



Potential of spinal GABA inhibition as a therapeutic target for chronic neuropathic pain: from transplantation to physical exercise

Emiko Senba^{1,2}, Katsuya Kami^{2,3}

¹Department of Physical Therapy, Osaka Yukioka College of Health Science, Ibaraki City, Osaka, Japan; ²Department of Rehabilitation Medicine, Wakayama Medical University, Wakayama City, Wakayama, Japan; ³Department of Rehabilitation, Faculty of Wakayama Health Care Science, Takarazuka University of Medical and Health Care, Wakayama City, Wakayama, Japan

Correspondence to: Emiko Senba, MD, PhD. Department of Physical Therapy, Osaka Yukioka College of Health Science, 1-1-41 Sojiji, Ibaraki City, Osaka 567-0801, Japan. Email: esenba@wakayama-med.ac.jp.

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Chronic neuropathic pain (NPP)

NPP is induced by damage to the somatosensory system, being the most intractable form among chronic pain diseases. More than 20 million individuals have NPP worldwide and suffer from a great economic burden on individuals and society, and lower quality of life with increased comorbidity such as depression and sleep disturbance (1). NPP is still highly refractive to existing pharmacological treatments. Therefore, chronic pain patients suffer drug tolerance, addiction and various side effects of taking overdoses of analgesics for long time (2). So, there is a high-priority demand to find alternate treatments based on the pathogenesis of NPP syndromes.

Although the etiology of NPP is broad from the periphery to the central nervous system, we focus on peripheral nerve injury-induced NPP in this manuscript. We intend to summarize recent scientific advances in the therapeutic strategies for chronic NPP targeting gamma-aminobutyric acid (GABA) interneurons in the spinal dorsal horn, trying to find future direction of NPP treatment.

Spinal inhibition in NPP

GABA is a major inhibitory neurotransmitter in the central

nervous system and especially in the spinal dorsal horn. GABA-containing interneurons are thickly distributed in superficial dorsal horn, constituting about 30% of lamina II neurons. GABAergic inhibitory system has been considered to play a critical role in the pain transmission system.

In order to elucidate the mechanisms of NPP, many studies have focused on spinal dorsal horn neurons. Among these mechanisms the strongest evidence exists for the dysfunction of GABAergic interneurons and altered synaptic transmission of pain signaling pathways in response to peripheral nerve damage. Preclinical studies hitherto have disclosed that peripheral nerve damage induces a selective reduction of GABAergic inhibition in the spinal dorsal horn (3). Apoptotic loss of GABAergic interneurons in the dorsal horn, which was most remarkable 1 week after surgery, has been considered to play a role in the preservation of NPP (4).

More directly, recent work has demonstrated that ablation or hM4Di-mediated silencing of inhibitory interneurons in the spinal dorsal horn causes spontaneous pain and hypersensitivity to thermal and mechanical stimuli (5).

The mechanisms how peripheral nerve injury depresses GABAergic inhibition

The existing line of evidence suggests the loss of inhibitory

tone due to NPP can be caused by mechanisms that can be categorized as: (I) decreased number of GABA neurons (apoptosis), (II) down regulation of GABA synthesizing enzyme, glutamic acid decarboxylase (GAD) (epigenetics), (III) decreased GABA release and/or dysfunction of GABA A/B receptors, (IV) abnormal cation-chloride cotransporters, such as K^+-Cl^- cotransporter-2 (KCC2) in spinal nociceptive neurons. All or some of these may contribute in a concerted manner to the establishment of NPP.

GABA neuron apoptosis

There has been a controversy about the cause of GABA neuron dysfunction. A question in controversy is whether GABA neurons are lost or only GABA synthesis/release is downregulated.

A recent work has clearly answered to this question, demonstrating that peripheral nerve injury evoked excitotoxic cell death of GABA interneurons in the superficial dorsal horn and contributed to chronic hyperalgesia (6). They clearly showed that elimination of NMDARs fully prevents cell death of GABA interneurons and NPP-like behavior in a mouse model of NPP. The finding that mice lacking proapoptotic Bax are protected against GABA neurons loss strongly indicates the causative relationship of apoptosis and GABA neuron loss. They also noticed that surviving neurons maintain GABA synthesis by means of epigenetics. In line with this, another group has reported that upregulated Cdh1 expression in the trigeminal caudalis nucleus attenuated mechanical allodynia by reducing apoptosis of GABA interneurons caused by chronic constriction injury (CCI) of infraorbital nerve. The Cdh1 inhibition is thought to trigger apoptotic cell death.

