Beneficial Effects of a Non-Steroidal Anti-Inflammatory Drug (NSAID) on Chemotherapy-Induced Behavioral Changes

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Hypothesis:

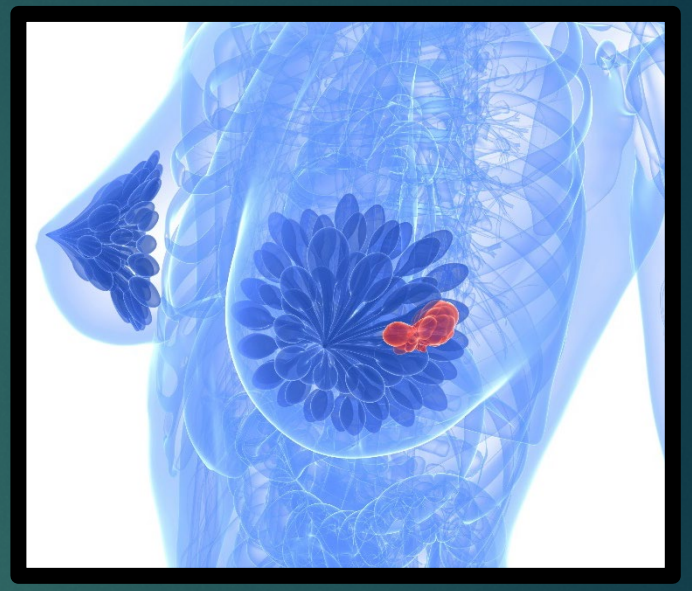
Naproxen, a nonsteroidal anti-inflammatory drug (NSAID), mitigates the behavioral changes produced by breast cancer chemotherapy agent, cyclophosphamide.

Background: Breast Cancer

- Among women, breast cancer is the most frequent cancer diagnosis– affecting 2.1 million women each year
- Median age of diagnosis = 62 years → post-menopausal females
- U.S. women have a 1-in-8 lifetime risk of being diagnosed with breast cancer
  - ↑ risk due to:
    - Increased life expectancy
    - Menopausal hormone use
    - Rising prevalence of obesity
    - Improved detection through mammography screening

Background: Chemotherapy

- Chemotherapy agents are used to limit the spread and growth of cancerous cells
- Cyclophosphamide is a mainstay chemotherapy agent in the treatment of breast cancer
  - Targets proliferating cells
  - Alkylating agent → interferes with DNA cross-linking → cell death
  - Crosses blood-brain-barrier (BBB)



Background: Depression

- **Chemo Brain”** symptoms:
  - Depression
  - Anxiety
  - Difficulty with memory
  - Changes in attention
- Depression is commonly reported by breast cancer patients undergoing chemotherapy treatment
  - A cause of significant mortality in these patients
  - Impacts patient’s quality of life, adherence to treatment plans and survival

Background: Inflammation

- Increased peripheral inflammatory markers seen in breast cancer patients up to 6 months after the completion of chemotherapy
  - 30% persist beyond 6 months
  - Inflammation in the CNS may contribute to:
    - Mood alterations (i.e. Depression & anxiety)
    - Behavioral changes
    - Fatigue
  - Anti-inflammatory treatment may be beneficial in reducing these symptoms
    - Naproxen is a nonsteroidal anti- inflammatory drug (NSAID)
      - Non-selective cyclooxygenase (COX) inhibitor
      - Crosses the blood-brain barrier
      - Oral administration

Experimental Design:

- Female Mice
  - C57 Bl/6 strain
  - Charles River Laboratories
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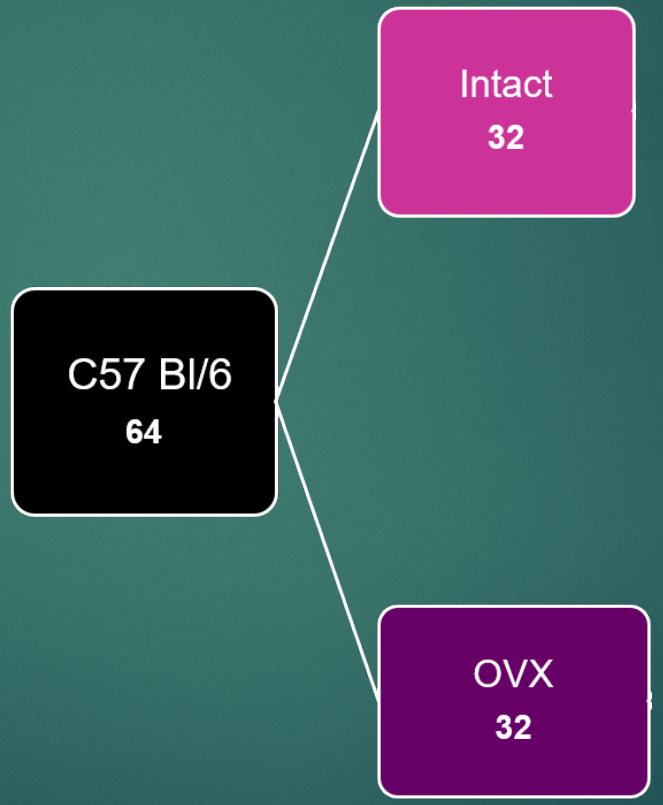

