R E V I E W

CNS Neuroscience & Therapeutics WILEY

Electro-acupuncture and its combination with adult stem cell transplantation for spinal cord injury treatment: A summary of current laboratory findings and a review of literature

Yuan-Shan Zeng[1,2,3,4,5] | Ying Ding[1,2,5] | Hao-Yu Xu[1] | Xiang Zeng[1,3,5] |
Bi-Qin Lai[1,3,4,5] | Ge Li[1,3,5] | Yuan-Huan Ma[1,3,5]

[1]Key Laboratory for Stem Cells and Tissue Engineering (Sun Yat-sen University), Ministry of Education, Guangzhou, China

[2]Department of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong Province, China

[3]Guangdong Provincial Key Laboratory of Brain Function and Disease, Guangzhou, Guangdong Province, China

[4]Co-innovation Center of Neuroregeneration, Nantong University, Nantong, China

[5]Institute of Spinal Cord Injury, Sun Yat-sen University, Guangzhou, Guangdong Province, China

Correspondence
Yuan-shan Zeng, Department of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, 74# Zhongshan 2nd Road, Guangzhou 510080, China.
Email: zengysh@mail.sysu.edu.cn

Abstract

The incidence and disability rate of spinal cord injury (SCI) worldwide are high, imposing a heavy burden on patients. Considerable research efforts have been directed toward identifying new strategies to effectively treat SCI. Governor Vessel electro-acupuncture (GV-EA), used in traditional Chinese medicine, combines acupuncture with modern electrical stimulation. It has been shown to improve the microenvironment of injured spinal cord (SC) by increasing levels of endogenous neurotrophic factors and reducing inflammation, thereby protecting injured neurons and promoting myelination. In addition, axons extending from transplanted stem cell-derived neurons can potentially bridge the two severed ends of tissues in a transected SC to rebuild neuronal circuits and restore motor and sensory functions. However, every single treatment approach to severe SCI has proven unsatisfactory. Combining different treatments—for example, electro-acupuncture (EA) with adult stem cell transplantation—appears to be a more promising strategy. In this review, we have summarized the recent progress over the past two decades by our team especially in the use of GV-EA for the repair of SCI. By this strategy, we have shown that EA can stimulate the nerve endings of the meningeal branch. This would elicit the dorsal root ganglion neurons to secrete excess amounts of calcitonin gene-related peptide centrally in the SC. The neuropeptide then activates the local cells to secrete neurotrophin-3 (NT-3), which mediates the survival and differentiation of donor stem cells overexpressing the NT-3 receptor, at the injury/graft site of the SC. Increased local production of NT-3 facilitates reconstruction of host neural tissue such as nerve fiber regeneration and myelination. All this events in sequence would ultimately strengthen the cortical motor-evoked potentials and restore the motor function of paralyzed limbs. The information presented herein provides a basis for future studies on the clinical application of GV-EA and adult stem cell transplantation for the treatment of SCI.

1 | INTRODUCTION

There is presently no effective treatment for severe spinal cord injury (SCI) because the microenvironment of the injured spinal cord (SC) tissue is not conducive to self-repair of disrupted neural pathways.[1] As such, improving the microenvironment of injured SC tissue, so that it promotes tissue repair, has been the focus of intense research.[2] Neurotrophic factors that have been used for this purpose include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin (NT-3).[3]

Both NGF and its receptor, tyrosine kinase (Trk)A, mainly act on sensory neurons,[4] with no obvious effects on motor neurons. BDNF and its receptor, TrkB, act on a limited number of neuron types.[5] NT-3 and its receptor, TrkC, play an important role in the development and differentiation of neurons, as well as in the survival and axonal regeneration of damaged central neurons (in the brain and SC).[6] Furthermore, NT-3 has also been shown to promote the regeneration of corticospinal tract nerve fibers after SCI,[7] although the effect is not maintained over a long period. To address this problem, our research team has adopted a neuromodulation technique, based on traditional Chinese medicine acupuncture combined with modern electrical stimulation (EA) to trigger the continuous secretion of endogenous NT-3 in the SC.

In traditional Chinese medicine, paraplegia caused by SCI is categorized as an "indolence," "wind," and "sputum" syndrome and is thought to result from damage to the Governor Vessel (GV),[8] which regulates the function of all yang medians. SCI is associated with GV damage, which prevents the flow of qi and blood, leading to atrophy and loss of muscle function.[9] In theory, EA at the GV acts directly on a diseased area such as the injured SC to replenish the yang medians and increase their accessibility, thereby alleviating paraplegia. Moreover, EA at the GV acupoints (located at the posterior midline of the trunk) is the preferred treatment for paraplegia caused by SCI.[8-12]

A key concept of GV-EA is that stimulation at specific somatic tissues/acupoints can modulate internal SC physiology. In GV-EA, a small low-frequency pulsed current is delivered through needles inserted at the GV acupoints, which ventilates the meridians to promote qi and blood flow.[8] Thus, this therapy has the dual effects of acupuncture and electrical stimulation. Furthermore, GV-EA has been shown to improve traumatic paraplegia.[13] Some aspects of the mechanism underlying this effect have been elucidated over the last two decades. Both EA and GV-EA can alleviate secondary damage after SCI, protect neurons, and stimulate nerve fiber regeneration and functional recovery by regulating the levels of neurotrophic factors, neurotransmitters, neuropeptides, and intracellular signaling pathway components.[14-17]

Our previous work has demonstrated that GV-EA improves the microenvironment of the injured SC and promotes the regeneration of damaged nerve fibers and functional recovery.[18] EA was shown to stimulate the migration, proliferation, and differentiation of endogenous neural stem cells (NSCs) at the injury site in the SC of rats.[19-21] Under normal conditions, these NSCs are induced to differentiate into astrocytes by factors in the microenvironment[22] that participate in the formation of glial scars for tissue stabilization and repair. NSCs rarely differentiate into neurons to replace those that have died due to injury; therefore, whereas GV-EA can promote SC repair, the depletion of endogenous NSCs, or their insufficient differentiation into neurons, prevents the restoration of neuronal circuits in the absence of any transplantation of exogenous NSCs.[23]

The transplantation of NSCs into the injured SC is a cell-based therapy that has been used for more than two decades.[1] When embryonic neural tissue is transplanted into the developing or adult SC, NSCs or newborn neurons replace lost nerve cells, contributing to the reconstruction of neuronal circuits and functional recovery.[24] NSCs can be induced to differentiate into neurons, astrocytes, and oligodendrocytes under appropriate conditions. NSCs for transplantation are mainly derived from embryonic and adult tissues. Adult NSCs repair SC injury by replacing dead neurons and thus facilitate the reconstruction of neuronal circuits[25]; the myelination of regenerated nerve fibers,[26] and secreting neurotrophic factors to improve the microenvironment of the injured SC and promote nerve fiber regeneration.[27]

Bone marrow mesenchymal stem cells (MSCs)—another type of adult stem cell that can be isolated from a variety of sources—rapidly proliferate in vitro and exhibit multidirectional differentiation potential. When transplanted into the body, MSCs can be induced to differentiate into chondroblasts, osteoblasts, adipocytes, myoblasts, cardiomyocytes, hepatocytes, oligodendrocytes, and neuron-like cells by factors in the local microenvironment.[28,29] As there are no ethical constraints to autologous transplantation of MSCs and no risk of immunologic rejection, MSCs can serve as substrates for cell and gene therapy in a variety of diseases[1] and represent a potential cellular source for SCI treatment. However, only a small number of adult NSCs differentiate into neurons following direct transplantation into the injury/graft site of the SC, whereas grafted adult MSCs are less likely to differentiate into neuron-like cells, and therefore cannot replace dead neurons. The efficiency of cell-based therapy for SCI can potentially be enhanced by other treatment strategies such as EA.

It is apparent from the literature that there are some critical issues and inherent limitations that need to be fully addressed whether we are to treat SCI more effectively. We consider the following strategies crucial for the better treatment of SCI: (1) to improve the

microenvironment of the damaged tissue so it is conducive for the enhancement of nerve regeneration after SCI; (2) to replace the dead neurons, oligodendrocytes, and other major functional cells, and (3) to promote the better integration of replacement functional cells into the original neural network of the SC. Collectively, these factors will eventually help to repair the damaged structure and restore function in the SC. In this review, we summarize the research conducted over the last two decades relating to the therapeutic potential of GV-EA in combination with adult stem cell transplantation for the repair of the injured SC, paying particular focus upon the data generated by our laboratory.

2 | GV-EA PROMOTES THE SURVIVAL OF INJURED SC NEURONS

The application of acupuncture and EA for the treatment of nervous system disorders has become widely accepted over recent years, particularly as the mechanistic basis of their effects have been elucidated.[30] Recent research revealed that the neuroprotection provided by EA was associated with activation of the parasympathetic nervous system in experimental stroke model animals.[31] EA pretreatment also can increase ambient endocannabinoid levels and result in activation of the ischemic penumbral astroglial cannabinoid type 1 receptor, which led to moderate upregulation of extracellular glutamate that protected neurons from cerebral ischemic injury.[32] In addition, EA can promote the survival and synaptic plasticity of hippocampal neurons and improve spatial memory disorders caused by sleep deprivation.[33] Interestingly, a previous research suggested that EA protected cerebral hippocampal neurons in vascular dementia by inhibiting the expression of p53 (a tumor suppressor) and Noxa (p53 downstream effector) in hippocampal CA1 region.[34] In response to EA, neurons synthesize and secrete specific proteins and neuropeptides that transduce the electrical signals. At different frequencies, EA can reportedly cause the central nervous system to release different types of neuropeptides that promote tissue recovery.[35] Neuropeptides are a class of neurotransmitters or neuromodulators that are characterized by slow induction and prolonged action in a manner that is comparable to the slow conduction of EA needles.

