Behavioral and neurobiological responses to a noxious heat stimulus in the snapping shrimp, *Alpheus angulosus*:

Exploring the potential for pain experience in a decapod crustacean

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Alpheus angulosus

INTRODUCTION

- **Determining that an animal is capable of pain experience is crucial for their welfare, often prompting ethical or legal considerations.**[1]
- **In the U.S., welfare policy only extends to vertebrate animals.**[2,3]
- **There is a growing body of evidence concerning pain experience in decapod crustaceans, animals widely and increasingly used in research and commercial practice.**[4]
- **However, there have been few attempts to explore pain experience in any shrimp species.**

Nociception → **Pain** → **Behavior**

Damaging or potentially damaging stimulus (e.g., extreme temperature, pH, or pressure) causes nociceptors to fire[5]

Signal moves to the brain, integration of sensory information, negative feeling at the site of nociception[5]

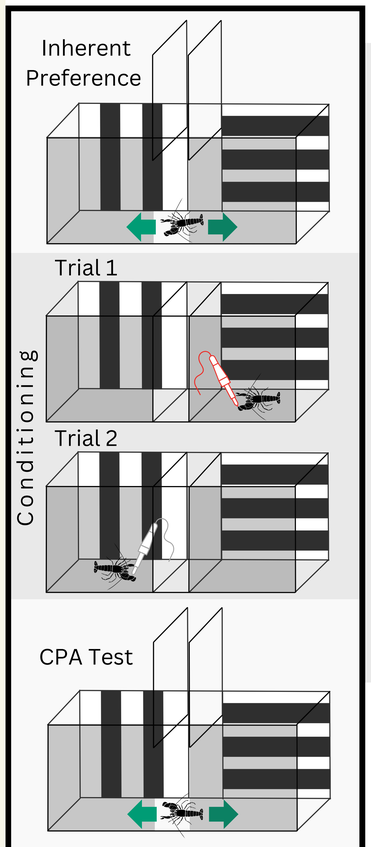
Motivational change, avoidance learning, heightened sensitivity, rest & recovery[6]

Conditioned Place Avoidance

Question: Can a shrimp learn to avoid a noxious heat stimulus?

Pain reinforces learning.[6] Learning occurs in the brain. If learning occurs as a result of a noxious heat stimulus, this indicates central processing of nociceptive information and cannot explained by reflex.

Conditioned place avoidance (CPA) is a method that has been used in mammals to study pain and the efficacy of anesthetic drugs.[7] Here it will be used to explore whether pain is likely to occur in a snapping shrimp.



Phase 1: Inherent preference test
- 10 minutes free exploration
- Compare time spent in each chamber

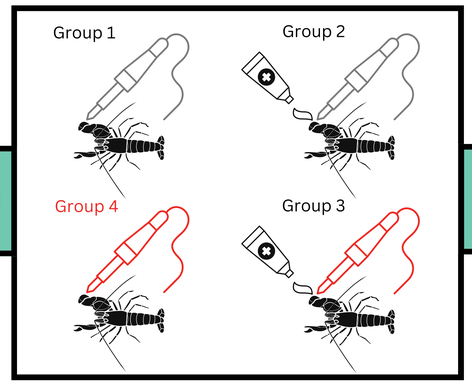
Phase 2: Conditioning
- Trial 1: Experimental treatment in preferred compartment
- Trial 2: Control treatment in non-preferred compartment

Phase 3: CPA test
- 10 minutes free exploration
- Compare time spent in each chamber
- Significant preference for control compartment is considered CPA

CPA in Group 4 suggests learned avoidance and central integration, which is consistent with the idea of pain.

METHOD

Behavioral



Neurobiological

Four experimental groups will be used in two experiments, one behavioral, and one neurobiological.

Groups of subjects will receive the following treatments applied to the major chela: 1) neutral soldering iron; 2) topical anesthetic + neutral iron; 3) topical anesthetic + hot iron; 4) hot iron.

Nitric Oxide Synthase in the CNS

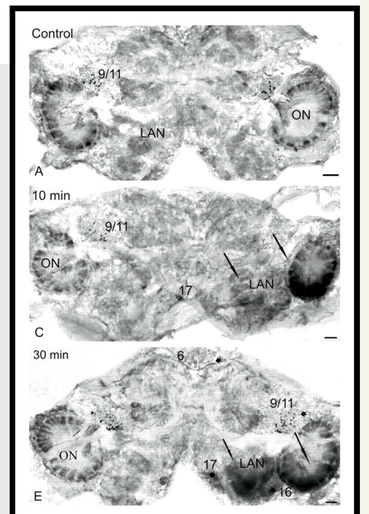
Question: Does a noxious heat stimulus result in a chemical signal to the shrimp brain?

Nitric Oxide (NO) is a biomarker that has been implicated in the transmission of nociceptive signals from the periphery to the brain in mammals and recently in a shore crab, *Hemigraspus Sanguineus*.[8,9] These studies find NO localized to areas of the CNS ipsilateral to injury.

NO can be visualized in the brain by staining for its synthesis enzyme, NADPH-d.[10] This study will quantify NADPH-d in the CNS of shrimp and compare experimental groups.

- Subjects will be treated, anesthetized, sacrificed, and dissected.
- The CNS will be sectioned and mounted on slides.
- Slides will be stained with an NADPH-d antibody and a blue stain for visualization.
- Alternate slides will be Nissl-stained to view brain structure.
- Slides will be imaged via light microscopy and NADPH-d will be quantified.
- A between-hemisphere difference score will be determined for each subject.

A significantly higher quantity of NADPH-d in the hemisphere ipsilateral to injury in Group 4 suggests the central integration of a nociceptive signal



Adapted from Dyuizen et al., 2012. NADPH-d density increased over time after a nociceptive stimulus in *H. sanguineus*. Optical density increased only in the hemisphere ipsilateral to injury in the olfactory lobe (ON), lateral antennular neuropil (LAN), and cell cluster 9/11. At 10 and 30 minutes after injury, optical density in treatment hemispheres significantly differed from control animals and from their own contralateral hemispheres.

SIGNIFICANCE

- **This study has the potential to show evidence consistent with the idea of pain in a shrimp species.**
- **This study will be the first to assess the role of NO in central nociceptive integration in any shrimp species.**
- **This study will be the first to behaviorally assess the effect of nociceptive stimuli on long term behavioral change in a shrimp species.**
- **This study will develop methods of anesthesia and humane sacrifice for this species which may be used in future studies.**

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