International Journal of
Translational Medicine

MDPI

Article

Systemic Administration of Docosahexaenoic Acid Suppresses Trigeminal Secondary Nociceptive Neuronal Activity in Rats

Hanano Takahashi, Yukito Sashide and Mamoru Takeda *

Laboratory of Food and Physiological Sciences, Department of Life and Food Sciences, School of Life and Environmental Sciences, Azabu University, 1-17-71, Fuchinobe, Chuo-ku, Sagamihara 252-5201, Japan; m23018@azabu-u.ac.jp (H.T.); me2401@azabu-u.ac.jp (Y.S.)
* Correspondence: m-takeda@azabu-u.ac.jp

Abstract: Background and Objectives: Docosahexaenoic acid (DHA) has been shown to modulate various voltage-gated ion channels and both excitatory and inhibitory synapses. Nonetheless, its exact effect on nociceptive signaling in the trigeminal system has yet to be elucidated. The purpose of the current investigation was to assess if acute DHA given intravenously to rats diminished the excitability of wide dynamic range spinal trigeminal nucleus caudalis (SpVc) neurons in response to mechanical stimulation in vivo. Methods: Single-unit extracellular activity was recorded from SpVc neurons in response to mechanical stimulation of the whisker pad in anesthetized rats. Responses to both non-noxious and noxious mechanical stimuli were analyzed in the present study. Results: The mean firing frequency of SpVc wide dynamic range neurons in response to both non-noxious and noxious mechanical stimuli was significantly dose-dependently inhibited by DHA, and the effect was seen within 5 min. After approximately 20 min, the inhibiting effects dissipated. Conclusions: These results suggest that, in the absence of inflammatory or neuropathic pain, the acute intravenous administration of DHA reduces the activity of trigeminal sensory neurons, including those responsible for pain, indicating that DHA could be utilized as an adjunct and alternative therapeutic agent for managing trigeminal nociceptive pain, including hyperalgesia.

Keywords: nociception; spinal trigeminal nucleus caudalis; single-unit recording; polyunsaturated fatty acids; mechanical stimulation; complementary alternative medicine

Academic Editor: Francesco Fornai

Received: 13 January 2025
Revised: 16 March 2025
Accepted: 18 March 2025
Published: 25 March 2025

Citation: Takahashi, H.; Sashide, Y.; Takeda, M. Systemic Administration of Docosahexaenoic Acid Suppresses Trigeminal Secondary Nociceptive Neuronal Activity in Rats. *Int. J. Transl. Med.* **2025**, *5*, 13. https://doi.org/10.3390/ijtm5020013

1. Introduction

The transmission of sensory information from the orofacial area relies heavily on the spinal trigeminal nucleus, underlining its role as an integral relay station. The nucleus is functionally subdivided into three nuclei (from rostral to caudal): oralis, interpolarlis, and caudalis [1]. The spinal trigeminal nucleus caudalis (SpVc), in addition to the upper cervical (C1–C2) dorsal horn, plays a crucial role as a relay station for trigeminal pain signals originating from inflammation and tissue damage [1,2]. Chronic pathological states, like tissue inflammation, can modify the somatic sensory pathway properties, causing hyperalgesia and allodynia [3]. Alterations in the excitability of primary afferent neurons (peripheral sensitization) modify how information is processed in the spinal trigeminal nucleus or higher centers [4]. Previous investigations have indicated that wide dynamic range (WDR) neurons in the SpVc region are key to the underlying mechanism of hyperalgesia/allodynia and/or the referred pain associated with orofacial pain [2,5].

Docosahexaenoic acid (DHA) is a key polyunsaturated fatty acid found in marine fish oils and the central nervous systems of humans and rats [6]. DHA possesses multiple

advantageous biological properties, including having antioxidant, anti-inflammatory, neuroprotective, anticancer, and cardioprotective effects [7,8]. Recent findings have highlighted the application of complementary alternative medicine (CAM) treatments, such as herbal solutions and acupuncture, for the treatment of clinical chronic pain [9–11], and diet and dietary supplements have been extensively researched for their potential impact on conditions linked to pain [12–14]. Since DHA is not known to have toxic side effects, it might serve as a potential CAM for pain therapy. Prior studies indicate that DHA influences the neuronal excitability of both the peripheral and central nervous systems through transient receptor potential (TRP) channels and voltage-dependent ion channels [15–19]. For example, the adenosine triphosphate-induced inward currents in primary sensory neurons are inhibited by DHA, which is also an effective inhibitor of TRP vanilloid 1 in vitro and in vivo [15,20], implying that DHA diminishes the generator potential in the primary afferent nerve endings. In addition, DHA influences the voltage-gated sodium (Nav) currents in dorsal root ganglion neurons involved in action potential generation [18]. Within the hippocampal slice preparation, DHA effectively reduces Nav and voltage-dependent calcium (Cav) currents, along with the repetitive firing of action potentials elicited by depolarization pulses in post-synaptic CA1 pyramidal neurons that contribute to excitatory synaptic transmission [16,19]. Alternatively, DHA reportedly lowers hippocampal neuron activity via a GABAergic inhibitory mechanism [21]. Neurons with DHA supplementation show a marked increase in spontaneous synaptic activity, mainly owing to increased glutamatergic synaptic functions [22]. Taken together, these findings strongly suggest that systemic DHA administration may suppress sensory transmission, including nociception, in both the peripheral and central nervous systems.

Previously, we discovered that the intravenous administration of the phytochemical, resveratrol, reduced trigeminal nociceptive SpVc WDR neuron activity, possibly via an inhibitory synaptic mechanism, may contribute to trigeminal referred pain [23]. Additionally, we observed that DHA administered locally weakened the nociceptive jaw-opening reflex, potentially through sodium channel blockade [24]. On the basis of these observations, we hypothesized that intravenous DHA administration would attenuate the noxious mechanical stimulation-induced excitability of SpVc neuronal activity through a central mechanism, as is the case for anesthetic and analgesic agents. Nonetheless, the immediate impact of DHA on trigeminal neuronal responses to both nociceptive and non-nociceptive mechanical stimuli in vivo is yet to be determined. Thus, the purpose of the current investigation was to assess if acute DHA given intravenously to rats diminished the excitability of SpVc WDR neurons in response to nociceptive and non-nociceptive mechanical stimulation in vivo.