The SC receives direct peripheral sensory input from the trunk and limbs, with peptidergic nerve fibers projecting to the dorsal horn. The fiber endings localized at the acupoints sense the needle and then release peptide substances (e.g., substance P [SP], vasoactive intestinal peptide [VIP], and neuropeptide [NP]Y).[36] Research suggests that GV-EA causes cells in the damaged SC to secrete acupoint-specific proteins and neuropeptides that promote neuronal survival. In our laboratory, we found that GV-EA increases the expression of acupoint-specific proteins, annexin (ANX)A5, and collapsin response-mediated protein (CRMP)2 in the injured SC tissue of rats.[16] Moreover, the application of EA at non-acupoints, and at the Huatuo-jiaji acupoint, has been shown to downregulate these proteins.

In addition, ANXA5 and CRMP2 have been shown to exert protective effects on injured neurons in the SC nucleus dorsalis.[16] In other studies, we showed that GV-EA, EA at non-acupoints, and EA at the Huatuo-jiaji acupoint, has distinct effects on calcitonin gene-related peptide (CGRP) levels in the injured SC tissue of rats; the application of GV-EA increased these expression levels.[37,38] Moreover, CGRP promotes the survival of injured neurons in the nucleus dorsalis and red nucleus of the midbrain. At the Huatuo-jiaji acupoint, GV-EA was also found to be more effective than EA in reducing the expression of nitric oxide synthase in injured neurons of the nucleus dorsalis, thus enhancing neuronal survival.[39] These findings provide evidence that EA can stimulate afferent nerve fibers in GV acupoints to induce the synthesis and secretion of specific proteins or neuropeptides by SC neurons to promote the survival of injured neurons.

3 | GV-EA STIMULATES THE SC VIA AFFERENT NERVE FIBERS OF THE MENINGEAL BRANCH

The use of GV-EA for the treatment of SCI in rats is generally performed at acupuncture points below the spinous processes of the vertebral column corresponding to the upper and lower segments of the injury site (at an insertion depth of about 5 mm that is achieved by twirling, Figure 1). The positive and negative electrodes deliver a low-frequency electrical pulse through upper and lower 0.30-mm diameter needles.[37] In our previous work, stimulation was performed with a sparse-dense wave alternating stimulation pulse (alternating between 50 Hz/1.05 s and 2 Hz/2.85 s, with a pulse width of 0.5 ms), at a current intensity of approximately 5 μA between the two acupoints flanking the lesion site (the intensity was determined based on the observation of a slight vibration of the rat's muscle); the retention time was 20 min, and the frequency was once every other day.[40]

In our previous studies, we speculated that GV-EA acts directly on the meningeal branch and perhaps also the dorsal (posterior) ramus of the spinal nerve of the SC. The acupuncture needle that penetrates the acupoint should pass through the skin, supraspinous ligament, interspinous ligament, and ligamentum flavum. It has been reported that the posterior longitudinal ligament of the vertebral bodies is also innervated by nerve endings of the meningeal branch.[41] The meningeal branch contains the sensory afferent nerve fibers from the dorsal root ganglion (DRG) and some sympathetic nerve fibers originating from the adjacent paravertebral ganglia.[41] We hypothesized that electrical stimulation would activate the GV acupoint to produce local sensory signals that are conveyed to the SC through afferent nerve fibers.[40]

To test this hypothesis, we injected red fluorescent cholera toxin B subunit CTB-555 into the GV acupoint region to mark afferent nerve fibers of the meningeal branch entering the SC across the DRG. Subsequently, we observed CTB-555 into the GV acupoint region to mark afferent nerve fibers of the meningeal branch entering the SC across the DRG. CTB-555 labeling was observed along
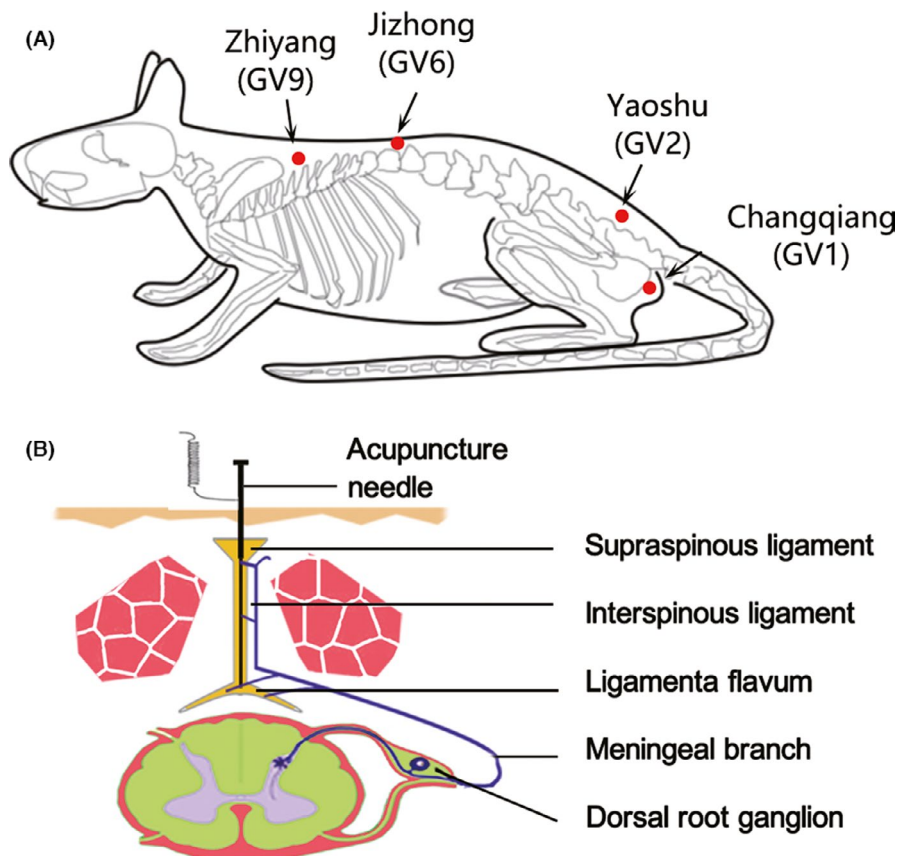
FIGURE 1 Showing two pattern diagrams of the location of the GV-EA, taken from Yang Yang et al., CNS Neurosci Ther (2021).[93] (A) A pattern diagram indicates selected electro-acupuncture acupoints located at GV in rat. Arrows show the four GV acupoints: Zhiyang (GV9), Jizhong (GV6), Yaoshu (GV2), and Changqiang (GV1). (B) Another pattern diagram of the afferent nerve pathway of GV-EA stimulation and the figure indicates that the stimulation information of GV-EA might have been transmitted first to the dorsal root ganglion (DRG) by peripheral branches of meningeal branch and finally transmitted to the spinal cord by the DRG's central branches

the needle trajectory and in nerve fibers of the meningeal branch in the posterior longitudinal ligaments of the vertebrae. Moreover, CTB-555-labeled sensory neurons were observed in the DRG, and sensory afferents labeled with red fluorescence were visible in the dorsal horn in corresponding segments.[40] These results support the ability of afferent nerve fibers of the meningeal branch, which ramify in GV acupoints, to transmit EA stimulation to the SC (Figure 2).

GV-EA increases the expression of CGRP in injured SC tissue; this may be attributable to electrical signals transmitted to the inside of the SC via the afferent nerve fibers of the meningeal branch of the SC.[37] SC tissue does not itself synthesize CGRP, which is thought to be mainly produced by DRG sensory neurons in the corresponding spinal segments and transmitted to the dorsal horn of the SC through afferent nerve fibers.[42] To investigate this possibility, we performed unilateral dorsal root transection combined with lidocaine injection at GV acupoints to block the local delivery of information by afferent nerve fibers in the meningeal branch of the SC. This strategy resulted in the downregulation of CGRP in the injured SC segment. Next, we speculated that electrical stimulation from GV-EA might be able to reach injury sites in the SC via afferent nerve fibers of the meningeal branch of the spinal nerve that are distributed at the GV acupoint. Subsequently, we demonstrated unequivocally that GV-EA can induce the upregulation of CGRP in the DRG cells and its subsequent release at the injury site of SC.[37,40] Collectively, these data indicate that the electrical signals generated by GV-EA stimulation are transmitted through afferent nerve fibers in the meningeal branches to the relevant spinal cord segment, in which their nerve endings secrete a neuropeptide (CGRP) to exert neurobiological effect.

