



Effect of 17 β -estradiol on nestin and aromatase B expression in larval zebrafish

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Introduction

The brains of teleost fish have very high regenerative capabilities when compared to the human brain. The abundance of radial glial cells (RGCs) that persist in the adult fish brain may be responsible for this ability to regenerate. Radial glial cells are neuronal progenitor cells and can thus differentiate into neurons.² In the teleost brain, aromatase B, the enzyme that converts androgens into estrogens, is expressed exclusively by RGCs.⁴ In addition, these progenitor cells are regulated by estrogens.³ Nestin is an intermediary filament expressed by neural progenitor cells. Radial glial cells have been shown to express nestin however, its expression is lost when the progenitor cells differentiate.¹ This intermediary filament is therefore critical to neurogenesis. The regulation of nestin expression remains unknown. We wanted to observe the effect of estradiol on RGCs during zebrafish brain development at the larval stage and tested the hypothesis that estradiol could stimulate nestin expression.

Methodology

17 β -estradiol solution :

- 17 β -estradiol (E2) powder was dissolved in ethanol to create a concentrated stock. The E2 stock was added to fresh embryo media to make a 10⁻⁷M solution.

Control solution :

- Pure ethanol was added to fresh embryo media.

Cyp19a1b-GFP :

- Embryos resulting from a cross between cyp19a1b-GFP and golden wild type fish were collected and the larvae were screened by fluorescent microscopy at 6 days post-fertilization (dpf).
- At 8 dpf, 6 fish were either exposed to the E2 solution or to the control solution.
- Fluorescent photos were taken and the solutions were refreshed every 24 hours.

Nestin-GFP :

- Embryos were collected resulting of a cross between nestin-GFP and golden wild type fish.
- At 10 hours post-fertilization (hpf) 30 fish were either exposed to the E2 solution or to the control solution.
- The solutions were refreshed every 24 hours.
- At 2 dpf the embryos were screened by fluorescent microscopy.
- Fluorescent photos were taken every 24 hours.
- To insure reproducibility of the data the nestin-GFP experiment was conducted twice.

Corrected Total Fluorescence (CTF)

- CTF = Integrated Density – (Area * Mean fluorescence of background readings)
- With the values determined with the “measure” function in ImageJ.

Results

Cyp19a1b-GFP exposure to estradiol :

