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Epigenetic modification of DRG neuronal gene expression subsequent to nerve injury: Etiological contribution to complex regional pain syndromes (Part II)

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Literature Search F
Funds Collection G

ABCDEFG 1,2 Fuzhou Wang
ABCDEF 3 George B. Stefano
ABCDEF 3 Richard M. Kream

1 Department of Anesthesiology and Critical Care Medicine, Affiliated Nanjing Maternity and Child Health Care Hospital, Nanjing Medical University, Nanjing, China
2 Division of Neuroscience, Bonoi Academy of Science and Education, Winston-Salem, NC, U.S.A.
3 Neuroscience Research Institute, State University of New York at Old Westbury, Old Westbury, NY, U.S.A.

Corresponding Authors: Fuzhou Wang, e-mail: zfwang50@njmu.edu.cn, fred.wang@basehq.org, George B. Stefano, e-mail: gstefano@sunynri.org, Richard M. Kream, e-mail: rmkream@sunynri.org

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Cumulating evidence indicated that nerve injury-associated cellular and molecular changes play an essential role in contributing to the development of pathological pain, and more recent findings implicated the critical role of epigenetic mechanisms in pain-related sensitization in the DRG subsequent to nerve injury. In this part of the dyad review (Part II), we reviewed and paid special attention on the etiological contribution of DGR gene expression modulated by epigenetic mechanisms of CRPS. As essential effectors to different molecular activation, we first discussed the activation of various signaling pathways that subsequently from nerve injury, and in further illustrated the fundamental and functional underpinnings of nerve injury-induced pain, in which we argued for the potential epigenetic mechanisms in response to sensitizing stimuli or injury. Therefore, understanding the specific mediating factors that influence individual epigenetic differences contributing to pain sensitivity and responsiveness to analgesics possesses crucial clinical implications.

MeSH Keywords: Chronic Pain • Complex Regional Pain Syndromes • Epigenomics • Ganglia, Spinal • Peripheral Nerve Injuries

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Background

Nerve injury was considered as the major cause of the genesis of complex regional pain syndrome (CRPS), and nerve injury-induced cellular and molecular changes were found playing an essential role in contributing to the development of pathological pain. Epigenetic modification mediated by mechanisms such as DNA methylation, histone acetylation, phosphorylation, imprinting and reprogramming regulates and produces flexible yet stable alterations in transcriptional activities of genes, which play an essential role in development and physiological processes and also are implicated in various pathologies over a long period [1]. Increasing evidence indicated that epigenetic regulation plays a key role in nerve injury-induced chronic pain. In the first part of thie dyad review (Part I), we mainly reviewed the nerve injury-related alterations in the molecular and cellular bases [2], and in this part (Part II), we will discuss nerve injury-induced activation of various singnaling casedes, and will review the epigenetic modification of DRG neuronal genes in the context of nerve injury-induced chronic pain, and give etiological suggestion on its pathogenic contribution to CRPS. It is believed that the epigenetic progress promises patient's hope for controlling the pain through modifying the gene expression of the pain related molecules that finally determines the patient's outcome.

Signaling Molecules and Nuclear Effectors After Nerve Injury

Understanding of how cellular pain signals are initiated and subsequently transmitted and perpetuated possesses critical role in interpreting the cellular features and the function of nociceptive neurons and giving insights on the complex integration of painful cellular events. Burgeoning literatures collectively indicated that pain-related intracellular pathways underline a pivotal functional role in the transduction of nociceptive information from the receptor binding of noxious molecules to the signaling effectors. We herein summarize the pain signaling pathways with a categorical manner.

Protein Kinases

By adding phosphate groups to substrate proteins, protein kinases (PKs) direct the activity, localization and overall function of many proteins, and serve to orchestrate the activity of the cellular processes. AGC kinases are the major types of PKs involving in the regulation of pain after nerve injury. The functional regulation of the NaV1.8 mRNA by PKA and PKCε in the DRG neurons is important for the development of the peripheral pro-nociceptive state induced by repetitive inflammatory stimuli and for the maintenance of the behavioral persistent hypernociception [3]. PKA mediates bradykinin sensitization of TRPA1 responsible for inflammatory pain. An increased activity of TRPV1 in DRG neurons contributes to opioid withdrawal-induced hyperalgesia through a sustained cAMP/PKA-dependent signaling mechanism [4]. Reversal of neuropathic allodynia by adenosine 2A receptor activation and estradiol (E2)-induced inhibition of voltage-gated Na^+ channels in DRG neurons are associated with PKA/PKC signaling. Furthermore, the cAMP-PKA signaling pathway is involved in the downregulating effect of progesterone on P2X3 receptors, the analgesic effects of gabapentin on thermal and mechanical hypersensitivity, the development of PGE2-induced mechanical hypernociception, and exogenous inflammatory cytokines-induced nociceptive responses in sensory neurons. PKA and PKCε signaling mechanisms are responsible for activin-associated contribution to acute thermal hyperalgesia, for bee venom (BV)-induced pain behaviors, and for AMPA/Kainate-mediated pain behavior in the thermal stimulus model. A-Kinase anchoring protein 150 (AKAP150) is required for the phosphorylation of TRPV1 by PKA or PKC in sensory neurons and, hence, affects TRPV1-dependent hyperalgesia under pain conditions, and in further, the activation of NMDA-induced TRPV1 sensitization involves phosphorylation level of serine 800 residues (p-S800) of TRPV1 in cell surface membrane in DRG neurons and that AKAP150 is required for NMDA- and PKC-mediated phosphorylation of TRPV1 S800 [5]. Spinal sigma-1 receptor-induced sensitization is mediated by an increase in nNOS activity, which is associated with an NO-induced increase in PKC-dependent pGluN1 expression, and the activation of spinal sigma-1 R enhances NMDA-induced pain via PKC- and PKA-dependent phosphorylation of the NMDA receptor NR 1 subunit. Kv3.4 channels help shape the repolarization of the nociceptor APs, and that modulation of Kv3.4 channel N-type inactivation by PKC regulates AP repolarization and duration suggesting that the dramatic modulation of high voltage-activated A-type K+ current (I_AHV) fast inactivation by PKC represents a novel mechanism of neural plasticity with potentially significant implications in the transition from acute to chronic pain [6]. PGE_2 binds to EP3 receptors, resulting in the activation of cAMP/PKA signaling pathway and leading to an enhancement of P2X3 homomeric receptor-mediated ATP responses in DRG neurons, and PGE_2-mediated cAMP formation and PGE_2-evoked CGRP release from cultured DRG neurons can be potentiated and augmented in a PKA-dependent manner in the context of sustained morphine treatment. The spontaneous augmentation of $Na_v1.9$ was regulated directly by PKA, and indirectly by PKC [7]. Neurokinin-1 (NK-1) receptor enhances TRPV1 activity in DRG neurons via PKCε and potentiates $Na_v1.8$ sodium current by PKCε-dependent signaling pathway, probably participating in the generation of inflammatory hyperalgesia [8]. *In vivo* chronic compression of DRG (CCD) or *in vitro* acute dissociation of DRG (ADD) treatment can activate the cGMP-PKG signaling pathway, and that continuing