2. Materials and Methods

Approval for the experiments described herein was granted by the Animal Use and Care Committee at Azabu University (No.200529-3) and the study adhered to the ethical guidelines set by the International Association for the Study of Pain [25]. Extensive actions were undertaken to decrease the use of animals and ease their suffering.

2.1. Extracellular Single-Unit Recording of WDR Neuronal Activity in the SpVc

Adult male Wistar rats (weighing 215–255 g) were kept under a fixed lighting schedule (lights on: 07:00–19:00). Temperature in the room was kept at 23 ± 1 °C. Food and water were available at all times. Recordings of electrophysiological activity were taken from 11 rats. Each rat was sedated with 3% isoflurane and maintained with additional doses of a mixture of anesthetics (0.3 mg/kg of medetomidine, 4.0 mg/kg of midazolam, and 5.0 mg/kg of butorphanol) at 0.25–0.45 mL/kg/h as required, through a cannula into the jugular vein. During the recording session, the absence of a reaction to paw

pinching confirmed the level of anesthesia. A homeothermic blanket maintained the rectal temperature at 37.0 ± 0.5 °C (Temperature Controller, 40-90-8D; FHC, Aspen, Tokyo, Japan) during recording. Throughout the experiments, the edges of all wounds were consistently covered with the local anesthetic 2% lidocaine (Xylocaine). The animals were subsequently situated in a stereotaxic device (SR-50; Narishige, Tokyo, Japan) and the neck muscles were divided along the animal's midline. The atlanto-occipital ligament and underlying dura mater were incised to access the medullary brain stem. A tungsten microelectrode (impedance 3–5 MΩ) was used to perform the extracellular recording of single-unit activity from the SpVc region in the ipsilateral medulla and moved forward or backward in 10 µm increments using a micromanipulator (SM-11 and MO-10; Narishige), according to the stereotaxic coordinates of the rat brain atlas of Paxinos and Watson [26]. The neuronal signals were amplified (DAM80; World Precision Instruments, Sarasota, FL, USA), filtered (0.3–10 KHz), and observed using an oscilloscope (SS-7672; Iwatsu, Tokyo, Japan), and data were recorded for future analysis using PowerLab and Chart v.5 software (ADI Instruments, Oxford, UK), as described previously [23].

2.2. Experimental Protocols

The process for analyzing extracellular single-unit SpVc WDR activity in response to whisker pad mechanical stimulation involved the following steps: To prevent the sensitization of the peripheral mechanoreceptors, the approximate receptor area of the receptive field in the left side of the whisker pad was identified swiftly using a paint brush as a search stimulus. Subsequently, the left side of the whisker pad was examined for individual units that reacted to a series of von Frey hairs (Semmes-Weinstein Monofilaments, North Coast Medical, Morgan Hill, CA, USA) with non-noxious (2, 6, 10 g) and noxious (15, 26, 60 g) mechanical stimulation for 5 s at intervals of 5 s [17,18]. The criteria for WDR neurons were previously identified as follows: graded mechanical stimulation, whether non-noxious or noxious, applied to the receptive field results in an increased firing frequency proportional to the stimulus strength. Following the identification of whisker pad-responsive nociceptive SpVc WDR neurons, we established the mechanical stimulation threshold and the size of the receptive field. The mapping of neurons' mechanical receptive fields was accomplished by applying von Frey hairs to the face and subsequently outlining them on a life-sized facial illustration [17,18]. The quantification of WDR neuronal discharges, due to mechanical stimulation, was achieved by removing the background activity from the evoked activity. Spontaneous discharge frequencies were determined over 2–5 min.

Previous research has shown that WDR neurons in the SpVc region play a crucial role in hyperalgesia and referred pain [2,3], so our study focused on the influence of DHA on nociceptive SpVc WDR neuronal activity, omitting the examination of nociceptive-specific neurons. Post-stimulus histograms (bin = 100 ms) were generated in response to each stimulus [2,5]. The effects of 1–10 mM DHA (Sigma-Aldrich, Milano, Italy), injected through a cannula into the jugular vein, were evaluated 5, 10, 20, and 30 min after administration, because peak effect and recovery were thought to occur during this period. DHA was dissolved in dimethyl sulfoxide and the stock solution was stored in small aliquots at −20 °C until use and then diluted in saline. The mean spontaneous and mechanical stimulation-induced discharge rates, as well as the mechanical threshold before and after the intravenous administration of DHA, were evaluated in the present study. The single-unit recording sites in the SpVc region were pinpointed using the micromanipulator, based on the distance from the obex, the medial point, and the depth from the surface of the medullary dorsal horn, with reference to the rat brain atlas, as described in our previous studies [23,26].

2.3. Data Analysis

The values are presented as the mean ± standard error. One-way repeated-measures analysis of variance was used for statistical evaluation, with the Tukey–Kramer or Dunnett tests administered as post hoc analyses, and Student's *t*-test administered for electrophysiological data (Excel Statcel 4). A two-sided p-value < 0.05 was considered to indicate a significant difference.

3. Results

3.1. Characteristics of SpVc WDR Neurons Innervating the Facial Skin

Extracellular recordings of single-unit activity from 11 neurons in the SpVc were obtained and examined to determine the effects of intravenous DHA injections. A representative example of the receptive field of SpVc neurons responding to both non-noxious and noxious mechanical inputs in the whisker pad is shown in Figure 1A, as described previously [23]. The recording sites were mostly found in the maxillary branches and layers III–VI of the SpVc (0.5 to 2.0 mm from the obex, Figure 1B). Examples of SpVc WDR neuronal unit responses are illustrated in Figure 1C. The most sensitive area of the receptive field was subjected to graded mechanical stimulation, which caused an increase in the firing activity of the SpVc WDR neurons in proportion to the stimulus intensity. Two out of eleven SpVc neurons showed a spontaneous discharge frequency. The mean mechanical stimulation-induced spike threshold was 1.9 ± 1.2 g. Every neuron recorded belonged to the WDR category of neurons (Figure 1D), as described previously [23].

