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Development of CGRP-dependent pain and headache related behaviours in a rat model of concussion: Implications for mechanisms of post-traumatic headache

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Abstract

Background and objective: Posttraumatic headache (PTH) is one of the most common, debilitating and difficult symptoms to manage after a mild traumatic brain injury, or concussion. However, the mechanisms underlying PTH remain elusive, in part due to the lack of a clinically relevant animal model. Here, we characterized for the first time, headache and pain-related behaviours in a rat model of concussion evoked by a mild closed head injury (mCHI) – the major type of military and civilian related trauma associated with PTH – and tested responses to current and novel headache therapies.

Methods: Concussion was induced in adult male rats using a weight-drop device. Characterization of headache and pain related behaviours included assessment of cutaneous tactile pain sensitivity, using von Frey monofilaments, and ongoing pain using the conditioned place preference or aversion (CPP/CPA) paradigms. Sensitivity to headache/migraine triggers was tested by exposing rats to low-dose glyceryl trinitrate (GTN). Treatments included acute systemic administration of sumatriptan and chronic systemic administration of a mouse anti-CGRP monoclonal antibody.

Results: Concussed rats developed cephalic tactile pain hypersensitivity that was resolved by two weeks post-injury and was ameliorated by treatment with sumatriptan or anti-CGRP monoclonal antibody. Sumatriptan also produced CPP seven days post mCHI, but not in sham animals. Following the resolution of the concussion-evoked cephalic hypersensitivity, administration of GTN produced a renewed and pronounced cephalic pain hypersensitivity that was inhibited by sumatriptan or anti-CGRP antibody treatment as well as a CGRP-dependent CPA. GTN had no effect in sham animals.

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Conclusions: Concussion leads to the development of headache and pain-related behaviours, in particular sustained enhanced responses to GTN, that are mediated through a CGRP-dependent mechanism. Treatment with anti-CGRP antibodies may be a useful approach to treat PTH.

Keywords

Cephalic allodynia; conditioned place preference; sumatriptan; Anti-CGRP monoclonal antibody

Introduction

Post-traumatic headache (PTH) is one of the most common and disabling symptoms following a concussion or mild traumatic brain injury, and resembles migraine or tension-type headache most closely (1). Reports suggest that 30–90% of individuals who sustain a concussion will report symptoms of acute PTH (1,2). PTH is defined as secondary headache that develops within seven days of a concussion (3), although longer intervals of headache occurrence have been reported (4,5). A higher prevalence of PTH is observed after a concussion as opposed to more moderate or severe head and brain injuries (1,6). Approximately 1.4 million people suffer a concussion annually in the U.S. alone, and in Europe concussion accounts for 235 per 100,000 hospitalizations (7,8). Therefore, the need for improved understanding of the pathophysiology of PTH is pressing. However, the ability to understand the pathophysiology is limited by the lack of well-characterized and clinically relevant animal models with strong predictive validity. Few studies exist on PTH in rodents, and the majority of current rodent models involve penetrative brain injuries such as those induced by the controlled cortical impact injury (CCI) and lateral fluid percussion injury models (9,10). However, as the majority of PTH cases are an outcome of closed head traumas (11), the development of a PTH rodent model that mimics this clinical scenario would facilitate further research in this area.

The present study employed a modified version of a previously developed concussion model in the rat that involves a mild closed head injury (mCHI) (12) in order to characterize putative PTH-related behaviors. We hypothesized that mCHI in rats will result in the development of a pain-related behavioral phenotype characterized by evoked and non-evoked nociceptive responses that would be indicative of headache/migraine. Patients who suffer from PTH commonly report decreased activity levels, due to exercise-induced exacerbation of headache, cranial hyperalgesia, and frequent headache attacks (13,14). Thus the initial aim was to perform an extensive post-injury behavioural characterization including assessment of spontaneous locomotor and exploratory activities, pericranial tactile hypersensitivity and investigation of the aversive state of pain using the conditioned place preference (CPP) paradigm, both of which have been previously proposed as pain-like behaviors in rodent headache and other pain models (15–17).

PTH usually resolves within 1–3 months following head trauma, but may become chronic in some individuals and persist for more than three months (18). The development of chronic PTH remains poorly understood, but may involve increased sensitivity to common headache triggering factors. We therefore tested the hypothesis that animals subjected to mCHI will

also develop a prolonged hypersensitivity to the headache and migraine trigger glyceryl trinitrate (GTN) (19,20).

