Similar to other signaling molecules, S1P has a short half-life. The levels of S1P are tightly controlled by its rapid degradation. S1P can be dephosphorylated by specific and nonspecific phosphatases back to sphingosine, which can then be reused for ceramide and sphingolipid biosynthesis (10). Alternatively, S1P can be irreversibly cleaved by S1P lyase in the final degradative step of sphingolipid metabolism (Figure 1). Constitutive levels of S1P in the nervous system are low, and this is probably due to the activity of S1P lyase (29). S1P levels in spinal cord, brainstem, cerebellum and cerebral cortex are  $179 \pm 10$  pmol/mg protein,  $132 \pm 14$  pmol/mg protein,  $80.5 \pm 8.0$  pmol/mg protein and  $36.2 \pm 2.1$  pmol/mg protein respectively (20, 21).

### Characteristics of S1PRs in the Nervous System

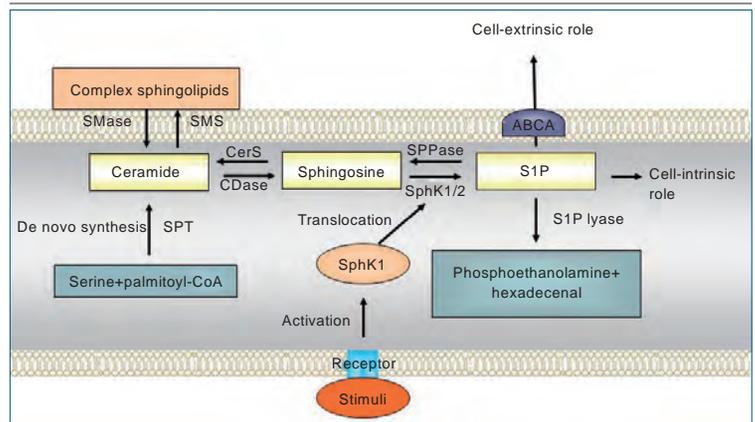
At present, five S1P receptors, S1P1-S1P5 (corre-

sponding to the endothelial differentiation genes EDG1, 5, 3, 6 and 8, respectively) show a preference and high-affinity for S1P (26). The nervous system exhibits high levels of S1PRs expression. The expressions of these receptors are also differentially regulated at key developmental stages. During the development of nervous system, in the rat brain, S1P1 mRNA is detected on embryonic day 15 (E15) in the neuroepithelium and steadily increases until reaching its highest level in adulthood, while S1P2 is highly expressed in migrating cranial neural crest cells and enteric neurons from E11.5 to E12.5 (30). Strikingly, S1P5 is expressed in E7 and E17 mice embryos, but not in E11 or E15 embryos (31). In situ hybridization has further revealed S1P5 to be associated with white matter tracts in the CNS (31, 32). In the adult nervous system, S1P1-3 are widely and region-specifically distributed with selective localization on neurons versus glia (33), while S1P5 is found primarily on oligodendrocytes, and S1P4 is absent in the brain (34-36).

Ligand exposure induces long-lasting receptors internalization from plasma membrane to the endosome. The internalized S1PRs can be recycled to plasma membrane or degraded (37-39), which depends on ligand properties (26, 38, 40). Downstream pathways mediated by S1PRs vary according to corresponding G proteins in the PNS and CNS (Table). S1P1 couples to Gi monogamously, while S1P2 and S1P3 couple to Gi, Gq or G12/13, and S1P4-5 couple to Gi or G12/13 (41). Activation of these G proteins would trigger different downstream cascades, such as phospholipase C activation, intracellular mobilization of calcium signaling and Akt and extracellular signal-regulated kinase (ERK)1/2 phosphorylation (42).

### S1P-S1PRs Alliance Signaling in the Peripheral and Central Sensitization

Following peripheral nerve injury, a series of events occur in primary afferents, unmyelinated (C-fibre) and thinly myelinated (A $\delta$ -fibre) nociceptive afferent fibers, causing peripheral sensitization. Central sensitization can be triggered by the ectopic action potentials as well as spontaneous activity in many types of pe-



**Figure 1. Formation and Transportation of S1P.**

S1P can be rapidly synthesized via the activation of an enzymatic cascade following the stimulation of various plasma-membrane receptors. SphK1/2 catalyzes the formation of S1P through the transfer of a phosphate group from ATP to the primary hydroxyl of sphingosine. S1P can be partly exported from the neural cell by a novel ABC transporter A to activate S1PRs in autocrine and/or paracrine manners. The synthesized S1P is irreversibly cleaved by S1P lyase and reversibly dephosphorylated to sphingosine by specific S1P phosphatases. During the process, bioactive sphingolipid metabolites such as ceramide, sphingosine and S1P are readily interconvertible.