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activation of cGMP-PKG pathway is required to maintain DRG neuronal hyperexcitability and/or hyperalgesia [9]. Neuromedin U (NMU) increases I_A via activation of NMUR1 that couples sequentially to the downstream activities of Gβγ of the G[o] protein, PKA, and ERK, which could contribute to its physiological functions including neuronal hypoexcitability in DRG neurons, and NMU inhibits T-type Ca^{2+} channel currents (T-currents) via pertussis toxin (PTX)-sensitive PKA pathway, which might contribute to its physiological functions including neuronal hypoexcitability in small DRG neurons. PKA/Fyn/GluN2B signaling plays an important role in triggering GluN2B R hyperfunction and pain hypersensitivity. Transient attenuation of G-protein coupled receptor kinase 2 (GRK2) produced neuroplastic changes in nociceptor function via PKCε- and cytoplasmic polyadenylation element binding protein (CPEB)-independent and is PKA- and Src tyrosine kinase (Src)-dependent mechanisms [10]. Low G-protein coupled receptor kinase 2 (GRK2) in DRG neurons switches epinephrine-induced signalling from a PKA-dependent toward a PKCε-dependent pathway that ultimately mediates prolonged epinephrine-induced hyperalgesia, and prolongs PGE_2 hyperalgesia via biased cAMP signaling to exchange proteins directly activated by cAMP (Epac)/Rap1, PKCε, and MEK/ERK [11].

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is serine/threonine-specific protein kinase that is regulated by the Ca^{2+}/calmodulin complex. Phosphorylation of CaMKII is necessary in the formation of long-term potentiation (LTP) that plays a central part in the persistence of neuropathic pain and CaMKII inhibitor attenuates neuropathic pain via down-regulating p-CREB. Recent evidence showed that CaMKII has an important role in cytosolic phospholipase A2 (cPLA2) activation following peripheral nerve injury through P2X3R or P2X2/3R and voltage-dependent Ca^{2+} channels (VDCCs) in DRG neurons [12]. Currents through voltage-gated Ca^{2+} channels (I_{Ca}) in DRG neurons is subject to both Ca^{2+}-dependent inactivation (CDI) or facilitation (CDF), and that hyperexcitability following nerve injury-induced loss of CDF may result from diminished CaMKII activity [13]. Administration of postsynaptic density protein 95 (PSD95) gene specific siRNAs attenuates the central sensitization through inhibiting CaMKIIα-related signaling cascades leading to the relief of neuropathic pain.

MAPKs play critical roles in the regulation of neural plasticity and inflammatory responses. They are activated by the phosphorylation on the activation loop containing a characteristic threonine-x-tyrosine (TxY) motif. Three major members of the MAPK family exist: ERK, JNK, and p38 MAPK. A huge number of data demonstrated that activation of these MAP kinases is a critical factor contributing to the development and maintenance of nerve injury-induced hypersensitivity. ERK signaling pathway is responsible for the analgesic effects of oxymatrine, curcumin, pioglitazone, an agonist of peroxisome proliferator-activated receptor gamma (PPARγ), zoledronic acid (ZOL), dexmedetomidine, and T-type calcium channel antagonist, and also ERK activation is involved in the pro-nociceptive effects of tissue plasminogen activator, PGE_2-induced brain-derived neurotrophic factor (BDNF) synthesis in DRG neurons [14], pro-inflammatory factors like TNF, CCL2, and neuregulin-1 [15]. Early intervention of ERK activation in the spinal cord can block initiation of peripheral nerve injury-induced neuropathic pain, and pERK was recommended as a marker superior to c-fos for neuronal activation following noxious stimulation and tissue injury reflecting the central sensitization [16].

Spinal nerve ligation (SNL) induces activation of JNK in different populations of DRG neurons, but in the spinal cord, JNK is activated persistently majorly in spinal astrocytes [17]. Interestingly, neuronal NMDAR-nNOS pathway participates in the activation of astrocytic JNK pathway [18]. The inhibition of all the JNK isoforms prevents the onset of neuropathic pain, while the deletion of a single splice JNK isoform mitigates established sensory abnormalities [19]. In further, JNK pathway is involved in the re-expression of Nav1.3 channel triggered by TNF-α, and the development of morphine tolerance [20]. These findings suggest that JNK signaling is an important part in contributing to the pain threshold regulation after peripheral nerve injury.

Evidence indicates that p38 MAPK signaling in microglia plays a critical role in the central sensitization and pathogenesis of neuropathic pain subsequent to nerve injury. Activation of p38 MAPK in DRG neurons is involved in the up-regulation of Nav1.3 channel in the stimulation of TNF-α, and inhibition of p38 MAPK reduces loss of DRG neurons after nerve transaction and attenuates the augmented angiotensin II/AT2 R signaling in the DRG subsequently alleviating neuropathic pain [21]. Moreover, activation of mitogen activated protein kinase phosphatase-1 (MKP-1), the natural regulator of p-p38, mediates resolution of the spinal cord pro-inflammatory milieu induced by peripheral nerve injury, resulting in prevention of chronic mechanical hypersensitivity [22]. p38 MAPK is also involved in the nociceptive regulation of cannabinoid type 2 receptor activation, sigma-1R mediated mechanical allodynia, melanocortin 4 receptor (MC4R), MIF [23], and P2X4R-evoked increase in Ca^{2+} and exocytotic release of BDNF from microglia [24]. The activated p38 MAPK contributes to inflammatory and neuropathic pain through a p38-mediated increase of $Na_v1.8$ current density in the DRG neurons. Taken together, p38 MAPK from DRG neurons and spinal microglia is an essential component of nerve injury-induced pain.