4 | GV-EA INDUCES THE SYNTHESIS AND SECRETION OF NEUROTROPHIC FACTORS BY CELLS IN THE INJURED SC

Following SC transection and demyelination, cells in the injured area and adjacent tissue undergo a number of pathophysiological changes, including the reduced synthesis and secretion of neurotrophic factors; this is one of the main factors underlying the inhibition of nerve repair. An important therapeutic mechanism of GV-EA in SCI is to induce cells in the SC to synthesize and secrete endogenous neurotrophic factors,[43] which creates a microenvironment that is conducive to neuronal survival and axonal regeneration.[14,44,45]

NT-3 is a neurotrophic factor that plays an important role in preventing the death of injured neurons, enhancing neuronal survival and axonal regeneration, and inducing endogenous oligodendrocyte precursor cells to differentiate into mature oligodendrocytes that can restore the myelin sheath.[46] GV-EA has been shown to stimulate the synthesis and secretion of NT-3 by various cell types in transected SC tissue,[47-49] including neurons, astrocytes, microglia/macrophages, and oligodendrocytes. The secretion of NT-3 by these cells progressively increases from Day 1 through 7 of GV-EA application; the level on Day 7 is significantly higher than that prior to the application of EA..[47] We also found that NT-3 levels were
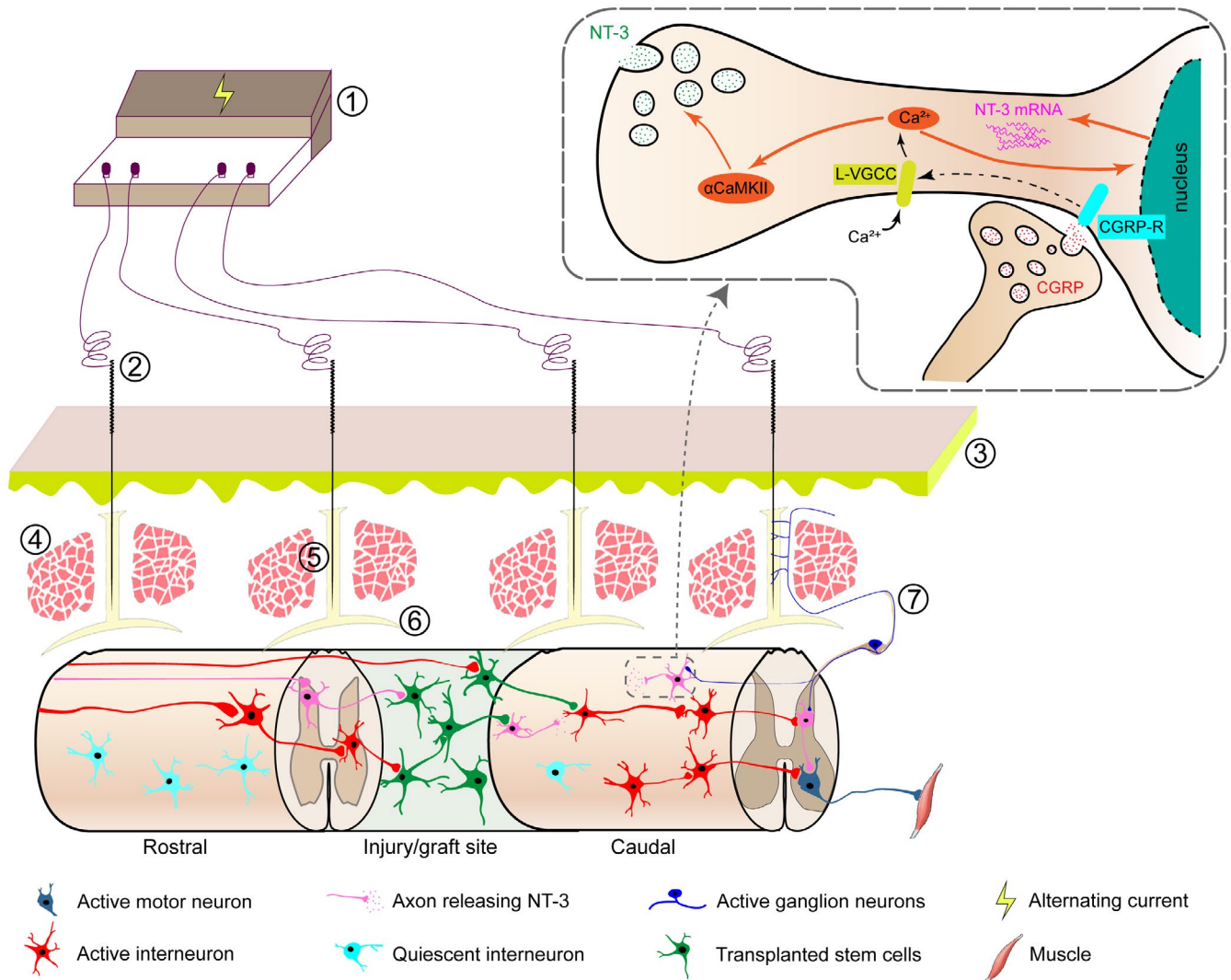
FIGURE 2 Schematic illustration of Governor Vessel electro-acupuncture (GV-EA) combined with stem cell transplantation for spinal cord injury (SCI) repair. ① EA therapeutic apparatus; ② acupuncture needle; ③ skin; ④ lumbus muscle; ⑤ supraspinous ligament, interspinous ligament, ligamenta flavum, and vertebral periosteum; ⑥ spinal dura mater; ⑦ meningeal branch

significantly higher in the EA group than in the control group on Days 14 and 28.[48,49] Other studies have confirmed that GV-EA can increase the synthesis and secretion of NT-3 in the demyelinated SC tissue of rats.[50-52]

GV-EA increases the expression of endogenous NT-3 in the SC, activates NT-3/TrkC/protein kinase B (AKT) signaling, and enhances TrkC-overexpressing NSC-derived neurons of transplanted neural network tissue to promote the establishment of synaptic connections with host neurons that secrete NT-3.[53] However, the mechanisms underlying these effects are unclear. GV-EA induces the depolarization of neurons,[54,55] thus leading to the opening of L-type voltage-gated Ca^{2+} channels (L-VGCC) and Ca^{2+} influx; this process activates calcium/calmodulin-dependent protein kinase (αCaMKII) signaling and the synthesis and secretion of neurotrophic factor (e.g., NT-3).[55]

Thus, GV-EA may directly stimulate the afferent nerve fiber endings of the meningeal branch of the SC in adjacent segments and

induce the release of CGRP by DRG neurons into the SC. CGRP acts on spinal neurons expressing receptor activity-modifying protein (RAMP)1, causing the opening of L-VGCC, and ultimately leading to the release of NT-3.[40] There is increasing evidence that epidural electrical stimulation or transcutaneous SC stimulation promotes the synthesis and secretion of neurotrophic factors in the injured SC, which improves the local microenvironment and activates neural circuits in the SC[56,57] through mechanisms similar to those of GV-EA.

It is important to note that following GV-EA, NT-3 is not just produced by neurons in the SC; there is evidence that endogenous NT-3 is also produced by the astrocytes. NT-3, along with microglia and macrophages may also contribute to the restoration of damaged SC tissue. NT-3, synthesized and secreted by reactive astrocytes in the rostral and caudal host tissues adjacent to the injury/graft site of SC, may protect the damaged neurons and facilitate axonal regeneration of transplanted NSC-derived neurons or MSC-derived neuron-like cells and host neurons.[46,58,59] In addition, NT-3 secreted by activated

microglia/macrophages may induce the polarization of proinflammatory M1-type microglia/macrophages to anti-inflammatory repair M2-type microglia/macrophages, thus reducing the inflammatory response.[60-63] However, the exact mechanisms by which NT-3 inhibits the inflammatory response for SCI repair have yet to be elucidated.

5 | GV-EA PROMOTES NT-3 SECRETION IN THE INJURED SC TO ALLEVIATE MUSCLE ATROPHY IN PARALYZED LIMBS

Studies have shown that sensory and motor functions are lost below the level of injury following severe SCI.[64] Due to the loss of innervation, the volume and weight of the muscle in the paralyzed limbs are reduced as the muscles undergo atrophy,[65,66] thus further hindering the recovery of motor function. Therefore, maintaining muscle function is essential for functional recovery from SC injury.[67] Although the ascending and descending neural pathways are transected in a thoracic segment SCI, below the injury level, the structural basis of the neural circuits that regulate the lower limbs remain in the lumbar segment of the SC.[68-70] These neural circuits may be functionally silent, and their reactivation can promote the nerve-muscle connection and prevent muscle atrophy.[65-70] We applied GV-EA to rats with a completely transected SC in the thoracic segment and found that muscle atrophy in the paralyzed hindlimbs was improved, which was associated with increased survival of motor neurons and NT-3 expression in the lumbar SC.[71] This may be attributed to the transmission of electrical stimulation to the SC segment through primary afferent nerve fibers.[40,66,72]

NT-3 also plays an important role in neuroprotection, axon regeneration, and synaptic plasticity.[73,74] The effects of GV-EA on the survival of motor neurons in the lumbar SC are speculated to be related to an increase in NT-3 levels.[71] GV-EA also stimulates the release of neurotransmitters (such as CGRP and acetylcholine, among others) in the lumbar SC, which enhances the excitability of specific neuronal circuits that lead to the contraction of target muscles innervated by the surviving motor neurons, thereby alleviating muscle atrophy.[40,71] Based on the above results, it is reasonable to suggest that electrical stimulation can improve muscle atrophy in paralyzed limbs in the early stages of SCI, laying the foundation for the next step of adult tissue stem cell transplantation to enhance the voluntary motor function of paralyzed limbs.

