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Synaptic activity-regulated Wnt signaling in synaptic plasticity, glial function and chronic pain

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Abstract

Wnt signaling pathways are master regulatory networks in a variety of developmental and oncogenic processes. In the nervous system, Wnt signaling pathways are known to regulate neuronal morphogenesis and synaptic differentiation. Aberrant Wnt signaling is implicated in various neurological diseases. Recent studies have revealed that the activity of Wnt signaling pathways is critically controlled by synaptic activity. The activity-regulated Wnt signaling plays an important role in the expression of synaptic plasticity and memory formation. Dysregulation of the activity-regulated Wnt signaling may have a significant impact on the function of the nervous system. In this article, we will review the identified mechanisms by which synaptic activity controls Wnt signaling in neurons and the neurological functions of the activity-regulated Wnt signaling under normal and specific disease conditions. In particular, we will discuss the role of Wnt signaling in the pathogenesis of chronic pain.

Keywords

Wnt; pain; synapse; glia; synaptic plasticity; neuroinflammation; NMDA receptor; mTOR; LTP; HIV-1

Introduction

The Wnt family proteins are secreted signaling molecules that are highly conserved during evolution and play key roles in a variety of developmental processes during embryogenesis (1–3). Dysregulation of Wnt signaling by mutations in Wnt genes or other components of the Wnt signaling pathways is implicated in many disease conditions, especially cancers (4, 5). Wnt proteins bind to their receptors, which include the Frizzled and LRP families, on the cell surface. This binding transduces signals to several cytoplasmic relay components to elicit intracellular signaling cascades (6, 7). There are many members in the Wnt family, including 5 in worms, 7 in flies, 15 in zebrafish, and 19 in mammals. A myriad of canonical Wnt receptors (also known as Frizzled receptors) have been identified, including 3 in worms, 5 in flies, 12 in fish, and 11 in mammals. Several non-conventional receptors such as RYK (related to receptor tyrosine kinase) and ROR2 (receptor tyrosine kinase-like orphan

receptor 2) have also been identified. These Wnt ligands and receptors can elicit complex intracellular signaling cascades to trigger diverse cellular processes. Among the Wnt signaling cascades, the Wnt/ β -catenin pathway that regulates the expression of Wnt target genes is best understood (6).

In the nervous system, Wnts have been found to play important roles in axon path-finding, dendritic development, synaptogenesis, synapse maturation, and plasticity and implicates in brain disorders (8, 9). During the development of neuromuscular junctions (NMJs), Wnts regulate neurotransmitter receptor clustering and the organization of specialized presynaptic and postsynaptic processes (10). At central synapses, Wnt signaling is implicated in the regulation of N-methyl-D-aspartate (NMDA) receptors, gamma-aminobutyric acid (GABA) receptors, and synaptic plasticity (11–14). Dysregulation of Wnt signaling has been implicated in a variety of neurological conditions.

Because of its important roles, the activity of Wnt signaling needs to be tightly regulated. Recent evidence indicates a critical role of synaptic activity in the regulation of Wnt signaling. The synaptic activity-regulated Wnt signaling likely plays a key role in the structural and functional changes of synapses that are induced by neuronal activity or experience. In this paper, we will review the studies on the neuronal activity-mediated regulation of Wnt signaling and its potential involvement in regulation of synaptic plasticity, glial cells, and the pathogenesis of pathological pain.

Synaptic activity-regulated Wnt signaling

After synthesis, Wnt proteins traffic through the ER, the Golgi complex and secretory vesicles to be secreted. During this trafficking process, Wnt protein is post-translationally modified (especially by glycosylation) and packaged into vesicles that eventually release the Wnt cargo by fusing with the plasma membrane. Porcupine/Sprinter plays a key role in glycosylation of Wnt protein (15, 16), and Wntless (Wls)/Evi is central for Wnt packaging into the vesicles (16–18). This secretory pathway is thought to be specifically dedicated to Wnt secretion. After reaching the plasma membrane following Wnt release, Wls is recycled to intracellular compartments via a retromer-mediated endocytic pathway (19–25). The roles of porcupine, Wls and retromer have been extensively reviewed in the literature (26–29) and will not be described in detail here. It is likely that there are other important regulators for Wnt secretion. For instance, recent studies have identified an important role of p24 in the export of Wnt from the endoplasmic reticulum (ER) (30, 31). Nadanaka et al. reported that chondroitin 4-O-sulfotransferase-1 (C4ST-1) regulates Wnt diffusion from Wnt-producing cells (32).

Wnt proteins are expressed in mature neurons. Intriguingly, Wnt3a is largely concentrated at synaptic regions of mature hippocampal neurons (11). Chen et al. observed that synaptic activation in hippocampal slices elicited rapid secretion of Wnt3a (11). This activity-induced Wnt3a release is dependent on NMDA receptors (NMDARs). These findings suggest that Wnt3a secretory vesicles are docked at synaptic regions (especially in the postsynaptic compartment) to be rapidly released after synaptic activation. This regulated secretion provides a mechanism by which synaptic activation is coupled to the activation of Wnt

signaling. The coupling may have significant implications in the regulation of synaptic plasticity and function, as well as the pathogenesis of neurological conditions (see below). In addition to central synapses, activity-regulated secretion was also observed at neuromuscular junctions (NMJs) in *Drosophila* (33). Wnt receptors, especially the Frizzled proteins, are likely located on both the pre- and post-synaptic membrane (11, 34, 35). Thus, the activity-regulated secretion of Wnt proteins may have a biological effect on both the pre- and post-synaptic compartments. Recent studies indicated that Wnts are secreted on exosomes. Gross et al. showed that after secretion, exosomes maintain Wnts on their surface to activate Wnt signaling in target cells (36). This extracellular exosome-mediated vesicular movement provides a mechanism by which secreted Wnts are transported to target cells from the source cells. At *Drosophila* NMJs, Wnt (Wingless) is secreted from the presynaptic terminus in exosome-like vesicles containing Wls, which then transport Wnt to the postsynaptic receiving cells (37).