Although c-kit, a type III receptor protein-tyrosine kinase, is not belonged to the classic MAPK family, we herein mention its critical role in nerve injury-associated hypersensitivity. In DRG neurons c-kit appears to be expressed in small and medium

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size neurons, which project to the superficial lamina I-II in the dorsal spinal cord, and functions as an important contribution to the nerve injury induced persistent pain [25]. Furthermore, the c-kit receptors are mainly expressed in peptidergic small-sized DRG neurons and are involved in pain regulation both peripherally and centrally through forming a functional stem cell factor (SCF)/c-kit receptor system.

G-protein Coupled Receptors

G-protein coupled receptors (GPCRs) are integral membrane proteins that possess seven membrane-spanning domains. The GPCRs are activated by external signals in the form of ligands. Emerging evidence convinced that GPCRs are implicated in multiple biological functions in health and diseases. The G-proteins are heterotrimers composed of α, β, and γ subunits, and according to the isoform of the Gα, G-proteins are classified four subtypes by their sequence homology: Gs, Gi/o, Gq/11, and G12/13. In general, the function of Gs is to activate but Gi/o is to inhibit cAMP pathway, Gq/11 is to activate PLC, and G12/13 is to regulate cell processes through the use of guanine nucleotide exchange factors [26]. In the nervous system, over half of the neurotransmitters take function through GPCR signaling transduction.

Ligands activating the Gs-coupled receptors (GsCRs) produce stimulating effects. In the context of pain, GsCRs activation is responsible for the pro-nociceptive effects of the transmitters, which means antagonizing the role of corresponding ligands possesses analgesic function. In contrast to the GsCRs, activation of the Gi-coupled receptors (GiCRs) will generate inhibitory effects like anti-nociceptive role suggesting that for these types of receptors, agonists would be potential analgesics.

Wnt, Notch and Hedgehog Signaling

Wnt proteins are a group of secreted glycoproteins that signals outside information into cells by binding to receptors of the Frizzled family and several coreceptors such as lipoprotein receptor-related protein (LRP). There are three Wnt signaling pathways have been characterized: the canonical Wnt/β-catenin pathway, the noncanonical Wnt/calcium pathway and planar cell polarity pathway. Recent studies demonstrated that Wnt signaling pathways are regulated by nociceptive input, and nerve injury induces a rapid-onset and long-lasting expression of Wnt, as well as activation of the canonical Wnt/Frizzled/β-catenin signaling in the DRG neurons and the spinal dorsal horn neurons, and stimulated production of the pro-inflammatory cytokines, and regulated the NR2B glutamate receptor [27] suggesting that Wnt signaling is a crucial player in the pathogenesis of nerve injury-induced pain.

Notch is a highly conserved molecular signaling protein including two ligands: Delta and Serrate (aka Jagged). Notch receptors are transmembrane proteins with large extracellular domains that consist primarily of epidermal growth factor (EGF)-like repeats. In the nerve system, notch exerts contributory function to the regeneration of the injured axons negatively [28]. Under the condition of pain, recent data highlighted that notch signaling plays an important role in the induction and maintenance of nerve injury-induced hyperalgesia.

Activation of the Hedgehog signaling by its three ligands including Sonic, Desert, and Indian Hedgehog takes a crucial part in governing a wide variety of processes during embryonic development and adult tissue homeostasis. Emerging evidence indicated that sonic hedgehog (SHH) is involved in the regulation of the function of the nervous system. The injury of motor neurons up-regulates SHH in Schwann cells through the induction of BDNF and then exerts protective effects [29]. Additionally, nerve injury and inflammation induce activation of SHH signaling that is involved in the regulation of PKA-associated signaling activity [30]. More recently, studies showed that SHH signaling acts in parallel to TNF signaling to mediate allodynia but distinct TRP channels-mediated allodynia and hyperalgesia, and intrathecal or peripheral administration of a specific inhibitor of SHH signaling can block the development of analgesic tolerance to morphine in inflammatory and neuropathic pain [31]. However, the precise role of Wnt, Notch, and Hedgehog signaling in different areas of the nervous system in CRPS warrants further investigation.

Nuclear Transcription Factors

Nuclear transcription factors are proteins that bind to specific DNA sequences, thereby controlling the flow of genetic information from DNA to messenger RNA (mRNA). A number of data demonstrated that activation of the nuclear transcription factors (NTFs) is an important step for the development and maintenance of pain subsequent to nerve injury, and they are functional effectors of extracellular signals in the context of pain. We herein review the NTFs that are found to be involved in the regulation of nerve injury-induced pain.

Nuclear factor kappa B (NF-κB) is the prototype of a family of dimeric transcription factors made from monomers which have approximately 300 amino-acid Rel regions that bind to DNA, interact with each other, and bind the inhibitor kappa B (I-κB). Cumulating evidence indicated that NF-κB pathway activation is involved in the TNF R1 induces IL-6 up-regulation and neuropathic pain, spinal PG-dependent hyperexcitability and allodynia, change in nNOS variants in the spinal cord, recombinant TNF (rTNF)-induced mechanical allodynia, glucocorticoid receptors (GR)-mediated glutamate transporter EAAC1