6 | GV-EA ALLEVIATES SCI BY PROMOTING THE INTEGRATION OF TRANSPLANTED STEM CELLS

Approximately half of all SCIs involve complete SC transection, with little or no remaining nerve tissue in the injured segment. Restoration of SC tissue—for example, through cell transplantation—is essential for the effective treatment of SCI.[1,75] NSCs or MSCs transplanted into the injured SC are induced to differentiate into neurons or neuron-like cells and glial cells that restore SC structure and function. Transplanted NSCs have been shown to differentiate into neurons and glia, thereby reducing secondary damage to the SC and improving spinal motor function.[76] However, they are also more likely to differentiate into astrocytes than neurons, which is a major barrier to the clinical application of cell-based therapies for SCI.

One way to overcome this problem is to use GV-EA in combination with NSC transplantation to treat SCI.[77] GV-EA has been shown to promote the survival, migration, and neuronal differentiation of NSCs transplanted into the injury/graft site of the SC.[77] This strategy can also enhance the survival of injured host neurons and nerve fiber regeneration, as well as evoked potentials in the cerebral cortex motor; these processes help to reduce muscle atrophy and immobility in paralyzed limbs.[49,78,79] These effects can be attributed to the GV-EA-induced stimulation of NT-3 secretion by cells in the injured SC tissue.

The NT-3-induced activation of intracellular TrkC/AKT signaling is known to promote the differentiation of NSCs into neurons; this process can be blocked by the application of TrkC/AKT inhibitors.[80] Thus, GV-EA can exert multiple therapeutic effects in SCI, including improving the local microenvironment, increasing endogenous levels of NT-3, activating TrkC/AKT signaling, enhancing the survival and differentiation of neurons, and promoting synapse-like junction formation by transplanted NSCs, as well as their integration in spinal neuronal circuits; collectively, these processes lead to the restoration of sensory and motor functions.[53]

However, it is important to consider that there are key ethical barriers to NSC transplantation that limit its translational potential. In clinical studies of spinal cord treatment, the embryonic neural stem cells (NSCs) that are used are generally derived from human embryonic brain tissue or human embryonic spinal cord tissue. The ethical debate over whether embryonic NSC technology is "life-damaging" was triggered by the destruction of embryos caused by selective abortion.[81] However, most researchers agree that the process of consent for human embryonic neural stem cell research, and its clinical application, is fully informed and voluntary.[82] Due to the fact that this is still an area associated with significant debate, the application of neural stem cells may be limited to translational clinical research for the time being. In contrast, there is no risk of immunological rejection or ethical issues associated with the transplantation of autologous MSCs; this process has been used successfully for the repair of bone, cartilage, tendon, and muscle tissues.[83] Thus, MSC transplantation may also be used to repair injured SC.[84,85] A previous *in vitro* study demonstrated that NT-3 induces MSCs to overexpress the NT-3 receptor (TrkC) to differentiate into neuron-like cells.[48,86] Within the last 10 years, studies in our laboratory, and others, demonstrated that the combination of EA and MSC transplantation provides a greater advantage than either procedure alone when applied to animals with SCI.[77,87-90]

Based on this observation, we used a combination of GV-EA and the transplantation of *TrkC* gene-modified MSCs to treat SCI.[48,87,91,92] This led to increased levels of NT-3 expression at the injury/graft site in the SC and adjacent tissue; enhanced survival of

neuron-like and oligodendrocyte-like cells differentiated from TrkC-overexpressing MSCs; a decreased number of glial fibrillary acidic protein (GFAP)-positive astrocytes; the reduced accumulation of the

nerve regeneration inhibitory factor, chondroitin sulfate proteoglycan (CSPG) in the extracellular matrix; the increased accumulation of the nerve regeneration-promoting factor laminin; an increased number of
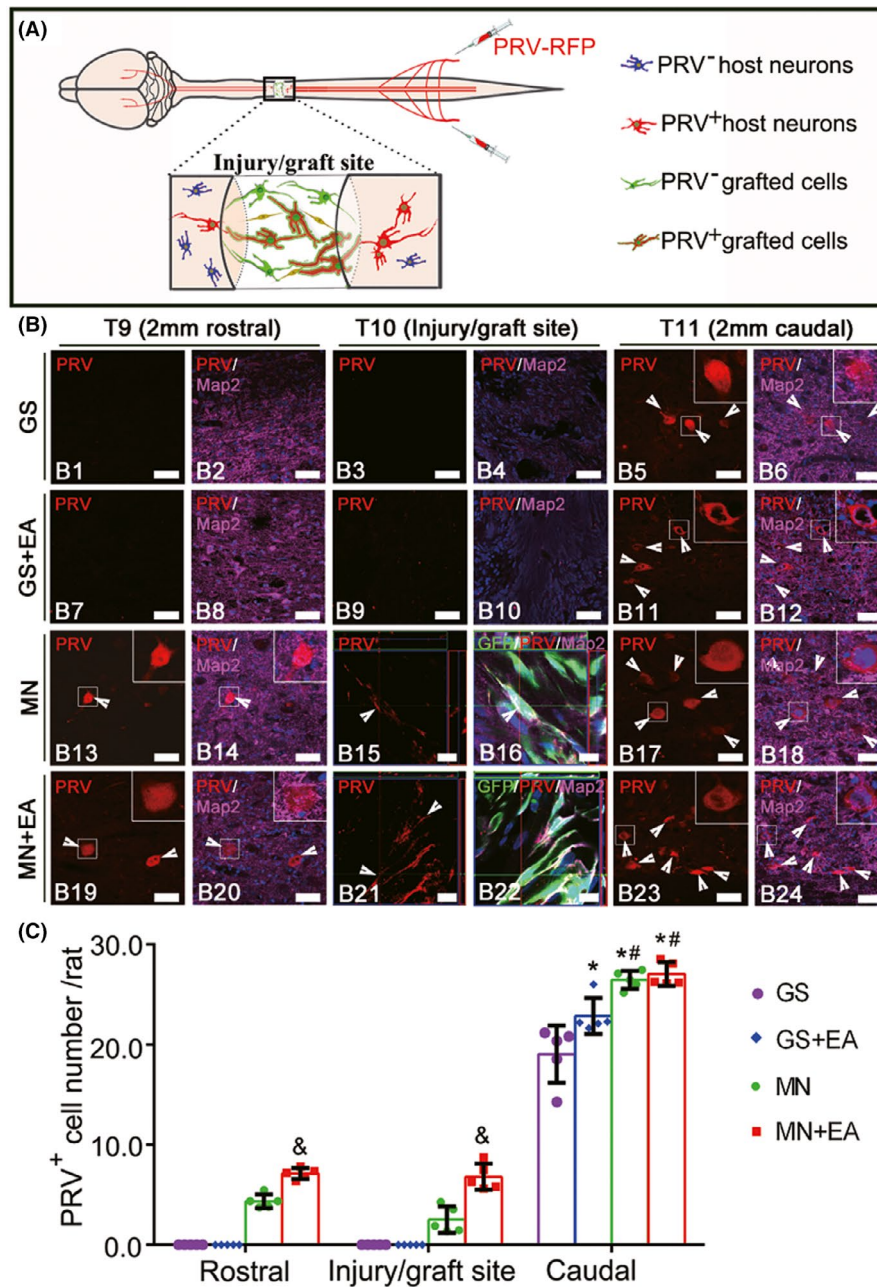

FIGURE 3 Pseudorabies virus (PRV) retrograde transsynaptic labeling confirmed the integration of transplanted mesenchymal stem cell (MSC)-derived neuron-like cells into the host spinal cord neuronal circuit, taken from Yang Yang et al.,[93] CNS Neurosci Ther (2021). (A) A schematic diagram showing that PRV that was injected into the sciatic nerve was transported from the caudal area to the rostral area through the injury/graft site of the spinal cord. (B) Representative images showing the host neurons or MSC-derived neuron-like cells retrogradely labeled with PRV (red, arrowheads) in the rostral and caudal regions relative to the graft tissue of spinal cord in the gelatin sponge scaffold (GS) group (B1–B6), GS+EA group (B7–B12), MSC-derived neural network (MN) group (B13–B18), and MN+EA group (B19–B24). The cell nuclei were counterstained with Hoechst33342 (Hoe). (C) Bar chart showing the number of PRV$^+$ neurons in the T9, T10, and T11 areas of the 4 groups. Values represent the mean $\pm$ SD. $n = 5$/group. $^*p < 0.05$, compared with the GS group, $^\#p < 0.05$, compared with the GS+EA group, and $^\&p < 0.05$, compared with the MN group by one-way ANOVA with LSD-t. Green fluorescent protein (GFP, green), PRV (red), microtubule-associated protein (Map2, white), and Hoe (blue). Scale bars =50 μm in (B1)–(B14), (B17)–(B20), (B23), and (B24); 10 μm in (B15) and (B16), (B21), and (B22). GS: gelatin sponge scaffold with no cells; GS+EA: GS combined electro-acupuncture; MN: MSC-derived neural network; MN+EA: MN combined with electro-acupuncture

TABLE 1 A summary of the combination strategy of EA and adult tissue stem cell transplantation in an animal model of SCI