Current migraine and headache treatment guidelines include 5-HT_{1B/1D} agonists (i.e. triptans), but in many patients these agents do not provide sufficient pain relief (21). Recent advances in the treatment of acute and chronic headache are focused on targeting the actions of the sensory neuropeptide calcitonin gene-related peptide (CGRP), in particular by employing a blocking monoclonal antibody (mAb) approach (22,23). We hypothesized that acute and chronic headache/pain related behaviors, observed following mCHI, would be attenuated by either an acute therapy with an established headache treatment (i.e. sumatriptan) and/or by a novel chronic anti-migraine therapy regimen (i.e. anti-CGRP mAb).

Materials and methods

Animals

All experiments were approved and conducted in compliance with the institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical Centre and Harvard Medical School and were in compliance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (24). A total of 128 male Sprague-Dawley rats (Taconic, USA), weighing 220–250 g at time of arrival, were used for the study. Animals were housed in pairs under a constant 12 hour light/dark (lights on at 07:00) cycle at room temperature. Food and water were available *ad libitum*. Experimental animals (n = 6–8 per group) were randomly assigned to either sham or mCHI groups as well as to the different treatment groups. Responses to different treatment were studied in a blinded fashion.

Experimental mild closed head injury

Experimental mild closed head injury (mCHI) was induced using the weight-drop concussive device as described previously (12,25). Briefly, rats were anesthetized with 3% isoflurane and placed chest down directly under a weight-drop concussive head trauma device. The device consisted of a hollow cylindrical tube (inner diameter 2.54 cm) placed vertically over the rat's head. To induce a head trauma, a 250 g weight was dropped through the tube from a height of 80 cm, striking the center of the head. To ensure consistency of hit location, animals were placed under the weight drop apparatus so that the weight struck slightly anterior to the center point between the ears. A foam sponge (thickness 3.81 cm, density 1.1 g/cm³) was placed under the animals to support the head while allowing some anterior-posterior motion without any rotational movement at the moment of impact. Immediately after the impact animals were returned to their home cages for recovery. All animals regained consciousness within two minutes of injury and were neurologically assessed in the early hours and days post-injury for any behavioral abnormalities suggestive of neurological impairment. Sham animals were anesthetized but not subjected to the weight drop. All animals subjected to PTH behavioral assessments did not display any major neurological deficits.

Behavioral testing

Open field monitoring system.—Activity Monitor SOF-811 (Med Associates, Vermont, USA) was used to measure locomotor activity in an open field environment. The system consists of a two areas setup and evaluates the movement of animals in the horizontal (X-Y axis) and vertical (Z-axis) planes. Each plane was monitored by 16 beams spaced 2.54 cm apart, and there was an infrared emitter and detector per beam located on each side of each arena to monitor movement across the width of the arena. Data were sent from the three sets of detector-emitters to a central computer displaying a range of pre-selected outputs, including total distance moved and vertical activity (rearing) during 60-second intervals, and were analysed as a total over 20 minutes. Each arena was lit with a single white LED bulb on a dimmer switch to maintain a homogenous lighting across the arenas (80 lux). The arenas were cleaned with mild detergent and dried to remove odour cues between successive rats. Testing was conducted at baseline (24 hours prior to head trauma) and then at 48 hours, 72 hours, seven and 14 days post mCHI.

Novel object recognition.—The novel object recognition test is designed to evaluate deficits in recognition memory in rodents, as a measure of the severity of the brain injury. The procedure used for the novel-object recognition test was similar to that described previously (26,27), with some modifications. The apparatus consisted of a Perspex arena (45 cm²). The “familiar” objects used were two identical shapes composed of Lego DUPLO building blocks. A third object, the “novel object”, consisted of a plastic bottle covered with green tape. Animals were habituated to the arena in the absence of objects for 30 minutes on the day before the test day. The test day comprised three stages: Habituation (five minutes’ exposure to the arena in the absence of objects), exposure 1 (five minutes’ familiarization with the identical objects) and exposure 2 (five minutes’ exposure to one familiar and one novel object). Habituation and exposure 1 were separated by a 5 minute inter-trial interval and exposures 1 and 2 by a further five minute interval. Exploration of an object was defined as any behavior directed toward it (i.e. sniffing, rearing, leaning or climbing). The three test stages were recorded for subsequent analysis, and object exploration was manually rated in a blind manner. The discrimination ratio for each object was calculated as time spent exploring either object/time spent exploring both objects. A Preference Index above 0.5 (i.e. 50%) indicates novel object preference, below 0.5 indicates familiar object preference, and 0.5 itself indicates no preference.

