

Research Article

Electroacupuncture at Hua Tuo Jia Ji Acupoints Reduced Neuropathic Pain and Increased GABA_A Receptors in Rat Spinal Cord

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Chronic constriction injury- (CCI-) induced neuropathic pain is the most similar model to hyperalgesia in clinical observation. Neuropathic pain is a neuronal dysfunction in the somatosensory system that may lead to spontaneous pain. In this study, electroacupuncture (EA) was applied at bilateral L4 and L6 of Hua Tuo Jia Ji points (EX-B2) for relieving neuropathic pain in rats. Eighteen Sprague-Dawley rats were randomly assigned to three groups: sham, 2-Hz EA, and 15-Hz EA groups. Following von Frey and cold plate tests, both the 2- and the 15-Hz EA groups had significantly lower mechanical and thermal hyperalgesia than the sham group. Western blot analysis results showed that γ -aminobutyric acid A (GABA_A), adenosine A1 receptor (A1R), transient receptor potential cation channel subfamily V member 1 (TRPV1), TRPV4, and metabotropic glutamate receptor 3 (mGluR3) were similar in the dorsal root ganglion of all three groups. Furthermore, levels of GABA_A receptors were higher in the spinal cord of rats in the 2- and 15-Hz EA groups compared with the sham control group. This was not observed for A1R, TRPV1, TRPV4, or mGluR3 receptors. In addition, all the aforementioned receptors were unchanged in the somatosensory cortex of the study rats, suggesting a central spinal effect. The study results provide evidence to support the clinical use of EA for specifically alleviating neuropathic pain.

1. Introduction

According to the International Association for the Study of Pain, neuropathic pain is defined as pain caused by a lesion or disease of the somatosensory system [1]. Pain resulting from neuropathic pain is always accompanied by depression, anxiety, and sleep disturbance [2, 3]. Neuropathic pain can be further subdivided into peripheral and central neuropathy. Peripheral neuropathy includes painful diabetic neuropathy, human immunodeficiency virus-associated neuropathy, chemotherapy-induced peripheral neuropathy, and postherpetic neuralgia. By contrast, central neuropathy includes spinal injury, central poststroke pain, and compressive myelopathy among other conditions. Unbalanced neurotransmitters or neuromodulators always mismatch painful

sensory inputs, which results in the generation of spontaneous painful sensations. In neuropathic pain, spontaneous mechanical or thermal hyperalgesia is often observed, and several central sensitizations are induced in the dorsal horn of the spinal cord (SC), such as sodium channels, *N*-methyl-d-aspartate (NMDA), γ -aminobutyric acid (GABA), and opioid receptors [4]. Medicines such as aspirin, acetaminophen, nonsteroidal anti-inflammation drugs, antidepressants, and opiates are currently used to treat neuropathic pain with limited success and side effects [5].

Adenosine is released from presynaptic terminals to bind postsynaptic A1, A2_A, A2_B, and A3 receptors for cardiovascular, immune, and nerve functions [6, 7]. Adenosine A1 receptor (A1R) is located mainly in peripheral sensory terminal [8], SC [9], and glial cells [10, 11]. Activation

of AIR produced an analgesic effect in inflammatory and neuropathic pain models [12, 13]. AIR is reported to activate Gi protein and inhibit the cyclic adenosine monophosphate-protein kinase A pathway [14]. Injection of adenosine can reliably attenuate allodynia and hyperalgesia in many pain symptoms [7, 15]. However, several side effects result from activation of adenosine A2 receptor (A2R). It may induce vasodilation, a reduced heart rate, and severe cardiovascular obstacles.

GABA receptors are the main inhibitory neurotransmitters in the mammalian central nervous system. GABA receptors can be further subdivided into GABA_A, GABA_B, and GABA_C. GABA_A is an ion channel that mediates fast inhibitory synaptic transmission and induces an influx of chloride, leading to stability of the neuronal membrane. Several factors can activate GABA_A for reducing neuronal excitability, and these are often used for anesthesia and pain management. Injection of GABA agonists is considered to be the most effective method of reducing pain signaling [16, 17]. In neuropathic pain, GABAergic interneurons initiate apoptosis via the caspase-3 pathway [18]. In addition, after nerve injury, GABA and glutamate decarboxylase 65 have been reported to be lower [19, 20].

The transient receptor potential (TRP) is a nonselective ion channel that can be activated following a tissue injury [21]. Transient receptor potential cation channel subfamily V member 1 (TRPV1) exists in nociceptors and the SC dorsal horn for pain signaling [22, 23]. TRPV1 can be activated by capsaicin, noxious heat (more than 43°C), and mechanical sensation [24–26]. TRPV1 is highly expressed in small C-fiber dorsal root ganglion (DRG) neurons and trigeminal and nodose ganglia [27]. Activation of TRPV1 induces sodium and calcium influx for neuronal depolarization [28, 29]. Depletion of TRPV1 gene results in insensitivity to noxious heat, radial heat, and hot-plate tests [30]. Inflammatory-material-induced thermal hyperalgesia was attenuated in TRPV1 knockout mice, suggesting the crucial role of TRPV1 in thermal pain sensation [31].

