The Effects of Chemotherapy on Cognitive Behavior and Neurogenesis in an Animal Model of Pre- and Post- Menopausal Females

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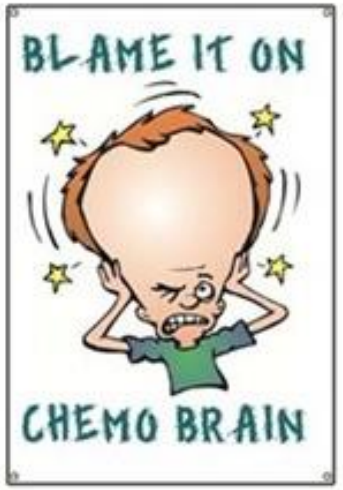
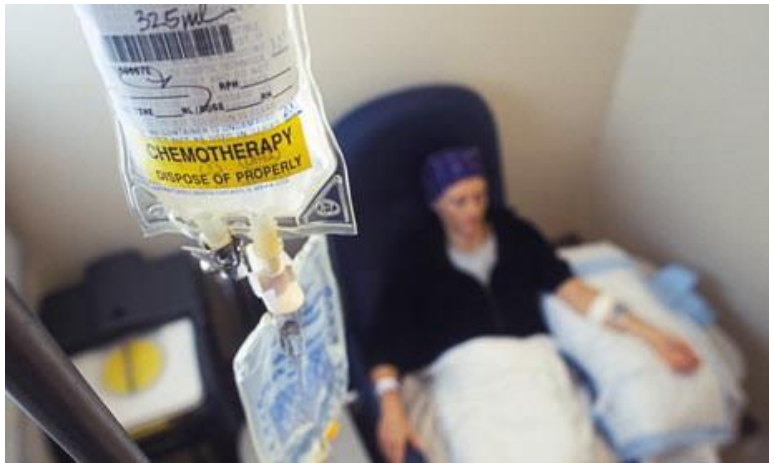
Background: Breast Cancer

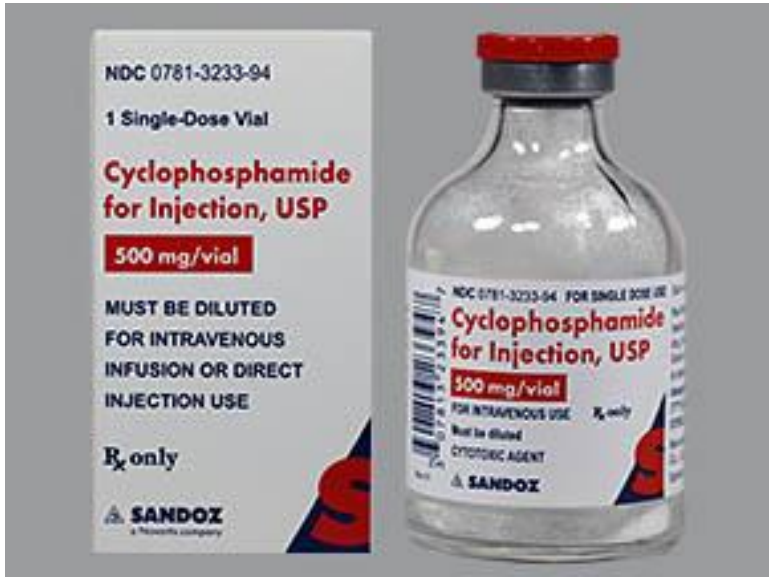
- Breast cancer is one of the most commonly diagnosed cancers
  - Median age of diagnosis is 61 years
- Patients on chemotherapy report cognitive disruptions and depressive-like symptoms, commonly known as “chemo brain”
  - These symptoms can persist after the completion of chemotherapy and are a cause of considerable distress



Background: Chemotherapy

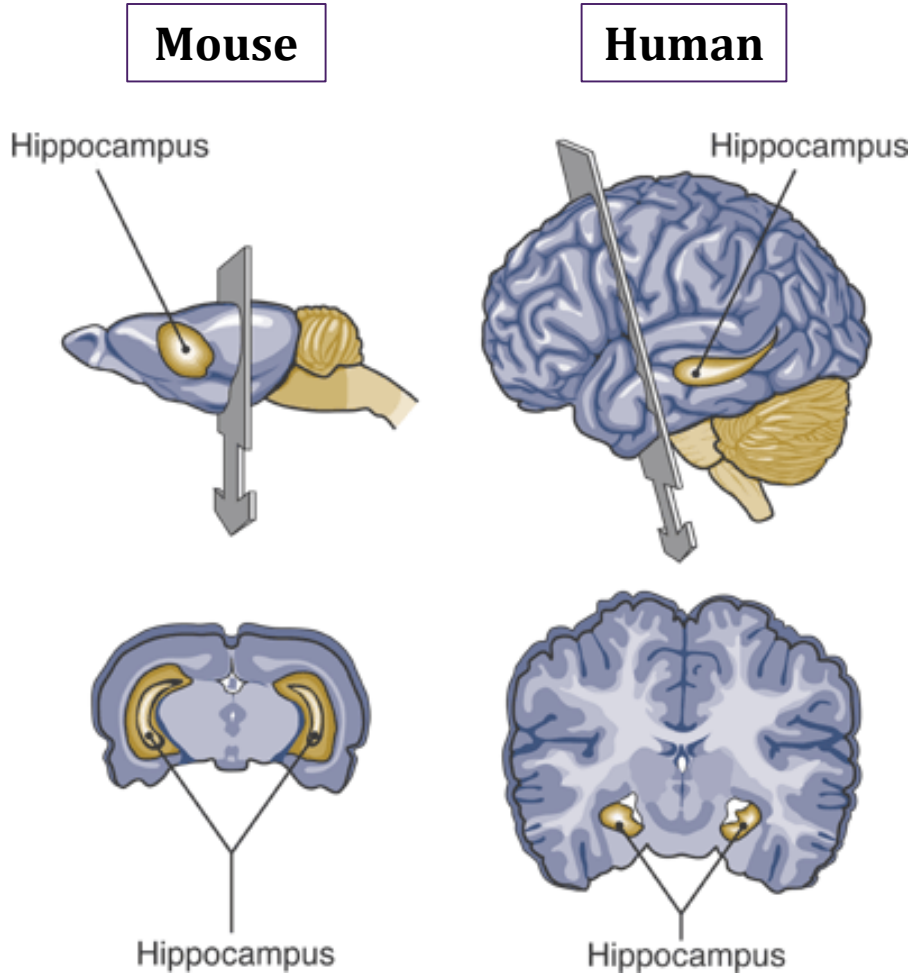
- Chemotherapy agents are used to target proliferating cell populations
- The effects of these drugs are nonspecific
  - In the central nervous system, progenitor cells in the hippocampus proliferate throughout our lifetimes
- Cyclophosphamide is an alkylating agent that crosses the blood-brain-barrier and interferes with DNA crosslinking, promoting apoptosis