Wnt3a accumulation at the synaptic region indicates that Wnt secretion at synapses is tightly controlled. Without synaptic activation, the secretion is blocked at a specific step. However,, there is a low level of basal secretion of Wnt that is independent of synaptic activity (our unpublished data from cultured neurons). It appears that the modes of activity-dependent and activity-independent (constitutive) Wnt secretion from neurons are needed to serve the different roles of Wnt signaling in resting and activated neurons. Understanding the mechanisms by which these secretion modes are gated is important but currently very limited. It is expected that Wnt secretion machinery interacts with and is regulated by proteins at synaptic regions. In this context, it is interesting that μ -opioid receptor (MOR) interacts with Wls and inhibits Wnt secretion (38). Stimulation of MOR causes redistribution of the Wls protein in neurons (39).

In addition to regulating Wnt secretion, neuronal activity also plays important roles in controlling the transcription of Wnt genes. Wnt-2 is an activity-dependent CREB-responsive gene, and neuronal activity enhances CREB-dependent transcription of Wnt-2 in neuron cultures. Wnt-2 expression mediates activity-stimulated dendritic arborization (40). The Wnt-mediated activity-regulated dendritic arborization likely involves β -catenin, a key effector protein in the canonical Wnt signaling pathway, and its interaction with cadherin (41). A recent study indicated that NMDAR activity up-regulates transcription of the β -catenin gene (42).

Besides transcription, synaptic activity also seems to stimulate Wnt protein synthesis. Li et al. reported that NMDAR activation induces rapid Wnt5a protein synthesis and secretion (43). Interestingly, this NMDAR activation-stimulated Wnt5a synthesis is not dependent on transcription, and thus is a result of stimulating translation from pre-existing Wnt5a mRNA. They also showed that the NMDAR-regulated Wnt5a translation requires MAPK signaling but not mTOR signaling (43).

Although the activity-regulated transcription and translation of Wnts cited above were based on in vitro studies with primary neuron cultures, this appears to remain true in vivo. For example, stimulation of nociceptors by injection of footpads with capsaicin causes rapid up-regulation of Wnt ligands (e.g. Wnt3a and Wnt5a) and receptors (Frizzled protein and

ROR2), as well as downstream effector proteins of Wnt signaling (e.g. β -catenin) in the spinal cord dorsal horn (44). Wnt signaling proteins are also up-regulated in other pain models (44–46).

Although many details remain to be worked out, the studies summarized above clearly indicate that neuronal activity can modulate Wnt signaling at multiple levels, including secretion, transcription and translation. Given the diverse biological function of Wnts, the activity-regulated Wnt signaling may provide an important molecular mechanism to couple neuronal activity with the structure and function of neural circuitry. It will be important to identify the biological processes modulated by activity-regulated Wnt signaling in the nervous system.

Activity-regulated Wnt signaling in synaptic plasticity

What is the biological function of the secreted Wnts in response to synaptic activation? Accumulating evidence indicates that they are involved in regulating synaptic plasticity. Chen et al. showed that tetanic stimulation, which induces long-term potentiation (LTP, a form of synaptic plasticity), evokes NMDAR-dependent synaptic Wnt3a release, nuclear β -catenin accumulation, and the activation of Wnt target genes (11). These findings suggest that LTP induction by tetanus elicits Wnt secretion and activates the Wnt/ β -catenin pathway. Importantly, inhibition of Wnt signaling impairs LTP expression, whereas stimulation of Wnt signaling facilitates LTP expression. These results provide the initial evidence for a critical role of Wnt signaling in synaptic plasticity. There are multiple potential molecular pathways by which Wnt signaling may contribute to synaptic plasticity. For example, activation of the Wnt/ β -catenin pathway may lead to the transcriptional activation of synaptic plasticity-related genes. Because β -catenin is enriched at synapses and interacts with cadherin (47), the stabilization of β -catenin by Wnt signaling may lead to structural plasticity of the synapse. More recently, Ma et al. reported that synaptic activity can activate mTOR (mammalian target of rapamycin) signaling to facilitate the expression of late-phase LTP (L-LTP), and this mTOR activation is mediated by Wnt signaling (48). Because mTOR plays important roles in regulating protein synthesis that is required for the expression of L-LTP (49), activity-regulated Wnt signaling thus may regulate LTP expression by control of the mTOR-protein synthesis pathway.

One potential way by which Wnt signaling may regulate synaptic plasticity is to modulate synaptic transmission. Avila et al. showed that nanomolar concentrations of purified Wnt3a protein were able to rapidly increase the frequency of miniature excitatory synaptic currents in cultured embryonic rat hippocampal neurons by stimulating fast Ca^{2+} influx and vesicle exocytosis in the presynaptic terminal. This occurs via the low density lipoprotein receptor-related protein 6 (LRP6) coreceptor (50). In another study, small molecule modulators of the Wnt pathway were used to determine their effect on synaptic transmission. The results showed that the small molecule modulators acutely enhance excitatory transmission in adult hippocampal neurons (51).

Later studies identified the specific activity of Wnt signaling in the regulation of synaptic receptors. Wnt-5a can acutely increase synaptic NMDAR currents in rat hippocampal slices

and thus facilitate LTP induction (12, 13). In contrast, Wnt-7a, a ligand for the canonical Wnt pathway, has no effect on NMDAR-mediated synaptic transmission (12). Cuitino et al. reported that Wnt-5a also stimulated surface expression and maintenance of the GABA(A) receptor at the postsynaptic membrane of the hippocampal inhibitory synapses. They proposed that Wnt-5a induced the recycling of functional GABA(A) receptors by activating CaMKII (14).

In addition to modulating synaptic receptors, activity-regulated Wnt signaling may also regulate the structural plasticity of synapses. For example, Wnt3a/Frizzled signaling was proposed to stimulate presynaptic differentiation. Overexpression of Frizzled-1 increased Bassoon scaffolding clusters, indicating the formation of active zones. Treatment with the extracellular cysteine-rich domain (CRD) of Frizzled-1 decreased Bassoon clustering. Furthermore, Wnt-3a also induced functional presynaptic recycling sites, which could be blocked by the CRD of Frizzled-1 (35). Ciani et al. showed that Wnt7a/Dishevelled-1 (Dvl1) signaling enhanced the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs). In addition, Wnt7a was able to increase the density and maturity of dendritic spines. Further, Wnt7a-Dvl1-deficiency caused defects in spine morphogenesis and hippocampal mossy fiber-CA3 synaptic transmission. The authors further demonstrated a role of CaMKII in mediating the effect of Wnt7a (52). Wnt7a was also reported to modulate presynaptic functions in the cerebellum in a retrograde fashion (53).

