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Gap Junctions, Pannexins and Pain

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Highlights

- Gap junctions increase in sensory ganglia and spinal cord after painful injury
- Gap junctions in sensory ganglia are found between neurons, between Satellite Glial cells and between neurons and glia
- Pannexin1 is increased in pain models and its deletion leads to hyposensitivity

Abstract

Enhanced expression and function of gap junctions and pannexin (Panx) channels has been associated with both peripheral and central mechanisms of pain sensitization. At the level of the sensory ganglia, evidence includes augmented gap junction and pannexin1 expression in glial cells and neurons in inflammatory and neuropathic pain models and increased synchrony and enhanced cross-excitation among sensory neurons by gap junction-mediated coupling. In spinal cord and in supraspinal areas, evidence is largely limited to increased expression of relevant proteins, although in several rodent pain models, hypersensitivity is reduced by treatment with gap junction/Panx1 channel blocking compounds. Moreover, targeted modulation of Cx43 expression was shown to modulate pain thresholds, albeit in somewhat contradictory ways, and

mice lacking Panx1 expression globally or in specific cell types show depressed hyperalgesia. We here review the evidence for involvement of gap junctions and Panx channels in a variety of animal pain studies and then discuss ways in which gap junctions and Panx channels may mediate their action in pain processing. This discussion focusses on spread of signals among satellite glial cells, in particular intercellular Ca^{2+} waves, which are propagated through both gap junction and Panx1-dependent routes and have been associated with the phenomenon of spreading depression and the malady of migraine headache with aura.

Keywords: Satellite Glial Cell; sensory neuron; Cx43; GJ: Panx1; ganglia; spinal cord; DRG; TG

1. Anatomy and physiology of Pain:

Pain is defined as an unpleasant or distressing experience that results in most cases from tissue injury and is usually localized to the injury site. It can be acute, resolving over minutes, hours, or days, or chronic, persisting with or without remissions. Some types of acute pain can be effectively managed with pharmacological approaches (e.g., local anesthetics), whereas treatment for chronic pain involves nonsteroidal anti-inflammatory drugs (NSAIDS) and opiates and is generally ineffective. Chronic pain thus represents a major unresolved health problem that affects hundreds of millions of people worldwide and needs new targets for alternative therapies.

1.1 *The pain pathways*

The sensation of pain can be elicited by stimulation of nociceptive endings in the skin or by injury of the sensory neuron axon or soma. Central nervous system processing, particularly in the spinal cord, can also contribute to pain.

Sensory neurons synapse upon interneurons within the dorsal horn of the spinal cord (Fig. 1), with different modalities projecting onto spatially distinct laminae and somatic and visceral sensory information also being processed in separate layers. The interneurons cross to the contralateral side of the spinal cord, relaying signals through the lateral spinothalamic tract to brainstem, midbrain and thalamus and then to the cortex. Principal sites where pain may be modulated before being transmitted to central relay and cortical regions in the CNS are the sensory neuron itself and at its first synapse within the spinal cord. Each of these sites has been the focus of research interest, with excitability of the primary nociceptive afferents being recognized as critically important in the generation and maintenance of chronic pain ([1] [2]).

Sensory ganglia are of particular interest because they are outside the blood-brain-barrier (BBB) and thus are accessible to BBB impermeant treatments. Neurons within sensory ganglia do not form synapses among themselves, yet are endowed with a variety of receptors for neurotransmitters and hormones. They are closely surrounded by a layer of Satellite Glial Cells (SGCs), with a variable number (on the order of 2-20 per neuron, depending on neuronal size). SGCs, together with the connective tissue space, serve to separate neuronal somata from one another (although there are occasional clusters of neurons surrounded by a single SGC sheath). SGCs have several properties that are more similar to astrocytes than to other types of glia, including abundant expression of the astrocyte gap junction protein connexin (Cx)43, the purinergic P2X7 receptor (P2X7R) and the astrocyte markers glutamine synthetase and glial fibrillary acidic protein (GFAP), the latter being expressed at a low basal level although it can be greatly induced by neuronal injury.

