



Evaluating the Side Effect Profile and Molecular Mechanism of a Novel Analgesic: An Arylepoxamide Receptor Agonist

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Background

- Novel analgesics, with minimal or no adverse effects, that can be used to combat injury-related pain on the battlefield are severely needed.
- Previously, we characterized a rat model of extremity trauma (ET) for spontaneous and reflexive pain behaviors and used this model to evaluate the novel arylepoxamide receptor (AEAR) agonist SBS1000, developed by Sparian Biosciences.
- The AEAR is a recently discovered cell receptor that is a splice variant of the OPRM1 gene (1-4).

Objectives

- Determine the side effect profile of SBS1000
- Determine SBS1000 effects on specific brain regions that are key to pain processing

Methods

- The ET model (n=17) consists of both soft tissue injury and a fibula fracture in anesthetized male Sprague-Dawley rats.
- Sham rats (n=16) were anesthetized but received no trauma.
- After completion of ET or sham, rats were allowed to recover. SBS1000 (1 mg/kg) was administered subcutaneously on day 3 post-ET.
- Control rats received vehicle (80% saline, 10% DMSO, 10% kolliphor EL).
- The effect of SBS1000 on sedation (rotarod) was measured at 1.5-, 3-, and 6- hrs. post-dosing.
- In a separate group of rats, effects of SBS1000 on reflexive pain (mechanical allodynia (MA) and thermal hyperalgesia (TH)) was measured followed by humane euthanasia and brain isolation.
- The contralateral and ipsilateral thalamus and prefrontal cortex were dissected, followed by total protein isolation, and a Luminex panel that measures the expression of inflammatory mediators.

