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Neuroinflammation and Synaptic Plasticity: New Insights into Postoperative Pain Mechanism

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Abstract

Postoperative pain, a common consequence of surgical procedures, is primarily attributed to tissue trauma and the ensuing inflammatory response. This response involves the release of inflammatory mediators such as prostaglandins, cytokines (IL-1β, TNF-α, IL-6), and chemokines, which sensitize nociceptors, enhancing pain perception. Although microglia are the main immune cells in the central nervous system, they play an important role in chronic pain by releasing pro-inflammatory cytokines and regulating synaptic transmission and plasticity, but their specific role in postoperative pain is still unclear. This study investigates synaptic changes and the expression of synaptic proteins like PSD-95 following incision surgery to identify potential therapeutic targets for pain management. Our findings reveal that the upregulation of cytokine IL-6 and TNF-α was activated microglia in the spinal cord, highlighting the neuroinflammation in regulating pain in incision pain model. We also studied PSD-95, a scaffolding protein essential for synaptic function and plasticity, is implicated in central sensitization and neuropathic pain. By evaluating synaptic plasticity and inflammatory reaction, we aim to clarify the neurobiological mechanisms behind pain-related cognitive and emotional disorders and potentially guide future pain treatment strategies. These insights emphasize the complex relationship between synaptic changes and pain-related changes in the central nervous system.

Keywords: Pain; Post-operative pain; Microglia; PSD-95; Synaptic function

Introduction

Postoperative pain is common following surgical procedures and is caused by the trauma to the tissues during the surgery [1]. Surgical incisions involve cutting through the skin, muscles, and sometimes deeper tissues, which directly causes pain due to the tissue damage [2]. After surgery, the body's natural inflammatory response to tissue injury releases various chemicals, such as prostaglandins and cytokines that can stimulate nerve endings and cause pain. It involves the release of inflammatory mediators such as prostaglandins, IL-1β, TNF-α and IL- 6 cytokines, and chemokines [3-6]. These mediators sensitize nociceptors making them more responsive to stimuli and resulting in pain. However, the mechanism remains unknown.

Microglia are the primary immune cells of the Central Nervous System (CNS) and play a crucial role in the development and maintenance of pain, particularly in the context of chronic pain [7,8]. The activated microglia can release pro-inflammatory cytokines and chemokines that contribute to the inflammatory response [9]. In addition, microglia can also produce other inflammatory mediators, which can enhance pain sensitivity. Microglia can also influence synaptic transmission and plasticity, which are critical in the modulation of pain. Microglia can release Brain-Derived Neurotrophic Factor (BDNF) that modify synaptic strength and neuronal excitability [10].

Synaptic functions play a critical role in the transmission and modulation of pain within the nervous system [11,12]. Pain signaling involves complex interactions at the synaptic level, particularly within the peripheral and central nervous systems. The pain signal begins at peripheral nociceptors after the incision surgery, and specialized neurons that detect harmful stimuli. When it was activated, pain signals were transmitted through the axons to the spinal cord. The first synapse occurs in the dorsal horn of the spinal cord, where primary afferent neurons release neurotransmitters like glutamate and substance P to activate secondary neurons [13]. Repeated or intense nociceptive input can lead to central sensitization, which is characterized by increased synaptic efficacy in the spinal dorsal horn. This makes the central nervous system more responsive to pain signals.

Postsynaptic density protein 95 (PSD-95) is a crucial scaffolding protein located in the

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Postsynaptic Density (PSD) of excitatory synapses, particularly at glutamatergic synapses [14]. It plays a significant role in synaptic function and plasticity, which are essential processes in the modulation of pain [15,16]. PSD-95 provides structural support to the postsynaptic density, anchoring receptors and other proteins which are necessary for synaptic signaling. In addition, PSD-95 organize and cluster neurotransmitter receptors and signaling molecules at the synapse, ensuring efficient synaptic transmission. PSD-95 is a key player in the modulation of synaptic function and plasticity, which is important to central sensitization and neuropathic pain. However, it is unclear whether PSD-95 also plays a role in postoperative pain via synaptic changes. Therefore, we studied the peripheral and central inflammation and synaptic changes after incision surgery, in order to find a new therapy target for pain treatment in future.

