Control of Neonatal Spinal Networks by Nociceptors: A Potential Role for TRP Channel Based Therapies

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ABSTRACT - Pediatric spinal cord injury (SCI) often leads to increased nociceptive input resulting in aberrant motor output like tremor and spasticity. Acute plasticity within spinal pain and motor networks following pediatric SCI may result in long-term sensorimotor disabilities. Despite this, pediatric SCI remains poorly understood. Part of the problem lies in the paucity of detailed studies aimed at defining sensorimotor control by nociceptors during development. This review provides an overview of work that highlights afferent control of sensorimotor networks by defined nociceptors in the developing spinal cord. Here, we focus on the well established and widely used neonatal sensorimotor model called sacrocaudal afferent (SCA) pathway. Until recently, the identity of specific subclasses of nociceptive afferents in the SCA pathway controlling developing sensorimotor networks was unknown. We highlight here the use of members of the Transient Receptor Potential (TRP) ion channels and mouse genetics to identify specific subsets of nociceptive afferents in the SCA pathway. In addition, we highlight the use of mouse genetics to map sensorimotor networks during development and potential future applications. A neonatal spinal cord model of central neuropathic pain via a defined set of nociceptors is presented as a probe into potential therapeutic avenues in neonatal SCI. Finally, knowledge translation from neonatal basic research to the pediatric population in the clinic is described. In conclusion, studies in neonatal models may lead to therapeutic strategies and pharmaceuticals for chronic pain and motor dysfunction after SCI during development.

INTRODUCTION

Spinal cord injury (SCI) in neonates results in long-term disabilities extending into adulthood[1,2]. Pediatric SCI can occur from motor vehicle accidents, falls or poor supervision [3,4,5,6]. The clinical symptoms of neonatal SCI include aberrant sensorimotor functions like pain, tremors and spasticity [7,8]. It is well established that nociceptive networks in neonates are functional, immature and prone to plasticity following injury [9,10,11]. There is general agreement that spinal circuits undergo plastic changes following SCI including sprouting of afferent collaterals, changes in synaptic transmission, and activation of microglia to name a few[12]. These changes in spinal networks ultimately lead to changes in the perception of pain and in motor output. A necessary first step in understanding the impact of these changes is to consider what is known about these spinal networks and in particular the role of afferents that project onto them.

Spinal circuits are under the influence of continuous sensory feedback relayed to the spinal cord through afferent pathways that intersect with and modulate spinal motor circuits [13,14,15,16]. Sensory information is carried to the spinal cord via primary afferents that innervate muscle and tendon receptors, cutaneous mechanoreceptors and nociceptors. The role of these inputs in controlling motor output is in some cases well defined. For example, the function of proprioceptive pathways and their connectivity under quiescent conditions [17] and during locomotion [18] is well understood. During locomotion, the afferent input projects onto the spinal circuits that generate locomotion and can alter the frequency along with the pattern of locomotion. The spinal network controlling locomotion has been termed the Central Pattern

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Generator (CPG). Compared to the proprioceptive afferents much less is known about the role of nociceptive afferents and their control of locomotion.

For the purposes of this review we will focus on the activation of spinal locomotor networks by painful stimuli. First we will discuss recent research that has generated new models and molecular tools that allow us to trace the nociceptive locomotor activating circuit in legged animals. We will then briefly discuss a form of chronic pain termed neuropathic pain. Neuropathic pain is distinct from acute pain as it usually occurs as a side effect of central nervous system trauma, and is chronic in nature. In contrast to acute pain, neuropathic pain serves no known survival value to the organism. It is a major concern since if nociceptive reflex pathways are chronically activated it could potentially affect recovery of locomotor network function. This has long-term translational potential considering that 60-80% of spinal cord injury (SCI) patients suffer from heightened acute pain reflex responses to non-painful stimuli or suffer from debilitating neuropathic pain [19].

The sacrocaudal afferent (SCA) pathway- a model for reflex pathways in the neonates

Aversive stimuli provide potent signals that can initiate fast escape responses in many species. These rapid responses allow prey to escape from predators. While escape networks have been relatively well worked out in fish, we do not fully understand how terrestrial mammals trigger locomotor networks following aversive stimuli. In general escape responses can be elicited by multiple modalities such as touch, pain, vision, and so on. Here we will concentrate on the role of nociceptive input from the limbs in modifying locomotor input.

