RAPAMYCIN REDUCED OROFACIAL NEUROPATHIC PAIN AND TNC GLIA ACTIVATION IN TRIGEMINAL NERVE-INJURED MICE

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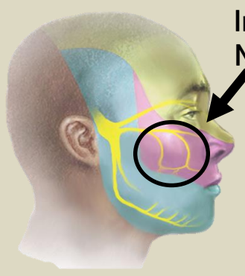
1. INTRODUCTION

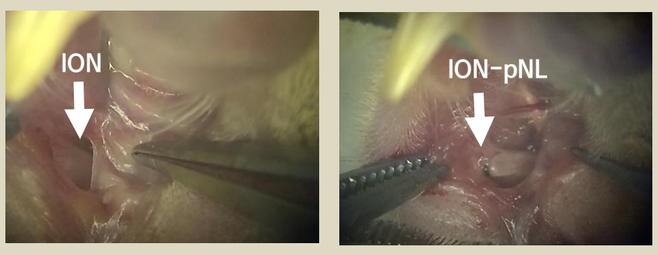
Neuropathic pain caused by trigeminal nerve injury is a typical refractory orofacial chronic pain accompanied by the formation of hyperalgesia and allodynia. This pain is also associated with glia activation in trigeminal nucleus caudalis (TNC). However, it is unclear whether glia activation is related to the activation of mTOR signaling.

2. MATERIALS AND METHODS

1. ANIMALS AND SURGERY

- Surgery : Infraorbital nerve partial nerve ligation(ION-pNL) using 8-0 silk





- Rapamycin (mTOR inhibitor), 0.1, 0.3, 1 mg/kg, i.p.

2. BEHAVIOR TESTS

- Mechanical allodynia test : 50% withdrawal threshold
- Cold allodynia test : Acetone test

3. WESTERN BLOT ASSAY

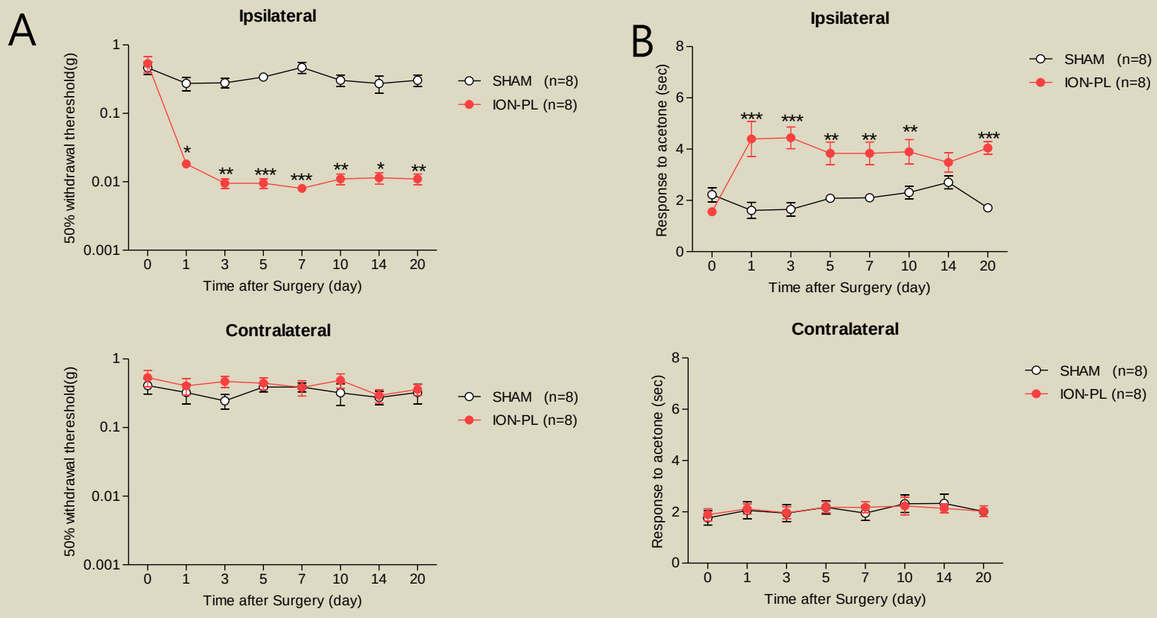
- Trigeminal nucleus caudalis (TNC), trigeminal ganglia (TG)
- Primary antibody : mTOR, p-mTOR (1:500, Cell signaling)
  S6, p-S6 (1:1k, Cell signaling)
  4EBP1, p-4EBP1 (1:1k, Cell signaling)
- Secondary antibody : anti-rabbit (1:1k)