SMase, sphingomyelinase; SMS, sphingomyelin synthase; SPT, serine palmitoyl transferase; CDase, ceramidase; CerS, ceramide synthase; SphK, sphingosine kinase; S1P, sphingosine-1-phosphate; SPPase, sphingosine phosphate phosphatase; ABCA, ATP-binding cassette (ABC) transporter A.

ripherally generated NPP. The central terminals of these damaged afferents exhibit spontaneous firing or alterations in their conduction/neurotransmitter properties, which would excite the second-order nociceptive neurons in the spinal dorsal horn (6). These exert long-term effects on dorsal horn excitability and/or alter the state of activation and plasticity of the CNS, causing central sensitization (Figure 2). The possible involvement of S1P-S1PRs alliance in NPP will be reviewed under the condition of peripheral nerve injury-induced NPP, involving its roles in the peripheral and central sensitization.

### S1P-S1PRs Alliance in Peripheral Sensitization

Peripheral sensitization more commonly results from inflammation-associated changes at the insult (54). S1P-S1PRs alliance is demonstrated to be an initiator of the local inflammatory environment and play important roles in the ion channel functions on the nociceptors.

**Table. Sphingosine-1-Phosphate Receptor Subtype Chromosome Location, the Type of G Protein Coupling, Distribution, Downstream Pathways and Receptor Subtype-Specific Actions in the Nervous System.**

Receptor	Chromosome location	Coupling	Distribution <sup>c</sup>	Downstream pathways	Functions
S1P1 (EDG1)	1p21a	Gi/o	Neuron <sup>a</sup> Astrocyte Oligodendrocyte Microcyte	Adenylyl cyclase inhibition MAPK activation PLC activation Rac activation Calcium mobilization	Neurogenesis (43, 44) Synaptogenesis (45) Glutamate release (46-48) Astrocyte migration Neuroinflammation (49) Hyperalgesia (14)
S1P2 (EDG5)	19p13.2a	Gi/o, Gq, G12/13	Neuron <sup>a</sup> Microcyte	Adenylyl cyclase stimulation MAPK activation PLC activation Rho activation Calcium mobilization	Cytoskeletal reorganization Neurite retraction (50) Excitatory postsynaptic currents inhibition (51) Neuroinflammation (49)
S1P3 (EDG3)	9q22.2a	Gi/o, Gq, G12/13	Neuron <sup>a</sup> Astrocyte	Adenylyl cyclase stimulation MAPK activation PLC activation Rho activation Calcium mobilization	Astrogliosis (52) Glutamate release (46-48)
S1P4 (EDG6)	19q22.1a	Gi/o, G12/13	Dorsal root ganglia	MAPK activation PLC activation	N/A <sup>b</sup>
S1P5 (EDG8)	19p13.2a	Gi/o, G12/13	Oligodendrocyte	Adenylyl cyclase inhibition MAPK inhibition PLC activation Calcium mobilization	Neurite inhibition Myelination, survival (53)

MAPK, mitogen-activated protein kinases.

<sup>a</sup> The central and peripheral neuron; <sup>b</sup> N/A, not available; <sup>c</sup> Predominant distribution of various S1PRs.

*The Inflammatory Chemical Milieu Mediated by S1P-S1PRs Alliance*

SphK1 could be activated by numerous stimuli, including pro-inflammatory cytokines, and promotes the formation of S1P (23). Downstream of binding to its receptors on the nociceptors, S1P increases the level of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-derived superoxide (O<sub>2</sub><sup>-</sup>) and nitric oxide synthase (NOS)-derived nitric oxide (NO). The reaction between O<sub>2</sub><sup>-</sup> and NO forms peroxynitrite (PN), which is a potent mediator of hyperalgesia (55-57). PN favors the in situ production of prostaglandin E2 (PGE2) through activating cyclooxygenase1/2 (COX1/2) and can also activate the mitogen-activated protein kinases (MAPK) pathway to increase the production of inflammatory mediators (IFMs), including TNF-α and IL-1β (58-61). Additionally, PN has potent chemotactic activity (62), and it may promote neutrophil infiltration that perpetuates the inflammatory cycle