TRPV4 is a polymodal receptor that acts as an osmotic, mechanical, and thermal receptor [32]. Activation of TRPV4 releases calcitonin gene-related peptide and substance P into the SC dorsal horn [33]. TRPV4 is widely expressed in hair cells, kidney, lung, and peripheral sensory ganglia [34]. TRPV4 expressed in heterologous systems is usually activated by osmotic stimuli, resulting in cell swelling [35]. TRPV4 may also participate in the reduction of visceral pain [32]. Coexpression of TRPV1 and TRPV4 may synergistically play a role in nociception [36].

Neuropathic pain from surgery may damage c- and A δ fibers to increase the release of glutamate. Glutamate is a major excitatory neurotransmitter in the mammalian central nervous system that is released from presynaptic terminals for binding on four receptor subtypes: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, NMDA, KA, and metabotropic glutamate receptor (mGluR) [37]. mGluR was indicated to be involved in neuropathic pain [38].

Acupuncture has been used for treating diseases for thousands of years. Increasing evidence suggests that electroacupuncture (EA) can be used to treat learning and

memory impairment in ischemia rats [39], epilepsy [40], body weight control [41], and pain [42, 43]. Accordingly, the aim of the present study was to identify whether EA applied at Hua Tuo Jia Ji acupoints could reduce chronic constriction injury- (CCI-) induced neuropathic pain in rat models. We further investigated whether AIR, GABA_A, TRPV1, TRPV4, and mGluR3 participate in the EA analgesic effect by evaluating DRG, SC, and somatosensory cortex (SSC) levels.

2. Materials and Methods

2.1. Animals. Eighteen male Sprague-Dawley rats weighing 201–250 g were purchased from BioLASCO (BioLASCO Taiwan Co., Ltd) and housed in the animal center of China Medical University (CMU). A 12-12-h light-dark cycle was maintained, and the room temperature was controlled at 25°C. Adequate food and water were provided. The Animal Care and Use Committee of CMU approved the use of these animals. In addition, all procedures were performed according to the *Guide for the Use of Laboratory Animals* (National Academy Press). The CCI model is often used to mimic clinical neuropathic pain by “tying 4-0 chromic gut sutures” in the sciatic nerve with the phenotype of hyperalgesia and allodynia. CCI was induced on the right sciatic nerve of rats, and their pain behavior was tested on day 5 to ensure the establishment of neuropathic pain. The sample size required an effect size of 0.6 in withdrawal threshold and an alpha of 0.05, and a power of 80% was estimated to be 6 animals per group. Rats were randomly subdivided into three groups ($n = 6/\text{group}$): (1) sham, (2) 2-Hz EA, and (3) 15-Hz EA groups. Rats were anesthetized with 3% isoflurane, and their right sciatic nerve was exposed. Furthermore, the nerve proximal to the trifurcation was ligated using four 4-0 chromic gut sutures. The surgical site was closed immediately using silk line before the rats were placed back in their cage.

2.2. EA Treatment. EA was applied at days 7, 8, and 9 using stainless steel needles (0.5[#], 32 G, Yu-Kuang, Taiwan) inserted into Hua Tuo Jia Ji acupoints at a depth of 2–3 mm, 5 mm from the spine at L4 and L6. EA was administered for 20 min immediately after the neuropathic injection. A stimulator (Trio 300, Ito, Japan) delivered 100- μ s square pulses of 2 mA for 15 min at 2 or 15 Hz. For the sham control group, we only inserted the needle and connected the animals to the stimulator without current input.

2.3. Animal Behavior. The von Frey test was adopted to examine mechanical hyperalgesia (IITC; Life Science Inc., USA). Rats were placed on a metal mesh and stimulated by applying a thin filament at the hind paw. The forces were recorded automatically when the rats withdrew their right hind paw. The cold plate test was used to assess thermal hyperalgesia during which the rats were placed on a cold plate apparatus (Panlab, Spain), with the temperature at 4°C. The total number of foot lifts was counted (right hind paw) for 5 min. The laboratory workers kept blind to treatment allocation during the experiments and analysis.

