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Inhibiting Nav1.7 channels in pulpitis: An *in vivo* study on neuronal hyperexcitability

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ABSTRACT

Pulpitis constitutes a significant challenge in clinical management due to its impact on peripheral nerve tissue and the persistence of chronic pain. Despite its clinical importance, the correlation between neuronal activity and the expression of voltage-gated sodium channel 1.7 (Nav1.7) in the trigeminal ganglion (TG) during pulpitis is less investigated. The aim of this study was to examine the relationship between experimentally induced pulpitis and Nav1.7 expression in the TG and to investigate the potential of selective Nav1.7 modulation to attenuate TG abnormal activity associated with pulpitis. Acute pulpitis was induced at the maxillary molar (M1) using allyl isothiocyanate (AITC). The mice were divided into three groups: control, pulpitis model, and pulpitis model treated with ProTx-II, a selective Nav1.7 channel inhibitor. After three days following the surgery, we conducted a recording and comparative analysis of the neural activity of the TG utilizing *in vivo* optical imaging. Then immunohistochemistry and Western blot were performed to assess changes in the expression levels of extracellular signal-regulated kinase (ERK), c-Fos, collapsin response mediator protein-2 (CRMP2), and Nav1.7 channels. The optical imaging result showed significant neurological excitation in pulpitis TGs. Nav1.7 expressions exhibited upregulation, accompanied by signaling molecular changes suggestive of inflammation and neuroplasticity. In addition, inhibition of Nav1.7 led to reduced neural activity and subsequent decreases in ERK, c-Fos, and CRMP2 levels. These findings suggest the potential for targeting overexpressed Nav1.7 channels to alleviate pain associated with pulpitis, providing practical pain management strategies.

1. Introduction

Pulpitis is characterized by persistent pain and hyperalgesia due to pulp inflammation [1]. Chronic pain resulting from pulpitis can lead to considerable discomfort and adversely affect the quality of life for patients. However, the mechanisms underlying pulpitis pain remain incompletely understood, and available treatment options and their long-term effects are currently limited [2]. It is widely acknowledged that prostaglandin metabolites secreted by pulpitis, alongside other inflammatory mediators, can intensify pain by amplifying peripheral and central sensitization [3]. Moreover, these inflammatory mediators interact with receptors involved in both peripheral and central sensitization, triggering intracellular signal transduction pathways.

Orofacial abnormalities resulting from pulpitis are mainly caused by pain signals that stimulate the trigeminal ganglion (TG). The TG governs sensation in the oral and facial areas and comprises the sensory innervation of primary afferent neurons within the pulp [4]. Therefore, it plays a central role in orchestrating neuroinflammation and hyperalgesic pain throughout the orofacial regions [5]. Electrical signals resulting from abnormal stimulation of the pulp are transduced into primary afferent neurons and odontoblasts of the tooth through molecular transducers known as mechanosensitive ion channels [6], and previous studies have shown that transient receptor potential (TRP) ion channels in particular have been implicated in pulpitis pain transmission and are highly expressed in the TGs and intrapulp odontoblasts [7]. Furthermore, inflammatory mediators can affect intracellular Ca^{2+} levels by lowering the activation threshold of transient receptor potential vanilloid type 1 (TRPV1) [8]. In our previous study, peripheral and central TRPV1 expressions after pulp inflammation were investigated [9]. Although results showed that TRPV1 channels play an important

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role in the peripheral transmission of toxic pulpitis pain, TRPV1 channels does not have the same effects in the central nervous system (CNS). Therefore, it is necessary to identify additional transducers that mediate signal transmission from pulp afferents to central targets.

Genetic evidence shows that the voltage-gated sodium channel (VGSC) Nav is crucial for pain in humans. In mammals, adult nociceptive neurons, which are sensory neurons detecting noxious signals, express Nav1.7, Nav1.8, and Nav1.9. Among these, Nav1.7, primarily expressed in dorsal root ganglion (DRG) neurons, plays a pivotal role in inflammatory pain and nociceptive hyperalgesia [10,11]. The VGSCs, notably Nav1.7, play essential roles in nociceptive signaling, amplify pain signals, participate in pathological pain conditions, and offer potential as targets for advancing pain management strategies. In a previous study, Nav1.7 expression was notably elevated in the TG of a pulpitis model compared to controls [12]. Additionally, administration of a Nav1.7 blocker suppressed hyperpolarizing activity, indicating the significant involvement of Nav1.7 in pathogenic pain [12]. This suggests a crucial role for Nav1.7 in pain mechanisms associated with pulpitis, underscoring the need for further elucidation of its underlying mechanisms.