1.2 Cross-excitation, cross-sensitization and referred pain.

One prominent feature in neuropathic pain is the ‘spread’ of pain sensation from the locus of the injured nerves to areas where the nerves are apparently undamaged [3]. Stimulation of sensory nerves was shown to result in subthreshold depolarization of unstimulated neurons that can sum with direct depolarization to generate action potentials; this “cross-excitation” appears to result from interactions between neurons within the ganglion (see Fig. 2; [4, 5]). Such depolarization might arise from elevated extracellular K^+ due to repetitive nerve or from transmitter release from either neurons or glia or through the spread of signals via GJ mediated neuron-neuron or neuron-SGC coupling (see Fig. 3).

Several early studies indicated potential consequences of the spread of signals within the ganglion and nerve injury. For example, retrograde labeling with Dil to mark damaged axons showed that expression of the astrocyte activation marker GFAP induced in SGCs by manipulation of individual teeth sometimes spread beyond the SGCs surrounding the neurons innervating the affected site to SGCs around neurons within all three divisions of the TG[6].

Transection of a component of the mandibular branch of the trigeminal nerve in rats was reported to induce allodynia in sensory areas innervated by the other two trigeminal branches. This phenomenon was associated with increased Cx43 expression and increased number of FluoroGold labeled neurons encircled by Cx43-expressing SGCs in regions of the trigeminal ganglion containing uninjured neurons [7]. . Infusion of a Cx mimetic peptide in the ganglion blocked allodynia and reduced FluoroGold labeling. The authors concluded that nerve injury signals released from damaged neurons activated SGCs, and that signals were then propagated throughout the ganglion by GJs spreading hyperexcitability.

2. Nerve injury increases GJ expression in sensory ganglia

Chronic pain can result from a number of distinct types of insult, including diabetes, multiple sclerosis, chronic inflammation and from nerve lesion. Early reports in invertebrates and in rat spinal cord showed that nerve section resulted in transient changes in electrical coupling among the injured motoneurons (e.g., [8] [9]). However, it was a great surprise that *astrocytes* were similarly affected. Nerve transection upregulated Cx43 in the ipsilateral facial nucleus in rats ([10]; this upregulation was rapid, occurring within 45-90 min after lesion ([11].

2.1. Gap junctions and intercellular coupling induced in pain models.

The idea that gap junctions may have a role in pain is quite new, and has received relatively little attention. One of the few reviews on this topic emphasized the role of gap junctions and hemichannels in sensory ganglia and spinal cord for spreading stress and toxic signals and noted the potential therapeutic role of blocking these pathways. [12] Studies exploring gap junction expression and dye coupling changes in sensory ganglia in various pain models were undertaken in collaboration between the Pannese and Hanani laboratories. Their initial study ([13] combined EL and dye coupling to show that two weeks after axotomy of mouse sciatic and saphenous nerves GJ plaques between SGCs in ipsilateral L4/L5 DRG were increased, as was Lucifer Yellow (LY) dye coupling both among SGCs surrounding individual neurons and also between the SGC sheaths of neighboring neurons.

Increased coupling among SGCs is a consistent feature of all pain models and all sensory ganglia that have been studied. For example, at 6-8 days following infraorbital nerve axotomy, a substantial increase in number of dye coupled SGCs was observed in mouse TG, both around individual neurons and between the SGC sheaths of neighboring neurons [14]. Likewise, intra- and inter-sheath dye coupling among SGCs in mouse L1 and S1 DRG increased following partial colonic obstruction [15]. Furthermore, LY spread among SGCs was most extensive when the injected SGC surrounded a neuron innervating the inflamed colon wall compared with randomly

injected cells. Similarly, in a model of colitis dye coupling among glial sheaths was most greatly enhanced around neurons projecting to the site of injury [16]. In this study the incidence of neuron-neuron coupling was also increased by almost 20-fold, and GJ channel blockers reduced neuronal excitability in this model and a single injection of blockers blunted behavioral hypersensitivity as well as pain-related hyperexcitability in the neurons.

Pain models in which Complete Freund's Adjuvant (CFA) is used to induce inflammation effectively increase SGC coupling. For example, injection of CFA into the hindfoot of mice increased LY coupling among L4/L5 DRG SGCs several-fold within one week after injection, and this increase persisted for up to one month ([17]. Intraperitoneal injection of the GJ channel blocker CBX decreased hypersensitivity at 2 weeks after CFA injection. Following sciatic nerve injection of CFA as a model of neuritis, coupling and GJs were increased between glial sheaths; moreover, coupling between neurons and SGCs and between neurons and neurons appeared ([18].