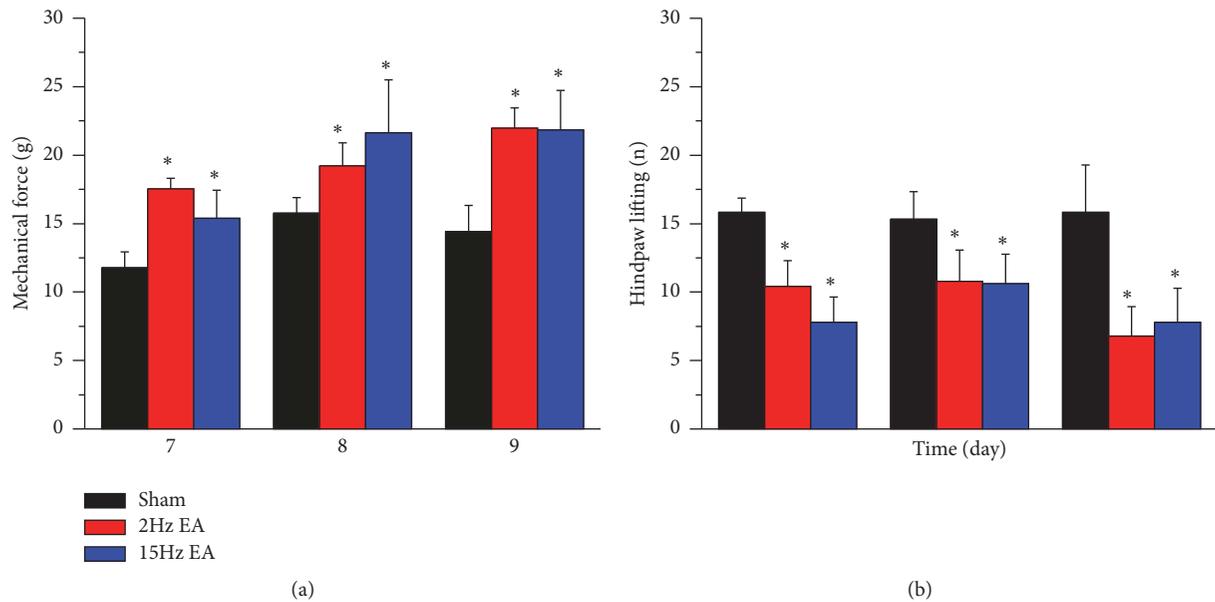


FIGURE 1: Withdrawal thresholds and hind paw lifting of rats in the sham ($n = 6$), 2-Hz EA ($n = 6$), and 15-Hz EA ($n = 6$) groups ($N = 18$) after CCI induction at days 7 to 9. (a) Mechanical forces of neuropathic pain rats on days 7 (7), 8 (8), and 9 (9). (b) Hind paw lifting of neuropathic pain rats on days 7 (7), 8 (8), and 9 (9). * $p < 0.01$ versus sham group. CCI: chronic constriction injury; EA: electroacupuncture.

2.4. Western Blot Analysis. Rat DRG, SC, and SSC were immediately excised at day 9 after behavior test for protein extraction. We followed the methods of Liao et al. 2016 [40]. Total protein was prepared by homogenizing the hippocampi for 1 h at 4°C in a lysis buffer containing 20 mmol/L imidazole-HCl (pH 6.8), 100 mmol/L KCl, 2 mmol/L MgCl₂, 20 mmol/L ethylene glycol tetraacetic acid (pH 7.0), 300 mmol/L sucrose, 1 mmol/L NaF, 1 mmol/L sodium vanadate, 1 mmol/L sodium molybdate, 0.2% Triton X-100, and a proteinase inhibitor cocktail. From each sample, 30 μg of protein was extracted and analyzed through a bicinchoninic acid protein assay. The extracted protein was subjected to 10%–15% sodium dodecyl sulfate-Tris-glycine gel electrophoresis and transferred onto a nitrocellulose membrane. The membrane was blocked with 5% nonfat milk in a TBST buffer (10 mmol/L Tris-buffered saline, pH 7.5; 100 mmol/L NaCl; and 0.1% Tween 20) and incubated overnight at 4°C with the anti-GABA_A antibody (1:1000, Alomone, Israel), anti-AIR antibody (1:1000, Alomone), anti-TRPV1 (1:1000, Alomone), anti-TRPV4 antibody (1:1000, Alomone), and anti-GluR3 antibody (1:1000, Alomone) in TBST containing bovine serum albumin. Peroxidase-conjugated antibody (1:500) was used as the secondary antibody. The membrane was assessed using the ECL-Plus protein detection kit.

2.5. Statistical Analysis. All data are presented as mean ± standard error. Statistically significant differences among the sham, 2-Hz EA, and 15-Hz EA groups were analyzed through one-way analysis of variance, followed by Tukey's *post hoc* test. A p value less than 0.05 was considered statistically significant.

3. Results

We first used the von Frey test to ensure the induction of CCI-induced mechanical hyperalgesia. Our data showed that the CCI had initiated mechanical hyperalgesia from day 7 to day 9 after induction in the sham group (Figure 1(a); 11.77 ± 1.16 g, 15.76 ± 1.15 g, 14.4 ± 1.92 g, respectively). We next discovered that the 2-Hz EA group had significantly lower mechanical hyperalgesia than the sham group (Figure 1(a); 17.53 ± 0.79 g, 19.21 ± 1.69 g, 21.98 ± 1.47 g, respectively). Similar results were also observed in the 15-Hz EA group (Figure 1(a); 15.39 ± 2.04 g, 21.63 ± 3.86 g, 21.84 ± 2.89 g, respectively). Next, we performed the cold plate test to determine whether thermal hyperalgesia was involved in the CCI-initiated neuropathic pain. Our results indicated that CCI reliably induced thermal hyperalgesia from day 7 to day 9 in the sham group (Figure 1(b); number of hind paw lifts: 15.83 ± 1.05, 15.33 ± 2.01, 15.83 ± 3.44, respectively). In both the 2-Hz group (Figure 1(b); 10.4 ± 1.9, 10.8 ± 2.28, 6.8 ± 2.13) and 15-Hz group (Figure 1(b); 7.8 ± 1.85, 10.6 ± 2.18, 7.8 ± 2.48), EA successfully attenuated thermal hyperalgesia. Accordingly, we suggest that 2- and 15-Hz EA can reliably attenuate both mechanical and thermal hyperalgesia.