In this study, our aim was to investigate the effects of Nav1.7 inhibitions on the physiological alterations and hyperalgesia associated with pulpitis. To achieve this goal, we used *in vivo* imaging with a voltage-sensitive dye to illustrate the inhibitory effects of Nav1.7 overexpression in the TG of a pulpitis model. Additionally, we validated these changes by analyzing the altered neural activation of the TG following Nav1.7 channel blockade. We investigated changes in nociceptive signaling within the TG through Western blot analysis and immunoreactivity on tissue samples. This study showed the clinical significance of Nav1.7 inhibition in pulpitis and indicated the potential of regulating Nav1.7 activity as a novel target for treating pulpitis-associated pain.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (6 weeks old; 20–25 g; Orient Bio, Seongnam, Gyeonggi, Korea) were used in this study. The animals were housed in groups of five per cage and maintained under a 12-h light/12-h dark cycle, with a constant temperature of 22 °C ± 2 °C and humidity of 55 % ± 5 %. Food and water were available to the mice, *ad libitum*. Animals were allowed to acclimate for 7 days after arrival at the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited Yonsei University College of Medicine Animal Care Facilities and These experiments were approved by the Institutional Animal Care and Use Committee (IACUC approval No. 2021-0173).

The animal model of pulpitis was established after mice were anesthetized with sodium pentobarbital (75 mg/kg, administered intraperitoneally). An upright hole was made in the right maxillary first molar (M1) of the mouse using a low-speed dental drill, and the pulp was exposed. Pulp inflammation was induced by exposing a dental paper tip (diameter, 0.15 mm; length, 20 mm) soaked in allyl-isothiocyanate (AITC, Sigma-Aldrich, Milwaukee, WI, USA) to the exposed pulp for 1 min. Control mice were treated with saline instead of AITC. Mice were randomly assigned to a saline-exposed pulp group (normal group; n = 6), an allyl isothiocyanate (AITC)-exposed pulp group (pulpitis group; n = 6), and an inflamed pulp group treated with ProTxll, a selective Nav 1.7 blocker (ProTxll group; n = 6). All experiments were performed to minimize the use of animals.

2.2. Optical imaging

Mice were anesthetized using sodium pentobarbital (75 mg/kg, i.p.) and mounted on a stereotaxic apparatus (Narishige Scientific Instrument Laboratory, Tokyo, Japan) three days after being exposed to AITC. A craniotomy was performed on the cortex and the dura was resected. The exposed brain was gently removed by suctioning with an aspirator until the trigeminal nerve was exposed. To stain the exposed TG, we directly applied a hydrophilic voltage-sensitive dye (VSD) (Di-2-ANEPEQ, 50 mg/ml in saline, Molecular Probes, Eugene, OR, USA) and left for 1 h. After staining, the TG was kept moist with saline. To verify the effects of Nav1.7 inhibition, we applied ProTx-II (a selective Nav1.7 blocker, 1 μM, Tocris Bioscience, Bristol, UK) directly on the TGs for 30 min. Optical signals were recorded from the TG, which is the origin of the mandibular nerve branch (V3). The TG was activated orthodromically by stimulating the mouse whisker pad with a stimulation electrode. Electrical stimulation was delivered via a needle stimulating electrode with a 200 ms delay, 1 ms pulse width, 3 s inter-stimulus intervals, and an intensity of 0.1–1 mA. The activated areas and amplitudes were analyzed upon electrical stimulation with 0.5 mA. The camera was positioned perpendicular to the surface of the TG, showing the V3 in the image field. Neuronal activity was visualized using a MiCAM02 system (BrainVision, Tokyo, Japan) by detecting changes in fluorescence of VSD. The system consisted of a high-resolution CCD camera with a maximum time of 3.7 ms per frame. Image acquisition is triggered by an electrocardiogram using the stimulus/non-stimulus subtraction method. To reduce background noise and artifacts, ten consecutive images were averaged in response to electrical stimulation of the whisker areas. The magnification was achieved using a 1 × objective and a 0.63 × projection lens (Leica Microsystems Ltd. Wetzlar, Germany). This resulted in a detector array of 192 × 128 pixels. For each color image, the activated area was analyzed and the converted area of each captured image was assessed as a percentage: activated area/whole captured area × 100. The optical intensity and activated area were analyzed with MetaMorph software (Universal Imaging Co., Pittsburg, PA, USA).