4. IMMUNOHISTOCHEMISTRY

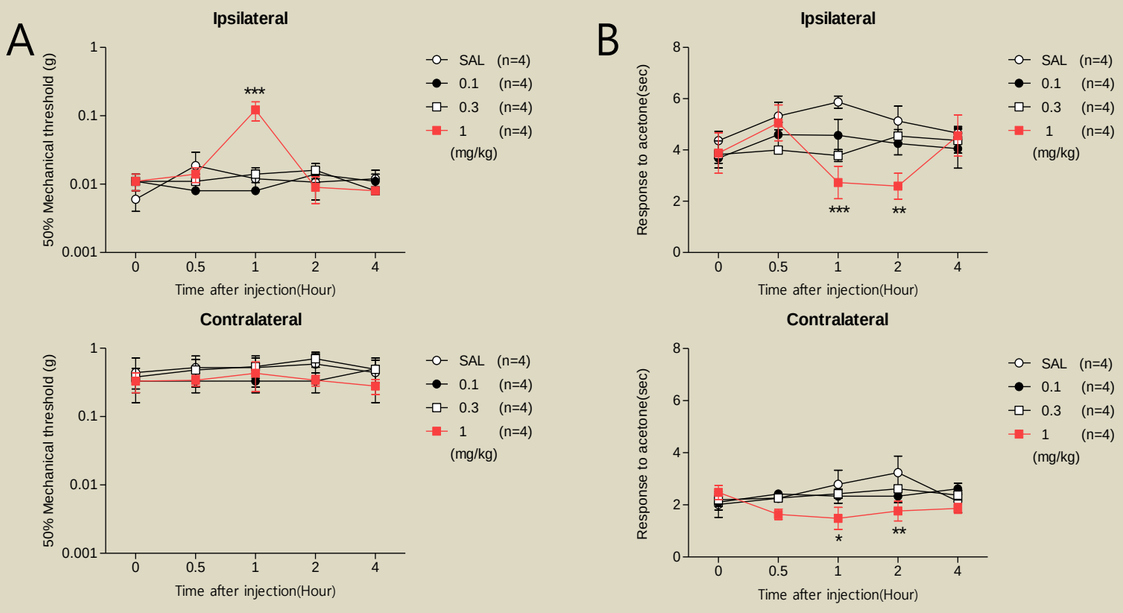
- Primary antibody : p-p38, p-S6, GFAP (1:1k, Cell signaling) /
  p-ERK, Iba-1 (1:500, Cell signaling)
- Secondary antibody : anti-rabbit with cy3 (1:600) /
  anti-mouse, rabbit with alexa 488 (1:500)