Western blot analysis was performed to clarify the involvement of receptors in the EA analgesia of CCI-induced neuropathic pain. Our results indicated that the protein levels of GABA_A receptors in rat DRG were not changed in the sham, 2-Hz EA, and 15-Hz EA groups (Figure 2(a); 100.01% ± 17.97%, 94.44% ± 25.91%, and 90.17% ± 14.62%, respectively; $p > 0.05$, $n = 6$). We next verified the effect of analgesic AIR on peripheral DRG neurons. We determined that there was no difference among the sham, 2-Hz EA, and 15-Hz EA groups

(Figure 2(b); $100.01\% \pm 7.6\%$, $86.01\% \pm 16.43\%$, and $93.51\% \pm 10.61\%$, respectively; $p > 0.05$, $n = 6$). The phenomenon was also observed for TRPV1 (Figure 2(c); $100.01\% \pm 11.15\%$, $94.61\% \pm 16.50\%$, and $96.81\% \pm 11.53\%$; $p > 0.05$, $n = 6$), TRPV4 (Figure 2(d); $100.01\% \pm 8.66\%$, $89.68\% \pm 8.41\%$, and $99.81\% \pm 10.95\%$; $p > 0.05$, $n = 6$), and mGluR3 (Figure 2(e); $100.04\% \pm 28.14\%$, $101.16\% \pm 19.23\%$, and $137.52\% \pm 45.89\%$; $p > 0.05$, $n = 6$) expression in rat DRG.

We further analyzed the levels of the aforementioned receptors in the central SC. Our data demonstrated that both 2- and 15-Hz EA significantly increased the protein levels of GABA_A receptors in rat SC (Figure 3(a); $124.79\% \pm 9.86\%$ and $129.29\% \pm 8.03\%$; $p < 0.05$, $n = 6$). We then evaluated whether analgesic AIR also participated in the EA process. Our results demonstrated that the potentiation was not observed in either the 2- or the 15-Hz EA group (Figure 3(b); $101.08\% \pm 17.19\%$ and $102.87\% \pm 23.1\%$; $p > 0.05$, $n = 6$). This phenomenon was also not observed in TRPV1 (Figure 3(c); $100.09\% \pm 20.92\%$ and 107.64 ± 34.72 ; $p > 0.05$, $n = 6$), TRPV4 (Figure 3(d); $115.83\% \pm 20.39\%$ and $104.05\% \pm 14.97\%$; $p > 0.05$, $n = 6$), or mGluR3 (Figure 3(e); $101.08\% \pm 14.34\%$ and $102.87\% \pm 22.65\%$; $p > 0.05$, $n = 6$) expression in SC.

Furthermore, we further tested whether these receptors were involved in EA analgesia in the rat SSC. Neither 2- nor 15-Hz EA altered the protein levels of GABA_A receptors in rat SSC (Figure 4(a); $112.84\% \pm 24.48\%$ and $99.81\% \pm 26.06\%$; $p > 0.05$, $n = 6$). We next showed that AIR did not participate in EA analgesia because the protein level was not altered in either the 2- or the 15-Hz EA group (Figure 4(b); $109.28\% \pm 22.05\%$ and $95.05\% \pm 17.43\%$; $p > 0.05$, $n = 6$). Protein levels remained unchanged in TRPV1 (Figure 4(c); $109.84\% \pm 4.55\%$ and $94.56\% \pm 6.13\%$; $p > 0.05$, $n = 6$), TRPV4 (Figure 4(d); $99.5\% \pm 2.23\%$ and $98.48\% \pm 3.76\%$; $p > 0.05$, $n = 6$), and mGluR3 (Figure 4(e); $109.73\% \pm 14.87\%$ and $114.98\% \pm 13.38\%$; $p > 0.05$, $n = 6$) expressions in the rat cortex.