2.3. Western Blotting

Targeted trigeminal ganglia (TGs) in each group were harvested and immediately frozen in liquid nitrogen. These tissues were homogenized in lysis buffer (PRO-PREP; Intron Biotechnology, Pyeongtaek, Korea) containing phosphatase and protease inhibitors (PhosSTOP; Roche, Mannheim, Germany). The lysates were centrifuged at 13,000 rpm for 15 min at 4 °C to separate the supernatants, and the proteins were separated by SDS-PAGE. The proteins were transferred to a PVDF membrane (Merck Millipore, Darmstadt, Germany). The membrane was blocked with 5 % skim milk for 1 h and incubated with anti-Nav1.7 (1457S, 1:1000; Cell Signaling, Danvers, MA, USA), anti-ERK (4695S, 1:1000; Cell Signaling), anti-phospho-ERK (4376S, 1:1000; Cell Signaling), anti-CRMP2 (9393S, 1:1000; Cell Signaling), anti-phospho-CRMP2 (9397S, 1:1000; Cell Signaling), and α-tubulin (ab7291, 1:5000; Abcam, Cambridge, UK) antibodies overnight at 4 °C. The blots were exposed to chemiluminescent substrate (GE Healthcare, Little Chalfont, UK) to detect the specific proteins and analyzed using the LAS system (LAS 4000; GE Healthcare).

2.4. Immunohistochemistry

After the experiment, the animals were perfused with saline and a 4 % paraformaldehyde solution and fixed for tissue extraction. To observe changes caused by AITC, the ipsilateral TG was extracted, fixed once more in a 30 % sucrose solution, and frozen in OCT compound. Horizontal sections were obtained using a cryosectioning (Microm HM 525; Thermo Scientific, Walldorf, Germany). To observe the expression of Nav1.7 and NeuN, the tissues were blocked in 5 % normal goat serum with 1 % Triton X-100 and incubated with rabbit anti-Nav1.7 primary antibody (1457S, 1:1000; Cell Signaling) and anti-goat NeuN (NBP3-5554, 1:1000; NOVUS, Denver, CO, USA) were incubated overnight at 4 °C. The samples were then reacted with Alexa Fluor 488 and 647 anti-rabbit/goat IgG secondary antibodies (1:1000; Jackson ImmunoResearch, West Grove, PA, USA) and counterstained with DAPI.

Immunofluorescence images were acquired using an LSM700 (Carl Zeiss, Jena, Germany) and analyzed with the ZEN program (Zen 2.3 black software, Carl Zeiss). The number of cells expressing Nav1.7 was quantified and analyzed with imageJ program (https://imagej.nih.gov/ij/).

2.5. Data analysis

All experimental values were presented as mean ± SEM and one-way ANOVA followed by Dunnett's multiple comparisons was conducted to compare the differences between groups in the VSD imaging, immunohistochemistry, and Western blot analysis. Statistical analyses were conducted with SPSS software (version 23.0; IBM Corporation, Armonk, NY, USA) and $p < 0.05$ were considered statistically significant.