Farias et al. reported that the Wnt-5a/JNK pathway modulated postsynaptic organization in hippocampal neurons. They found that Wnt-5a induced short-term changes in the clustering of PSD-95 by promoting the recruitment of PSD-95 from a diffuse dendritic cytoplasmic pool to form new PSD-95 clusters in dendritic spines. They also showed that the effect of Wnt-5a required JNK signaling (54). In addition, Wnt5a can also increase dendritic spine density (13).

Although the results outlined above were mainly collected from neurons in culture or from brain slices, activity-regulated Wnt signaling may also regulate synaptic plasticity in vivo in various model systems. Maguschak and Ressler reported that either antagonizing or activating Wnt/ β -catenin signaling with Dkk-1 or Wnt1, respectively, in the adult amygdala during fear learning impaired long-term fear memory consolidation without affecting short-term memory. These data suggest that the Wnt/ β -catenin signaling during consolidation needs to be deliberately controlled during fear memory formation (55). In a separately study, these authors showed that amygdala-specific deletion of β -catenin prevented the conversion of short-term fear learning into long-term memory (56).

In the nervous system of *Caenorhabditis elegans*, Jensen et al. discovered that a Wnt-signaling pathway regulated synaptic strength by controlling the translocation of acetylcholine receptors (AChRs) to synapses. They showed that mutations of CWN-2 (Wnt ligand), LIN-17 (Frizzled), CAM-1 (ROR receptor tyrosine kinase), or the downstream effector DSH-1 (disheveled) caused subsynaptic accumulations of ACR-16/ α 7 AChRs, a synaptic current reduction, and behavioral defects (57). Lim et al. examined the role of Wnt signaling in regulating visual experience-dependent plasticity of receptive fields (RFs) of tectal cells in the developing *Xenopus* optic tectum. They found that repetitive exposure to

unidirectional moving visual stimuli caused varying degrees of shift in the RFs in different regions of the tectum. By acute perfusion of exogenous antagonists and inducible transgene expression, they showed that Wnt secretion from tectal cells are specifically responsible for the enhanced visual stimulation-induced changes in neuronal responses and RFs in the tectum. Based on these findings, the author concluded that Wnt signaling contributes to region-specific plasticity of visual circuit functions (58).

Patterned stimulation of *Drosophila* neuromuscular junctions (NMJs) causes rapid changes to synaptic structure and function. Ataman et al. showed that the activity-induced NMJ plasticity was regulated by a bidirectional Wnt/Wingless signaling pathway. They suggested that activity in response to stimulation elicited Wnt1/Wg release from synaptic boutons, which stimulated plasticity at both the pre- and post-synaptic sides (33).

Activity-regulated Wnt signaling and regulation of glial cells

Neurons intimately interact with glial cells. Thus, rapid secretion of Wnt ligands from neurons due to neuronal activity likely affects the biology of glial cells. Consistent with this idea, recent results revealed a critical role of Wnt5A, a prototypic Wnt ligand that activates the non-canonical Wnt signaling pathway, in regulating the expression of cytokines such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) in neuron/glia co-cultures (59). In primary cultures of microglia, Hallskog et al. showed that Wnt5A induced extensive proinflammatory responses, including increased expression of inducible nitric oxide synthase (NOS), cyclooxygenase-2 (COX2), cytokines, and chemokines, as well as enhanced invasive capacity and proliferation. The author further identified that Wnt5a-induced and G protein-dependent signaling to ERK1/2 is important for the proinflammatory responses (60). The same group showed in another study that Wnt3A, the prototypic Wnt ligand for the canonical Wnt signaling pathway, stimulated the expression of proinflammatory immune response genes in microglia and exacerbated the release of de novo IL-6, IL-12, and TNF- α (61). More recently, the authors investigated the potential anti-inflammatory modulation of lipopolysaccharide (LPS)-activated microglia by Wnt3A and Wnt5A. Interestingly, they showed that while Wnt3A and Wnt5A could up-regulate COX2, a generic pro-inflammatory microglia marker, Wnts were able to inhibit LPS-induced COX2 protein and mRNA expression in a dose-dependent manner. They proposed that Wnts have pro- and anti-inflammatory effects on microglia, depending on the context (62). However, in a different study by another laboratory, Wnt3a was reported not to induce a neurotoxic, pro-inflammatory phenotype in primary microglia. Instead, Wnt3a was found to induce the release of exosomes from cultured microglia, and the exosomes were loaded with proteinaceous cargo (63). While clearly Wnt signaling regulates microglial function, work remains to determine the exact conditions under which Wnt signaling may have different effects. Emerging studies indicate an association of neuroinflammation with various neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease; it will be interesting to determine the potential contribution of Wnt signaling-regulated neuroinflammation to the pathogenesis of these diseases.

Astrocytic processes wrap the synapses, and thus are ideally-positioned to sense activity-induced Wnt that is released from the synaptic region. Although direct evidence for the Wnt

effect on astrocytes is still scarce, observations to support this have accumulated, especially from studies on animal models of chronic pain and spinal cord injury (see below).

Recent mounting evidence suggests that oligodendrocyte differentiation and myelination are intimately controlled by Wnt signaling. Several proteins in the Wnt signaling pathway have been shown to play important roles in regulating oligodendrocyte biology. One study reported that inhibition of Wnt signaling in the embryonic mouse neocortical ventricular zone before the usual onset of oligodendrocyte precursor cell (OPC) production caused early production of OPCs. This finding indicates that Wnt signaling controls the timing of OPC production in the developing telencephalon (64). During spinal cord development, oligodendrocytes are generated from a restricted region of the ventral ventricular zone before migrating to other spinal regions. In the dorsal region, Wnt proteins were identified as inhibitory factors for oligodendrocyte development, and Wnt/ β -catenin signaling prevents the differentiation of OPCs to an immature oligodendrocyte state. Addition of rmFz-8/Fc, a Wnt scavenger, promoted the formation of immature oligodendrocytes in the spinal cord explant culture (65). Another study showed that loss of function of a Wnt receptor, Frizzled-8a, perturbed the proliferation and organization of radial glial cells that give rise to OPCs in the ventral precursor region of the spinal cord. Furthermore, activation of Wnt signaling blocks the differentiation of OPCs and the formation of mature oligodendrocytes. These findings suggest that Frizzled-8a plays a critical role in the specification and maturation of OPCs in the ventral spinal cord (66).