4. Discussion

EA has been widely used for pain relief. A recent study [44] indicated that high-frequency (100-Hz) EA obtained superior results to low-frequency (2-Hz) EA in the rat ankle sprain pain model. EA can relieve acute pain by releasing opiates to activate μ -, δ -, and κ -opioid receptors. By contrast, EA can regulate persistent pain by activating μ - and δ -opioid receptors [45]. Abuaisa et al. [46] suggested that acupuncture is a potential treatment for chronic painful peripheral diabetic neuropathy. Cruccu et al. [47] stated that pharmacological relief of neuropathic pain is always insufficient. Electrical neurostimulation is therapeutic in chronic neuropathic pains. One study suggested that EA is more favorable than high-frequency transcutaneous electrical nerve stimulation. Our previous study [48] demonstrated that EA at Zusanli and Shangjuxu acupoints significantly reduced both mechanical and thermal hyperalgesia by reducing cerebral TRPV4 expressions but not TRPV1 expression.

EA at 2 Hz can reduce pain depending on the noradrenergic descending pathway and spinal GABAergic modulation.

By contrast, high-frequency EA at 100 Hz mainly acts on GABA_B mechanisms [49]. EA at 2 Hz can reliably decrease pain signaling through GABA_A in the dorsal anterior pre-tectal nucleus. Furthermore, 100-Hz EA can attenuate pain via μ -opioid and 5-hydroxytryptamine 1 (5-HT₁) receptors in the ventral anterior pre-tectal nucleus [49]. Fusumada et al. [50] suggested that EA at the ST36 acupoint may regulate GABAergic transmission on descending pathways in periaqueductal areas. Injection of a GABA receptor agonist, gabazine or saclofen, attenuated the therapeutic effect of EA on cold allodynia in rats. These results indicate the crucial role of both GABA_A and GABA_B receptors in neuropathic rats at spinal levels [51]. Xing et al. [52] demonstrated that neuronal hyperactivity in spinal pain transmission was enhanced after nerve injury and further developed into neuropathic pain. EA at 2 Hz was curative in neuropathic pain with the expression of LTD in the C-fiber in SNL rats. The phenomenon could be blocked by NMDA and an opioid receptor antagonist. By contrast, 100-Hz EA induced LTP in SNL rats, which was mainly mediated by endogenous GABAergic and serotonergic inhibitory systems [52]. In this study, EA administered at Hua Tuo Jia Ji acupoints reduced neuropathic pain by increasing the protein level of GABA_A receptor in the SC.

Goldman et al. [53] determined that adenosine was released during manual acupuncture to activate adenosine A1 but not A2 receptors. They also showed that direct injection of AIR agonist could mimic the analgesic effect of acupuncture. Inhibition of adenosine-degradation enzymes enhanced the concentration of adenosine that was positively related to pain relief [53]. Acupuncture and adenosine agonist injected into Zusanli acupoints significantly reduced electrical pain signals in the anterior cingulate cortex. The phenomenon was majorly mediated by the activation of AIR but not A2R. A recent study [54] also showed that interstitial adenosine concentration was increased through acupuncture at the ST36 acupoint for 30 minutes. The phenomenon was only observed at acupoints with rotation, but not when the needle was delivered without rotation or with rotation at a nonacupoint. Intrathecal injection of adenosine reduced spontaneous pain in humans with neuropathic pain [55]. By contrast, AIR but not A2R activation at the spinal level attenuated spontaneous pain [56]. Our data indicated that AIR receptor was unaltered when EA was delivered at Hua Tuo Jia Ji acupoints. Thus, we suggest that AIR is crucial for local analgesia.

TRPV1 was increased in a complete Freund's adjuvant-induced inflammatory pain model from day 1 to day 21. Subcutaneous or intrathecal injection of TRPV1 antagonist capsazepine attenuated thermal hyperalgesia in an inflammatory pain model [57]. Wu et al. [58] indicated that injection of capsaicin, a TRPV1 agonist, into the ST36 acupoint relieved inflammatory pain. This suggested that capsaicin replicated the analgesic effect of acupuncture [58]. TRPV4 participated in inflammatory thermal and mechanical hyperalgesia [59]. Chemotherapy-induced neuropathic pain was ameliorated by TRPV4 antisense oligodeoxynucleotide [60]. TRPV4 plays an important role in several pain syndromes such as acute inflammatory, chronic, neuropathic, and chemotherapy-mediated pain. Chen et al. [61] indicated that EA at bilateral ST36 acupoints reduced the potentiation of TRPV1 and