3. Results

3.1. Relief the pulpitis pain by Nav1.7 inhibition in TG

To assess the alleviation of pulpitis pain, we monitored neuronal activity via changes in membrane potentials using the VSD imaging method. The spatiotemporal characteristics of *in vivo* TG neuronal activity following electrical stimulation of the whisker pad were explored. VSD images depicted a representative response to electrical stimulation of 0.5 mA in the TG (Fig. 1A). Comparative analysis across experimental groups revealed broader and more pronounced patterns of activation in the pulpitis group. Temporal changes in neuronal activation were quantified and illustrated in Fig. 1B. The extent of activation measured by the area under the curve (AUC) of color-changed pixels was analyzed (Fig. 1C). The TGs from the pulpitis group exhibited significantly heightened neuronal activity compared to the normal group (Normal: 41.54 ± 3.65, Pulpitis: 113.90 ± 17.21, ProTx-II: 58.95 ± 7.64). To validate the pain-relieving effects on pulpitis-induced neuronal activity, we compared the spatiotemporal dynamics of neuronal activitis after 30 min of ProTx-II application. In the ProTx-II group, the activated pixels within TGs were significantly reduced compared to mice in the pulpitis group (Pulpitis: 113.90 ± 17.21, ProTx-II: 58.95 ± 7.64). To investigate whether the increased neural activities induced by pulpitis and the decreased color-changed pixels induced by ProTx-II were related, we analyzed the higher activation pixels of the TG responses in the VSD experiment using red and green color pixels (Fig. 1D). The results exhibited significant differences in the activation colors red and green among the normal, pulpitis, and ProTx-II groups (Green: Normal 2.71 ± 2.03, Pulpitis 7.45 ± 1.08, ProTx-II 2.76 ± 1.56; Red: Normal 3.41 ± 1.48, Pulpitis 7.39 ± 1.36, ProTx-II 5.51 ± 1.22). Our findings demonstrate that ProTx-II inhibited hyper-excitable neuronal activity by reducing optical signaling density by approximately 40–50 % compared to pulpitis group. These results suggest that increased hyper-activity of neurons in pulpitis pain can be effectively mitigated by Nav1.7 channel inhibition.

3.2. Comparison of Nav1.7 expressions after ProTx-II, a Nav1.7 inhibitor, treatment in the TG

In order to compare the expressions of Nav1.7 in the TG, Nav1.7 expression images in each group were selected and analyzed. Fig. 2A shows the representative images of immunostained TGs in each group. The 10 sections of each group of TGs, we counted 1026 neurons in normal group. However, only 228 neurons showed Nav1.7 expressions (22.2 %). In the pulpitis group, 1163 neurons were counted and 1017 neurons were shown to expresse Nav1.7 positive (87.4 %). In the ProTx-ll treated TGs, 1178 neurons were counted and 953 neurons showed Nav1.7 expressions (80.9 %) (Fig. 2B). Following inflammation, there was a notable elevation in Nav1.7 channel expressions within the TG in the pulpitis group (87.4 %), contrasting with the saline-treated group (22.2 %). Nevertheless, ProTx-II treatment did not exhibit a significant