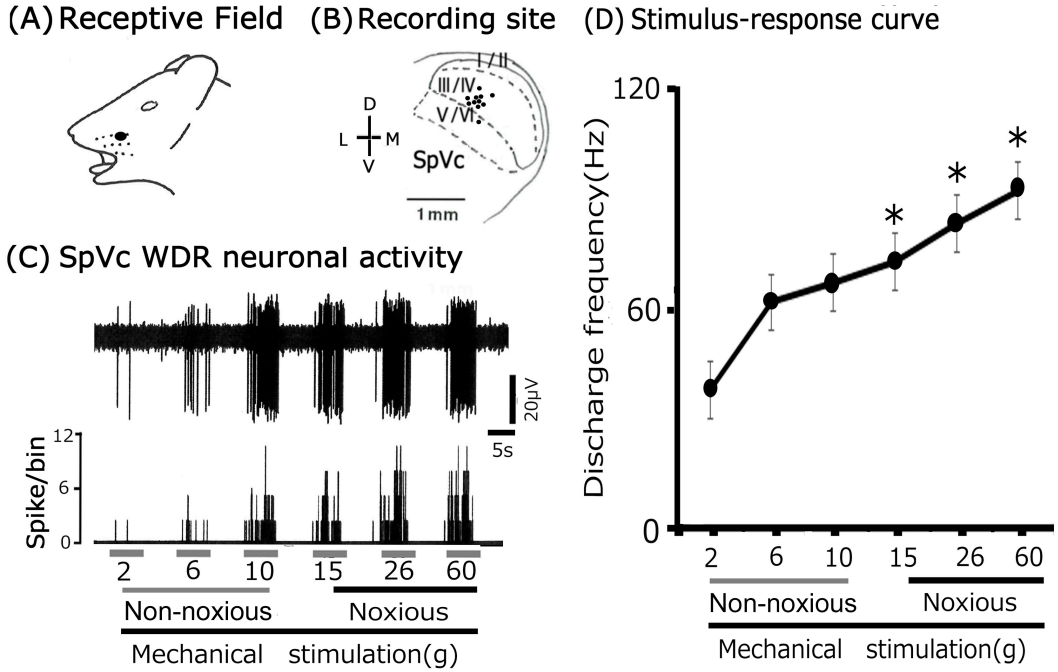

Figure 1. Basic properties of activity in spinal trigeminal nucleus caudalis (SpVc) wide dynamic range (WDR) neurons due to mechanical stimulation. (**A**) Receptive area of the whisker pad on the facial skin. The shaded region shows the position and dimensions of the receptive field. (**B**) Distribution of SpVc WDR neurons reacting to both the non-noxious and noxious mechanical stimulation of the facial skin (n = 11). (**C**) Example of SpVc WDR neuronal activity induced by the non-noxious (2, 6, 10 g) and noxious mechanical stimulation (15, 26, 60 g) of the orofacial skin. Upper trace, SpVc WDR neuronal activity; lower trace, post-stimulus histogram. (**D**) Stimulus–response curve for SpVc WDR neurons (n = 11). * p < 0.05, for the comparison of 2 g vs. 15 g, 26 g, and 60 g.

3.2. Changes in Excitability of SpVc WDR Neurons Due to Intravenous DHA in Response to Non-Noxious and Noxious Stimuli

In Figure 2, a representative example is shown of the impact of intravenous DHA (10 mM) on the excitability of SpVc WDR neurons reacting to non-noxious stimulation. Five minutes following an intravenous injection of 10 mM DHA, non-noxious (2–10 g) mechanical stimulation-induced SpVc activity was inhibited, with activity returning to control levels within approximately 20 min. There was no substantial alteration in the receptive field size prior to or following DHA administration, as previously described [23]. No apparent changes in the mechanical threshold were evident after administering DHA. Figure 3 summarizes how DHA affects the SpVc WDR neuron activity evoked by non-noxious mechanical stimulation. Following DHA injection, there was a significant reduction in the mean firing rates of non-noxious mechanically stimulated (10 g) SpVc WDR neurons compared to their prior rates ($p < 0.05$), and rates returned to control levels within 20 min ($p < 0.05$). No significant changes in spontaneous firing were observed post-intravenous injection of DHA.

