Effects of rapamycin on mTOR signaling in the insular cortex of neuropathic rats

Minjee Kwon

Department of Medical Science

The Graduate School, Yonsei University

Effects of rapamycin on mTOR signaling in the insular cortex of neuropathic rats

Directed by Professor Bae Hwan Lee

The Doctoral Dissertation

submitted to the Department of Medical Science,

the Graduate School of Yonsei University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Minjee Kwon

June 2017

This certifies that the Doctoral

Dissertation of Minjee Kwon is approved.

Thesis Supervisor : Bae Hwan Lee

Thesis Committee Member #1 : Joong Woo Leem

Thesis Committee Member #2 : Insop Shim

Thesis Committee Member #3 : Ji Hwan Ryu

Thesis Committee Member #4 : Je Wook Yu

The Graduate School

Yonsei University

June 2017

ACKNOWLEDGEMENTS

‘생명의 원리’ 라는 다소 추상적인 꿈을 안고 대학원에 들어온 지 어느덧 6년째가 됩니다. 그리고 이제 미약한 흔적에 작은 마침표를 찍고 새로운 선에 서게 되었습니다. 먼저 이 모든 것을 허락하시고 이끌어주신 하느님께 감사를 드립니다.

과학이라는 것에 대해 문외한이었던 제게 생리학을 할 수 있도록 배움의 기회를 주시고 제 연구에 대해 믿음을 갖고 끝까지 응원해 주셨던 지도 교수님, 이배환 교수님께 진심으로 감사드립니다. 좋지 않은 머리로 낑낑대던 제가 예비 과학자로서 성장할 수 있었던 원동력은 교수님의 가르침입니다. 아직까지도 부족한 점이 많은 논문이지만 아낌없이 조언해 주시고 지도해 주신 존경하는 임중우 교수님, 심인섭 교수님, 유지환 교수님, 유제욱 교수님께 이 자리를 빌어 깊은 감사의 인사를 드립니다. 지도해 주신대로, 더 열심히 정진하는 과학자가 되도록 노력하겠습니다.

가족보다 많은 시간을 함께 보내며 동고동락했었던 생리학교실 식구들께 고마움과 감사를 드립니다. 항상 따뜻한 조언과 응원을 해

주신 이경희 선생님께 감사의 말씀을 드립니다. 그리고 우리 실험실이 성장해오는 데 많은 노력과 공헌을 하셨던 차명훈 박사님께 감사의 말을 전합니다. 또한 실험에 대해 아무것도 몰랐던 저를 하나하나 꼼꼼히 가르쳐 주시고 챙겨주신 김은정 선생님께 깊이 감사드립니다. 선생님들께서 계셨기에 즐겁게 실험실 생활을 할 수 있었고, 학문적으로 더 크게 성장할 수 있었습니다. 실험실에서 같이 생활하면서 격려와 용기를 주셨던 사영희 선생님께도 감사의 마음을 전합니다.

저의 첫 번째 후배였던 정수에게 진심으로 고맙다는 말을 전하고 싶습니다. 함께 논문을 읽고 토론하고 실험 결과에 대해 고민하면서 많은 것을 배울 수 있었습니다. 좋은 연구 결과와 우수한 논문으로 학위를 같이 마칠 수 있게 되어 참 기쁩니다. 효진이와 민지, 선우 그리고 실험실에 들어온 지 얼마 안 된 막내 경민이에게도 고맙다는 말을 아울러 전합니다. 힘들 때마다 함께 고민을 들어주고, 위로해 준 후배들이 있었기에 힘을 낼 수 있었습니다. 모두들 좋은 결과로 연구를 잘 마무리 할 수 있길 바랍니다. 생리학교실에서 함께 생활하면서 얻은 특별한 인연, 보람이와 정원이 그리고 박사과정

으로 다시 입학해 신입생이 된 명지에게도 고맙다는 말을 전하고 싶습니다. 처음 수업을 같이 들었던 시간부터, 석사 과정을 지나 박사 과정으로, 그리고 논문을 마무리 할 때까지 어려운 시간이 올 때마다 큰 힘이 되어주었습니다. 열심히 실험하는 모습에 저도 함께 최선을 다할 수 있었습니다. 함께 웃고 껴안을 수 있는 시간이 기다려지고, 기대됩니다.

지금은 계시지 않지만 지난 2년 동안 함께 해주셨던 곽용호 박사님과 김봉수 박사님께도 진심으로 감사 드립니다. 선배 과학자로서 좋은 연구하시는 모습이 항상 존경스러웠습니다. 실험실 생활 초기에 많은 도움을 주셨던 두 분께 다시 한 번 감사 드립니다.

마지막으로 큰 사랑과 인내심으로 학위 과정을 마칠 수 있도록 물심양면으로 도와주신 부모님께 너무나 감사 드립니다. 처음 대학원에 입학했을 때 쉽지 않은 길을 택한 것 같아 늘 걱정이셨다던 부모님의 아낌없는 희생과 믿음, 응원이 아니었다면 여기까지 오기는 힘들었을 것입니다. 아버지, 어머니 감사합니다. 항상 노력하는 과학자가 되도록 끊임없이 정진하겠습니다. 동생인 태현이에게도 고마

움을 전하고 싶습니다. 부족한 누나지만 항상 곁에서 응원해주고, 위로해주고, 힘든 순간엔 오빠처럼 든든하게 지켜줘서 너무 고맙습니다. 갓난아기 시절부터 지금까지 제 옆에서 묵묵히 저를 보살펴 주신 이모님들께도 진심으로 감사드립니다. 학위과정 동안 항상 의지할 수 있는 든든한 마음의 버팀목이었습니다. 고맙고 감사한 마음을 잊지 않고, 자랑스러운 딸, 조카, 누나가 되도록 더욱 노력하겠습니다.

박사과정 동안 많은 분들의 도움으로 학위를 마칠 수 있었습니다. 저를 믿고, 도와주시고 응원해주셨던 모든 분들께 깊이 감사드립니다. 신경과학자로서 새로운 첫발을 내딛는 순간이 설레기도 하지만 큰 책임감도 느껴집니다. 어떠한 여정이 기다리고 있을 지는 알 수 없으나 가르쳐주신 대로 과학을 하는 즐거움을 만끽하면서 더 큰 꿈을 가지고 앞으로 나아가겠습니다.

2017 년 목련 피는 4월 밤에

권 민 지

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Effects of rapamycin on mTOR signaling in the insular cortex of neuropathic rats.

Minjee Kwon

Department of Medical Science

The Graduate School, Yonsei University

(Directed by Professor Bae Hwan Lee)

Injury of peripheral nerves can trigger neuropathic pain, producing allodynia and hyperalgesia via peripheral and central sensitization. Neuropathic pain is related to physiological changes in the primary afferent nerves, including spinal cord and brain. Recent studies have focused on the role of the insular cortex in neuropathic pain. Because the insular cortex is thought to store pain-related memories, translational regulation in this structure may reveal novel targets for controlling chronic pain. In neurons, many proteins that are involved in the inducement of synaptic activity and the expression of synaptic plasticity are converged at synapses especially. Signaling via mammalian target of rapamycin (mTOR), which is known to control mRNA translation and influence synaptic plasticity, may be involved in the development of chronic pain. The activity of mTOR and its downstream effectors have been detected in peripheral and central regions including pain transmission. mTOR has been studied at the spinal level in

neuropathic pain, but its role in the insular cortex under these conditions remains elusive. Therefore, this study was conducted to determine the role of mTOR signaling in neuropathic pain and to assess the potential therapeutic effects of rapamycin, an inhibitor of mTOR, in the insular cortex of rats with neuropathic pain. Mechanical allodynia was assessed in adult male Sprague-Dawley rats after neuropathic surgery and following microinjections of rapamycin in the insular cortex on post-operative days 3 and 7. To evaluate the effective pain prevention of pre-administration with rapamycin in the insular cortex, the animals were microinjected rapamycin into the insular cortex before nerve injury. Rapamycin reduced mechanical allodynia and downregulated the expression of mTOR signaling pathway, postsynaptic density protein 95 and *N*-methyl-D-aspartate receptor, thereby inhibiting neuropathic pain-induced synaptic plasticity. Finally, pre-microinjection of rapamycin effectively alleviated development mechanical allodynia but failed to the down regulated mTOR signaling pathway. These findings suggest that mTOR signaling in the insular cortex may be a critical molecular mechanism modulating neuropathic pain.

Key words: insular cortex, neuropathic pain, mammalian target of rapamycin (mTOR), post synaptic density 95 (PSD95), *N*-methyl-D-aspartate receptor (NMDAR), synaptic plasticity, rapamycin

Effects of rapamycin on mTOR signaling in the insular cortex of neuropathic rats

Minjee Kwon

Department of Medical Science

The Graduate School, Yonsei University

(Directed by Professor Bae Hwan Lee)

I. INTRODUCTION

Pain is a survival mechanism that plays a role as a warning sign of ongoing or imminent tissue damage. While pain is physiologically phenomenon, neuropathic pain is a pathological condition triggered by detrimental changes to the sensory pathway after damage or dysfunction of the nervous system.[1,2] It is characterized by allodynia, hyperalgesia and spontaneous pain. For patients with neuropathic pain, the suffering can be severe and have a disturbingly ordinary life.[3] Despite progress in clinical and advanced animal model for research, the mechanisms of neuropathic pain remain ambiguous. Because of the complex pathologic mechanisms involved, the development of new drugs for such pain has stayed elusive. Thus, understanding the cellular, molecular changing mechanisms underlying neuropathic pain states will be a decisive phase in the development of new drugs to specifically target the distinct mechanisms of neuropathic pain.

Neuroplasticity in the form of protein transcription and translation may contribute to the development of chronic pain including neuropathic pain.[4] Since neuropathic pain is mediated by

both peripheral and central synaptic plasticity, the molecules implied in the synaptic plasticity may serve as the important target for the neuropathic pain.[5,6] Mammalian target of rapamycin (mTOR), a serine/threonine protein kinase that controls protein synthesis and balance, influences extensive range of biological functions and regulates neuronal activity including cell growth, memory formation, and pain processing.[7-9] mTOR belongs to the phosphatidylinositol 3-kinase-related kinase protein family and forms at least 2 multiprotein complexes known as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2).[10] The activation of mTORC1, especially in a sensitive to rapamycin, promotes the phosphorylation of downstream molecules, including eukaryotic initiation factor 4E-binding protein (4EBP) and p70ribosomal S6 protein kinase (p70S6K), which can lead to initiation of new protein synthesis.[11-14] Increasing evidence has supported that mTOR plays an important role in the modulation of neuronal excitability and controls learning and memory-associated with long-term plasticity.[12,15-17] It has been demonstrated that P70S6K or 4EBP knock out mice results in deficiency of long-term memory and synaptic plasticity.[14,17,18] Similarity between the molecular mechanisms of long-term memory and pain-related plasticity suggested that mTOR is a potential target for the induction of pathological states including neuropathic, inflammatory and cancer-related pain.[19-23] One study showed that mTOR signaling regulated pain-related synaptic plasticity in the entorhinal-hippocampal pathway in a model of persistent peripheral nociception induced by peripheral bee venom injections.[24] Moreover, the levels of mTOR and extracellular signal-regulated kinase (ERK) were increased in the cerebrospinal fluid-contacting nucleus of the brainstem after nerve injury.[25] In the spinal cords of rats with chronic constriction injury, inhibition of mTOR with rapamycin reduces the expression of postsynaptic density protein 95 (PSD95, also known as SAP90).[26] PSD95 is an abundant post synaptic scaffolding protein regulating the formation, strength and plasticity of excitatory synapses.[27] Furthermore, PSD95 has multiple protein-protein interaction domains that are shown

by PSD95-dependent changes in the gating of N-methyl-D-aspartate (NMDA) receptors.[28-31] Previous studies reported that NMDA receptors triggers plastic changes during persistent inflammation-related pain.[32,33] Thus, PSD95 may have a role in synaptic plasticity and pain hypersensitivity.[34-38]

The insular cortex (IC) is an integrating region of temporal cortex that has been involved in sensory and cognitive process such as learning, memory and sensory perception.[39,40] For instance, conditioned taste aversion and long term potentiation (LTP) in the IC have similar molecular mechanisms, which are the activation of ERK1/2,[41] NMDA receptor dependence,[42] and protein synthesis.[43] Clinical and basic studies persistently indicate critical roles of the IC in pain processing.[44-46] One study reported plastic changes and LTP in the IC after injury.[39] Moreover, human fMRI studies report a relationship between the IC and chronic neuropathic pain conditions,[47,48] and interestingly, rostral regions of the IC are activated during noxious somatosensory stimulation.[49,50] The rostral agranular IC (RAIC) in rats is essential for modulating nociception[51] and is implicated in pain behavior.[52,53] Recently, it has been found that lesion in RAIC diminished pain-related behaviors in neuropathic pain models.[54] These results suggest that the rostral part of the IC plays a crucial role in pain processing.

Therefore, the aim of this study was to investigate the role of the mTORC1 pathway in the IC after nerve injury. In particular this research has been focused on the relationship between the mTORC1 signaling and PSD95 or NMDA receptor 2B (NMDAR2B), which has been implicated to be associated with synaptic plasticity. In the present study, based on these lines of evidence, the effective pain prevention of pre-administration with rapamycin in the IC was performed. Using behavioral test, immunohistochemistry, and western blot study, the effects of rapamycin was verified. Lastly, the changes in neuropathic pain and synaptic plasticity after microinjection of rapamycin were observed. The findings from this study may provide the foundation for a new

molecular target, the mTOR pathway, for understanding neuropathic pain and may contribute to potential therapeutic applications.

II. MATERIALS AND METHODS

1. Neuropathic pain model

A. Animals

Adult male Sprague-Dawley rats, weighing 250-280 g (Harlan, Koatec, Pyeongtaek, Korea) were house in plastic cages and allowed to acclimate to the colony room for 7 days after arrival. Rats were maintained under 12 hrs light/dark cycles with pellets and water provided *ad libitum*. All experimental protocols in this study were in compliance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee of Yonsei University Health System.

B. Surgical procedure

Under sodium pentobarbital (50 mg/kg, intraperitoneal, [i.p.]) anesthesia, branches of the left sciatic nerve were exposed. In the neuropathic group, The tibial and sural nerves were tightly ligated with 4-0 black silk and cut, whereas the common peroneal nerve was left intact (nerve injured, NP group).[55] Animals in the sham group underwent the same procedure for exposing the sciatic nerve but without injury.

2. Cannula implantation

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and in a stereotaxic frame. Guide cannulae (28 gauge) were bilaterally implanted into the RAIC (AP: +0.6 mm, ML: ±4.7 mm, DV: -5.8 mm). [56] [51,54] Rats were allowed to recover for 1 wk after cannula implantation.

3. Drug microinjection into the insular cortex

Rapamycin (LC Laboratories, Woburn, MA, USA) was prepared in 0.06% dimethyl-sulfoxide (DMSO) diluted in normal saline (0.9% NaCl). Microinjections were performed on post-operative day (POD) 3, POD7 and before nerve injury. Rapamycin (0.5 μl of 600 nM) or an equivalent amount of vehicle solution was infused into the IC bilaterally through the injection cannulae using Hamilton syringes and PE-10 tubing. The injection cannula was maintained in position for at least 1 min to allow for drug absorption.

4. Behavioral test

A. Measuring response threshold

Mechanical hypersensitivity of the hind paw was assessed before nerve injury and on POD 1, 3 and 7 by a researcher blinded to the experimental conditions. Rats were habituated for 30 mins to the test chambers, a metal mesh floor under plastic domes (8 x 8 x 18 cm). Mechanical allodynia was measured by assessing thresholds for hind paw withdrawal to stimulation by an electrical von Frey filament (UGO Basile, Varese, Italy). Licking or rapid withdrawal of the hind paw was considered as a positive response. The responses were measured eight times in 2-3 mins

intervals. The mechanical forces were recorded for each withdrawal of the hind paw.