Development of tactile pain hypersensitivity using Von Frey filaments.—The method used was previously used to study headache-related behaviors (28–30) and was also recently employed in our laboratory (31). Briefly, animals were placed in a transparent flat-bottomed acrylic holding apparatus (20.4 cm × 8.5 cm). The apparatus was large enough to enable the animals to escape the stimulus. Animals were habituated to the arena for 15 minutes prior to testing. In order to determine if the animals developed pericranial tactile hypersensitivity following mCHI, the skin region, including the midline area above the eyes and 2 cm posterior, was stimulated with different von Frey (VF) filaments (0.6 g–10 g) (18011 Semmes-Weinstein Anesthesiometer kit). Development of hindpaw hypersensitivity was tested by stimulating, using the VF filaments, the mid-dorsal part of the hindpaw. Changes in tactile skin sensitivity were evaluated similarly to previous studies in our

laboratory (32) by recording four behavioral responses adapted from Vos et al. (33), as follows: 0) *No response*: Rat did not display any response to stimulation 1) *Detection*: Rat turned its head towards the stimulating object and the latter is explored, usually by sniffing; 2) *Withdrawal*: Rat turned its head away or pulled it briskly away from the stimulating object (usually followed by scratching or grooming of the stimulated region); 3) *Escape/Attack*: Rat turned its body briskly in the holding apparatus in order to escape stimulation or attacked (biting and grabbing movements) the stimulating object. Starting with the lowest weight, each filament was applied three times with an intra-application interval of five seconds, and the behavior that was observed at least twice was recorded. For statistical analysis, the score recorded was based on the most aversive behavior noted. The force that elicited three consecutive withdrawal responses was considered the response threshold. To evaluate pain behavior in addition to changes in threshold, for each rat, at each time point, a cumulative response score was determined by combining the individual scores (0–3) for each one of the VF filaments tested. All tests were conducted and evaluated in a blinded manner. Responses to von Frey stimuli were tested at baseline and also at 48 hours, 72 hours as well as at seven and 14 days post mCHI.

Conditioned place preference using sumatriptan.—The method employed was adapted from previously published reports (34). Two CPP boxes (Med Associates, Vermont, USA), consisting of two separate chambers, were used to assess chamber preference before and after the drug-conditioning phase. Chambers were discriminated between based on visual (walls) and tactile (floors) cues. Sumatriptan was chosen because it has previously demonstrated efficacy with CPP in a pre-clinical migraine model (16). On the pre-conditioning day (day 6 post mCHI), sham or mCHI rats were placed into CPP boxes with access to both chambers for a period of 20 minutes and time spent in each chamber was recorded. CPP data were analyzed only from animals that spent >30% of their time in one of the chambers during the preconditioning day. A total of five animals were excluded. On the morning of the conditioning day (day seven post mCHI), animals received a vehicle control (saline i.p.) and two hours later were confined to the vehicle-paired chamber for 20 minutes. In the afternoon, four hours after vehicle injection, animals received sumatriptan and two hours later were confined to the opposite chamber (the sumatriptanpaired chamber) for 20 minutes. A two hour period was chosen between administration of sumatriptan and chamber confinement, as data suggests that optimal efficacy of sumatriptan is observed two hours post administration (35). Twenty four hours later (day 8 following mCHI), animals were placed into the CPP apparatus with free access to both chambers for 15 minutes, and time spent in each chamber was recorded.

GTN-evoked tactile pain hypersensitivity.—Low dose GTN (100 µg/kg) was administered on day 15 and 30 post mCHI to investigate if any increased susceptibility to a common headache/migraine trigger existed in mCHI animals following resolution of the cephalic pain hypersensitivity two weeks post mCHI. Development of pericranial and hindpaw mechanical hypersensitivity was assessed as above at one hour and four hours post GTN administration. Acute administration of sumatriptan, or chronic blockade of CGRP action using a mouse anti-CGRP mAb, were investigated for their ability to attenuate the GTN-induced pain hypersensitivity.

GTN induced conditioned place aversion.—Conditioned place aversion to GTN was tested using the two CPP boxes as indicated above. The protocol included a pre-condition day (day 13 post mCHI), followed by a conditioning day (day 14) and a post conditioning day 24 hours later. On the morning of the conditioning day, animals were first confined for 20 minutes to one chamber, prior to GTN administration (pre-GTN-paired chamber). Animals were then administered with GTN and four hours later were confined to the opposite chamber (GTN-paired chamber) for 15 minutes. On the postconditioning day animals again had free access to both chambers. Aversion to GTN was determined by calculating the difference in scores between the times spent in the different chambers during the preconditioning and post-conditioning days for animals treated with the anti-CGRP mAb or the isotype control IgG.