As early as 1910, Sherrington demonstrated that nociceptive input could either facilitate or interfere with hindlimb stepping in decorticate cats depending on the site in the hindquarters that the stimulus was applied [20]. Later work by Schomburg's group showed that chemical activation of fine group III and IV muscle afferents by bradykinin or electrical stimulation of the sural nerve could make the rhythm more robust [21]. More recent studies demonstrated that administration of noxious heat could dramatically improve a L-DOPA elicited rhythm or, in some cases, elicit a rhythm [22]. Furthermore, when μ-opioid receptor agonist [d-Ala(2), N-Me-Phe(4), Gly(5)-ol]-enkephalin acetate salt (DAMGO), which acts presynaptically to reduce afferent synaptic transmission and postsynaptically on second-order neurons, was administered to cats it attenuated or completely blocked a locomotor rhythm evoked by stimulating high-threshold afferents [23]. Lev-Tov and colleagues extended these findings to the neonatal rat by demonstrating that noxious radiant heat delivered to the skin can elicit bouts of locomotor activity in the tail-attached isolated spinal cord preparations [24]. The sacral segments of the spinal cord contain relay circuits that can activate a rostrally located locomotor CPG that control movements of the hindlimbs [25]. This model system offers the ability to localize sacral afferent input from activation of thoracolumbar CPGs. Lev-Tov and colleagues found that application of μ-opioid agonist DAMGO to the isolated spinal cord of the rat resulted in modest changes in the pharmacologically-induced locomotor rhythm [24].

On the other hand, when the μ-opioid agonist (DAMGO) was applied to spinal cord segments containing the SCA dorsal roots, a complete suppression of SCA-evoked locomotion occurred which could be reversed by application of the μ-opioid antagonist naloxone [24]. These results concur with findings from the cat where high-threshold afferent pathways were depressed by the μ-opioid agonist (DAMGO) following activation of the CPG by L-DOPA [23]. Lev-Tov and colleagues suggested that the SCA recruitment of the lumbar CPG involves recruitment of sacral interneurons by sacral afferents [25,26,27]. These intercalated interneurons project axons into the ventral (VF), ventrolateral (VLF) and lateral (LF) funiculus [27] (Figure 1). Using the neonatal mouse model of SCA-evoked locomotion, we have suggested that afferents projecting through the tracts of Lissauer can contribute to recruitment of the lumbar CPG [28]. This suggests that nociceptive afferents from sacral segments extend collaterals that project rostrally for multiple segments and which can activate the lumbar CPG either directly or indirectly via lumbar relay interneurons (Figure 1).

Molecular insights to pain afferent control of spinal CPG using mouse genetics

Work in the cat, rat, and mouse suggests that thermoreceptors contribute to activation of spinal CPGs. The thermal phenotypes of the C and Aδ nociceptive afferent subsets known to be sensitive to specific temperature thresholds have been identified [29].