B. Behavioral test schedule

(A) Mechanical allodynia test on POD3 and POD7

After 7 days of acclimation periods, the animals were conducted pre-behavioral test. Then, neuropathic pain surgery was performed. On the next day, which was POD1, the pain thresholds were measured. In the same manner, in POD3 and POD7, behavioral test was conducted.

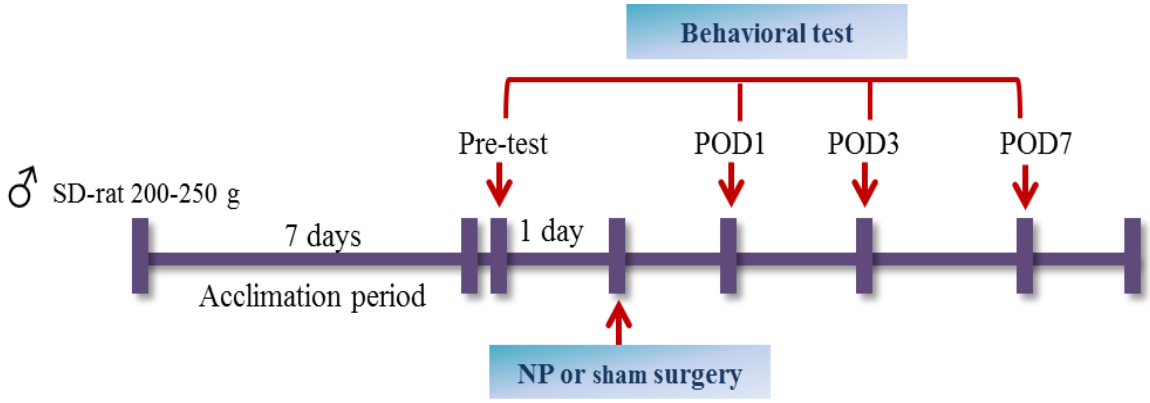


Figure 1. Schematic procedure of nerve injury and behavioral test on POD3 and POD 7.

(B) Pre-administration of drug and behavioral test

The cannulation surgery was performed following one week acclimation periods. After cannulation surgery, the animals were provided with 7 days of recovery. The rapamycin or vehicle solutions were injected in the IC. Then after 10 mins, neuropathic pain surgery was performed. The behavioral test was measured from on POD1 to on POD7.

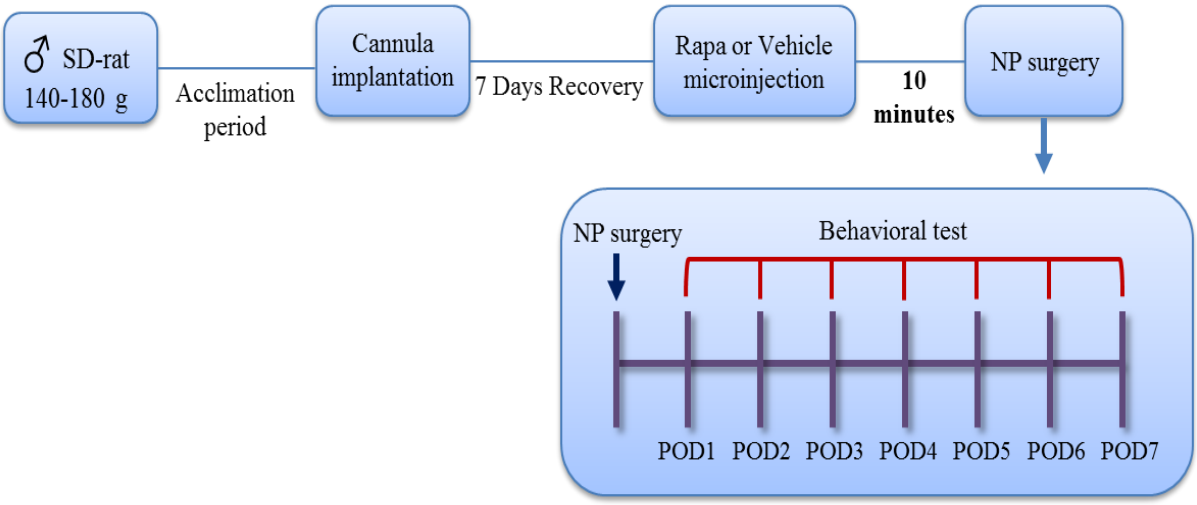


Figure 2. Schematic procedure of pre-administration with drug in the IC and behavioral test.

(C) Post-microinjection and behavioral test on POD3 and POD7

After cannulation surgery and recovery periods, the rats were conducted the neuropathic pain surgery subsequent to recovery periods. Testing was performed before and at 0.5, 1, 2, 4, 8, 12, 24 and 48 hrs after drug microinjection on POD3 and POD7.

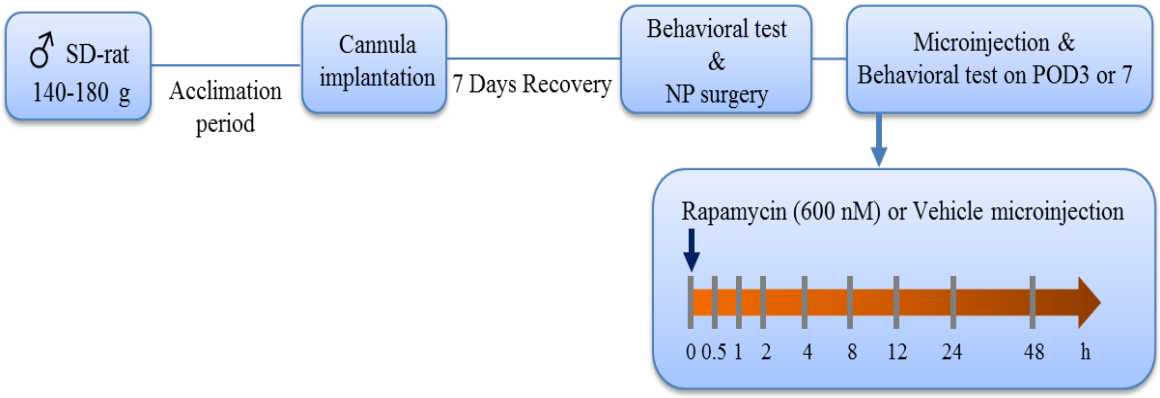


Figure 3. Schematic highlighting the time points of drug-microinjection and behavioral test on POD 3 and POD 7.

5. Histology

Rats were anesthetized with urethane (1.25 g/kg, i.p.) and perfused transcardially with normal saline followed by a 4% solution of formaldehyde in 0.1 M sodium phosphate buffer (pH 6.8). The brains were extracted and post-fixed overnight at 4°C before cryoprotecting in 30% sucrose in phosphate-buffered saline (PBS, pH 7.4) for 24 hours. Coronal tissue sections (30 μm) were cut using a cryostat (Thermo Scientific, Waltham, MA, USA). The sections were incubated with PBS containing 1% normal horse serum for 30 minutes, and then incubated overnight in anti-c-Fos (1:4,000, Merck Millipore, Darmstadt, Germany) and phospho (p)-ERK (1:2,000, Cell Signaling Technology, Danvers, MA, USA) antibodies at 4°C. The sections were washed with PBS and incubated for 30 minutes in biotinylated antibody (1:50, Vector Laboratories, Burlingame, CA, USA). The slides were then washed again and incubated for 30 minutes with PBS containing the avidin-biotinylated horseradish peroxide complex (ABC kit, Vector Laboratories, Burlingame, CA, USA). After washing, sections were incubated for 5-7 minutes in a PBS solution containing 0.1% 3,3'-diaminobenzidine tetrahydrochloride (DAB), 0.1% ammonium nickel sulfate, and 10 μl of H_2O_2. The reaction was terminated by washing with PBS. The slides were then dehydrated with ethanol, cleared in xylene and covered with Permount (Thermo Scientific, Waltham, MA, USA). c-Fos- and p-ERK-labeled cells in the ipsilateral and contralateral sites of the rostral IC defined by the rat brain atlas[57] were observed under light-field microscopy (20x objective; Olympus, Tokyo, Japan). To quantify c-Fos- and p-ERK-positive cells in the IC, a maximum of eight representative sections of the IC were chosen at random from each rat and counted by an observer blinded to the experimental conditions. The numbers of positive cells from each section were averaged to represent the cell count of each animal. Regions of interest (ROIs) were set for the ipsilateral and contralateral sides.

6. Western blot analysis

As described above, animals were anesthetized with enflurane for decapitation and IC tissue sample collection. The ipsilateral and contralateral rostral ICs were quickly dissected, frozen on dry ice, and stored at -70°C. For protein extraction, samples were homogenized in lysis buffer (Intron Biotechnology, Pyeongtaek, Korea) containing phosphatase inhibitor (Roche, Mannheim, Germany). Samples were the centrifuged at 12,000x g for 20 mins at 4°C, and the supernatants were collected. Total protein concentrations were assessed with a spectrophotometer (Thermo Scientific), and 30 μg of protein per well were denatured and run on 10% gels (Bio-Rad, Herculues, CA, USA). Proteins were transferred onto a polyvinylidene difluoride membrane (Merck Millipore), and the membranes were blocked by incubating in 3% skim milk. Membranes were incubated with primary antibodies against mTOR (1:1,000, Cell Signaling Technology, Danvers, MA, USA), p-mTOR (Ser 2448, 1:500, Cell Signaling Technology, Danvers, MA, USA), P70S6K (1:1,000, Cell Signaling Technology Danvers, MA, USA), p-P70S6K (Thr 389, 1:500, Cell Signaling Technology, Danvers, MA, USA), 4EBP (1:1,000, Cell Signaling Technology, Danvers, MA, USA), p-4EBP (Thr37/46, 1:500, Cell Signaling Technology, Danvers, MA, USA), PSD95 (1:1,000, Cell Signaling Technology, Danvers, MA, USA), NMDAR2B (1:1,000, Cell Signaling Technology, Danvers, MA, USA) and ß-actin (1:10,000, Cell signaling Technology, Danvers, MA, USA). Membranes were then incubated with the appropriate anti-rabbit or anti-mouse horseradish peroxidase-conjugated secondary antibodies (1:10,000, Cell Signaling Technology, Danvers, MA, USA). Proteins were visualized by applying a chemiluminescent substrate (GE Healthcare, Little Chalfont, UK) and observed using the LAS system (GE healthcare, Little Chalfont, UK). The signals for phosphorylated proteins were normalized to those from the nonspecific forms. ß-actin was used as the loading control.

7. Statistical analysis

Data from the behavioral test were analyzed by two-way analysis of variance (ANOVA) with repeated measures, with group and POD as factors, and by unpaired *t* test for *post hoc* comparisons between groups. Unpaired *t* tests were performed for the analyses of immunohistochemistry and western blotting data. Statistical analyses were performed by SPSS 20.0 software (IBM Corporation, Armonk, NY, USA). All values are expressed as means ± SEM. *P* value less than 0.05 were considered statistically significant.

III. RESULTS

1. The activation of pain marker, mTOR signal pathway, PSD95 and NMDA receptor 2B in the insular cortex after neuropathic surgery

A. Development of mechanical allodynia

It was examined that the time course of behavioral changes in neuropathic pain model[55] using the threshold of the injured paws at 1, 3 and 7 days after the neuropathic pain surgery. From POD1 to POD7, the thresholds between NP (n=6) and sham groups (n=6) were significantly different (group, $F_{(1,10)}$ = 118.917, P < 0.001; POD, $F_{(3,30)}$ = 16.159, P < 0.001; group and POD interaction, $F_{(3,30)}$ = 29.5337, P < 0.001; two-way repeated measured ANOVA). The mechanical thresholds of the peripheral nerve injury group were decreased compared with those of the shams on POD1, POD3 and POD7 (unpaired t test, P < 0.001; Figure 4).

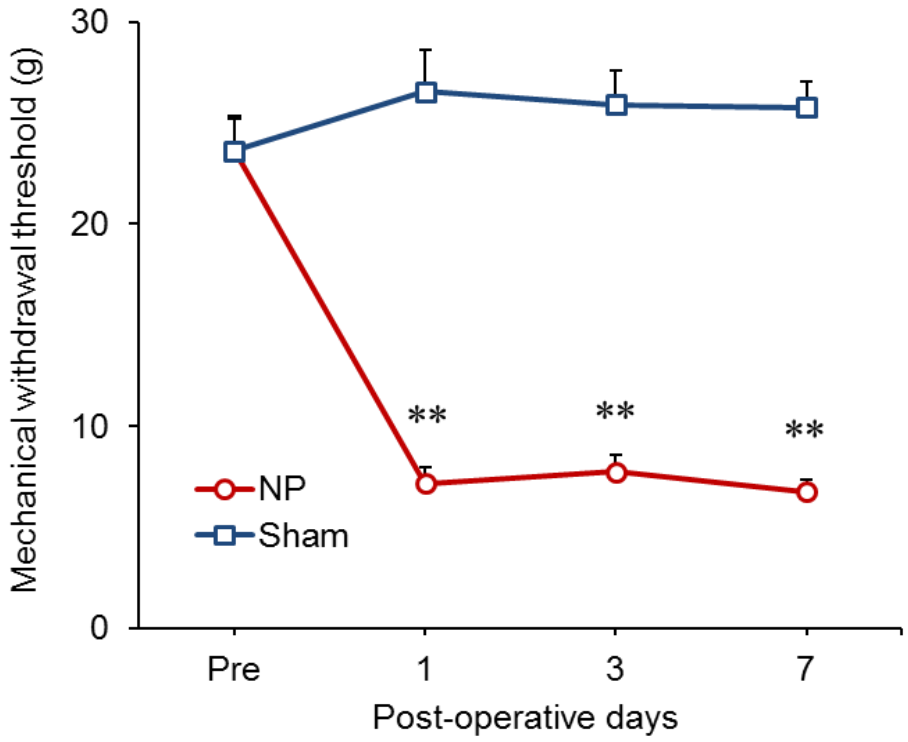


Figure 4. Development of mechanical allodynia in nerve-injured (NP, n=6) and sham-injured (Sham, n=6) rats. After nerve injury, rats developed significant neuropathic pain on post-operative day 1 (POD1), POD3 and POD7 compared with the sham group. Data presented as means ± SEM, $^{**}P < 0.01$.

B. Increased in pain marker expression induced by nerve-injury

Several authors have proposed that the IC is activated under various type of pain, including neuropathic pain.[58,59] Figure 5A shows histological confirmation of rostral IC with Paxinos and Watson's rat atlas.[57] To confirm that the rostral IC is involved with neuropathic pain, immunohistochemistry was performed to assess the numbers of cells expressed c-Fos and p-ERK, which are important markers for nociceptive neuronal activation in the CNS.[60] On POD3, rats in the NP group had higher numbers of c-Fos- (Figure 5B, the first row microphotographs and Figure 5C, the graph of c-Fos-positive neurons, NP group (n=5), Sham group (n=5), $P < 0.001$) and p-ERK- (Figure 5 B, the second raw microphotographs and Figure 5D, the graph of p-ERK positive cells NP group (n=4), Sham group (n=4), $P < 0.05$, scale bar: 200 μm) positive cells than sham animals. The numbers of cells positive for c-Fos (Figure 5B, the third row microphotographs and Figure 5E, the graph of c-Fos-positive cells, NP group (n=6), Sham group (n=6), $P < 0.01$) and p-ERK (Figure 5B, the fourth row microphotographs and Figure 5F, the graphs of p-ERK-positive cells, NP group (n=6), Sham group (n=6), $P < 0.01$) in nerve-injured rats were also significantly higher than in sham controls on POD7.