Drugs

Sumatriptan (Sigma, USA) was freshly dissolved in 0.9% saline and administered intraperitoneally (i.p.) at a dose of 1 mg/kg in a volume of 1 ml/kg. All behavioural testing was conducted two hours after sumatriptan administration. Drug dose and times of administration were based on the pharmacokinetics of the drugs as well as in-house pilot work and published studies demonstrating their efficacy in animal models of trigeminal pain (9,36). A mouse anti-CGRP mAb and a corresponding isotype control IgG were provided by Teva Pharmaceuticals and were administered i.p. at a dose of 30 mg/kg in a volume of 0.54 ml/100 g. Mouse antibody was used in this study to ensure no immune response would be mounted against the drug during the chronic dosing regimen. Isotype control antibody was used to ensure any effects of the anti-CGRP antibody are not attributable to the effector functions of the Fc region. The first administration was delivered immediately after mCHI induction and every six days subsequently. GTN (American Reagent, USA) was freshly dissolved in 0.9% saline and administered i.p. at a dose of 100 µg/kg in a volume of 1 ml/kg. The final vehicle concentration for the GTN was 0.6% propylene glycol, 0.6% ethanol and 0.9% saline. Previous work has shown no effect on mechanical thresholds with 6% propylene glycol and ethanol vehicle concentration (37).

Data analysis

Data are presented as mean + standard error of the mean. Statistical analyses were conducted using Statview (SAS Institute, New York, NY, USA). Normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene tests, respectively. Two-way repeated measures analysis of variance (ANOVA) was performed to determine the main effects of time, head trauma, and drug treatment, or their interaction, on locomotor activity in the open field, or the development of pericranial hypersensitivity using the von Frey apparatus. Bonferroni *post hoc* test or Student's unpaired 2-tailed t-tests were used, as appropriate, to assess differences between the groups and across time points. CPP/aversion data were analyzed using Mann-Whitney U test; $p < 0.05$, or lower, based on Bonferroni corrected values, were considered statistically significant.

Results

Reduced vertical rearing activity but no evidence of cognitive deficits in mCHI/concussed animals

Following mCHI, animals displayed reduced vertical exploratory (rearing) activity in the open field (RM ANOVA time: $F_{(4, 56)} = 7.03$, $p < 0.01$, injury $F_{(1, 14)} = 21.31$, $p < 0.01$). Student's unpaired two-tailed t-tests revealed that the mCHI group displayed consistently decreased vertical exploratory activity compared to the sham group at 48 hours, 72 hours and day seven post mCHI ($p < 0.01$) (Figure 1a), indicating mild traumatic brain injury induced deficits in exploratory behaviour (39). At these time points, however, no changes were detected in total distance travelled, centre zone exploration or thigmotaxis (all $p > 0.05$, data not shown). There was also no evidence of major cognitive deficits in mCHI animals at seven days post-injury as measured by the novel object recognition test ($p > 0.05$) (Figure 1b).

Development of tactile hypersensitivity in mCHI animals

Using von Frey filaments, we identified a time-dependent development of cephalic tactile hypersensitivity in mCHI, but not in sham animals. Repeated measures ANOVA revealed a significant effect of time ($F_{(4, 48)} = 7.54$, $p < 0.001$) and a time x injury interaction ($F_{(4, 48)} = 5.71$, $p < 0.001$). Student's unpaired two-tailed t-tests revealed that response thresholds were significantly reduced and nociceptive scores in the mCHI group were significantly increased at 72 hours and day seven (both $p < 0.05$) compared to the sham group (Figure 2a and 2b). mCHI did not lead to development of hindpaw tactile hypersensitivity at any of the time points tested (Figure 2c and 2d).