Evidence that coupling between neurons and SGCs could increase rapidly following painful stimuli was provided by experiments in which capsaicin injection in whisker fields led to neuron-SGC spread of the small fluorescent dye True Blue that had been injected 7 days previously into fields innervated by separate branches of the TG ([19]. Before capsaicin injection, the dye that had been transported to the neuronal somata was confined to neurons innervating the whisker area, but within 15 min thereafter dye was observed in adjacent SGCs. Moreover, at later times the dye was detected in SGCs in regions supplied by other trigeminal nerve branches, providing evidence for rapid and long range signal spread by neuron-SGC-SGC coupling initiated by the painful stimulus.

Further support for involvement of GJs in hyperalgesia has come from studies targeting GJ gene expression and from pharmacological inhibitors of function. In one such study Cx43

dsRNA was infused into the trigeminal ganglion of rats following chronic constriction injury (CCI) of the infraorbital nerve [20] [21]). Although reducedCx43 levels in the TG was analgesic in the CCI model, this treatment in control rats led to pain-like behavior similar to that of CCI. Mechanisms responsible for the unexpected opposite remain to be resolved.

In another pain model involving the trigeminal ganglia, a single injection of CFA into the submandibular skin of mice resulted in long-lasting allodynia (up to 7 weeks) and chronic hypersensitivity to tactile stimuli [22]. Tactile thresholds returned to uninjured levels following single i.p. injection of CBX.

Dye coupling in DRG SGCs is also induced in a model of chemotherapy-induced neuropathy (CINP). Injection of taxol or oxaliplatin induced tactile hypersensitivity within one week that resolved two weeks later and was paralleled by increased GFAP staining and strongly increased LY coupling [23]. The GJ blockers CBX and palmitoleic acid restored pain threshold to normal levels. In a follow-up study, dye coupling was examined in cultured TG SGCs treated with oxaliplatin for 2 hours [24]. Although altered Cx43 expression was not detected, dye coupling was increased, which was restored to control levels by CBX.

Two other pain models have revealed the apparent generality of the coupling increase within sensory ganglia. In the mouse model of multiple sclerosis (experimental autoimmune encephalomyelitis) GFAP staining and LY coupling in L4/L5 DRG SGCs were elevated ([25]).. Following systemic inflammation induced in mice by i.p. injection of lipopolysaccharide (LPS), tactile sensitivity and GFAP expression in L4/L5 DRG were enhanced within one week [26] This hypersensitivity was reversed by both CBX and palmitoleic acid. Dye coupling within the ganglion increased substantially at 3-7 days after LPS injection, with neuron-neuron coupling appearing and coupling of SGCs surrounding different neurons increasing 4-fold. Electron microscopy revealed an approximately 3-fold increase in number of GJs between SGCs, and Ca^{2+} imaging

revealed heightened sensitivity to ATP. GFAP immunoreactivity, dye coupling and SGC gap junctions as well as mechanical hypersensitivity were all still increased at 30 days after LPS injection [27]. In nodose ganglia, which contain vagal sensory neurons innervating the viscera, responses of SGCs to ATP were significantly enhanced at 1 day to 2 weeks after LPS injection [28]. At 1, 3, and 7 days after LPS dye coupling between SGC sheaths was increased whereas Cx43 immunostaining was decreased at 7 days after LPS, and the authors suggested that the increased coupling could be due to expression of another GJ protein.

The studies described above indicate that GJs and LY dye coupling are upregulated in SGCs in each of the pain models that has been investigated. Moreover, in several studies LY coupling has been detected between neurons and between neurons and SGCs. This naturally raises the issue of the identity of the gap junction proteins that are upregulated. Several studies have shown changes in Cx43, which is the major GJ protein in astrocytes and is abundant in SGCs (e.g., [20]), whereas others have reported detection of other Cxs. For example, Cx26 between SGCs and neurons was reported to be transiently upregulated in TG following peripheral capsaicin injection, and was sustained in the CFA model [29]. Cx36 was also upregulated in neurons, but Cx43 changes were not detected. This topic deserves further study because, as Cxs can be therapeutic targets, different Cxs may be blocked by different drugs ([30].