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expression, and the analgesic effects of epidural glucocorticoid. Lentivirus [LV]-mediated delivery of short-hairpin RNA (shRNA) targeting NF-κB p65 for gene silencing inhibited the expression of NF-κB p65 and pro-inflammatory cytokines like TNF-α, IL-1β and IL-6, and alleviated mechanical allodynia and thermal hyperalgesia after nerve injury [32]. In addition, NF-κB decoy was conveyed and transduced into DRG that reduced mechanical allodynia and thermal hyperalgesia in the rat inflammatory pain [33]. Furthermore, transgenic glial fibrillary acidic protein (GFAP)-I κBα-dn mice had lower duration of formalin-induced paw-licking behavior supporting a role of glial NF-κB inhibition in reducing pain after peripheral nerve inflammation and in the same transgenetic animals, glial NF-κB inhibition reduces galanin and CGRP expression, which are neuropeptides that correlate with pain behavior and inflammation after peripheral nerve injury. Inhibitor kappa B kinase (IKK) β-mediated NF-κB stimulation in injured DRG neurons promotes cytokine and chemokine production and contributes to the development of chronic pain [34]. Meanwhile, NF-κB is involved in the regulation of Nav1.3 in primary sensory neurons in ventral root injured neuropathic pain, and the enhancement of K^+ currents by the chemokine CXCL1/growth related oncogene in small diameter DRG neurons [35]. In chronic postischemia pain (CPIP) animal, a model exhibiting many features of human CRPS-I, NF-κB is involved in the development of allodynia after a physical injury indicating that NF-κB plays a potential role for in human CRPS [36]. In sum, NF-κB exerts function in nociceptive transmission and processing and that substances that can inhibit the NF-κB-activating cascade are capable of reducing the nociceptive response suggesting that a modulation of specific participants in the NF-κB signal transduction might be a useful approach for the development of new analgesics.

Nuclear factor of activated T-cells (NFATc) is a transcriptional nuclear factor regulated by the Ca^{2+}-dependent protein phosphatase calcineurin. New study found that peripheral nerve injury causes a time-dependent change in NFATc expression in the DRG neurons, and the calcineurin-NFATc-mediated expression of pro-nociceptive factor C-C chemokine receptor type 2 (CCR2) contributes to the transition from acute to chronic pain after nerve injury [37].

Cyclic AMP response element-binding protein (CREB) functions through binding to certain DNA sequences called cAMP response element (CRE), thereby increasing or decreasing the transcription of the downstream genes. CREB-associated gene transcription is involved in the pro-nociceptive regulation of c-fos, BDNF, tyrosine hydroxylase (TH), enkephalin, corticotropin-releasing factor (CRF), and VGF. While phosphorylated CREB (pCREB) has been reported to be activated by nerve injury and is commonly used as a marker for pain-related changes in neuronal plasticity in somatosensory pathways, more interesting thing is that cortical CREB-mediated transcription contributes to chronic behavioral allodynia in inflammatory and neuropathic states rather than the acute condition [38]. In further, of inhibiting spinal CREB directly using antisense oligonucleotide (ASO) attenuated tactile allodynia caused by partial sciatic nerve ligation suggesting that phosphorylation of CREB is an essential contributing event in the central plasticity and the pathogenesis of neuropathic pain [39].

Menin, the product of the multiple endocrine neoplasia type 1 (*MEN1*) gene, predominantly is a nuclear protein that has roles in transcriptional regulation, genome stability, cell division and proliferation. In the nervous system, menin functions as a synaptogenic factor that is critically involved in a general postsynaptic mechanism of synapse formation between central neurons. Recent evidence demonstrated that menin mediates the pathogenesis of nerve injury-induced hypersensitivity though potentiating synaptic plasticity [40]. Besides, the up-regulated spinal menin contributes to the imbalanced glutamate/GABA production epigenetically through altering the level of glutamic acid decarboxylase 65 (GAD65) [41].

Activator protein 1 (AP1) is a heterodimer composed of proteins belonging to the c-Fos and c-Jun family, and is activated in response to a variety of stimuli, including cytokines, growth factors and stress [42]. AP1 response element is the effector of NGF in encoding acid-sensing ion channel 3 (ASIC3), a depolarizing sodium channel gated by protons during tissue acidosis (ASIC3) gene promoter.

Specificity protein 1 (Sp1) is a transcription factor that contains a zinc finger protein motif, by which it binds directly to GC-rich motifs of many promoters and then enhances gene transcription. TRPV1 expression is dependent on Sp1-like transcription factors with Sp4 playing a predominant role in activating TRPV1 RNA transcription in DRG neurons. The expression of both *KCNQ2* and *KCNQ3* encoding respective Kv7.2 and Kv7.3, two potassium channel subunits playing a key role in stabilizing neuronal activity, needs to be activated at their promoters suggesting that the changes in M-current density and excitability of neurons are implicated in the genesis of pain and potential therapeutics [43].

Heat shock proteins (HSPs) are a group of functionally related proteins involved in the folding and unfolding of other proteins. Production of high levels of HSPs can be triggered by exposure to different kinds of environmental stress conditions like hypoxia, inflammation, nerve injury, and pain. The expression of HSP 27 is up-regulated in the DRG neurons after peripheral nerve injury, as well as in the spinal cord in response to spinal cord injury [44]. Interestingly, the co-inducer of HSPs BRX-220 can lead to reduction in pain-related behavior after 4 weeks oral application rather than the rapid consumption suggesting that induction of HSPs either producing

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a slowly developed analgesia or enhancing the recovery processes [45]. Further evidence indicated that HSPs are important in neuroprotection after a variety of stresses or injuries through regulating a broad range of endogenous responses to peripheral nerve injury.

Hypoxia-inducible factors (HIFs) are transcription factors that respond to changes in the hypoxia environment. Recent data showed that hypoxia is a novel sensitization mechanism for TRPV1 by inducing HIF up-regulation. A low-level laser can modulate HIF-1α activity indicating that it can be used as a clinically applicable therapeutic approach for the improvement of tissue hypoxia/ischemia and inflammation in nerve neuropathy, as well as for the promotion of nerve regeneration [46]. HIF-1 is a key mediator in both spontaneous recovery and HA-induced neuroprotection after traumatic brain injury (TBI) [47]. In the context of diabetes, HIF-1α and target genes encountered transient expression in peripheral nerves suggesting that HIF-1α is responsible for the alterations in nerve function and regeneration that characterize the diabetic neuropathy [48]. HIF-2α has a role in NGF-promoted survival of sympathetic neurons indicated that HIF-2α is implicated in the neuroprotective mechanisms of prolyl hydroxylase inhibitors and in an endogenous cell survivor activated by NGF.

Novel Factors Associated with Nerve Injury

Besides abovementioned molecules that are strongly involved in the regulation of nerve injury-induced pain, we in this review highlights another two molecules – BDNF and oxytocin as the novel factors which possess direct therapeutic implications and promise the control of pain.