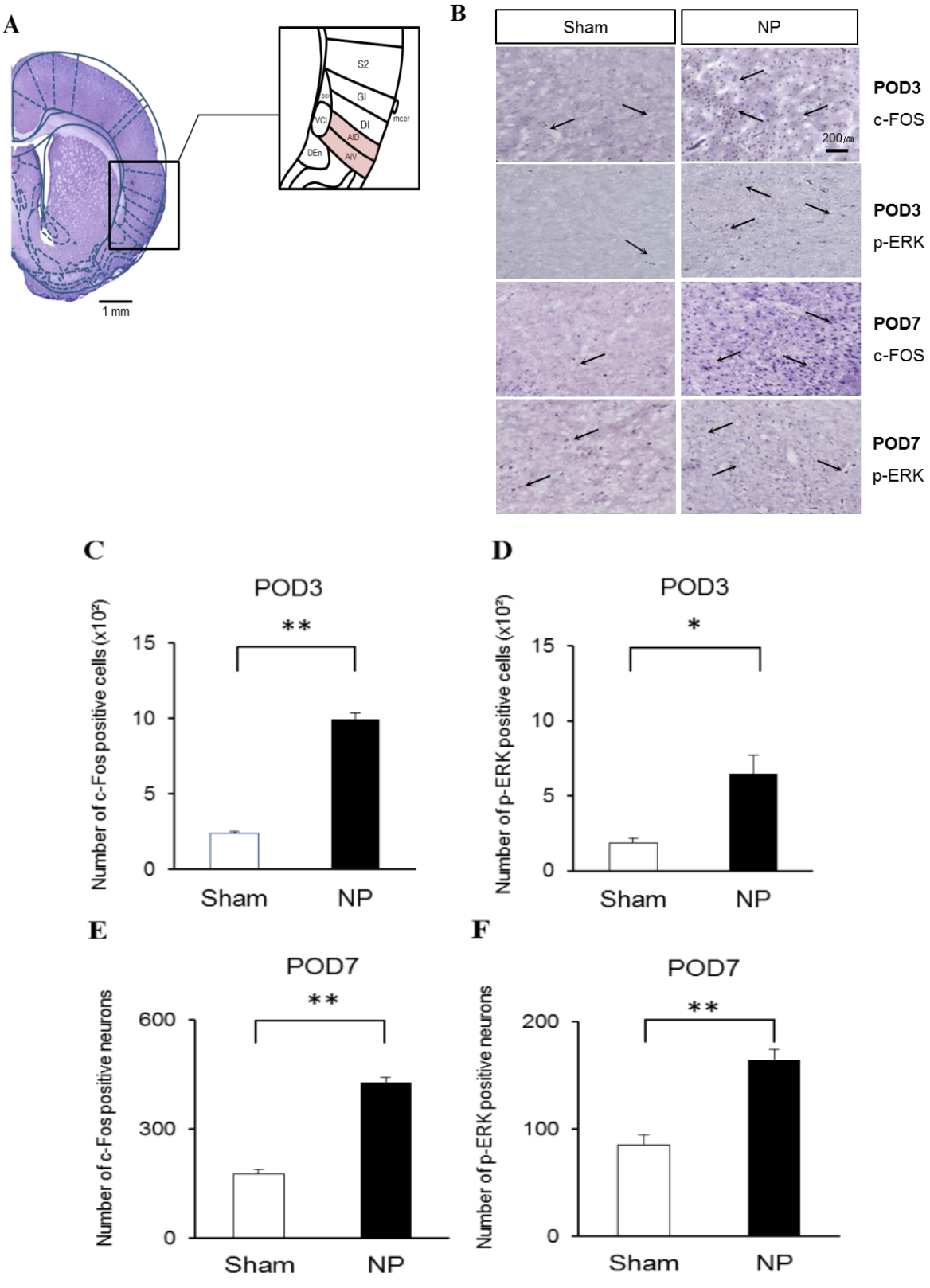


Figure 5. c-Fos and phospho (p)-extracellular signal-regulated kinase (ERK) expression in NP and Sham rats. (A) Histological clarification of rostral IC with rat atlas. Subdivisions of the IC were included in the black square box. c-Fos- and p-ERK-positive cells in the AIV and AID were analyzed. *Abbreviations*: AIV, agranular insular cortex ventral; AID, agranular insular cortex dorsal; DI, dysgranular insular cortex; GI, granular insular cortex; S2, secondary somatosensory cortex; Den, dorsal endopiriform nucleus; VCI, ventral part of claustrum; DCI, dorsal part of claustrum. (B) Microphotographs of c-Fos and p-ERK in the rostral IC (AIV and AID areas). The arrows mean positive cells for c-Fos or p-ERK. (C) Quantification of c-Fos- and (D) p-ERK-positive cells on POD3. (E) Quantification of c-Fos- and (F) p-ERK-positive cells on POD7. Scale bars, 200 μm. Data are presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

C. Changes in mTOR, P70S6K, 4EBP, PSD95 and NMDA receptor 2B activity after neuropathic pain

On the basis of the above results, it was hypothesized that chronic pain derived from nerve injury alters mTOR signaling in the IC. To test this, I measured protein and phosphorylation levels of mTOR and its downstream targets, P70S6K and 4EBP, as well as PSD95 and NMDA receptor 2B (NMDAR2B) expression in the IC at 3 and 7 days after nerve injury. The results indicate that on POD3, p-mTOR was upregulated in the NP group (n=5) compared with that in the sham group (n=8, $P < 0.05$, Figure 6A), with no difference in the total amounts of mTOR (Figure 6B). Furthermore, phosphorylation levels of its downstream targets P70S6K and 4EBP were upregulated significantly in the NP group compared with those in shams (p-P70S6K:NP, n=5; sham, n=5, $P < 0.05$, Figure 6C, p-4EBP: NP, n=4; sham, n=4, $P < 0.01$, Figure 6E). There was no changes in total P70S6k (Figure 6D) and 4EBP levels (Figure 6F). Additionally, the expression of PSD95 in the NP group (n=5) was higher than in the sham group (n=5, $P < 0.05$, Figure 6G). The level of NMDAR2B was increased in NP group (n=7) compared with the sham group (n=7, $P < 0.05$, Figure 6H). Similarly, mTOR signaling activity was elevated in the NP groups compared with that in the shams on POD7. Levels of p-mTOR (Figure 7A) and p-P70S6K (Figure 7C) in the NP rats (n=4) were higher than in the sham group (n=4, $P < 0.01$). 4EBP activity measured by p-4EBP expression was also increased in the NP group (n=4 each, $P < 0.05$, Figure 7E). There were no differences in total levels of mTOR (Figure 7B), P70S6K (Figure 7D), and 4EBP (Figure 7F). The levels of PSD95 on POD7 were significantly higher in NP rats than in sham controls (n=4 each, $P < 0.05$, Figure 7G). Finally, the expression level of NMDAR2B was increased significantly in the NP group (n=6) compared with the sham group (n=6, $P < 0.05$, Figure 7H). These results suggest that mTOR pathway in the IC strongly is related to PSD95 and NMDAR, and lead to neuropathic pain.

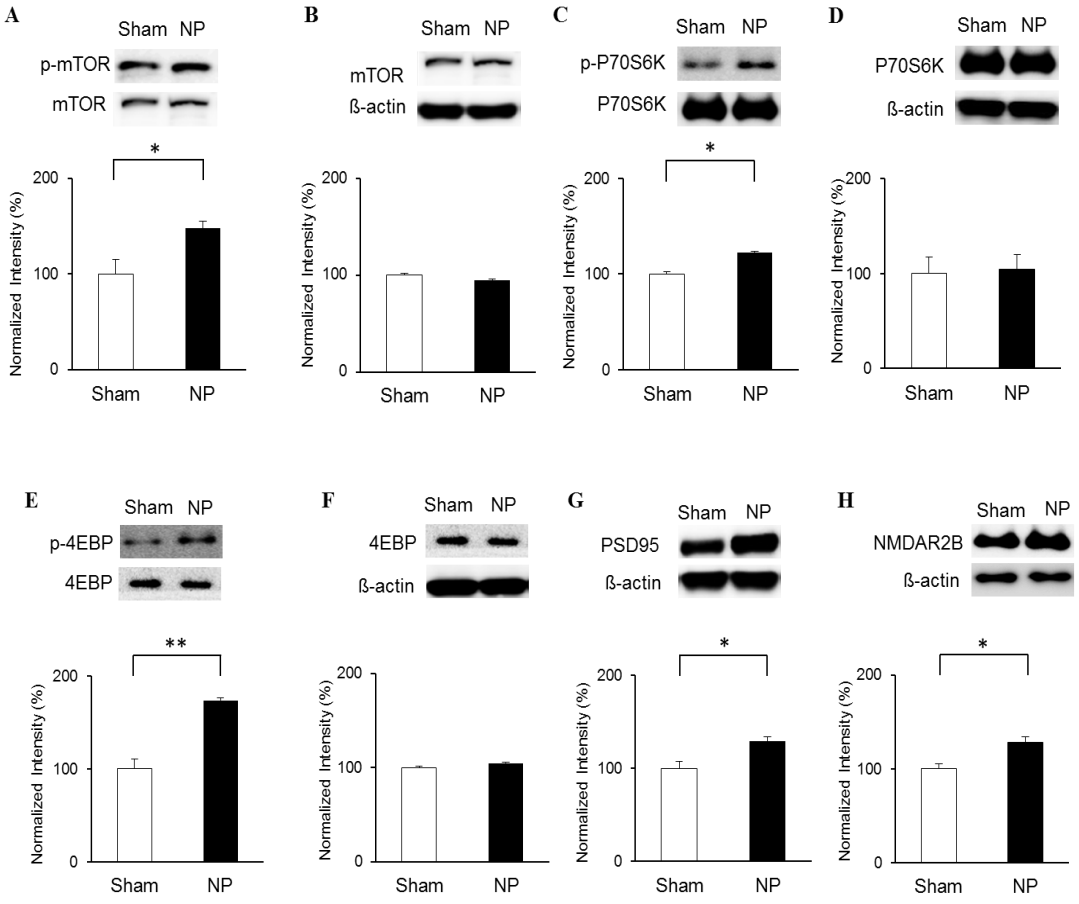


Figure 6. Phosphorylation of mammalian target of rapamycin (mTOR), p70 ribosomal S6 protein kinase (P70S6K) and 4E binding protein (4EBP), and expression of postsynaptic density protein 95 (PSD95) and NMDAR2B in the IC on the day 3 after nerve injury. (A, B) p-mTOR increased in the NP group (n=5) compared with the sham group (n=8), but total mTOR levels were not significantly different on POD3. (C, D) p-P70S6K increased in the NP group (n=5) compared with the sham group (n=5), but total P70S6K levels were not different. (E, F) p-4EBP increased in the NP group (n=4) compared with the sham group (n=4), but total 4EBP levels were not different. (G, H) PSD95 and NMDAR2B levels increased significantly in the NP group (n=5, 7 respectively) compared with the sham group (n=5, 7 respectively). The

intensity of the phosphor-form band was normalized to that of the total form, and the total form bands were normalized to ß-actin protein. Data are presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

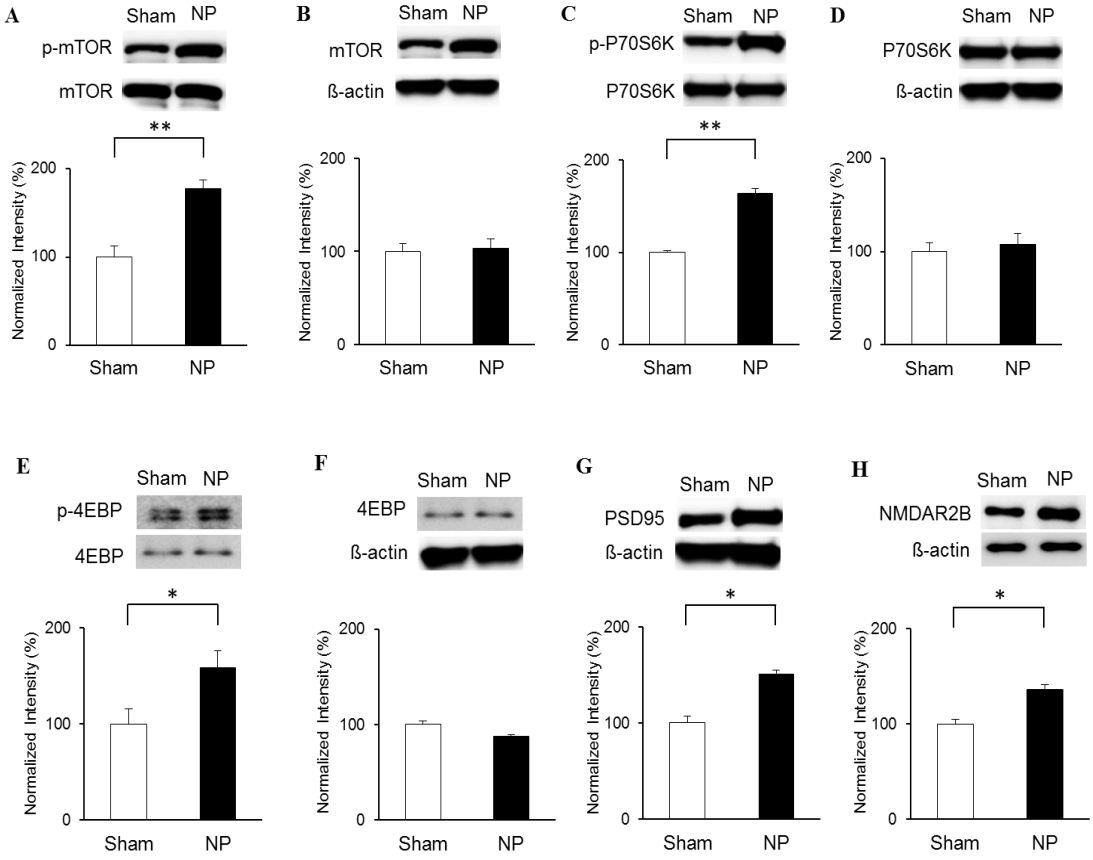


Figure 7. Phosphorylation of mTOR, P70S6K and 4EBP, and expression of PSD95 and NMDAR2B in the IC on the day 7 after nerve injury. (A, B) p-mTOR increase in the NP group (n=4) compared with the sham group (n=4), but total mTOR levels were not significantly different on POD7. (C, D) p-P70S6K increased in the NP group (n=4) compared with the sham group (n=4), but total P70S6K levels were not different. (E, F) p-4EBP increased in the NP group (n=4) compared with the sham group (n=4), but total 4EBP levels were not different. (G, H) PSD95 and NMDAR2B levels increased significantly in the NP group (n=4, 6 respectively) compared with the sham group (n=4, 6 respectively). The intensity of the phosphor-form band was normalized to that of the total form, and the total form bands were normalized to ß-actin protein. Data are presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

2. Effective pain prevention of pre-administration with rapamycin in the insular cortex

A. Changes in pain thresholds induced by pre-rapamycin injection

To determine whether rapamycin could prevent the development of the induction stage of mechanical allodynia, the animals were microinjected rapamycin or vehicle solution via a cannula implanted 10 mins before neuropathic surgery. The behavioral responses were recorded from POD1 to POD7 (Figure 2). After treatment with drugs, the pain threshold in pre-administration of rapamycin (Pre-Rapa group) showed significant change compared to pre-administration of vehicle solution (Pre-Vehicle group) at day 1, 2 and 3 after nerve-injury (group, $F_{(1,16)} = 65.134$, $P < 0.001$; POD, $F_{(7,112)} = 1324.317$, $P < 0.001$; POD and group interaction, $F_{(7,112)} = 14.918$, $P < 0.001$; rapamycin, n=11; vehicle, n=11 two-way repeated measured ANOVA; Figure 8) This result indicated that rapamycin could possibly alleviate the neuropathic pain induced by peripheral nerve injury in the induction stage. Data are presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

B. Alteration of c-Fos and p-ERK expression induced by pre-rapamycin injection

Whether pre-administration of rapamycin reduces the expression of markers of nociceptive activation in the IC on POD2 after pre-drug injections, the immunohistochemistry was assessed (Figure 9A, B). However, unlike the result of behavioral test, rapamycin could not decrease the expressions of c-Fos (Figure 9C, the first row microphotographs and Figure 9D, the graph of c-Fos-positive cells, $P > 0.05$) and p-ERK (Figure 9C, the second row microphotographs and Figure 9E, the graph of p-ERK-positive cells, $P > 0.05$) in the IC of neuropathic rats.