2.2 Coupled activation of neurons in sensory ganglia in response to painful stimuli

Confocal imaging on anesthetized mice with genetically encoded Ca^{2+} indicators in DRG neurons has provided a new level of understanding of the role of gap junctions in DRG pain processing [31]. With this method it was possible to detect activity patterns from ~1600 DRG sensory neurons in response to mechanical stimulation in pain models (CFA injection of the hindpaw and CCI of sciatic nerve). At 2 days after injury both insults produced behavioral hyperalgesia and hyperactivity of neurons in the L4 DRG; a high percentage of the activated neurons were very

close to one another, and they were termed “coupled-activated”. To assess junctional coupling directly, the authors injected rhodamine into neurons in DRG from control and CFA-treated mice. Incidence of neuron-neuron dye coupling was higher in intact DRG from CFA injected mice, and dual whole cell recordings from freshly dissociated DRG revealed that about half the neuron-neuron and neuron-SGC pairs were coupled. Further evidence that GJs were involved in coupled activation of neurons came from findings of reduced hyperalgesia and fewer coupled activated cells after heptanol and either local or systemic CBX treatment. In addition, coupled activation was less in mice with targeted deletion of Cx43 in SGCs.

2.3. What causes the increased coupling after nerve injury?

The studies summarized above showed that dye coupling among SGCs increases in a variety of chronic pain states. Moreover, coupling between SGCs and neurons and between neurons is more frequent in pain models, and hyperalgesia can be suppressed by treatment with GJ blocking agents or by reducing Cx43 mediated coupling. These findings lead to the question of the mechanism responsible for the increased coupling. Sustained changes in coupling within the sensory ganglia are likely mediated by growth factors and hormones released by the tonically activated sensory neurons, but local changes could also play a role, in particular mediating the early events in peripheral sensitization. TGJ coupling in SGCs is increased by alkaline intracellular pH and elevated extracellular K^+ [32], indicating that local ionic changes that occur within the ganglion following nerve injury can modulate SGC coupling. Whether these effects on coupling are mediated directly by known actions of cell acidification and increased intracellular Ca^{2+} on glial GJs (see [33] [34]) or result from depolarization of sensory neurons and release of factors that modulate GJs remains to be determined,

3. Involvement of gap junctions and Panx1 in spinal cord processing of pain information

Several studies indicated that GJs in the CNS contribute to pain . A common finding in such studies is that intrathecal administration of GJ blocker attenuates pain behavior. For example, intrathecal CBX attenuated nociceptive behavior and medullary dorsal horn central sensitization induced by partial infraorbital nerve transection [35] and by spinal nerve ligation (SNL) in rats [36]. Surprisingly, the latter group reported that spinal Cx43 was **reduced** in SNL, and intrathecal application of Cx43 siRNA alleviated SNL hypersensitivity [37]. This apparent paradox was explained by proposing that the **function** of Cx43 channels increased despite their lower expression levels. A major caveat of all studies using intrathecal application of drugs is that they readily diffuse to the sensory ganglia [38]. Thus, such application may not distinguish between central and peripheral drug actions.

As mentioned above, oxaliplatin treatment leads to neuropathic pain and sensitization in sensory ganglia. It also leads to gap junction changes in the spinal cord, with reactive gliosis and upregulated Cx43 all appearing as hypersensitivity develops between 1-2 weeks after treatment [39]. These effects were prevented by intrathecal CBX pre-treatment, but not by CBX administration after CINP was established. In a rat sciatic inflammatory neuropathy (SIN) model, intrathecal CBX dose-dependently reversed allodynia and mirror-image pain, while in the CCI model CBX only affected mirror-image pain [40]. Further insight into mirror-image pain has come from a recent study showing that spinal cord carrageenan injection led to bilateral pain responses and bilaterally increased Cx43 expression in spinal cord [41]. The mechanism appears to be inflammation-related.

As many as 80% of spinal cord injury (SCI) patients suffer from pain and it has been proposed that changes in spinal cord GJs contribute to the spread and severity of the damage; for review see [42] and for recent work on animal models see [43].