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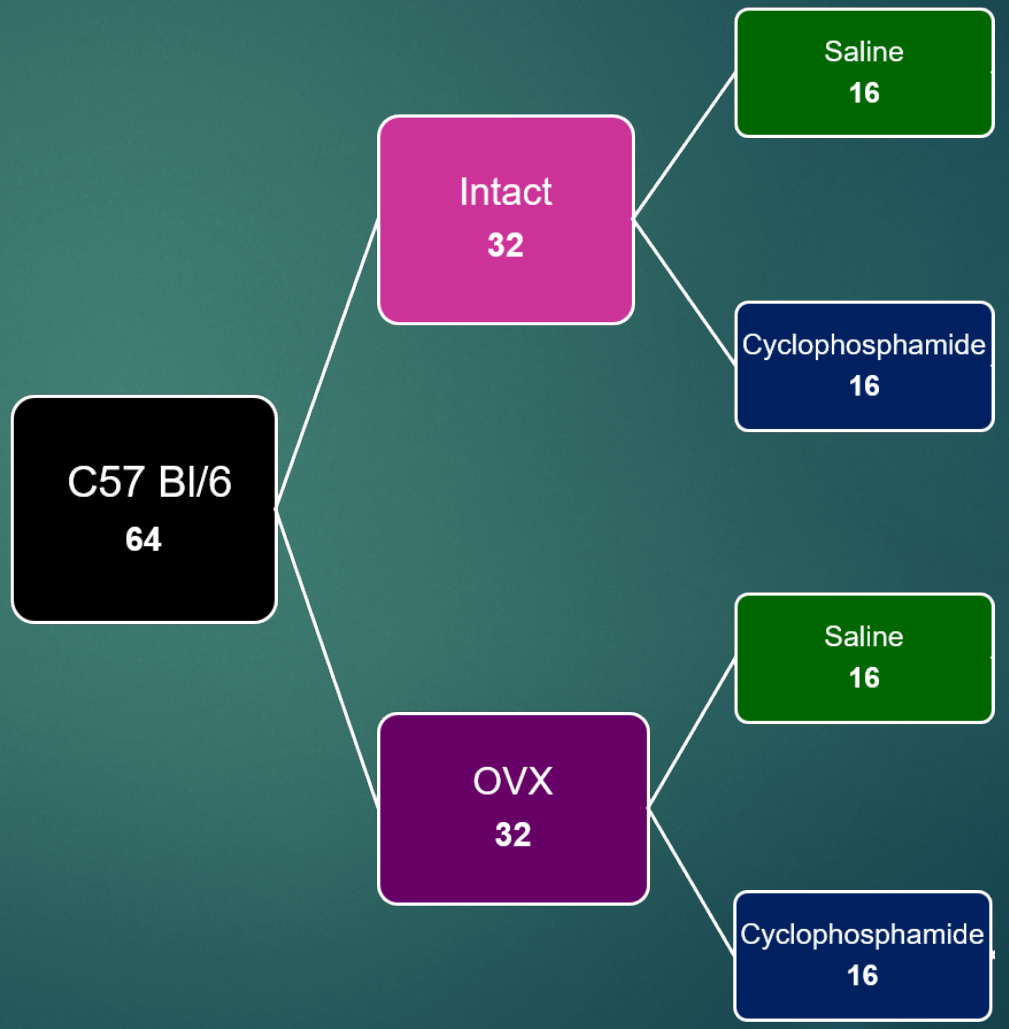

