

Cannabinoid receptor type-2 modulates nociceptive signaling molecules in a model of post-concussion headache

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Abstract

The goal of this study was to investigate the role of the cannabinoid receptor type-2 (CB₂R) in the trigeminal pain pathway in a model of post-concussion headache. Sprague Dawley rats were randomized to receive either a repeated mild closed head injury (CHI) or served as incision controls. Changes in CGRP, nNOS, and IBA-1 were assessed in the trigeminal nucleus caudalis (TNC) and trigeminal ganglia via IHC. A subset of CHI rats received either a cannabinoid receptor type 2 (CB₂R) anti-inflammatory agonist (JWH133), an NSAID (Ketorolac), or vehicle and underwent von Frey testing for trigeminal allodynia. An in vitro brain slice study was performed on TNC and cerebrum slices incubated with capsaicin, capsaicin plus a or media control solutions for 24hrs; CGRP and PGE₂ were assessed via ELISA. Repeated CHI showed increases in CGRP and PGE₂, and altered nNOS and IBA-1 immunoreactivity in the trigeminal ganglia and TNC, respectively. JWH-133 blocked capsaicin-induced increases in CGRP and PGE₂ in the TNC and cerebrum slices. Findings show the CB₂R modulates trigeminal pain in a model of concussion, although the mechanisms eliciting analgesia warrant a more in depth investigation.

Introduction

A common symptom of post-concussion syndrome is headache, which can be persistent in a substantial number of patients contributing to a poor quality of life. Compounding this issue is the fact that current treatments are contraindicated or lack efficacy, thus there exists a pressing need to develop novel treatments for post-traumatic headache. Trigeminal pain signaling has been shown to be altered in models of post-traumatic headache via the use of nociceptive and behavioral markers. More specifically traumatic brain injury in animal models has been shown to increase the expression of calcitonin gene related peptide (CGRP). A potential therapeutic target for post-traumatic headache is the endogenous cannabinoid system, with the cannabinoid receptor type-2 (CB₂R) being an ideal target. It is an ideal target due it being devoid of psychotropic properties, involved in nociceptive pathways, and expressed in microglia.

Methods

Model of Traumatic Brain Injury

Mild traumatic brain injury (TBI) was induced in rats using a repeated closed head injury (rCHI) injury model as described previously by our laboratory[1,2,3]. Animals were anesthetized with isoflurane (3% induction; 2-2.5% maintenance). CHI was induced using an electromagnetic stereotaxic impactor (Leica Biosystems) at 3.0 mm depth, 5.0 m/sec at a 40° impact angle to the calvarium. Controls received incision only without injury.

Experimental Design and Outcomes:

Repeated CHI animals were treated with either vehicle control, a CB₂ agonist (JWH133; 5mg/kg), or an NSAID (Ketorolac; 0.4mg/kg). Sensory behavior indicative of headache, trigeminal allodynia was performed using von Frey thresholds (Macolino et al., 2014).

The effects of rCHI were examined through immunohistochemistry for microglia (IBA-1), CGRP, and nNOS comparing repeated CHI to an incision control on either trigeminal nucleus caudalis or trigeminal ganglia, respectively. In vitro brain slice experiments were performed on 300µm slices of TNC and cerebrum. Brain slices were incubated with capsaicin, capsaicin + JWH133, or media control solutions for 24hrs. The media was then collected and levels of CGRP and PGE₂ were assessed with an ELISA.