(63, 64) (Figure 3). The majority of IFMs in turn regulate S1P metabolism mainly via modulation of the signaling of certain innate receptors on peripheral non-neural cells, such as Toll-like receptor (TLR), TNF receptor (TNFR) and Protease-activated receptor 1 (PAR1) (65, 66). Engagement of TLR, TNFR and PAR1 results in activation of SphK1. Such as, TNF-α, a major effector of innate immunity and inflammation induces a 14-fold increase in SphK1 activity and enhances its membrane affinity through increasing SphK1 phosphorylation specifically on a single serine, Ser-225 (67). The plasma membrane selectivity presumably enhances its interaction with membrane acidic phospholipids, which facilitates the formation of S1P (67, 68). S1P can either bind and activate intracellular targets such as TNF receptor associated factor 2 or be transported to the outside, associating with one or more pro-algesic receptors (S1PRs) on the nociceptors and allowing a broad range of possible downstream tar-

gets on the pathology of NPP (69).

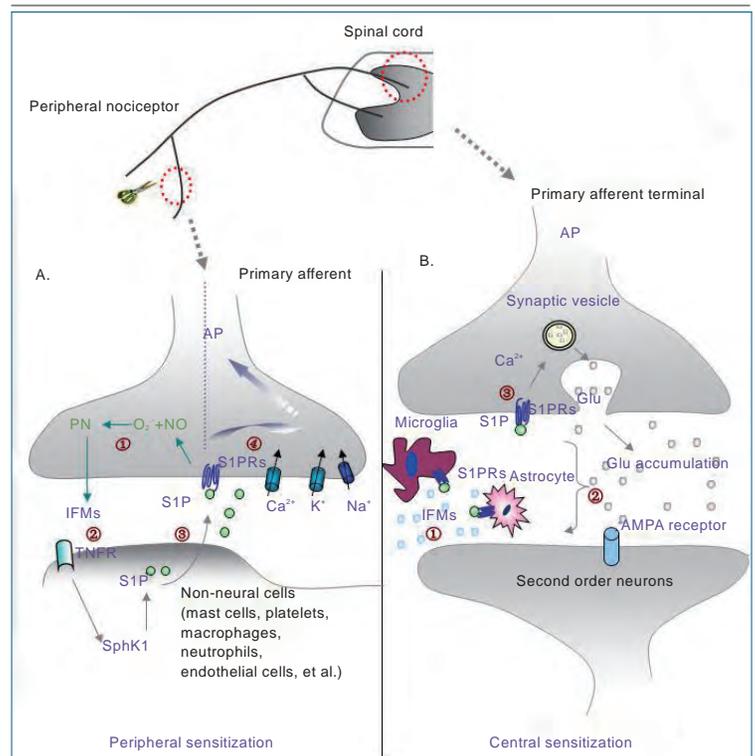
**Ion Channel Function Mediated by S1P-S1PRs Alliance**  
Robust hypersensitivity to heat can develop with inflammation or after injection of S1P. Whole-cell patch clamp measurements manifested that S1P caused a significant dose dependent increase in peak amplitude of heat-evoked excitatory inward currents ( $I_{\text{heat}}$ ) and an approximately  $2^{\circ}\text{C}$  decrease in the threshold activation temperatures of  $I_{\text{heat}}$  (16). While, after the use of G protein blocker, S1P-induced increment in action potentials (APs) was completely abolished (11). S1P-S1PRs alliance affects thermal hyperalgesia via enhancing transient receptor potential cation channel, subfamily V1 (TRPV1) functions and MAPK activity, resulting in enhanced inward tetrodotoxin-resistant sodium current ( $I_{\text{Na}^+}$ ) and calcium current ( $I_{\text{Ca}^{2+}}$ ), and decreased total outward potassium current ( $I_{\text{K}^+}$ ) (Figure 3). So, ion channel function mediated by S1P-S1PRs alliance may provide a supplementary way leading to peripheral sensitization.

### S1P-S1PRs Alliance in Central Sensitization

Central sensitization is a state of hyperexcitability established in the central nervous system, characterized as enhanced processing of nociceptive messages (70). This review focuses on two mechanisms implicated in central sensitization that mediated by S1P-S1PRs alliance: alteration in glial-neuronal interactions and glutamatergic neurotransmission on the spinal cord.