BDNF

BDNF is one of the potent NGFs exerting a wide range of functions from trophic effect on neurons in the nervous system to orchestrating the transmission and plasticity of sensory neurons. BDNF is the extensively studied factor in the field of pain. Surgical incision induces segmental upregulation of BDNF in the DRG and spinal cord through somatic afferent nerve transmission contributing to the pain hypersensitivity. Endogenous BDNF is involved in spinal sensitization following inflammation and that blockade of BDNF reduces central sensitization mostly due to deficient BDNF input in the spinal cord from primary afferents [49], and the released BDNF from the sensitized primary afferents increases the excitability of substantia gelatinosa (SG) neurons through its action on the presynaptic terminals, thereafter induces monosynaptic Aβ afferents to the SG, thereby developing hyperalgesia and/or allodynia during inflammation [50]. These altered actions of BDNF on excitatory and inhibitory neurons contribute to a global increase in dorsal horn network excitability and hence to the onset of neuropathic pain. In addition the excitability of small diameter capsaicin-sensitive sensory neurons is modulated by BDNF through activating p75 neurotrophin receptor and its downstream sphingomyelin signalling cascade.

The up-regulated BDNF in DRG, in addition to its post-synaptic effect in spinal dorsal horn, acts as an autocrine/paracrine signal to activate the pre-synaptic TrkB receptor and regulate synaptic excitability in pain transmission, thereby contributing to the development of hyperalgesia, and this autocrine/paracrine effect also performs protective role selectively in dopaminergic neurons against the loss of trophic support from the target striatum [51]. Besides, the increased BDNF activity in medium and large DRG neurons contributes to the reduction in Kv channel function through TrkB receptor stimulation in painful diabetic neuropathy [52]. In the context of nerve injury, the levels of BDNF in DRG and spinal cord neurons are significantly increased, which contributes to the reduced big conductance Ca^{2+}-activated K^+ channel activity in DRG neurons, and to the enhanced excitatory synaptic drive to excitatory neurons but decreased that to inhibitory neurons in the spinal cord.

The *de novo* synthesized or released BDNF after surgical incision, inflammation [49], nerve injury, as well as TNFα stimulation drives spinal noradrenergic sprouting and enhanced α2 AR-associated analgesia following nerve injury [53], and the increased reliance of spinal α2 ARs on cholinergic stimulation to cause analgesia after nerve injury reflects a shift from direct inhibition to direct excitation through interacting with Gs proteins and BDNF in spinal cholinergic neurons [54]. Moreover, NGF-induced up-regulation of BDNF was mediated by ERK1/2, and BDNF in turn functions as a key regulator of atypical PKC synthesis and phosphorylation and an essential mediator of the maintenance of a centralized chronic pain state [55]. Cumulating evidence indicates that BDNF is an essential mediator functioning as a final common path for a convergence of perturbations that culminate in the generation of neuropathic pain [56]. More recent study using chromatin immunoprecipitation (ChIP) assay revealed that nerve injury promotes histone H3 and H4 acetylation at BDNF promoter I at day 1 post-injury in the DRG, and the levels of histone acetylation remain elevated for at least 7 days suggesting that an initial increase in BDNF exon I expression controlled by epigenetic mechanisms might play a crucial role in the development of neuropathic pain [57].

Oxytocin

Oxytocin (OXT), a nine amino acid neuropeptide, is regarded as the “love hormone” found to be involved in a wide variety

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of physiological and pathological functions. Upon its effect on pain regulation and nociception, although the precise mechanisms have not yet been well elucidated, oxytocin attracts attention for its potential therapeutic target in different pain conditions. Over two decades ago, OXT was identified expressed in DRG neurons [58], and thereafter OXT was found present in the PNS and specifically in DRG neurons along with arginine vasopressin (AVP). OXT inhibits ATP-mediated currents in DRG neurons through the activation of PKA and the elevation of intracellular Ca^{2+} signaling in a dose- and PKC-dependent mechanism [59]. It was further found that OXT-induced analgesia and scratching are mediated by the AVP-1A receptor in the DRG suggesting that AVP-1A receptor expressed in DRG might represent a previously unrecognized target for the analgesic action of OXT and AVP [60].

Hypothalamic paraventricular nucleus (PVN), a long-been-known neuronal nucleus in the hypothalamus, projects neural fibers not only to the posterior pituitary gland, but also to other brain areas and the spinal cord functioning as an important source of both OXT and AVP under stressful and nociceptive conditions. The elevated OXT content is responsible for a tonic analgesia exerted on both mechanical and thermal modalities that is mediated by an OXT receptor-mediated stimulation of neurosteroidogenesis, which leads to an increase in $GABA_A$ receptor-mediated synaptic inhibition in lamina II spinal cord neurons [61]. Although OXT was involved in the antinociceptive process through the OXT receptor in the periaqueductal grey (PAG) and caudate nucleus (CdN), in the hypothalamic supraoptic nucleus (SON), only OXT rather than AVP regulates SON-mediated antinociception [62]. When systemically administered, OXT and AVP modulate nociception [low dose antinociceptive versus high dose pronociceptive] and windup plasticity suggesting that OXT and AVP possess neurohormonal role in pain responses [63], and when applied in CNS, OXT reduces hyperalgesia via affecting cannabinoid and opioid systems [64]. While OXT is widely used in reproductive medicine, its intriguing role in contributing post-Cesarean chronic pain disclosed that in the postpartum period rather than pregnancy protects against chronic hypersensitivity from peripheral nerve injury and that this protection may reflect sustained OXT signaling in the CNS during this period.

Although the contribution of OXT to the regulation of diverse cognitive and physiological functions including nociception has been identified when administered directly into the brain, the spinal cord, or systemically, the epigenetic regulation on OXT under different pain conditions are still not demonstrated clearly. There are studies illustrated the epigenetic regulation of OXT and its receptors in different pathological conditions like autism, social stimulation, and psychopathy, and the epigenetic modification of genes involved in OXT signaling was considered to be involved in the mechanisms mediating the long-term influence of early adverse experiences on socio-behavioral outcomes [65], whereas understanding the pathophysiological role of OXT in DRG neurons and possible epigenetic modulation under various pain settings remains an important challenge.