Strategies [Ref.]	Species/Age and sex of animals	Cellular source of the stem cells	Neural cell differentiation of transplanted stem cells in the injury/ graft site of spinal cord	Regenerating nerve fibers on in the injury/ graft site
EA+grafted NSCs[77]	Rat/Adult/Female	Rat hippocampus	Showing neuron-like cells and astrocyte-like cells	No nerve fiber regeneration shown
EA+grafted MSCs[87]	Rat/Adult/Female	Rat hippocampus	Showing neuron-like cells and oligodendrocyte-like cells	Showing nerve fiber regeneration
EA+grafted BMSCs[88]	Rat/Adult/Male	Rat bone marrow	Showing neuron-like cells and astrocyte-like cells	No nerve fiber regeneration shown
EA+grafted MSCs[92]	Rat/Adult/Female	Rat bone marrow	No cell differentiation was shown	Showing nerve fiber regeneration
EA+grafted BMSCs[89]	Rat/Adult/Male	Rat bone marrow	No cell differentiation was shown	No nerve fiber regeneration shown
EA+grafted MSCs[91]	Rat/Adult/Female	Rat bone marrow	Showing neuron-like cells and oligodendrocyte-like cells	Showing nerve fiber regeneration
EA+grafted TrkC-MSCs[48]	Rat/Adult/Female	Rat bone marrow	Showing neuron-like cells and oligodendrocyte-like cells	Showing nerve fiber regeneration
EA+grafted NR-MSCs[47]	Rat/Adult/Female	Rat bone marrow	Showing neuron-like cells and oligodendrocyte-like cells	No nerve fiber regeneration shown
EA+grafted TrkC-MSCs[51]	Rat/Adult/Female	Rat bone marrow	Showing oligodendrocyte-like cells	No nerve fiber regeneration shown
EA+grafted NR-MSCs[52]	Rat/Female	Rat bone marrow	Showing oligodendrocyte-like cells	No nerve fiber regeneration shown
EA+grafted SCED[90]	Dog/Adult/Male and female	Canine exfoliated deciduous teeth	No cell differentiation was shown	No nerve fiber regeneration shown
EA+grafted NN[53]	Rat/Adult/Female	Rat hippocampus	Showing neurons, astrocytes, and oligodendrocytes	Showing nerve fiber regeneration
EA+grafted MN[93]	Rat/Adult/Female	Rat bone marrow	Showing neuron-like cells	Showing nerve fiber regeneration

Note: Abbreviations: BBB score, Basso, Beattie, and Bresnahan (BBB) open-field locomotor test; BMSCs, bone marrow mesenchymal stromal cells; CMEP, cortical motor-evoked potentials; EA, electro-acupuncture; MEPs, motor-evoked potentials; MN, TrkC gene-modified bone marrow mesenchymal stem cell (TrkC-MSC)-derived neural network; MSCs, bone marrow mesenchymal stem cells; NN, NT-3 and TrkC gene-overexpressing neural stem cell (NSC)-derived neural network; NR-MSCs, neurotrophin-3 (NT-3) and retinoic acid (RA) preinduced bone marrow mesenchymal stem cells; NSCs, neural stem cells; SCED, stem cells from canine exfoliated deciduous teeth; SCEPs, spinal cord evoked potentials; SSEP, somatosensory evoked potentials; TrkC-MSCs, tyrosine receptor kinase C (TrkC) gene-modified bone marrow mesenchymal stem cells.

CGRP-positive nerve fibers (ascending), as well as 5-hydroxytryptamine (5-HT)-positive and corticospinal tract nerve fibers (both descending), and the formation of synapse-like junctions; and strengthened cortical motor-evoked potentials. Previous researches have showed that the MSC-derived neuron-like cells in the injury/graft site of spinal cord were integrated into the host neural circuitry; therefore, they can receive neural input from the rostral and caudal areas of the injury/graft site in the transected SC and transmit the information to host neurons on both sides of the gap (Figure 3).[3,93] As reported previously, the aim of the this particular strategy is to induce the transdifferentiation of _TrkC_ gene-modified MSCs into neuron-like cells that can serve as a relay mechanism upon transplantation into a gap in the SC.[94-96] To sum up, these processes promoted the regeneration of SC tissue and improved motor function in paralyzed limbs.

Although these transdifferentiated cells are neuron-like, they express most neuron markers including cytoskeleton proteins (neurofilament [NF]-200, NF-H, microtubule-associated protein [MAP]2, and β-tubulin III); synaptic structure proteins (postsynaptic density

[PSD]-95 and synaptophysin); neurotransmitters (γ-aminobutyric acid [GABA] and choline acetyltransferase [ChAT]); and doublecortin (DCX). We have also shown that some of the _TrkC_ gene-modified MSC-derived cells exhibit electrophysiologic properties in vitro, such as postsynaptic currents and action potentials in the presence of Schwann cells overexpressing NT-3.[93,95] Importantly, the cells form extensive connections or contacts with host neurons and descending 5-HT-positive nerve fibers. This unique cell type, with features of both neurons and MSCs (e.g., expression of hypoxia inducible factor [HIF]-1α, prostaglandin [PG]-E2, and vascular endothelial growth factor [VEGF]), may have specific roles in the repair of the injured SC.[95] Nevertheless, this has yet to be demonstrated experimentally.

Collectively, these studies have shown that GV-EA can improve the microenvironment surrounding the injury/graft area, enhance the survival rate of transplanted neural network tissue cell, and maintain their synaptic connections, thus promoting the functional integration of the donor tissue cells with the SC intrinsic neural circuit. Therefore, under the EA stimulation, the transplanted stem

Myelination in the injury/ graft site	Synapse-like junction in the injury/graft site	Integration of grafted stem cells with host neural circuit in the injury/graft site	Clinical electrophysiological evaluation	Locomotion evaluation
No myelination shown	No synapse-like junction shown	No integration shown	No electrophysiological evaluation shown	No BBB score shown
Showing myelination	No synapse-like junction shown	No integration shown	Showing strengthened SCEPs	Showing a higher score
No myelination shown	No synapse-like junction shown	No integration shown	No electrophysiological evaluation shown	No BBB score shown
No myelination shown	No synapse-like junction shown	No integration shown	Showing strengthened MEPs	Showing a higher BBB score
No myelination shown	No synapse-like junction shown	No integration shown	Showing strengthened SSEP	Showing a higher BBB score
No myelination shown	No synapse-like junction shown	No integration shown	No electrophysiological evaluation shown	Showing a higher BBB score
Showing myelination	Showing synapse-like junction	Showing the integration	Showing strengthened MEPs	Showing a higher BBB score
No myelination shown	No synapse-like junction shown	No integration shown	No electrophysiological evaluation shown	No BBB score shown
Showing myelination	No synapse-like junction shown	No integration shown	Showing strengthened CMEP	Showing a lower error footsteps score
Showing myelination	No synapse-like junction shown	No integration shown	Showing strengthened CMEPs	No BBB score shown
No myelination shown	No synapse-like junction shown	No integration shown	No electrophysiological evaluation shown	Showing a higher functional evaluation score
No myelination shown	Showing synapse-like junction	Showing the integration	Showing strengthened CMEP and SSEP	Showing a higher BBB score
No myelination shown	Showing synapse-like junction	Showing the integration	Showing strengthened CMEP	Showing a higher BBB score

cell-derived neural network tissue plays a role of preferably restoring SC structure and function as the neuronal relay in the injury/graft area.[97]

7 | GV-EA STIMULATES THE REPAIR BY TRANSPLANTED MSCS OF DEMYELINATION INJURY IN THE SC

Both mechanical and chemical SCI can lead to nerve fiber demyelination.[98] We have previously shown that GV-EA promotes the synthesis and secretion of NT-3 in demyelinated areas of the SC and increases the number of endogenous oligodendrocyte precursor cells.[50,99] However, the number of such cells is limited, and even MSCs transplanted into the injury site rarely differentiate into oligodendrocyte-like cells.[87,92]

NT-3 and TrkC activates intracellular signaling pathways that promote the survival and differentiation of oligodendrocyte precursor cells into myelin-forming cells in the SC.[100-102] GV-EA increases NT-3 levels in demyelinated areas of the SC and induces the differentiation of TrkC-overexpressing MSCs pretreated with retinoic acid into oligodendrocyte-like cells that form a myelin sheath around regenerating nerve fibers, and thereby restores nerve conduction pathways in the SC.[51,52,86] These findings suggest a new strategy for the treatment of not only SCI, but also of demyelinating diseases such as multiple sclerosis.[103] Table 1 summarizes the combination strategy of EA and adult stem cell transplantation in an animal model of SCI.

8 | PERSPECTIVES

A large number of studies have investigated the applicability of GV-EA in the treatment of SCI over the last two decades. Our research has revealed the mechanisms by which GV-EA can be combined with adult stem cell transplantation to improve the microenvironment of

the injured SC to promote the reconstruction of neuronal circuits. In this review, we discussed the multiple effects of GV-EA, including stimulation of afferent nerve fibers of the meningeal branch of the SC for the transmission of information in the completely transected SC. We also described how GV-EA can induce NT-3 synthesis and secretion by cells in the injured SC and enhance the survival, differentiation, and migration of TrkC-overexpressing NSCs and MSCs transplanted into the injury/graft site and their integration into existing neuronal circuits. We also describe how GV-EA can assist in the replacement of injured host neurons, improve the microenvironment surrounding the injured tissue, stimulate nerve fiber regeneration and myelination, enhance cortical motor-evoked potentials, and restore motor function in paralyzed limbs. The current evidence indicates that the combination of GV-EA and gene-modified adult stem cell transplantation is an innovative strategy for SCI treatment that warrants further development and testing in clinical settings (Figure 2).

