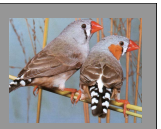
Sex Differences in Cell Proliferation Following Estrogen Depletion in the Adult Zebra Finch



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INTRODUCTION

- Estrogens, produced in the male and female brain via aromatase (estrogen synthase), influence neuronal proliferation and survival.
- In songbirds (e.g., zebra finches), aromatase is expressed in high levels throughout the telencephalon, including the hippocampus.
- Following hippocampal injury in adult female zebra finches, aromatase is upregulated, possibly creating a locally synthesized, estrogen-rich, microenvironment conducive to cell proliferation and brain repair (Peterson et al., 2007).
- The aromatase inhibitor fadrozole suppresses proliferation in the female hippocampus and stem cell-rich subventricular zone (SVZ; Lee, Fernando, Peterson, Allen, & Schlinger, 2007). This effect is reversed with estrogen treatment.
- Comparatively, males have more hippocampal aromatase (Peterson, Yarram, Schlinger, & Saldanha, 2005) and injury-induced cell proliferation following a hippocampal lesion (Law et al., 2006). To determine if this robust response is due to their higher levels of aromarase or its estrogen products, the proposed study will investigate sex differences in cell proliferation following estrogen depletion.
- This study also proposes to examine sex differences in neuronal proliferation.

METHODS

- Thirty two adult male zebra finches and thirty two adult female zebra finches will be purchased from a local vendor, individually housed, and randomly assigned to one of 8 groups (*n*/group = 8).
- In order to deplete estrogen, male and female zebra finches will be fed either fadrozole or saline daily for 18 days.
- Birds will receive either no lesion, or a unilateral lesion to the right hippocampus on day 16.
- To label mitotic cells, all birds will receive one intramuscular injection of BrdU on day 17.
- On day 18, all birds will be euthanitized and transcardially perfused with 0.1 M phosphate buffered saline followed by 4% paraformaldehyde. Brains will be postfixed in 4% paraformaldehyde for 24 hours, transferred to 0.1M phosphate buffer, embedded in 8% gelatin, and then cut into 5 equivalent sets of 40μm coronal sections using a vibratome.
- BrdU immunohistochemistry (IHC) will be used to visualize newly born cells. Following standard IHC procedures previously reported by Lee et al. (2007), primary incubation will be achieved with 1:500 anti-BrdU (Roche). Secondary incubation with 1:200 biotinylated horse anti-mouse IgG (Vector) will be followed by incubation in 1:200 avidin-biotin-peroxidase complex (Vector) and detection will be accomplished using diaminobenzidine (Sigma)
- BrdU-IR cells will be visualized using DIC illumination on a Nikon E-800 microscope using NeuroLucida software (MicroBrightField, Inc.) and will be counted in the hippocampus, septum, proximal SVZ (pSVZ; adjacent to the hippocampus), and distal SVZ (dSVZ; not adjacent to the HP).
- To determine phenotype of BrdU-positive cells, tissue will be processed with fluorescent tags recognizing BrdU and Neuronal Nuclei (NeuN; a neuronal marker). Cells double-labeled with BrdU (green) and NeuN (red) will denote newly born neurons (yellow) and will be verified using confocal microscopy.

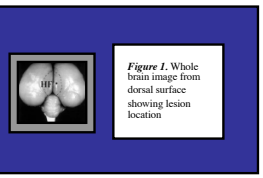

Figure 1. Whole brain image from dorsal surface showing lesion location

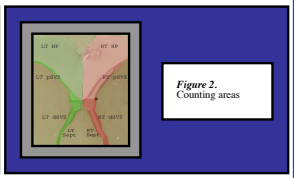

Figure 2. Counting areas

Group	Sex	Days 1-15	Day 16	Day 17	Day 18
1	Male	Fadrozole	Fadrozole + HP Lesion	Fadrozole + BrdU	Fadrozole + Perfusion
2	Male	Saline	Saline + HP Lesion	Saline + BrdU	Saline + Perfusion
3	Male	Fadrozole	Fadrozole + Sham	Fadrozole + BrdU	Fadrozole + Perfusion
4	Male	Saline	Saline + Sham	Saline + BrdU	Saline + Perfusion
5	Female	Fadrozole	Fadrozole + HP Lesion	Fadrozole + BrdU	Fadrozole + Perfusion
6	Female	Saline	Saline + HP Lesion	Saline + BrdU	Saline + Perfusion
7	Female	Fadrozole	Fadrozole + Sham	Fadrozole + BrdU	Fadrozole + Perfusion
8	Female	Saline	Saline + Sham	Saline + BrdU	Saline + Perfusion