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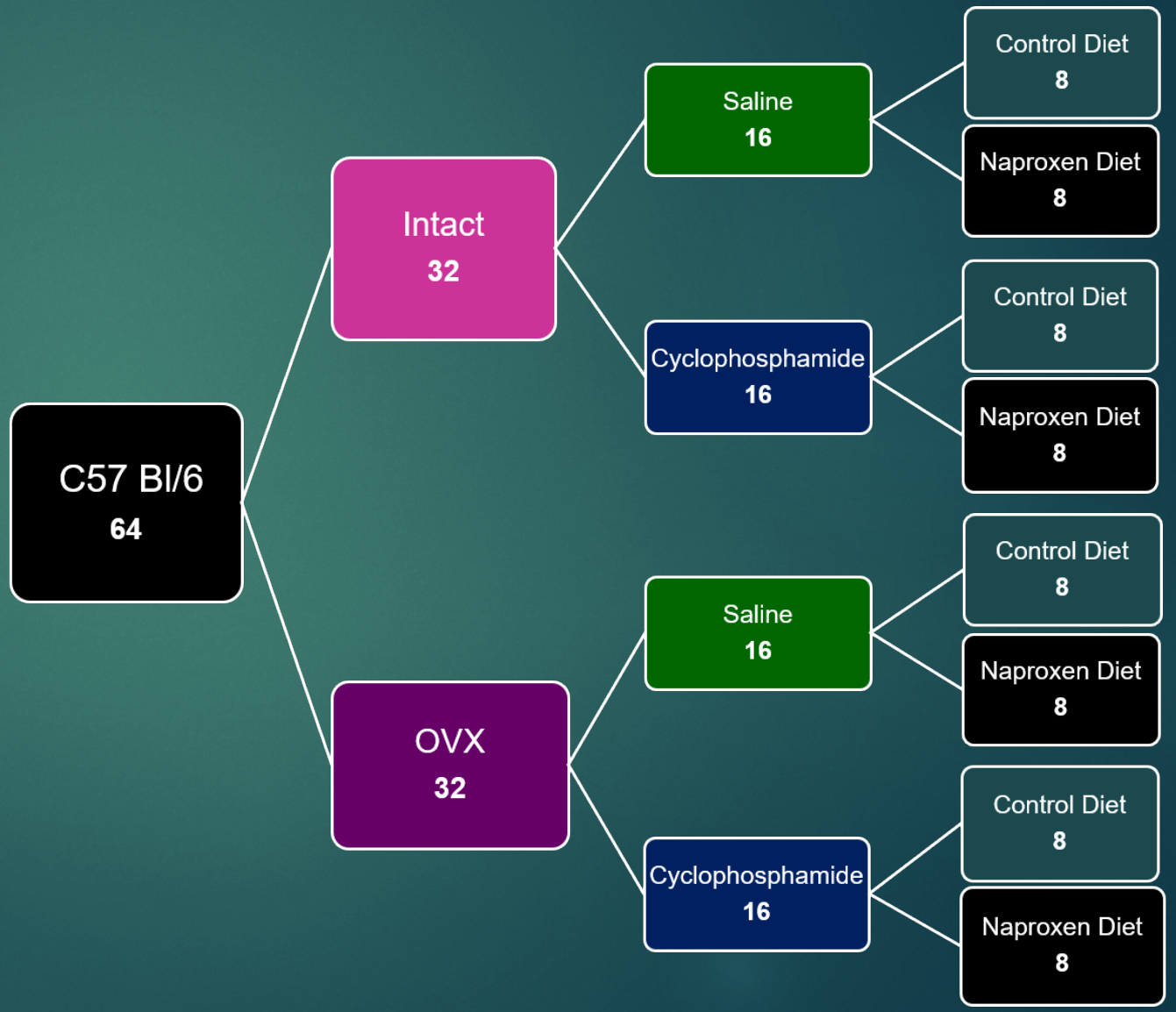


Experimental Design:

- **Phase 1:** Drug & Diet Administration
- **Phase 2:** Behavioral Assays
- **Phase 3:** Analysis of Inflammatory Markers

Drug + Diet Administration

1. Control Diet or Naproxen Diet
2. Cyclophosphamide (CP) or Saline Injections (IP)

Behavior

Activity: Locomotor Chamber

Depression: Tail-Suspension Test (TST)

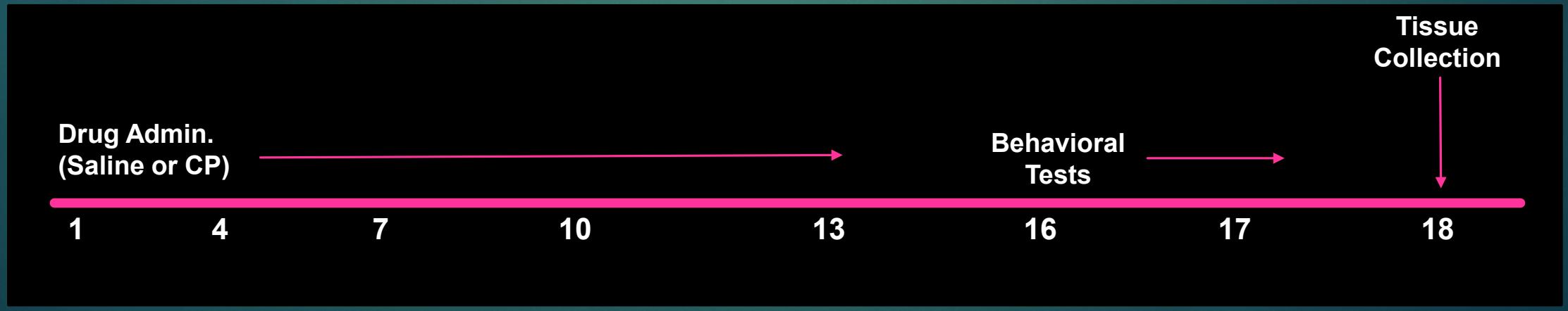
Anxiety: Elevated Zero Maze (EZM)