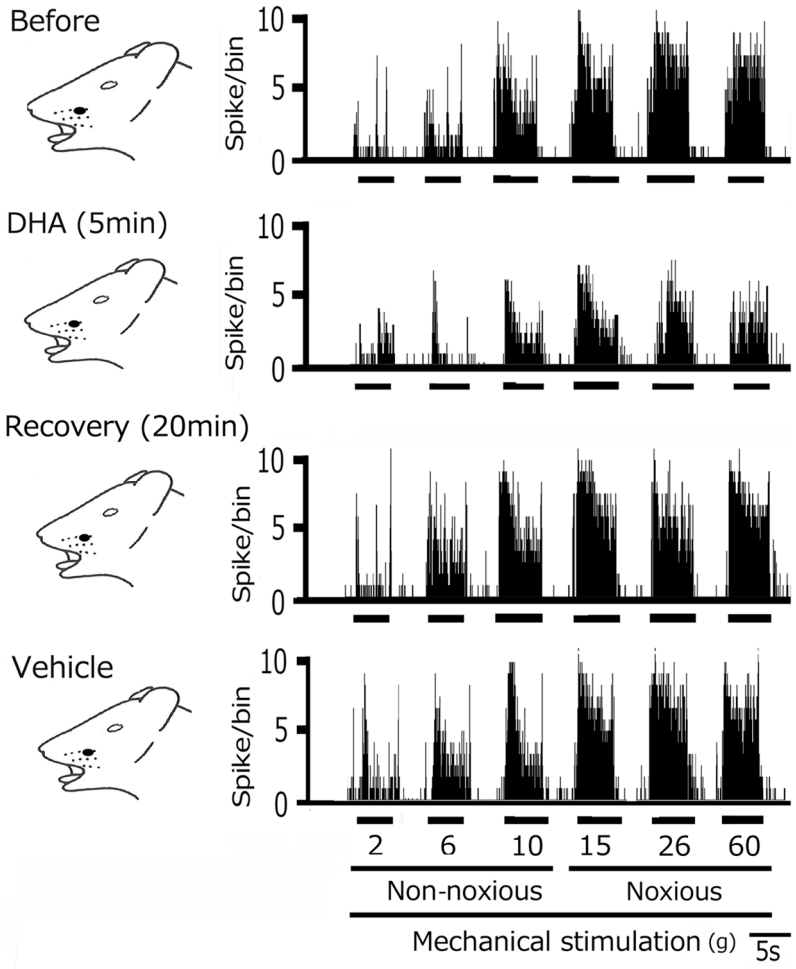

Figure 2. Impact of 10 mM of intravenous docosahexaenoic acid (DHA) on SpVc WDR neuronal responses to non-noxious and noxious mechanical stimuli. Illustrations of SpVc WDR neuronal reactions to non-noxious and noxious mechanical stimuli are shown before and 5 and 20 min after DHA application. The effects of the intravenous administration of the vehicle on SpVc WDR neuronal activity are also shown. The blackened area indicates the location and size of the receptive field.

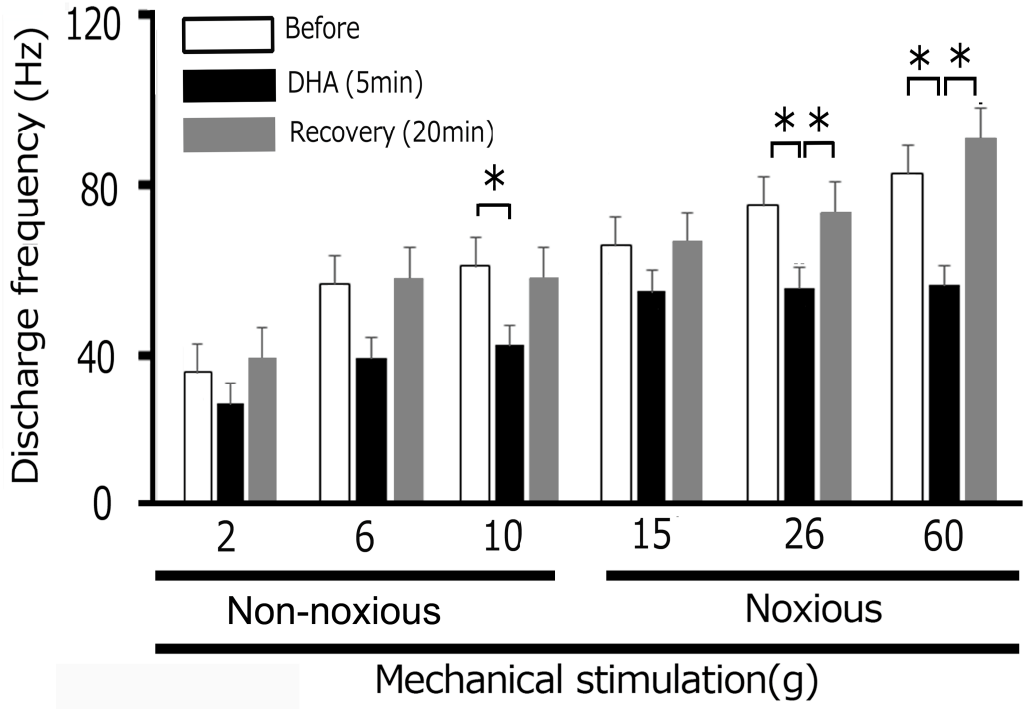

Figure 3. Effects of 10 mM of DHA administered intravenously on the average firing frequency of SpVc WDR neurons reacting to non-noxious and noxious mechanical stimuli over time. * $p < 0.05$, before vs. 5 min after DHA administration, and * $p < 0.05$, 5 min vs. 20 min after DHA administration ($n = 7$).

Figure 2 also shows a representative example of the impact of intravenous DHA (10 mM) on the excitability of SpVc WDR neurons in reaction to noxious mechanical stimulation (15–60 g). SpVc WDR neuron activity elicited by noxious mechanical (15–60 g) stimulation was reduced 5 min after DHA was administered, yet the activity later resumed to previous levels within approximately 20 min (Figure 2). The average firing rates of SpVc WDR neurons induced by noxious mechanical stimuli (26 and 60 g) significantly diminished following DHA injection compared with controls ($p < 0.05$; $n = 7$; Figure 3). There was no notable alteration in the size of the receptive field before or after DHA administration. The intravenous injection of the vehicle (dimethyl sulfoxide) did not significantly affect spontaneous or mechanical stimulation-induced SpVc WDR neuron responses ($n = 3$; Figure 2).

DHA (1–10 mM) significantly suppressed SpVc WDR neuronal firing induced by non-noxious mechanical stimulation in a dose-dependent manner (1 mM vs. 10 mM, $p < 0.05$; Figure 4). DHA also exhibited a significant dose-dependent (1–10 mM) suppression of noxious mechanical stimulation-induced SpVc WDR neuron firing (1 mM vs. 10 mM, $p < 0.05$; Figure 4).

3.3. Response of SpVc WDR Neurons to Noxious Stimuli Compared to Non-Noxious Stimuli Following DHA Treatment

We assessed the relative suppressive effect of a 10 mM intravenous dose of DHA on the reactions to non-noxious and noxious stimuli. There was no notable difference between non-noxious and noxious stimuli in terms of the mean magnitude of inhibition of the discharge frequency caused by DHA (non-noxious vs. noxious, 30.0% ± 1.3% vs. 27.3% ± 2.1%, NS).