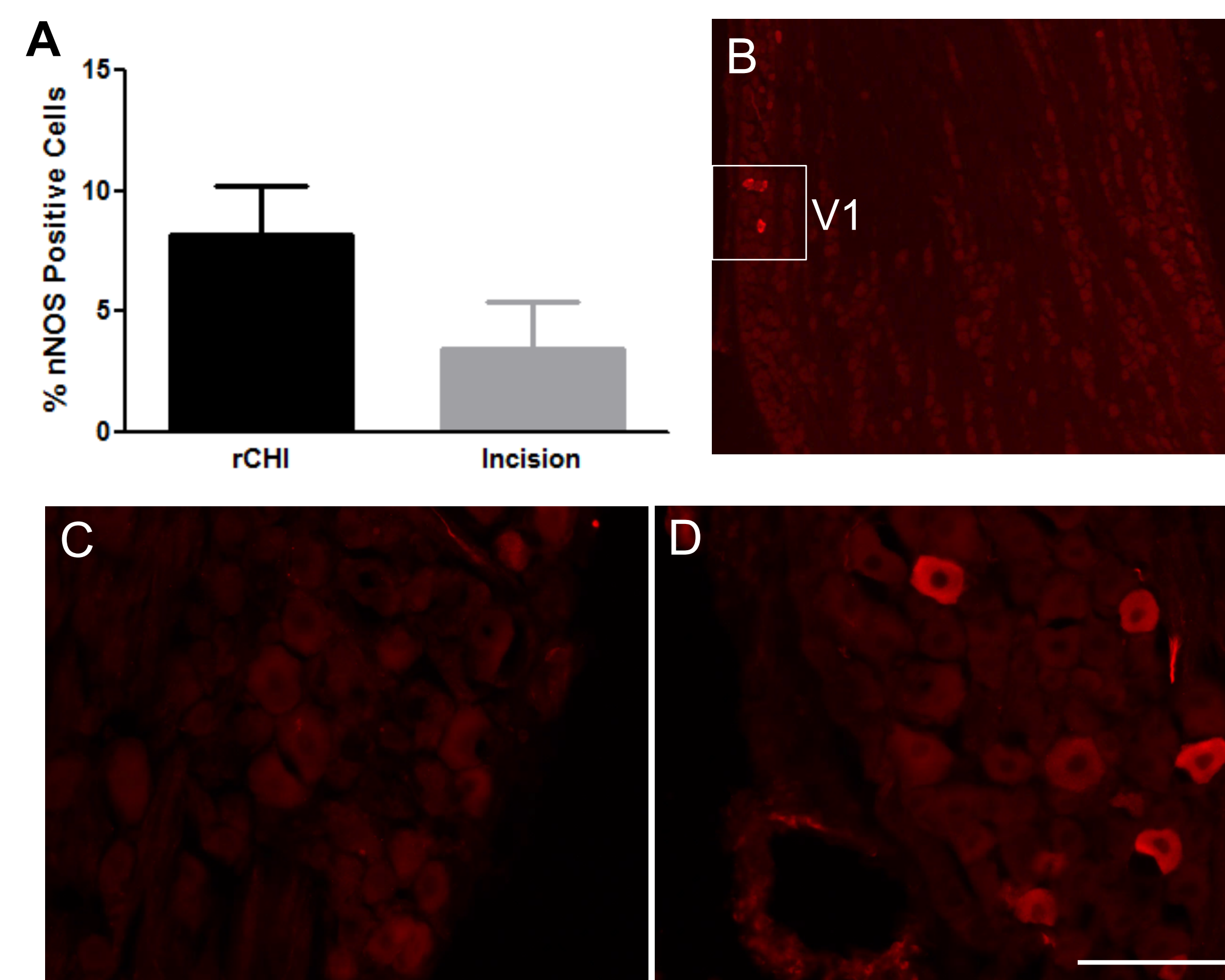


Figure 1. nNOS immunoreactivity in trigeminal ganglia. (A) nNOS positive cells one week post-rCHI injury. (B) Low magnification image with the ophthalmic V1 region of interest (box) indicated. Representative high magnification images in (C) incision control and (D) rCHI rats. Scale bar = 100µm