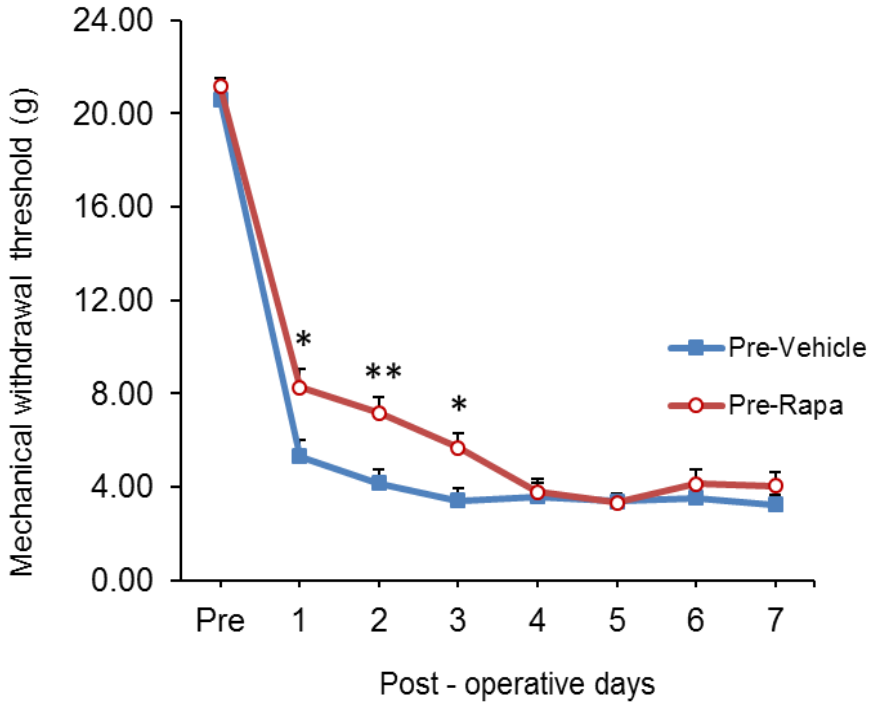
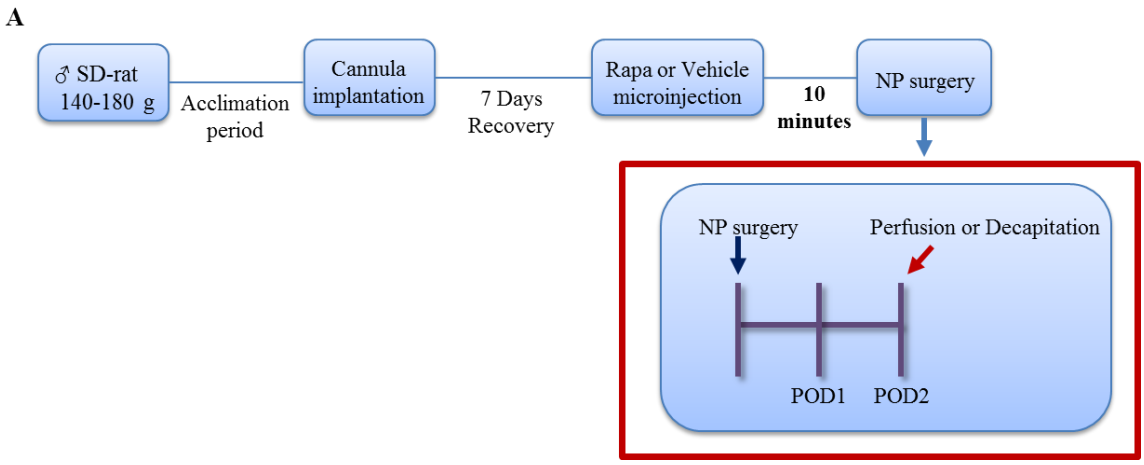
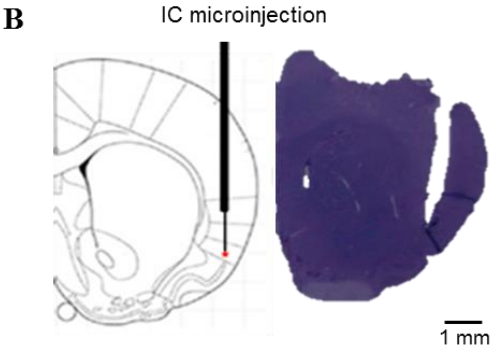
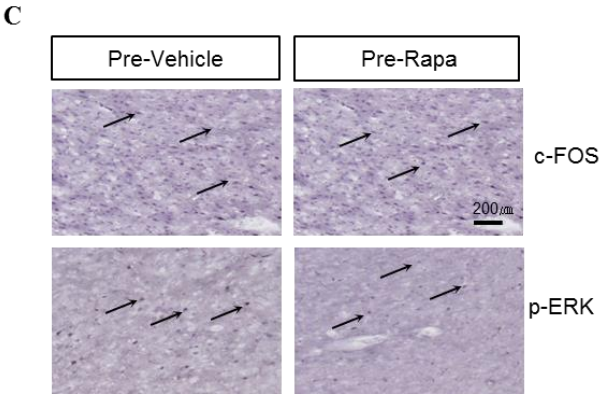
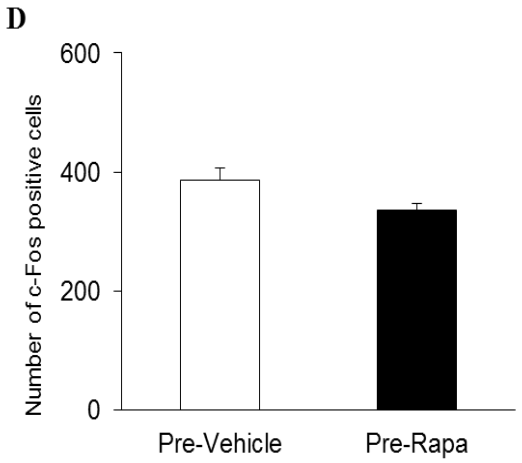


Figure 8. Changes in pain threshold to mechanical stimulation after pre-administration of rapamycin on POD1 POD2 and POD3. Intra-IC microinjection of rapamycin could alleviate on the induction stage of nerve-injury induced neuropathic pain in rats (POD1, ($P < 0.05$); POD2, ($P < 0.01$); POD3, ($P < 0.05$).









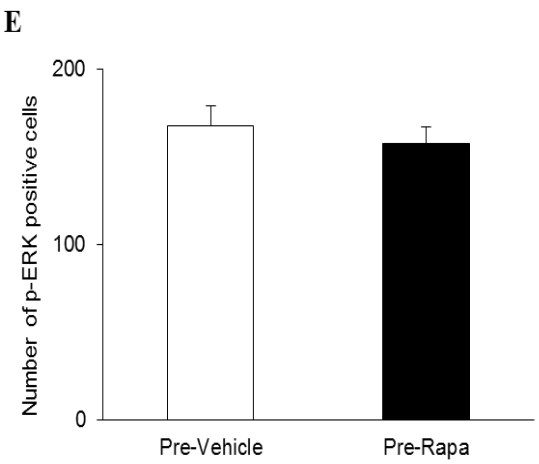


Figure 9. Pre-microinjection of rapamycin does not reduce pain markers. (A) Schematic procedure of pre-administration with drug in the IC and the day of tissue extraction. (B) Microinjection site into the IC (left) and a representative photograph of a coronal section (right). Scale bar, 1 mm. (C) Microphotographs of c-Fos and p-ERK in the rostral IC. The arrows mean positive cells for c-Fos and p-ERK on POD2. On POD2, (D) the numbers of c-Fos-positive and (E) p-ERK-positive cells did not decrease significantly in the pre-Rapa group (n=6) compared with in the pre-Vehicle group (n=6).

C. Change in mTOR, P70S6K, 4EBP, PSD95 and NMDA receptor 2B activity after pre-rapamycin injection

The western blot was performed whether pre-microinjection of rapamycin effects mTOR signaling pathway, PSD95, NMDAR2B expressions on POD2. Like the results of immunohistochemistry, the phosphorylation of mTOR was not decrease significantly on POD2 in rats pre-receiving rapamycin (Figure 10A, n=5). Similarly, phosphorylation levels of P70S6K (Figure 10C, n=6) and 4EBP (Figure 10E, n=5) in the rapamycin-pre-treated group were not significantly different ($P > 0.05$). The expression levels of total mTOR (Figure 10B, n=5), P70S6K (Figure 10D, n=6) and 4EBP (Figure 10F, n=5) were not different ($P > 0.05$). Furthermore, the expressions of PSD95 (Figure 10G, n=5) and NMDAR2B (Figure 10H, n=5) in the pre-Rapa group were not significantly decrease ($P > 0.05$).

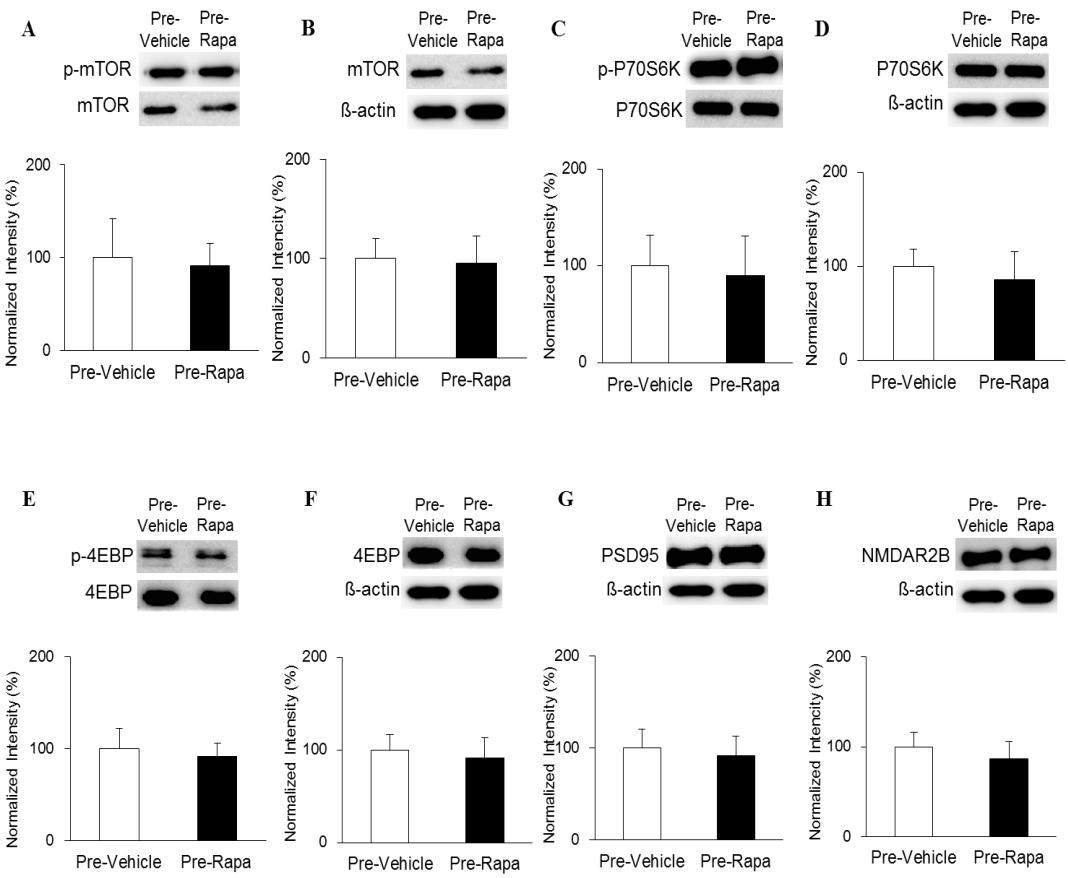


Figure 10. Pre-microinjection of rapamycin cannot inhibit mTOR signaling activity in the IC. (A, B) p-mTOR and total mTOR levels did not change in the Pre-Rapa group (n=5) compared with in the Pre-Vehicle group (n=5). (C, D) Like mTOR, p-P70S6K levels did not decreased in the Pre-Rapa group (n=6) compared with in the Pre-Vehicle group (n=6), and total P70S6K levels were not different. (E, F) The expression levels of p-4EBP and 4EBP did not significantly different in the Pre-Rapa group (n=5) compared with in the Pre-Vehicle group (n=5). (G) PSD95 and (H) NMDAR2B levels were not decrease in the Pre-Rapa group (n=5) compared with in the Pre-Vehicle group (n=5, $P > 0.05$). The intensity of the phosphor-form band was normalized to that of the total form, and the total form bands were normalized to ß-actin protein.

3. Effects of intracranial administration with rapamycin on neuropathic pain model induced nerve-injury

A. Changes in mechanical hypersensitivity induced by rapamycin microinjection of intra-IC on POD3 and POD7

Previous studies demonstrated that intrathecal or systemic rapamycin administration reduces neuropathic pain.[61,62] To determine if this is due to effects in the IC (Figure 9 B), rats with neuropathic pain received microinjections of rapamycin (600 nM, n=12) or the vehicle (n=11) after mechanical allodynia testing on POD3 (Figure 11A) and POD7 (Figure 11B). Microinjection of rapamycin into the IC significantly affected withdrawal thresholds on POD3 (group, $F_{(1,20)} = 18.171$, $P < 0.001$; time, $F_{(8,160)} = 10.821$, $P < 0.001$; group and time interaction, $F_{(8,160)} = 5.191$, $P < 0.001$; n=11 each, two-way repeated measured ANOVA). The analgesic effect of rapamycin in comparison with the vehicle persisted for up to 24 hrs after the microinjection as measured by mechanical allodynia (0.5 hrs, $P < 0.01$; 1 hr, $P < 0.01$; 2 hrs, $P < 0.01$; 4 hrs, $P < 0.01$; 8 hrs, $P < 0.01$; 12 hrs, $P < 0.01$, 24 hrs, $P < 0.05$). However, there was no difference in the withdrawal thresholds between the rapamycin and vehicle groups 48 hrs after injection ($P > 0.05$, respectively). Similar results were observed when microinjections were performed on POD7. Figure 8B shows that microinjection of rapamycin on POD7 decreased mechanical allodynia significantly in comparison with rats receiving the vehicle (group, $F_{(1,19)} = 14.546$, $P < 0.01$; time, $F_{(8,152)} = 6.510$, $P < 0.001$; group and time interaction, $F_{(8,152)} = 3.223$, $P < 0.01$; rapamycin, n=12; vehicle, n=9, two-way repeated measured ANOVA). Mechanical thresholds in the rapamycin-treated group increased compared with the vehicle group from 1 hr to 24 hrs after the injections (0.5 hrs, $P > 0.05$; 1 hr, $P < 0.05$; 2 hrs, $P < 0.01$;

4 hrs, $P < 0.001$; 8 hrs, $P < 0.01$; 12hrs, $P < 0.05$, 24 hrs, $P < 0.05$; 48 hrs, $P > 0.05$).

