Non-psychotropic cannabinoid based therapy modulates nociceptive signaling molecules, microglia, and pain behavior in a model of post-concussion headache

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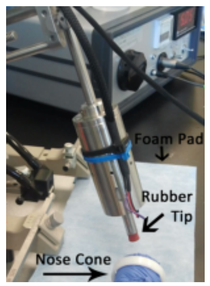
Background & Objectives

Headache is a common symptom of post-concussion syndrome which may persist in a substantial portion of patients contributing to a poor quality of life and disability. There is a pressing need to develop novel treatments for post-traumatic headache as current treatments have a number of side effects, are contraindicated or lack efficacy. The Elliott laboratory first established models of post-traumatic headache demonstrating sensitization of trigeminal neurons in rodent models of post-traumatic headache using well-known nociceptive and behavioral markers in the pain and migraine fields[1-3]. Traumatic brain injury in mice increases expression of calcitonin gene related peptide (CGRP) and inducible nitric oxide synthase (iNOS) in the trigeminal pathway, changes that are accompanied by persistent headache behavior, trigeminal allodynia[1-3].

The endogenous cannabinoid (eCB) system is a potential therapeutic target for post-traumatic headache. In a series of studies, our laboratory showed CB_2R-mediated anti-inflammatory actions in a model of TBI, whereby the reduction of iNOS mRNA and protein and substance P immunoreactivity have implications for headache[4-6]. The cannabinoid receptor type-2 (CB_2R) is an ideal analgesic target as it is devoid of psychoactive properties, shows anti-nociceptive actions upon stimulation, and regulates immune function. What is unclear, is precisely how anti-nociceptive actions are elicited by the CB_2R, and if CB_2R stimulation will be effective at alleviating trigeminal hypersensitivity in model of post-traumatic concussion or closed head injury. The objective of this study was to determine the role of the CB_2R in the trigeminal pain pathway in a model of post-concussion headache, while identifying mechanisms underlying trigeminal pain after mild TBI.

Methods



Model of Traumatic Brain Injury Mild traumatic brain injury (TBI) was induced in Sprague Dawley rats utilizing a mild closed head injury (CHI) injury model using a modified electromagnetic impactor device (Leica Biosystems Inc.). Animals were anesthetized with isoflurane (3% induction; 2-2.5%) and had their body supported on a foam pad to prevent skull fracture. An incision was performed to expose the calvarium and CHI was induced using a 10 mm diameter rubber tip at 5.0 mm depth, 5.0 m/sec at a 40° impact angle to the calvarium. Controls received incision only without injury.

Experimental Design and Outcomes:
At 2 weeks after injury repeated CHI animals (rCHI) were treated two consecutive days with either vehicle control, a CB2 agonist (JWH133; 1 mg/kg), or an NSAID (Ketorolac; 0.4mg/kg). Sensory behavior indicative of headache, trigeminal allodynia was performed using von Frey thresholds and photosensitivity testing[1, 3, 5]. Western blot experiments for proteins were then performed to examine expression of GFAP in the right cortex (ipsilateral to injury). GAPDH protein was used as protein loading control. GFAP was measured using ELISA on serum samples and reported as pg/mL. The effects of CHI were examined through immunohistochemistry labeling astrocytes (GFAP), calcitonin receptor-like receptor, CRLR and inducible nitric oxide synthase (iNOS) in the trigeminal nucleus caudalis (TNC). In vitro brain slice experiments were performed using 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 PGE_2 were assessed with an ELISA.

Results

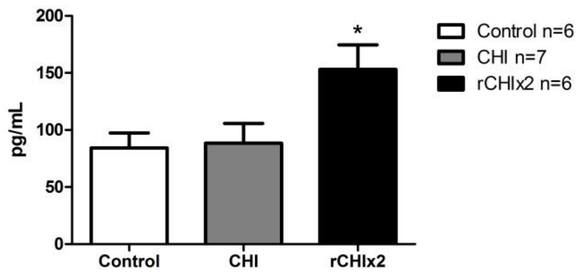


Figure 1: Serum glial fibrillary acidic protein (GFAP). GFAP was measured using enzyme linked immunosorbent assay for incision, single closed head injured (CHI), and repeated (rCHI) rats. RCHI shows an increase in serum GFAP levels compared to incision control, * p<0.05. n=6-7