In summary, there is considerable evidence that expression of Cx43 increases in dorsal horn following injury and that blockade of gap junctions relieves the hypersensitivity. As noted, few

studies thus far have examined functional changes in gap junctions in the spinal cord, although available evidence indicates that GJs in spinal cord may play a role in chronic pain.

4. Pannexin1 in sensory ganglia

Pannexins (Panx) are membrane channels related to GJ, but they do not form cell-to-cell channels and they are highly permeable to ATP ([44, 45]. Panx1 expression and impact on peripheral pain sensitivity were reported at scientific meetings in 2012 and summarized in a review [46]. However, the first published account of Panx1 expression in sensory ganglia was the report of high levels of Panx1 expression in TG, where it was localized to both sensory neurons and SGCs [47]. Panx1 increased in TG in the CFA orofacial pain model [48]. Moreover, mice in which Panx1 was globally deleted did not develop allodynia following CFA; selective Panx1 deletion in glia (GFAP-cre/Panx1^{ff}) prevented hypersensitivity similarly to the global knockout, whereas targeting neuronal deletion (NFH-cre) only resulted in a slight delay in onset of hypersensitivity that did not persist or develop into allodynia. Measurement of Ca²⁺ signaling in SGCs or neurons revealed that both cell types were hyperactive, and their response to ATP was exaggerated in the pain model. Thus, glial and neuronal Panx1 appear to play major, but distinct, roles in development of allodynia in this pain model.

There have been several other reports associating Panx1 expression and/or function with pain. In a study on nodose ganglion, Panx1 and Cx43 were reported to be exclusively expressed in neurons and glia, respectively [49]. Both gap junction and Panx1 drugs modified vagal sensory nerve activity, from which they concluded that SGC Cx43 hemichannels played a role in neuronal excitation. However, , reduced activity of Panx1 channels through pharmacological blockade or in the Panx1 null mouse also attenuated the response, suggesting that Panx1 likely also plays a role in excitation. Moreover, Panx1 was subsequently detected in nodose ganglion SGCs as well

as in sensory neurons, and its expression increased in the LPS injection model of systemic pain [28].

SNL in rats led to upregulated Panx1 mRNA and protein level in DRG but not in spinal cord, and immunostaining revealed increased Panx1 in DRG neurons ([50]. Although it was not emphasized, Panx1 labeling in SGCs was also increased in this pain model. Intrathecal injection of Panx1 blockers or Panx1-specific siRNA reduced hypersensitivity induced by SNL.

Using CCI and SNL pain models. Panx1 null mice were also found to be resistant to chronic pain [51]. In contrast to studies cited above [48], however, the authors found no protection when targeting deletion to either neurons or glia, but profound analgesia when inflammation was blocked by targeting Panx1 deletion to hematopoietic cells. This disagreement could have resulted from differential recombination in the two studies, and the finding of protection by deletion in hematopoietic cells could reflect general suppression of inflammasome activation.

Evidence summarized above that spinal cord GJs play a role in neuropathic pain emphasized analgesia produced by administration of GJ inhibitors. However, most of these drugs are even more potent on Panx1 channels, and tPanx1 blockers (CBX, probenecid and a peptide inhibitor) reduced hypersensitivity in a sural nerve transection neuropathic pain model [52]. This finding suggests that targeted blockade of spinal cord Panx1 might be effective for pain relief, although Panx1 expression in dorsal horn was not changed in this pain model, making it less likely that Panx1 is a key component of central pain sensitization. However, because delivery was intrathecal, blockers would be expected to act both on spinal cord and at DRG

5. How does intercellular interaction affect pain?

Intercellular signaling in the nervous system is mediated by the release of transmitters and by direct GJ-mediated ion and small molecule diffusion. For neurons, transmission speed is optimized through vesicular release and by axonal conduction. For non-neuronal cells, intercellular signals spread more slowly, mediated by regenerative release of Ca^{2+} from

intracellular stores and diffusion of molecules. This mode of signal propagation has been termed the “calcium wave” and involves both GJs and chemical signaling, largely by ATP acting on P2 receptors. Such waves have been described both for astrocytes [53] and cultured sensory ganglia [54]. For this mechanism, Panx1 channels enable the release of ATP and likely also other transmitters, which then act on both ionotropic and metabotropic receptors to admit extracellular Ca^{2+} or release it from intracellular stores.