Adenomatous polyposis coli (APC) is a member of the protein destruction complex that negatively regulates the Wnt/ β -catenin pathway. APC is transiently induced in the oligodendroglial lineage during both normal myelination and remyelination in different demyelination murine models. Studies by conditional deletion of APC from the oligodendroglial lineage showed that APC is essential for oligodendrocyte differentiation in a cell-autonomous manner. APC regulates oligodendrocyte differentiation through both β -catenin-independent and β -catenin-dependent mechanisms (67).

AXIN2 is a transcriptional downstream target of the Wnt/ β -catenin signaling pathway, and causes negative feedback to the pathway that promotes β -catenin degradation. AXIN2 is expressed in immature OPCs and is essential for remyelination. The small molecule tankyrase inhibitor, XAV939, can stabilize Axin2 in OPCs and consequently promote their differentiation and myelination after hypoxic and demyelinating injury (68).

TCF7L2 (aka TCF4) is an oligodendrocyte-restricted transcription factor in the Wnt/ β -catenin signaling pathway. Several studies revealed a critical role of TCF7L2 in oligodendrocyte differentiation. In human patients with demyelination diseases, TCF7L2 protein increased in regions undergoing active remyelination. In a mouse demyelination model induced by a dietary toxin, TCF7L2 protein is expressed specifically during the active remyelination phase, mainly in non-dividing OPCs. TCF7L2 can form a protein complex with Olig2, a key transcription factor involved in OPC production and differentiation (69). TCF7L2/TCF4 also functions as a bipartite co-effector of β -catenin to regulate oligodendrocyte differentiation. Targeted disruption of the Tcf7l2 gene in mice causes defects in oligodendrocyte maturation, while expression of a dominant-repressive form of

TCF7L2 promotes precocious oligodendrocyte specification in developing chick neural tube (70). Fancy et al. reported that in rodent demyelination/remyelination models, TCF7L2 in OPCs is specific to lesioned (but not normal) adult white matter. They found that β -catenin signaling is active during oligodendrocyte development and remyelination in vivo. Deregulation of Wnt/ β -catenin signaling in OPCs, by either conditional activation of β -catenin in the oligodendrocyte lineage in vivo or down-regulation of APC in mice, leads to profound delays of both developmental myelination and remyelination. These findings suggest an important role of Wnt/ β -catenin signaling in myelination/remyelination in the mammalian CNS (71).

Wnt signaling in chronic (pathological) pain

The involvement of Wnt signaling in the regulation of pain behaviors is suggested by the expression of Wnt ligands, Frizzled receptors and their downstream effector proteins in the dorsal root ganglion (DRG) sensory neurons and pain processing neurons in the spinal cord dorsal horn (SDH). Immunofluorescent staining reveals that both Wnt3a and Wnt5a are expressed in DRG sensory neurons and in the SDH in mice (44). In fact, these Wnts are predominantly expressed in neurons in these regions. Using a transgenic mouse that expresses the Frizzled 10-LacZ reporter, Hu et al. showed that Frizzled 10 is expressed in the pain neural pathway, including DRG neurons and SDH neurons (72). In addition to the Wnt ligands and their receptor, β -catenin, are expressed in the SDH, with a high level in the layer II (44).

Persistent activation and/or sensitization of pain sensory and transmission neural circuits lead to the manifestation of chronic pain. As discussed above, because the expression and secretion of Wnts are tightly controlled by neuronal activity, one may expect that the expression of Wnts is stimulated in the pain neural pathway by painful stimulation. Indeed, we showed that Wnt3a, Wnt5a and β -catenin are up-regulated in the SDH of multiple pain models, including capsaicin-induced pain, neuropathic pain, and HIV-1 gp120-induced pain (44). These Wnt signaling proteins are also up-regulated in the SDH of a multiple sclerosis mouse model, which develops chronic pain (45). We found that Wnts are rapidly up-regulated in the SDH by administration of capsaicin into mouse footpads or by gp120 intrathecal injection in mouse perispinal regions (44). More recently, up-regulation of Wnt signaling proteins in the SDH was also reported in a cancer bone pain model (46). In addition, we also observed the increase of proteins in Wnt signaling pathways in the SDH of HIV-1 human patients who developed pain syndromes, compared with HIV-1 patients who did not develop chronic pain (73). These findings suggest that Wnt signaling is activated in the pain neural pathway in both animal models and human patients with chronic pain. The activation of Wnt signaling may play an important role in regulating pain behavior. Emerging direct evidence outlined below appears to support this notion.

Many multiple sclerosis (MS) patients develop pathological pain. Mice with experimental autoimmune encephalomyelitis (EAE) are widely used as an MS model. EAE mice develop chronic pain, as manifested by mechanical hyperalgesia and allodynia in forepaws and hindpaws (45). Proteins in the canonical pathway, including Wnt3a and β -catenin are significantly increased in the SDH of the EAE mice. In addition, Wnt5a, a prototypic Wnt

ligand that activates the non-canonical pathway, and its receptor (co-receptor) ROR2 also increase in the SDH of the EAE mice. Importantly, antagonizing Wnt5a with Box5 (a Wnt5a-derived hexapeptide) or inhibiting β -catenin with indomethacin attenuated mechanical allodynia in the EAE mice, indicating a critical role of Wnt signaling in the development of EAE-related chronic pain (45), with the potential caveat of other effects of indomethacin besides inhibiting β -catenin.

Over 50% of HIV-1 infected patients develop chronic pain. As mentioned above, Wnt signaling proteins, including Wnt5a, are up-regulated in the SDH of both the HIV-1 patients who developed pathological pain and the i.t. gp120 mouse model, which exhibits mechanical allodynia. We observed that antagonizing Wnt5a in the spinal cord with either Box5 or anti-Wnt5a antibody impaired the development of mechanical allodynia (unpublished observation). These findings indicate that Wnt5a is important for the expression of chronic pain in this model of HIV-associated pain.

More recently, Zhang et al. investigated the potential role of Wnt signaling in rodent models of neuropathic pain and bone cancer pain. Similar to the findings summarized above, the authors found that nerve injury and bone cancer induced the activation of Wnt/ β -catenin signaling in the SDH, as well as primary sensory neurons. They also observed that spinal inhibition of Wnt signaling pathways inhibited the development and persistence of neuropathic pain (46).

How would Wnt signaling contribute to the development of pathological pain? As discussed above, it is well established that Wnt signaling plays important roles in synaptic plasticity. One potential mechanism is to regulate the synaptic plasticity in the pain-processing neural pathway. Although this is theoretically possible, direct evidence is still lacking.