Finally, recent advances in genetic engineering techniques and an improved knowledge of gene expression in adult stem cells have led to the demonstration that gene-modified adult stem cells exhibit stronger therapeutic properties in some disease models compared with wild-type adult stem cells. For example, a previous study investigated repair in a completely transected SCI and found that if wild-type adult stem cells were transplanted directly, then the number of differentiated neurons would be smaller and they would not be able to effectively replace neurons that had died due to injury. Hence, gene-modified adult stem cells may represent a useful option for transplantation to better differentiate into neurons and repair the neural circuits of the host SC. However, there remains an obvious gap between experimental models and clinical trials, along with a host of limitations related to the safety of gene-modified adult stem cell transplantation for therapeutic use. It would be possible to avoid ethical issues if we were able to transplant another type of stem cell-human induced pluripotent stem cells (hiPSCs). This is because the preparation of hiPSCs does not involve embryos and germ cells that are harvested from early humans.[104,105] More importantly, hiPSCs have unlimited capacity to expand; this could solve the problems associated with cell source and number, thus facilitating the advancement of clinical translational research. Studies have shown that the differentiation of hiPSC-derived neural progenitor cells (NPCs) into neurons can be observed after the transplantation of NPCs into animals with a completely transected spinal cord.[106] Differentiated neurons have been observed to develop long cellular processes to form synaptic connections with host neurons and promote the recovery of motor function after spinal cord injury.[106,107] Collectively, the available literature suggests whether it is possible to repair the structure and function of the injured spinal cord effectively if we apply the combination of electro-acupuncture and the transplantation of hiPSC-derived NPCs. More clinical studies are clearly desirable to ensure the safety profile of such treatment and to support the translation of this treatment model into a wide range of clinical settings. In addition, some researches indicated that sex differences exist a certain effect on the repair of central nervous system injury.[108-114] Hence, future study should pay attention to the potential influence of sex differences on electro-acupuncture and its combination with adult stem cell transplantation for spinal cord injury treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Yuan-shan Zeng was responsible for literature review, investigation, draft preparation and editing; Ying Ding was responsible for literature review and editing; Hao-yu Xu was responsible for drawing and editing; Xiang Zeng, Bi-Qin Lai, Ge Li, and Yuan-Huan Ma were responsible for manuscript review. All authors have read and agreed to the published version of manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Yuan-Shan Zeng 🔟 https://orcid.org/0000-0003-3804-5792

REFERENCES

1. Assinck P, Duncan GJ, Hilton BJ, Plemel JR, Tetzlaff W. Cell transplantation therapy for spinal cord injury. *Nat Neurosci.* 2017;20(5):637-647.
2. O'Shea TM, Burda JE, Sofroniew MV. Cell biology of spinal cord injury and repair. *J Clin Investig.* 2017;127(9):3259-3270.
3. Lin XY, Lai BQ, Zeng X, et al. Cell transplantation and neuroengineering approach for spinal cord injury treatment: a summary of current laboratory findings and review of literature. *Cell Transplant.* 2016;25(8):1425-1438.
4. Webber CA, Salame J, Luu GL, et al. Nerve growth factor acts through the TrkA receptor to protect sensory neurons from the damaging effects of the HIV-1 viral protein, Vpr. *Neuroscience.* 2013;252:512-525.
5. Matyas JJ, O'Driscoll CM, Yu L, et al. Truncated TrkB.T1-mediated astrocyte dysfunction contributes to impaired motor function and neuropathic pain after spinal cord injury. *J Neurosci.* 2017;37(14):3956-3971.
6. Lu P, Yang H, Jones LL, Filbin MT, Tuszynski MH. Combinatorial therapy with neurotrophins and cAMP promotes axonal regeneration beyond sites of spinal cord injury. *J Neurosci.* 2004;24(28):6402-6409.
7. Taylor SJ, McDonald JW 3rd, Sakiyama-Elbert SE. Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. *J Control Release.* 2004;98(2):281-294.
8. Longhurst JC. Defining meridians: a modern basis of understanding. *J Acupunct Meridian Stud.* 2010;3(2):67-74.
9. Xu JP, Wang MJ, Liu XR, Wang ZM, Chen ZG, Zhang JJ. Clinical observation of 80 cases of treatment on spinal cord injury with GV

acupoints electroacupuncture. *J Clin Acupuncture and Moxibustion (Chinese)*. 1994;10(6):13-15.

10. Wang W, Xie J, Fang J. The research progress about Spinal cord injury treatment of Du channel electroacupuncture. *J Clin Acupunct Moxibustion (Chinese)*. 2005;20(1):60-62.

11. He GH, Ruan JW, Zeng YS, Zhou X, Ding Y, Zhou GH. Improvement in acupoint selection for acupuncture of nerves surrounding the injury site: electro-acupuncture with Governor vessel with local meridian acupoints. *Neural Regen Res*. 2015;10(1):128-135.

12. Alvarado-Sanchez BG, Salgado-Ceballos H, Torres-Castillo S, et al. Electroacupuncture and curcumin promote oxidative balance and motor function recovery in rats following traumatic spinal cord injury. *Neurochem Res*. 2019;44(2):498-506.

13. Liu Z, Ding Y, Zeng YS. A new combined therapeutic strategy of governor vessel electro-acupuncture and adult stem cell transplantation promotes the recovery of injured spinal cord. *Curr Med Chem*. 2011;18(33):5165-5171.

14. Liu SJ, Zheng SS, Dan QQ, Liu J, Wang TH. Effects of Governor Vessel electroacupuncture on the systematic expressions of NTFs in spinal cord transected rats. *Neuropeptides*. 2014;48(4):239-247.

15. Li BL, Zhou BB, Jin X, et al. Effect of electroacupuncture on the recovery of lower limbs of rats after spinal cord injury on the contrast of Jiaji and Du Meridian. *J Basic Chin Med*. 2016;22(12):1676-1680.

16. Li WJ, Pan SQ, Zeng YS, et al. Identification of acupuncture-specific proteins in the process of electro-acupuncture after spinal cord injury. *Neurosci Res*. 2010;67(4):307-316.

17. Shi SH, Lyu W, Qin LN, et al. Effects of governor channel electroacupuncture treatment on MEK2, p-ERK1 after 1, 7 days on spinal cord injury based on Raf/Ras/ERK signal pathway. *J Tradit Chin Med Pharm (Chinese)*. 2017;32(7):2919-2923.

18. Guo JS, Zeng YS, Chen YL, et al. Experimental study on treatment of spinal cord transection by electroacupuncture at the GV in the rat. *Chin Acupunct Moxibustion (Chinese)*. 2003;23(6):351-354.

19. Cui XJ, Li YW, Chen DF, Zhang J, Li H, Shen YP. Effect of Du Channel electrotherapy on neural stem cell following injuried spinal cord. *Anat Res (Chinese)*. 2002;24(3):180-183.

20. Zhang XM, Gao WB, Teng XY, Gao YP. The Effect of Jiaji electroacupuncture and preconditioning on endogenous neural stem cells of rats with spinal cord injury. *J Clin Acupunct Moxibustion (Chinese)*. 2011;27(11):53-56.

21. Teng XY, Wang DS, Zhang XM, et al. Effect of Jiaji electroacupuncture in transected rat spinal cord. *Chin Sci Bull (Chinese)*. 2012;57(7):762-768.

22. Lukovic D, Stojkovic M, Moreno-Manzano V, et al. Concise review: reactive astrocytes and stem cells in spinal cord injury: good guys or bad guys? *Stem Cells*. 2015;33(4):1036-1041.

23. Zheng Y, Mao YR, Yuan TF, Xu DS, Cheng LM. Multimodal treatment for spinal cord injury: a sword of neuroregeneration upon neuromodulation. *Neural Regen Res*. 2020;15(8):1437-1450.

24. Bonner JF, Steward O. Repair of spinal cord injury with neuronal relays: from fetal grafts to neural stem cells. *Brain Res*. 2015;1619:115-123.

25. Muheremu A, Peng J, Ao Q. Stem cell based therapies for spinal cord injury. *Tissue Cell*. 2016;48(4):328-333.

26. Mothe AJ, Kulbatski I, Parr A, Mohareb M, Tator CH. Adult spinal cord stem/progenitor cells transplanted as neurospheres preferentially differentiate into oligodendrocytes in the adult rat spinal cord. *Cell Transplant*. 2008;17(7):735-751.

27. Garbossa D, Boido M, Fontanella M, Fronda C, Ducati A, Vercelli A. Recent therapeutic strategies for spinal cord injury treatment: possible role of stem cells. *Neurosurg Rev*. 2012;35(3):293-311.

28. Akiyama Y, Radtke C, Kocsis JD. Remyelination of the rat spinal cord by transplantation of identified bone marrow stromal cells. *J Neurosci*. 2002;22(15):6623-6630.

29. Ding DC, Shyu WC, Lin SZ. Mesenchymal stem cells. *Cell Transplant*. 2011;20(1):5-14.

30. Ju DH, Yang JB, Song KY, Luo YF. Experimental Studies on the neuro-endocrine- immune regulating circuit of acupuncture analgesia. *J Basic Chin Med (Chinese)*. 1998;4(2):29-34.

31. Chi L, Du K, Liu D, Bo Y, Li W. Electroacupuncture brain protection during ischemic stroke: a role for the parasympathetic nervous system. *J Cereb Blood Flow Metab*. 2018;38(3):479-491.

32. Yang C, Liu J, Wang J, et al. Activation of astroglial CB1R mediates cerebral ischemic tolerance induced by electroacupuncture. *J Cereb Blood Flow Metab*. 2021;41(9):2295-2310.