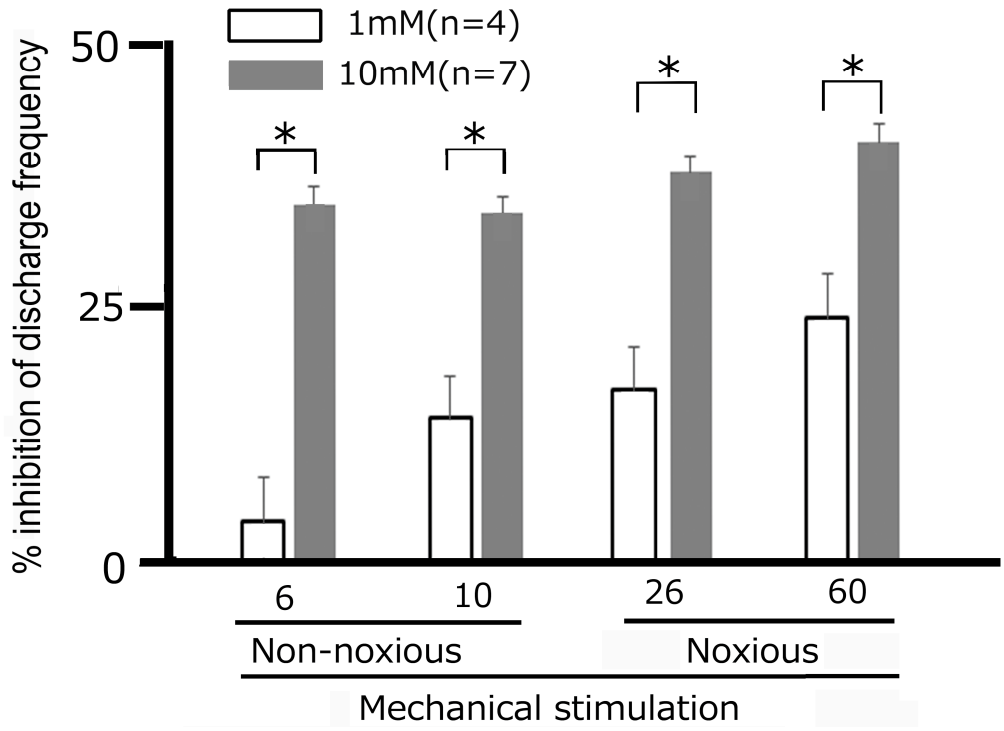

Figure 4. DHA-induced dose-dependent reduction of the average firing rate of SpVc WDR neurons in response to non-noxious and noxious mechanical stimuli. * $p < 0.05$, 1 mM ($n = 4$) vs. 10 mM of DHA ($n = 7$).

4. Discussion

4.1. SpVc WDR Neuron Excitability Is Inhibited by Intravenous DHA

The aim of the current investigation was to assess if acute DHA given intravenously to rats diminished the excitability of SpVc WDR neurons in response to nociceptive and non-nociceptive mechanical stimulation in vivo. The key findings of this research are as follows: (i) the SpVc WDR neuronal firing rate in response to both non-noxious and noxious mechanical stimuli was inhibited in a dose-dependent manner by DHA (1–10 mM, i.v.); (ii) the suppression of the discharge frequency in reaction to both non-painful and painful mechanical stimuli was reversible and took place within 20 min; and (iii) the vehicle injection did not significantly influence SpVc WDR neuronal activity in response to either non-noxious or noxious mechanical stimulation. A prior investigation found that the application of 50 μM of DHA markedly suppressed the repetitive action potential firing in hippocampus slice preparations [19]. In the present study, after the systemic administration of 10 mM of DHA, the drug entered the bloodstream and was diluted to a calculated concentration of approximately 10 μM, and this concentration still had a significant effect on the nociceptive transmission in the SpVc. These findings imply that acute intravenous DHA administration reduces trigeminal pain transmission in in vivo conditions within the SpVc, at the level of the secondary neurons.

4.2. Mechanisms Underlying DHA's Inhibition of the Excitability of SpVc WDR Neurons

The mechanism of nociceptive sensory signaling depends on the following four general processes: (i) transduction from peripheral terminals that transduce external stimuli; (ii) the generation of action potentials; (iii) the propagation of action potentials along axons; and (iv) transmission to central terminals, which form the pre-synaptic elements of the first synapses in the sensory pathways in the central nervous system [2]. DHA alters the excitability of neurons in both the peripheral and central nervous systems via TRP channels and voltage-dependent ion channels [15–19]. For example, DHA inhibits adenosine

triphosphate-induced inward currents in the primary sensory neurons, demonstrating its potency as a TRP vanilloid 1 inhibitor in vitro and in vivo [20], suggesting that DHA decreases the generator potential in the terminals of the primary afferent nerves. In addition, DHA affects the Nav currents in the dorsal root ganglion neurons that are involved in the generation of action potentials [18]. On the basis of these results, it may be presumed that DHA diminishes the excitability of the peripheral terminals of the trigeminal nerve by modifying the processes involved in both the generation of generator potentials and the initiation of action potentials. Additional research is necessary to clarify the exact mechanisms through which DHA operates.

According to Young et al. [19], DHA considerably diminished Nav and Cav currents in hippocampal slice preparations and reduced the repeated generation of action potentials triggered by depolarization pulses in post-synaptic CA1 pyramidal neurons [19] that contributed to excitatory synaptic transmission. DHA influenced the inhibitory GABAergic mechanism by boosting $GABA_A$ receptor activity at inhibitory synapses under in vitro conditions [21,27–29]. DHA contributed notably to the development of the hippocampus and synaptic function in mice [22], and altered the activity of excitatory and inhibitory synapses in hippocampal networks, thus modulating the excitability of neural circuits [21]. Thus, it is possible that DHA administered systemically might reduce trigeminal sensory transmission, including pain perception, through reducing excitatory synaptic signals and boosting inhibitory synaptic signals within the trigeminal secondary neuronal network.

