Comparison of rat models of vascular headache and trigeminal sensitization in male and female rats

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Background

Approximately half of the adult population suffers from headache annually, and migraine represents one of the most common headache disorders affecting approximately 16% of the adult population with a greater prevalence in females compared to males. Current treatments for headache and migraine often provide inadequate pain relief in a substantial number of patients. The National Institutes of Health Helping to End Addiction Long-term Initiative, or NIH HEAL Initiative, Preclinical Screening Platform for Pain (PSPP) program aims to accelerate the discovery and development of novel non-opioid, non-addictive pain therapeutics for pain disorders including headache and migraine. Here, we describe the characterization of rat models of vascular headache and trigeminal sensitization to evaluate novel compounds and mechanisms for the treatment of headache and migraine disorders. An additional focus was to compare the pain phenotype in male and female rats given the greater prevalence of headache disorders in females compared to males.

Methods

Animals: Adult male and female Sprague Dawley rats (200 – 300 g, Envigo) were used in all studies. All housing and testing of animals were in accordance with the Principles of Laboratory Animal Care and the approval of the PsychoGenics Inc., Institutional Animal Care and Use Committee in AAALAC-accredited facilities.

Vascular headache model: Rats were acclimated to Bowman restrainers and were tested for baseline (BSL) facial mechanical sensitivity. Rats then received a single injection of the nitric oxide donor isosorbide dinitrate (ISDN; 10 mg/kg, 10 mL/kg, IP) or vehicle (saline; 10 mL/kg, IP) and effects on facial mechanical sensitivity were determined. In studies evaluating the effects of reference compounds, reference compounds were administered 5 min prior to ISDN injection, and effects on facial mechanical allodynia were determined.

Trigeminal sensitization model: Rats received a surgically implanted stainless steel screw guide cannula (P1 Technologies, Roanoke, VA) over the dura at the junction of the superior sagittal and transverse sinus. After recovery, rats received either a single dural infusion or daily dural infusion over 5 consecutive days of inflammatory soup (IS; 2 mM histamine, bradykinin, serotonin, 0.2 mM prostaglandin E2; pH 5) or vehicle (aCSF; pH 7.4) under brief isoflurane anesthesia (2-3%). During each infusion, 10 μL of IS was delivered at 2.5 μL/min through the screw guide cannula using an infusion pump. In studies evaluating the effects of reference compounds, reference compounds were administered 5 min prior to the single dural infusion of IS, and effects on facial mechanical allodynia were determined.

Measurement of facial mechanical sensitivity: Rats were placed in Bowman restrainers for facial sensitivity testing. Von Frey filaments (1 – 8 g) were applied in ascending order to the forehead above the eyes to determine the facial sensitivity threshold. A positive response was defined as either a recoil of the head, stroking of the face, vocalization, or aggressive behavior towards the filament, and a positive response in 2/3 trials was defined as the facial sensitivity threshold. If a positive response in 2/3 trials was not found for any filament, a maximum facial sensitivity threshold of 10 g was assigned. Rats displaying baseline facial sensitivity thresholds <6.0 g were excluded from the study.

Reference compounds: Morphine sulfate (6 mg/kg, SC; mu opioid receptor agonist), SNC80 (30 mg/kg, SC; delta opioid receptor agonist), sumatriptan (1 mg/kg, IP; 5-HT1B/1D receptor agonist), olcegepant (1 mg/kg, IP; CGRP receptor antagonist), naproxen (30 mg/kg, PO; NSAID), dipraglurant (50 mg/kg, IP; mGluR5 NAM).