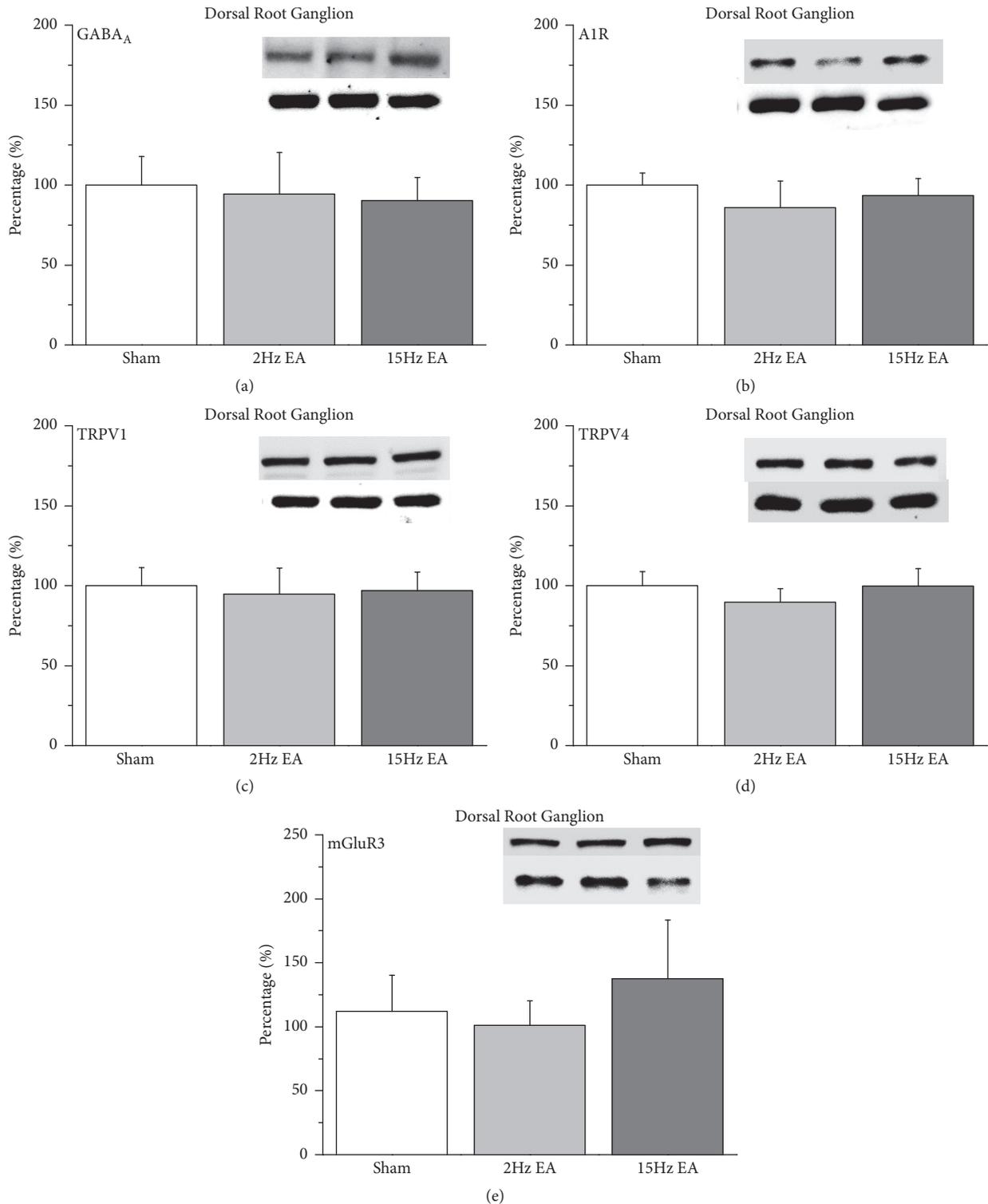


FIGURE 2: Expression levels of GABA_A, AIR, TRPV1, TRPV4, and mGluR3 receptors in rat DRG. (a) GABA_A, (b) AIR, (c) TRPV1, (d) TRPV4, and (e) mGluR3 expression levels in DRG from the sham, 2-Hz EA, and 15-Hz EA groups (from left to right). Sham: neuropathic pain rats with sham EA; 2 Hz: neuropathic pain rats that received 2-Hz EA; 15 Hz: neuropathic pain rats that received 15-Hz EA. * $p < 0.05$ compared with the sham group. The Western blot bands at the top of each panel show the target protein. The lower bands are internal controls (β -actin). AIR: adenosine A1 receptor; DRG: dorsal root ganglion; EA: electroacupuncture; GABA: γ -aminobutyric acid; TRPV1: transient receptor potential cation channel subfamily V member 1; mGluR3: metabotropic glutamate receptor 3; TRPV4: transient receptor potential cation channel subfamily V member 4.