We propose that the increased gap junction and Panx1 expression seen in sensory ganglia in the setting of chronic pain models play a determinant role in the hyperexcitability that is responsible for peripheral sensitization (Fig. 2A). For GJs between SGCs, the role is to increase intercellular signal spread both within the envelopes that closely appose each neuron and also between neuron-SGC units. The GJs between SGCs and neurons and between neurons induced under painful states likely contribute to enhanced excitability, due either to exchange of second messengers or to a wave of depolarization accompanying the second messenger diffusion in SGCs. For Panx1, the role is likely enhanced ATP release from SGCs and/or neurons with activation of nearby neurons and SGCs. As noted above, injury enhances both Cx43 and Panx1 activity as a consequence of elevated extracellular K^+ and intracellular Ca^{2+} ([15] [55, 56], contributing to excitatory drive. A likely consequence of such activation is spread of Ca^{2+} signals from SGCs surrounding directly injured neurons to other SGCs (Fig. 2B); to the extent that such activity achieves suprathreshold “cross excitation”, this recruitment could contribute to allodynia. Although changes in coupling and patterns of Ca^{2+} activation are less well understood in dorsal horn, it is likely that similar mechanisms could operate to provide local modulation at the point of convergence of peripheral sensory information.

Some aspects of intercellular Ca^{2+} wave spread are similar to the phenomenon of spreading depression and associated with migraine headache [57]. These depolarization waves, followed by electrical inhibition, are evoked by transient hypoxia and other types of brain injury

and spread slowly across the cortex and other brain regions. Spreading depression is similar to Ca^{2+} wave spread among astrocytes with regard to velocity and sensitivity to GJ/Panx1 inhibitors [58], although the mechanism is generally believed to involve elevated K^+ and glutamate release, rather than ATP [59]. Both GJs [60] and Panx1 channels [61] have been implicated in migraine headache and spreading depression, and a putative GJ channel blocker (tonabersat) was initially touted for migraine relief [62], although clinical trials were unsuccessful and its effects on coupling have been questioned (see [63, 64]).

A model conceptually similar to spreading depression entitled the 'ignition theory' has been proposed to explain allodynia ([65] [4]), where a self-sustaining mechanism (positive feedback) must exist in sensory ganglia to maintain paroxysmal pain. This mechanism is envisioned to include two elements: "electrical cross talk" and "crossed after discharge", which requires chemical communication between neurons. Although this theory was applied to trigeminal neuralgia, it can be generalized to other pain types. We propose specific elements for this model: The electrical cross talk likely involves the GJs found in SGCs and neurons in pain models, and the after discharge correlates with the augmented release of ATP, one major pathway of which is Panx1, and increased sensitivity of purinergic receptors. The ignition theory did not include SGCs, which appear to be key players in this scheme.

6. Conclusions. Glia in sensory ganglia and CNS may modulate activity of adjacent neurons by the release of ATP and other substances through Panx1 channels. In addition, GJs among glia can provide long-range spread of signals and GJs induced by injury between neurons and between glia and neurons may directly depolarize or provide second messenger modulation. A large body of evidence now indicates that GJs and Panx1 channels might be therapeutically targeted to provide alternatives to opioid analgesia. FDA approved pharmaceuticals exist for both connexins and Panx1, and it is likely that efficacy of these and other agents in pain management will soon be evaluated.

Conflict of interest statement

The authors declare no conflicts of interest.

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Figure legends.

Figure 1. Peripheral and central pathways of pain. Nerve fibers (labeled red, blue and green) innervate skin and other organs. The cell bodies of these sensory neurons are surrounded by Satellite Glial Cells (SGCs, gray encircling neuron somata) located in sensory ganglia, and for most body areas the neurons are in dorsal root ganglia (DRG). Sensory axons form their first synapses in the dorsal horn of the spinal cord. From here, post-synaptic neurons cross the spinal cord and are relayed via ascending tracts to central areas (brainstem, thalamus, cortex).