Inflammation

1. Sacrifice
2. Plasma Collection
3. Brain Tissue Collection
4. Immunohistochemistry
5. ELISA assay

Phase 1: Diet + Administration

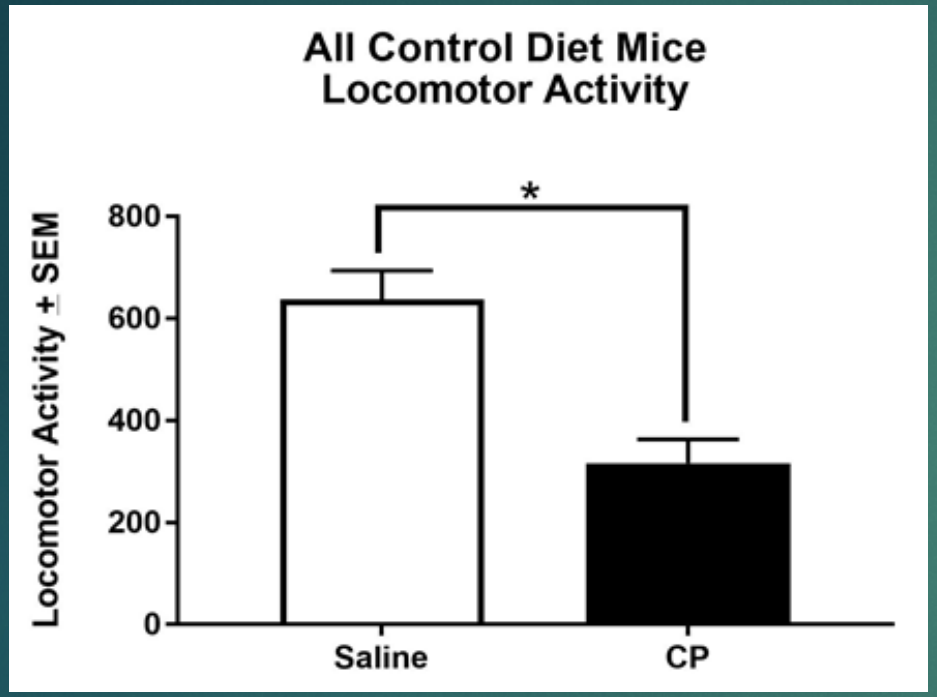
- **NSAID: Naproxen**
  - Administration of Naproxen Diet (375 mg) or Control Diet
- **Chemotherapy agent: Cyclophosphamide (CP)**
  - Administration of CP (100 mg/kg i.p.) or saline
  - 5 doses over a 2-week period: one dose every 3rd day

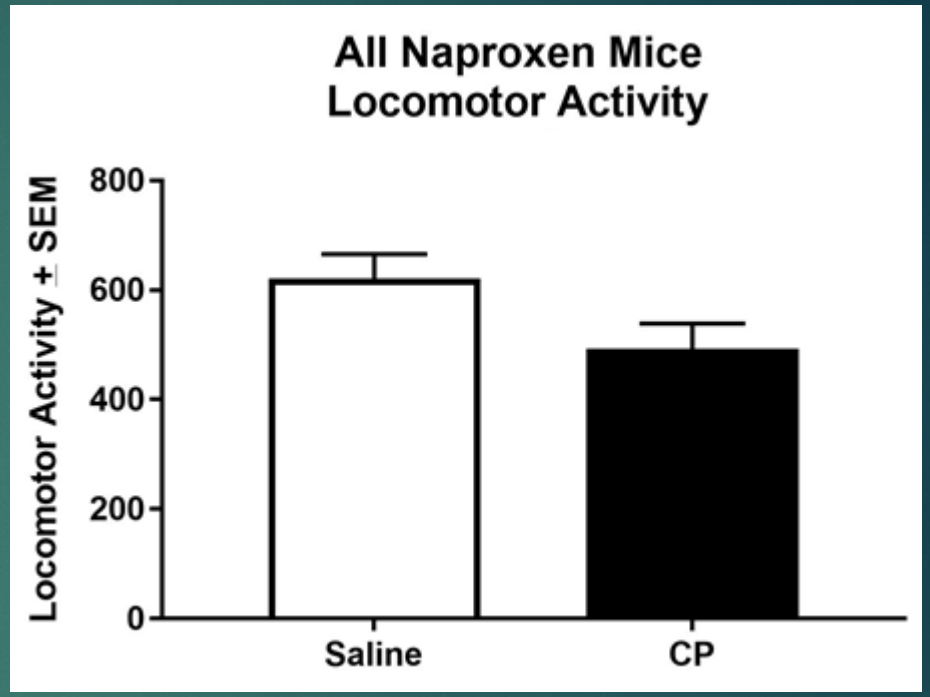


Phase 2: Behavioral Assays

- **Locomotor Activity: Chamber**
- 90 minute trial
- Infrared beam-sensor monitored the number of movements
- Analysis:
  - General Activity