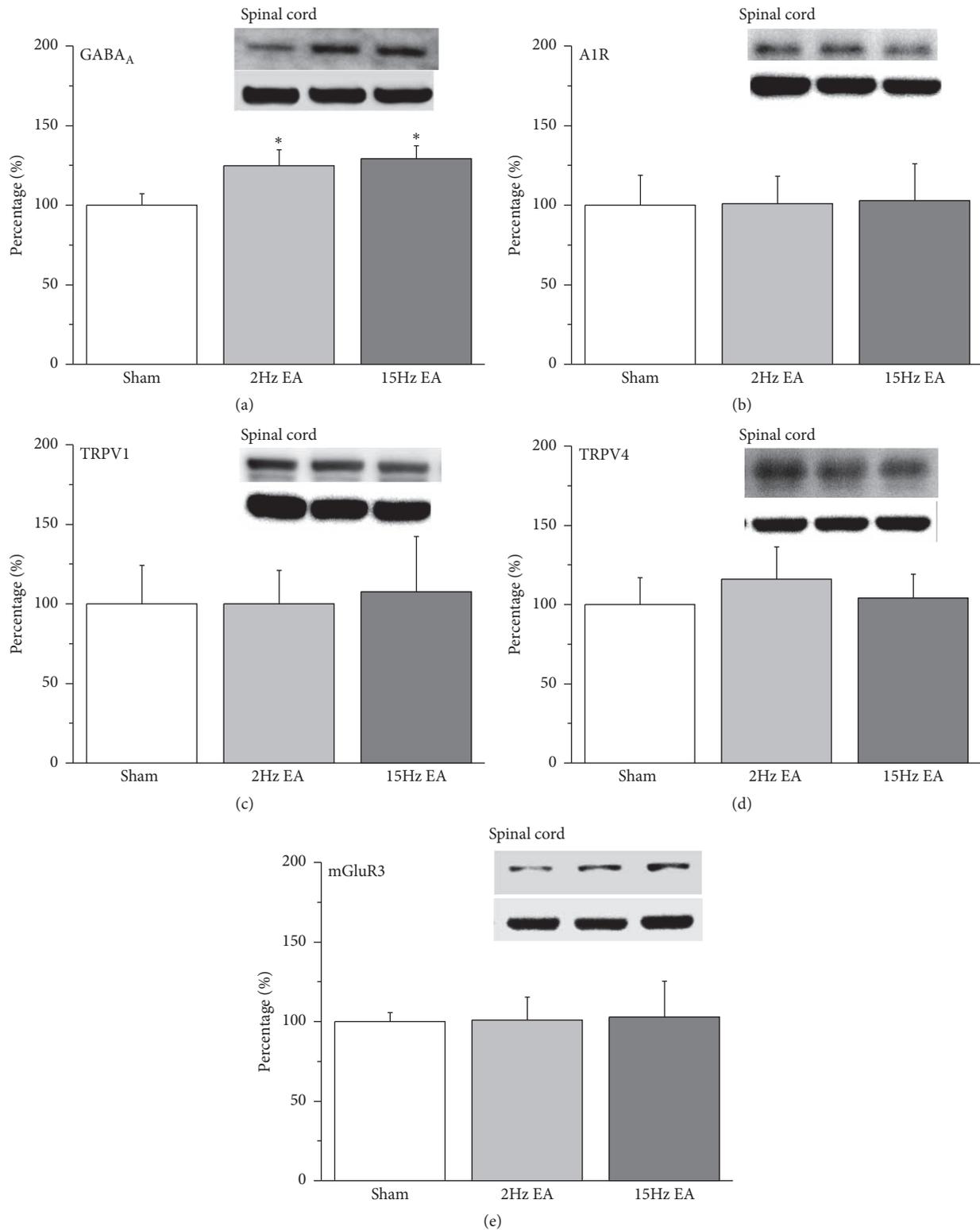


FIGURE 3: Expression levels of GABA_A, A1R, TRPV1, TRPV4, and mGluR3 receptors in rat SC. (a) GABA_A, (b) A1R, (c) TRPV1, (d) TRPV4, and (e) mGluR3 expression levels in the SC of the rats from the sham, 2-Hz EA, and 15-Hz EA groups (from left to right). Sham: neuropathic pain rats with sham EA; 2 Hz: neuropathic pain rats that received 2-Hz EA; 15 Hz: neuropathic pain rats that received 15-Hz EA. * $p < 0.05$ compared with the sham group. The Western blot bands at the top of each panel show the target protein. The lower bands are internal controls (β -actin). A1R: adenosine A1 receptor; DRG: dorsal root ganglion; EA: electroacupuncture; GABA: γ -aminobutyric acid; TRPV1: transient receptor potential cation channel subfamily V member 1; mGluR3: metabotropic glutamate receptor 3; TRPV4: transient receptor potential cation channel subfamily V member 4.