Methods

Animal

For our experiments, we selected adult male C57 mice and stratified them into two distinct groups: control group undergoing sham surgery and the surgery group subjected to an incision pain model. This model is frequently employed to simulate post-surgical pain, thereby providing valuable insights into the molecular and cellular mechanisms of pain. Animals were randomly assigned to each experimental group. All the animal procedures were approved by the Institutional Animal Care and Use Committee of Tianjin Medical University, China.

Incision pain model

The incisional postoperative pain model of mice followed the procedures described by Brennan [17]. Mice were anesthetized with isoflurane by a nose mask under sterile conditions. A longitudinal incision was made through the skin, starting at from the edge of the heel and extending toward the toes of the right mice hind paw. Using forceps elevated the plantaris muscle, leaving the muscle origin and insertion intact. After hemostasis with gentle pressure, the skin was closed and covered with erythromycin ointment. Animals with sham operation underwent the same procedure without incision.

Behavioral test

All animals were habituated to the testing environment for at least 2 days before the behavior testing. All the animal behaviors were tested blindly. Von Frey test was conducted for examining mechanical pain.

Western blotting

The spinal cord was quickly removed and cryopreserved in liquid nitrogen. The sample was homogenized mechanically in RIPA buffer that contained PMSF (Abcam, Cambridge, UK). The bicinchoninic acid test method was used to assess the amount of protein present. Using a membrane coated with a monoclonal mouse anti-β-actin antibody (1:5000; Sigma-Aldrich), the loading and blotting of an identical quantity of total proteins were confirmed. Following resolution on a 10% SDS-PAGE gel, the samples were transferred to nitrocellulose membranes, and probed with rabbit antibodies against PSD-95 (1:2000, ab238135, Abcam). After which secondary antibodies coupled with horseradish peroxidase (1:2000, Jackson Immuno Research, West Grove, PA, USA) were incubated. Enhanced chemiluminescence (Thermo Scientific, Rockford, IL, USA) was used to visualize the membrane-bound secondary antibodies, and Media Cybernetics Inc.'s Image-Pro Plus software (Version 6.0) was used to quantify the Results.

Immunofluorescence

Mice spinal cord was harvested and postfixed in 4% PFA overnight. Next, dehydrate the DRG tissue and embed the dehydrated tissue for cryostat sectioning. Incubate the sections with primary antibodies against IBA1 (Rabbit, 1:200, ab178846, Abcam) at 4°C overnight. Then, incubate with secondary antibodies (1:500) including donkey anti-rabbit (IHC, Jackson, West Grove, PA, 711-165-152) for 1hour at Room Temperature (RT). Images were collected using a fluorescence microscope (Olympus, Japan). Images were analyzed by Fiji.

ELISA

ELISA kits were also used to detect the levels of IL-6 (BMS603-2, Invitrogen), TNF-α (BMS607-3, Invitrogen) in the L4-L5 spinal cord and serum. Take an appropriate amount of sample, dilute it appropriately with the sample diluent provided in the kit, then add 100 μL of the diluted sample or standard to each well, cover the plate, and incubate at 37°C for 1-2 hours. Subsequently, the plate was washed for 3-5 times to remove unbound samples. Enzyme-labeled secondary antibody (HRP-labeled) was added at a concentration of 100 per well, and the incubation was continued at 37°C for 1 hour. Wash the plate for another 3-5 times, then add 100 liters of chromogenic substrate solution (such as TMB) to each well, and incubate it in the dark at room temperature for 10 minutes to 30 minutes until a visible color change occurs. Add 50 L of stopping solution (such as 2 m sulfuric acid) to each well to stop the reaction, and immediately read the absorbance at 450 nm with an enzyme-labeled instrument.

According to the standard curve, the concentration of the target substance in the serum sample is calculated. The statistics and standardization process are the same as above.