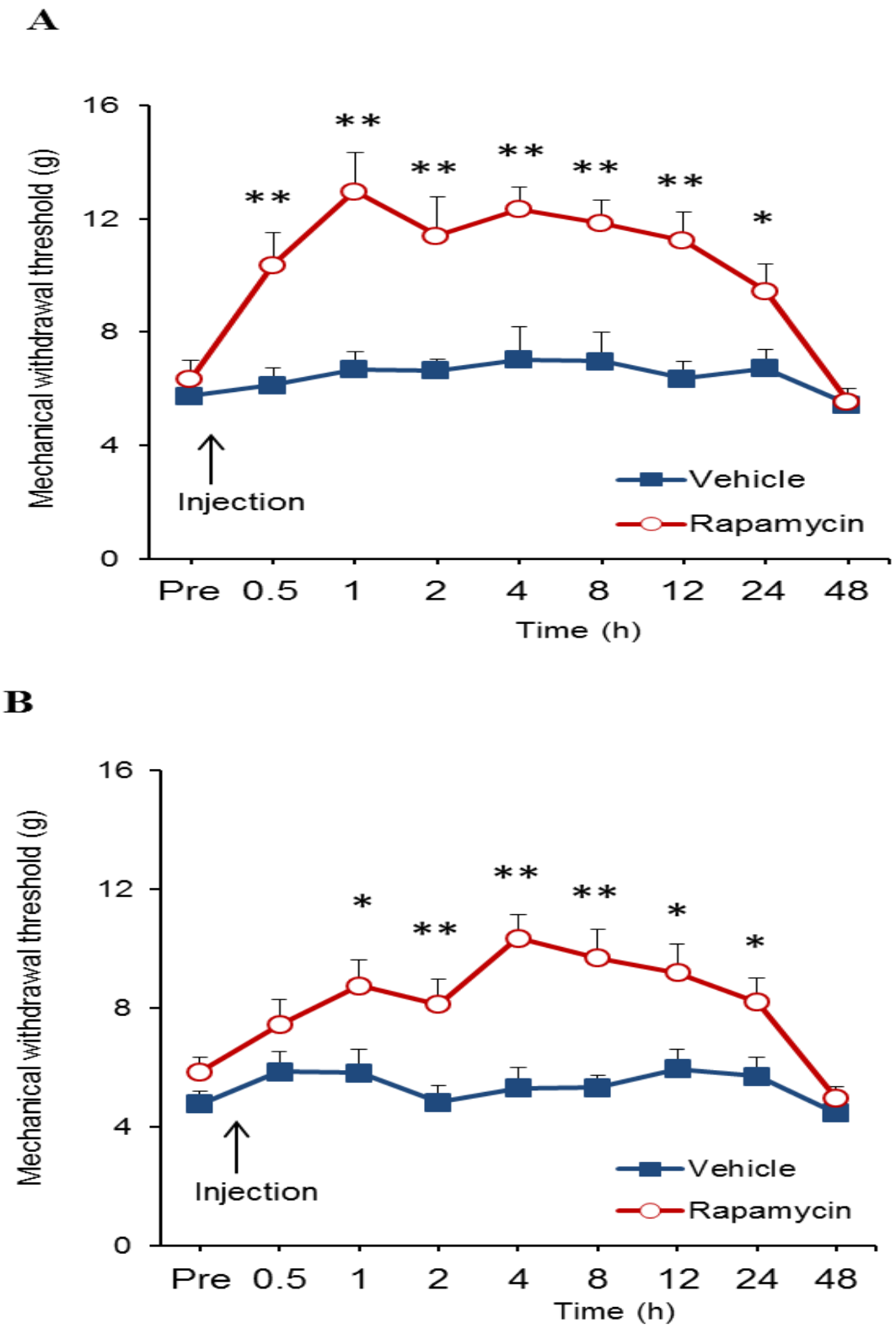
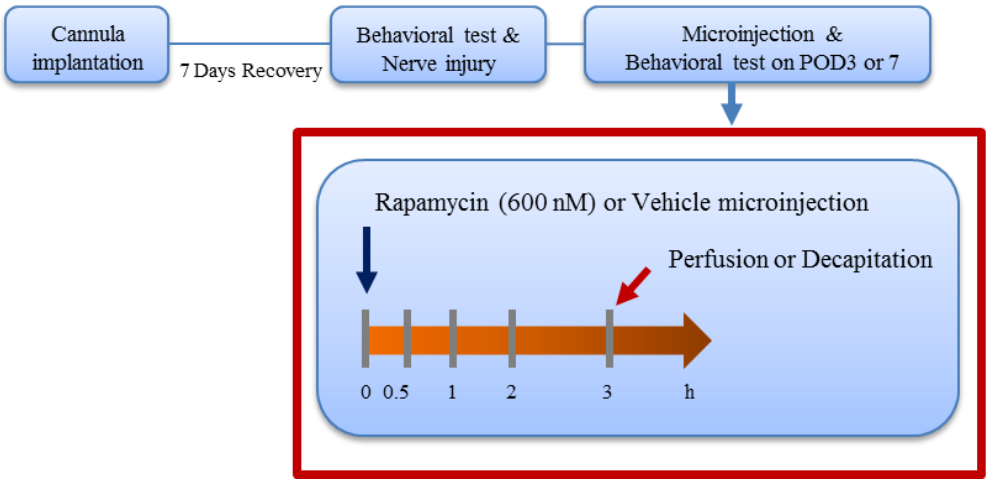


Figure 11. Intracranial administration of rapamycin attenuates mechanical allodynia. (A) Changes in paw withdrawal thresholds to mechanical stimulation after microinjection of rapamycin (Rapa group, Rapa) or vehicle (Vehicle group, Vehicle) on POD3. The arrow indicates the time point of microinjection. Significant differences between the rapamycin (n=12) and vehicle groups (n=11) were observed between 0.5 hr and 24 hrs after microinjection. (B) Changes in paw withdrawal thresholds to mechanical stimulation after microinjection of rapamycin (n=12) or vehicle (n=11) on POD7. The arrow indicates the time point of microinjection. Similarly, the rapamycin and vehicle groups were significantly different between 1 hr and 24 hrs after microinjection. Data are presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

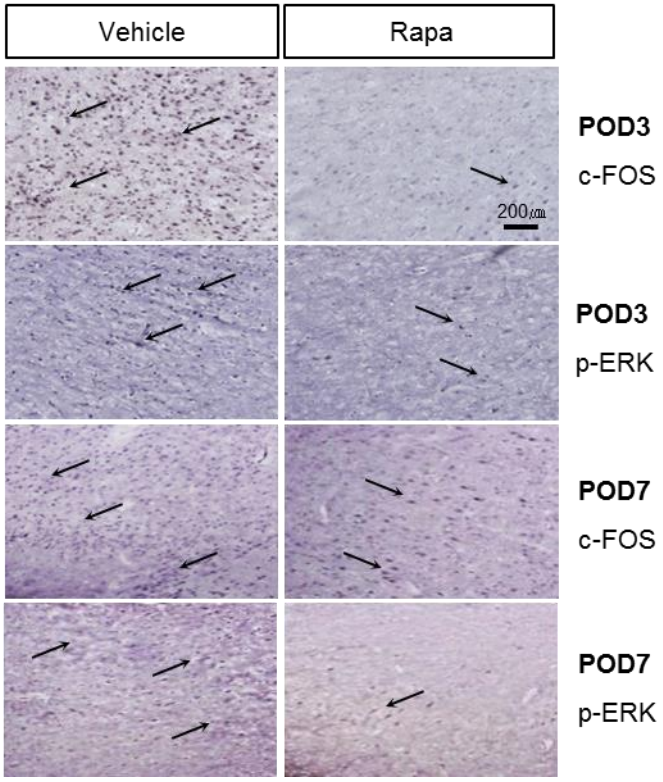
B. Changes in the number of c-Fos and p-ERK immune-positive cells induced by rapamycin microinjection on POD3 and POD7

This research was tested whether the inhibition of mTOR by rapamycin reduces the expression of markers of nociceptive activation in the IC 3 hrs after rapamycin or vehicle injections (Figure 12A). On POD3, rats receiving rapamycin injection had reduced number of cells positive for c-Fos (Figure 12B, the first row microphotographs and Figure 12C, the graph of c-Fos positive neurons, Rapa group (n=6), Vehicle group (n=6), $P < 0.01$) and p-ERK (Figure 12B, the second row microphotographs and Figure 12D, the graph of p-ERK positive cells, Rapa group (n=6), Vehicle group (n=6), $P < 0.05$) in the IC compared with in rats receiving the vehicle. Similarly, on POD7, rats receiving rapamycin injection had reduced numbers of cells positive for c-Fos (Figure 12B, the third row microphotographs and Figure 12E, the graph of c-Fos positive neurons, Rapa group (n=4), Vehicle group (n=4), $P < 0.001$) and p-ERK (Figure 12B, the fourth row microphotographs and Figure 12F, the graph of p-ERK positive neurons, Rapa group (n=4), Vehicle group (n=4), $P < 0.001$).

A



B



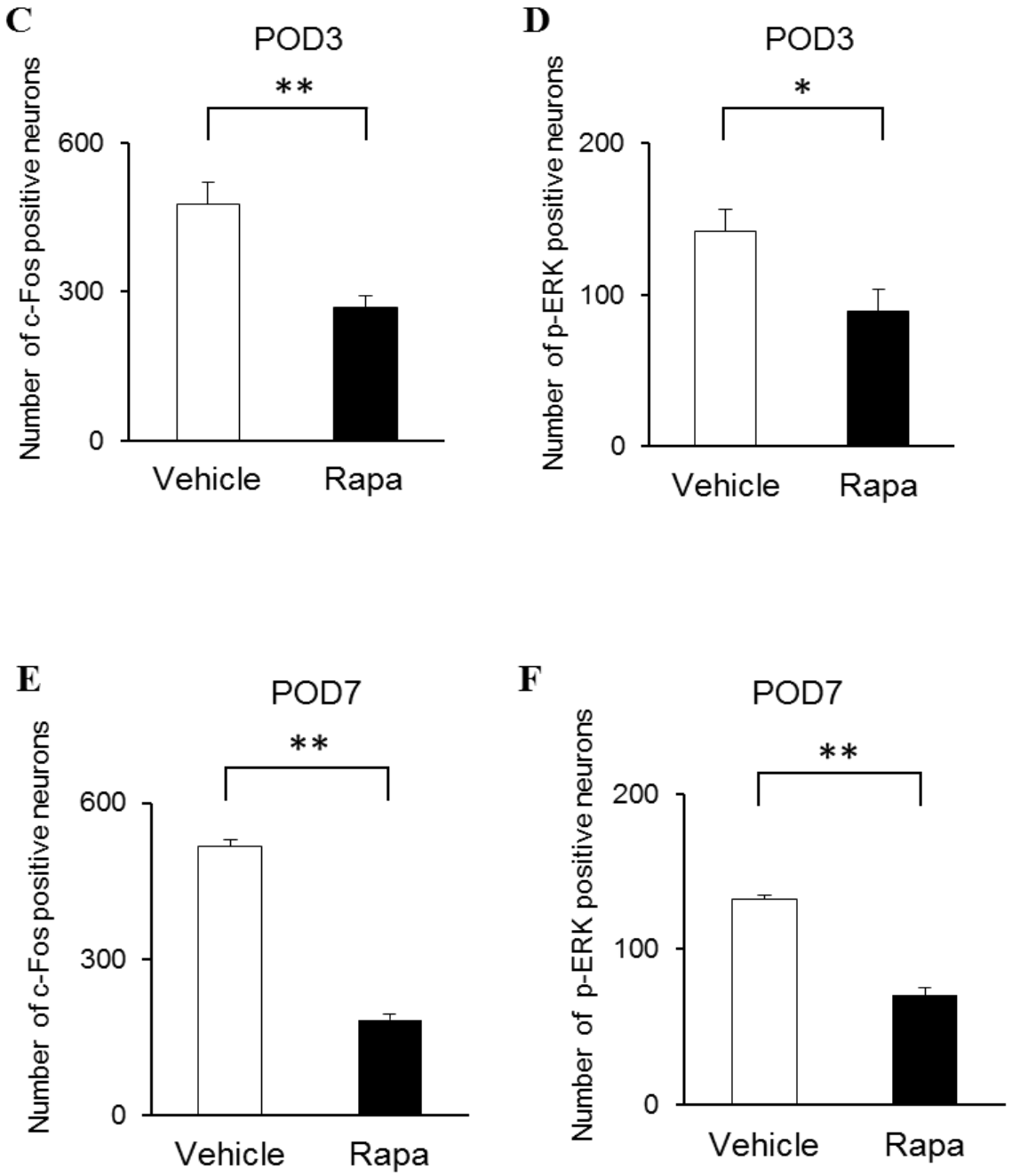


Figure 12. Microinjection of rapamycin reduces the markers of nociceptive activation. (A) Experimental schematic highlighting the time points of cannula implantation, nerve injury, microinjection and perfusion. (B) Microphotographs of c-Fos and p-ERK in the rostral IC. The arrows mean positive cells for c-Fos or p-ERK. On POD3, (C) the numbers of c-Fos-positive and (D) p-ERK-positive cells decreased significantly in the Rapa group compared with in the Vehicle group. On POD7, (E) the numbers of c-Fos-positive and (F) p-ERK-positive cells decreased significantly in the Rapa group compared with in the Vehicle group. Data are presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

C. Changes in p-mTOR, p-P70S6K, p-4EBP, PSD95 and NMDA receptor 2B expression induced by rapamycin microinjection on POD3 and POD7

It was also performed that the levels of mTOR activation in the IC 3 hrs after rapamycin or vehicle injections (Figure 12A). On POD3, rats receiving rapamycin injections had lower expression of p-mTOR than vehicle-treated rats (Figure 13A, n=5 each, $P < 0.05$), as well as decreased phosphorylation of P70S6K (Figure 13C, n=4, $P < 0.01$) and 4EBP (Figure 13E, n=4, $P < 0.01$). However, the levels of mTOR (Figure 13B, $P > 0.05$), P70S6K (Figure 13D, $P > 0.05$) and 4EBP (Figure 13F, $P > 0.05$). Additionally, the expressions of PSD95 (Figure 13G, n=5, $P < 0.05$) and the NMDAR2B (Figure 13H, n=5, $P < 0.05$) were decreased significantly with rapamycin compared with the vehicle.

On POD7, rats received microinjections of rapamycin or vehicle and mTOR activity and PSD95 expression were measured. The phosphorylation of mTOR was decreased significantly on POD7 in rats receiving rapamycin (Figure 14A, n=4, $P < 0.001$). Similarly, phosphorylation levels of P70S6K (Figure 14C, n=4, $P < 0.05$) and 4EBP (Figure 14E, n=4, $P < 0.01$) in the rapamycin-treated group were downregulated compared with in the vehicle-treated group. The expression levels of total mTOR (Figure 14B, $P > 0.05$), P70S6K (Figure 14D, $P > 0.05$) and 4EBP (Figure 14F, $P > 0.05$). In addition, the expressions of PSD95 (Figure 14G, n=4, $P < 0.01$) and the NMDAR2B (Figure 14H, n=5, $P < 0.05$) in the rapamycin group were significantly decreased on POD7 compared with in the vehicle group.