Implications for CRPS

Although the complex pathophysiological mechanisms of CRPS are not yet clearly illustrated, the cellular and molecular changes as set forth are significative to be hinted at for the pathogenesis of CRPS. Spontaneous pain and hyperexcitability to subthreshold stimuli are two key characteristics of CRPS, which not only indicate CRPS a self-sensitizing disorder, but also imply the sensitized neural responses which underlie the potential mechanisms of CRPS. Change in transmission capacity of ion channels, shift in the function of neurotransmitters, facilitation of synaptic transmission by inflammatory mediators, and alterations in signaling transduction in different locations of the CNS in different contexts of pain may also be applicable to the situation of CRPS.

Epigenetic Changes in DRG Neurons After Nerve Injury

Studies on the epigenetic mechanisms of pain after nerve injury are a newly developing area that is considered as a promise of elucidating the original underlying mechanisms of pain and subsequently as novel targets of pain management. The currently available evidence indicated that peripheral nerve injury causes a variety of epigenetically modified alterations in pain-related gene expression in DRG neurons, which underlie the neural plasticity in neuropathic pain.

Potassium channels are important components that were found to be involved in the pain processing, and increasing data indicated that the DRG K^+ channels were extensively regulated by epigenetic mechanisms after peripheral nerve injury. ChIP analysis revealed that nerve injury reduces the mRNA expression of Kv4.3 gene, which contains a conserved neuron-restrictive silencer element (NRSE), a binding site for neuron-restrictive silencer factor (NRSF), and increases the direct NRSF binding to Kv4.3-NRSE in the DRG. It is further showed that acetylation of histone H4, but not H3, at Kv4.3-NRSE is markedly reduced at day 7 post-injury, and this injury-induced Kv4.3 downregulation could be blocked by antisense-knockdown of NRSF suggesting that nerve injury causes an epigenetic silencing of Kv4.3 gene mediated through transcriptional suppressor NRSF in the DRG [66]. Additionally, the expression of Kv7.2 subunit of membrane potential-stabilizing M channel, which is encoded by *Kcnq2* gene, a major *Kcnq* gene transcript

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in small-diameter nociceptive DRG neurons, is down-regulated substantially, but the level of *Kcnq2* suppressor repressor element 1-silencing transcription factor (REST) is up-regulated in response to nerve injury [67]. Moreover, *Kcna2* antisense RNA, a conserved long noncoding RNA (lncRNA) responsible for a voltage-dependent potassium channel Kv1.2, largely located in first-order sensory neurons of the DRG is markedly increased in injured DRG through activation of myeloid zinc finger protein 1, a transcription factor that binds to the *Kcna2* antisense RNA gene promoter, contributing to the development and maintenance of neuropathic pain [68]. It is well known that the expression of big conductance Ca^{2+}-activated K^+ (BK) channels in combination of its activity in small and medium DRG neurons are profoundly reduced after nerve injury. More recent studies demonstrated that the reduced BK channel activity in DRG neurons after nerve injury is mediated by the increased BDNF through epigenetic and transcriptional mechanisms, and this BDNF-associated epigenetic regulation on pain is strongly related to the histone H3 and H4 acetylation at BDNF promoter I in the DRG that initiated at day 1 post-injury and lasted for 7 days [57].

DRG sodium channels, another essential part of the pain mediators, were also involved in and regulated by epigenetic modification subsequent to nerve injury. In a study, nerve injury promotes NRSF binding to the NRSE within μ-opioid receptor (MOP) and Nav 1.8 genes, thereby causing epigenetic silencing [69]. In the same study, NRSF knockdown significantly blocked nerve injury-induced down-regulations of MOP and Nav 1.8 gene expressions, C-fiber hypoesthesia, and the losses of peripheral morphine analgesia and Nav 1.8-selective blocker-induced hypoesthesia. In addition, these nerve injury-induced down-regulations of DRG Nav 1.8 and C-fiber-related hypoesthesia were reversed by histone deacetylase (HDAC) inhibitors, which increases histone acetylation at the regulatory sequence of Nav 1.8 [70].

MicroRNAs (miRNAs) comprise of short noncoding RNA that regulate gene expression post-transcriptionally. Recent studies have demonstrated that epigenetic mechanisms are involved in the regulation of the expression of miRNAs. Conversely, another subset of miRNAs takes charge of the expression of important epigenetic regulators including DNA methyltransferases and histone deacetylases [71]. In DRG after entrapment neuropathy, 6 miRNAs including miR-9, miR-320, miR-324-3p, miR-672, miR-466b, and miR-144 were significantly downregulated, and only the first three are downregulated after decompression suggesting that entrapment neuropathy results in different microRNA expression patterns from denervation injury and implying that epigenetic regulation is different in these two conditions.

The mGlu2 receptors are coupled to Gi/o proteins and their activation reduces neuronal excitability by inhibiting cAMP production which mediates the inhibition of voltage-gated Ca^{2+} entry with the ensuing reduction of glutamate release. *In vitro* study mGlu2 receptors are transcriptionally regulated by the NF-κB pathway, and hyperacetylation of p65/RelA by the acetylating drug L-acetylcarnitine (LAC) enhances the expression of mGlu2 receptors in cultured DRG neurons suggesting that the expression of mGlu2 receptors in the DRG is regulated by acetylation mechanisms [72], and these findings also imply that the LAC and HDAC inhibitor-induce analgesia relies on the up-regulation of mGlu2 receptors in the DRG through an epigenetic mechanism involving the acetylation of the NF-κB transcription factor.

In collection, the direct evidence of epigenetic modifications on DRG gene expression including Kv and Nav channels, miRNA, and mGlu2 receptors in response to nerve injury combined with the indirect implications of emerging cellular and molecular changes in the nervous system after various injuries as described in the previous section give us an overall view that pain-associated alterations in the expression of different molecules underlie the pathogenesis of nerve injury-induced hypersensitivity.

Epigenetic Modification of DRG Neuronal Genes: Etiological Contribution to CRPS?

CRPS is a chronic neuropathic pain condition that is usually characterized as pain and dysfunction of the sympathetic nervous system in one region of the body, usually an extremity. A large body of data indicates that the pathophysiological changes in the DRG play a contributory role to the development of CRPS, and successful treatment of CRPS of the foot by spinal cord stimulation (SCS) of the DRG convey an advantage to DRG stimulation over conventional SCS suggesting that the pathological changes in DRG possesses a more crucial role in determining the outcomes of CRPS than the spinal cord [73].