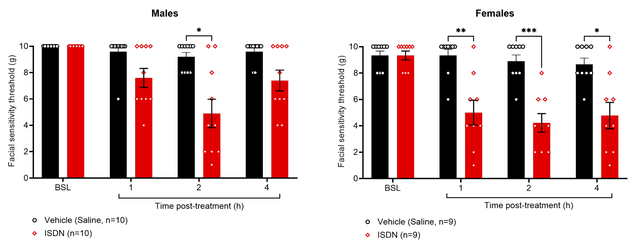
Data and statistics: Results were graphed and analyzed using GraphPad Prism version 10.2.2. Data are represented as individual animal responses and mean ± SEM and analyzed using two-way repeated measures ANOVA with post-hoc Bonferroni's test.

Rigor

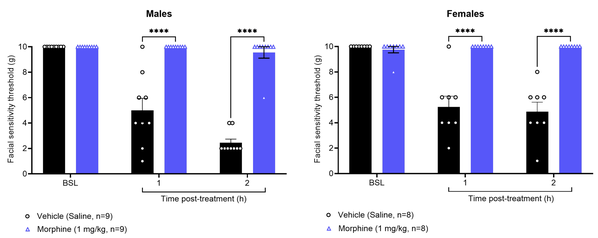
- Experimenters were blinded to treatments
- Inclusion/exclusion criteria were applied according to baseline responses
- Groups were balanced according to baseline responses

Vascular Headache Model

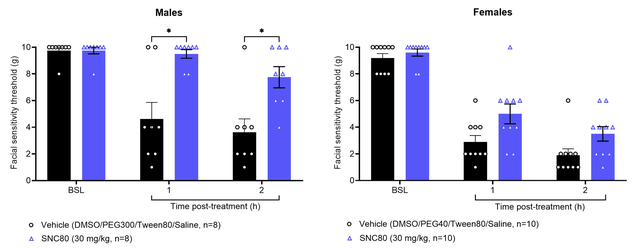
A) Facial mechanical allodynia following ISDN (10 mg/kg, IP)



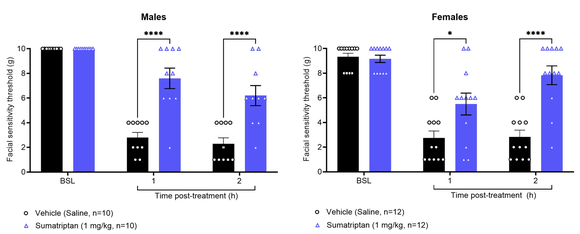
B) Effect of morphine (6 mg/kg, SC)



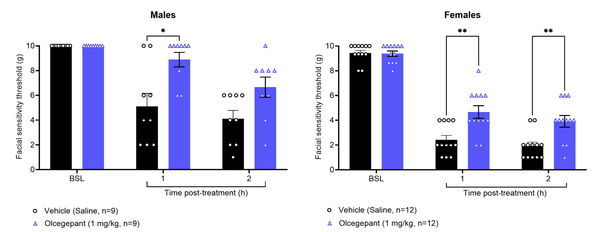
C) Effect of SNC80 (30 mg/kg, SC)



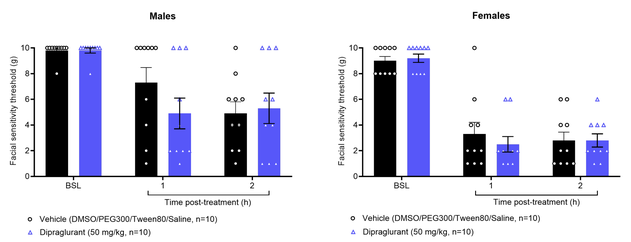
D) Effect of sumatriptan (1 mg/kg, IP)



E) Effect of Olcegepant (1 mg/kg, IP)



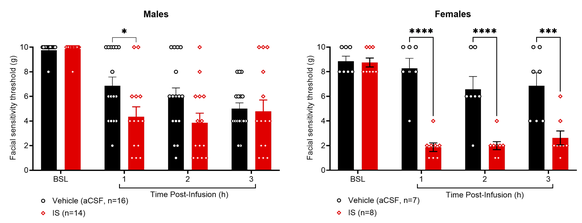
F) Effect of dipraglurant (50 mg/kg, IP)