Taken together, these studies clearly show that peripheral nerve damage induced apoptosis of GABAergic neurons is the primary cause of NPP in animal models.

KCC2 reduction in nociceptive neurons

Another mechanism of NPP is a reduction of GABAergic inhibition due to a depolarizing shift in chloride equilibrium, which is induced by a 50% reduction of KCC2 expression in dorsal horn neurons following peripheral nerve injury. KCC2 is a transporter which maintains intracellular Cl^- concentration at low level (7). Reduction of KCC2 in dorsal horn neurons have been demonstrated in NPP model animals (8), suggesting that reduction of KCC2

expression seriously affects the potency of GABAergic inhibition. GABAergic interneurons receiving A-beta inputs and supplying feed-forward inhibition to nociceptive lamina I projection neurons may paradoxically cause excitation of these projection neurons when KCC2 contents of these neurons are downregulated.

Therapeutic approaches using a surgical procedure

Given the established role of GABAergic dysfunction in the etiology of NPP, restoration of this function represents an attractive and promising therapeutic approach. At least three approaches targeting spinal cord using a surgical procedure have been attempted in preclinical and clinical studies: (I) intrathecal injection of GABA mimetic drugs, (II) spinal cord stimulation and (III) cell transplantation.

Intrathecal injection

Since Eaton *et al.* (9) reported that intrathecal injection of GABA in an animal model of NPP permanently reversed NPP symptoms, a variety of analgesics, including GABA A/B agonists, and even less studied agents has been tried with novel gene-based intrathecal targeting methodologies. However, as Eaton *et al.* (9) has reported, when GABA was administered any later than 2–3 weeks after nerve injury, it was unsuccessful to prevent such NPP symptoms, indicating that this approach is not suitable for chronic NPP.

Spinal cord stimulation (SCS)

After 40 years of clinical practice, it has been proved that SCS is an accepted and cost-effective therapy for the treatment of intractable chronic pain. Preclinical and clinical studies that used high frequency-SCS for a variety of chronic pain syndromes have been reported. The effects of SCS on the spinal GABAergic neurons have been studied in a rat model of NPP (10). In animals that respond to SCS, a significant increase of GAD 65 was observed in superficial dorsal horn, probably reflecting an increased GABA production following SCS. Recently, López-Álvarez *et al.* (11) used a rat model of sciatic nerve transection and repair to show that chronic stimulation protocols restored the amount of KCC2, which had been significantly decreased by nerve injury, in the dorsal horn of these rats.

Cell transplantation of GABAergic interneurons

A different approach to augmenting inhibitory tone, spinal transplantation of GABA neurons, may be a promising NPP therapy. The idea of transplanting cells that secrete GABA or GABAergic neurons directly into the spinal cord of NPP models has been paid much attention.

This approach was first demonstrated in rodent models in which chronic pain behavior was attenuated by grafting GABAergic neurons or cells modulated to secrete GABA by bioengineering (12,13). Then, fetal mouse GABAergic cells or human neural precursor cells (NPCs) differentiated to show a GABAergic phenotype were transplanted into the spinal cord 10 days after spinal nerve ligation surgery (14). The transplants successfully attenuated allodynia induced by peripheral nerve injury.

In the next stage, immature mouse telencephalic GABA progenitor cells were transplanted into the spinal cord of NPP model animals. Jergova *et al.* induced NPP in rats via CCI of the sciatic nerve, then grafted GABAergic precursor cells developed from E14 lateral ganglionic eminence (LGE) cells into the ipsilateral lumbar spinal cord 1 week after the constriction injury (15).

Another study examined the effects of transplanting of GABAergic progenitors from E12.5–13.5 mouse medial ganglionic eminence (MGE) into the spinal cord of mice manifesting NPP due to spared sciatic nerve injury (16). Only 1–3% of grafted cells survived but they could differentiate into GABAergic neurons, constructing significant functional contacts with both primary afferent fibers and dorsal horn neurons. The density of GABA producing enzymes GAD65/67 was also increased in the spinal cord, and mice received these grafts showed a complete loss of mechanical allodynia due to peripheral nerve damage.

In another study from the same group, GABAergic cell grafting in a paclitaxel induced chemotherapy model of NPP showed beneficial effects. In this study, transplanting of E12.5–13.5 mouse MGE progenitors into the spinal cord 1 week after the development of hyperalgesia attenuated NPP symptoms. Interestingly, such symptoms were not attenuated when MGE cells from mice lacking vesicular GABA transporter (VGAT) gene were grafted, indicating that anti-hyperalgesic effects of MGE cell transplantation are dependent on GABA release by grafted interneurons.