In normal state, the sensory neurons in the DRG are not directly innervated by the sympathetic nervous system because the sympathetic fibers normally are associated only with blood vessels. Currently we know that CRPS is exacerbated by sympathetic activity. In animal models, sympathetic fibers sprout into the DRG after peripheral nerve injury, forming abnormal connections with sensory neurons. In addition, the sustained pain from the sensitization of DRG neurons may trigger increased release of NE systemically and in the local area of injury in the context of CRPS. Based on these observations, a "cocktail" of antagonists of NE and ATP receptors, or the sympathetic release blocker bretylium, or pre-cutting the grey ramus through which sympathetic fibers coursed to the ligated DRG can effectively reduce or eliminate the enhanced spontaneous activity in large and medium diameter neurons, and

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the reduced rheobase in large neurons after repeated stimulation of the dorsal ramus. After nerve injury, α1A, α1B, α2C, α2A, α2D, and β2 ARs mRNA within DRG neurons are presented, and the activation of these receptors potentiate P2X(2/3) receptor currents through activating PKC. Furthermore, NA stimulates ATP release from DRG neurons as mediated via β3 ARs linked to Gs protein involving PKA activation [74], and also stimulates capsaicin-evoked SP release through activation of α ARs [75]. Interestingly, an interaction between AR activation and epigenetic modulation has been revealed. Acute β AR/PKA activation protects against hypertrophic signaling by delaying Gq-mediated transcriptional activation via HDAC5 nuclear export, and upstream histone demethylase JHDM2a [76]. The expression of α1D ARs is under control of the methylation-dependent disruption of Sp1 binding in a cell-specific manner [77]. Collectively, these evidences indicate that: 1) nerve injury produces a substantial increase in the number of DRG neurons evidencing the presence of ARs; 2) the expression of ARs is regulated by epigenetic mechanisms suggesting that epigenetic-modulated presence of ARs in DRG neurons after nerve injury may be an essential contributor to the CRPS.

The endogenous opioid system, one of the most studied innate pain-relieving systems, is consisted of endorphin, enkephalin, and dynorphin acting as neurotransmitters and neuromodulators at the three major classes of opioid receptors to produce analgesia. In F344 rats, the most resistant strain to neuropathic pain, a significant up-regulation of prodynorphin gene expression occurred only in the injured DRG, but Δ-opioid receptor (DOR) was found to be markedly down-regulated only in injured DRG of Lewis rats, and proenkephalin gene was down-regulated in both F344 and Lewis strains suggesting that nerve injury-induced changes in the opioid system in the DRG is an essential underlying mechanism of neuropathic hyperalgesia [78]. Patients with CRPS showed reduced opioid receptor binding potential (OR-BP) in contralateral amygdala and parahippocampal gyri and increased OR-BP in contralateral prefrontal cortical areas. When OR-BP in the midcingulate cortex and the ipsilateral temporal cortex was low, the McGill pain rating index was high demonstrating that the central opioidergic neurotransmission in CRPS undergoes alteration [79]. Under peripheral nerve injury, epigenetic silencing of MOP gene in the DRG neurons results in long-lasting down-regulation of MOP causing pathological and pharmacological dysfunction of C-fibers. In consideration of the significant association between the deficit in social-emotion recognition and the affective dimension of pain, and a disrupted ability to recognize others' mental and emotional states in patients with CRPS [80], and the relevance of affiliative contact behavior between conspecifics that is responsible for the activation of the endogenous opioid system which induces nociceptive threshold increase indicating that CRPS-related deficit of social recognization is considerably associated with the dysfunction of endogenous opioid system, and such social stressful sequelae may be the consequence of epigenetically modified opioid system [81]. In sum, epigenetic modifications of opioidergic gene expression in the DRG resulted from the nerve injury itself and stressful social deficiency may underlie the genesis of CRPS and function as an etiological contributor to CRPS.

As mentioned above, ionic channels are the anatomic bases of action potential formation, electrical signal transmission, and excitability determination. Nerve injury-induced changes in the expression of ion channel genes are considered as one of the basic underlying mechanisms of neuropathic pain such as CRPS. First, an intravenous (*i.v.*) lidocaine infusion has been shown to be effective in uncontrolled trials for reducing spontaneous and evoked pain with CRPS, and the topical application of 5% lidocaine patch has also been reported to produce clinically significant pain relief under the condition of CRPS suggest the involvement of Na+ channels in the pathological genesis of CRPS. In neuropathic injury, Nav1.8 encountered decrease in its distribution in DRG neurons, but increase in its local expression in the peripheral axons of DRG neurons both facilitate nociceptive signal generation and propagation [82] endorse the contribution of Na^+ channels to CRPS. Second, in a study with 59 CRPS patients, the administration of calcium channel blocker nifedipine showed improvement in patients' outcomes especially in the early stage indicates the association of Ca^{2+} channels with CRPS [83]. A more recent study using spinal nerve injury model revealed that the current density of T-type Ca^{2+} channels in small DRG neurons and the percentage of T-type Ca^{2+} channels in medium and large DRG neurons are significantly increased, and the expression of the mRNA levels of Cav3.2 and Cav3.3 in DRGs substantially up-regulated. Moreover, SNL significantly increases mRNA and protein levels of the Cavβ3 subunit in the DRG and the high voltage-activated calcium channels (HVACC) currents in small DRG neurons [84] suggesting that the levels and function of Ca^{2+} channels in DRG encounter significant alteration after nerve injury evidencing the occurrence of CRPS. Third, leakage potassium channels are of importance for neuronal excitability, and multiple leakage channels are expressed in DRG neurons. Under the condition of neuropathic injury, robust expression of TWIK1 was found in both large and medium size DRG neurons, but TASK1 and TASK3 are selectively expressed only in small cells. TWIK1 expression is much higher than TASK1 and TASK3 and is strongly decreased 1, 2 and 4 weeks after neuropathic injury, but TASK3 expression decreases 1 week after surgery but reverts to baseline by 2 weeks, and TASK1 shows no significant change at any time point [85] indicating that these background leakage K^+ channels are pivotal in the development and maintenance of neuropathic pain. As showed that nerve injury causes an epigenetic silencing of Kv4.3 gene mediated through transcriptional suppressor NRSF in the DRG [66], and Kv9.1 dysfunction leads to spontaneous and evoked