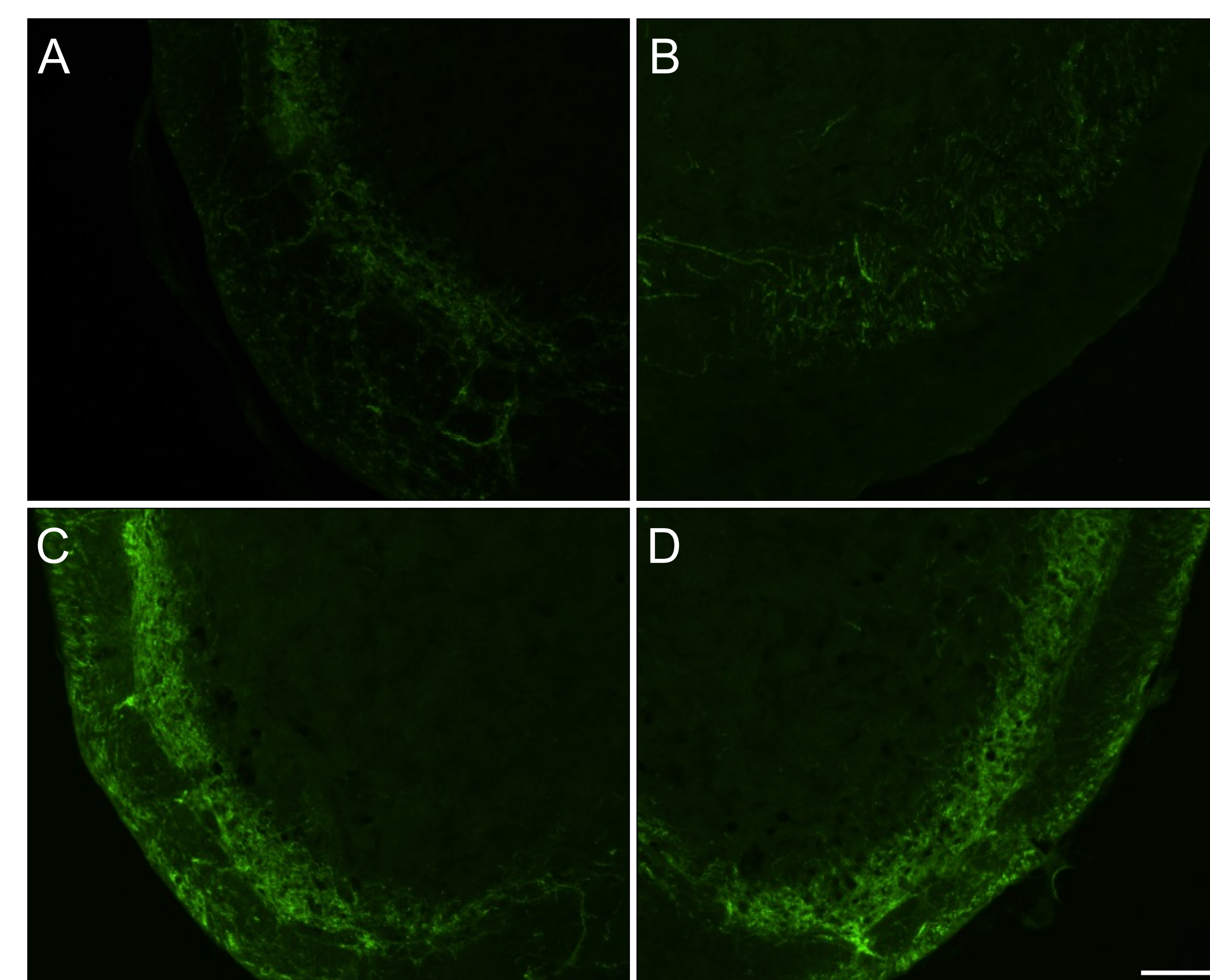


Figure 2: CGRP immunoreactivity in the TNC for (A-B) Incision controls and (C-D) rCHI. Images in anatomical orientation (A,C) left TNC and (B,D) right TNC. Scale bar = 100 µm