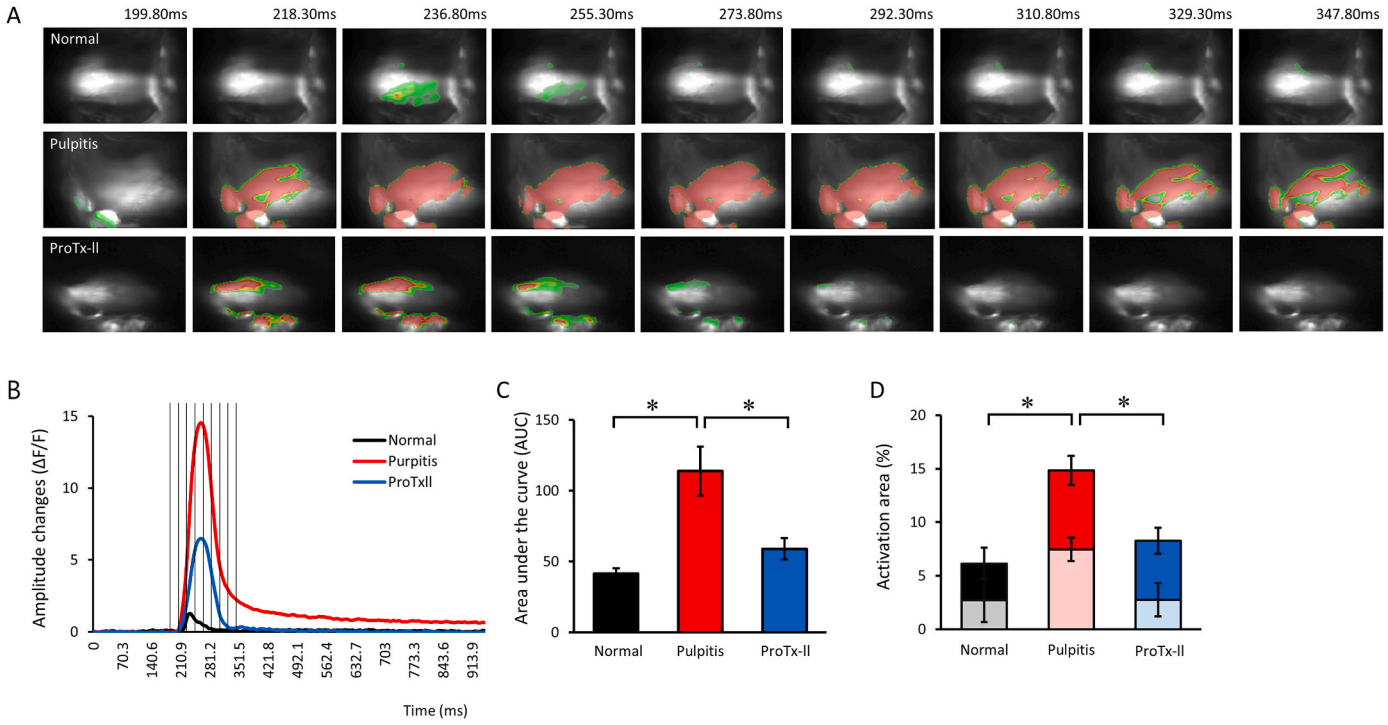

Fig. 1. Comparison of spatiotemporal neural activation in *in vivo* recording of the TGs. (A) Comparison of neural activation patterns in normal, pulpitis, and ProTx-II group. Representative images from each group are shown from 199.80 ms to 347.80 ms. (B) Comparison of time-dependent changes in neural activity following stimulation in the whisker pad. The nine vertical line indicates the timeline of the images in (A). (C) Comparison of total activated areas. (D) Comparison of the activated areas shown in green/red. Data are presented as mean ± SEM. *$p < 0.05$, one-way repeated measures ANOVA followed by Dunnett's multiple comparisons, n = 6 per group. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