Effects of acute sumatriptan treatment and chronic CGRP blockade on mCHI-induced cephalic tactile hypersensitivity

Overall, acute sumatriptan treatment at 72 hours post-CHI attenuated cephalic tactile hypersensitivity. (Oneway ANOVA $F_{(2, 17)} = 8.79$, $p < 0.05$) (Figure 3a and 3b). We further tested the ability of chronic blockade of CGRP using injections of a blocking mAb or isotype control IgG, starting immediately after mCHI and every six days subsequently, on the development of mCHI-related cephalic tactile hypersensitivity. Overall, CGRP blockade attenuated mCHI-induced tactile hypersensitivity (RM ANOVA time: $F_{(3, 39)} = 17.76$, $p < 0.001$). Student's unpaired two-tailed t-tests revealed that the anti-CGRP mAb treated group had a significantly increased response threshold and reduced nociceptive score compared to the isotype control IgG-treated group at day seven post mCHI (Figure 3c and 3d, $p < 0.05$). Chronic treatment with the anti-CGRP mAb did not have any effect on the tactile response threshold in sham animals (data not shown), in agreement with the current finding of Kopruszinski et al., who also employed an anti-CGRP mAb (38).

Sumatriptan-related conditioned place preference in mCHI animals

To investigate the aversive nature of PTH pain in mCHI animals, we used the CPP paradigm to determine whether the anti-migraine drug sumatriptan could produce CPP in mCHI rats but not in sham controls at seven days post-head injury. Difference scores (post-conditioning

– pre-conditioning time difference for each chamber) for individual rats indicate that mCHI rats displayed significantly increased time ($p < 0.01$) in the sumatriptan chamber compared to sham controls (Figure 4b), suggesting that systemic sumatriptan treatment alleviated the aversive, headache-like effect of mCHI.

Development of tactile hypersensitivity following administration of GTN to asymptomatic mCHI animals

By day 14 post-mCHI, no significant differences in cephalic tactile sensitivity existed between sham and mCHI groups. To determine if rats subjected to mCHI developed enhanced tactile pain sensitivity to a headache/migraine-triggering event, following the resolution of the acute behavioural symptoms, we tested changes in cephalic and extra-cephalic responses to mechanical stimulation following the administration of the headache/migraine trigger GTN on days 15 and 30 post mCHI. On day 15, administration of GTN resulted in renewed and pronounced cephalic tactile hypersensitivity in mCHI animals (RM ANOVA time: $F_{(1,14)} = 38.68$, $p < 0.001$, time $\times$ GTN treatment $F_{(2,14)} = 5.18$, $p < 0.05$) but not in sham animals. Bonferroni *post-hoc* tests revealed that the mCHI group displayed a significantly reduced cephalic response threshold and increased nociceptive score at one hour ($p < 0.01$ and $p < 0.05$ respectively) and at four hours ($p < 0.001$ and $p < 0.01$ respectively) post GTN when compared to sham controls (Figure 5a and 5b). On day 30 post mCHI, GTN also produced pronounced cephalic hypersensitivity (RM ANOVA treatment: $F_{(1,13)} = 9.7$, $p < 0.05$, time $\times$ GTN treatment: $F_{(2,26)} = 3.24$, $p < 0.05$). Bonferroni *post-hoc* tests revealed that the mCHI group displayed a significantly reduced cephalic response threshold at one hour and four hours post-GTN (Figure 5c, $p < 0.05$ for both) compared to sham controls, and an increased nociceptive score at four hours post-GTN (Figure 5d, $p < 0.05$).

As Figure 6 depicts, GTN administration also induced hindpaw tactile hypersensitivity. That was, however, limited to reduced thresholds, selectively in mCHI animals on day 15 only (RM ANOVA time: $F_{(2,26)} = 8.27$, $p < 0.01$, and GTN treatment: $F_{(1,14)} = 8.12$, $p < 0.05$). *Post-hoc* analyses revealed that the mCHI group displayed significantly reduced mechanical thresholds at four hours post GTN (Figure 6a, $p < 0.05$) on day 15. On day 30 there was no difference between the sham and mCHI groups, neither at one hour nor at four hours post GTN.

Effects of acute sumatriptan treatment and chronic CGRP blockade on GTN-evoked tactile hypersensitivity in mCHI animals

On day 15 post mCHI, acute sumatriptan treatment attenuated the GTN-evoked delayed tactile cephalic hypersensitivity (at four hours) when administered 1 h after GTN. Student's unpaired two-tailed *t*-tests revealed that acute sumatriptan treatment significantly reduced both the decrease in response threshold and the increase in nociceptive score compared to vehicle treated animals (Figure 7a and 7b, $p < 0.001$ and $p < 0.05$ respectively.). When compared to vehicle, sumatriptan treatment did not attenuate the GTN-evoked hindpaw hypersensitivity in mCHI animals (data not shown). Chronic treatment with the anti-CGRP mAb prevented GTN-induced cephalic tactile hypersensitivity on day 15 post-mCHI. Student's unpaired two-tailed *t*-tests revealed that the anti-CGRP mAb group displayed

significantly increased response thresholds and reduced nociceptive scores compared to the isotype control IgG group (Figure 7c and 7d, $p < 0.01$, $p < 0.05$ respectively). When compared to treatment with the control IgG, anti-CGRP mAb treatment was also effective at attenuating the GTN-induced reduced cephalic mechanical thresholds at 30 days post-injury ($p < 0.05$, Student's unpaired two-tailed t-tests, data not shown). Anti-CGRP mAb treatment, however, was ineffective in blocking the GTN-evoked hindpaw hypersensitivity in mCHI animals (data not shown).