Figure 2. Cross-excitation in sensory ganglia, in which excitation spreads from one excited sensory neuron to depolarize another. This figure depicts one of several ways in which this phenomenon has been demonstrated. A. Brief tetanic electrical stimulation is applied to a nerve root containing the red but not either blue or green axon, and a microelectrode records intracellularly from the blue neuron while repeatedly applying subthreshold current pulses. As in Fig. 1 gray semicircles around neurons are SGCs, which are interlinked to one another via gap junctions (GJs); stimulated neuron releases K^+ and it and SGCs release ATP. B. Electrical recordings from the neuron during this experiment. Lower recording shows neuron response to tetanic stimulation, while recordings labeled 1-3 correspond to responses to neuron depolarization at selected times. 1) Subthreshold neuron depolarization does not generate an action potential in the neuron. 2) During nerve stimulation, which slightly depolarizes the neuron as indicated in the lower recording, the current pulse in the neuron sums with the small depolarization due to nerve stimulation to fire action potentials. 3) The small depolarization due to nerve stimulation declines and no longer is sufficient to reach threshold in the neuron. Modified from [5].

Figure 3. Hypothesized roles of GJs and Panx1 in intercellular communication in sensory ganglia. A. Under normal conditions, SGCs surrounding each neuron (N1, N2) are coupled to one another weakly but not to SGCs of neighboring sheaths. B. When neuron N1 (red) is activated/injured, K^+ is released which sustains neuronal depolarization and activates Panx1 channels to release other signaling molecules including ATP. ATP binds with receptors (P2X7 receptors, located only in SGCs, are illustrated as blue symbols, but other P2X ionotropic and P2Y metabotropic receptors are located on both neurons and glia, providing positive feedback). Gap junctions are strengthened between SGCs (white bars linking yellow SGCs surrounding individual neurons and turquoise between SGCs surrounding different SGCs), between neurons and glia (green bars)

and, in lower number, between neurons. In addition, neurons and SGCs become hyper-responsive to purinergic stimulation (not illustrated).

Figure 4. Model of GJ and Panx1 roles in intercellular communication in sensory ganglia and likely other neural pain processing centers. DRG is illustrated here as a matrix of circular neurons surrounded by gray SGCs. A. When they are injured, neurons within sensory ganglia become active (red). B. Excitation spreads from neurons to their surrounding SGCs, indicated by the local SGCs becoming yellow, as a result of ATP released by Panx1 and acting on P2 receptors and also GJ mediated signal spread. C. With prolonged stimulation, signals such as Ca^{2+} waves are relayed between SGCs surrounding adjacent neurons. D. Ultimately, additional neurons (pink) are recruited into the neural response, likely contributing to the phenomenon of allodynia.