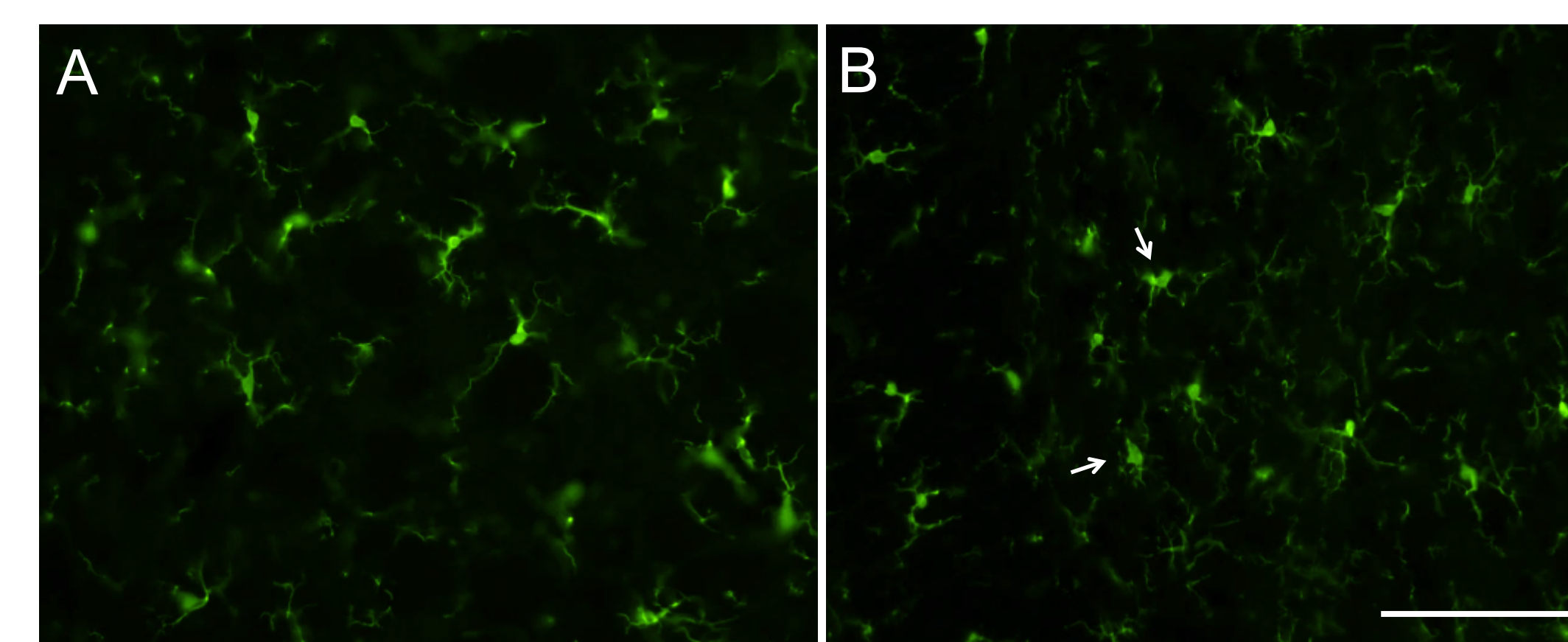


Figure 3. Microglia IBA-1 immunoreactivity in the TNC for rat. (A) Incision control and (B) rCHI. Microglial phenotype changes were observed; ramified microglia with long thin processes seen in control, while rCHI display retracted and/or bushy processes and altered shape of soma denoted with arrow. Scale bar=100 µm