#### Glial-Neuronal Interactions Mediated by S1P-S1PRs Alliance

Glial cells located at the level of the spinal dorsal horn are emerging as possible additional players in the initiation and maintenance of NPP (71, 72). These glial cells interact with neurons closely and exert particular impact on pain transmission under pathophysiological conditions (73). Within hours of peripheral nerve injury, microglia release a series of mediators including pro-inflammatory cytokines and chemokines, which aggravate neuronal central sensitization and nerve injury-mediated persistent pain. In vitro studies show that SphK1 and S1PRs are expressed in the mouse BV2 microglia and suppression of SphK1 results in decreased mRNA ex-



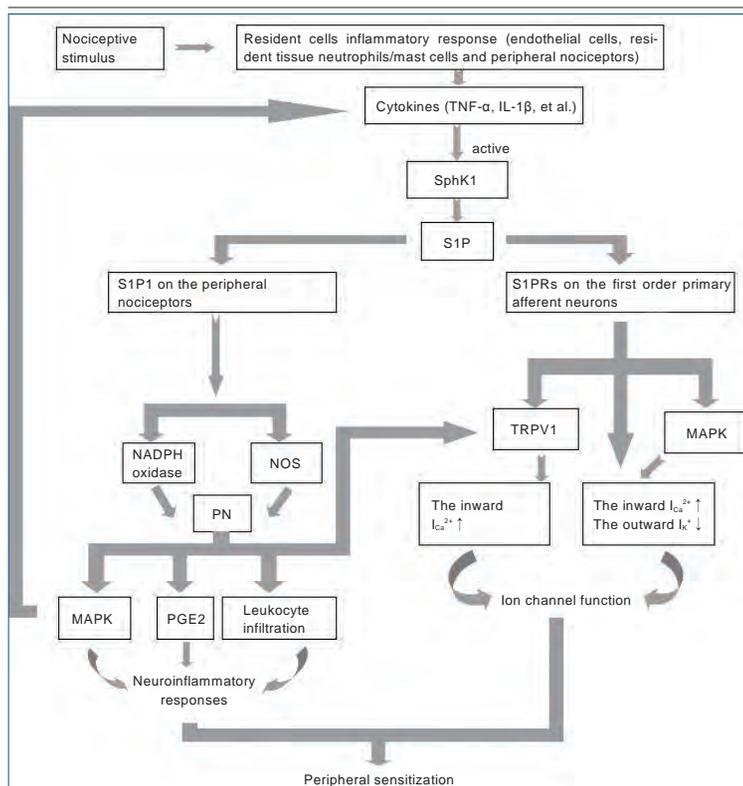
**Figure 2. S1P-S1PRs Alliance in NPP Processing.**

A. Peripheral sensitization evoked by S1P-S1PRs alliance: ① S1P interacts with its receptors on the nociceptor, increasing the level of superoxide ( $\text{O}_2^-$ ) and nitric oxide (NO); ② The reaction between  $\text{O}_2^-$  and NO forms peroxynitrite (PN), which enhances inflammatory mediators (IFMs) release at the site of nerve injury; ③ IFMs in turn increase S1P levels via modulation of the signaling of certain innate receptors on non-neural cells, which sustain a permanent inflammatory milieu; ④ In addition, synthesized S1P interacts with its receptors on the first order primary afferent neurons and affects ion channel functions, producing an enduring APs and peripheral hypersensitivity.

B. Central sensitization evoked by S1P-S1PRs alliance: ① S1P-S1PRs alliance can activate glial cells and enhance the release of IFMs at the spinal level; ② S1PRs expressing on glial cells play an important role in keeping the extracellular glutamate (Glu) homeostasis; ③ S1P-S1PRs alliance could also enhance glutamatergic neurotransmission through increasing intracellular  $\text{Ca}^{2+}$  level. The accumulated Glu at synapses level contributes to the long-term potentiation (LTP) of field potentials.

Depolarization of primary afferent neuron can also cause the activation of S1P1 on the presynapses of sensory neurons from the spinal dorsal horn which mediates another way of Glu accumulation. Those maladaptive processing contributes to synaptic re-plasticity and central sensitization. AMPA receptor,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor; TNFR, TNF- $\alpha$  receptor; APs, action potentials.

pression of TNF- $\alpha$ , IL-1 $\beta$  and iNOS. The addition of exogenous S1P to activated microglia further intensifies their pro-inflammatory responses (74). Microglia could propagate the neuroinflammation by recruiting other microglia and



**Figure 3. Illustration of Proposed Downstream Signal Pathway in Peripheral Sensitization Evoked by S1P-S1PRs Alliance.**

S1P-S1PRs alliance regulates peripheral sensitization in two ways, regulating neuroinflammatory response and enhancing ion channel function.