Results: Locomotor Activity



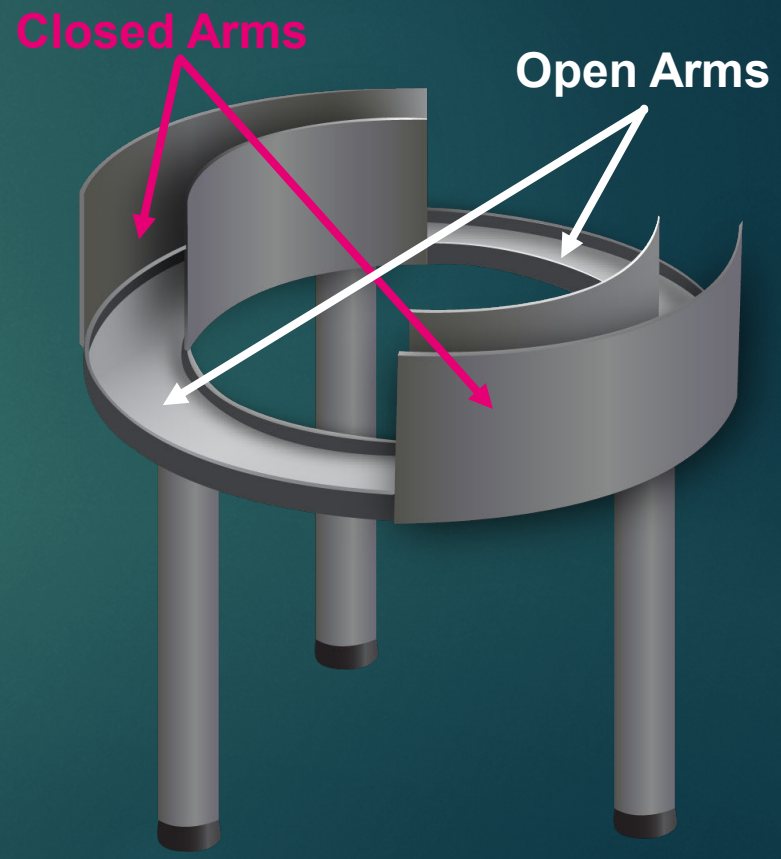


- Cyclophosphamide (CP) treated mice on the control diet had significantly reduced locomotor activity than the mice given saline and on control diet
- Naproxen diet “normalized” the locomotor activity

Phase 2: Behavioral Assays

Elevated Zero Maze (EZM) → Anxiety-like Behavior

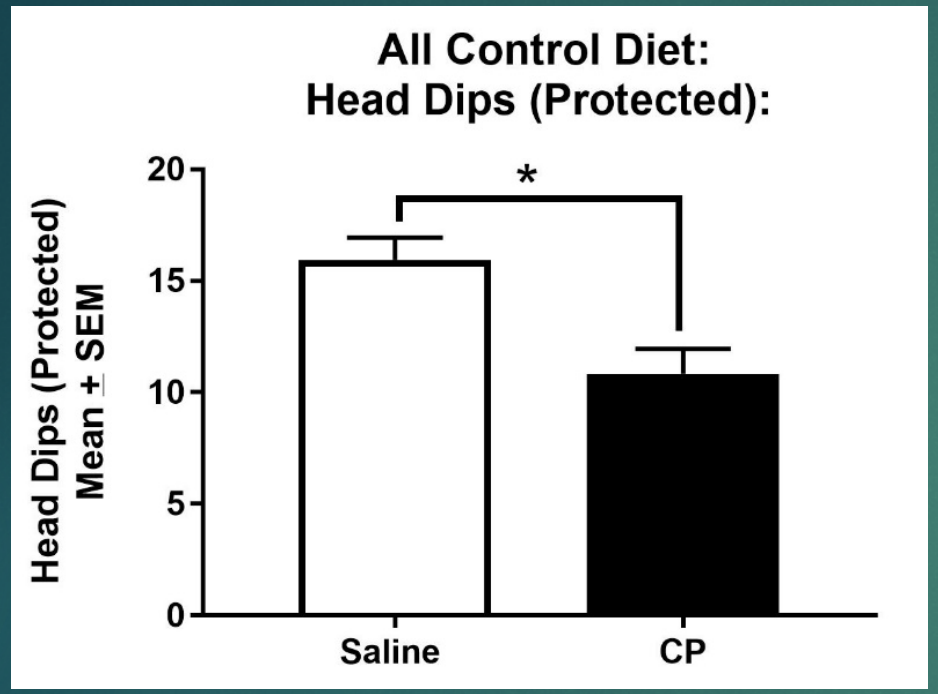
- 5 minute trial
- Analysis:
  - Time spent in closed arms
  - Arm entries
  - Protected head dips
- Increased time spent in the closed arms and decreased number of head dips may indicate an anxiety-like behavior phenotype