33. Pei W, Meng F, Deng Q, et al. Electroacupuncture promotes the survival and synaptic plasticity of hippocampal neurons and improvement of sleep deprivation-induced spatial memory impairment. *CNS Neurosci Ther*. 2021;27(12):1472-1482.

34. Zhu Y, Zeng Y. Electroacupuncture protected pyramidal cells in hippocampal CA1 region of vascular dementia rats by inhibiting the expression of p53 and Noxa. *CNS Neurosci Ther*. 2011;17(6):599-604.

35. Han JS. Induction of the release of central neuropeptides by peripheral electrical stimulation. *J Peking Univ (Health Sciences)*. 2002;34(5):408-413.

36. Pan CC, Shen DK, Jin WX, Liu XG. The correlation of neuropeptides with needling sensation response of acupoints. *Mcta Academiae Medicinae Wannan (Chinese)*. 1993;12(2):82-85.

37. Li WJ, Li SM, Ding Y, et al. Electro-acupuncture upregulates CGRP expression after rat spinal cord transection. *Neurochem Int*. 2012;61(8):1397-1403.

38. Li SM, Li WJ, Zeng YS, Ruan JW. Effects of different local points of electroacupuncture on the expression of calcitonin gene-related peptide in rats with spinal cord injury. *Chin J Rehabil Med (Chinese)*. 2010;25(9):823-827.

39. Ding Y, Zeng YS, Chen YY, Zhang YQ, Chen SJ. A comparison of the effects of electricacupuncture stimulation between Dumai and Jiaji on the neuronal survival and expression of nitric oxide synthase at Clarke's nucleus in injured spinal cord. *Chin J Rehabil Med (Chinese)*. 2006;21(1):8-10.

40. Xu H, Yang Y, Deng QW, et al. Governor Vessel electro-acupuncture promotes the intrinsic growth ability of spinal neurons through activating calcitonin gene-related peptide/α-calcium/calmodulin-dependent protein kinase/ neurotrophin-3 pathway after spinal cord injury. *J Neurotrauma*. 2021;38(6):734-745.

41. Standring S. *Spinal Cord and Spinal Nerves: Gross Anatomy/Gray's Anatomy: The Anatomical Basis of Clinical Practice*, 41st ed. Elsevier Limited; 2016:762-772.

42. Iyengar S, Ossipov MH, Johnson KW. The role of calcitonin gene-related peptide in peripheral and central pain mechanisms including migraine. *Pain*. 2017;158(4):543-559.

43. Shin HK, Lee SW, Choi BT. Modulation of neurogenesis via neurotrophic factors in acupuncture treatments for neurological diseases. *Biochem Pharmacol*. 2017;141:132-142.

44. Wang TH, Wang XY, Li XL, Chen HM, Wu LF. Effect of electroacupuncture on neurotrophin expression in cat spinal cord after partial dorsal rhizotomy. *Neurochem Res*. 2007;32(8):1415-1422.

45. Mo YP, Yao HJ, Lv W, et al. Effects of Electroacupuncture at Governor Vessel acupoints on neurotrophin-3 in rats with experimental spinal cord injury. *Neural Plast*. 2016;2016:2371875.

46. Harvey AR, Lovett SJ, Majda BT, Yoon JH, Wheeler LP, Hodgetts SI. Neurotrophic factors for spinal cord repair: Which, where, how and when to apply, and for what period of time? *Brain Res*. 2015;1619:36-71.

47. Zhang K, Liu Z, Li G, et al. Electro-acupuncture promotes the survival and differentiation of transplanted bone marrow mesenchymal stem cells pre-induced with neurotrophin-3 and retinoic acid in gelatin sponge scaffold after rat spinal cord transection. *Stem Cell Rev Rep*. 2014;10(4):612-625.

48. Ding Y, Yan Q, Ruan JW, et al. Electroacupuncture promotes the differentiation of transplanted bone marrow mesenchymal stem cells overexpressing TrkC into neuron-like cells in transected spinal cord of rats. *Cell Transplant*. 2013;22(1):65-86.

49. Li XB, Zeng YS, Chen YL, Chen YY, Guo JS, Chen SJ. A study on the combination of Goremor Vessel electroacupuncture and transplanted neural stem cells to promote the survival and axonal regeneration of injured neurons in completely transected spinal cord in rats. *Acta Anatomica Sinica (Chinese)*. 2006;37(1):30-35.

50. Huang SF, Ding Y, Ruan JW, et al. An experimental electroacupuncture study in treatment of the rat demyelinated spinal cord injury induced by ethidium bromide. *Neurosci Res*. 2011;70(3):294-304.

51. Ding Y, Zhang RY, He B, et al. Combination of electroacupuncture and grafted mesenchymal stem cells overexpressing TrkC improves remyelination and function in demyelinated spinal cord of rats. *Sci Rep*. 2015;5:9133.

52. Liu Z, He B, Zhang RY, et al. Electroacupuncture promotes the differentiation of transplanted bone marrow mesenchymal stem cells preinduced with neurotrophin-3 and retinoic acid into oligodendrocyte-like cells in demyelinated spinal cord of rats. *Cell Transplant*. 2015;24(7):1265-1281.

53. Jin H, Zhang YT, Yang Y, et al. Electroacupuncture facilitates the integration of neural stem cell-derived neural network with transected rat spinal cord. *Stem Cell Rep*. 2019;12(2):274-289.

54. Quiroz-González S, Segura-Alegría B, Guadarrama-Olmos JC, Jiménez-Estrada I. Cord dorsum potentials evoked by electroacupuncture applied to the hind limbs of rats. *J Acupunct Meridian Stud*. 2014;7(1):25-32.

55. Patel NB, Poo MM. Perturbation of the direction of neurite growth by pulsed and focal electric fields. *J Neurosci*. 1984;4(12):2939-2947.

56. Harvey LA, Glinsky JV, Bowden JL. The effectiveness of 22 commonly administered physiotherapy interventions for people with spinal cord injury: a systematic review. *Spinal Cord*. 2016;54(11):914-923.

57. Teoli D, An J. Transcutaneous electrical nerve stimulation. In: *StatPearls*. StatPearls Publishing Copyright © 2021, StatPearls Publishing LLC.; 2021.

58. Lin CH, Cheng FC, Lu YZ, Chu LF, Wang CH, Hsueh CM. Protection of ischemic brain cells is dependent on astrocyte-derived growth factors and their receptors. *Exp Neurol*. 2006;201(1):225-233.

59. Qian C, Tan D, Wang X, et al. Peripheral nerve injury-induced astrocyte activation in spinal ventral horn contributes to nerve regeneration. *Neural Plast*. 2018;2018:8561704.

60. Choi DC, Lee JY, Moon YJ, Kim SW, Oh TH, Yune TY. Acupuncture-mediated inhibition of inflammation facilitates significant functional recovery after spinal cord injury. *Neurobiol Dis*. 2010;39(3):272-282.

61. Zhao J, Wang L, Li Y. Electroacupuncture alleviates the inflammatory response via effects on M1 and M2 macrophages after spinal cord injury. *Acupunct Med*. 2017;35(3):224-230.

62. Wang J, Lu S, Yang F, et al. The role of macrophage polarization and associated mechanisms in regulating the anti-inflammatory action of acupuncture: a literature review and perspectives. *Chin Med*. 2021;16(1):56.

63. Li G, Che MT, Zhang K, et al. Graft of the NT-3 persistent delivery gelatin sponge scaffold promotes axon regeneration, attenuates inflammation, and induces cell migration in rat and canine with spinal cord injury. *Biomaterials*. 2016;83:233-248.

64. Moore CD, Craven BC, Thabane L, et al. Lower-extremity muscle atrophy and fat infiltration after chronic spinal cord injury. *J Musculoskelet Neuronal Interact*. 2015;15(1):32-41.

65. Dougherty KJ, Zagoraiou L, Satoh D, et al. Locomotor rhythm generation linked to the output of spinal shox2 excitatory interneurons. *Neuron*. 2013;80(4):920-933.

66. Huang XQ, Xu JS, Ye XR, Chen X. Wide pulse width electroacupuncture ameliorates denervation-induced skeletal muscle atrophy in rats via IGF-1/PI3K/Akt pathway. *Chin J Integr Med (Chinese)*. 2021;27(6):446-454.

67. Pelletier R, Higgins J, Bourbonnais D. Addressing neuroplastic changes in distributed areas of the nervous system associated with chronic musculoskeletal disorders. *Phys Ther*. 2015;95(11):1582-1591.

68. Adams MM, Ditor DS, Tarnopolsky MA, Phillips SM, McCartney N, Hicks AL. The effect of body weight-supported treadmill training on muscle morphology in an individual with chronic, motor-complete spinal cord injury: a case study. *J Spinal Cord Med*. 2006;29(2):167-171.

69. Talbot LA, Brede E, Metter EJ. Effects of adding neuromuscular electrical stimulation to traditional military amputee rehabilitation. *Mil Med*. 2017;182(1):e1528-e1535.

70. Carraro U, Kern H, Gava P, et al. Recovery from muscle weakness by exercise and FES: lessons from Masters, active or sedentary seniors and SCI patients. *Aging Clin Exp Res*. 2017;29(4):579-590.

71. Zhang YT, Jin H, Wang JH, et al. Tail nerve electrical stimulation and electro-acupuncture can protect spinal motor neurons and alleviate muscle atrophy after spinal cord transection in rats. *Neural Plast*. 2017;2017:7351238.