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neuronal hyperexcitability in myelinated fibers, coupled with development of neuropathic pain behaviors. Functional K_ATP channels like SUR1, SUR2, and Kir6.2 but not Kir6.1 subunits are present in normal DRG neurons, wherein they regulate resting membrane potential (RMP), and the alterations of these channels may be involved in the pathogenesis of neuropathic pain following peripheral nerve injury suggesting that K_ATP channels in DRG remain available for therapeutic targeting against established neuropathic pain [86]. No matter of the channels of Na^+, Ca^{2+}, and K^+, their genetic expression in the DRG neurons after nerve injury all evidenced alteration, and respective epigenetic modifications on each type of channels underlie the potential pathological mechanism of CRPS [66,69,70].

The balance between excitatory and inhibitory neurotransmitters is the basis of neural homeostasis. The expression of VGLUTs has been detected using specific riboprobes in all adult DRG neurons suggesting that glutamate is used as transmitter by the DRG neurons. Glutamate expression is increased in the DRG neurons of all diameters in response to nerve injury, and this increase occurs and is present in the somata of neurons with injured axons as well as in somata of neighboring uninjured neurons indicating that glutamate can be released within the sensory ganglion and the glutamatergic transmission within the ganglion impacts nociceptive threshold [87]. In line with the pharmacological evidences showing that activation of mGluR7 inhibits nociceptive reception, the expression of mGluR7 was down-regulated in both peptidergic and large DRG neurons after peripheral nerve injury implying the anti-excitatory effect of mGluR7 on pathological hypersensitivity [88]. However, mGluR5 protein increased after sciatic nerve section in ipsilateral L4 and L5 DRG neuronal profiles, with most of the increase occurring in myelinated A-fiber somata [89] indicating that different subtypes of glutamate receptors in the DRG play different roles. Also clinical evidence using ketamine, the NMDA receptor antagonist, as an adjuvant to sympatholytic blocks produced marked relief in heat allodynia and positive cognitive symptoms of CRPS [90]. Currently, emerging evidence disclosed the epigenetic modulation of glutamate-associated factors after nerve injury. Methods focusing on the transcriptional regulation of mGlu2 receptors via the acetylation-promoted activation of the p65/RelA transcription factor using LAC and HDAC inhibitors produced marked alleviation of pain [91]. Besides, the CpG islands have been found within the promoter or proximal regions of the NMDA receptor genes and demethylation occurred by 5-aza-2βdeoxycytidine unmasked the promoter region and led to expression of NR2B transcripts [92]. Collectively, different responses of glutamate and its receptors in DRG neurons to nerve injury play an important role in promoting or conquering the overexcited neuronal transmission that may be substantially associated with epigenetic modifications of corresponding gene expression that functions as another pathological contributor to the development and maintenance of CRPS.

As the counterpart of excitatory transmission, GABA, the major inhibitory neurotransmitter, plays a pivotal role in contributing to the adjustment of neural homeostasis. Spinal nerve ligation significantly reduced the $GABA_B$ receptors in the DRG neurons in combination with the effective prevention of hyperalgesia in rats subjected to a sciatic nerve crush injury after applying muscimol and gaboxadol, two potent GABAA agonists, directly to the ipsilateral DRG [93] suggesting that GABA inhibition after peripheral nerve injury is an critical cause of hypersensitivity, and means activating GABA receptors is a promise of controlling neuropathic pain. GAD functions as the major enzyme that catalyzes glutamate to GABA, an important step that reduces the excitability but increases the inhibition. When the GAD65-expressing rAAV2 was applied to DRG and sciatic nerve, the pain symptoms were dramatically reduced and persistently controlled by inducing inhibitory transmitter of GABA release in the spinal cord. In further, nonreplicating herpes simplex virus (HSV)-based vectors coding for proenkephalin or GAD have been used to transduce DRG neurons *in vivo* to produce enkephalin or GABA, which both reduce pain-related behavior in rodent models of peripheral neuropathic pain, and further such vector inoculation given subcutaneously (*s.c.*) also results in a substantial and significant reduction in mechanical allodynia in a model of lumbar radiculopathy by ligation of the dorsal and ventral lumbar roots proximal to the DRG [94]. More interestingly, neuropathic injury epigenetically suppresses *Gad2*, encoding GAD65, transcription through HDAC-mediated histone hypoacetylation, resulting in impaired GABA synaptic inhibition. In clinics, $GABA_B$ receptor stimulation by baclofen (ITB), a derivative of GABA, exerts differential antinociceptive effects on specific pain qualities in CRPS patients with dystonia. Summarily, nerve injury-induced changes in GABAergic transmission in the DRG are related with the content balance between glutamate and GABA regulated by GAD65 and with the alteration of GABA receptor expression, of which the epigenetic modulation underlies both content of GABA and receptor expression responsible to nerve injury that may function as a potential etiological contributor to CRPS.

Collectively, as mentioned above, the newly sprouted sympathetic fibers in the DRG after nerve injury in the context of CRPS in combination with substantial changes in the homeostasis of different neurotransmitters and ionic channels all play roles in causing CRPS. Even paradoxical results exist regarding the basic and clinical data on the CRPS pathologies, and emerging evidence focusing on the epigenetic mechanisms has led us to a new understanding on the pathogenesis of CRPS. The currently available data on the epigenetic modifications of the DRG gene expression are only a tip of the iceberg, and extensive studies are needed to decipher the context-based functional repertoire of epigenetic-related pathophysiological alterations in CRPS.

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Conclusions

Studies on the epigenetic modifications of DRG gene expression after nerve injury are growing to be a rapidly developing research direction that may provide a breakthrough in understanding the thorny pathology of CRPS and pose hope for developing novel epigenetic-associated therapeutics for CRPS. In addition, the complex syndrome of CRPS itself needs to be investigated on its etiological origins that depend on the precise understanding of the epigenetic contribution to the expression of different genes in the DRG after nerve injury.

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