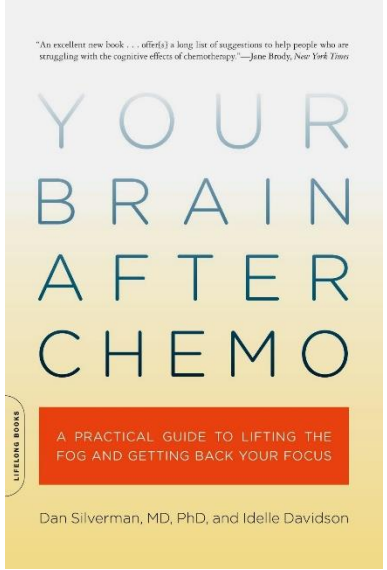
Background: Hippocampal Neurogenesis

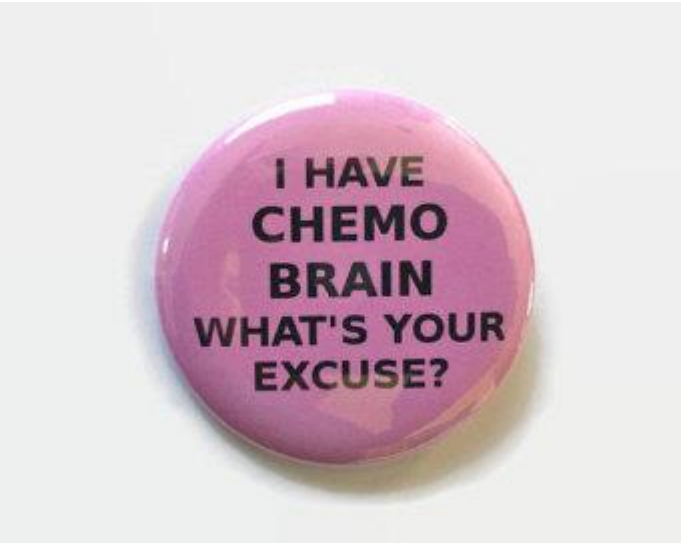
- Within the **dentate gyrus** of the hippocampus, new neurons are generated from neural stem cells in a process known as neurogenesis
- Hippocampal neurogenesis plays a major role in hippocampus-dependent functions which includes learning, and memory.
- This is the only area in the adult brain where neurogenesis occurs
- Decreased hippocampal neurogenesis is associated with cognitive deficits



Why animals?

- The independent contributions of chemotherapy drugs to cognitive disruptions remain poorly understood
- Psychosocial features associated with cancer diagnosis are confounding variables
  - Using an animal model without cancer will exclude these
  - This allows for direct analysis of potential link between chemotherapy, cognitive deficits and hippocampal neurogenesis

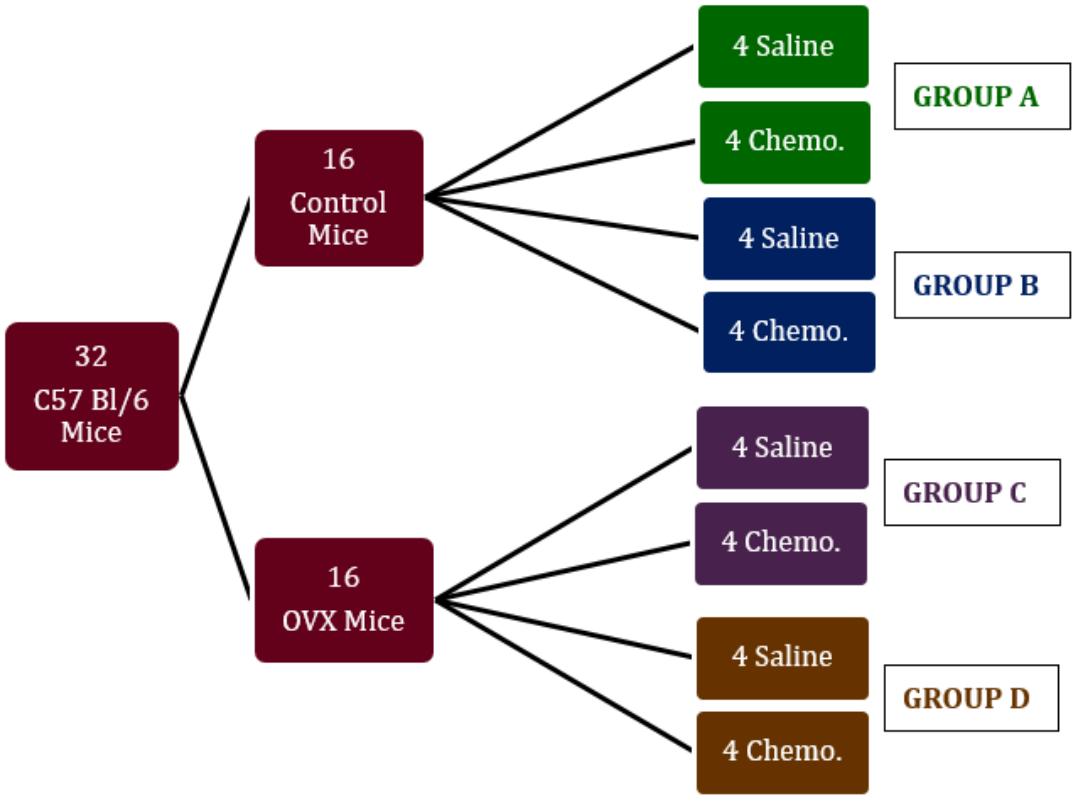




Hypothesis

Cyclophosphamide, a breast cancer chemotherapy agent, impairs cognitive function and decreases adult hippocampal neurogenesis in a mouse model without cancer

Experimental Design



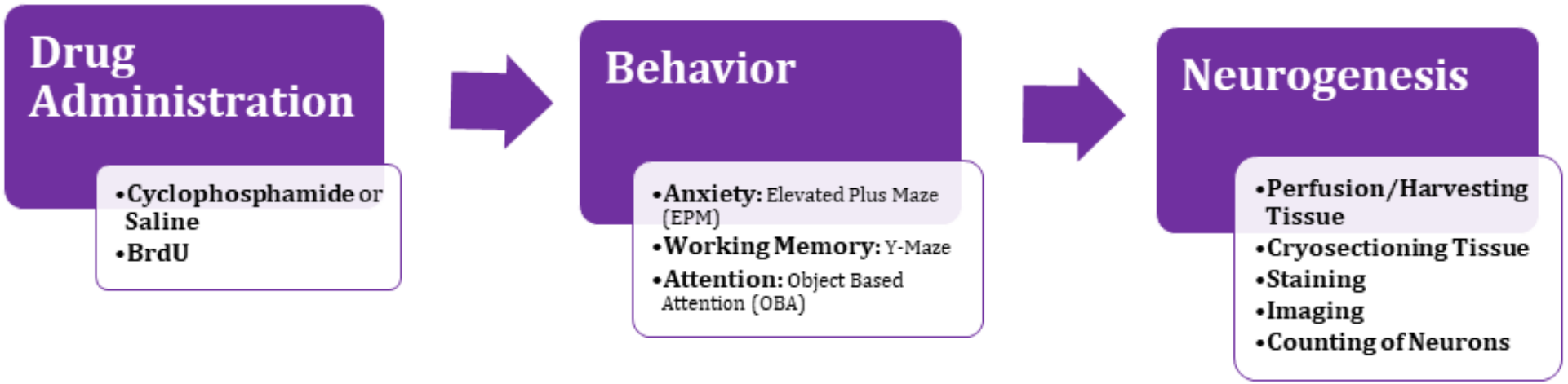
- **Animals:**
  - Species: Female mice
  - Strain: C57 Bl/6
  - Source: Charles River Laboratory
  - Age: 8 weeks
  - Control (C57) → Non ovariectomized, pre-menopausal model
  - Ovariectomized C57 (OVX) → Post-menopausal model

Statistics:

- Differences among the experimental groups were analyzed using a two-way analysis of variance test (2-way ANOVA).
- Differences between two groups were analyzed post hoc using Tukey's Multiple Comparison Test.
- A p value of <0.05 was considered to indicate statistical significance.