Golgi staining

Mice were utilized for the Golgi staining procedure to visualize the morphology of neurons in the spinal dorsal horn. After perfusion, the spinal cord was carefully dissected, with the emphasis on the lumbar segments (L4-L5) of the spinal cord. The extracted tissue was immediately put in a 2.5% potassium dichromate solution at room temperature and stored in the dark for 14 days. In the meantime, the solution is updated every 48 hours to maintain the best conditions for tissue impregnation and prevent dichromate precipitation. After the dichromate treatment was completed, the tissue was transferred to a 1% silver nitrate solution for another 72 hours, and it was also kept in the dark at room temperature. This step facilitated the impregnation of neurons, where silver ions replaced the dichromate ions, forming a precipitate within the neuronal structures. After impregnation, the spinal cord segments were embedded in paraffin. Serial sections were cut using a microtome at a thickness of 80 micrometers to 100 micrometers. Slices are then carefully fixed on gelatin coated glass slides to ensure adhesion in subsequent processing steps. Slices were dehydrated by fractional ethanol series (70%, 95% and 100%) and clarified in xylene to be ready for microscopic examination.

Statistical analysis

All the data in the figures were expressed as mean ± SEM. We analyzed the biochemical or survival data using Prism GraphPad 10.0. One-Way ANOVA with Bonferroni post hoc or unpaired t-test were used. $p<0.05$ was considered statistically significant.

Results

Inflammation cytokines was increased in the spinal cord after incision pain mice model

To examine the central change of inflammatory factors, we

checked level of inflammatory cytokines in the spinal cord by Enzyme-Linked Immunosorbent Assay (ELISA). The expression of inflammatory cytokines IL-6 and TNF-α were elevated after incision surgery group compared with sham group (Figure 1A,1B). The result suggested that incision surgery caused central inflammation.

The activation of microglia in the spinal cord after surgery

Microglia as a central immune cell can response to injury and inflammation. To test microglia, we stained with IBA-1. The result shows that IBA-1 microglia were increased compared with the sham group after incision surgery (Figure 2A,2B). This result indicates microglia is participate in the incision pain regulation.

Synaptic plasticity plays a crucial role via PSD-95 in the modulation of pain sensitivity

Here, we employed the Golgi-Cox staining of dendritic spine morphology, to evaluate synaptic plasticity and neuronal health. The result revealed a marked reduction in the overall density of dendritic spines within the spinal cord that underwent the incisional pain procedure compared to their sham-operated counterparts (Figure 3A,3B). Postsynaptic density-95 (PSD-95) is a critical synaptic marker, which plays a critical role in regulating synaptic transmission. Its upregulation could lead to aberrant processing of pain signals, thereby impacting pain behavioral responses in mice. PSD-95 protein expression in the spinal cord showed a statistically significant elevation of PSD-95 level in the incisional pain group compared to the sham group (Figure 3C,3D). This result indicated that synaptic plasticity is indeed altered in the spinal cord in response to pain stimuli caused by incision surgery. The elevated PSD-95 expression is indicative of potential modifications in synaptic architecture and functionality, which could influence the mechanisms of signal transduction and neural information processing related to pain perception. Whereas modification of synaptic strength and organization, potentially facilitating the heightened perception and retention of pain stimuli.

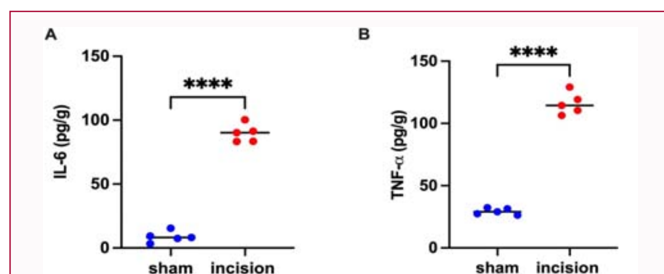

Figure 1: The expression of inflammation cytokines in mice spinal cord with incision pain mice model.
(A-B) Spinal cord IL-6 levels and (B) TNF-α levels, n=5 in each group. Statistical significance was determined by two-tailed Students *t*-test.