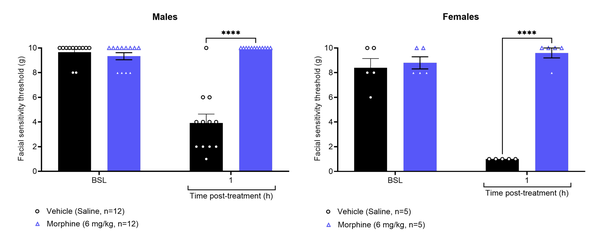
A single administration of ISDN (10 mg/kg) produced facial mechanical allodynia which was more robust in female rats compared to male rats **(A)**. Pretreatment with morphine **(B)**, sumatriptan **(D)**, and olcegepant **(E)** reduced ISDN-induced facial mechanical allodynia in both sexes. Pretreatment with SNC80 **(C)** reduced ISDN-induced facial mechanical allodynia in male, but not female rats. Pretreatment with dipraglurant **(F)** had no effect on ISDN-induced facial mechanical allodynia in male or female rats . **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05 Bonferroni's test.

Trigeminal Sensitization Model

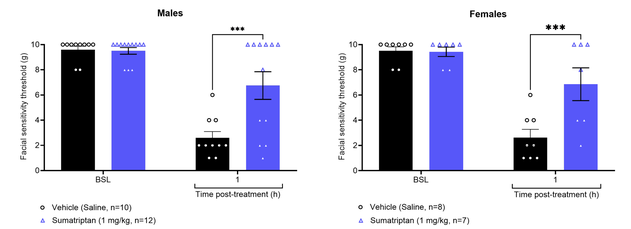
A) Facial mechanical allodynia following single dural infusion of IS



B) Effect of morphine (6 mg/kg, SC)

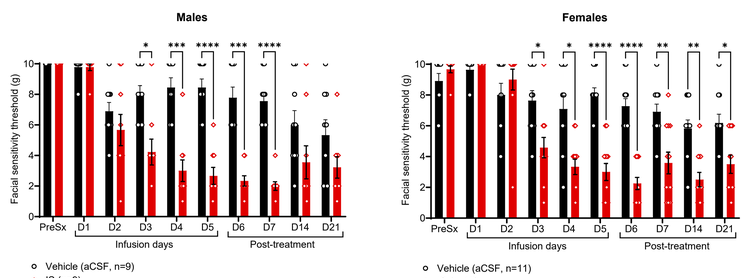


C) Effect of sumatriptan (1 mg/kg, IP)



A single dural infusion of IS produced facial mechanical allodynia which was more robust with a longer duration of action in female rats compared to male rats **(A)**. Pretreatment with morphine **(B)** and sumatriptan **(C)** reduced IS-induced facial allodynia in both sexes. **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05 Bonferroni's test.

D) Facial mechanical allodynia following 5 daily dural infusions of IS



Five repeated IS infusions produced persistent facial allodynia with progressive intensities in both sexes **(D).** Facial mechanical allodynia persisted for the maximum 21-day testing time following the initial infusion in female rats. **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05 Bonferroni's test.

Conclusion

- Facial mechanical allodynia is more robust and persists for a longer duration in female rats compared to male rats in both the vascular headache and trigeminal sensitization models.
- Clinically effective treatments for headache and migraine, including 5-HT1B/1D receptor agonists and CGRP receptor antagonists, can prevent the development of facial allodynia in both the vascular headache and trigeminal sensitization models in male and female rats.
- Additional studies are currently being performed to further understand the pharmacology associated with these models, and potential future studies will be designed to further characterize behavioral phenotype associated with these models (e.g. photophobia and/or phonophobia).
- Characterization of the pharmacology and behavioral phenotype associated with these models will support the use of these models to accelerate the development of novel treatments for headache and migraine.

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