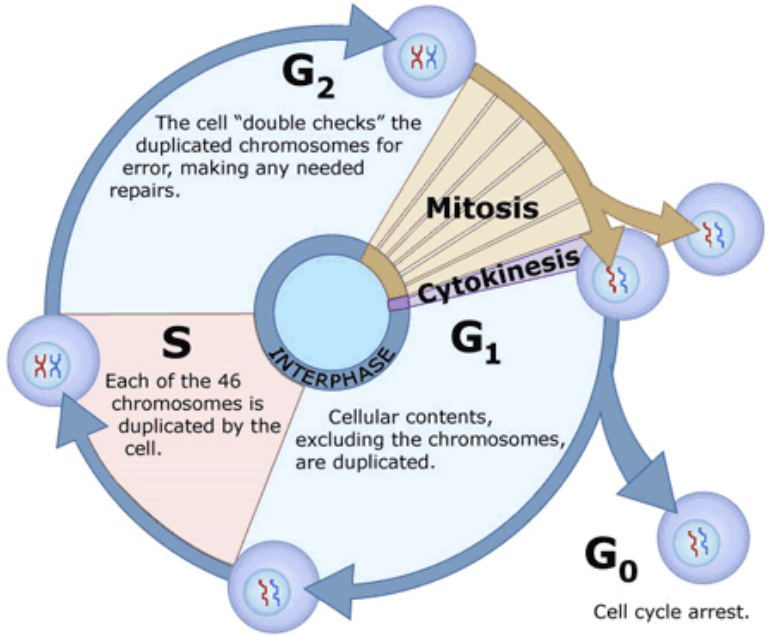
Experimental Design

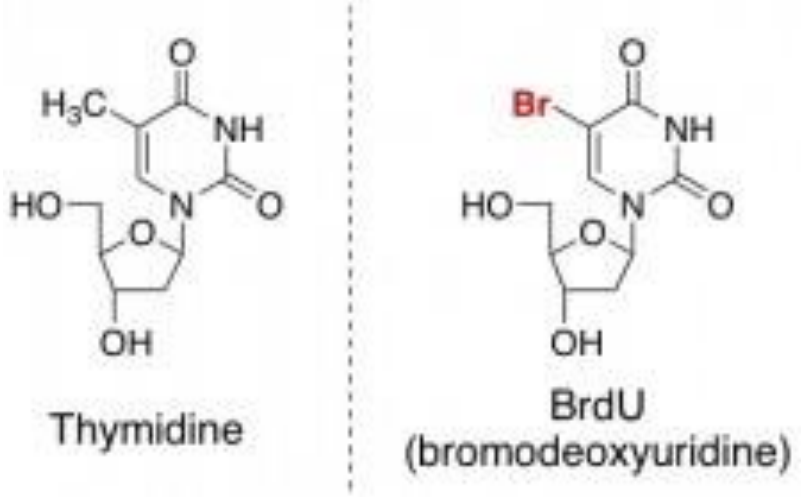
- **Phase 1:** Drug Administration+ BrdU
- **Phase 2:** Analysis of Cognitive Behaviors
- **Phase 3:** Analysis of Hippocampal Neurogenesis



Phase 1: Drug Administration + BrdU

- **Chemotherapy agent: Cyclophosphamide (CP)**
  - Administration of CP (50 mg/kg) or saline
  - 5 doses every 3rd day for 2 weeks
  - Intraperitoneal (IP) route of administration
- **S-phase labeling agent: Bromodeoxyuridine (BrdU)**
  - BrdU (50 mg/kg) was administered at the conclusion of the drug administration
  - 5 doses every 12 hours for 2.5 days
  - Intraperitoneal (IP) route of administration