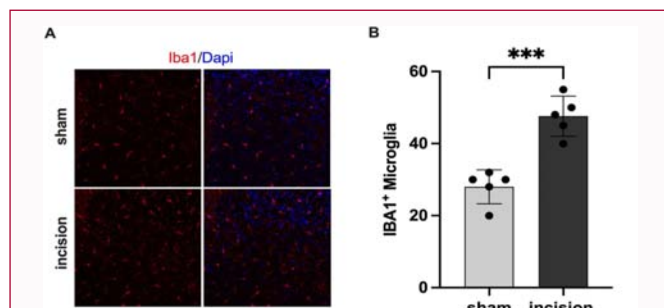

Figure 2: The Microglia was increased in spinal cord after incision surgery.
(A) The representative images of Iba1 expression in spinal cord (B) The qualification of Iba1⁺ microglia cell numbers. n=5 in each group. Statistical significance was determined by two-tailed Students *t*-test.

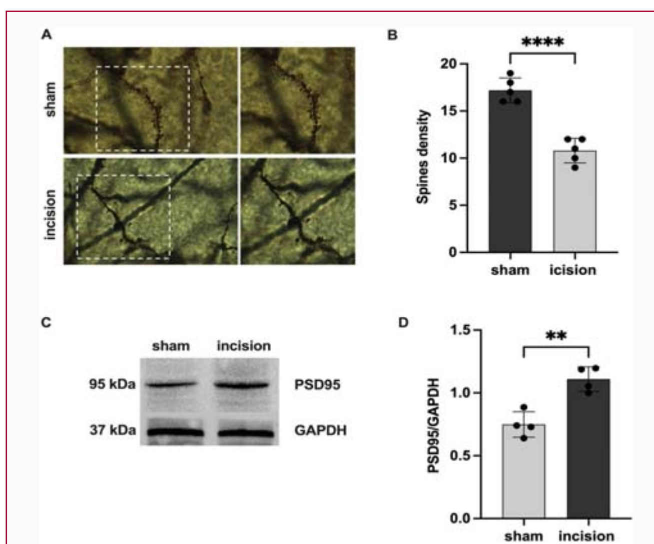

Figure 3: The Synaptic changes in spinal cord after incision surgery.
(A) The representative images of Golgi staining in spinal cord (B) The spines density qualification of Golgi staining. n=5 in each group. (C-D) The PSD95 WB representative image and qualification of PSD95 WB. n=4 in each group. Statistical significance was determined two-tailed Students *t*-test.

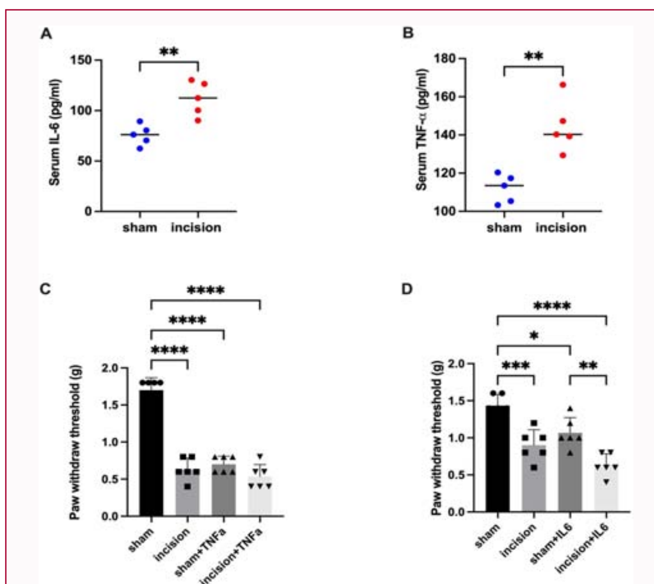

Figure 4: The BWT behaviour of TNF-α and IL-6 injection.
(A-B) IL-6 levels of serum and (B). TNF-α levels of serum n=5 in each group. Statistical significance was determined by two-tailed Students *t*-test. (C) BWT pain behaviour after IL-6 injection. n=6 in each group. Statistical significance was determined by one way ANOVA.