Emerging evidence indicates that Wnt signaling-regulated neuroinflammation may critically contribute to the pathogenesis of chronic pain. It is generally considered that unresolved persistent neuroinflammation is a key factor that establishes and maintains chronic pain. An activity of Wnt signaling in the regulation of inflammation was initially suggested by work in *Drosophila*, which revealed that WntD is an inhibitor of the toll/NF-kappa B pathway (74). Later, several studies in mammalian systems also suggested a role of Wnt signaling in the regulation of inflammation in peripheral tissues. For example, Wnt5a and Frizzled-5 were found to be drastically up-regulated in synovial tissues from rheumatoid arthritis (RA) patients (75). In another study, Pereira et al. identified a critical role of the Wnt5A/Frizzled-5/CaMKII signaling pathway in macrophage inflammatory activation (76).

In addition to regulating peripheral inflammation, recent findings suggest that Wnt signaling also plays critical roles in neuroinflammation. Using neuron-glia co-cultures, we showed that Wnt5a mediates amyloid- β -elicited proinflammatory cytokine expression (59). In another study, Wnt3a was reported to stimulate the expression of proinflammatory immune response genes in microglia and exacerbate the release of de novo IL-6, IL-12, and tumor necrosis factor α (TNF- α) (61).

More recent work provides strong evidence for a critical role of Wnt signaling in regulating neuroinflammation in vivo. This is indicated by the up-regulation of Wnt signaling proteins

under conditions of CNS neuroinflammation. HIV-1 patients may or may not develop chronic pain conditions; but the patients who have chronic pain often manifest prominent neuroinflammatory phenotypes in the SDH, including the reaction of astroglia and drastic up-regulation of pro-inflammatory cytokines (77). Interestingly, Wnt ligands (e.g., Wnt3a and Wnt5a) and the Wnt/ β -catenin pathway are specifically up-regulated in the SDH of the pain-positive patients (73). This observation suggests a possible association between the up-regulation of Wnt signaling and neuroinflammation in the SDH of human HIV patients. Consistent with this notion, mouse intrathecal injection (i.t.) of HIV-1 gp120, an HIV-1 coat glycoprotein that evokes strong neuroinflammatory responses in the CNS, rapidly up-regulates Wnts and their receptors in the SDH (44). Furthermore, we demonstrated that Wnt5a, which is up-regulated in the mouse SDH after i.t. gp120 administration, is critical for gp120 to induce pro-inflammatory cytokine expression (78). This Wnt5a activity is mediated by the activation of CaMKII and JNK signaling pathways (78). Our further studies revealed that, functionally, the Wnt5a/JNK signaling is important for gp120 to induce mechanical allodynia (Yuan et al., unpublished). Multiple sclerosis (MS) is a prominent disease of CNS inflammation. In the EAE mouse model of MS, Wnt signaling is also up-regulated in the SDH, and antagonizing Wnt5a attenuates mechanical allodynia in this model (45). Consistent with a role of Wnt signaling in chronic pain-related neuroinflammation, Zhang et al. also showed in a recent study that Wnt/ β -catenin signaling is activated in neuropathic pain models and that Wnt signaling activation stimulates cytokine expression (46).

Conclusion

Converging lines of evidence suggest that Wnt signaling in neurons is intimately controlled by synaptic activity. Synaptic activation may enhance Wnt signaling via multiple mechanisms, including stimulation of Wnt secretion and up-regulation of Wnt transcription and translation. Activity-induced Wnt signaling is expected to have a broad range of biological effects in the nervous system. In neurons, Wnt signaling is known to play a critical role in regulating synaptic plasticity, including activity-induced alterations of synaptic transmission and structures. Thus, Wnt signaling is likely involved in experience-stimulated remodeling of functional neural circuits. Wnt secretion evoked by synaptic activity may also induce biological responses from glial cells that are interacting with the stimulated neurons. One potential glial (especial microglial and astroglial) response to secreted Wnts is to express pro-inflammatory phenotypes by changing their morphologies and releasing pro-inflammatory mediators. The pro-inflammatory responses of the reactive glia may in turn modulate the function of the neurons and the plasticity of the related neural circuits. This neuronal activity-evoked and Wnt signaling-mediated bidirectional interaction between neurons and glia may play a key role in the pathogenesis of various neurodegeneration diseases such as chronic pain. A clearer understanding of the mechanism and pathological significance of the Wnt signaling-regulated neuron-glia interaction will provide new perspectives to design novel therapies to intervene in the disease processes.

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Abbreviations

AChRs	acetylcholine receptors
COX2	cyclooxygenase-2
EAE	experimental autoimmune encephalomyelitis
GABA	gamma-aminobutyric acid
IL-1β	interleukin-1 β
JNK	C-Jun N-terminal kinase
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
μ-MOR	opioid receptor
mTOR	mammalian target of rapamycin
NMDA	N-methyl-D-aspartate
NMDAR	NMDA receptor
NMJ	neuromuscular junction
SDH	spinal cord dorsal horn
TNF-α	tumor necrosis factor- α

References

1. Hikasa H, Sokol SY. Wnt Signaling in Vertebrate Axis Specification. Cold Spring Harbor Perspectives in Biology. 2012; 5(1)doi: 10.1101/cshperspect.a007955
2. Harrison-Uy SJ, Pleasure SJ. Wnt Signaling and Forebrain Development. Cold Spring Harbor Perspectives in Biology. 2012; 4(7)
3. Wang J, Sinha T, Wynshaw-Boris A. Wnt Signaling in Mammalian Development: Lessons from Mouse Genetics. Cold Spring Harbor Perspectives in Biology. 2012; 4(5)
4. Polakis P. Wnt Signaling in Cancer. Cold Spring Harbor Perspectives in Biology. 2012; 4(5)doi: 10.1101/cshperspect.a008052
5. Zimmerman ZF, Moon RT, Chien AJ. Targeting Wnt Pathways in Disease. Cold Spring Harbor Perspectives in Biology. 2012; 4(11)
6. MacDonald BT, He X. Frizzled and LRP5/6 Receptors for Wnt/ β -Catenin Signaling. Cold Spring Harbor Perspectives in Biology. 2012; 4(12)
7. van Amerongen R. Alternative Wnt Pathways and Receptors. Cold Spring Harbor Perspectives in Biology. 2012; 4(10)
8. Salinas PC. Wnt Signaling in the Vertebrate Central Nervous System: From Axon Guidance to Synaptic Function. Cold Spring Harbor Perspectives in Biology. 2012; 4(2)
9. Inestrosa NC, Arenas E. Emerging roles of Wnts in the adult nervous system. Nat Rev Neurosci. 2010; 11(2):77–86. [PubMed: 20010950]
10. Koles K, Budnik V. Wnt Signaling in Neuromuscular Junction Development. Cold Spring Harbor Perspectives in Biology. 2012; 4(6)