Anti-Nociceptive Testing and Side Effect Profiling

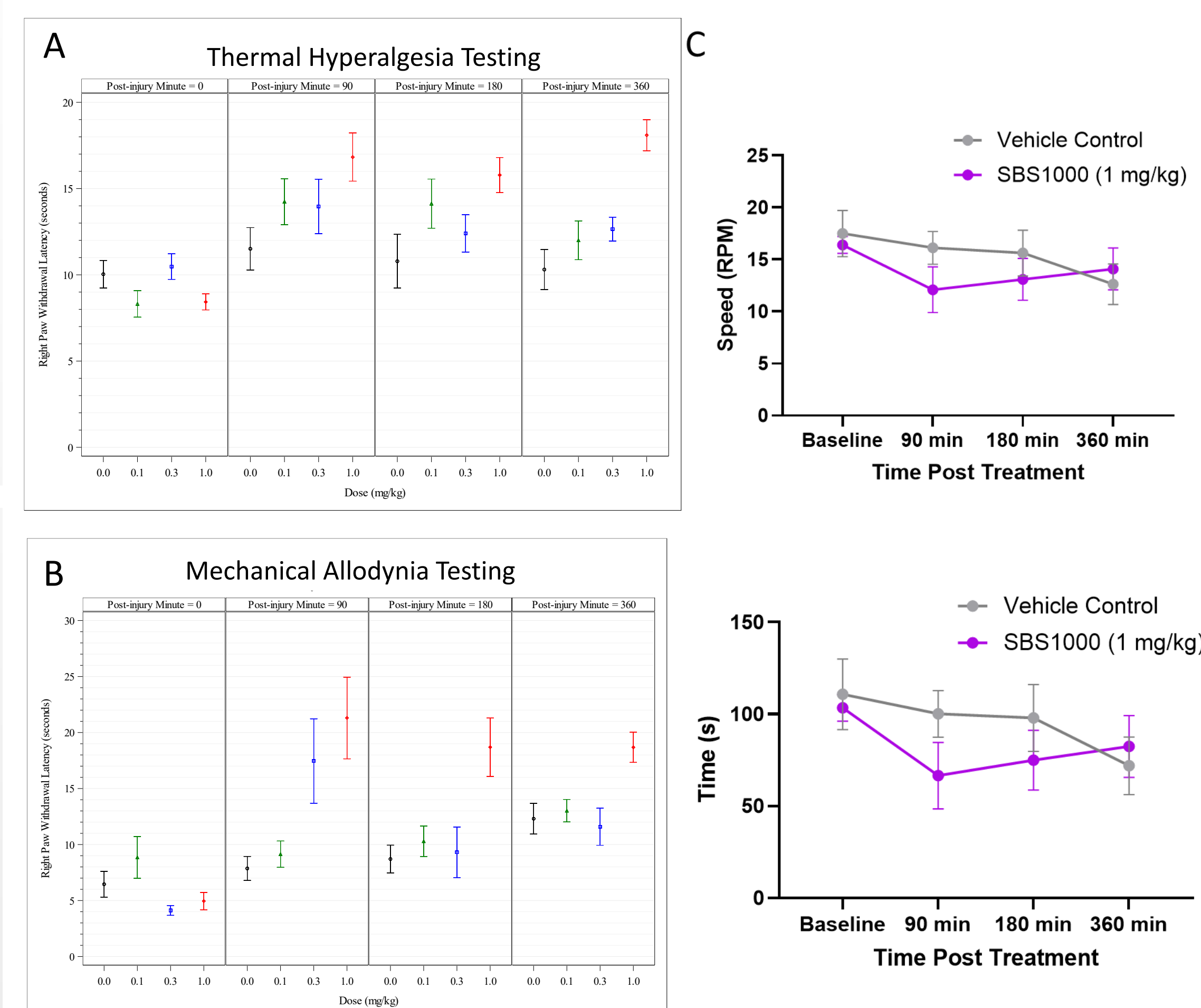


Figure 1. Effects of SBS1000 on Thermal Hyperalgesia, Mechanical Allodynia, and Rotarod Testing.

- A. For thermal hyperalgesia testing, the 1.0 mg/kg SBS1000 groups' paw withdrawal latency was markedly improved compared to the Vehicle group at 90 Minutes (46.2%, p=.0042), 180 Minutes (46.3%, p=.0069), and 360 Minutes (75.6%, p=.0001).
- B. For mechanical allodynia testing, the 1.0 mg/kg SBS1000 groups' paw withdrawal latency was markedly improved compared to the Vehicle group at 90 Minutes (170.8%, p=.0001), 180 Minutes (114.7%, p=.0029), and 360 Minutes (51.8%, p=.0538).
- C. For rotarod testing, there was no distinct differences in either speed of the rotarod or latency to fall between vehicle and SBS1000 treated groups.