Table 1: Group Descriptions

PREDICTED RESULTS

Injury-Induced Cell Proliferation

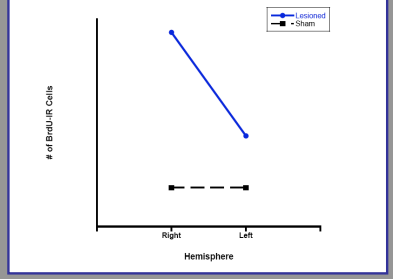

Figure 4. Lesioned hippocampus > Unlesioned hippocampus as long as estrogens are being synthesized

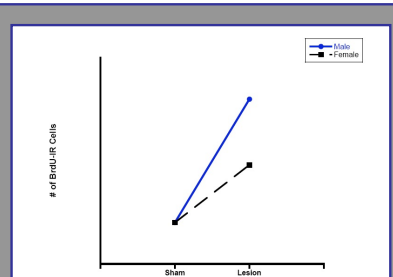

Figure 5. Lesioned males > Lesioned females as long as estrogens are being synthesized.

Estrogen Depletion

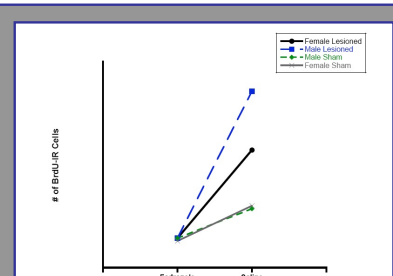

Figure 6. Estrogen depletion inhibits cell birth and eliminates sex differences.

TISSUE IMAGES

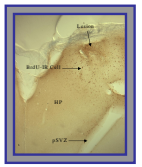

Figure 7. Photomicrograph of a hippocampal lesion following BrdU IHC

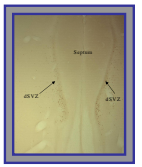

Figure 8. Photomicrograph of the dSVZ and Septum following BrdU IHC

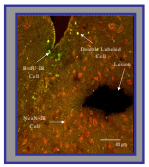

Figure 9. Photomicrograph of a hippocampal lesion following BrdU/NeuN fIHC

CONCLUSIONS

Injury-Induced Cell Proliferation

- Injury to the hippocampus is expected to result in an increase in cell proliferation in saline-fed birds in all regions examined.
- Moreover, a unilateral hippocampal lesion should result in an increase in cell proliferation in both the injured and uninjured hemispheres.

Fadrozole

- Fadrozole is expected to suppress cell proliferation in both sexes, regardless of lesion. Thus, fadrozole-fed birds should have significantly fewer new cells than saline-fed birds, indicating that cell proliferation is highly estrogen-dependent.

Sex Differences

- Supporting previous research, unlesioned males and females should have equal rates of cell proliferation.
- While hippocampal injury is expected to increase cell proliferation in both sexes, saline-fed males are expected to have higher rates of cell proliferation than saline-fed females. This would indicate that males and females respond differently to brain injury.
- If males show more injury-induced cell proliferation due to higher levels of hippocampal aromatase, administration of fadrozole should eliminate that sex difference. This would indicate that estrogen creates sex differences in response to brain injury.

Implications

- Brain injury is subject to sex differences and hormonal regulation. Examination of these sex differences holds profound implications for designing estrogen-based therapies to minimize loss of function due to injury or disease, and facilitate recovery.
- For example, females may benefit more from estrogen-based therapies following brain injury possibly due to their preexisting lower levels of aromatase.

REFERENCES

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ACKNOWLEDGEMENTS

Supported by NSF DBI-0116477 and NIH SCORE S06-GM063119 (DWL).