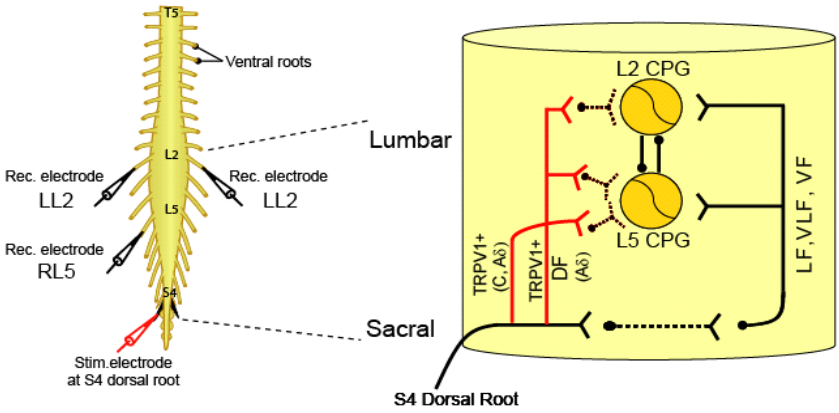

Figure 1. The sacrocaudal afferent pathways that recruit lumbar central pattern generators in the neonatal murine spinal cord. The schematic on the left shows a neonatal mouse spinal cord preparation representing the activation of sacrocaudal afferents (SCA) by electrical stimulation of the dorsal rootlet of the sacral segment 4 (S4). The motor output is recorded as neurograms from the left and right ventral rootlets of the lumbar segment 2 (L2) and left ventral rootlet of lumbar segment 5 (L5). The schematic on the right shows the ascending tracts of SCA pathways that recruit lumbar central pattern generator (CPG) networks. The ascending tracts include primary afferents synapsing onto interneurons in the ventral (VF), ventrolateral (VLF), lateral (LF) funiculi. Ascending tracts recruiting lumbar CPG also include a subset of direct projections of the TRPV1+ (C and Aδ) afferent collaterals projecting into multiple segments in the dorsolateral Lissauer's tract and a subset of TRPV1^{+} (Aδ) afferent collaterals in the medial dorsal funiculus (DF). NOTE: This figure is modified from Mandadi S et al., 2013.[28]

These new molecular tools are extremely useful since they can allow afferent transmission to be controlled and dorsal horn circuits to be targeted. The exciting possibility exists to put these tools to identify a spinal circuit that can modulate locomotion. While the focus has been on the role of nociceptive afferents here it is clear that stimulation of SCA will recruit multiple classes of afferents. Indeed, following administration of DAMGO [24], which attenuates afferent transmission, or capsaicin that produces desensitization of the afferent terminal [30], evoked stimulation can still activate CPGs when the stimulus intensity is increased.

Using genetic approaches we can identify the subpopulations of nociceptive afferents that project onto dorsal horn interneurons. This is an exciting development since it has been recognized for some time that nociceptors are polymodal and when stimulated can produce bouts of stepping activity [20]. Characterizing and identifying subsets of C and Aδ afferents with specific sensory modalities is an important step in examining the spinal circuitry underlying nociceptive-evoked locomotion [30]. The molecular identity of transducers of nociception that characterize subsets of C and Aδ afferents remained unclear until the discovery of a new superfamily of ion channels termed Transient Receptor Potential (TRP) [31]. Identification of temperature-sensitive members of the TRP family (TRPV1 > 43 ºC, TRPV2 > 52 ºC, TRPV3 ~ 37 ºC, TRPV4 ~ 34 ºC, TRPM8 ~ 17 ºC and TRPA1 < 10 ºC) expressed in subsets of C and Aδ afferents have been extremely important for the somatosensation and pain field [29]. The discovery of these members as molecular detectors of temperature with distinct thermal thresholds has allowed for the development of tools to identify sensory afferent pathways involved in the transduction of a wide variety of sensory modalities. Recent work has made use of these tools, to identify subclasses of C and Aδ afferents that express TRPV1 and TRPM8, that contribute to the excitation and inhibition of spinal CPGs, respectively [30]. Consistent with their role in enhancing nociceptive responses, TRPV1 are activated by temperatures greater than 43 ºC and by capsaicin, the noxious component of hot peppers [32].

On the other hand, TRPM8 is expressed in predominantly non-TRPV1 subsets of C and Aδ afferents, detects cold temperatures (< 25 ºC) and can be activated by menthol, the natural ingredient in mentha plants [33,34]. Using *trpv1*$^{-/-}$, *trpm8*$^{-/-}$ and transgenic mice where GFP expression was under control of the TRPM8 promoter, evidence for CPG modulation by both centrally and peripherally expressed TRPV1 and TRPM8 in neonatal mouse spinal cord preparations has recently been obtained [30] (Figure 2).