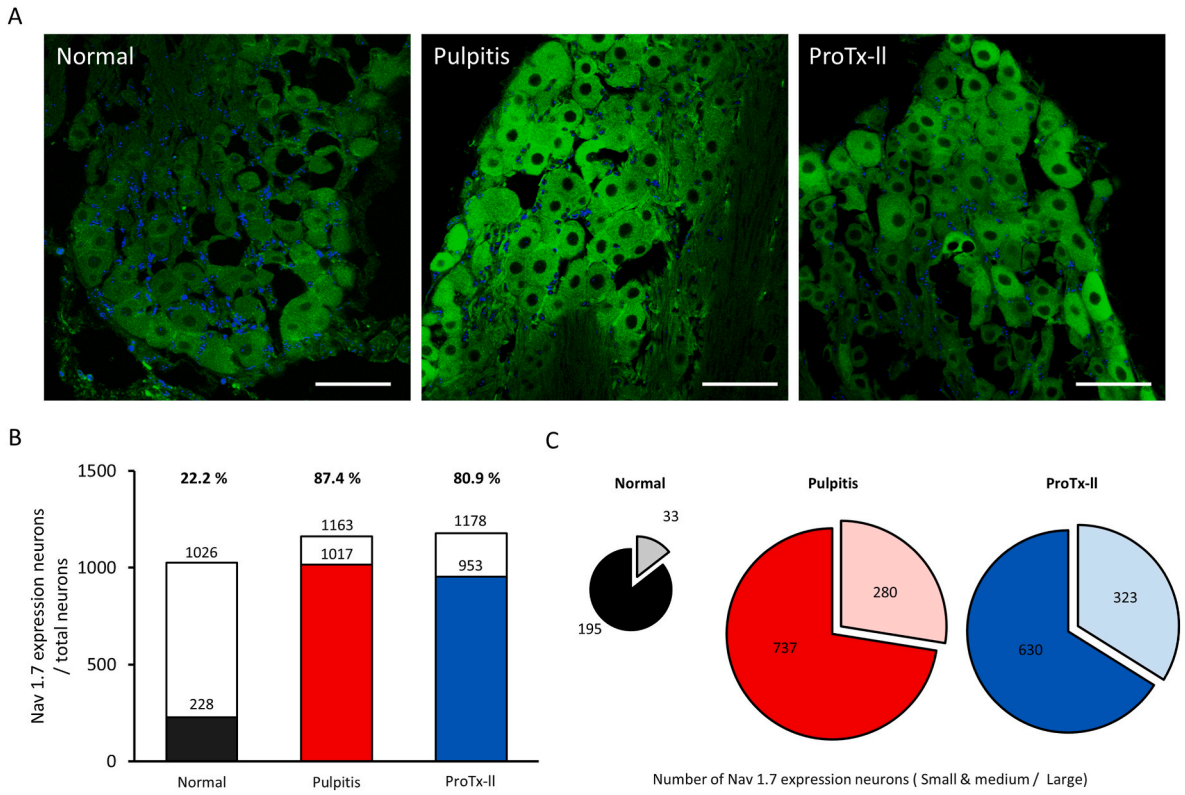

Fig. 2. Expression of Nav1.7 in the TGs. (A) Immunostaining showing Nav1.7 expressions in the TG. Expressions of Nav1.7 were increased in the TG in pulpitis and ProTx-II group. (B) Analysis of Nav1.7 positive neurons. The figure shows the total number of neurons expressed in each group and the number of Nav1.7-positive neurons were calculated and shown as a percentage. (C) Classification of Nav1.7-positive neurons according to cell size. Nav1.7-positive neurons from each group were divided and analyzed according to size (small and medium-diameter ~45 μm, scale bar 50 μm).

decrease compared to the pulpitis group (80.9 %). To identify the neuronal populations expressing Nav1.7, we quantified Nav1.7-positive cells based on size, revealing that the majority of Nav1.7-expressing cells within the trigeminal ganglion were of small to medium size (Normal: small & medium 195, large 33, pulpitis: small & medium 737, large 280, and ProTx-II: small & medium 630, large 323; (small and medium-diameter ~45 μm) (Fig. 2C). These results indicated that pulpitis increases the expression of Nav1.7 channels in small and medium-sized neurons of the TG, and that ProTx-II does not reduce the expression of Nav1.7 channels.

3.3. Expressions of pain signaling molecules in pulpitis model TGs

To delineate alterations in Nav1.7 channels and associated signaling pathways triggered by pulpitis, we conducted Western blot analysis to ascertain whether changes in pain signaling were induced in TGs. The

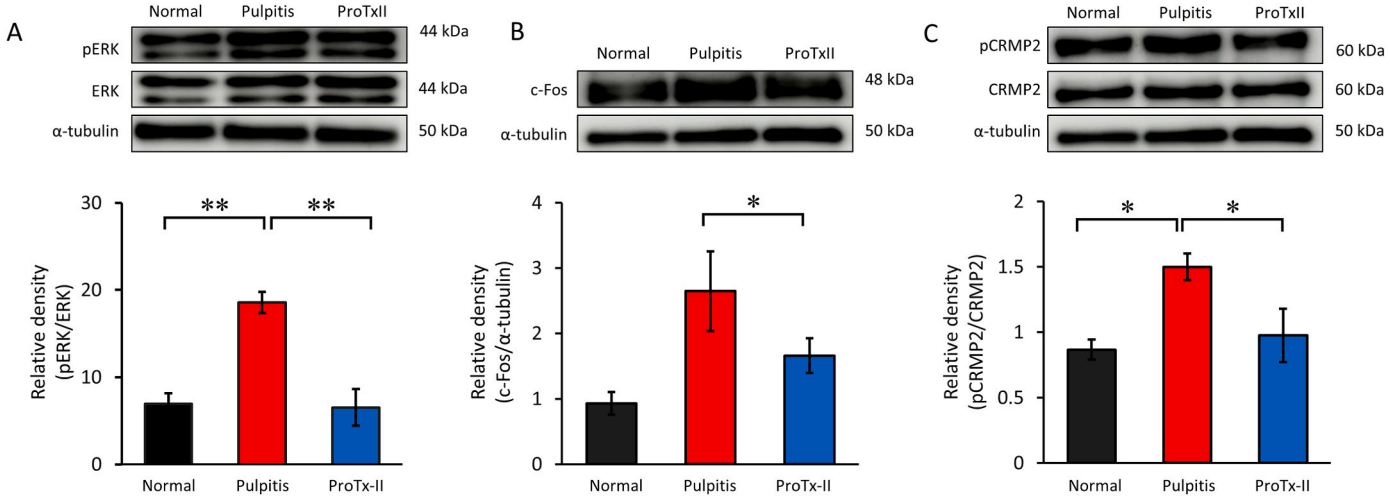

Fig. 3. Changes in signaling molecules in each group (A) Changes in pERK/ERK. A significantly increased pERK observed in pulpitis compared to normal, and this change was reduced after ProTx-II treatment. (B) Between-group changes in c-FOS. Increased c-fos changes were observed in pulpitis, and ProTx-II decreased them. (C) Changes in pCRMP2/CRMP2. The induction of inflammation and changes in pCRMP2 after ProTx-II observed. n = 6 per group. Data are presented as mean ± SEM. $*p < 0.05$ and $**p < 0.01$, one-way repeated measures ANOVA followed by Dunnett's multiple comparisons.