Effects on Inflammatory Mediators

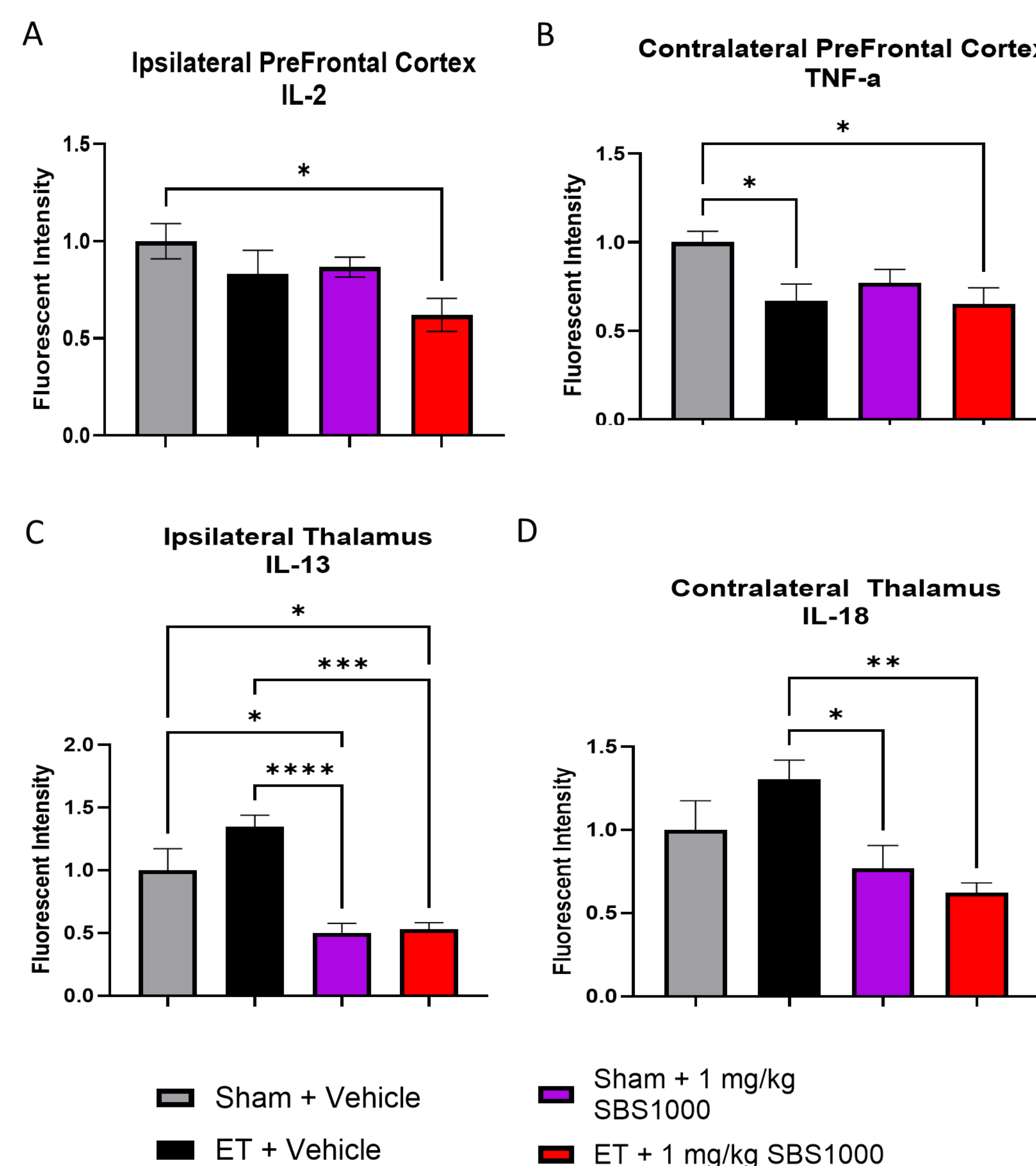


Figure 2. Effects of ET and SBS1000 Treatment on Inflammatory Mediators in the Brain.

- A. For the ipsilateral prefrontal cortex, the IL-2 protein expression in the ET + 1 mg/kg SBS1000 group was reduced compared to the Sham + Vehicle group.
- B. For the contralateral prefrontal cortex, TNF-α protein expression was reduced in the ET + vehicle and ET + 1 mg/kg SBS1000 groups compared to the Sham + Vehicle group.
- C. For the ipsilateral thalamus, IL-13 protein expression was decreased in both SBS1000 treated groups compared to the Sham + Vehicle and ET + Vehicle groups.
- D. For the contralateral thalamus, IL-18 protein expression was decreased in both SBS1000 treated groups compared to the ET + vehicle group.

Conclusions

- SBS1000 has anti-nociceptive properties and a minimal side effect profile.
- ET injury did not have a significant effect on inflammatory mediators in the contralateral or ipsilateral thalamus or the ipsilateral prefrontal cortex whereas it did significantly reduce inflammatory mediators in the contralateral prefrontal cortex.
- SBS1000 treatment significantly reduced several inflammatory mediators in both the contralateral and ipsilateral thalamus and prefrontal cortex.

Discussion

- ET effects on inflammatory mediators: ET causes all 23 inflammatory mediators tested to increase in the L4-6 spinal cord. Whereas, in the prefrontal cortex, several inflammatory mediators decreased in response to ET.
- SBS1000 effects on inflammatory mediators: SBS1000 causes reduction of all inflammatory mediators tested in the L4-6 spinal cord. SBS1000 decreases majority of inflammatory mediators tested in the prefrontal cortex and thalamus.
- This data indicates that specific mechanisms that underly both ET-induced reflexive pain and SBS1000 analgesic efficacy have yet to be fully explored.
- An in-depth understanding of battlefield-induced pain and the novel analgesics that can combat this pain will greatly enhance the military's ability to effectively treat battlefield pain in future conflicts.

References

- Lu, Z., et al., Mediation of opioid analgesia by a truncated 6-transmembrane GPCR. *J Clin Invest*, 2015. 125(7): p. 2626-30.
- Lu, Z., et al., Truncated mu-Opioid Receptors With 6 Transmembrane Domains Are Essential for Opioid Analgesia. *Anesth Analg*, 2018. 126(3): p. 1050-1057.
- Majumdar, S., et al., Truncated G protein-coupled mu opioid receptor MOR-1 splice variants are targets for highly potent opioid analgesics lacking side effects. *Proc Natl Acad Sci U S A*, 2011. 108(49): p. 19778-83.
- Majumdar, S., et al., Synthesis and evaluation of aryl-naloxamide opiate analgesics targeting truncated exon 11-associated mu opioid receptor (MOR-1) splice variants. *J Med Chem*, 2012. 55(14): p. 6352-62.

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Animal Statement: Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by AAALAC.