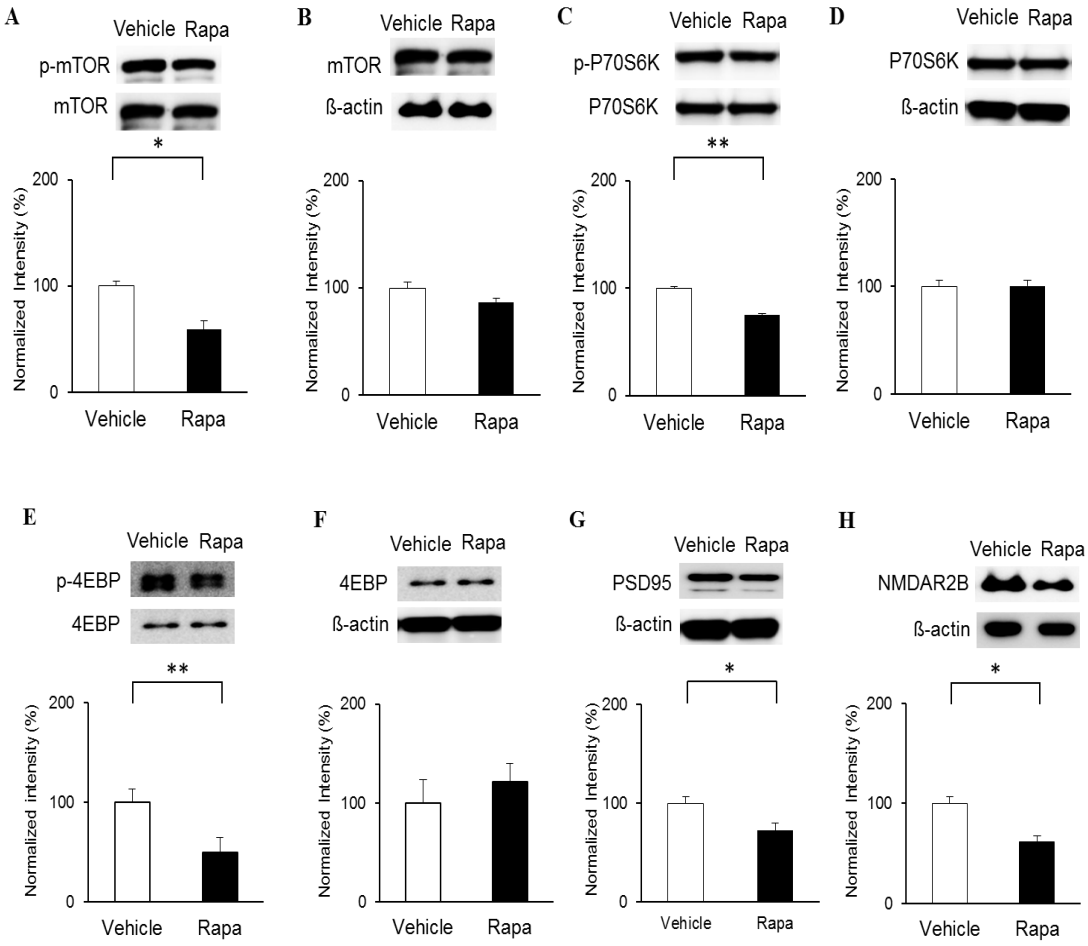


Figure 13. Microinjection of rapamycin reversed the upregulation of the mTOR pathway, PSD95 and NMDAR2B expression on POD3. (A, B) p-mTOR levels decreased in the Rapa group (n=5) compared with in the Vehicle group (n=5), but total mTOR levels were not significantly defferent. (C, D) p-P70S6K levels decreased in the Rapa group (n=4) compared with in the Vehicle group (n=4), but total P70S6K levels were not different. (E, F) p-4EBP levels decreased in the Rapa group (n=5) compared with in the Vehicle group (n=5), but total 4EBP levels were not different. (G) PSD95 and (H) NMDAR2B levels decreased in the Rapa group (n=5) compared with in the Vehicle group (n=5). Data are presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

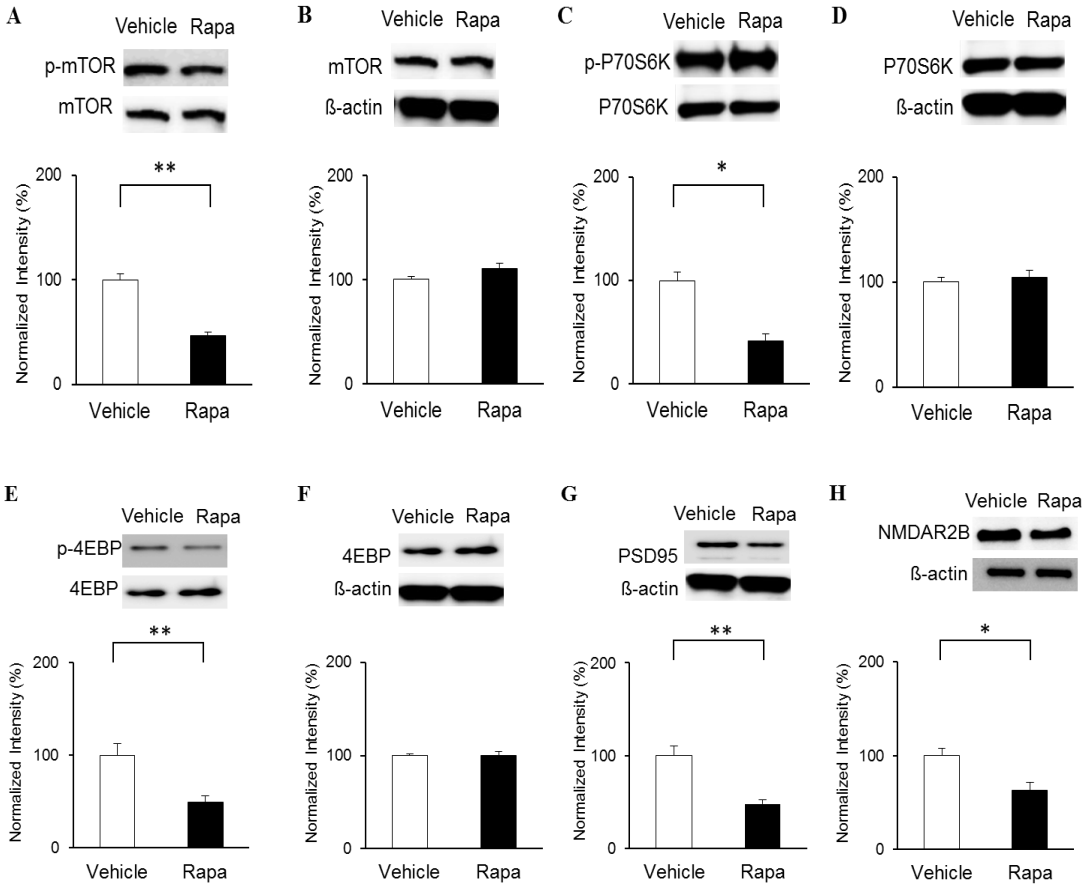


Figure 14. Microinjection of rapamycin reversed the upregulation of the mTOR pathway, PSD95 and NMDAR2B expression on POD7. (A, B) p-mTOR levels decreased in the Rapa group (n=4) compared with in the Vehicle group (n=4), but total mTOR levels were not significantly different. (C, D) p-P70S6K levels decreased in the Rapa group (n=4) compared with in the Vehicle group (n=4), but total P70S6K levels were not different. (E, F) p-4EBP levels decreased in the Rapa group (n=4) compared with in the Vehicle group (n=4), but total 4EBP levels were not different. (G) PSD95 and (H) NMDAR2B levels decreased in the Rapa group (n=4, 5 respectively) compared with in the Vehicle group (n=4, 5 respectively). The intensity of the phosphor-form band was normalized to that of the total form, and the total form bands were normalized to ß-actin protein. Data are presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

IV. DISCUSSION

Many studies have focused on the processes involved in neuropathic pain, and controlling neuropathic pain remains unclear.[3] This study demonstrates that neuropathic pain after nerve injury is accompanied by considerable changes in the biochemistry and neuronal activity of the IC. More specifically, nerve injury increases the activation of mTOR in the IC. To my knowledge, this is the first study to investigate the role of the mTOR signaling pathway and the potential effects of rapamycin in the IC using a neuropathic pain model.

mTOR has been extensively studied in tumors,[63] neurodegenerative[64] and psychiatric disorders.[20,65] Recently, emerging evidence has indicated that mTOR plays a role in pain processing, and it is becoming clear that mTOR is important in the regulation of nociception, in both peripheral and central nervous system. mTOR belongs to the phosphatidylinositol 3-kinase-related kinase (PI3K) protein family, which integrates signals from neuronal activity, growth factors, and nutrient levels to regulate the initiation of protein synthesis.[66] The activation of mTOR triggers phosphorylation of downstream effectors, such as P70S6K and 4EBP, to regulate mRNA translation and protein synthesis.[67-69] The formation of long-term memory requires protein synthesis.[70] Controlling of protein synthesis plays an important role in regulating synaptic plasticity in the CNS. The long-lasting synaptic strength is known as long- term potentiation (LTP). LTP exhibits two distinct phases; early-phase LTP (E-LTP) and late-phase LTP (L-LTP). L-LTP requires transcription of gene and protein synthesis. LTP is generally considered to be the cellular model for learning and memory, as both LTP and memory formation share similar molecular and cellular mechanisms. L-LTP and long-term memory can be impaired by inhibition of protein synthesis or the mTOR pathway, supporting the critical role of translation control downstream of the mTOR pathway in regulating long-lasting synaptic plasticity. Despite accumulating evidence

supporting the role of mTOR signaling in pain-related memory processing,[26,71] little is known about the molecular mechanisms of this pathway in the brain.

Rapamycin, an inhibitor of mTOR, has been developed and widely used as immunosuppressant in patients undergoing transplantation surgery, and has also been used as anticancer drug.[72-74] Because the side effects from rapamycin are relatively low compared to with other drugs, it has also been widely used in chronic pain treatment,[62,75-77] including the possible development of complex regional pain syndrome (CRPS).[78,79] Intrathecal, intraplantar and systemic injection of rapamycin alleviated mechanical hypersensitivity in formalin-induced pain models[80] as well as capsaicin-induced hyperalgesia.[22] Rapamycin may also reduce mechanical allodynia under inflammatory pain conditions.[81] In this study, behavioral, immunohistochemical test and western blot were performed to demonstrate that mTOR signaling in the IC contributes to neuropathic pain and regulates mechanical hypersensitivity. Inhibition of this pathway by microinjections of rapamycin increased the pain threshold and reduced mechanical hypersensitivity on POD3 and POD7. Our findings are compatible with the results of previous data regarding mTOR.[23,26,61] However, the analgesic effect did not persist beyond 24 hours after microinjection, and further studies are needed to assess the duration of rapamycin's efficacy.

The IC is involved in several sensory and cognitive processes, such as learning, memory and perception,[82,83] and connects with other regions to influence other higher-level functions, such as pain-perception and decision-making.[39] While the IC plays an important role in pain processing,[45,58,84] the plasticity of this region in pain-related animal models remains unclear. It was shown that nociceptive stimuli activate neurons in the IC, as evidenced by increases in c-Fos,[60] an immediate early marker of activation.[85-87] A previous study indicated that the role of the p-ERK was cell proliferation and differentiation.[88] Additionally, p-ERK has been implicated in synaptic plasticity related to memory and pain hypersensitivity.[19,89-91] c-Fos and p-ERK levels are increased

in the IC following nerve injury, suggesting that this brain region is closely related to pain state. Importantly, these changes were attenuated by microinjections of rapamycin, indicating the mTOR signaling pathway as a mechanism for plastic changes regulating neuropathic pain.

Glutamate is predominant neurotransmitter to mediated excitatory synaptic responses in the nociceptive pathway. Glutamate also binds to NMDA receptors which are consecutively blocked by Mg^{2+}, but can be relieved from Mg^{2+} blockade under pathological states (Figure 15).[92] Following intense stimulation or persistent injury, activated C and Aδ nociceptors release a variety of neurotransmitters including substance P, glutamate and calcitonin-gene related peptide (CGRP).[93,94] As a consequence, normally silent NMDA receptors located in the postsynaptic neuron can now signal increase intracellular Ca^{2+} and activate Ca^{2+} dependent signaling pathways and second messengers including protein kinase C, Akt and mitogen-activated protein kinase.[53,95] This cascade of events will increase the excitability of the output neuron and facilitate the transmission of pain signals to the brain. Specifically, the IC receives afferent projections from thalamic nuclei, including spinothalamic and spinoreticulothamic tracts, and it forms mutual connections with amygdala, anterior cingulate cortex and cortical association areas.[96] An increased in Ca^{2+} following NMDA receptor activation might activate the PI3K/Akt pathway in the IC. Phosphorylated Akt can activate mTOR in the IC neurons, and peripheral nerve injury lead to activation of PI3K/Akt/mTOR signaling pathway. Interestingly, there is a report that mTOR activation required activation of NMDA receptors in a bone cancer-induced pain model.[68] This study demonstrated that bone cancer pain–induced dorsal horn activation of the mTOR pathway participates in NMDA receptor triggered dorsal central sensitization, which indicates that the NMDA receptor/mTOR signaling pathway is involved in pain processing. In a neuropathic pain model, microinjection of an NMDA receptor antagonist into the IC significantly reduced pain behavior.[46] Furthermore, LTP of synaptic responses in the IC is NMDA receptor dependent,[46] and

inhibition of protein synthesis or of the mTOR pathway can diminish LTP and memory.[97] These data suggest that LTP in the IC regulates neuropathic pain. My research data support this, as NMDA receptor expression in the IC was increased after peripheral nerve injury and attenuated by a microinjection of rapamycin.

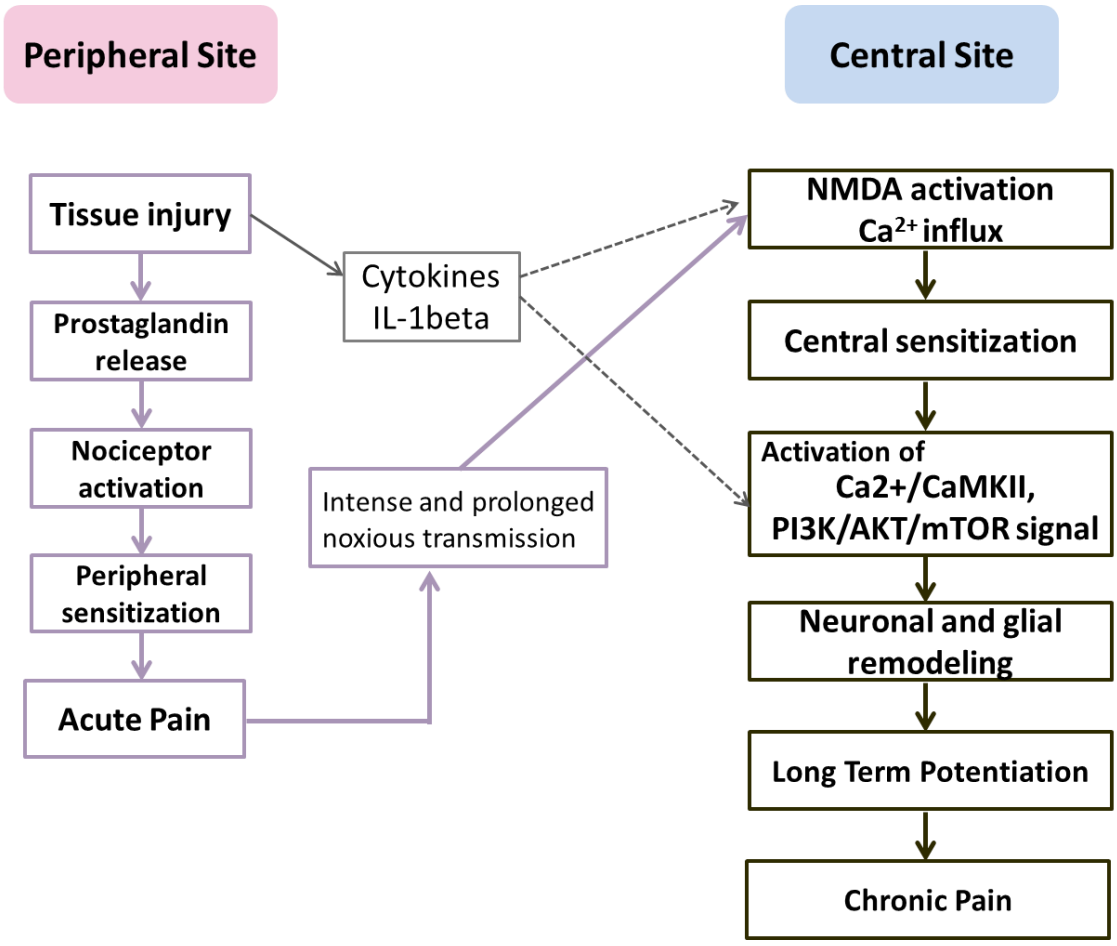


Figure 15. Peripheral and central sites in pain perception and sensitization. Normally, the NMDA receptor is blocked by its Mg^{2+} ion, however, under continuous stimulation it is removed. This enhanced NMDA receptor activation plays a role in pain-related synaptic plasticity, and results in the exacerbation of chronic pain.