Although these previous studies have demonstrated that fetal GABA neuron progenitors can effectively reduce NPP, the multipotent nature of these progenitor cells makes

them unsuitable for clinical application. Multipotent MGE progenitors can differentiate into other cell types, therefore elevating the risk of cancer generation.

Here, Manion *et al.* (17) has recently overcome this problem by showing that terminally differentiated human induced pluripotent stem cell-derived GABAergic (iGABAergic) neurons transplanted into the spinal cord significantly reduced NPP symptoms of mice subjected to spinal nerve injury. They found that iGABAergic neurons can survive in the spinal cord of NPP mice, and these cells were able to alleviate pain long term (~2 months) with a single treatment, demonstrating that this translational strategy is a viable option for the alleviation of established NPP. Differentiated iGABAergic neurons can be used for autologous transplants and it is obvious that this approach has far greater clinical relevance than previous ones.

However, the only caveat, if any, of this approach is that it can be applied to only a limited number of patients because it requires advanced technology and equipment, in addition to a great health care cost.

Non-surgical approach: physical exercise therapy

In this context, recently non-surgical and cost-effective procedure was found to retrieve the reduced GABAergic inhibition in the nerve-injured spinal cord. We demonstrated using mice subjected to partial sciatic nerve ligation (PSL) that treadmill running effectively blocked PSL-induced decrease of GABA and GAD65/67 immunoreactivities, leading to the preservation of GABA in neurons and neuropils in the dorsal horn of these animals (18) (*Figure 1A*). Mechanical hypersensitivity due to PSL was significantly reduced in PSL-Runner mice compared to that in PSL-sedentary mice. Thresholds of pain behavior positively correlated with GABA and GAD65/67 levels and numbers of GABA interneurons in the ipsilateral dorsal horn of PSL-Runner mice. Thus, we concluded that the improvement of impaired GABAergic inhibition constitutes a new mechanism that explains exercise-induced hypoalgesia (EIH).

Effectiveness of physical exercise in attenuating NPP has been established in preclinical and clinical researches. Many studies have attempted to clarify the underlying mechanisms of EIH at various levels of peripheral and central nervous systems [for review see (19)].

The cutting-edge therapy of transplantation surgery will be applied to only a limited number of patients, while physical exercise is a first-line therapy for every patient