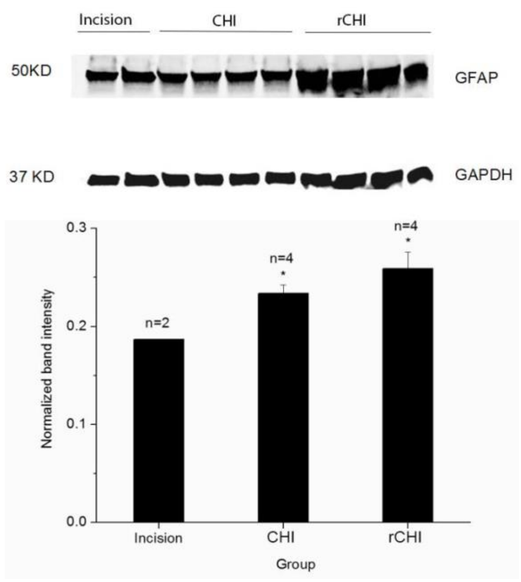


Figure 2. Western blot GFAP expression. GFAP expression for the ipsilateral somatosensory cortex of incision, single closed head injured (CHI), and repeated (rCHIx3) rats. Analysis revealed progressively increased GFAP in CHI (14.5%) and rCHI (48.3%) rats on post-operated day 7 compared with controls; $p < 0.05$; n = 4-6). Results demonstrate that the injury promotes gliosis in the sensory cortex, which is a prominent marker for concussion.

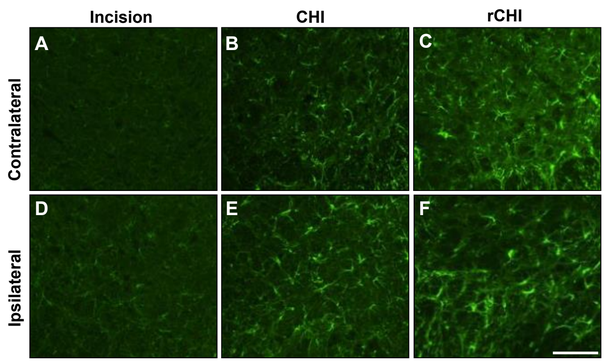


Figure 3: GFAP immunoreactivity in the TNC. GFAP immunoreactivity for (A,D) Incision, (B,E) single CHI, and (C,F) repeated rCHI. Increases in GFAP immunoreactivity can be seen in both CHI and rCHI when compared with incision control. Scale bar = 100µm

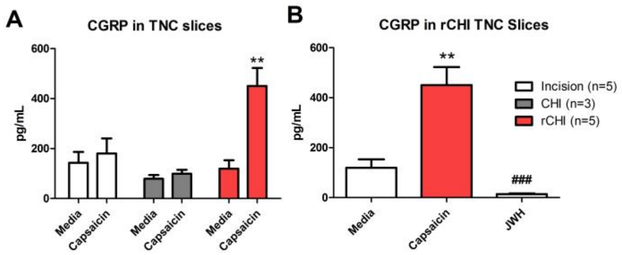


Figure 6: CGRP levels measured by ELISA for TNC brain slices. CGRP levels from incision control and repeated CHI (rCHIx2) rats incubated with media, capsaicin, or capsaicin + JWH133. (A) **p<0.01 repeated CHI vs. Incision and single CHI, (B) **p<0.01 vs. media, (B) ###p<0.001 vs. capsaicin .

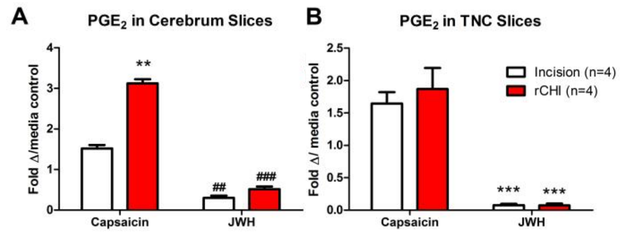


Figure 7: PGE_2 levels measured by ELISA for TNC and cerebrum brain slices. PGE_2 from incision control and repeated CHI rats incubated with media, capsaicin, or capsaicin + JWH133. (A) Cerebrum slices **p<0.01 repeated CHI vs. incision control; ##p<0.01, ###p<0.001 vs. capsaicin, (B) TNC slices ***p<0.001 vs. capsaicin.