Results: Elevated Zero Maze

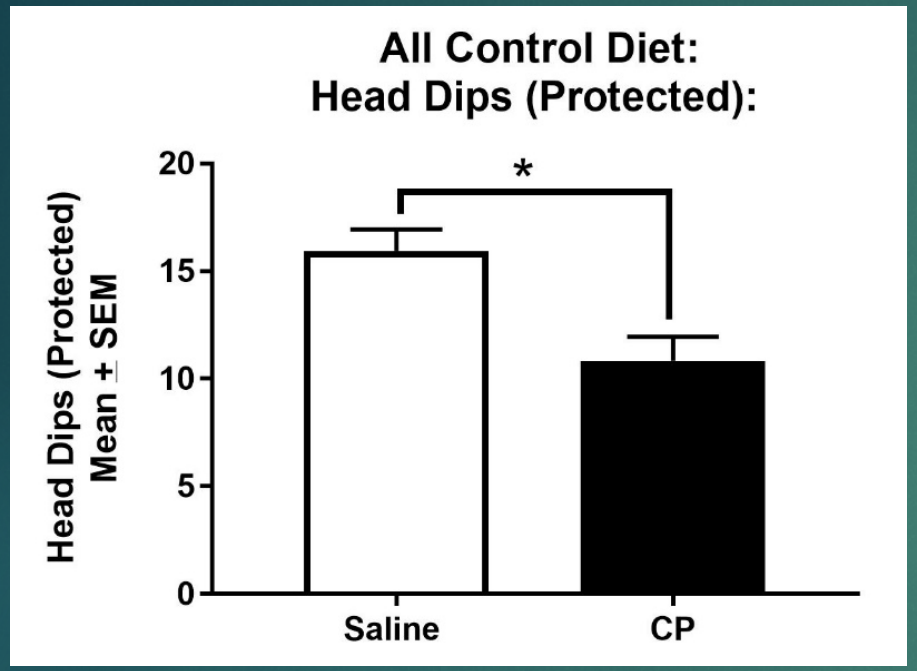
- Cyclophosphamide (CP) and naproxen did not influence arm entries

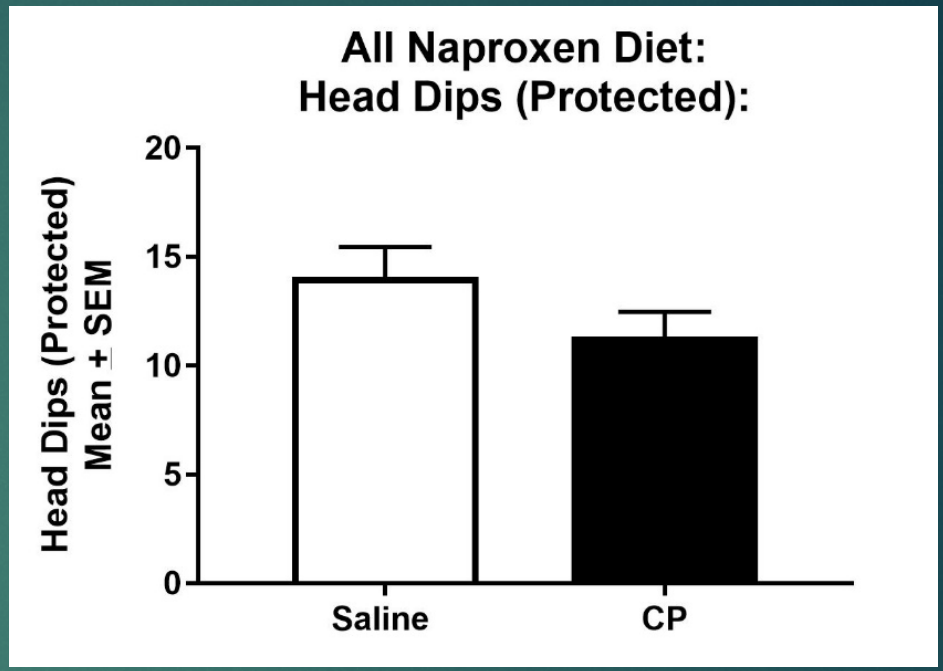
Results: Anxiety-like Behavior



- CP treated mice on the control diet made significantly fewer head dips compared to saline control mice on control diet.
- Naproxen diet “normalized” or “rescued” this behavior
- Cyclophosphamide (CP) and naproxen did not influence arm entries

Results: Anxiety-like Behavior





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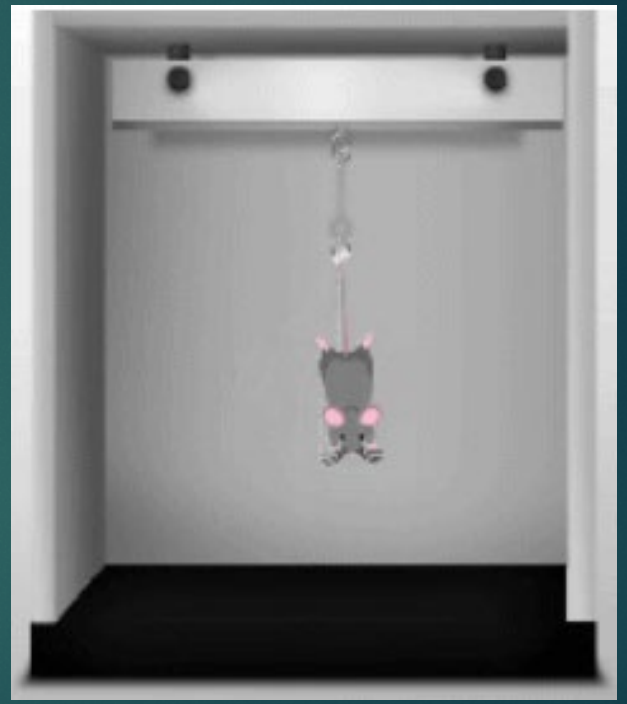
Phase 2: Behavioral Assays