Effect of chronic CGRP blockade on conditioned place aversion to GTN

We finally tested, using the conditioned place aversion paradigm, whether GTN administration following mCHI could induce ongoing pain-like behavior that is responsive to anti-CGRP mAb treatments. Administration of GTN to mCHI animals on day 14 post mCHI resulted in a conditioned place aversion in animals treated with the control IgG (Figure 8b, $p < 0.05$), suggesting GTN-evoked pain in mCHI animals at a time when the cephalic pain hypersensitivity was already resolved. There was no evidence of a similar GTN-evoked conditioned place aversion in mCHI animals treated with the anti-CGRP mAb (Figure 8b), suggesting that GTN-evoked pain in mCHI animals is CGRP-dependent.

Discussion

The present study employed an experimental rat model of concussive closed head injury, the most common type of trauma associated with PTH (11), to investigate the development of headache and pain related behaviors, as well as their responses to currently used headache abortive medications (sumatriptan) and an emerging biological treatment currently in testing for headache prevention (anti-CGRP mAb). The main findings of the study are: 1) Concussion via mCHI was associated with an acute phase of reduced rearing (exploratory activity), but not with major motor or cognitive deficits, suggesting a mild transient form of traumatic brain injury (39); 2) Concussed animals developed cephalic tactile pain hypersensitivity that was resolved by two weeks post-injury, and which was attenuated by acute treatment with sumatriptan and prolonged inhibition of CGRP via a blocking mAb; 3) Animals subjected to mCHI displayed spontaneous/ongoing pain-like behavior in the CPP paradigm when using systemic sumatriptan treatment as an analgesic treatment; 4) Following resolution of the headache/pain behaviors, administration of the headache/migraine trigger GTN resulted in a renewed and pronounced cephalic hypersensitivity as well as conditioned place aversion that was inhibited by sumatriptan and anti-CGRP mAb treatments.

Development of cephalic cutaneous tactile hypersensitivity following mCHI

Cephalic cutaneous allodynia is a common feature of migraine headache (40–43), and has also been reported in PTH patients (13). In our study, rats subjected to concussive mCHI developed cephalic cutaneous tactile pain hypersensitivity that was resolved by two weeks post-injury. The underlying mechanism of cephalic allodynia in PTH is not known, but is likely to involve a peripheral mechanism given the lack of extra-cephalic hypersensitivity in PTH patients (13). In migraine, cephalic tactile allodynia is thought to be a consequence of inflammatory-related activation and sensitization of meningeal afferents and the ensuing

sensitization of central trigeminal dorsal horn neurons, which receive their input from meningeal as well as the scalp and skin afferents (40,41). The finding that triptan treatment, which has been shown to attenuate migraine-related cephalic hypersensitivity in animals (42) and to inhibit its development in humans (43), ameliorated the cephalic pain hypersensitivity in mCHI animals suggests a link between this behavior and the headache of PTH, in particular PTH with migrainous features. In our previous studies, using a similar model of mCHI in mice, we documented trauma-related inflammatory process within the cranial meninges (44) and the calvarial periosteum (45). Periosteal inflammation can lead to the activation of periosteal afferents and, similarly to meningeal inflammation, to cephalic pain hypersensitivity (31). Chronic periosteal inflammation was also documented in chronic migraine with cephalic hypersensitivity (46). While further work is needed in order to determine the extent of the injury to the meningeal and periosteal tissues in the present rat mCHI model, our previous finding in a mouse mCHI model, together with the current behavioural data, including the lack of extra-cephalic hypersensitivity, point to the possibility that trauma-related peripheral cephalic changes, in particular ongoing activation of meningeal and potentially calvarial periosteal afferents, play an important role in mediating the pain behavior in mCHI animals and potentially the allodynia in PTH patients. It should be noted that cephalic tactile hypersensitivity was also reported previously in two rat models of penetrative brain injury (9,10). These models, however, also involved a craniotomy, which in itself damages the calvarial periosteum and cranial meninges and thus could have led to the cephalic allodynia in these studies (22). To our knowledge, the present study is the first to report the development of cephalic tactile hypersensitivity in a mild traumatic brain injury (concussion) model, which does not involve a craniotomy. This is an important consideration given that mCHI, rather than penetrative brain injuries, is the main trauma associated with PTH (1).