Accordingly, this research was found that nerve injury increased the expression of PSD95, and essential scaffold protein in the post synaptic density of excitatory synapses.[26,98] Dendrites are the sites at which neurons receive information from multiple presynaptic factors. Dendritic function is critically dependent on dendritic growth and small protrusions called dendritic spines.[99] A previous study showed that the expression of PSD95 significantly increases when mTOR-dependent pathways are activated under stress conditions.[100] This research results similarly demonstrated that neuropathic pain increases mTOR signaling and PSD95 expression. Moreover, levels of PSD95 in the IC were decreased by inhibition of mTOR with rapamycin. Interestingly, the knockdown of PSD95 in the spinal cord delays the progression of neuropathic pain.[98,101] phosphatidylinositol 3-kinase (PI3K)/AKT (protein kinase B)/mTOR signaling has emerged as key regulator of dendritic size and dendritic complexity as well as of dendritic spine density.[99] Specifically, the PSD95 is largely localized in spines and plays an important role in regulating dendritic spine size and shape.[27,102] Thus, changes in dendritic morphology and synaptic-associated proteins may affect synaptic plasticity. In my studies, mechanical allodynia as a result of peripheral nerve injury appears to be regulated by mTOR-dependent synthesis of PSD95 in the IC. Taken together, these data suggest that mTOR signaling maintains chronic pain conditions via regulation of synaptic proteins.

Based on these evidences, I hypothesized that NMDA receptor-mediated increase in calcium concentration which stimulates AKT activation by PI3K calcium/calmodulin-dependent protein kinase 2.[103-105] Once activated, mTOR and its downstream were activated. As a result, synaptic proteins at the synapse such as PSD95, synapsin 1 were translated (Figure 16).[106]

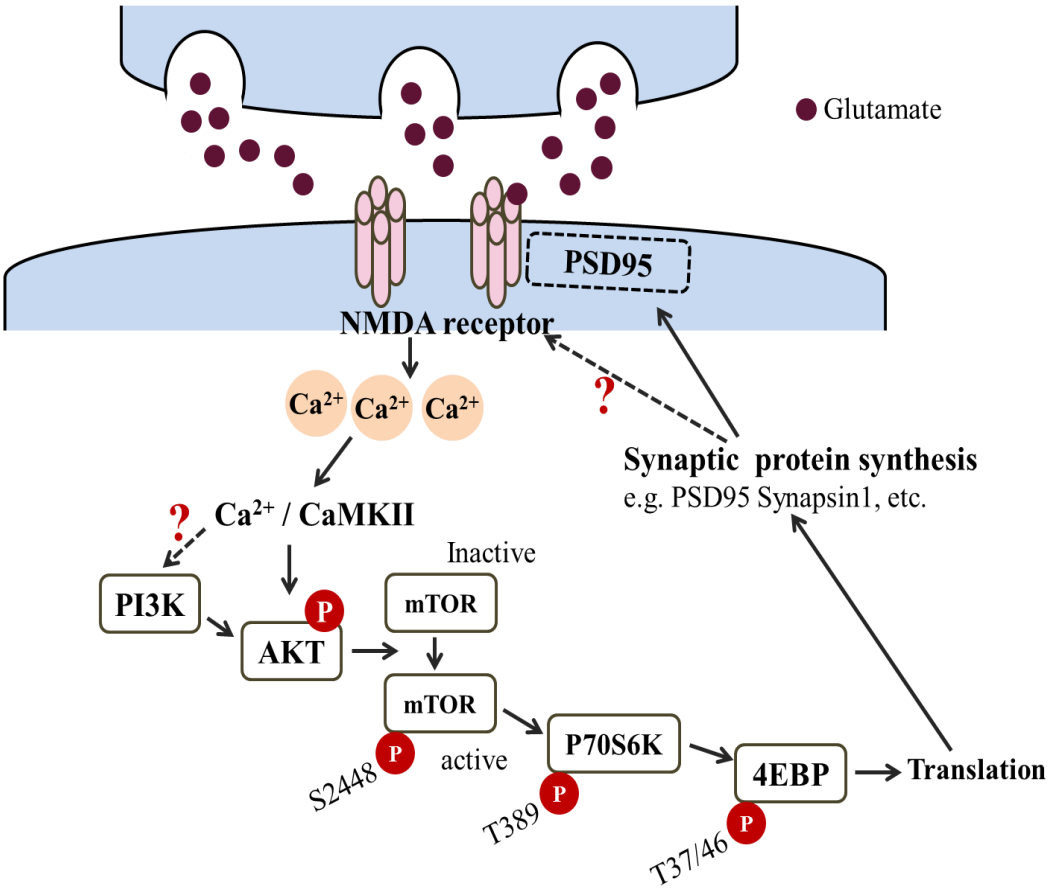


Figure 16. Overview of possible mechanisms involved in mTOR signaling mediated synaptic plasticity in the IC of neuropathic pain model. Nerve injury results in the excessive release of gutamate leading to the over activation of NMDA receptors and releasing calcium ions. Increased calcium ions stimulate Ca2+/CaMKII, which is important kinase that is well documented in the fields of learning and menory process. Phosphorylation of AKT was induced by activated Ca2+/CaMKII and PI3K. Activation of AKT stimulates the phosphorylation of mTOR, a central signaling hub that has multiple downstream effectors. Activated mTOR phosphorylates p70S6K and 4EBP, thereby stimulate translation. This process facilitates synpatic protein synthesis and transport such as PSD95, synapsin 1, and NMDA receptors. *Abbreviations*: PI3K, phosphatidylinositol 3-kinase; AKT, known

as PKB, Protein kinase B; CaMKII, calmodulin-dependent protein kinase II; mTOR, mammlian target of rapamycin; P70S6K, known as S6K, ribosomal protein S6 kinase; 4EBP, eukaryotic translation initiation factor 4E binding protein; PSD95, postsynaptic density protein 95.

The present study used pre-administration with rapamycin in the IC, to effective neuropathic pain prevention. The behavioral test result showed that significantly attenuated the nociceptive behaviors induced by nerve-injury. In contrast, no statistical significance was detected compared to the Pre-Vehicle group in immunohistochemistry and western blot analysis. This presumably assumes that the prevention effects of the pain might have another signal pathway different from the mTOR-P70S6K-4EBP pathway as mentioned above. Additional research will be needed to interpret my findings.

This study suggests that the mTOR pathway is a critical molecular signaling pathway regulating synaptic plasticity in the IC and mechanical hypersensitivity after peripheral nerve injury and neuropathic pain.

V. CONCLUSION

This is the first study to investigate the effects of mTOR signaling activation in the IC. Author found that neuropathic pain induced nerve injury can activate the mTOR signaling pathway and increase synaptic protein PSD95, and NMDAR2B. Increased levels of mTOR signaling, PSD95 and NMDAR2B were blocked by the rapamycin, the mTOR inhibitor.

This research provided potent results using behavioral test, immunohistochemistry and western blot analysis that mTOR signaling contributes to neuronal plasticity and pain-like behavior in the IC of neuropathic pain model. Furthermore, these data suggest that mTOR signaling pathway may be significantly important to sustain chronic pain conditions via mRNA translation and protein synthesis of memory formation required for modulating pain sensation in the IC.

Hence, the author can infer that the activation of mTOR signaling is a promising target for developing novel neuropathic pain drugs.

REFERENCES

1. Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the International Association for the Study of Pain, Subcommittee on Taxonomy. Pain Suppl 1986;3:S1-226.

2. Treede RD, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, et al. Neuropathic pain: redefinition and a grading system for clinical and research purposes. Neurology 2008;70:1630-5.

3. Ro LS, Chang KH. Neuropathic pain: mechanisms and treatments. Chang Gung Med J 2005;28:597-605.

4. Mao J, Mayer DJ. Spinal cord neuroplasticity following repeated opioid exposure and its relation to pathological pain. Ann N Y Acad Sci 2001;933:175-84.

5. Voscopoulos C, Lema M. When does acute pain become chronic? Br J Anaesth 2010;105 Suppl 1:i69-85.

6. Ling YZ, Li ZY, Ou-Yang HD, Ma C, Wu SL, Wei JY, et al. The inhibition of spinal synaptic plasticity mediated by activation of AMP-activated protein kinase signaling alleviates the acute pain induced by oxaliplatin. Exp Neurol 2017;288:85-93.

7. Jacinto E, Hall MN. Tor signalling in bugs, brain and brawn. Nat Rev Mol Cell Biol 2003;4:117-26.

8. Maiese K, Chong ZZ, Shang YC, Wang S. mTOR: on target for novel therapeutic

strategies in the nervous system. Trends Mol Med 2013;19:51-60.

9. Izumi Y, Sasaki M, Hashimoto S, Sawa T, Amaya F. mTOR signaling controls VGLUT2 expression to maintain pain hypersensitivity after tissue injury. Neuroscience 2015;308:169-79.

10. Magnuson B, Ekim B, Fingar DC. Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. Biochem J 2012;441:1-21.

11. Jaworski J, Sheng M. The growing role of mTOR in neuronal development and plasticity. Mol Neurobiol 2006;34:205-19.

12. Costa-Mattioli M, Sossin WS, Klann E, Sonenberg N. Translational control of long-lasting synaptic plasticity and memory. Neuron 2009;61:10-26.

13. Lu B, Jiang J, Sun J, Xiao C, Meng B, Zheng J, et al. Inhibition of mammalian target of rapamycin activation in the rostral anterior cingulate cortex attenuates pain-related aversion in rats. Behav Brain Res 2016;310:51-8.

14. Wang J, Feng DY, Li ZH, Feng B, Zhang H, Zhang T, et al. Activation of the Mammalian Target of Rapamycin in the Rostral Ventromedial Medulla Contributes to the Maintenance of Nerve Injury-Induced Neuropathic Pain in Rat. Neural Plast 2015;2015:394820.

15. Martin KC, Casadio A, Zhu H, Yaping E, Rose JC, Chen M, et al. Synapse-specific,

long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. Cell 1997;91:927-38.

16. Sutton MA, Schuman EM. Dendritic protein synthesis, synaptic plasticity, and memory. Cell 2006;127:49-58.

17. Banko JL, Poulin F, Hou L, DeMaria CT, Sonenberg N, Klann E. The translation repressor 4E-BP2 is critical for eIF4F complex formation, synaptic plasticity, and memory in the hippocampus. J Neurosci 2005;25:9581-90.

18. Antion MD, Merhav M, Hoeffer CA, Reis G, Kozma SC, Thomas G, et al. Removal of S6K1 and S6K2 leads to divergent alterations in learning, memory, and synaptic plasticity. Learn Mem 2008;15:29-38.

19. Ji RR, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci 2003;26:696-705.

20. Hoeffer CA, Klann E. mTOR signaling: at the crossroads of plasticity, memory and disease. Trends Neurosci 2010;33:67-75.

21. Jimenez-Diaz L, Geranton SM, Passmore GM, Leith JL, Fisher AS, Berliocchi L, et al. Local translation in primary afferent fibers regulates nociception. PLoS One 2008;3:e1961.

22. Geranton SM, Jimenez-Diaz L, Torsney C, Tochiki KK, Stuart SA, Leith JL, et al. A

rapamycin-sensitive signaling pathway is essential for the full expression of persistent pain states. J Neurosci 2009;29:15017-27.

23. Norsted Gregory E, Codeluppi S, Gregory JA, Steinauer J, Svensson CI. Mammalian target of rapamycin in spinal cord neurons mediates hypersensitivity induced by peripheral inflammation. Neuroscience 2010;169:1392-402.

24. Lyu D, Yu W, Tang N, Wang R, Zhao Z, Xie F, et al. The mTOR signaling pathway regulates pain-related synaptic plasticity in rat entorhinal-hippocampal pathways. Mol Pain 2013;9:64.

25. Li G, Lu X, Zhang S, Zhou Q, Zhang L. mTOR and Erk1/2 Signaling in the Cerebrospinal Fluid-Contacting Nucleus is Involved in Neuropathic Pain. Neurochem Res 2015;40:1053-62.

26. Zhang W, Sun XF, Bo JH, Zhang J, Liu XJ, Wu LP, et al. Activation of mTOR in the spinal cord is required for pain hypersensitivity induced by chronic constriction injury in mice. Pharmacol Biochem Behav 2013;111:64-70.

27. Han K, Kim E. Synaptic adhesion molecules and PSD-95. Prog Neurobiol 2008;84:263-83.

28. Sheng M, Kim MJ. Postsynaptic signaling and plasticity mechanisms. Science 2002;298:776-80.

29. Kornau HC, Seeburg PH, Kennedy MB. Interaction of ion channels and receptors with PDZ domain proteins. Curr Opin Neurobiol 1997;7:368-73.

30. Niethammer M, Kim E, Sheng M. Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. J Neurosci 1996;16:2157-63.

31. Lin Y, Jover-Mengual T, Wong J, Bennett MV, Zukin RS. PSD-95 and PKC converge in regulating NMDA receptor trafficking and gating. Proc Natl Acad Sci U S A 2006;103:19902-7.

32. Li S, Han J, Wang DS, Yang Q, Feng B, Kang WB, et al. Sinomenine attenuates chronic inflammatory pain in mice. Metab Brain Dis 2017;32:211-9.

33. Zhuo M. Glutamate receptors and persistent pain: targeting forebrain NR2B subunits. Drug Discov Today 2002;7:259-67.

34. Kalia LV, Kalia SK, Salter MW. NMDA receptors in clinical neurology: excitatory times ahead. Lancet Neurol 2008;7:742-55.

35. Kessels HW, Malinow R. Synaptic AMPA receptor plasticity and behavior. Neuron 2009;61:340-50.

36. Liu XJ, Salter MW. Glutamate receptor phosphorylation and trafficking in pain plasticity in spinal cord dorsal horn. Eur J Neurosci 2010;32:278-89.

37. Yasaka T, Hughes DI, Polgar E, Nagy GG, Watanabe M, Riddell JS, et al. Evidence against AMPA receptor-lacking glutamatergic synapses in the superficial dorsal horn of the rat spinal cord. J Neurosci 2009;29:13401-9.

38. Zhou HY, Chen SR, Pan HL. Targeting N-methyl-D-aspartate receptors for treatment of neuropathic pain. Expert Rev Clin Pharmacol 2011;4:379-88.

39. Zhuo M. Contribution of synaptic plasticity in the insular cortex to chronic pain. Neuroscience 2016;338:220-9.

40. Angeles-Duran S, Ramos-Languren LE, Escobar ML. PKMzeta inhibition prevents the metaplastic change induced by conditioned taste aversion on insular cortex long-term potentiation in vivo. Rev Neurosci 2012;23:473-80.

41. Jones MW, French PJ, Bliss TV, Rosenblum K. Molecular mechanisms of long-term potentiation in the insular cortex in vivo. J Neurosci 1999;19:Rc36.

42. Escobar ML, Alcocer I, Chao V. The NMDA receptor antagonist CPP impairs conditioned taste aversion and insular cortex long-term potentiation in vivo. Brain Res 1998;812:246-51.

43. Moguel-Gonzalez M, Gomez-Palacio-Schjetnan A, Escobar ML. BDNF reverses the CTA memory deficits produced by inhibition of protein synthesis. Neurobiol Learn Mem 2008;90:584-7.

44. Burkey AR, Carstens E, Jasmin L. Dopamine reuptake inhibition in the rostral agranular insular cortex produces antinociception. J Neurosci 1999;19:4169-79.