S1P, sphingosine-1-phosphate; S1PRs, sphingosine-1-phosphate receptors; NOS, nitric oxide synthase; NO, nitric oxide; NADPH, nicotinamide adenine dinucleotide phosphate; MAPK, mitogen-activated protein kinase; TRPV1, transient receptor potential cation channel, subfamily V1; PGE2, prostaglandin E2; PN, peroxynitrite.

eventually activates nearby astrocytes. IL-1β and possibly interleukin-18 (IL-18) released from activated microglia bind to their receptors located on the astrocyte membrane, inducing more pro-inflammatory cytokines secretion to sustain the inflammatory cycle and prolong the pain state. The expression of S1P1 subtype on astrocytes is certified by FTY720, whose anti-NPP role is ineffective in mice with selective deficiency of the S1P1 on astrocytes (17). While over-expression of S1PRs in C6 glioma cells leads to a significant enhancement of high density lipoprotein (HDL)-induced ERK activation and calcium mobilization, which are known to be involved in the development and/or maintenance of pain (28, 76).

The intracellular transduction pathways by

which S1P-S1PRs alliance modulates IFMs from glia has not been well illuminated, but a few possibilities from different fields are proposed based on existing publications. S1P-S1PRs alliance has been shown to activate nuclear factor-κB (NF-κB) and several MAPK including p38 and ERK1/2 (77-80). P38 activation through the transcription factor NF-κB results in increased expression of secreted intestinal myofibroblast (IMFs)/growth factors (e.g., cytokines and brain-derived neurotrophic factor [BDNF]). When released, these mediators would sensitize nociceptive dorsal horn neurons via presynaptic and postsynaptic mechanisms, causing persistent pain hypersensitivity. Collectively, the NF-κB and/or MAPK (p38 kinase)-dependent manner (Figure 4) may contribute to the S1P-S1PRs alliance-mediated release of IFMs from glial cells.

*Glutamatergic Neurotransmission Mediated by S1P-S1PRs Alliance*

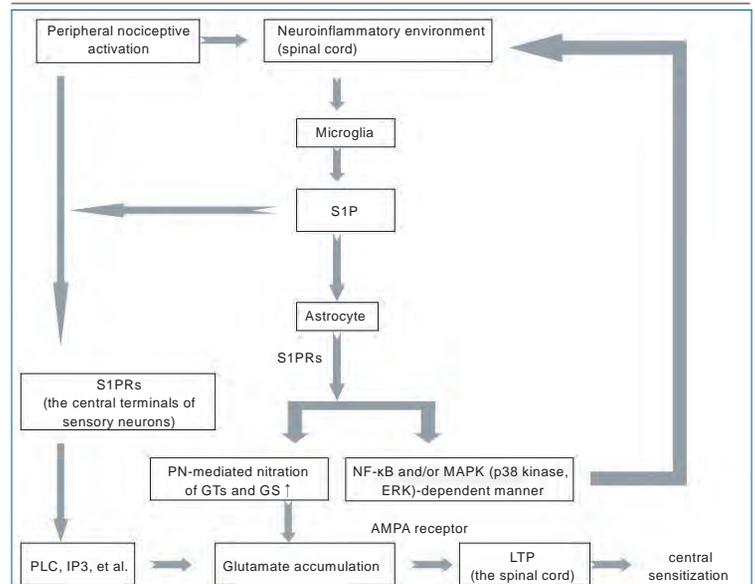
Glutamate is the main excitatory neurotransmitter in nervous system and the spinal cord with its content increases during chronic pain (81). Normally, extracellular glutamate homeostasis is tightly regulated by sodium-dependent high-affinity glutamate transporters (GTs) in the plasma membranes of both neurons and glia (82, 83), while the intracellular metabolic fate of glutamate is regulated by glutamine synthetase (GS) (84, 85). Through feedback regulation, a decrease in the activity of GS can reduce the activity of GTs, implicating the reciprocal interaction between these two pathways. Nitration of these glial cell proteins underlying S1P-S1PRs alliance leads to the decrease activities of GS and GTs and increases the glutamate accumulation (Figure 4). SphK1 inhibitor could reduce the level of glutamate to baseline, thus restoring optimal glutamatergic neurotransmission and normal neuronal activities (86).