- **Tail-Suspension Test (TST) → Depression-like Behavior**
- 6 minute trial
- Analysis:
  - Total Time Immobile
  - Latency to 1St Immobility



- Increased time spent immobile may indicate learned helplessness – surrogate measure for depression-like phenotype
- Learned helplessness is a behavior in response to an uncontrollable and aversive stress

Results: Depression- like Behavior

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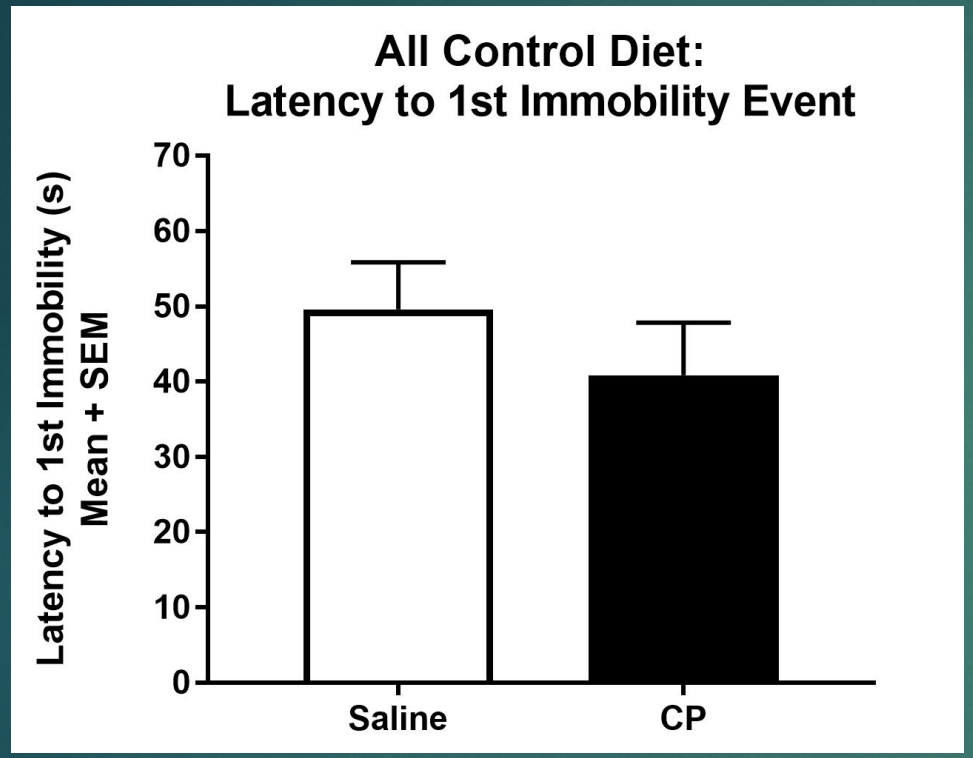
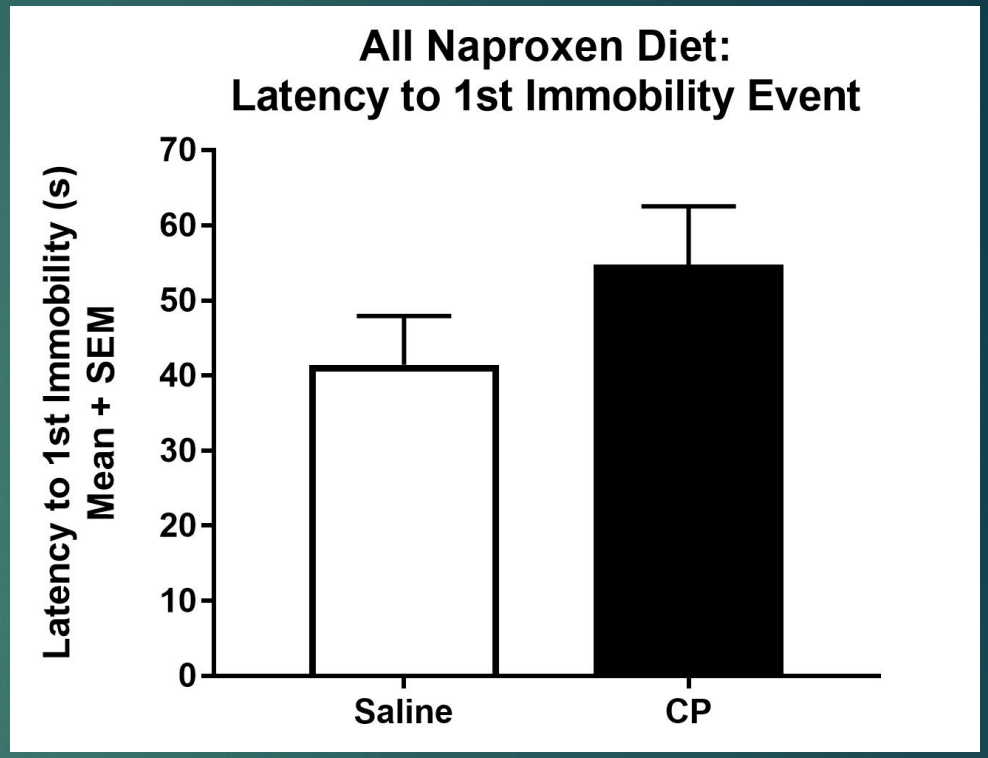
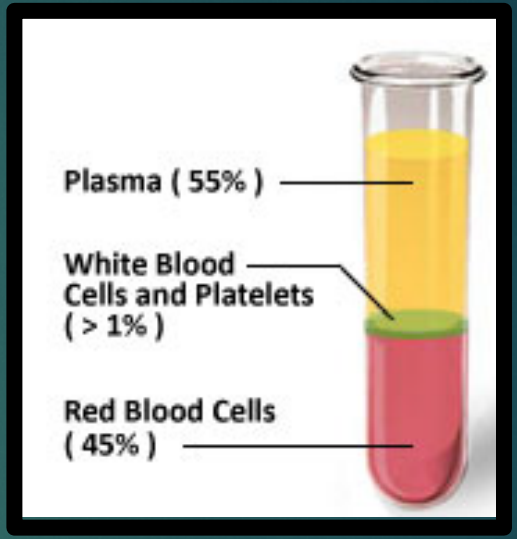