Alternatively, studies have revealed that the response of rats to noxious stimuli, resulting in a withdrawal reflex, is due to the secretion of β-endorphin from the hypothalamus. The administration of DHA to the central nervous system blocks the secretion of β-endorphin from the hypothalamus, and this occurs via receptor 40 (GPR40) [30,31]. Furthermore, L-type Cav channel functions are controlled by insulin secretion from pancreatic β cells via the GPR40 receptor using a polyvalent fatty acid (oleic acid) similar to DHA [32]. These facts indicate that DHA might influence neuronal activity in the central nervous system by blocking voltage-gated ion channels and excitatory synaptic transmission via GPR40. However, further investigations must be conducted to elucidate the specific ways in which DHA functions.

4.3. The Functional Impact of DHA in Reducing the Excitability of SpVc Neurons Triggered by Nociceptive Stimulation

Ultimately, treatments like herbal medicines and acupuncture, categorized under CAMs, have been applied to address clinical chronic pain [9–11]. Studies have been conducted into how diet and nutritional supplements might influence the conditions linked to pain [12–14], including polyunsaturated fatty acids, DHA, and eicotapentanoic acid (EPA) [24,33,34]. By possibly blocking Nav channels, DHA and EPA diminish the nociceptive jaw-opening reflex, which strongly supports the hypothesis that DHA and EPA function as pain inhibitors, firmly promoting the notion that DHA and EPA could serve as possible therapeutic agents and CAMs for preventing acute trigeminal nociception [24,34]. Nakazaki et al. [33] demonstrated that the chronic administration of DHA attenuated inflammation-induced mechanical hyperalgesia and also showed that long-term DHA administration reduced inflammation-induced mechanical hyperalgesia associated with the suppression of the hyperexcitability of SpVc WDR neurons via the inhibition of the activity of the prostaglandin E2 production enzyme, cyclooxygenase-2. These results suggest that DHA could be used as a therapeutic agent in local anesthetics and inflammatory pain treatments within CAM for alleviating trigeminal inflammatory pain hypersensitivity. This evidence, along with the results of our current experiment, show that DHA may be able to transiently relieve inflammatory pain when administered intravenously.

Surgical incisions can lead to intense pain, and surgery is known to be a significant contributor to chronic pain [35,36]. DHA may be effective in reducing clinical pain, such as postoperative pain [37,38]. The present research shows that administering DHA intravenously in acute doses can reduce trigeminal nociceptive transmission without inflammatory or neuropathic pain. Consequently, DHA could serve as a potential therapeutic agent for addressing trigeminal nociceptive pain, including clinical pain. Finally, the present study examines the effects of a single intravenous administration of DHA on nociceptive pain in naïve rats, whereas the effects on pathological pain, such as inflammatory or neuropathic pain, require further investigation. Therefore, future experiments should focus on investigating the effects on pathological pain. Furthermore, experiments on the continuous intravenous administration of DHA are necessary to examine the duration of its analgesic effects.

5. Conclusions

The current investigation shows that, in the absence of inflammatory or neuropathic pain, acute intravenous DHA reduces trigeminal sensory transmission, including nociception, via the possible inhibition of Cav and Nav channels (Figure 5). Hence, DHA might be viewed as a potential CAM agent for managing trigeminal nociceptive pain.

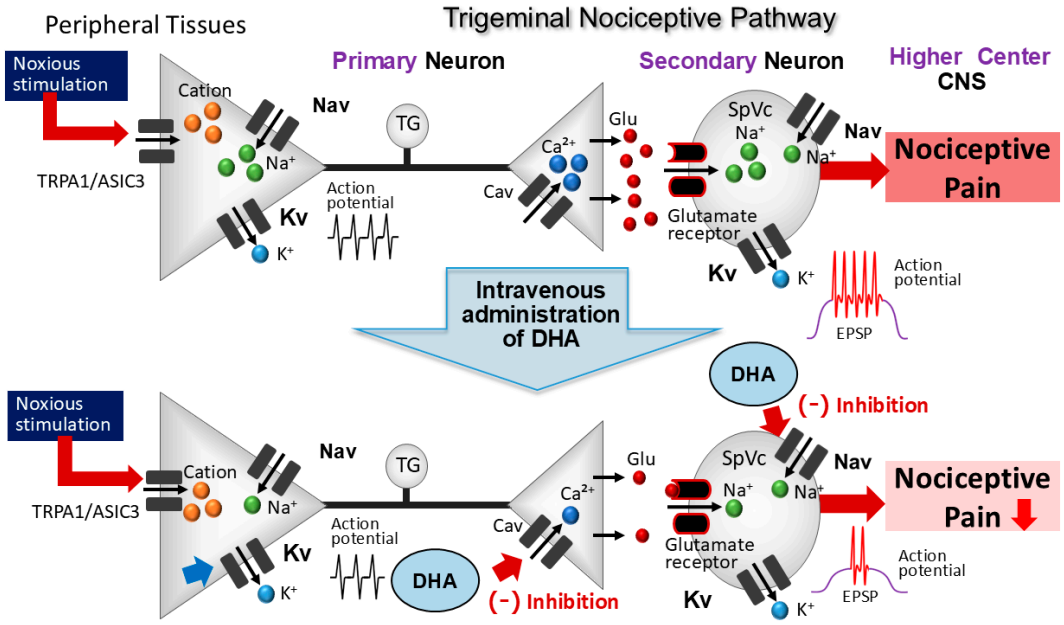

Figure 5. Schema showing one potential mechanism by which DHA inhibits SpVc WDR neuronal discharge in response to nociceptive mechanical stimulation. The application of nociceptive mechanical stimulation to the skin causes mechanosensitive ion channels (TRPA1/ASIC3) to open, initiating a generator potential. As a result of this depolarization, voltage-gated sodium (Nav) and potassium (Kv) channels are further opened, producing action potentials that are conveyed via primary afferent fibers to the central terminals of the nociceptive neurons in the SpVc. As the action potential reaches the central end of the nerve terminal, voltage-gated calcium (Cav) channels at that site open, resulting in depolarization, and permitting the entry of Ca^{2+} ions. An increase in the intracellular Ca^{2+} concentration triggers the release of excitatory neurotransmitters like glutamate (Glu) from the pre-synaptic terminal into the synaptic cleft, enabling cations to pass into the cell by triggering ionotropic glutamate receptors on the secondary sensory neurons. The activation of glutamate receptors results in cations entering the cell, generating an excitatory post-synaptic potential (EPSP). Once this EPSP attains a certain membrane potential, the triggering of further action potentials is initiated. Administering DHA intravenously decreases the excitability of SpVc WDR neurons by blocking Cav channels at the pre-synaptic terminal in the trigeminal ganglion (TG) and

inhibiting the Nav channels in SpVc neurons, leading to a reduction in the firing rate of the action potentials of SpVc WDR neurons transmitting pain signals to higher brain centers. TRPA1, transient receptor potential ankyrin 1; ASIC3, acid sensing ionic channel 3; and CNS, central nervous system.