expression levels of phosphorylated ERK (pERK), ERK, phosphorylated CRMP (pCRMP), CRMP, and c-Fos in each experimental group were assessed (Fig. 3). Our findings revealed a significant increase in pERK expression within the TG of the pulpitis group compared to the normal group, and ProTx-II treatment effectively modulated pERK levels (Normal: 6.96 ± 1.18; pulpitis: 18.56 ± 1.22; ProTx-II: 6.53 ± 2.10) (Fig. 3A). Furthermore, c-Fos protein levels, which were elevated in the pulpitis group, exhibited a decrease following ProTx-II application (Normal: 0.93 ± 0.17; pulpitis: 2.64 ± 0.61; ProTx-II: 1.66 ± 0.26). Additionally, the expression of *p*-CRMP was found to be elevated in the pulpitis group compared to the normal group, but significantly decreased after ProTx-II treatment (Normal: 0.87 ± 0.08; pulpitis: 1.51 ± 0.10; ProTx-II: 0.98 ± 0.20). These results highlight the effectiveness of ProTx-II, a Nav1.7 inhibitor, in significantly reducing *p*-ERK and *p*-CRMP protein levels within the TG, thereby indicating a reduction in c-Fos, a marker of neuronal activation.

4. Discussion

Building upon our previous study, which identified aspects of orofacial pain associated with inflammatory responses through, this study aimed to investigate alterations in Nav1.7 channels within the TG, known to regulate nociceptive signal processing in pulpitis pain. We further analyzed changes in pERK, c-Fos, and pCRMP2 levels in the TG to assess the relevance of Nav1.7 channel inhibition and neuronal activation in pulpitis-induced pain. Our findings revealed that pulp inflammation induces hyperexcitatory neuronal activity in the TG, and treatment with a selective Nav1.7 inhibitor, ProTx-II, effectively suppressed neuronal excitability. These results strongly suggest that inhibition of the Nav1.7 channel could serve as an effective strategy for controlling nociceptive signal processing in pulpitis pain.

Pulpitis induces the activation of intracellular signaling pathways which lead to increased membrane excitability of VGSCs [13]. The presence of Nav1.7 amplifies the generator potentials and act as a threshold channel, setting the gain of nociceptor [14]. Neuronal excitability regulated by VGSCs has spatiotemporally distinct electrophysiological properties and may contribute to the pathophysiology of inflammatory pulpitis pain. Elevated levels of VGSCs associated with inflammatory pain in the TG are recognized contributors to central sensitization. This phenomenon occurs through enhanced synaptic efficacy and long-term potentiation [15,16]. In our previous study, we confirmed the same results in a pulpitis model [12]. And present study focused to investigate the synaptic plasticity and pain signaling mechanisms associated with alterations in Nav1.7 activities in the pulpitis model of the TG. In the cases of pulpitis pain, there is an elevation in the expression of Nav channels, notably Nav1.7, following pulpitis [17]. This heightened expression correlates with the severity of the pain experienced. In addition, the expression of Nav1.7 involved in the ectopic activity or abnormal firing of afferent nerves after inflammation [17]. Importantly, Nav1.7 channels are predominantly localized to sensory nerve endings and serve a crucial role in transmitting painful information processing. This assertion is bolstered by evidence demonstrating that nerve injury-induced pain hypersensitivity remained unaffected following conditional knockout of Nav1.7 in the DRG [18]. Consistent with the results of the our previous study, the upregulation of Nav1.7 resulted in the reduction of the threshold for spike initiation and the generation of hyper-excitability, characterized by high-frequency discharges in nociceptive TG neurons [12].

Nav1.7 channels are sensitive to blockade even at nanomolar concentrations of tetrodotoxin (TTX). It has been demonstrated that the Nav1.7 α-subunit can induce the activation and deactivation of inward currents with rapid kinetics, a process promptly halted by TTX in whole-cell patch clamp [19]. Previous studies have demonstrated that ProTx-II effectively reduces inflammatory-induced hyperalgesia and neuronal hyper-excitability [12,20]. Furthermore, through *in vivo* optical recording, this study reaffirmed that treatment with ProTx-II effectively suppressed hyperpolarized activity following electrical stimulation in the TG of the pulpitis model.