Figure 1

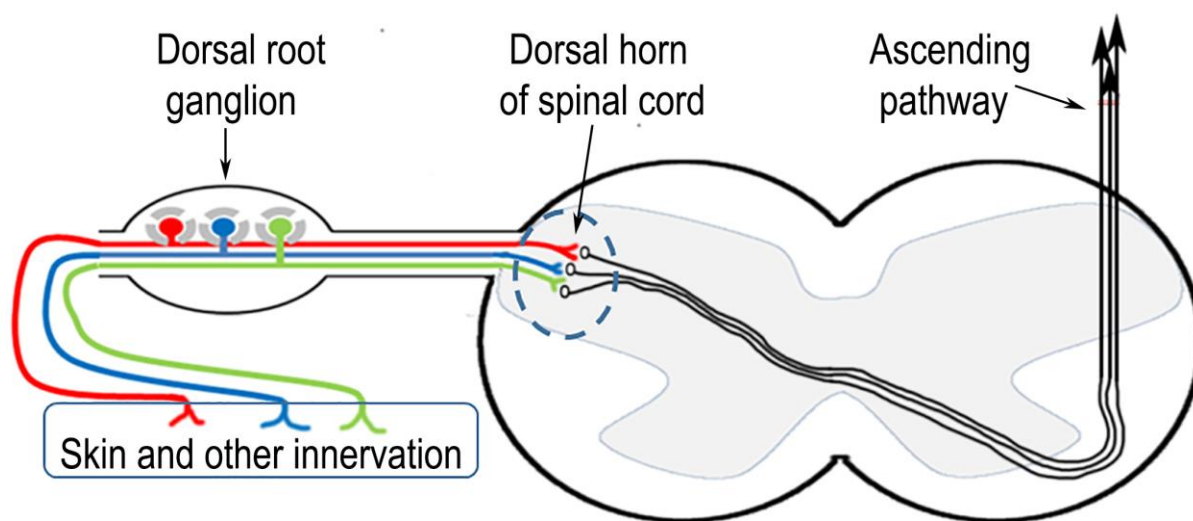


Figure 2

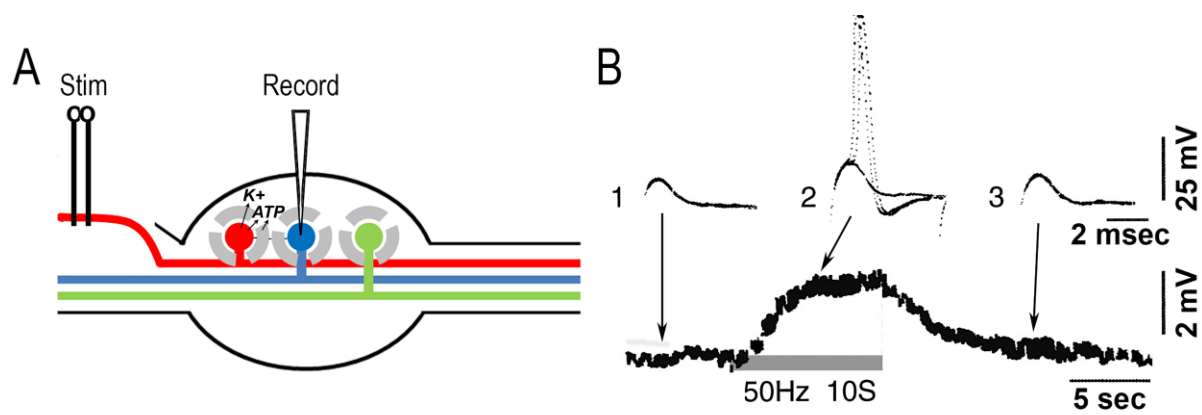


Figure 3

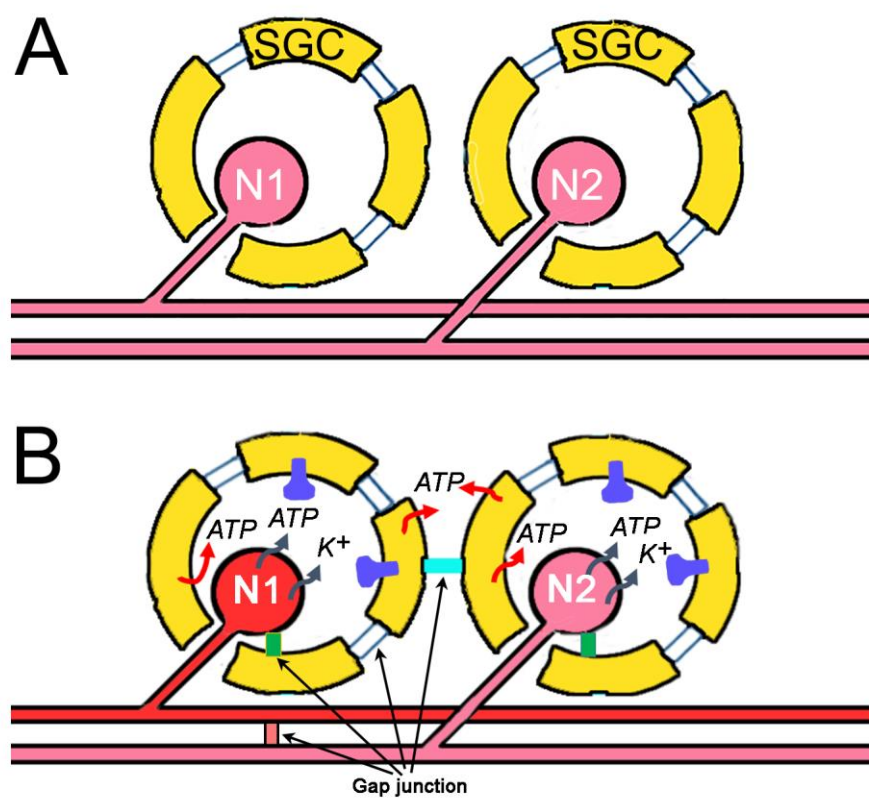


Figure 4