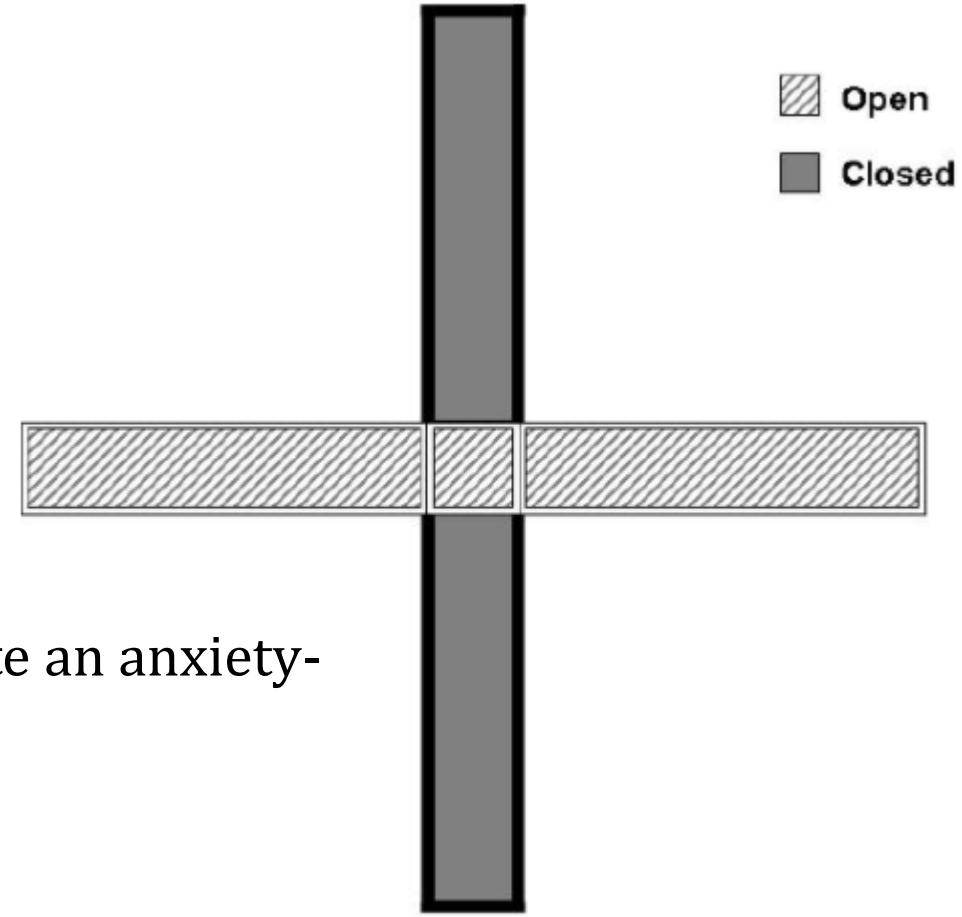




Phase 2: Analysis of Cognitive Behavior

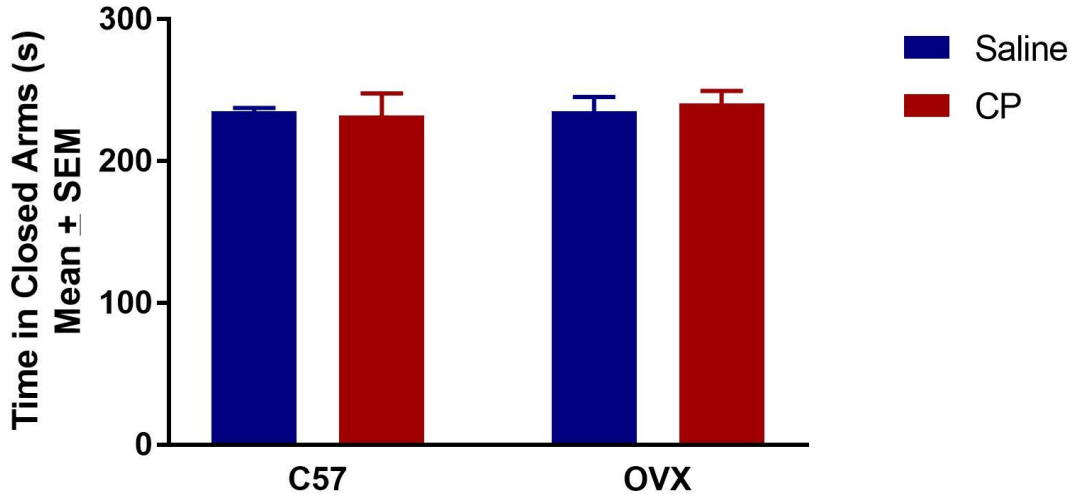
Elevated Plus Maze (EPM) → Anxiety-like Behavior

- 5 minute trial
- Analysis:
  - Time spent in:
    - Open arms
    - Closed arms
  - Number of arm entries in:
    - Open arms
    - Closed arms
- Increased time spent in the closed arms may indicate an anxiety-like behavior phenotype

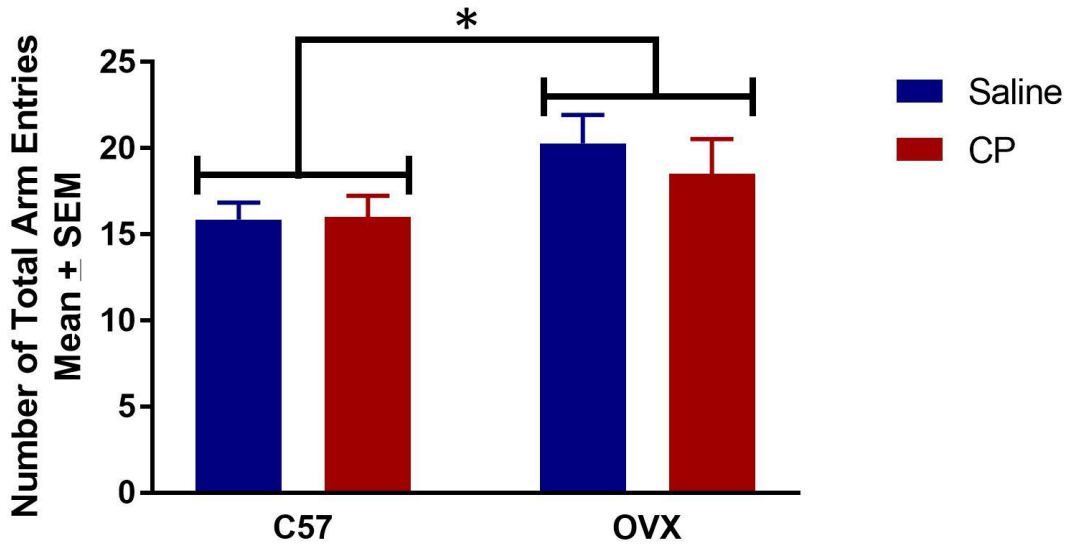


Results: Elevated Plus Maze (Anxiety-like Behavior)

Time in Closed Arms



Total Arm Entries

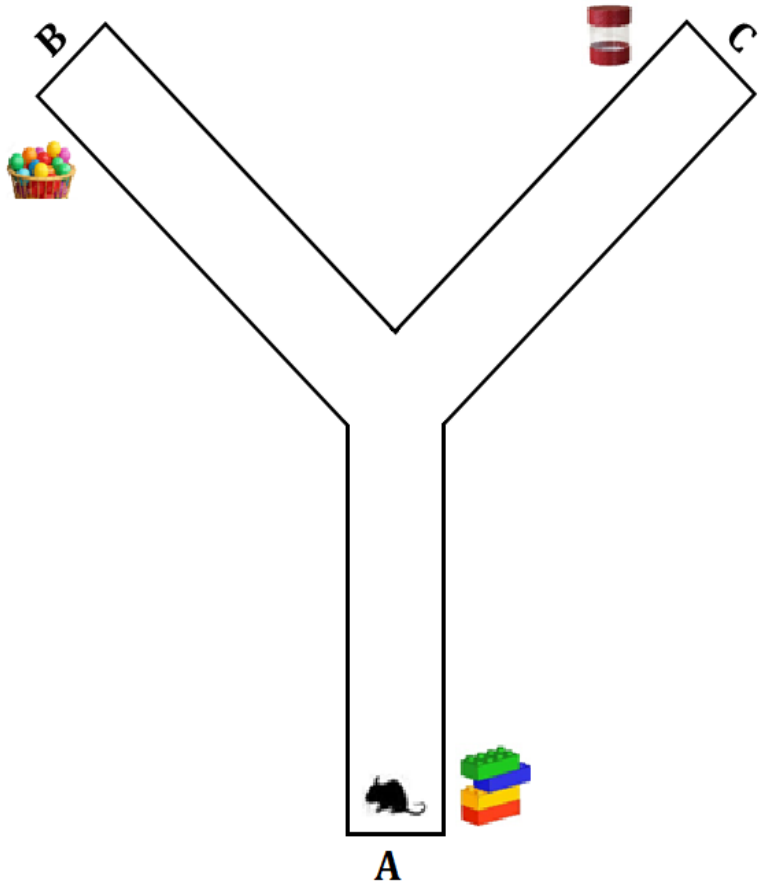


- Cyclophosphamide did not influence anxiety-like behavior or arm entries
- OVX mice made significantly greater number of total arm entries compared to C57 control mice, regardless of treatment