3. RESULTS

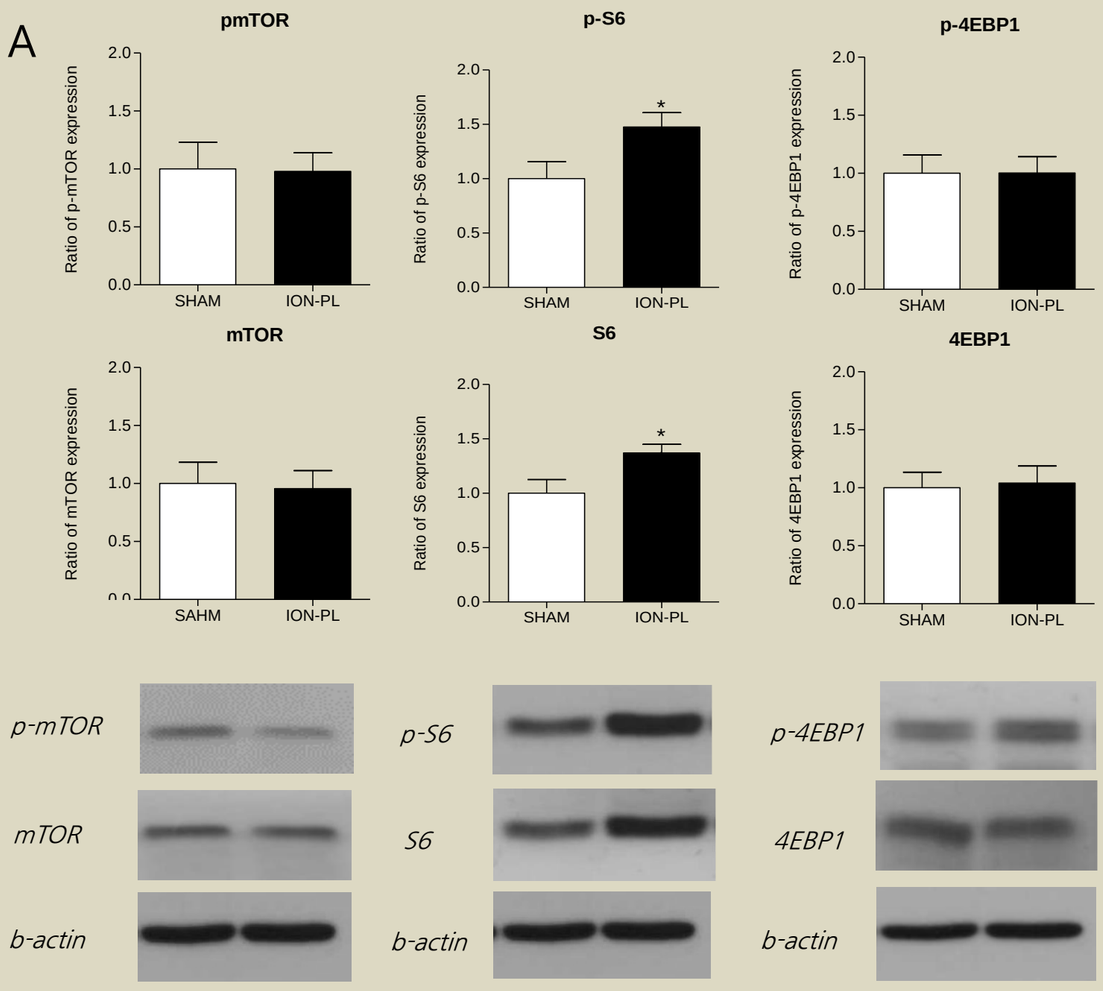
1. Development of chronic mechanical(A) and cold allodynia(B) by infraorbital nerve injury