11. Chen J, Park CS, Tang SJ. Activity-dependent synaptic Wnt release regulates hippocampal long term potentiation. *J Biol Chem.* 2006; 281(17):11910–6. [PubMed: 16501258]
12. Cerpa W, Gambrell A, Inestrosa NC, Barria A. Regulation of NMDA-Receptor Synaptic Transmission by Wnt Signaling. *The Journal of Neuroscience.* 2011; 31(26):9466–9471. [PubMed: 21715611]
13. Varela-Nallar L, Alfaro InE, Serrano FG, Parodi J, Inestrosa NC. Wingless-type family member 5A (Wnt-5a) stimulates synaptic differentiation and function of glutamatergic synapses. *Proceedings of the National Academy of Sciences.* 2010; 107(49):21164–21169.
14. Cuitino L, Godoy JA, Farás GG, Couve As, Bonansco C, Fuenzalida M, et al. Wnt-5a Modulates Recycling of Functional GABAA Receptors on Hippocampal Neurons. *The Journal of Neuroscience.* 2010; 30(25):8411–8420. [PubMed: 20573888]
15. Proffitt KD, Virshup DM. Precise Regulation of Porcupine Activity Is Required for Physiological Wnt Signaling. *Journal of Biological Chemistry.* 2012; 287(41):34167–34178. [PubMed: 22888000]
16. Goodman RM, Thombre S, Firtina Z, Gray D, Betts D, Roebuck J, et al. Sprinter: a novel transmembrane protein required for Wg secretion and signaling. *Development.* 2006; 133(24):4901–4911. [PubMed: 17108000]
17. Bartscherer K, Pelte Ng, Ingelfinger D, Boutros M. Secretion of Wnt Ligands Requires Evi, a Conserved Transmembrane Protein. *Cell.* 2006; 125(3):523. [PubMed: 16678096]
18. Banziger C, Soldini D, Schutt C, Zipperlen P, Hausmann G, Basler K. Wntless, a Conserved Membrane Protein Dedicated to the Secretion of Wnt Proteins from Signaling Cells. *Cell.* 2006; 125(3):509. [PubMed: 16678095]
19. Harterink M, Port F, Lorenowicz MJ, McGough IJ, Silhankova M, Betist MC, et al. A SNX3-dependent retromer pathway mediates retrograde transport of the Wnt sorting receptor Wntless and is required for Wnt secretion. *Nat Cell Biol.* 2011; 13(8):914. [PubMed: 21725319]
20. Eaton S. Retromer Retrieves Wntless. *Developmental Cell.* 2008; 14(1):4. [PubMed: 18194646]
21. Franch-Marro X, Wendler F, Guidato S, Griffith J, Baena-Lopez A, Itasaki N, et al. Wingless secretion requires endosome-to-Golgi retrieval of Wntless/Evi/Sprinter by the retromer complex. *Nat Cell Biol.* 2008; 10(2):170. [PubMed: 18193037]
22. Port F, Kuster M, Herr P, Furger E, Banziger C, Hausmann G, et al. Wingless secretion promotes and requires retromer-dependent cycling of Wntless. *Nat Cell Biol.* 2008; 10(2):178. [PubMed: 18193032]
23. Belenkaya TY, Wu Y, Tang X, Zhou B, Cheng L, Sharma YV, et al. The Retromer Complex Influences Wnt Secretion by Recycling Wntless from Endosomes to the Trans-Golgi Network. *Developmental Cell.* 2008; 14(1):120. [PubMed: 18160348]
24. Yang P-T, Lorenowicz MJ, Silhankova M, Coudreuse DYM, Betist MC, Korswagen HC. Wnt Signaling Requires Retromer-Dependent Recycling of MIG-14/Wntless in Wnt-Producing Cells. *Developmental Cell.* 2008; 14(1):140. [PubMed: 18160347]
25. Pan C-L, Baum PD, Gu M, Jorgensen EM, Clark SG, Garriga G. C. elegans AP-2 and Retromer Control Wnt Signaling by Regulating MIG-14/Wntless Developmental. *Cell.* 2008; 14(1):132.
26. Hausmann G, Banziger C, Basler K. Helping Wingless take flight: how WNT proteins are secreted. *Nat Rev Mol Cell Biol.* 2007; 8(4):331. [PubMed: 17342185]
27. Gross JC, Boutros M. Secretion and extracellular space travel of Wnt proteins. *Current Opinion in Genetics & Development.* 2013 in press.
28. Port F, Basler K. Wnt Trafficking: New Insights into Wnt Maturation, Secretion and Spreading. *Traffic.* 2010; 11(10):1265. [PubMed: 20477987]
29. Lorenowicz MJ, Korswagen HC. Sailing with the Wnt: Charting the Wnt processing and secretion route. *Experimental Cell Research.* 2009; 315(16):2683. [PubMed: 19559695]
30. Port F, Hausmann G, Basler K. A genome-wide RNA interference screen uncovers two p24 proteins as regulators of Wingless secretion. *EMBO Rep.* 2011; 12(11):1144. [PubMed: 21886182]
31. Buechling T, Chaudhary V, Spirohn K, Weiss M, Boutros M. p24 proteins are required for secretion of Wnt ligands. *EMBO Rep.* 2011; 12(12):1265. [PubMed: 22094269]