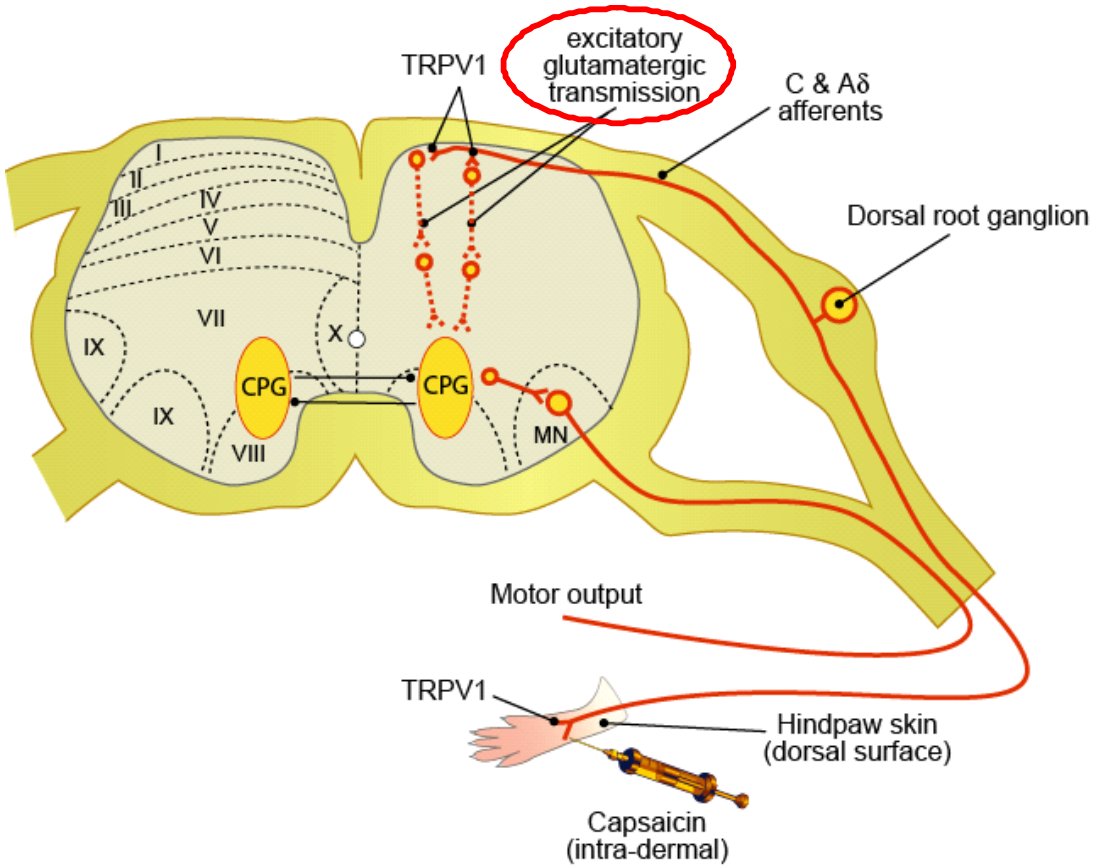

Figure 2. Sensorimotor integration in a neonatal spinal cord. The schematic shows the integration between the TRPV1$^+$ afferents projecting onto the dorsal horn and the locomotor CPG networks in the ventral horn of a neonatal spinal cord. A nociceptive input by activation of TRPV1$^+$ afferents by capsaicin (TRPV1 agonist) can significantly alter the locomotor CPG networks function via polysynaptic connectivity. NOTE: This figure is modified with permission from Mandadi S et al., 2009.[30]

The next step in deciphering the spinal circuit is to identify the target neurons of TRPV1 and TRPM8 afferents. Several classes of dorsal horn interneurons that receive afferent input have been identified over the last decade [35,36]. With regard to pain, in the embryonic spinal cord, subpopulations of neurons that express the *Lbx1* transcription factor receive nociceptive afferent input [36]. *Lbx1* expression in the dorsal horn neurons renders them inhibitory (GABAergic), whereas supression of *Lbx1* by another transcription factor *Tlx3* promotes the development of excitatory (glutamatergic) neurons. The ability of nociceptive afferents to correctly project onto these second-order relay interneurons in the dorsal horn is also under the control of specific transcription factors. For example, the runt-domain transcription factor *Runx1* is necessary for projection of nociceptive afferents to more dorsal laminae of the spinal cord [37]. *Runx1* also determines nociceptive sensory neuron phenotype and its expression during development is required for thermal and neuropathic pain [38]. These genetic approaches allow identified interneurons to be tagged using various fluorescent proteins (GFP, YFP etc), which allows population activation to be measured or individual cells to be targeted for intracellular recordings [39,40]. In an exciting development, identified cells can also be activated or inactivated, allowing the design of experimenters to test whether their roles are necessary and/or sufficient for circuit activity [39,41]. Activation and inactivation can be achieved using pharmacological or optogenetic approaches [42,43].