In the immunohistochemistry results, we observed the TG serving as a crucial relay station for processing trigeminal nociceptive inputs originating from inflammation and tissue injury. Shukai et al. [17] reported that phosphorylation of ERK 1/2 occurs within the trigeminal nervous system during inflammatory pulpitis pain in rats. Additionally, a study utilizing an animal model of pulpitis-induced inflammation demonstrated that treatment with the ERK antagonist PD98059 significantly reduced the levels of Nav1.7. This ERK1/2 phosphorylation is associated with the regulation of trigeminal ganglion Nav1.7 in hyperalgesia linked to inflamed temporomandibular joints. These findings suggest a correlation between acute pERK1/2 inhibitions and a shift in depolarizing activation and rapid inactivation of Nav1.7. In this study, we additionally demonstrated that the expression level of pERK1/2 was significantly decreased following ProTx-II treatment in the TG. These findings suggest that modulating Nav1.7 can regulate the MEK/ERK pathway in pulpitis, consequently influencing the levels of neurotrophic factors and proinflammatory cytokines [21]. The pERK1/2 and c-Fos are recognized markers of neural sensitization and are closely associated with the intensity of pain generation [22]. The expression of c-Fos serves as an indicator of physiological activity, highlighting specific neural pathways in the brain, and can undergo rapid and substantial upregulation following pain-related neural activity [23]. Notably, experimental dental pain has been shown to induce the expression of c-Fos in the TG [24]. In our study, the detection of markedly elevated levels of c-Fos in the TG of a pulpitis pain model, along with its significant alterations following ProTx-II treatment, underscores the pivotal role of Nav1.7 channels in pain-related processes. Previous studies have shown that pCRMP2 is increased in chronic pain situations [25,26]. These results indicate that pCRMP2 can affect the transmission of neural signals and is associated with synaptic strengthening. In this study, changes in pCRMP2 in the TG by pulpitis and inhibition of increased pCRMP2 expression levels by ProTx-II were observed. Previous studies have reported that the non-phosphorylated form of CRMP2 is involved in promoting microtubule assembly and reducing the amount of NMDA receptor subtype 2B (NR2B) in neuronal signaling due to the insensitivity of neurons to glutamate [27]. The other research has indicated that CRMP2 serves as a dual regulator of N-type voltage-gated calcium (Cav2.2) and Nav1.7 VGSCs [28]. When phosphorylated CRMP2 interacts with Cav2.2 or Nav1.7 sodium channels at the presynaptic terminals, it stimulates calcium currents and vesicular fusion. Consequently, this enhances neurotransmitter release, potentially involving glutamate, which can modulate AMPA and NMDA receptors [29]. Moreover, our Western blot results suggest that the reduction of Nav1.7 expression could effectively block peripheral nociceptive signaling mediated by VGSCs, thereby preventing the onset of spontaneous pain induced by inflammatory pulpitis [20]. Central sensitization in inflammatory conditions is facilitated by ongoing activities of primary afferents exposed to inflammatory irritants [22]. Consequently, if the disease process is adequately controlled by sodium channel blockers, pain hypersensitivity could return to normal once the initiating event has healed. However, the use of sodium channel blockers as analgesic candidates is currently limited in clinical trials due to their non-selectivity, relatively narrow therapeutic windows, and associated adverse side effects [30,31].

Taken together, our study demonstrates that inhibition of Nav1.7 activities in the TG during pulpitis significantly alleviates neuronal sensitization and signaling changes induced by pulpitis. The reduction in Nav1.7 overexpression and alterations in molecules associated with pain and synaptic changes are closely correlated with pain responses elicited in pulpitis. These findings establish a neurophysiological causal relationship between the regulation of Nav1.7 and the relief of pulpitis pain.

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Data availability

The data that support the findings of this study are available from the corresponding authors, MC and BHL, upon reasonable request.

CRediT authorship contribution statement

Kyung Hee Lee: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Un Jeng Kim:** Formal analysis, Investigation. **Myeounghoon Cha:** Conceptualization, Writing – review & editing. **Bae Hwan Lee:** Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2024.150044.

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