32. Nadanaka S, Kinouchi H, Taniguchi-Morita K, Tamura J-i, Kitagawa H. Down-regulation of Chondroitin 4-O-Sulfotransferase-1 by Wnt Signaling Triggers Diffusion of Wnt-3a. *Journal of Biological Chemistry*. 2011; 286(6):4199–4208. [PubMed: 21123170]
33. Ataman B, Ashley J, Gorczyca M, Ramachandran P, Fouquet W, Sigrist SJ, et al. Rapid Activity-Dependent Modifications in Synaptic Structure and Function Require Bidirectional Wnt Signaling. *Neuron*. 2008; 57(5):705. [PubMed: 18341991]
34. Varela-Nallar L, Ramirez VT, Gonzalez-Billault C, Inestrosa NC. Frizzled receptors in neurons: From growth cones to the synapse. *Cytoskeleton*. 2012; 69(7):528. [PubMed: 22407911]
35. Varela-Nallar L, Grabowski C, Alfaro I, Alvarez A, Inestrosa N. Role of the Wnt receptor Frizzled-1 in presynaptic differentiation and function. *Neural Development*. 2009; 4(1):41. [PubMed: 19883499]
36. Gross JC, Chaudhary V, Bartscherer K, Boutros M. Active Wnt proteins are secreted on exosomes. *Nat Cell Biol*. 2012; 14(10):1036. [PubMed: 22983114]
37. Korkut C, Ataman B, Ramachandran P, Ashley J, Barria R, Gherbesi N, et al. Trans-Synaptic Transmission of Vesicular Wnt Signals through Evi/Wntless. *Cell*. 2009; 139(2):393. [PubMed: 19837038]
38. Jin J, Kittanakom S, Wong V, Reyes B, Van Bockstaele E, Stajlar I, et al. Interaction of the mu-opioid receptor with GPR177 (Wntless) inhibits Wnt secretion: potential implications for opioid dependence. *BMC Neuroscience*. 2010; 11(1):33. [PubMed: 20214800]
39. Reyes BAS, Vakharia K, Ferraro TN, Levenson R, Berrettini WH, Van Bockstaele EJ. Opiate agonist-induced re-distribution of Wntless, a mu-opioid receptor interacting protein, in rat striatal neurons. *Experimental Neurology*. 2012; 233(1):205. [PubMed: 22001156]
40. Wayman GA, Impey S, Marks D, Saneyoshi T, Grant WF, Derkach V, et al. Activity-Dependent Dendritic Arborization Mediated by CaM-Kinase I Activation and Enhanced CREB-Dependent Transcription of Wnt-2. *Neuron*. 2006; 50(6):897. [PubMed: 16772171]
41. Yu X, Malenka RC. Beta-catenin is critical for dendritic morphogenesis. *Nat Neurosci*. 2003; 6:1169–1177. [PubMed: 14528308]
42. Wan X-Z, Li B, Li Y-C, Yang X-L, Zhang W, Zhong L, et al. Activation of NMDA Receptors Upregulates A Disintegrin and Metalloproteinase 10 via a Wnt/MAPK Signaling Pathway. *The Journal of Neuroscience*. 2012; 32(11):3910–3916. [PubMed: 22423111]
43. Li Y, Li B, Wan X, Zhang W, Zhong L, Tang S-J. NMDA receptor activation stimulates transcription-independent rapid wnt5a protein synthesis via the MAPK signaling pathway. *Molecular Brain*. 2012; 5(1):1. [PubMed: 22217152]
44. Shi Y, Yuan S, Li B, Wang J, Carlton S, Chung K, et al. Regulation of Wnt signaling by nociceptive input in animal models. *Molecular Pain*. 2012; 8(1):47. [PubMed: 22713358]
45. Yuan S, Shi Y, Tang SJ. Wnt Signaling in the Pathogenesis of Multiple Sclerosis-Associated Chronic Pain. *J Neuroimmune Pharmacology*. 2012; [Epub ahead of print]. doi: 10.1007/s11481-012-9370-3
46. Zhang Y-K, Huang Z-J, Liu S, Liu Y-P, Song AA, Song X-J. WNT signaling underlies the pathogenesis of neuropathic pain in rodents. *The Journal of Clinical Investigation*. 2013; 123(5):2268. [PubMed: 23585476]
47. Murase S, Mosser E, Schuman EM. Depolarization Drives β -Catenin into Neuronal Spines Promoting Changes in Synaptic Structure and Function. *Neuron*. 2002; 35(1):91. [PubMed: 12123611]
48. Ma T, Tzavaras N, Tsokas P, Landau EM, Blitzer RD. Synaptic Stimulation of mTOR Is Mediated by Wnt Signaling and Regulation of Glycogen Synthetase Kinase-3. *The Journal of Neuroscience*. 2011; 31(48):17537–17546. [PubMed: 22131415]
49. Tang SJ, Reis G, Kang H, Gingras A-C, Sonenberg N, Schuman EM. A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. *Proceedings of the National Academy of Sciences*. 2002; 99(1):467–472.
50. Avila ME, Sepúlveda FJ, Burgos CF, Moraga-Cid G, Parodi J, Moon RT, et al. Canonical Wnt3a Modulates Intracellular Calcium and Enhances Excitatory Neurotransmission in Hippocampal Neurons. *Journal of Biological Chemistry*. 2011; 285(24):18939–18947.