72. Boncompagni S, Kern H, Rossini K, et al. Structural differentiation of skeletal muscle fibers in the absence of innervation in humans. *Proc Natl Acad Sci USA*. 2007;104(49):19339-19344.

73. Joo W, Hippenmeyer S, Neurodevelopment LL. Dendrite morphogenesis depends on relative levels of NT-3/TrkC signaling. *Science*. 2014;346(6209):626-629.

74. Ernfors P, Lee KF, Kucera J, Jaenisch R. Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. *Cell*. 1994;77(4):503-512.

75. Heimburger RF. Return of function after spinal cord transection. *Spinal Cord*. 2005;43(7):438-440.

76. Zhang X, Zeng Y, Zhang W, Wang J, Wu J, Li J. Co-transplantation of neural stem cells and NT-3-overexpressing Schwann cells in transected spinal cord. *J Neurotrauma*. 2007;24(12):1863-1877.

77. Chen YY, Zhang W, Chen YL, Chen SJ, Dong H, Zeng YS. Electroacupuncture improves survival and migration of transplanted neural stem cells in injured spinal cord in rats. *Acupunct Electrother Res*. 2008;33(1–2):19-31.

78. Li XB, Zeng YS, Chen YL, Chen SJ. The combination of Goremor Vessel electroacupuncture and neural stem cells transplantation to promote the survival of injured motoneuron of anterior horn and to reduce the myatrophy of hind limbs in rat spinal cord transected completely. *Chin J Rehabil Med (Chinese)*. 2006;21(2):104-107.

79. Li XB, Zeng YS, Chen YL, Guo JS, Li XJ. The combination of Goremor Vessel electroacupuncture and neural stem cells transplantation to promote the Recovery of motor function of hind limbs in rat spinal cord transected completely. *Acta Anatomica Sinica (Chinese)*. 2004;35(6):582-588.

80. Lai BQ, Che MT, Du BL, et al. Transplantation of tissue engineering neural network and formation of neuronal relay into the transected rat spinal cord. *Biomaterials*. 2016;109:40-54.

81. Aboul-Soud MAM, Alzahrani AJ, Mahmoud A. Induced pluripotent stem cells (iPSCs)-roles in regenerative therapies, disease modelling and drug screening. *Cells*. 2021;10(9)2319.

82. Goldstein RS, Pomp O, Brokhman I, Ziegler L. Generation of neural crest cells and peripheral sensory neurons from human embryonic stem cells. *Methods Mol Biol*. 2010;584:283-300.

83. Wang LT, Ting CH, Yen ML, et al. Human mesenchymal stem cells (MSCs) for treatment towards immune- and inflammation-mediated diseases: review of current clinical trials. *J Biomed Sci*. 2016;23(1):76.

84. Qu J, Zhang H. Roles of mesenchymal stem cells in spinal cord injury. *Stem Cells Int*. 2017;2017:5251313.

85. Tang H, Guo Y, Zhao Y, et al. Effects and mechanisms of acupuncture combined with mesenchymal stem cell transplantation on neural recovery after spinal cord injury: progress and prospects. *Neural Plast*. 2020;2020:8890655.

86. Damasceno PKF, de Santana TA, Santos GC, et al. Genetic engineering as a strategy to improve the therapeutic efficacy of mesenchymal stem/stromal cells in regenerative medicine. *Front Cell Dev Biol*. 2020;8:737.

87. Ding Y, Yan Q, Ruan JW, et al. Electro-acupuncture promotes survival, differentiation of the bone marrow mesenchymal stem cells as well as functional recovery in the spinal cord-transected rats. *BMC Neurosci*. 2009;10:35.

88. Sun Z, Li X, Su Z, Zhao Y, Zhang L, Wu M. Electroacupuncture-enhanced differentiation of bone marrow stromal cells into neuronal cells. *J Sport Rehabil*. 2009;18(3):398-406.

89. Liu H, Yang K, Xin T, Wu W, Chen Y. Implanted electro-acupuncture electric stimulation improves outcome of stem cells' transplantation in spinal cord injury. *Artif Cells Blood Substit Immobil Biotechnol*. 2012;40(5):331-337.

90. Prado C, Fratini P, de Sá Schiavo Matias G, et al. Combination of stem cells from deciduous teeth and electroacupuncture for therapy in dogs with chronic spinal cord injury: a pilot study. *Res Vet Sci*. 2019;123:247-251.

91. Yan Q, Ruan JW, Ding Y, Li WJ, Li Y, Zeng YS. Electro-acupuncture promotes differentiation of mesenchymal stem cells, regeneration of nerve fibers and partial functional recovery after spinal cord injury. *Exp Toxicol Pathol*. 2011;63(1–2):151-156.

92. Ding Y, Yan Q, Ruan JW, et al. Bone marrow mesenchymal stem cells and electroacupuncture downregulate the inhibitor molecules and promote the axonal regeneration in the transected spinal cord of rats. *Cell Transplant*. 2011;20(4):475-491.

93. Yang Y, Xu HY, Deng QW, et al. Electroacupuncture facilitates the integration of a grafted TrkC-modified mesenchymal stem cell-derived neural network into transected spinal cord in rats via increasing neurotrophin-3. *CNS Neurosci Ther*. 2021;27(7):776-791.

94. Wu GH, Shi HJ, Che MT, et al. Recovery of paralyzed limb motor function in canine with complete spinal cord injury following implantation of MSC-derived neural network tissue. *Biomaterials*. 2018;181:15-34.

95. Zeng X, Qiu XC, Ma YH, et al. Integration of donor mesenchymal stem cell-derived neuron-like cells into host neural network after rat spinal cord transection. *Biomaterials*. 2015;53:184-201.

96. Yang EZ, Zhang GW, Xu JG, et al. Multichannel polymer scaffold seeded with activated Schwann cells and bone mesenchymal stem cells improves axonal regeneration and functional recovery after rat spinal cord injury. *Acta Pharmacol Sin*. 2017;38(5):623-637.

97. Lai BQ, Zeng X, Han WT, et al. Stem cell-derived neuronal relay strategies and functional electrical stimulation for treatment of spinal cord injury. *Biomaterials*. 2021;279:121211.

98. Khan AA, Huat TJ, Al Mutery A, et al. Significant transcriptomic changes are associated with differentiation of bone marrow-derived mesenchymal stem cells into neural progenitor-like cells in the presence of bFGF and EGF. *Cell Biosci*. 2020;10:126.

99. Yang XH, Ding Y, Li W, et al. Effects of electroacupuncture and the retinoid X receptor (RXR) signalling pathway on oligodendrocyte differentiation in the demyelinated spinal cord of rats. *Acupunct Med*. 2017;35(2):122-132.

100. Kumar S, Kahn MA, Dinh L, de Vellis J. NT-3-mediated TrkC receptor activation promotes proliferation and cell survival of rodent progenitor oligodendrocyte cells in vitro and in vivo. *J Neurosci Res*. 1998;54(6):754-765.

101. McTigue DM, Horner PJ, Stokes BT, Gage FH. Neurotrophin-3 and brain-derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord. *J Neurosci*. 1998;18(14):5354-5365.

102. Rubio N, Rodriguez R, Arevalo MA. In vitro myelination by oligodendrocyte precursor cells transfected with the neurotrophin-3 gene. *Glia*. 2004;47(1):78-87.

103. Gugliandolo A, Bramanti P, Mazzon E. Mesenchymal stem cells in multiple sclerosis: recent evidence from pre-clinical to clinical studies. *Int J Mol Sci*. 2020;21(22):8662.

104. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131(5):861-872.

105. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007;318(5858):1917-1920.

106. Lu P, Woodruff G, Wang Y, et al. Long-distance axonal growth from human induced pluripotent stem cells after spinal cord injury. *Neuron*. 2014;83(4):789-796.

107. Ito S, Nagoshi N, Kamata Y, et al. LOTUS overexpression via ex vivo gene transduction further promotes recovery of motor function following human iPSC-NS/PC transplantation for contusive spinal cord injury. *Stem Cell Reports*. 2021;16(11):2703-2717.

108. Fukutoku T, Kumagai G, Fujita T, et al. Sex-related differences in anxiety and functional recovery after spinal cord injury in mice. *J Neurotrauma*. 2020;37(21):2235-2243.

109. Theis T, Kumar S, Wei E, et al. Myristoylated alanine-rich C-kinase substrate effector domain peptide improves sex-specific recovery and axonal regrowth after spinal cord injury. *Faseb J*. 2020;34(9):12677-12690.

110. Kim T, Chelluboina B, Chokkalla AK, Vemuganti R. Age and sex differences in the pathophysiology of acute CNS injury. *Neurochem Int*. 2019;127:22-28.

111. Bramble MS, Vashist N, Vilain E. Sex steroid hormone modulation of neural stem cells: a critical review. *Biol Sex Differ*. 2019;10(1):28.

112. Yasuda K, Maki T, Kinoshita H, et al. Sex-specific differences in transcriptomic profiles and cellular characteristics of oligodendrocyte precursor cells. *Stem Cell Res*. 2020;46:101866.

113. Kerr N, Dietrich DW, Bramlett HM, Raval AP. Sexually dimorphic microglia and ischemic stroke. *CNS Neurosci Ther*. 2019;25(12):1308-1317.

114. Chandra PK, Cikic S, Baddoo MC, et al. Transcriptome analysis reveals sexual disparities in gene expression in rat brain microvessels. *J Cereb Blood Flow Metab*. 2021;41(9):2311-2328.