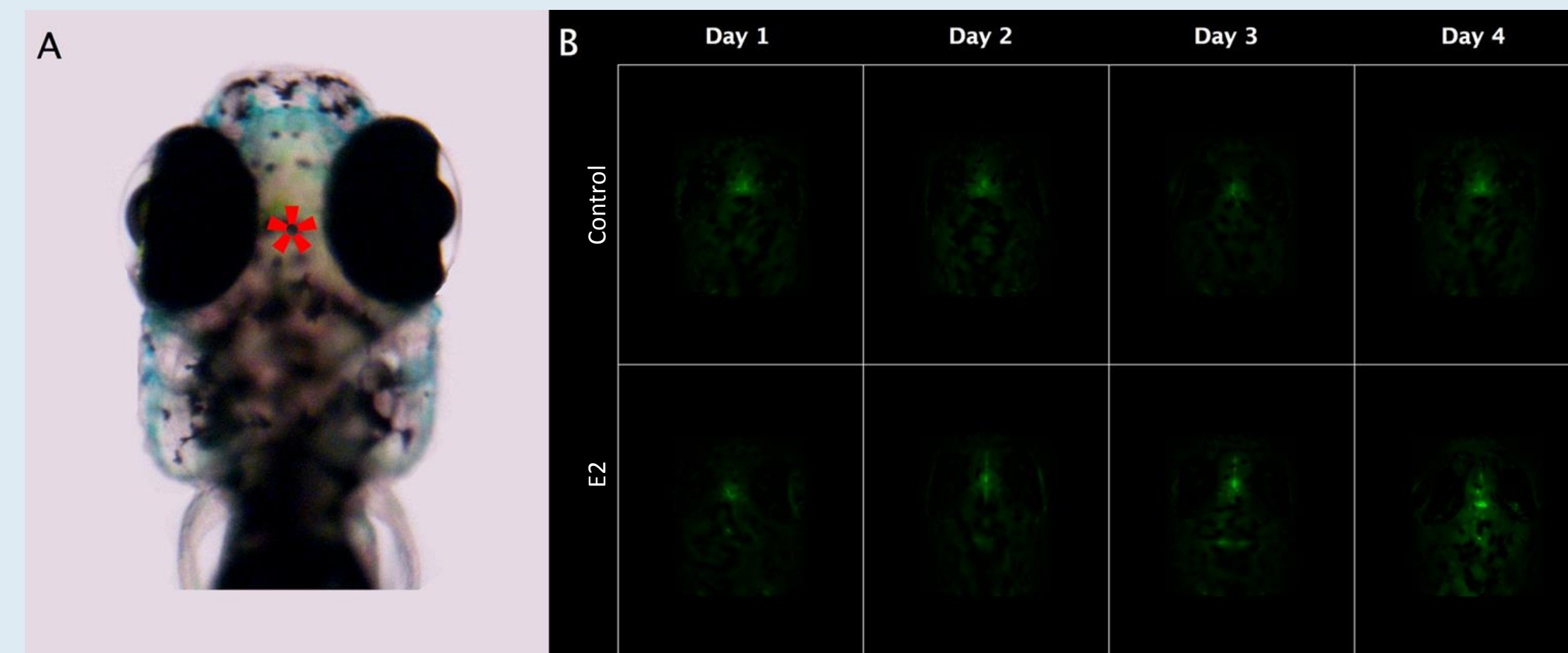


Figure 1. Effect of estradiol on the fluorescent expression of cyp19a1b in the brain of cyp19a1b-GFP transgenic zebrafish larvae. A) Zebrafish head under emitted light. The red asterisk denotes the area of interest. B) Fluorescent view of control and estradiol treated zebrafish at each day of exposure.

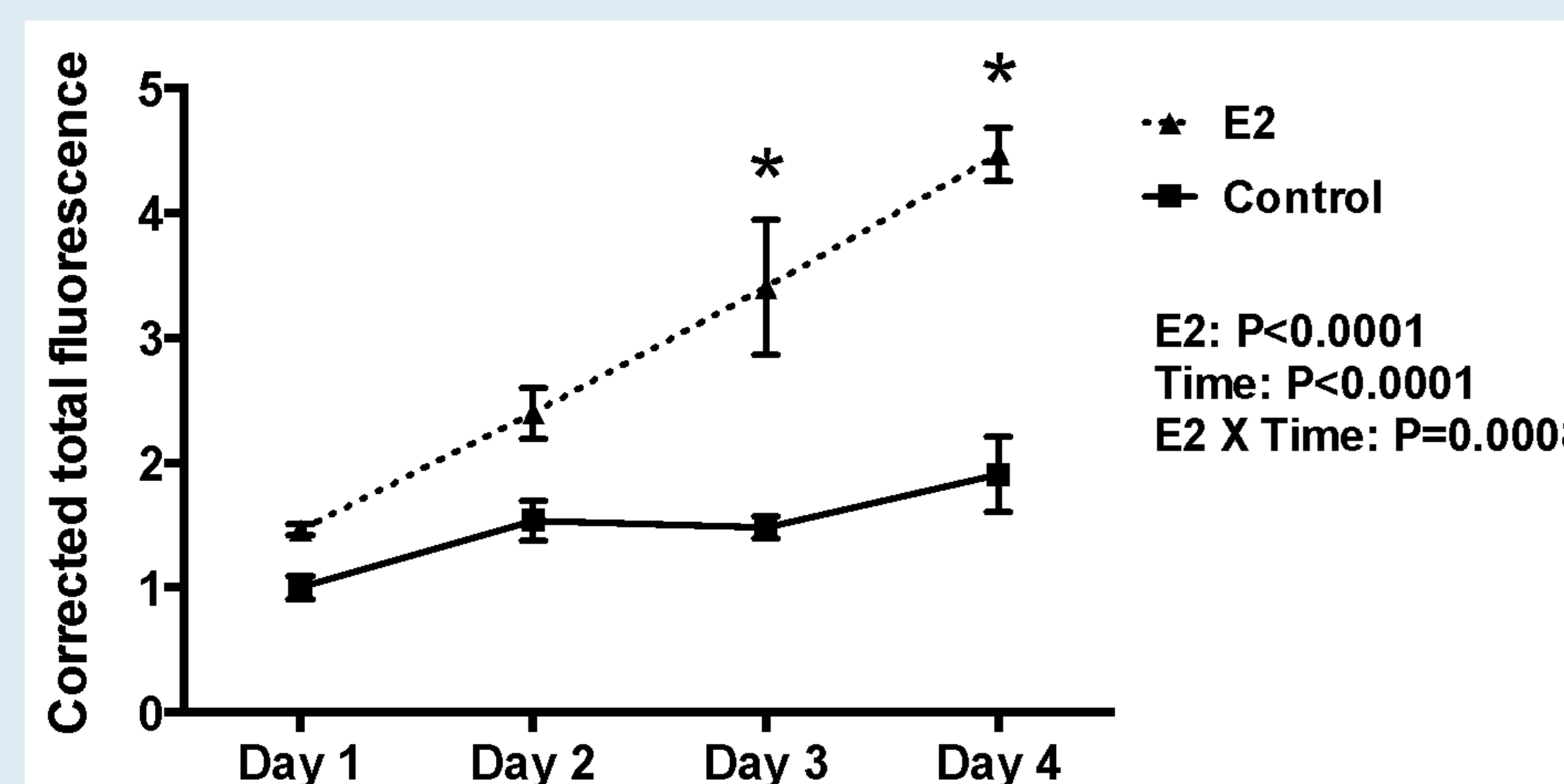


Figure 2. Effect of estradiol on fluorescent intensity of cyp19a1b in the brain of cyp19a1b-GFP transgenic zebrafish larvae. Zebrafish exposed to estradiol at 3 and 4 days had a significant increase in fluorescence. A two-way ANOVA test was performed to determine the effects of estradiol on cyp19a1b expression. Treatment groups marked by an asterisk are significantly different compared to control groups. Error bars represent SEM. Days 1-3 n=6; Day 4 n=5 control and n=4 treated.