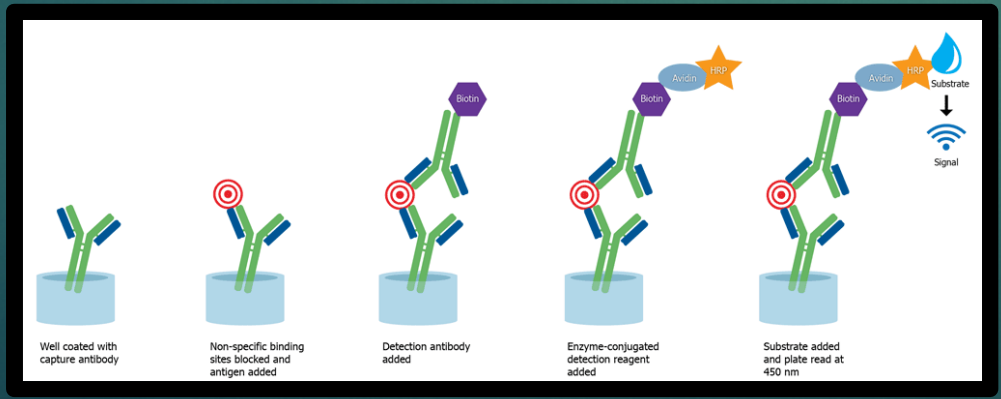


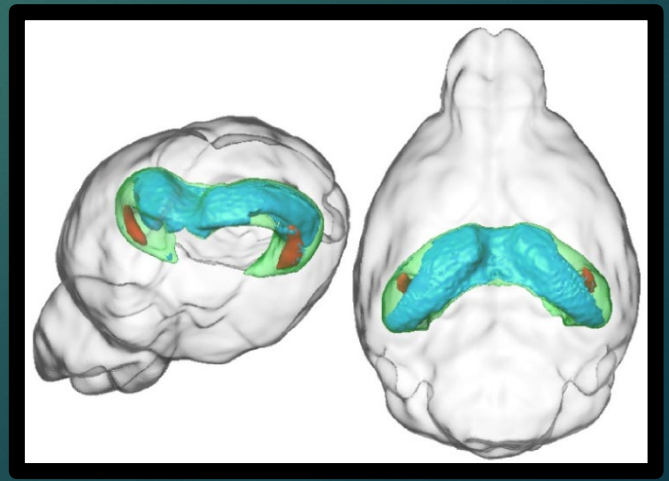
- Cyclophosphamide (CP) treated mice on the control diet showed a trend toward shorter latency to first immobility compared to the mice given saline and on control diet, indicating that they “gave up” and became helpless over a shorter interval.
- CP treated mice on the naproxen diet did not show this trend. Instead these mice showed a trend toward longer latency to first immobility, indicating that they took longer before “giving up”

Phase 3: Inflammatory Analysis

- **Sacrifice**
  - Isoflurane Anaesthesia
  - Rapid Decapitation
- **Trunk Blood Collection**
- **Hippocampi Dissection**
- **Inflammatory Marker Analysis**
  - Analyze cytokine content (ELISA)
    - IL-1, IL-6, IL-10, TNF-α, NF-kB
  - Immunohistochemistry







Results: Inflammatory Markers

Conclusion:

- Cyclophosphamide produced significant effects in EZM and Locomotor activity test
  - CP mice on control diet made a fewer head dips indicating anxiety-like behavior. (EZM) and displayed reduced locomotor activity than the mice given saline and on control diet
- Naproxen restored baseline behaviors in EZM and Locomotor Activity tests in CP-treated mice
- CP mice on control diet had a shorter latency to 1st immobility while CP treated mice on naproxen diet displayed a longer latency to 1st immobility.
  - The trend indicates that naproxen restored or even may have improved performance in the tail suspension test in CP-treated mice

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