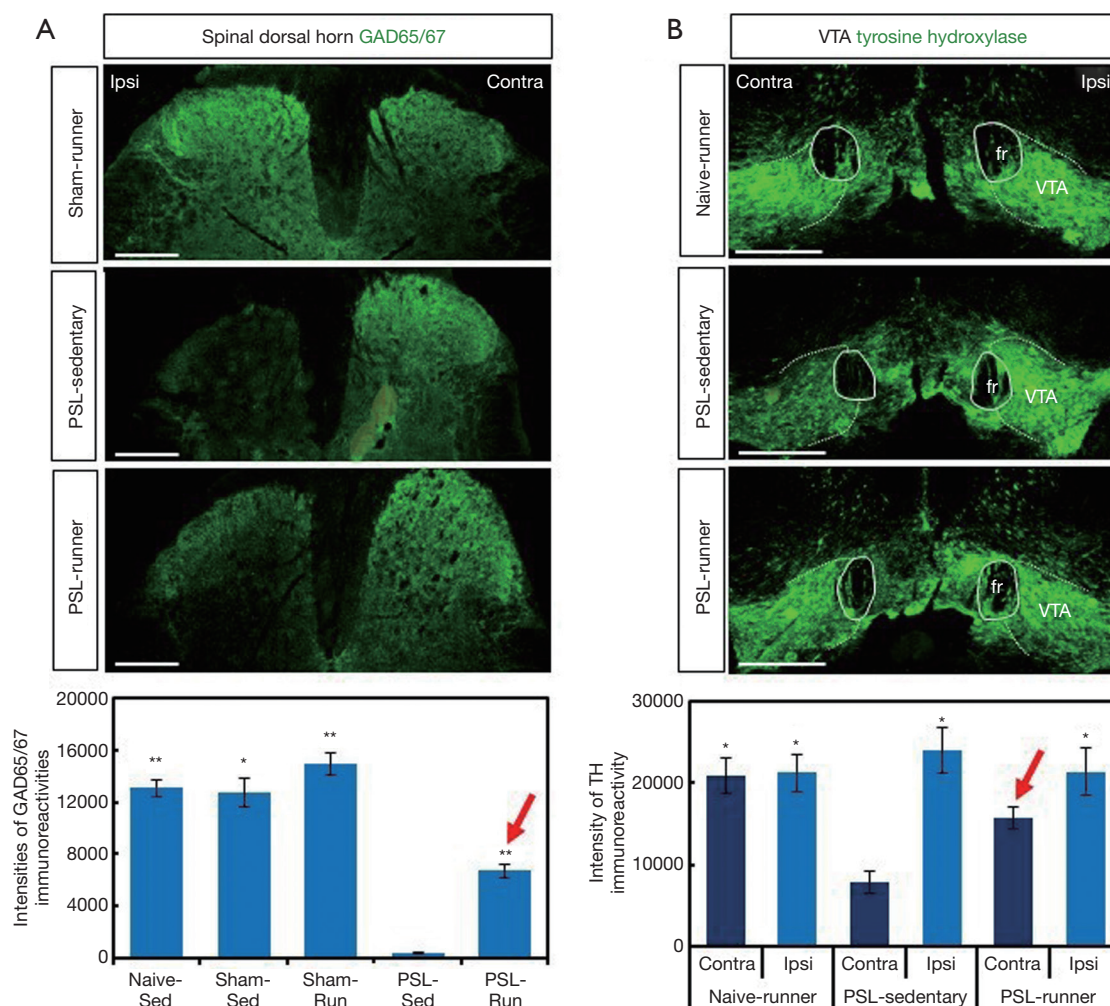


Figure 1 Effects of exercise on spinal GABA synthesis and dopamine synthesis in the VTA. (A) PSL reduced GAD65/67 immunoreactivities in the ipsilateral (ipsi) dorsal horn (L4–5) of PSL-sedentary mice, but exercise by treadmill running prevented it in PSL-Runner mice (indicated by an arrow). Scale bar: 200 μ m. A bar chart shows that GAD65/67 immunoreactivity was significantly weaker in PSL-Sedentary mice compared to the other groups. The significance of differences among groups was calculated by a one-way ANOVA and Tukey's post hoc test (*vs.* PSL-sedentary; *, $P < 0.01$; **, $P < 0.001$; $n = 6$); (B) TH immunoreactivity in the VTA was decreased by PSL surgery in PSL-sedentary mice but voluntary running restored it in PSL-Runner mice (indicated by an arrow). fr: fasciculus retroflexus, Scale bar: 300 μ m. A bar chart shows that TH-immunoreactivities were significantly augmented by voluntary wheel running, while TH-immunoreactivities were significantly decreased in the contralateral (contra) side of the lateral VTA in PSL-sedentary mice compared to the other groups (*vs.* PSL-sedentary contra; *, $P < 0.01$; $n = 5$). Quantified data are presented as the mean \pm SEM. GABA, gamma-aminobutyric acid; VTA, ventral tegmental area; PSL, partial sciatic nerve ligation; GAD, glutamic acid decarboxylase; ANOVA, analysis of variance; TH, tyrosine hydroxylase; SEM, standard error of the mean.

suffering chronic pain. Physical exercise is a safe and cost-effective therapy because it does not require any surgical procedure, any special equipment and can be carried out in anywhere in the world. More recently we have elucidated that physical exercise not only reduces pain by restoring GABAergic inhibition in the spinal cord (18), but also

activates brain reward system, i.e., dopaminergic neurons in the ventral tegmental area (VTA), restoring dopamine synthesizing enzyme tyrosine hydroxylase (TH), which was decreased by PSL surgery (20,21) (Figure 1B).

Voluntary exercise has been considered as a strong natural reward, and the activated mesolimbic reward system

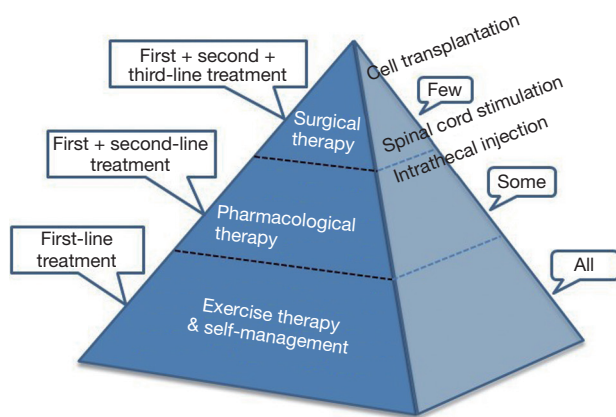


Figure 2 Treatment pyramid for NPP targeting spinal GABA neurons. All patients should be proposed exercise and self-management as first-line treatment, while some will need drug therapy as second-line treatment, and few will need surgical interventions including cell transplantation as third-line treatment [modified from *Figure 1* of ref (25)]. NPP, neuropathic pain; GABA, gamma-aminobutyric acid.