IL-6 and TNF-a administration increased postoperative incision pain

To further investigated the role of IL-6 and TNF-a, we tested the expression of plasma IL-6 and TNF-a after surgery. The results showed that both IL-6 and TNF-a are elevated in the incision pain group compared with sham group (Figure 4A,4B). We performed the pain behavior after the IL-6 and TNF-a injection. Compared with sham group, PWT was decreased after incision surgery (Figure 4C,4D). The TNF-a injection was decreased compared with sham

group (Figure 4C). The IL-6 injection was dramatically lowered PWT compared with sham group, PWT of incision+IL-6 group was reduced compared with the sham+IL-6 group (Figure 4D), which indicate that IL-6 could be an activator of incision pain.

Discussion

Pain is a complex disease process due to injury or disease affecting the somatosensory nervous system [18]. Pain that persists after the surgical wound has healed is the consequence either of ongoing inflammation or a manifestation of Neuropathic Pain (NP) from surgical injury to peripheral nerves. Neuroinflammation is a well-controlled physiological process that serves to promote regeneration and healing [19]. In this study, we evaluated the expression of IL-6 and TNF-a in both the spinal cord and serum. The results shows that IL-6 and TNF-a cytokines are not only increased in the peripheral serum but also elevated in the brain. These findings are illustrated the incision pain correlated with inflammation. Proinflammatory cytokines are related with pain syndrome and they have an important role in pain through different mechanisms in several sites of pain transmission pathways [19,20]. And we performed pain behavior with IL-6 and TNF-a administration. But our results cannot indicate whether cytokines enter the brain through the blood or whether the increase of cytokines in the brain is directly caused by surgery. This mechanism will be further elucidated in our next studies.

However, the results showed that the neuroinflammation and the increase of inflammatory cytokines play an important role in postoperative pain.

Pro-inflammatory cytokines and microglia are closely interlinked in their roles within the Central Nervous System (CNS), particularly concerning their contributions to pain and neuroinflammation. Microglia, as the resident immune cells of the CNS, play a key role in producing and responding to these cytokines, which modulate pain pathways and contribute to both acute and chronic pain conditions [21]. Cytokines activate microglia through specific receptor-mediated signaling pathways [22]. When pain persists, microglia may become activated and release various cytokines and chemokines, which can affect synaptic function and plasticity. Our results demonstrated the changes of microglial function and morphology contributed to incision surgery pain. The activated microglia in the dorsal horn of the spinal cord are necessary for synaptic alterations in pain hypersensitivity after injury [23]. Microglia activation could alter the strength and efficacy of synaptic connections, leading to changes in neural circuitry that might manifest as alterations in cognitive and emotional processing.

Synaptic plasticity, the ability of synapses to strengthen or weaken over time, is fundamental to learning and memory [24]. Thus, persistent pain signals may induce a typical change in synaptic plasticity in this area, affecting these essential functions in further. This reduction was not uniform across all spine types. Specifically, there was a significant decrease in the proportion of mushroom-shaped dendritic spines, which are generally associated with stable synaptic connections and are indicative of mature neuronal networks. The decline in mushroom-shaped spines suggests a disruption in synaptic stability and a potential shift towards a less mature synaptic state, which could reflect underlying alterations in synaptic efficacy and circuitry plasticity. These changes are critical as they might directly impact the ability to perform its roles in cognitive processing and emotional regulation. Understanding the relationship between PSD-95 expression and pain processing may provide insights into the neurobiological underpinnings of pain-related cognitive and emotional disturbances.

These findings highlight the intricate relationship between synaptic protein expression and the cognitive and behavioral manifestations of pain, underscoring the role of synaptic changes in the pathophysiology of pain-related alterations in central nerves system.

Thus, it is imperative to investigate whether synaptic function changes in the spinal cord, and lead to pain sensation in incision model. To address this question, we employed an incisional pain mice model and evaluated PSD-95 levels in the spinal cord at 24h post-injury. This surgery model leaded to the upregulation of inflammation cytokins and the activation of nociceptive pathways in the spinal cord. In addition, we detected the mechanical behavior test following IL-6 and TNF-a injection to confirm the role of inflammation in incisional pain.

These findings underscore the significant impact of incision pain conditions, on the structural plasticity of neurons in the central nerves system.

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