Development of spontaneous pain following mCHI

Not all PTH patients experience allodynia, and a major component of PTH is non-evoked continuous pain, which resembles migraine or TTH in characteristics (14). Thus it is important to investigate and characterise the multidimensional nature of PTH pain in order to better understand its pathophysiology and to improve the translational validity of animal models for this condition. We employed the CPP paradigm, which has been adapted to the pain field, under the premise that relief from pain is intrinsically rewarding (47). Our finding of sumatriptan-induced CPP in mCHI animals, but not in sham controls, strongly suggests that the current head trauma model is associated with triptanresponsive ongoing/spontaneous headache pain that is perceived as aversive. The previous finding of sumatriptan-induced CPP in a pre-clinical headache model, involving application of inflammatory mediators to the dura mater (16), further supports the notion that mCHI gives rise to spontaneous headache, migraine-like pain.

Development of prolonged hypersensitivity to the headache/migraine trigger GTN following mCHI

A key finding from the current study is that following the resolution of the mCHI-evoked cephalic hypersensitivity, systemic administration of GTN resulted in renewed and pronounced cephalic tactile hypersensitivity in concussed animals, but had no effect on sham

controls. The development of trauma-related persistent hypersensitivity to GTN in animals subjected to mCHI may be of great importance to the understanding of the mechanisms underlying chronic PTH; it points to the possibility that head trauma gives rise to a migraineous or tension-type headache phenotype in patients, including an exquisite sensitivity to common triggers (48,49). Whether this mCHI-related hypersensitivity is unique to GTN or also exists for other headache/migraine triggers, whether endogenous or exogenous, remains to be determined. The enhanced response to GTN observed in mCHI animals is reminiscent of the earlier finding of Oshinsky and Gommonchareonsiri, who showed enhanced GTN-evoked cephalic allodynia in animals subjected to chronic administration of inflammatory mediators to the meninges, a model of chronic migraine (28). The prolonged hypersensitivity to GTN in mCHI animals also resembles the phenomenon of hyperalgesic priming documented in the rat's hindpaw (50), in which an initial local inflammatory response results in a subsequent state of localized increased susceptibility to noxious chemical stimuli lasting several weeks beyond the initial acute injury. Whether the heightened responses to GTN in mCHI animals are mediated through similar mechanisms will require further studies. Another important observation was that while mCHI in itself did not provoke hindpaw hypersensitivity, such extra-trigeminal pain hypersensitivity developed in mCHI animals following GTN administration, although it was less robust than the evoked cephalic hypersensitivity. Importantly, while the GTN-evoked cephalic hypersensitivity was ameliorated by pre-treatment with sumatriptan, or chronic treatment with the anti-CGRP mAb (see also below) the GTN-evoked hindpaw hypersensitivity did not respond to these treatments, suggesting the heightened response seen at the hindpaw is mediated by a different mechanism than that which underlies the cephalic hypersensitivity or the conditioned place aversion to GTN.

Effect of chronic treatment with anti-CGRP mAb on PTH-related behaviors

Currently, there are no evidence-based treatment guidelines for PTH management, thus it is often treated similarly to the primary headache disorder it resembles, with studies showing the efficacy of sumatriptan in some PTH patients with migraineous features (51–53). However, because a significant proportion of patients do not respond to triptan treatment (21), other therapy options are needed. Based on the hypothesized role of CGRP in migraine and headache (54,55), a promising emerging therapy strategy targets CGRP or its receptor using blocking mAbs (56). Phase IIb trials of one such mAb, TEV-48125, have demonstrated favorable results in terms of safety, efficacy and tolerability of this agent (22,57). Because PTH may share pathophysiological mechanisms with migraine (58), CGRP may also play a role in PTH. The results from the current study indicate that inhibiting CGRP, by using a mouse anti- CGRP mAb, can reduce the headache and pain-related symptoms evoked by mCHI, suggesting the involvement of CGRP in mediating PTH pain. It is, however, noteworthy that the anti-CGRP mAb treatment was not effective in inhibiting the cephalic hypersensitivity at 72 hours post mCHI, while acute sumatriptan treatment was effective at that time point. These findings point to the possibility that at the 72 hour post-mCHI time point, this headache-like behavior is mediated by other mediators apart from CGRP. Alternatively, it is possible that in this model CGRP is acting rather as a neuromodulator, and any effects of its sequestration by the mAb may take time to become apparent, hence the lack of efficacy at 72 hours post mCHI.