Author Contributions: Conceptualization, H.T. and M.T.; Writing—original draft, H.T. and M.T.; Methodology, H.T., Y.S. and M.T.; Data curation, H.T. and Y.S.; Formal analysis, H.T. and Y.S.; Investigation. H.T. and Y.S.; Funding acquisition, M.T.; Project administration, M.T.; Resources, M.T.; Supervision, M.T.; Validation, M.T.; Writing—review and editing. M.T. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by a Grant-in-Aid for Scientific Research (C) from the Japanese Society for the Promotion of Science (No. 22K10232).

Institutional Review Board Statement: All experiments reported herein were approved by the Animal Use and Care Committee of Azabu University on 08 February 2024 (No. 230120-11).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare that they have no conflicts of interest related to this work.

References

1. Sessle, B.J. Acute and chronic craniofacial pain: Brainstem mechanisms of nociceptive transmission and neuroplasticity and their clinical correlates. *Crit. Rev. Oral Biol. Med.* **2000**, *11*, 57–91. [CrossRef] [PubMed]
2. Takeda, M.; Matsumoto, S.; Sessle, B.J.; Shinoda, M.; Iwata, K. Peripheral and central mechanisms of trigeminal neuropathic pain and inflammatory pain. *J. Oral Biosci.* **2011**, *53*, 318–329. [CrossRef]
3. Scholz, J.; Woolf, C.J. Can we conquer pain? *Nat. Neurosci.* **2002**, *5* (Suppl. S11), 1062–1067.
4. Millan, M.J. The induction of pain: An integrative review. *Prog. Neurobiol.* **1999**, *57*, 1–164.
5. Iwata, K.; Takeda, M.; Oh, S.; Shinoda, M. Neurophysiology of orofacial pain. In *Contemporary Oral Medicine*; Farah, C.S., Balasubramaniam, R., McCullough, M.J., Eds.; Springer International Publishing: New York, NY, USA, 2017; pp. 1–23.
6. Crawford, M.A.; Casperd, N.M.; Sinclair, A.J. The long-chain metabolites of linoleic and linolenic acids in liver and brain in herbivores and carnivores. *Comp. Biochem. Physiol.* **1976**, *54*, 395–401. [CrossRef] [PubMed]
7. Hashimoto, M.; Hossain, S. Neuroprotective and ameliorative actions of polyunsaturated fatty acids against neuronal diseases: Beneficial effect of docosahexaenoic acid on cognitive decline in Alzheimer's disease. *J. Pharmacol. Sci.* **2011**, *116*, 150–162.
8. Kim, Y.J.; Chung, H.Y. Antioxidative and anti-inflammatory actions of docosahexaenoic acid and eicosapentaenoic acid in renal epithelial cells and macrophages. *J. Med. Food* **2007**, *10*, 225–231. [PubMed]
9. Rao, J.K.; Mihaliak, K.; Kroenke, K.; Bradley, J.; Tierney, W.M.; Weinberger, M. Use of complementary therapies for arthritis among patients of rheumatologists. *Ann. Intern. Med.* **1999**, *131*, 409–416.
10. Konvicka, J.J.; Meyer, T.A.; McDavid, A.J.; Roberson, C.R. Complementary/alternative medicine use among chronic pain clinic patients. *J. Perianesth. Nurs.* **2008**, *23*, 17–23. [CrossRef]
11. Rosenberg, E.I.; Genao, I.; Chen, I.; Mechaber, A.J.; Wood, J.A.; Faselis, C.J.; Kurz, J.; Menon, M.; O'Rorke, J.; Panda, M.; et al. Complementary and alternative medicine use by primary care patients with chronic pain. *Pain Med.* **2008**, *9*, 1065–1072.
12. Shir, Y.; Raja, S.N.; Weissman, C.S.; Campbell, J.N.; Seltzer, Z.E. Consumption of soy diet before nerve injury preempts the development of neuropathic pain in rats. *Anesthesiology* **2001**, *95*, 1238–1244. [CrossRef] [PubMed]
13. Ernst, E. Complementary medicine. *Curr. Opin. Rheumatol.* **2003**, *15*, 151–155. [CrossRef] [PubMed]
14. Tall, J.M.; Raja, S.N. Dietary constituents as novel therapeutics for pain. *Clin. J. Pain* **2004**, *20*, 19–26. [CrossRef] [PubMed]
15. Matta, J.A.; Miyares, R.L.; Ahern, J.P. TRPV1 is a novel target for omega-3 polyunsaturated fatty acids. *J. Physiol.* **2007**, *578*, 397–411. [CrossRef]
16. Vreugdenhil, M.; Bruehl, C.; Voskuyl, R.A.; Kang, J.X.; Leaf, A.; Wadman, W.J. Polyunsaturated fatty acids modulate sodium and calcium currents in CA1 neurons. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 12559–12563. [CrossRef] [PubMed]
17. Hong, M.-P.; Kim, H.I.; Shin, Y.K.; Lee, C.S.; Park, M.; Song, J.-H. Effects of free fatty acids on sodium currents in rat dorsal root ganglion neurons. *Brain Res.* **2004**, *1008*, 81–91. [CrossRef]
18. Xiao, Y.F.; Kang, J.X.; Morgan, J.P.; Leaf, A. Blocking effects of polyunsaturated fatty acids on Na+ channels of neonatal rat ventricular myocytes. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 11000–11004. [CrossRef]