may play a part in suppression of tonic pain. As a matter of fact, we demonstrated that voluntary exercise increases the expression of phosphorylated CRE binding (pCREB) protein in VTA dopamine neurons, which would enhance the transcription of TH gene and then dopamine synthesis, thereby contributing to the activation of the mesolimbic reward pathway in a mouse model of NPP. Voluntary running in PSL mice on a running wheel equipped in each cage for 15 days after PSL surgery remarkably attenuated NPP behaviors compared to those of PSL-sedentary mice and the recovery was in proportion to the total running distance and the levels of pCREB and TH in the VTA of these mice. Activation of brain reward system may also improve depression and sleep disturbance, and eventually improve patients' quality of life. We further elucidated the mechanisms how voluntary exercise can activate VTA dopamine neurons; glutamatergic /cholinergic neurons in the latero-dorsal tegmental nucleus and orexin neurons in the lateral hypothalamic area are activated by exercise and then project to the VTA (21).

Not only the spinal mechanisms, but also extensive plasticity and sensitization of the central nervous system have been implicated in the etiology of chronic NPP. Therefore, chronic pain patients were shown to suffer severe dysfunction in various brain regions involved in perception, emotion, and cognition, and eventually in pain

behavior (22). Cell transplantation approach mainly targets spinal mechanisms, while physical exercise can influence more extensive central brain regions, especially regions mediating pain emotion, in which cortico-limbic system and meso-limbic reward system play pivotal roles. It has been demonstrated that chronic NPP causes dysfunction of cortico-limbic system, such as medial prefrontal cortex, amygdala, nucleus accumbens and hippocampus (23), and VTA dopamine neurons are inhibited via activated glutamatergic inputs from the lateral habenula. Recently we have demonstrated that physical exercise can effectively counteract these chronic NPP-induced dysfunctions and inhibition (Kami *et al.*, in submission). Through these central mechanisms, physical exercise can exert both analgesic and emotional (anti-depressive) influence on chronic pain patients, eventually and effectively improving their quality of life.

Future prospect: NPP treatment strategy

Various surgical and non-surgical therapeutic approaches, targeting the potentiation of GABA interneurons in the spinal cord have been reviewed based on recent publications, focusing on the possibility of future clinical application. Especially, spinal transplantation of iGABAergic cells and physical exercise seem to be most practical and promising, from a viewpoint of safety and effectiveness.

To fully understand and utilize these treatment strategies, mechanistic and translational investigations will be essential, with intimate communication between basic science and clinical researchers for the better experimental designs and clinical trials, to optimize the potential of these therapeutic technologies. It should be emphasized that these two strategies are not conflicting in nature, but mutually complementing, as shown in a recent study that physical exercise and GABAergic neuronal progenitor cell transplants showed mutually beneficial effects in a rodent model of spinal cord injury (24).

The exercise therapy can be carried out alone or combined with pharmacotherapy and surgical treatment. *Figure 2* shows the NPP treatment pyramid, a principle strategy of therapies targeting spinal GABA interneurons, modified from that proposed for osteoarthritis treatment (25). NPP will be best controlled using education and exercise, which requires an active lifestyle alteration by the patient. Exercise and self-management are not only effective but also cost-effective. In a meta-analysis study in which the

cost-effectiveness of first-line treatments were investigated, exercise therapy was assessed to be most cost saving. All the NPP patients should be proposed to exercise as first-line treatment, and drug therapy and surgical intervention will be added when it is necessary. Patients who need second-line treatment might have a fear of dependency on analgesic drugs such as opioids, but the knowledge of the safe and effective third-line therapy will give them more confidence and hope. We hope that the evidence-based information will help patients and medical staffs to communicate each other for the best practice treatments. Multidisciplinary, patient-centered and stepped care strategy is essential for the execution of such clinical guidelines and the selection of suitable non-surgical or surgical treatment options for chronic pain patients.

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