Results

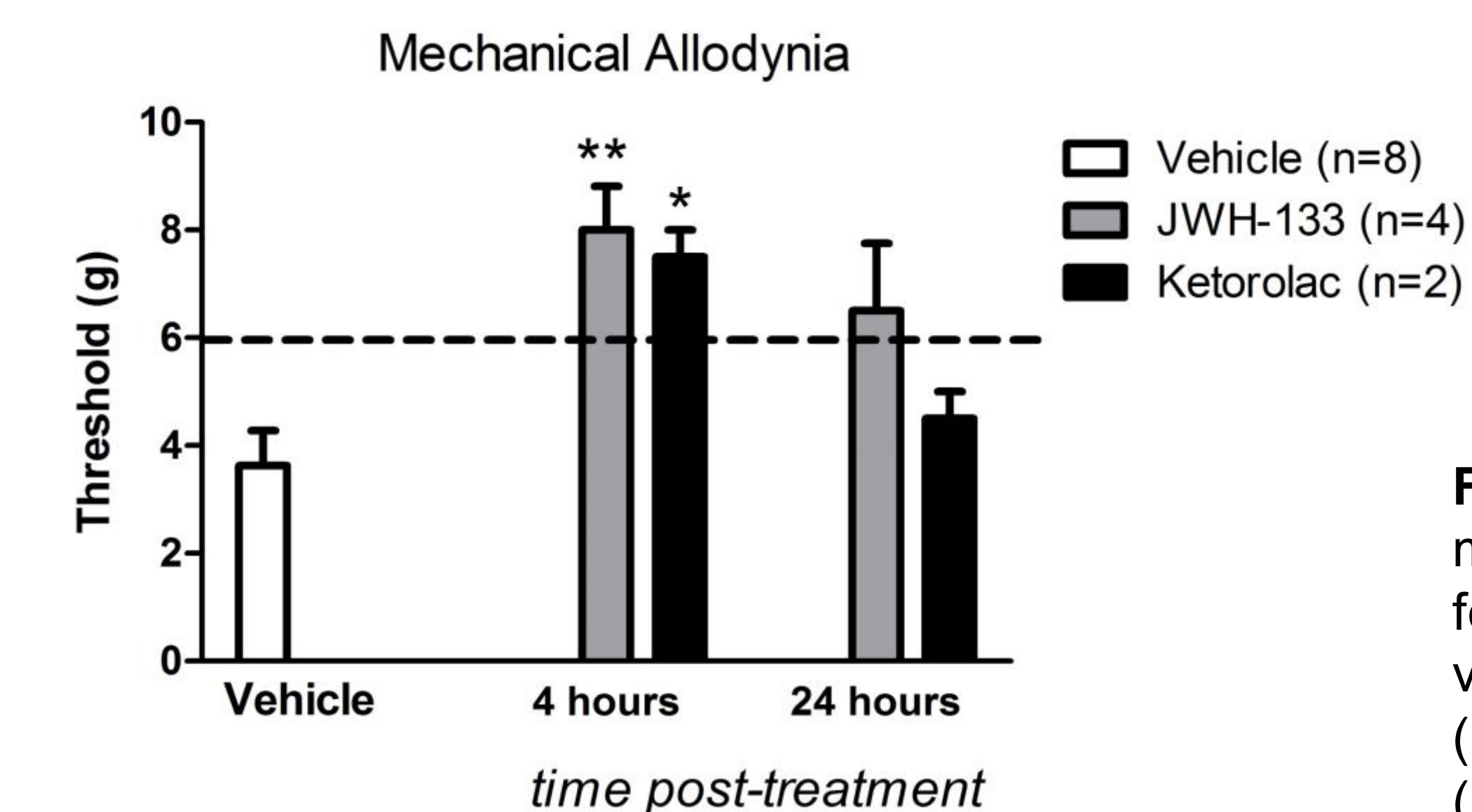


Figure 4: Trigeminal allodynia measured with Von frey filaments for rats with rCHI treated with vehicle control (n=8), JWH133 (5mg/kg) (n=4) and Ketorolac (0.4mg/kg) (n=2). Dashed line represents allodynic thresholds at ≤ 6g. *p<0.05 and **p<0.01