19. Hong, M.-P.; Kim, H.I.; Shin, Y.K.; Lee, C.S.; Park, M.; Song, J.-H. Docosahexaenoic acid inhibits synaptic transmission and epileptiform activity in the rat hippocampus. *Synapse* **2000**, *37*, 90–94.
20. Eto, K.; Arimura, Y.; Mizuguchi, H.; Nishikawa, M.; Noda, M.; Ishibashi, H. Modulation of ATP-induced inward currents by docosahexaenoic acid and other fatty acids in rat nodose ganglion neurons. *J. Pharmacol. Sci.* **2006**, *102*, 343–346. [CrossRef]
21. Taha, A.Y.; Zahid, T.; Epps, T.; Trepanier, M.-O.; Burnham, W.; Bazinet, R.P.; Zhang, L. Selective reduction of excitatory hippocampal sharp waves by docosahexaenoic acid and its methyl ester analog ex-vivo. *Brain Res.* **2013**, *537*, 9–17. [CrossRef]
22. Cao, D.; Kevala, K.; Kim, J.; Moon, H.; Jun, S.B.; Lovinger, D.; Kim, H. Docosahexaenoic acid promotes hippocampal neuronal development and synaptic function. *J. Neurochem.* **2009**, *111*, 510–521. [CrossRef] [PubMed]
23. Takehana, S.; Kubota, Y.; Uotsu, N.; Yui, K.; Iwata, K.; Shimazu, Y.; Takeda, M. The dietary constituent resveratrol suppresses nociceptive transmission via NMDA receptor. *Mol. Pain* **2017**, *13*, 1744806917697010. [CrossRef] [PubMed]
24. Mitome, K.; Takehana, S.; Oshima, K.; Shimazu, Y.; Takeda, M. Local anesthetic effect of docosahexaenoic acid on the nociceptive jaw-opening reflex in rats. *Neurosci. Res.* **2018**, *137*, 30–35. [CrossRef]
25. Zimmermann, M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* **1983**, *16*, 109–110. [CrossRef]
26. Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*, 2nd ed.; Academic Press: New York, NY, USA, 1986.
27. Hammno, H.; Nabekura, J.; Nishikawa, M.; Ogawa, T. Docosahexaenoic acid reduces GABA response in substantia nigra neurons of rat. *J. Neurophysiol.* **1996**, *75*, 1264–1270. [CrossRef]
28. Nabekura, J.; Noguchi, K.; Witt, M.R.; Nielsen, M.; Akaike, N. Functional modulation of human recombinant gamma aminobutyric acid type A receptor by docosahexaenoic acid. *J. Biol. Chem.* **1998**, *273*, 1156–1161. [CrossRef]
29. Søgaard, R.; Werge, T.M.; Bertelsen, C.; Lundbye, C.; Madsen, K.L.; Nielsen, C.H.; Lundbæk, J.A. GABAA receptor function is regulated by lipid bilayer elasticity. *Biochemistry* **2006**, *45*, 13118–13129. [CrossRef]
30. Nakamoto, K.; Nishinaka, T.; Matsumoto, K.; Kasuya, F.; Mankura, M.; Koyama, Y.; Tokuyama, S. Involvement of long-chain fatty acid receptor GPR40 as a novel pain regulatory system. *Brain Res.* **2012**, *1432*, 74–83. [CrossRef] [PubMed]
31. Nakamoto, K.; Nishinaka, T.; Sato, N.; Mankura, M.; Koyama, Y.; Kasuya, F.; Tokuyama, S. Hypothalamic GPR40 signaling activated by free long chain acids suppresses CFA-induced inflammatory chronic pain. *PLoS ONE* **2013**, *8*, e81563. [CrossRef]
32. Fujiwara, K.; Maekawa, F.; Yada, T. Oleic acid interacts with GPR40 to induce Ca^{2+} signaling in rat islet beta-cells: Mediation by PLC and L-type Ca^{2+} channel and link to insulin release. *Am. J. Physiol. Endocrinol. Metab.* **2005**, *289*, E670–E677. [CrossRef]
33. Nakazaki, S.; Tadokoro, K.; Takehana, S.; Syoji, Y.; Shimazu, Y.; Takeda, M. Docosahexaenoic acid attenuates inflammation-induced hyperexcitability of trigeminal spinal nucleus caudalis neurons associated with hyperalgesia in rats. *Eur. J. Oral Sci.* **2018**, *126*, 458–465. [PubMed]
34. Osaki, H.; Mori, M.; Oshima, K.; Shimazu, Y.; Takeda, M. Effect of local administration of eicosapentaenoic acid on the jaw-opening reflex in rats. *Eur. J. Oral Sci.* **2023**, *131*, e12917. [CrossRef] [PubMed]
35. Perkins, F.M.; Kehlet, H. Chronic pain as an outcome of surgery. A review of predictive factors. *Anesthesiology* **2000**, *93*, 1123–1133. [PubMed]
36. Kehlet, H.; Jensen, T.S.; Woolf, C.J. Persistent postoperative pain: Risk factors and prevention. *Lancet* **2006**, *367*, 1618–1625.
37. Locher-Claus, M.T.; Erickson, T.E.; Law, A.S.; Johnson, W.T.; Gebhart, G.F. Effect of pre-emptive morphine, ibuprofen or local anesthetic on fos-expression in the spinal trigeminal nucleus following tooth pulp exposure in rat. *J. Endod.* **2005**, *31*, 578–583.
38. Tillu, D.V.; Melemedjian, O.K.; Asiedu, M.N.; Qu, N.; De Felice, M.; Dussor, G.; Price, T.J. Resveratrol engages AMPK to attenuate ERK and mTOR signaling in sensory neurons and inhibits incision-induced acute and chronic pain. *Mol. Pain* **2012**, *8*, 5.