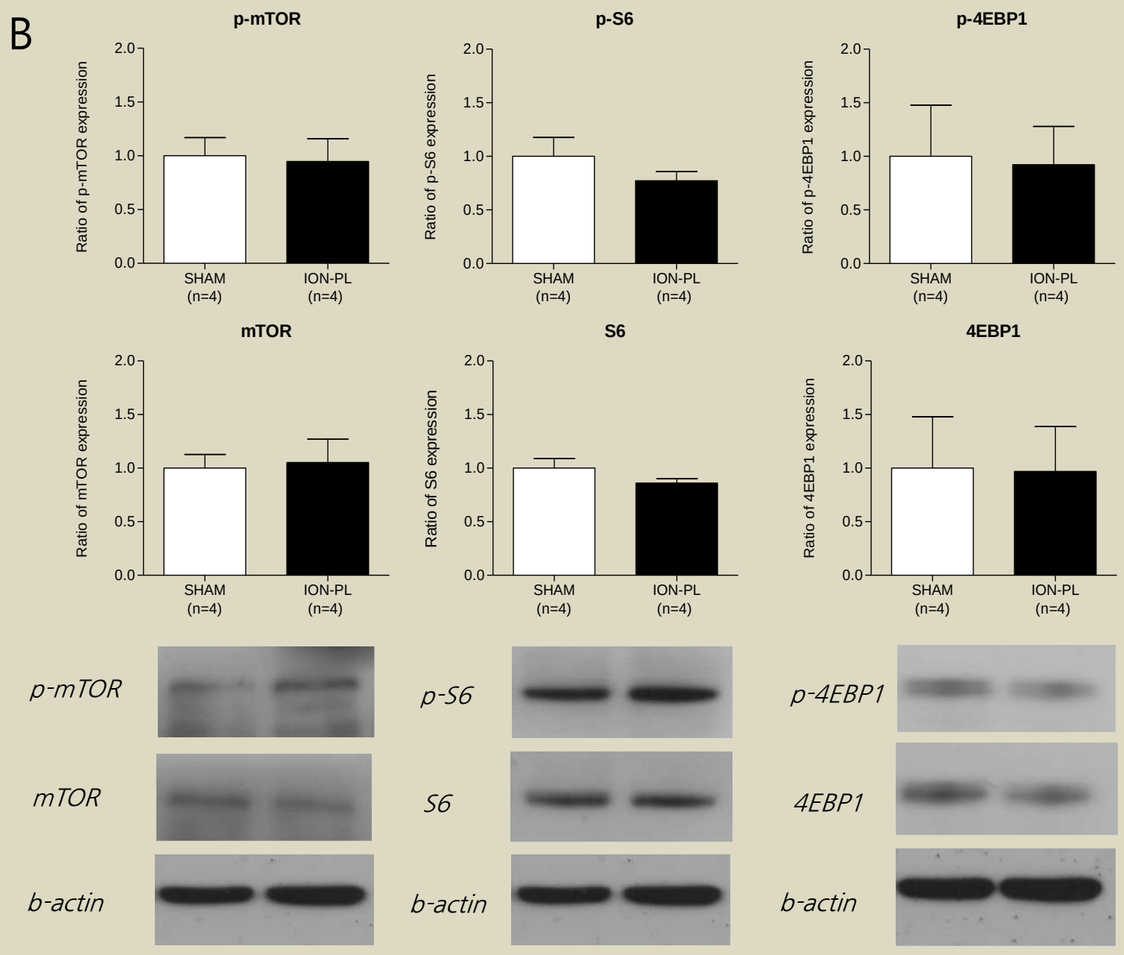


2. Dose-dependent effect of rapamycin on mechanical(A) and cold allodynia(B) in orofacial neuropathic pain mice

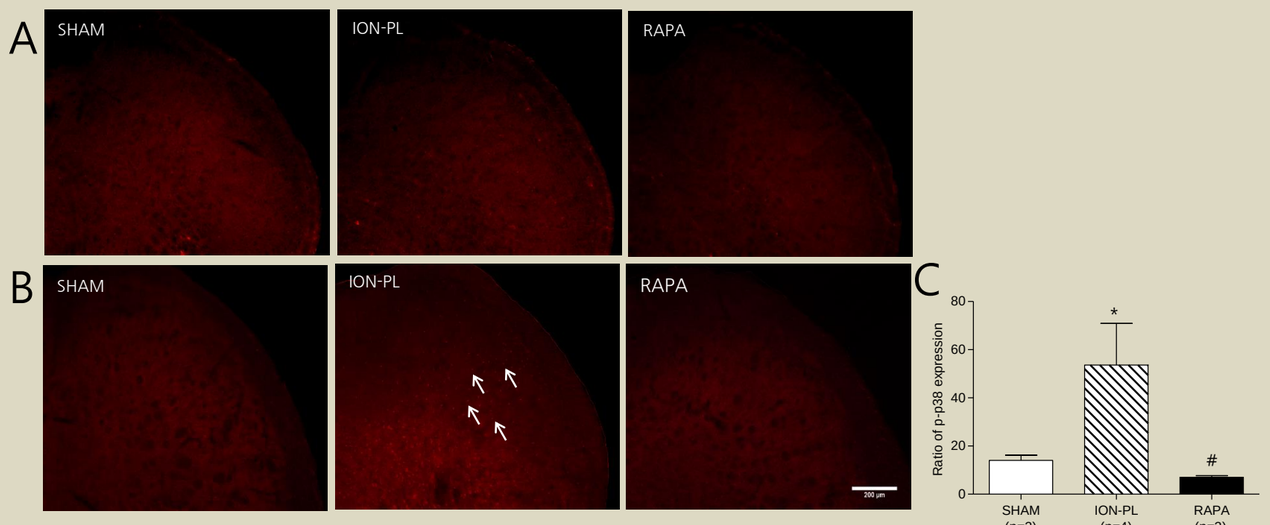


3. Expression of mTOR signaling in TG(A) and TNC(B)

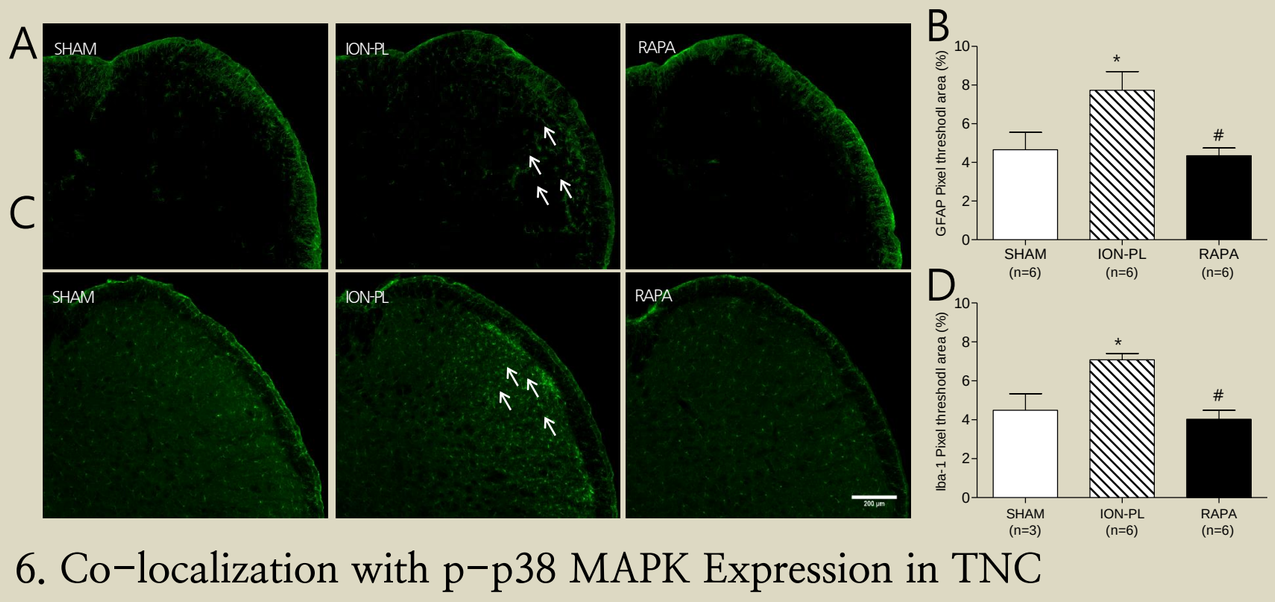




4. Effect of rapamycin on p-ERK(A) and p-p38(B) MAPK in TNC



5. Effect of mTOR inhibition on GFAP(A) and Iba-1(C) in TNC



6. Co-localization with p-p38 MAPK Expression in TNC

4. CONCLUSION

These findings demonstrated that mTOR signaling related changes (the increase of S6 and p-S6) were observed after trigeminal nerve injury, and that the inhibition of mTOR signaling with rapamycin could reduce both mechanical and cold allodynia. Moreover, this anti-allodynic effect of rapamycin was closely associated with the modulation of microglia activation in TNC.