Phase 2: Analysis of Cognitive Behavior

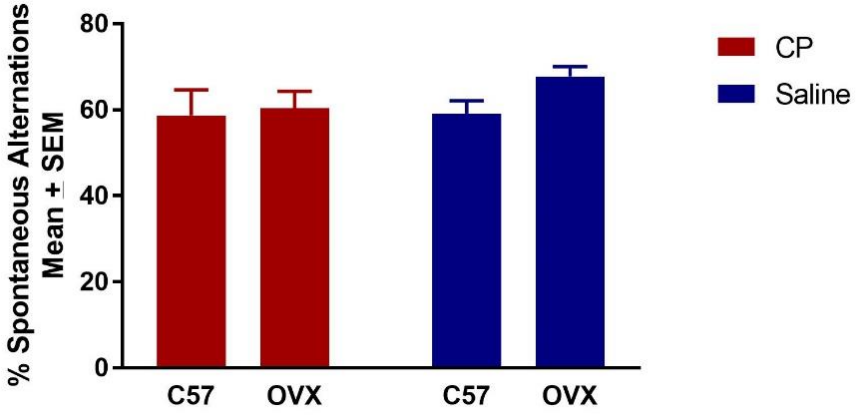
Y- Maze → Spatial Working Memory

- 6 minute trial
- Three arms at 45 ˚ with spatial cues
- Mice with intact spatial working memory will explore an arm but will not immediately reenter it = <u>correct alternation</u> (ABC, CBA, BCA, etc.)
- Measures:
  - Arm entries
  - Correct alternations
  - $\%\ Spontaneous\ Alternations = \frac{Correct\ alternations}{Total\ arm\ entries} x\ 100$

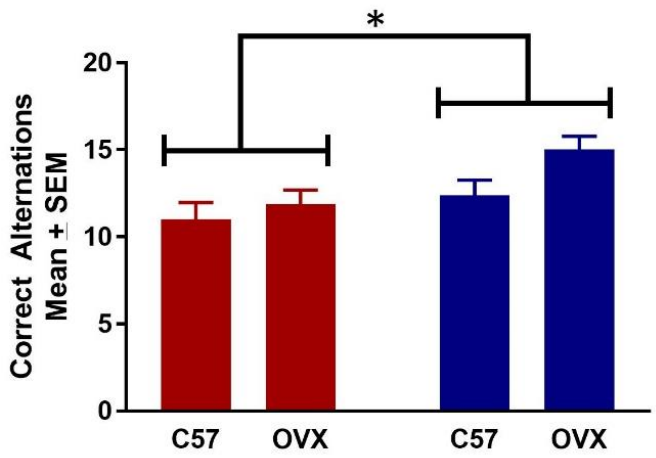


Results: Y-Maze (Spatial Working Memory)

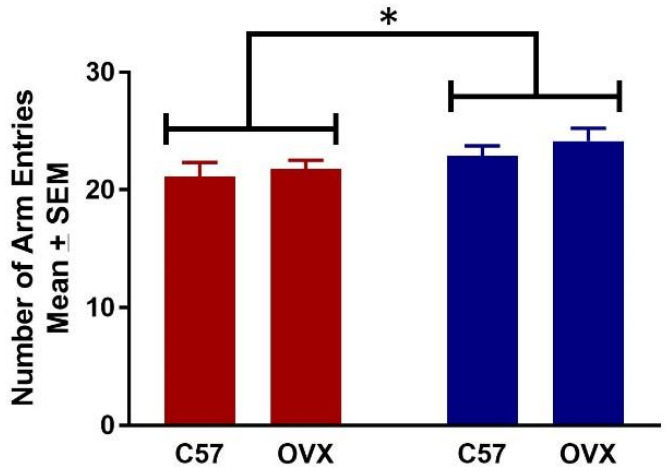
% Spontaneous Alternations



Correct Alternations

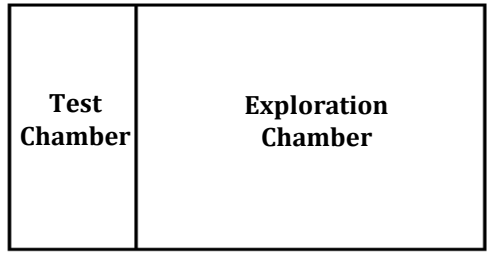


Arm Entries



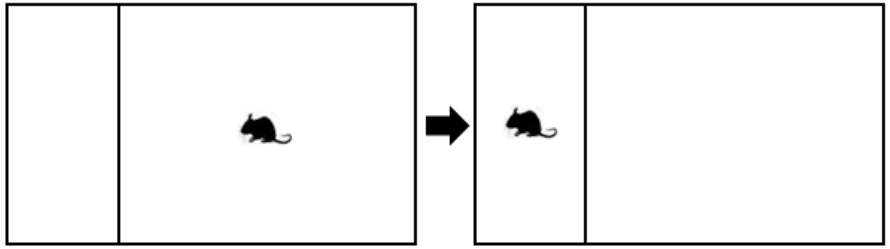
- Cyclophosphamide did <u>not</u> produce significant effects on spontaneous alternations
- CP treated mice made significantly <u>fewer correct alternations</u> and <u>fewer arm entries</u> than saline treated mice, regardless of ovariectomy
- OVX did not produce significant effects on these measurements

Phase 2: Analysis of Cognitive Behavior

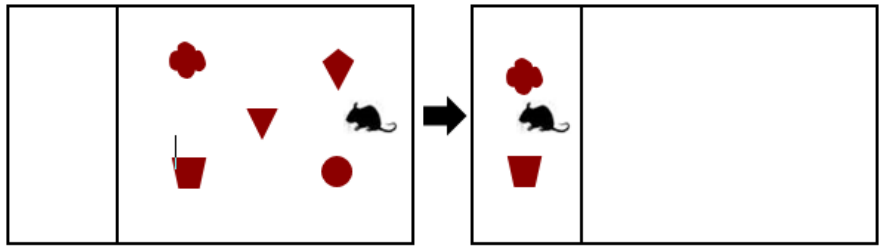


- **Object Based Attention Test (OBA)**
- $Recognition\ Index = \frac{Time\ spent\ with\ Novel\ Object}{Total\ time\ spent\ with\ objects\ in\ test\ chamber}\ x\ 100$