Moreover, at the synaptic level, S1P-S1PRs alliance could enhance glutamatergic neurotransmission through increasing intracellular Ca<sup>2+</sup> level. One of the early events triggered by many S1PRs is the elevation of free Ca<sup>2+</sup> level ([Ca<sup>2+</sup>]<sub>i</sub>) in presynaptic environment. Fluctuations in [Ca<sup>2+</sup>]<sub>i</sub> play diverse roles in synaptic functional regulation, especially in excitability modulation (87, 88). SphKs convert sphingosine, which inhibits the store-operated calcium release-activat-

ed calcium current (SOCC)  $I_{CRAC}$ , to S1P which would lower sphingosine levels, leading to the disinhibition of  $I_{CRAC}$  and increase calcium influx (89). S1P itself not only mobilizes calcium through a second messenger role on the endoplasmic reticulum (ER) in the absence of plasma membrane S1PRs (known as IP<sub>3</sub>-independent way) (89, 90), but also promotes calcium mobilization under the activation of S1PRs on the plasma membrane (known as Gi-PLC-IP<sub>3</sub> way) (91). S1P1 and S1P3 collaboratively mediate  $Ca^{2+}$  current engendered by S1P (92). The elevation of intracellular  $Ca^{2+}$  level facilitates the pre-synaptic neurotransmitter release (93). S1P, at a nanomolar level, could elicit glutamate secretion from hippocampal neurons even when the  $Na^+$ -channel is blocked. While, at a picomolar level, S1P potentiates depolarization-evoked glutamate secretion and after S1P3 is silenced, the glutamate secretion is limited (46-48). Reversely, S1P2 may play a role in inhibiting excitatory transmission, as part of S1P2-null mice display significant increases in excitatory postsynaptic currents (51). Therefore, maybe there is an equilibrium state of S1P1-3 in the neuronal excitability regulation. Depolarization can also cause the activation of S1P1 on the pre-synapses of hippocampal neurons and induce rapid and transient formation of intracellular S1P in PC12 cells, which forms a positive feedback circle in the neural excitability regulation (94). Therefore, S1P-S1PRs alliance plays an important role in promoting transmitter release and producing the LTP of field potentials at synapses level (95-97).

### Summary and Perspective

Due to a relatively limited understanding of the pivotal molecular mechanisms in NPP processing, effective treatments for NPP is still lacking. In this review, we reckon that S1P-S1PRs alliance may be the intriguer and the promoter of the "Trojan horse" model of NPP. In the peripheral nervous system, S1P-S1PRs alliance contributes to the inflammatory chemical milieu formation and ion channel function, which increase the ectopic action potentials of sensory neurons. One of the most important roles of this alliance in peripheral is facilitating the PN accumulation at the insult. PN activates the MAPK pathway and fa-



**Figure 4. Illustration of Proposed Downstream Signal Pathway in Central Sensitization Evoked by S1P-S1PRs Alliance.**

Following peripheral nociceptive activation, microglia secrete proinflammatory cytokines and S1P which propagate the neuroinflammation and neuroexcitability by recruiting astrocytes. S1P-S1P1 alliance on the presynapses of sensory neurons from the spinal dorsal horn mediates the release of glutamate. The cumulative glutamate could activate  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in pain transmission pathway directly, which greatly contribute to the LTP and central sensitization.

iNOS, inducible nitric oxide synthetase; GTs, glutamate transporters; GS, glutamine synthetase; PLC, phospholipase C; IP<sub>3</sub>, inositol 1, 4, 5-triphosphate; LTP, the long-term potentiation.

vors the pro-inflammatory cytokine release. In addition, S1P-S1PRs alliance affects thermal hyperalgesia through enhancing inward tetrodotoxin-resistant  $I_{Na^+}$  and decreasing total outward  $I_K^+$  on the nociceptors. In the central nervous system, S1P-S1PRs alliance facilitates the neuroinflammatory response and glutamate accumulation at the "first pain synapse", which leads to persistent central hypersensitization. One of the early events triggered by S1PRs is the elevation of  $[Ca^{2+}]_i$  in presynaptic environment. Fluctuations in  $[Ca^{2+}]_i$  enhance glutamate release and produce the LTP. Furthermore, S1P-S1PRs alliance could disequilibrate the extracellular glutamate via reducing the activities of GS and GTs, which are the key players in glutamate metabolism. So, targeting S1P-S1PRs alliance might be an "Achilles' heel" of the "Trojan horse" model of NPP.

No potential conflict of interests relevant to this review was reported.

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