It is necessary to identity the downstream CPG neurons to which these intercalated neurons project. In that regard identified populations of interneurons in the spinal cord that may contribute to CPG function have been identified using genetic approaches [41,44,45,46]. Much progress has been made in targeting ventral horn interneurons that contribute to the CPG network by identifying transcription factors that specify interneuronal

subtypes [45]. For example, V0 interneurons that are necessary for left-right alternation in a locomotor rhythm can be targeted by altering the expression of a transcription factor *Dbx1* [47]. *Dbx1* mutant mice lacking these commissural interneurons showed a disrupted left-right rhythm. This is one of the many examples of the homeodomain and the helix-loop-helix classes of transcription factors that control expression of anatomically specific populations of interneurons in the ventral horn of the spinal cord [45]. Cells expressing the Hb9 transcription factor located on the midline show oscillatory properties and other intrinsic properties consistent with these cells contributing to network function [48]. Although they are only one of many classes of cells that contribute to network function [49] they form a convenient cluster for the investigation of connectivity. In these cells, both monosynaptic and polysynaptic excitatory postsynaptic currents (EPSCs) can be recorded following stimulation of the dorsal roots. Given that vesicular glutamate transporter 1 (VGlut1) is expressed on fibres contacting the Hb9 cells it is likely that some are from group I afferents [50]. The longer latency responses could come from slowly conducting fibres or from strong disynaptic connections. Given that most C and Aδ fibres terminate in lamina I/II, it seems reasonable to speculate that some second-order interneurons project to interneurons that comprise the CPG.

Neuropathic pain

Neuropathic pain is often observed following central trauma. For example, it affects about 60-80% of individuals with a spinal cord injury [51,52]. Neuropathic pain is often described as a burning or stabbing sensation by patients and can be severely debilitating. Unfortunately this form of chronic pain is permanent and current treatments are not very effective. A comprehensive review of the mechanisms underlying neuropathic pain is beyond the scope of this article. There is some consensus that cytokines at the injury site activate microglia leading to central sensitization of primary afferents onto second order interneurons in the spinal cord. Given the data presented thus far on the effects of acute pain on spinal motor circuits it is reasonable to assume that chronic pain could also interfere with operation of spinal cord networks. Preliminary results on spinal cord preparations from the neonatal mice support this conclusion [53]. When the spinal cord is injured, multiple cytokines and neurotrophic factors are released. Bradykinin and glial cell-derived neurotrophic factor (GDNF) are two pro-nociceptive agents that are released following injury. We have found that intrathecal application of GDNF upregulates the bradykinin receptor B1R (Figure 3). Activation of the upregulated B1R induced sensitization of nociception to noxious heat via TRPV1 and disrupted ongoing locomotor activity. The disruption of locomotor activity suggests upregulation of the TRPV1 afferent connection onto polyneuronal pathways that modulate CPGs (Figure 3). Acute activation of spinal B2R bradykinin receptors resulted in disruption of locomotor network function via TRPV1 (Figure 3). These data suggest that chronic pain conditions can impair operation of nociceptor control of motor networks during development.

Clinical translation

Defining the changes that occur in neuropathic pediatric pain is critical to reach a well-defined management plan. Identifying the pathways, receptors, and chemicals released during this process will help guide therapeutic strategies in the future. The development of the sensory nervous system and pain pathways occur during mid-gestation, and for up to three months post partum[54]. However we continue to see neuropathic pain neglected in clinical practice. The misconception is that most frequent neuropathic pain conditions witnessed in adults rarely occur in the neonates [55]. Over the last two decades the diagnosis of neuropathic pain in neonates has increased. Unfortunately, there is a lack of epidemiological studies to confirm the incidence of neuropathic pain in pediatrics [55].

So far, the clinical data that have been published for neuropathic pain have mainly come from case reports. These case reports describe the intervention used for the neuropathic pain observed in children [55]. It is important to be cautious while reading these case reports, because there has been a lack of clinical trials to support the use of medications in neuropathic pain in children. This is why more research is needed for development of a therapeutic approach for neuropathic pain in the pediatric population. This review demonstrates the function of nociceptors found in spinal pathways that play a role in controlling sensorimotor function. Their role in the pathophysiology of neuropathic pain via TRP channels during development may offer a therapeutic target for the future.