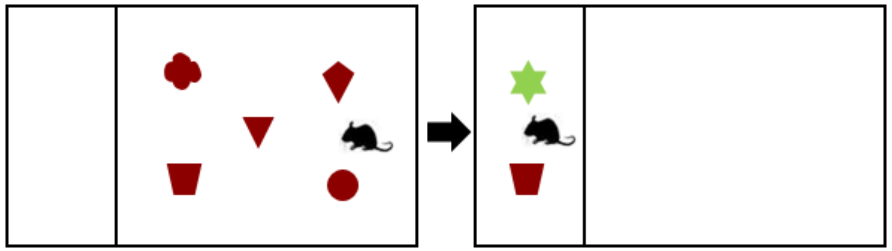
Day 1 **Chamber Habituation**



Day 2 **Object Habituation**



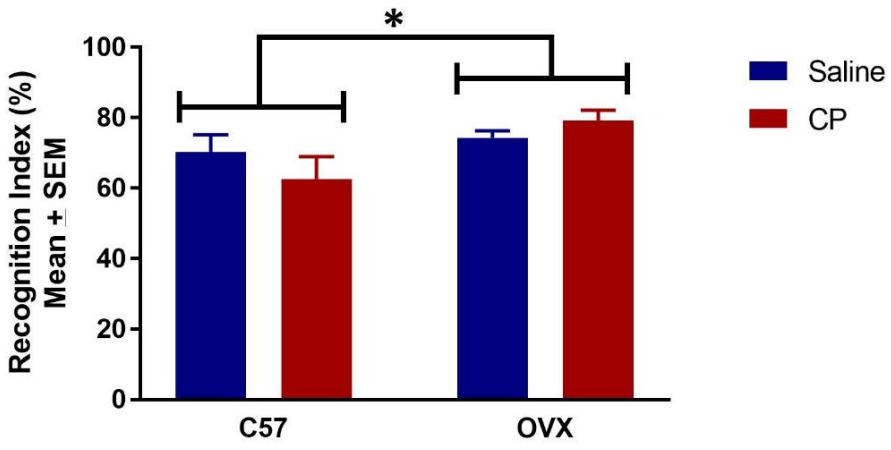
Day 3 **Test Day**



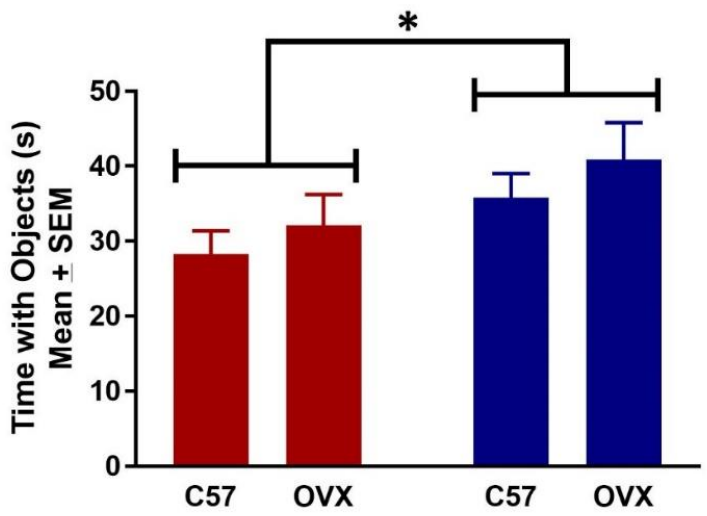
Results: Object Based Attention (OBA)

Recognition Index

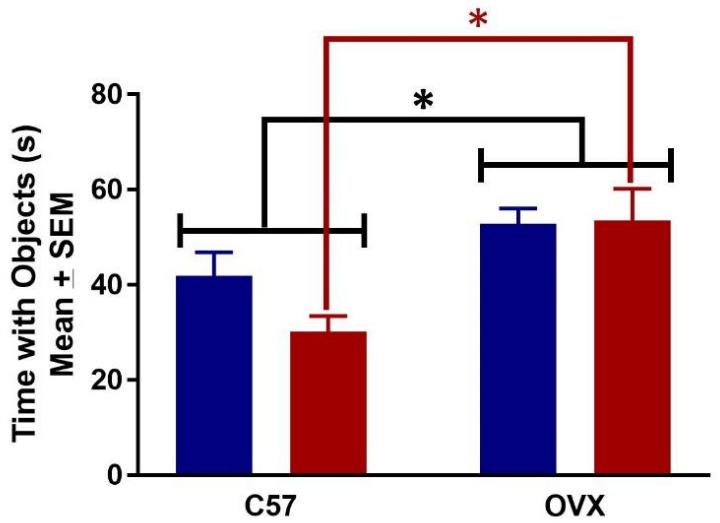
* Measure of attention



Test Day



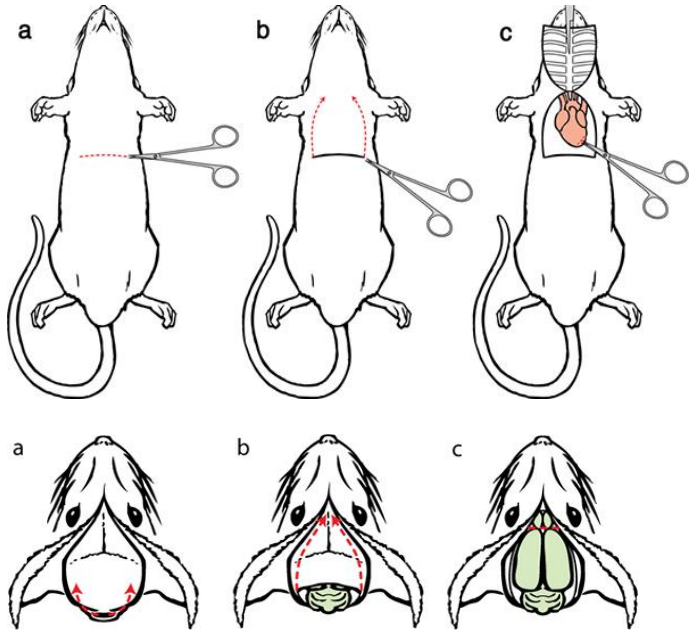
Object Habituation

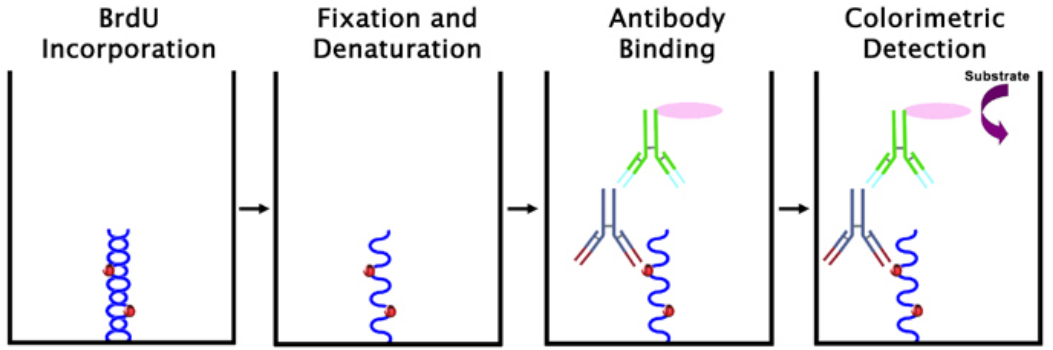


- Cyclophosphamide (CP) did <u>not</u> produce significant effects on recognition index, regardless of ovariectomy.
  - CP treated mice spent significantly shorter periods of time interacting with the objects on days 2 and 3
- Ovariectomized (OVX) mice showed a significantly greater recognition index, regardless of CP treatment.
  - OVX mice spent significantly longer periods of time interacting with the objects on day 2 in both chambers, regardless of CP treatment.