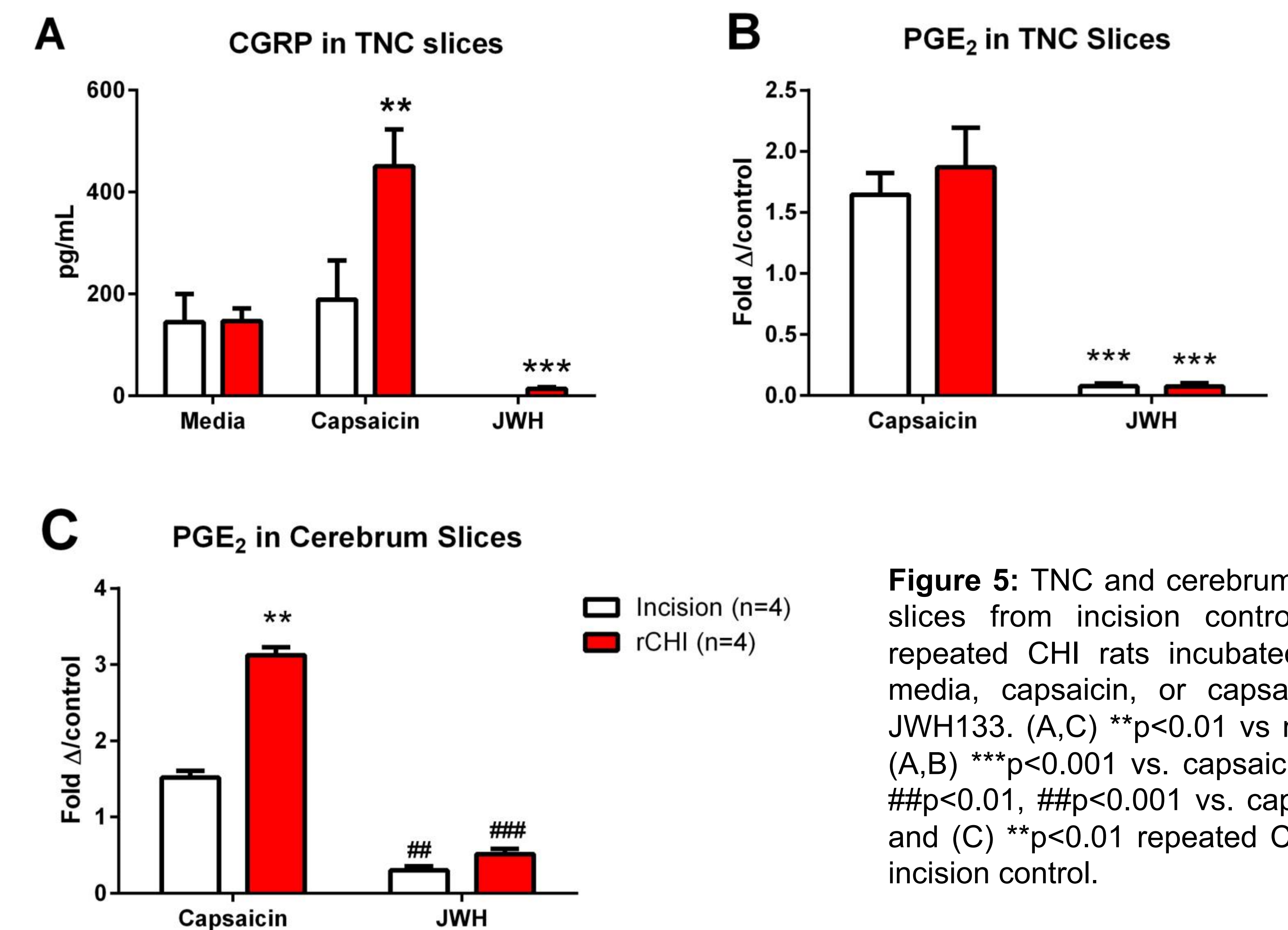


Figure 5: TNC and cerebrum brain slices from incision control and repeated CHI rats incubated with media, capsaicin, or capsaicin + JWH133. (A,C) **p<0.01 vs media, (A,B) ***p<0.001 vs. capsaicin, (C) ##p<0.01, ###p<0.001 vs. capsaicin and (C) **p<0.01 repeated CHI vs. incision control.

Summary and Conclusions

- Increased expression of nNOS and CGRP were observed within the trigeminal pathway, concordant with previous findings in mild focal TBI.
- rCHI microglial phenotype shifts from M1 to M2 morphologically in the TNC.
- Treatment with JWH133 showed a significant decrease in trigeminal allodynia, similar to the NSAID, Ketorolac.
- rCHI injured rats showed increased capsaicin-induced CGRP in TNC slices.
- rCHI injured rats showed increased capsaicin-induced PGE₂ in cerebrum, but not TNC slices, indicating potential for other pain mediators to be important in this pain region.
- A CB₂R agonist, JWH133, blocked capsaicin-induced increases in CGRP and PGE₂ in TNC and cerebrum slices incubated separately.
- CB₂R plays a role in the trigeminal nociceptive pathway, although the mechanisms of analgesia will require more in depth investigation.

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References: 1) Elliott MB et al. Headache. 2012, 2) Amenta et al., J Neuroinflammation 2014, 3) Macolino et al., J Neuroscience Methods, 2014, Russo et al., 2009 Mol Cell Pharmacol