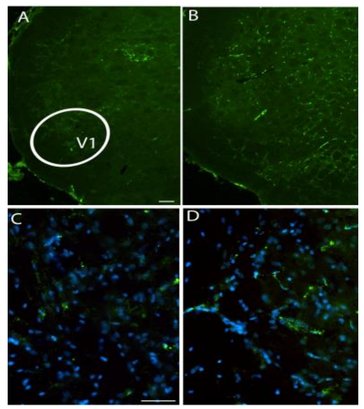


Figure 4: Calcitonin receptor-like receptor, CRLR, immunoreactivity in the TNC. CRLR for (A-C) Incision control and (B-D) rCHIx2. Scale bar = 20µm (A,B) and 50 µm (C,D).

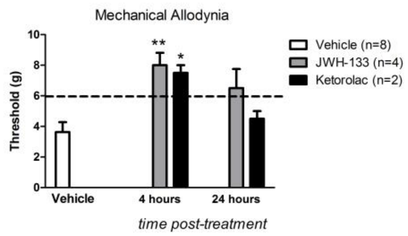


Figure 8: Trigeminal allodynia thresholds (grams). Trigeminal allodynia for rCHI rats treated with vehicle control (n=8), JWH133 (5mg/kg) (n=4) and Ketorolac (0.4mg/kg) (n=2). *Dashed line represents allodynic thresholds at ≤ 6g* *p<0.05 and **p<0.01.

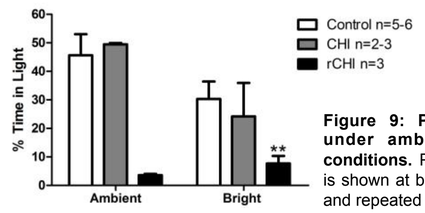


Figure 9: Photosensitivity testing under ambient and bright light conditions. Percentage of time in light is shown at baseline control, single CHI and repeated CHI rats.

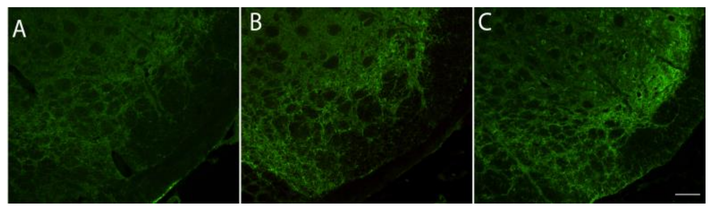


Figure 5: Inducible nitric oxide synthase (iNOS) immunoreactivity in the TNC. Inducible NOS immunoreactivity for incision control (A), single CHI (B), and repeated CHIx3 (C). Noted pattern of iNOS IR appears to be from trigeminal afferents and in the soma of second order TNC neurons. Scale bar = 100µm

Summary and Conclusions

- Repeated CHI increased GFAP in the serum and ipsilateral cortex indicating reactive gliosis. GFAP is currently one of the only utilized biomarkers for patients with concussion.
- Increased expression of GFAP and iNOS were observed within the trigeminal pathway in a graded manner respective to the number of injuries and concordant with previous findings in mild focal TBI.
- CHI altered the calcitonin receptor-like receptor, CRLR, immunoreactivity in the TNC in which a vascular and global response was noted.
- Treatment with JWH133 showed a significant decrease in trigeminal allodynia, similar to the NSAID, Ketolorac. Light testing showed changes in repeated CHI compared to controls that were not seen in single CHI.
- Repeated CHI injured rats showed increased capsaicin-induced CGRP in TNC slices, while CHI did not result in a triggered CGRP increase over controls.
- A CB_2R agonist, JWH133, blocked capsaicin-induced increases in CGRP and PGE_2 in TNC and cerebrum slices incubated separately. CHI injured rats showed increased capsaicin-induced PGE_2 in cerebrum, but not TNC slices, indicating potential for other pain mediators to be important in this pain region.
- CB_2R plays a role in the trigeminal nociceptive pathway, although the mechanisms of analgesia will require more in depth investigation.

References:

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