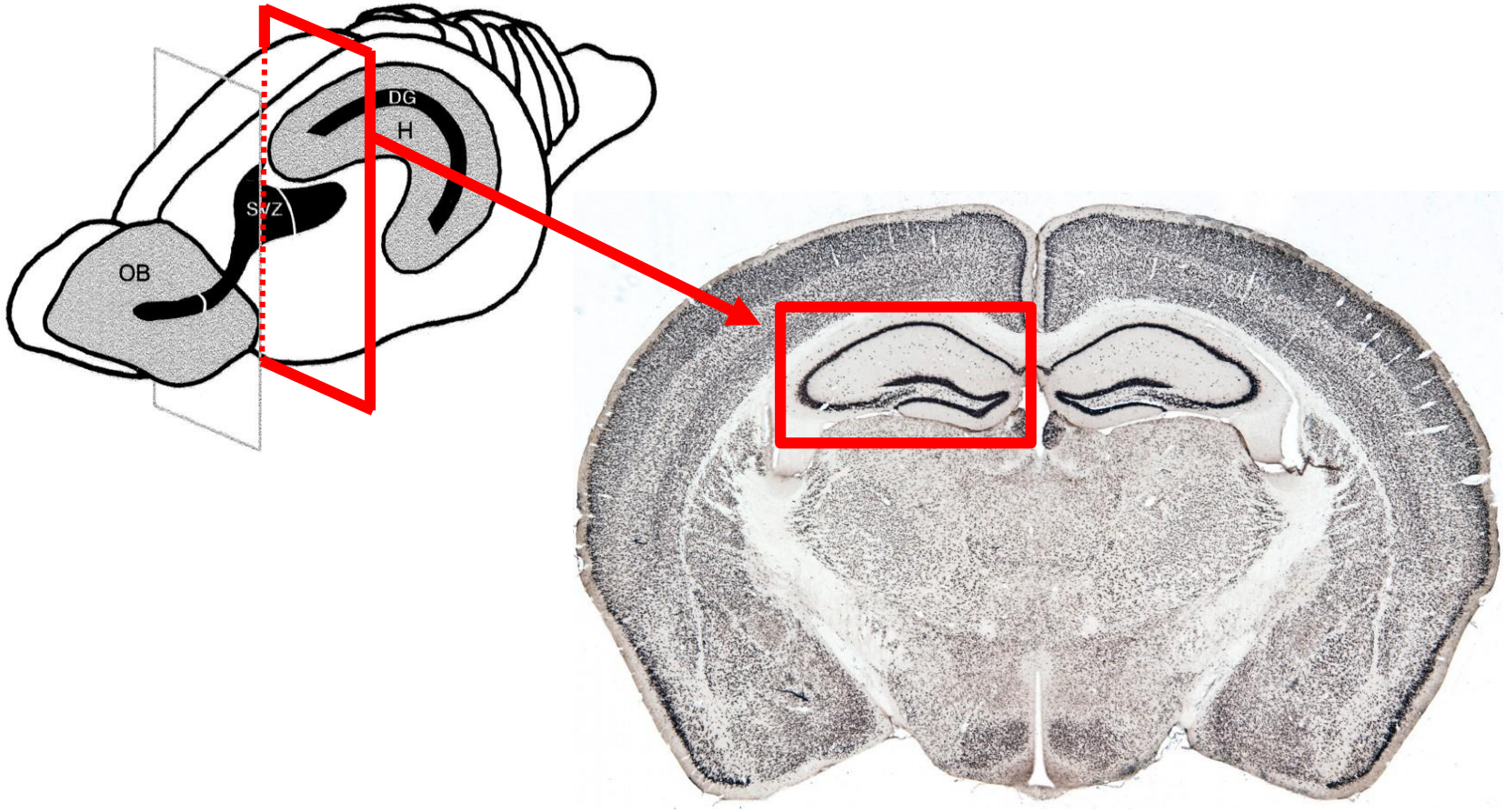
Phase 3: Analysis of Neurogenesis

- **Harvesting of Tissue**
  - Transcardial perfusion with 4% paraformaldehyde (PFA)
- **Cryosectioning Tissue**
  - Microtome: 35 µm coronal sections
- **Immunohistochemistry**
  - BrdU Primary Antibody
  - Cy3 Secondary Antibody (Red)
- **Imaging**
  - Laser Confocal Microscope

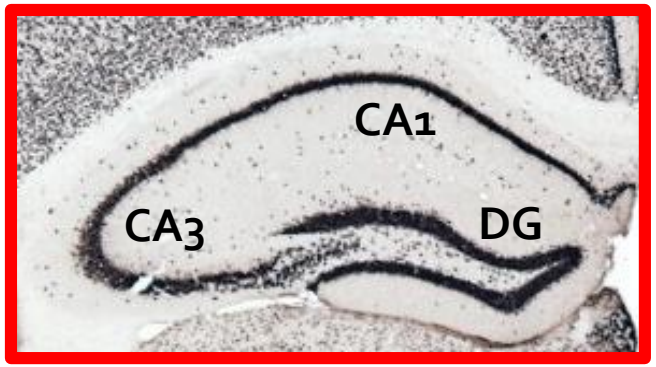




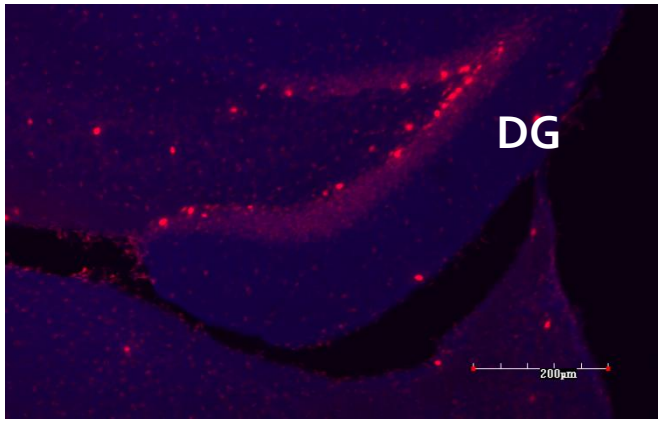
Results: Analysis of Neurogenesis



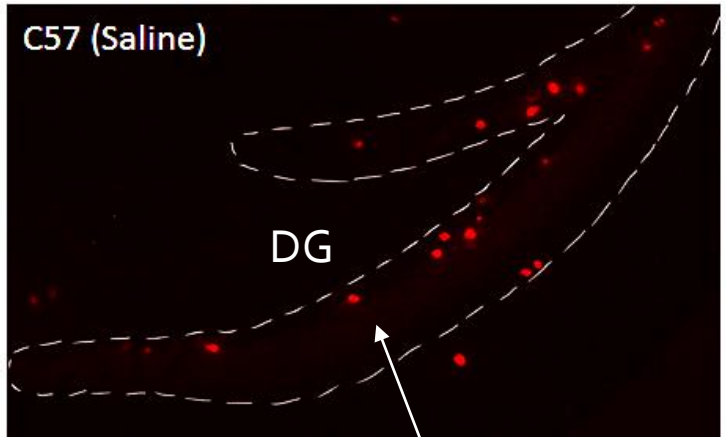
Coronal section through adult mouse brain