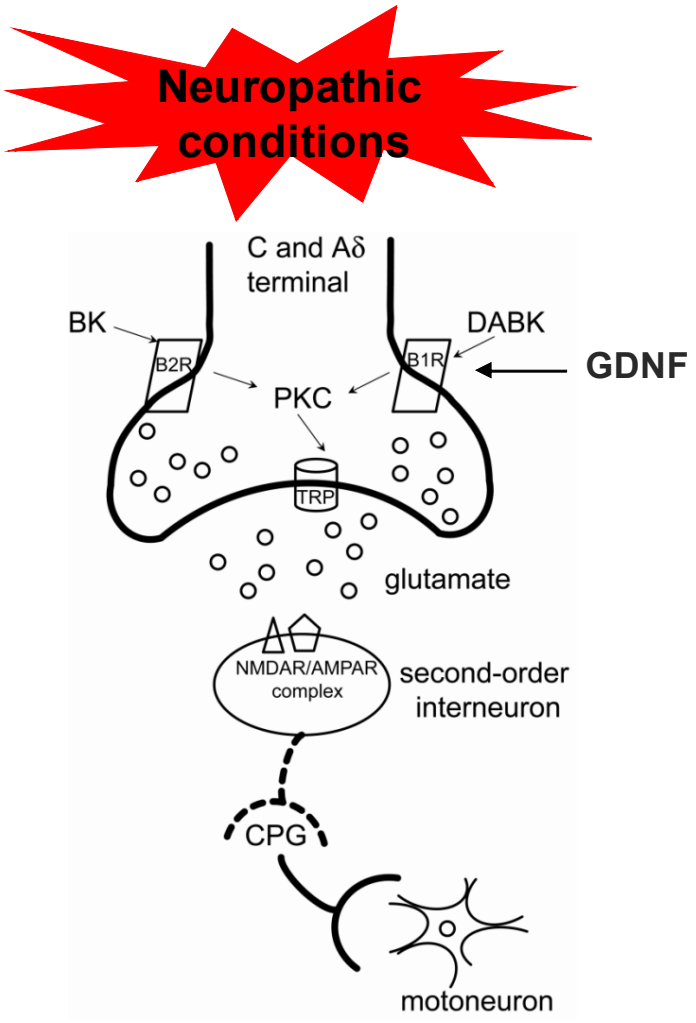

Figure 3. Mechanisms of interaction between kinin receptors and transient receptor potential ion channels in the dorsal horn terminals of the C and Aδ nociceptors . The schematic shows under neuropathic conditions, interactions are mediated by PKC following activation of B1R by des-Arg-BK (DABK) and B2R by bradykinin (BK). PKC action on TRP channels results in modulation of TRP-mediated release of the neurotransmitter glutamate from the terminals. Modulation of glutamate release will alter excitatory glutamatergic mechanisms via NMDA/AMPA receptors in the second-order interneurons in the dorsal horn. This has downstream effects between second-order interneurons and the CPG. For example, an increase in excitatory input from the dorsal horn via TRPV1 can disrupt rhythmic output from the locomotor networks[30].

SUMMARY

There is little doubt that the identification of genes allowing the identification and control of nociceptive receptors, and second-order neurons has revolutionized sensorimotor research. These mouse models can be used to probe function of individual genes through the analysis of gene targeting or specific point mutations. The identification and analysis of genes related to pain pathways in injury models is urgently required to provide new research tools. Furthermore, the application of new optogenetic tools with identified neurons and afferents provides an approach to acutely activate or deactivate neural circuits within the spinal cord [56,57].

It is clear that acute pain can modulate the locomotor networks. Many in the sensorimotor field have made use of radiant heat to activate nociceptors since it is a relatively pure noxious stimulus compared to mechanosensory modalities. This is significant for those with spinal cord injury since thermoreceptors that normally respond to noxious heat/cold alter their sensitivity following peripheral and central trauma. It is plausible that changes in nociceptive afferent input could increase the potential for plasticity of the spinal cord CPGs following injury. Therefore, it is interesting to

speculate that chronic pain in incomplete SCI patients may have some benefit in terms of promoting activity-based recovery of function.

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LIST OF ABBREVIATIONS

Spinal cord injury (SCI), sacrocaudal afferents (SCA), transient receptor potential (TRP), central pattern generator (CPG), ventral funiculus (VF), ventrolateral funiculus (VLF), lateral funiculus (LF), dorsal funiculus (DF), transient receptor potential vanilloid 1 (TRPV1), transient receptor potential melastatin 8 (TRPM8), Ladybird homeobox 1 (*Lbx1*), T-cell leukemia homeobox 3 (*Tlx3*), Runt-related transcription factor 1 (*Runx1*), developing brain homeobox protein 1 (*Dbx1*), glial cell-derived neurotrophic factor (GDNF), bradykinin receptor 1 (B1R), bradykinin receptor 2 (B2R), green fluorescent protein (GFP), yellow fluorescent protein (YFP), gamma-aminobutyric acid (GABA).

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