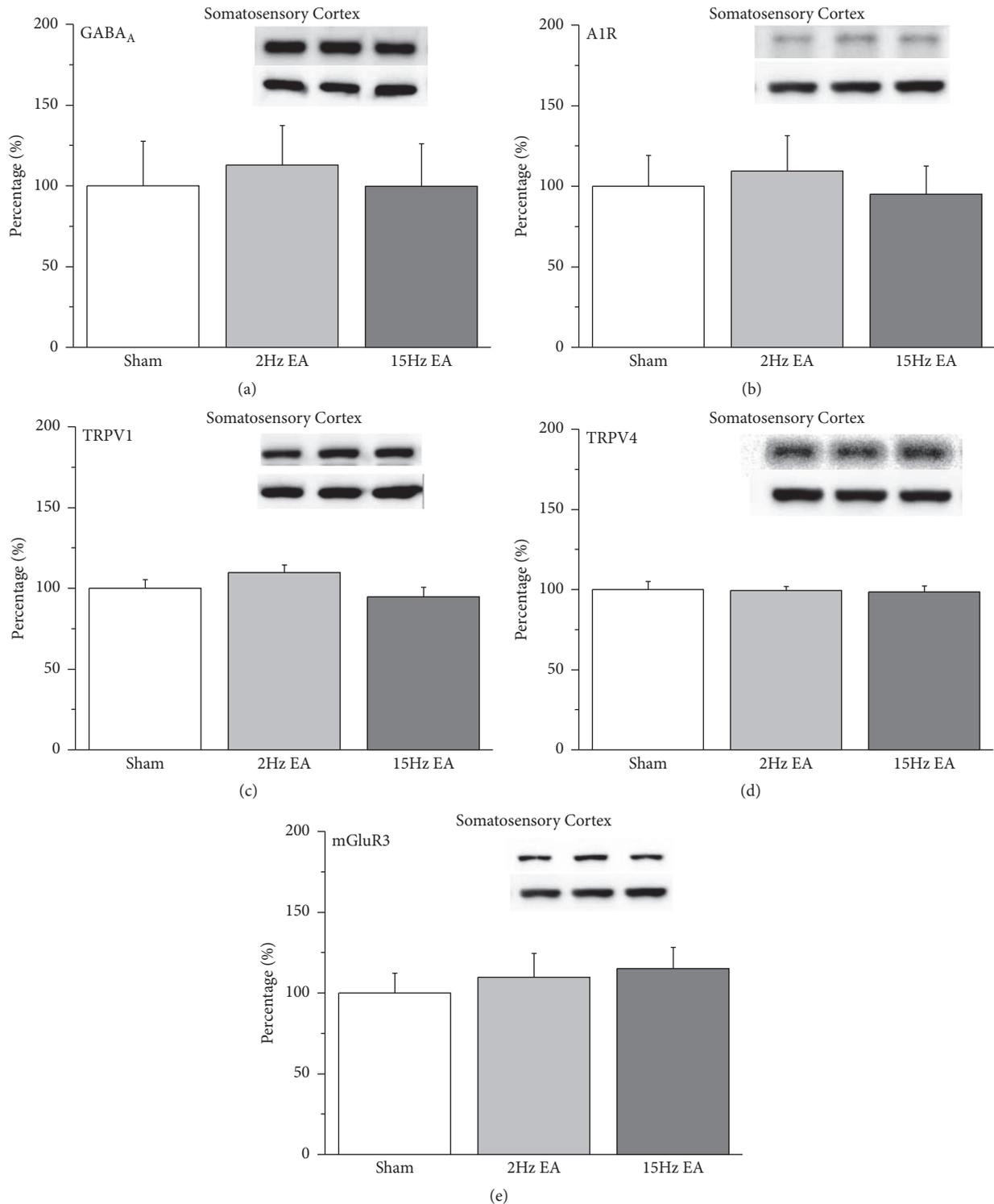


FIGURE 4: Expression levels of GABA_A, A1R, TRPV1, TRPV4, and mGluR3 receptors in rat SSC. (a) GABA_A, (b) A1R, (c) TRPV1, (d) TRPV4, and (e) mGluR3 expression levels in the SSC of rats from the sham, 2-Hz EA, and 15-Hz EA groups (from left to right). Sham: neuropathic pain rats with sham EA; 2 Hz: neuropathic pain rats that received 2-Hz EA; 15 Hz: neuropathic pain rats that received 15-Hz EA. **p* < 0.05 compared with the sham group. The Western blot bands at the top of each panel show the target protein. The lower bands are internal controls (β -actin). A1R: adenosine A1 receptor; DRG: dorsal root ganglion; EA: electroacupuncture; GABA: γ -aminobutyric acid; TRPV1: transient receptor potential cation channel subfamily V member 1; mGluR3: metabotropic glutamate receptor 3; TRPV4: transient receptor potential cation channel subfamily V member 4.

TRPV4 in mice with inflammatory pain. Another study [38] demonstrated that activation of mGluRs attenuated glutamatergic input, which explains the analgesic effect of its agonists on neuropathic pain. Chiechio et al. [62] suggested that activation of mGlu2/3 could reduce neuropathic pain [62]. Our data showed that TRPV1, TRPV4, and mGluR3 were not altered by EA performed at Hua Tuo Jia Ji acupoints.

In summary, we conclude that EA at bilateral L4 and L6 of Hua Tuo Jia Ji acupoints significantly reduces rat neuropathic pain, as determined by measuring mechanical and thermal hyperalgesia through von Frey and cold plate tests. We also determined that spinal GABA_A receptor—but not AIR, TRPV1, TRPV4, or mGluR3—responded to EA analgesia.

Conflicts of Interest

The authors declare that there are no conflicts of interest associated with this manuscript and there was no significant financial support that could have influenced the outcome.

Authors' Contributions

S.-W. Jiang performed the animal experiments; Y.-W. Lin participated in discussions and provided help; C.-L. Hsieh participated in protocol design and wrote the manuscript text. S.-W. Jiang and Y.-W. Lin contributed equally to this work. All authors have reviewed and approved the manuscript.

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