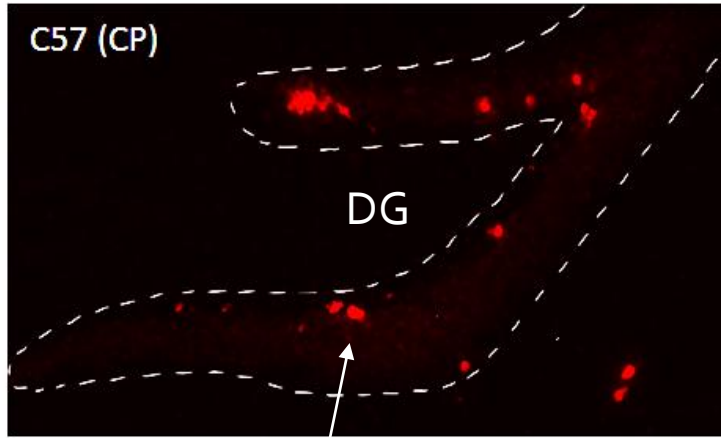


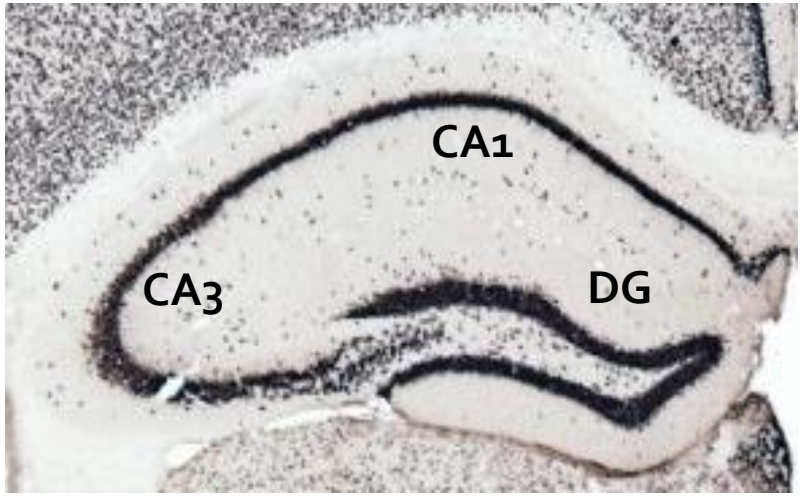
Dentate gyrus (DG) of the hippocampus



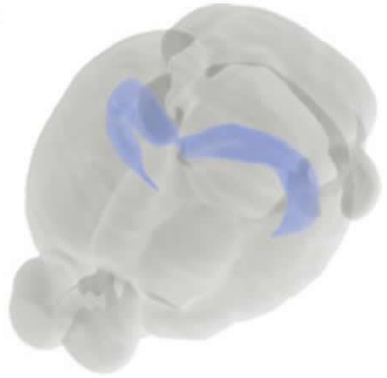
Results: Analysis of Neurogenesis

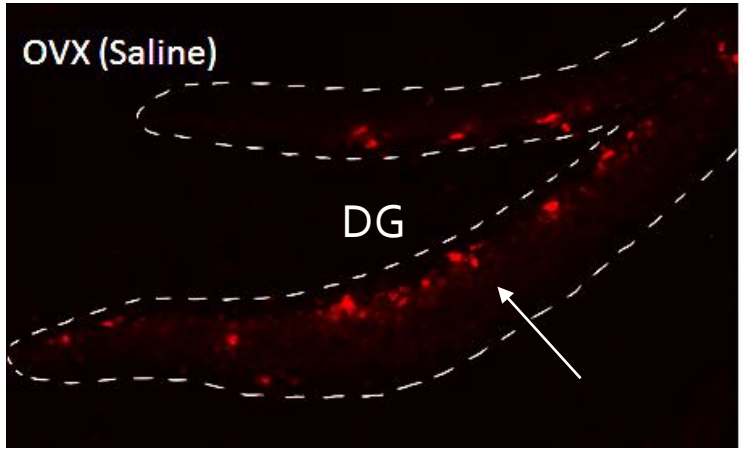


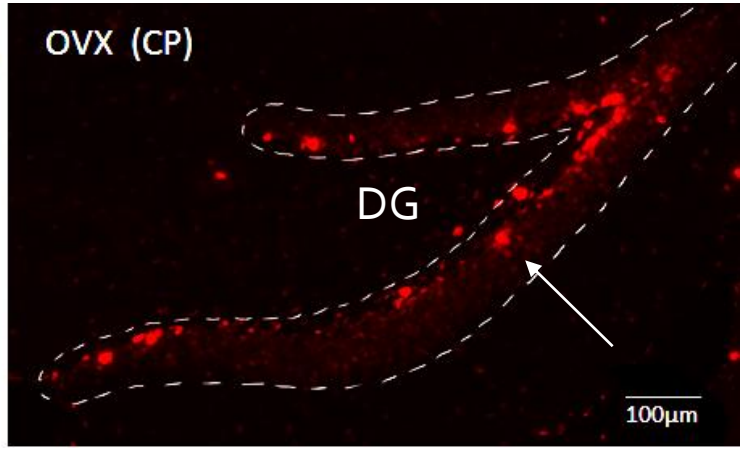




BrdU labeled cells in the dentate gyrus (DG) of the hippocampus (arrows)







Conclusion

- CP did not produce significant effects on EPM, Y-Maze, or OBA
  - CP mice were less exploratory (Y-Maze) and less interactive with objects (OBA), regardless of ovariectomy.
- OVX significantly increased the recognition index (77%) in the OBA, independent of cyclophosphamide treatment.
  - Mice spent significantly more time interacting with the objects
    - This may suggest ovariectomy-induced changes in object exploration pattern or strategy.
  - OVX mice made significantly greater number of arm entries in the EPM, which may suggest increased activity or exploratory behavior.

Clinical Significance

- Collectively, my data shows that chemotherapy and ovariectomy may produce independent effects on cognitive behaviors in the absence of cancer.
- This suggests that the cognitive deficits may be in part due to the chemotherapy treatment
- Ovariectomy alone may alter cognitive behavior, suggesting that pre- and post-menopausal females may have different cognitive deficits when undergoing chemotherapy treatment.

Future Aims

1. Further examine changes in hippocampal neurogenesis
2. Evaluate hippocampus-dependent behaviors such as reversal learning and depressive-like phenotypes.
3. Examine the role of inflammation in hippocampal neurogenesis
    i. Immunohistochemistry staining of inflammatory mediators (i.e. interleukins) and cells (i.e. activated microglia)
    ii. If inflammation is present, examine the role of treatment with non-steroidal anti-inflammatory drugs (NSAIDs) in mediating the effects of CP on hippocampal neurogenesis and related behaviors

Acknowledgements

- Dr. Pradeep Bhide
- Ms. Deirdre McCarthy
- Dr. Pascal Jean-Pierre
- Ms. Melissa Martin
- Dr. Lataisia Jones and Dr. Lin Zhang
- Dr. Mohamed Kabbaj and Katherine Wright
- Dr. Elizabeth Foster and the Office of Student Research
- Ms. Suzanne Baker and Ms. Carson Taylor

Funding:

- FSU College of Medicine Summer Research Fellowship; Jim and Betty Ann Rodgers Chair Fund

Thank You!

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