51. Beaumont V, Thompson S-A, Choudhry F, Nuthall H, Glantschnig H, Lipfert L, et al. Evidence for an enhancement of excitatory transmission in adult CNS by Wnt signaling pathway modulation. *Molecular and Cellular Neuroscience*. 2007; 35(4):513. [PubMed: 17588772]
52. Ciani L, Boyle KA, Dickins E, Sahores M, Anane D, Lopes DM, et al. Wnt7a signaling promotes dendritic spine growth and synaptic strength through Ca²⁺/Calmodulin-dependent protein kinase II. *Proceedings of the National Academy of Sciences*. 2011; 108(26):10732–10737.
53. Ahmad-Annuar A, Ciani L, Simeonidis I, Herreros J, Fredj NB, Rosso SB, et al. Signaling across the synapse: a role for Wnt and Dishevelled in presynaptic assembly and neurotransmitter release. *The Journal of Cell Biology*. 2006; 174(1):127–139. [PubMed: 16818724]
54. Farias GG, Alfaro InE, Cerpa W, Grabowski CP, Godoy JA, Bonansco C, et al. Wnt-5a/JNK Signaling Promotes the Clustering of PSD-95 in Hippocampal Neurons. *Journal of Biological Chemistry*. 2009; 284(23):15857–15866. [PubMed: 19332546]
55. Maguschak KA, Ressler KJ. Wnt Signaling in Amygdala-Dependent Learning and Memory. *The Journal of Neuroscience*. 2011; 31(37):13057–13067. [PubMed: 21917789]
56. Maguschak KA, Ressler KJ. [beta]-catenin is required for memory consolidation. *Nat Neurosci*. 2008; 11(11):1319. [PubMed: 18820693]
57. Jensen M, Hoernkli FdrJ, Brockie PJ, Wang R, Johnson E, Maxfield D, et al. Wnt Signaling Regulates Acetylcholine Receptor Translocation and Synaptic Plasticity in the Adult Nervous System. *Cell*. 2012; 149(1):173. [PubMed: 22464329]
58. Lim BK, Cho S-j, Sumbre G, Poo M-m. Region-Specific Contribution of Ephrin-B and Wnt Signaling to Receptive Field Plasticity in Developing Optic Tectum. *Neuron*. 2010; 65(6):899. [PubMed: 20346764]
59. Li B, Zhong L, Yang X, Andderson T, Huang M, Tang SJ. Wnt5a signaling contributes to Abeta-induced neuroinflammation and neurotoxicity. *PLoS ONE*. 2011; 6:e22920. [PubMed: 21857966]
60. Halleskog C, Dijksterhuis J, Kilander MB, Becerril-Ortega J, Villaescusa J, Lindgren E, et al. Heterotrimeric G protein-dependent WNT-5A signaling to ERK1/2 mediates distinct aspects of microglia proinflammatory transformation. *Journal of Neuroinflammation*. 2012; 9(1):111. [PubMed: 22647544]
61. Halleskog C, Mulder J, Dahlström J, Mackie K, Hortobágyi T, Tanila H, et al. WNT signaling in activated microglia is proinflammatory. *Glia*. 2011; 59(1):119. [PubMed: 20967887]
62. Halleskog C, Schulte G. WNT-3A and WNT-5A counteract lipopolysaccharide-induced pro-inflammatory changes in mouse primary microglia. *Journal of Neurochemistry*. 2013; [Epub ahead of print]. doi: 10.1111/jnc.12250
63. Hooper C, Sainz-Fuertes R, Lynham S, Hye A, Killick R, Warley A, et al. Wnt3a induces exosome secretion from primary cultured rat microglia. *BMC Neuroscience*. 2012; 13(1):144. [PubMed: 23173708]
64. Langseth AJ, Munji RN, Choe Y, Huynh T, Poznaniak CD, Pleasure SJ. Wnts Influence the Timing and Efficiency of Oligodendrocyte Precursor Cell Generation in the Telencephalon. *The Journal of Neuroscience*. 2010; 30(40):13367–13372. [PubMed: 20926663]
65. Shimizu T, Kagawa T, Wada T, Muroyama Y, Takada S, Ikenaka K. Wnt signaling controls the timing of oligodendrocyte development in the spinal cord. *Developmental Biology*. 2005; 282(2):397. [PubMed: 15950605]
66. Kim S, Kim S-H, Kim H, Chung A-Y, Cha YI, Kim C-H, et al. Frizzled 8a function is required for oligodendrocyte development in the zebrafish spinal cord. *Developmental Dynamics*. 2008; 237(11):3324. [PubMed: 18924237]
67. Lang J, Maeda Y, Bannerman P, Xu J, Horiuchi M, Pleasure D, et al. Adenomatous Polyposis Coli Regulates Oligodendroglial Development. *The Journal of Neuroscience*. 2013; 33(7):3113–3130. [PubMed: 23407966]
68. Fancy SPJ, Harrington EP, Yuen TJ, Silbereis JC, Zhao C, Baranzini SE, et al. Axin2 as regulatory and therapeutic target in newborn brain injury and remyelination. *Nat Neurosci*. 2011; 14(8):1009. [PubMed: 21706018]
69. Fu H, Kesari S, Cai J. Tcf7l2 is Tightly Controlled During Myelin Formation. *Cellular and Molecular Neurobiology*. 2012; 32(3):345–352. [PubMed: 22160878]

70. Ye F, Chen Y, Hoang T, Montgomery RL, Zhao X-h, Bu H, et al. HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the [beta]-catenin-TCF interaction. *Nat Neurosci*. 2009; 12(7):829. [PubMed: 19503085]
71. Fancy SPJ, Baranzini SE, Zhao C, Yuk D-I, Irvine K-A, Kaing S, et al. Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. *Genes & Development*. 2009; 23(13):1571–1585. [PubMed: 19515974]
72. Hu C, Liu J, Zhang Y, Li Y, Xie W, Zhao C. A useful transgenic mouse line for studying the development of spinal nociceptive circuits. *Neuroscience Letters*. 2009; 450(2):211. [PubMed: 19063941]
73. Shi Y, Shu J, Gelman BB, Lisinicchia JG, Tang SJ. Wnt signaling in the pathogenesis of human HIV-associated pain syndromes. *J Neuroimmune Pharmacology*. 2013 in press.
74. Gordon MD, Dionne MS, Schneider DS, Nusse R. WntD is a feedback inhibitor of Dorsal/NF-[kappa]B in Drosophila development and immunity. *Nature*. 2005; 437(7059):746. [PubMed: 16107793]
75. Sen M, Lauterbach K, El-Gabalawy H, Firestein GS, Corr M, Carson DA. Expression and function of wingless and frizzled homologs in rheumatoid arthritis. *Proceedings of the National Academy of Sciences*. 2000; 97(6):2791–2796.
76. Pereira C, Schaer DJ, Bachli EB, Kurrer MO, Schoedon G. Wnt5A/CaMKII signaling contributes to the inflammatory response of macrophages and is a target for the antiinflammatory action of activated protein C and interleukin-10. *Arterioscler Thromb Vasc Biol*. 2008; 28(3):504–10. [PubMed: 18174455]
77. Shi Y, Gelman BB, Lisinicchia JG, Tang S-J. Chronic-pain-associated astrocytic reaction in the spinal cord dorsal horn of HIV-infected patients. *J Neurosci*. 2012; 32:10833–10840. [PubMed: 22875918]
78. Li B, Shi Y, Shu J, Gao J, Wu P, Tang S-J. Wingless-type Mammary Tumor Virus Integration Site Family, Member 5A (Wnt5a) Regulates Human Immunodeficiency Virus Type 1 (HIV-1) Envelope Glycoprotein 120 (gp120)-induced Expression of Pro-Inflammatory Cytokines via the Ca²⁺/Calmodulin-dependent Protein Kinase II (CaMKII) and c-Jun N-terminal Kinase (JNK) Signaling Pathways. *Journal of Biological Chemistry*. 2013; 288(19):13610–13619. [PubMed: 23539626]