Treatment with the anti-CGRP mAb was also effective in attenuating the GTN-related cephalic tactile hypersensitivity in mCHI animals, and attenuated conditioned place aversion to GTN, pointing to CGRP also being a mediator of the hyperalgesic priming-like effect evoked by the head trauma, in particular the headache-like allodynia and ongoing pain induced by GTN. As the anti-CGRP mAb is unlikely to cross the blood-brain-barrier (due to its large molecular weight), its mechanism of action in mCHI animals likely involves a peripheral site, potentially the interruption of mCHI-evoked meningeal or periosteal inflammatory response. Because the anti-CGRP mAb treatment was ineffective in ameliorating the development of GTN-evoked hindpaw mechanical sensitization, this extrace-phalic pain response in mCHI animals is also likely to be mediated by a CGRP-independent mechanism.

Conclusions

In conclusion, the present study reports the development and characterisation of headache and pain related behaviors in a rat model of concussion evoked by a mild head trauma. The finding of cephalic mechanical hypersensitivity, evidence of ongoing/spontaneous pain behavior and a latent persistent susceptibility to a common headache/migraine trigger that are mitigated by both established and novel anti-migraine therapies may be exploited to investigate possible mechanisms of acute and chronic PTH.

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Article highlights

- Mild concussive head trauma in rats results in the development of a pain-related behavioral phenotype, analogous to post-traumatic headache (PTH).
- Pain and headache related behaviors in concussed rats can be alleviated by acute treatment with sumatriptan or by chronic treatment with a mouse anti-CGRP monoclonal antibody, suggesting a peripheral CGRP-dependent process as an underlying mechanism.
- Following the resolution of PTH-like pain symptoms, concussed rats display CGRP-dependent persistent pain hypersensitivity to glyceryl trinitrate (GTN), suggesting that hypersensitivity to headache triggers may be a putative cause of persistent PTH.

Figure 1. Effect of mCHI on (a) vertical exploratory activity and (b) novel object recognition. Data are mean + SEM (n = 8) * $p < 0.05$ vs sham.

Figure 2.

Cephalic and hindpaw response thresholds (a and c) and cumulative nociceptive scores (b and d) to mechanical stimulation over 14 days following mCHI. Data are mean + SEM (n = 6) * $p < 0.05$ vs sham.

Figure 3.

Effect of acute sumatriptan (a and b) or chronic anti-CGRP mAb (c and d) treatment on response threshold and cumulative nociceptive score to mechanical stimulation following mCHI. Data are mean + SEM ($n = 6-8$) * $p < 0.05$ Sumatriptan vs vehicle, # $p < 0.05$ anti-CGRP mAb vs isotype control IgG.

Figure 4. (a) Treatment protocol for conditioned place preference during conditioning day; (b) Sumatriptan produces CPP in animals at seven days post mCHI but not in sham animals. Data are mean + SEM (n = 8) ** $p < 0.001$ saline paired vs sumatriptan paired chambers.

Figure 5. Effect of GTN administration at day 15 and day 30 post mCHI on cephalic mechanical sensitivity. Data are mean + SEM (n = 8) * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$ vs sham.

Figure 6.
Effect of GTN administration at day 15 and day 30 post mCHI on hindpaw mechanical sensitivity. Data are mean + SEM (n = 8) * $p < 0.05$ vs sham.

Figure 7.

Effect of acute sumatriptan treatment or chronic administration of anti-CGRP mAb and their respective control treatments on the percentage decrease in cephalic response thresholds (a and c) or percentage increase in nociceptive scores (b and d) at 4 h post-GTN compared to corresponding pre-GTN day 14 post mCHI values. Data are mean + SEM (n = 8) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs GTN+Veh or GTN+IgG.

Figure 8.

(a) Treatment protocol for conditioned place aversion during conditioning day; (b) GTN produced place aversion in the IgG injected mCHI animals on day 14 post-injury, but not in the mCHI animals treated with the anti-CGRP mAb. Data are mean + SEM ($n = 8$). * $p < 0.05$ pre-GTN-paired chamber vs GTN-paired chamber.