45. Coffeen U, Manuel Ortega-Legaspi J, Lopez-Munoz FJ, Simon-Arceo K, Jaimes O, Pellicer F. Insular cortex lesion diminishes neuropathic and inflammatory pain-like behaviours. Eur J Pain 2011;15:132-8.

46. Liu MG, Kang SJ, Shi TY, Koga K, Zhang MM, Collingridge GL, et al. Long-term potentiation of synaptic transmission in the adult mouse insular cortex: multielectrode array recordings. J Neurophysiol 2013;110:505-21.

47. Garcia-Larrea L, Peyron R. Pain matrices and neuropathic pain matrices: a review. Pain 2013;154 Suppl 1:S29-43.

48. Tseng MT, Chiang MC, Chao CC, Tseng WY, Hsieh ST. fMRI evidence of degeneration-induced neuropathic pain in diabetes: enhanced limbic and striatal activations. Hum Brain Mapp 2013;34:2733-46.

49. Coghill RC, Sang CN, Maisog JM, Iadarola MJ. Pain intensity processing within the human brain: a bilateral, distributed mechanism. J Neurophysiol 1999;82:1934-43.

50. Brooks JC, Nurmikko TJ, Bimson WE, Singh KD, Roberts N. fMRI of thermal pain: effects of stimulus laterality and attention. Neuroimage 2002;15:293-301.

51. Wu WY, Liu CY, Tsai ML, Yen CT. Nocifensive behavior-related laser heat-evoked

component in the rostral agranular insular cortex revealed using morphine analgesia. Physiol Behav 2016;154:129-34.

52. Burkey AR, Carstens E, Wenniger JJ, Tang J, Jasmin L. An opioidergic cortical antinociception triggering site in the agranular insular cortex of the rat that contributes to morphine antinociception. J Neurosci 1996;16:6612-23.

53. Jasmin L, Rabkin SD, Granato A, Boudah A, Ohara PT. Analgesia and hyperalgesia from GABA-mediated modulation of the cerebral cortex. Nature 2003;424:316-20.

54. Jung HH, Shin J, Kim J, Ahn SH, Lee SE, Koh CS, et al. Rostral Agranular Insular Cortex Lesion with Motor Cortex Stimulation Enhances Pain Modulation Effect on Neuropathic Pain Model. Neural Plast 2016;2016:3898924.

55. Lee BH, Won R, Baik EJ, Lee SH, Moon CH. An animal model of neuropathic pain employing injury to the sciatic nerve branches. Neuroreport 2000;11:657-61.

56. Han J, Kwon M, Cha M, Tanioka M, Hong SK, Bai SJ, et al. Plasticity-Related PKMzeta Signaling in the Insular Cortex Is Involved in the Modulation of Neuropathic Pain after Nerve Injury. Neural Plast 2015;2015:601767.

57. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates: Hard Cover Edition: Elsevier Science; 2006.

58. Alvarez P, Dieb W, Hafidi A, Voisin DL, Dallel R. Insular cortex representation of

dynamic mechanical allodynia in trigeminal neuropathic rats. Neurobiol Dis 2009;33:89-95.

59. Qiu S, Chen T, Koga K, Guo YY, Xu H, Song Q, et al. An increase in synaptic NMDA receptors in the insular cortex contributes to neuropathic pain. Sci Signal 2013;6:ra34.

60. Gao YJ, Ji RR. c-Fos and pERK, which is a better marker for neuronal activation and central sensitization after noxious stimulation and tissue injury? Open Pain J 2009;2:11-7.

61. Asante CO, Wallace VC, Dickenson AH. Mammalian target of rapamycin signaling in the spinal cord is required for neuronal plasticity and behavioral hypersensitivity associated with neuropathy in the rat. J Pain 2010;11:1356-67.

62. Melemedjian OK, Khoutorsky A, Sorge RE, Yan J, Asiedu MN, Valdez A, et al. mTORC1 inhibition induces pain via IRS-1-dependent feedback activation of ERK. Pain 2013;154:1080-91.

63. Fruman DA, Rommel C. PI3K and cancer: lessons, challenges and opportunities. Nat Rev Drug Discov 2014;13:140-56.

64. Ghavami S, Shojaei S, Yeganeh B, Ande SR, Jangamreddy JR, Mehrpour M, et al. Autophagy and apoptosis dysfunction in neurodegenerative disorders. Prog Neurobiol 2014;112:24-49.

65. Abelaira HM, Reus GZ, Neotti MV, Quevedo J. The role of mTOR in depression and antidepressant responses. Life Sci 2014;101:10-4.

66. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 2012;149:274-93.

67. Klann E, Dever TE. Biochemical mechanisms for translational regulation in synaptic plasticity. Nat Rev Neurosci 2004;5:931-42.

68. Shih MH, Kao SC, Wang W, Yaster M, Tao YX. Spinal cord NMDA receptor-mediated activation of mammalian target of rapamycin is required for the development and maintenance of bone cancer-induced pain hypersensitivities in rats. J Pain 2012;13:338-49.

69. Zhuang F, Li M, Gao X, Wang Y, Wang D, Ma X, et al. The antidepressant-like effect of alarin is related to TrkB-mTOR signaling and synaptic plasticity. Behav Brain Res 2016;313:158-71.

70. Squire LR, Davis HP. The pharmacology of memory: a neurobiological perspective. Annu Rev Pharmacol Toxicol 1981;21:323-56.

71. Lutz BM, Nia S, Xiong M, Tao YX, Bekker A. mTOR, a new potential target for chronic pain and opioid-induced tolerance and hyperalgesia. Mol Pain 2015;11:32.

72. Dancey J. mTOR signaling and drug development in cancer. Nat Rev Clin Oncol

2010;7:209-19.

73. Delgoffe GM, Powell JD. mTOR: taking cues from the immune microenvironment. Immunology 2009;127:459-65.

74. Populo H, Lopes JM, Soares P. The mTOR signalling pathway in human cancer. Int J Mol Sci 2012;13:1886-918.

75. Lee L, Sudentas P, Dabora SL. Combination of a rapamycin analog (CCI-779) and interferon-gamma is more effective than single agents in treating a mouse model of tuberous sclerosis complex. Genes Chromosomes Cancer 2006;45:933-44.

76. Alamo JM, Barrera L, Casado MD, Bernal C, Marin LM, Suarez G, et al. Efficacy, tolerance, and safety of mammalian target of rapamycin inhibitors as rescue immunosuppressants in liver transplantation. Transplant Proc 2009;41:2181-3.

77. Gao M, Yan X, Weng HR. Inhibition of glycogen synthase kinase 3beta activity with lithium prevents and attenuates paclitaxel-induced neuropathic pain. Neuroscience 2013;254:301-11.

78. Molina MG, Diekmann F, Burgos D, Cabello M, Lopez V, Oppenheimer F, et al. Sympathetic dystrophy associated with sirolimus therapy. Transplantation 2008;85:290-2.

79. Witzig TE, Geyer SM, Ghobrial I, Inwards DJ, Fonseca R, Kurtin P, et al. Phase II

trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. J Clin Oncol 2005;23:5347-56.

80. Asante CO, Wallace VC, Dickenson AH. Formalin-induced behavioural hypersensitivity and neuronal hyperexcitability are mediated by rapid protein synthesis at the spinal level. Mol Pain 2009;5:27.

81. Obara I, Tochiki KK, Geranton SM, Carr FB, Lumb BM, Liu Q, et al. Systemic inhibition of the mammalian target of rapamycin (mTOR) pathway reduces neuropathic pain in mice. Pain 2011;152:2582-95.

82. Bermudez-Rattoni F. Molecular mechanisms of taste-recognition memory. Nat Rev Neurosci 2004;5:209-17.

83. Craig AD. Significance of the insula for the evolution of human awareness of feelings from the body. Ann N Y Acad Sci 2011;1225:72-82.

84. Qiu S, Zhang M, Liu Y, Guo Y, Zhao H, Song Q, et al. GluA1 phosphorylation contributes to postsynaptic amplification of neuropathic pain in the insular cortex. J Neurosci 2014;34:13505-15.

85. Greenberg ME, Ziff EB, Greene LA. Stimulation of neuronal acetylcholine receptors induces rapid gene transcription. Science 1986;234:80-3.

86. Hunt SP, Pini A, Evan G. Induction of c-fos-like protein in spinal cord neurons

following sensory stimulation. Nature 1987;328:632-4.

87. Coggeshall RE. Fos, nociception and the dorsal horn. Prog Neurobiol 2005;77:299-352.

88. Widmann C, Gibson S, Jarpe MB, Johnson GL. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol Rev 1999;79:143-80.

89. Ji RR, Woolf CJ. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. Neurobiol Dis 2001;8:1-10.

90. Price TJ, Inyang KE. Commonalities between pain and memory mechanisms and their meaning for understanding chronic pain. Prog Mol Biol Transl Sci 2015;131:409-34.

91. Page G, Khidir FA, Pain S, Barrier L, Fauconneau B, Guillard O, et al. Group I metabotropic glutamate receptors activate the p70S6 kinase via both mammalian target of rapamycin (mTOR) and extracellular signal-regulated kinase (ERK 1/2) signaling pathways in rat striatal and hippocampal synaptoneurosomes. Neurochem Int 2006;49:413-21.

92. Luo C, Kuner T, Kuner R. Synaptic plasticity in pathological pain. Trends Neurosci 2014;37:343-55.

93. Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. Cell 2009;139:267-84.

94. Millan MJ. Descending control of pain. Prog Neurobiol 2002;66:355-474.

95. Yang JX, Hua L, Li YQ, Jiang YY, Han D, Liu H, et al. Caveolin-1 in the anterior cingulate cortex modulates chronic neuropathic pain via regulation of NMDA receptor 2B subunit. J Neurosci 2015;35:36-52.

96. Price DD. Psychological and neural mechanisms of the affective dimension of pain. Science 2000;288:1769-72.

97. Melemedjian OK, Khoutorsky A. Translational control of chronic pain. Prog Mol Biol Transl Sci 2015;131:185-213.

98. Tao F, Tao YX, Mao P, Johns RA. Role of postsynaptic density protein-95 in the maintenance of peripheral nerve injury-induced neuropathic pain in rats. Neuroscience 2003;117:731-9.

99. Kumar V, Zhang MX, Swank MW, Kunz J, Wu GY. Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. J Neurosci 2005;25:11288-99.

100. Yang PC, Yang CH, Huang CC, Hsu KS. Phosphatidylinositol 3-kinase activation is required for stress protocol-induced modification of hippocampal synaptic plasticity. J

Biol Chem 2008;283:2631-43.

101. Tao F, Tao YX, Gonzalez JA, Fang M, Mao P, Johns RA. Knockdown of PSD-95/SAP90 delays the development of neuropathic pain in rats. Neuroreport 2001;12:3251-5.

102. Ehrlich I, Klein M, Rumpel S, Malinow R. PSD-95 is required for activity-driven synapse stabilization. Proc Natl Acad Sci U S A 2007;104:4176-81.

103. Niciu MJ, Ionescu DF, Mathews DC, Richards EM, Zarate CA, Jr. Second messenger/signal transduction pathways in major mood disorders: moving from membrane to mechanism of action, part I: major depressive disorder. CNS Spectr 2013;18:231-41.

104. Lau CG, Takeuchi K, Rodenas-Ruano A, Takayasu Y, Murphy J, Bennett MV, et al. Regulation of NMDA receptor Ca2+ signalling and synaptic plasticity. Biochem Soc Trans 2009;37:1369-74.

105. Hou L, Klann E. Activation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for metabotropic glutamate receptor-dependent long-term depression. J Neurosci 2004;24:6352-61.

106. Patel S, Grizzell JA, Holmes R, Zeitlin R, Solomon R, Sutton TL, et al. Cotinine halts the advance of Alzheimer's disease-like pathology and associated depressive-like

behavior in Tg6799 mice. Front Aging Neurosci 2014;6:162.

ABSTRACT (in Korean)

신경병증성 통증 모델의 뇌섬엽에서 rapamycin이 mTOR 신호전달에 미치는 영향

< 지도교수 이 배 환 >

연세대학교 대학원 의과학과

권 민 지

말초신경 손상은 신경병증성 통증을 유발하여 말초 및 중추 감작을 통한 이질통과 통각과민을 일으키는 것으로 알려져 있다. 신경병증성 통증은 척수와 뇌를 포함한 일차 구심성 신경의 병리적인 변화를 의미한다. 최근 여러 연구들은 신경병증성 통증과 관련하여 뇌 섬엽 피질의 역할에 초점을 맞추어 왔다. 뇌섬엽 피질은 통증과 관련된 기억을 형성하고 저장하는 것으로 생각되어 왔기 때문에, 뇌 섬엽 피질에서 기억 형성과 연관된 단백질 합성을 조절하는 요인은 만성통증을 완화시키는 데에 기여할 수 있다. mRNA에서 단백질로 번역이 되는 과정을 조절하고, 시냅스 가소성에 영향을 끼치는 것으로 알려져 있는 mTOR 신호전달체계는 신경병증성 통증 모델의 척수 수준에서 연구가 진행되어 있다. 척수 수준 이상의 중추신경계인 뇌에서의 연구는 거의 이루어지지 않은 상태이다. 따라서 이 연구는 신경병증성 통증 모

델의 뇌섬엽 피질에서 mTOR 신호전달의 역할을 규명하고 mTOR의 억제제인 rapamycin을 뇌섬엽 피질 내로 투여하여 통증예방 혹은 경감효과를 관찰함으로써 rapamycin의 잠재적인 치료 효과를 평가하기 위해 수행되었다.

정상 백서의 뇌 섬엽 피질 내로 rapamycin을 미세주입 한 후 신경손상을 유도하여 신경병증성 통증을 예방할 수 있는 지를 관찰하였다. 또한 rapamycin의 통증감소효과를 검증하기 위해 백서를 사용하여 신경통증 모델을 형성, 수술 후 1일, 3일, 7일에 기계적 이질통이 발생한 것을 확인하였다. 수술 후 3일과 7일째 각각 뇌 섬엽에 rapamycin을 미세주입하여 이질통의 감소효과를 평가하였다.

그 결과, rapamycin의 사전 미세주입은 점차적으로 발달되는 기계적 이질통을 효과적으로 완화시키기는 하였으나 mTOR 신호전달과 관련된 요인들의 발현량을 감소시키는 데에는 효과가 없었다. 이와는 반대로 통증모델 형성 이후 미세 주입된 rapamycin은 기계적 이질통을 감소시키고 mTOR와 그 하위 경로에 있는 분자요인들, 시냅스 말단에 다량 존재하는 단백질인 post synaptic density 95와 NMDA 수용체의 발현을 감소시켜 신경병증으로 인한 통증유발과 관련된 시냅스 가소성을 억제했다. 이러한 결과는 뇌 피질의 mTOR 신호가 신경병증통증을 조절할 수 있는 중요한 분자 기전일 수 있음을 시사한다.

핵심되는 말: 뇌 섬엽피질, 신경병증성 통증, mammalian target of rapamycin (mTOR), post synaptic density 95 (PSD95), *N*-methyl-D-aspartate receptor (NMDAR), 시냅스 가소성, rapamycin

PUBLICATION LIST

1. **Kwon M**, Han J, Kim UJ, Cha M, Um SW, Bai SJ, Hong SK, Lee BH. Inhibition of mammalian target of rapamycin (mTOR) signaling in the insular cortex alleviates neuropathic pain after peripheral nerve injury. Front Mol Neurosci. 2017 Mar 21; 10:79

2. Han J, Cha M, **Kwon M**, Hong SK, Bai SJ, Lee BH. In vivo voltage-sensitive dye imaging of the insular cortex in nerve-injured rats. Neurosci Lett. 2016 Nov 10;634:146-152

3. Han J, **Kwon M**, Cha M, Tanioka M, Hong SK, Bai SJ, Lee BH. Plasticity-related PKMζ signaling in the insular cortex is involved in the modulation of neuropathic pain after nerve injury. Neural Plast. 2015; 2015:601767