Nestin-GFP exposure to estradiol :

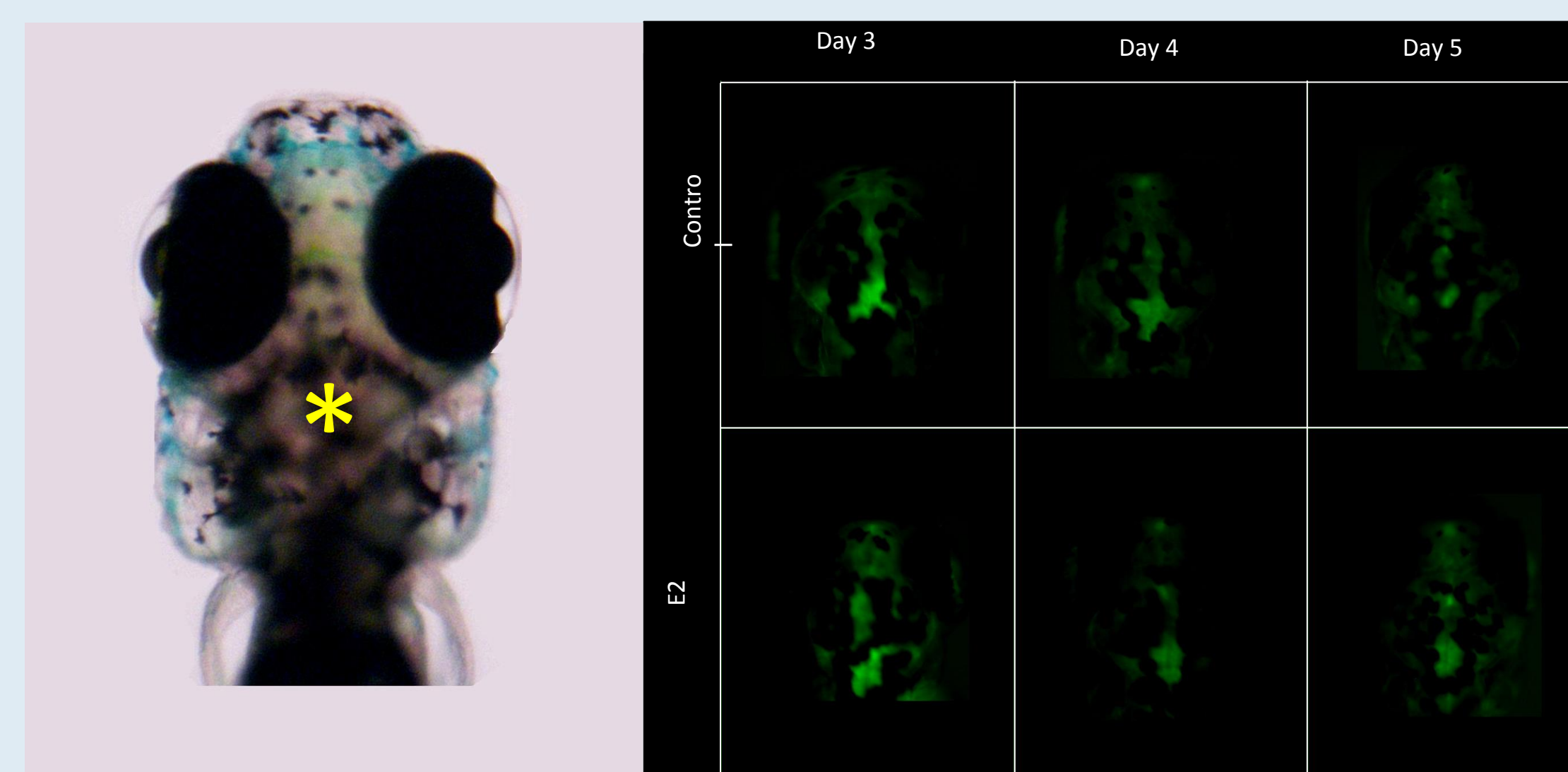


Figure 3. Effect of estradiol on the fluorescent expression of nestin in the brain of nestin-GFP transgenic zebrafish larvae. A) Zebrafish head under emitted light. The yellow asterisk denotes the area of interest. B) Fluorescent view of control and estradiol treated zebrafish at each day of exposure.

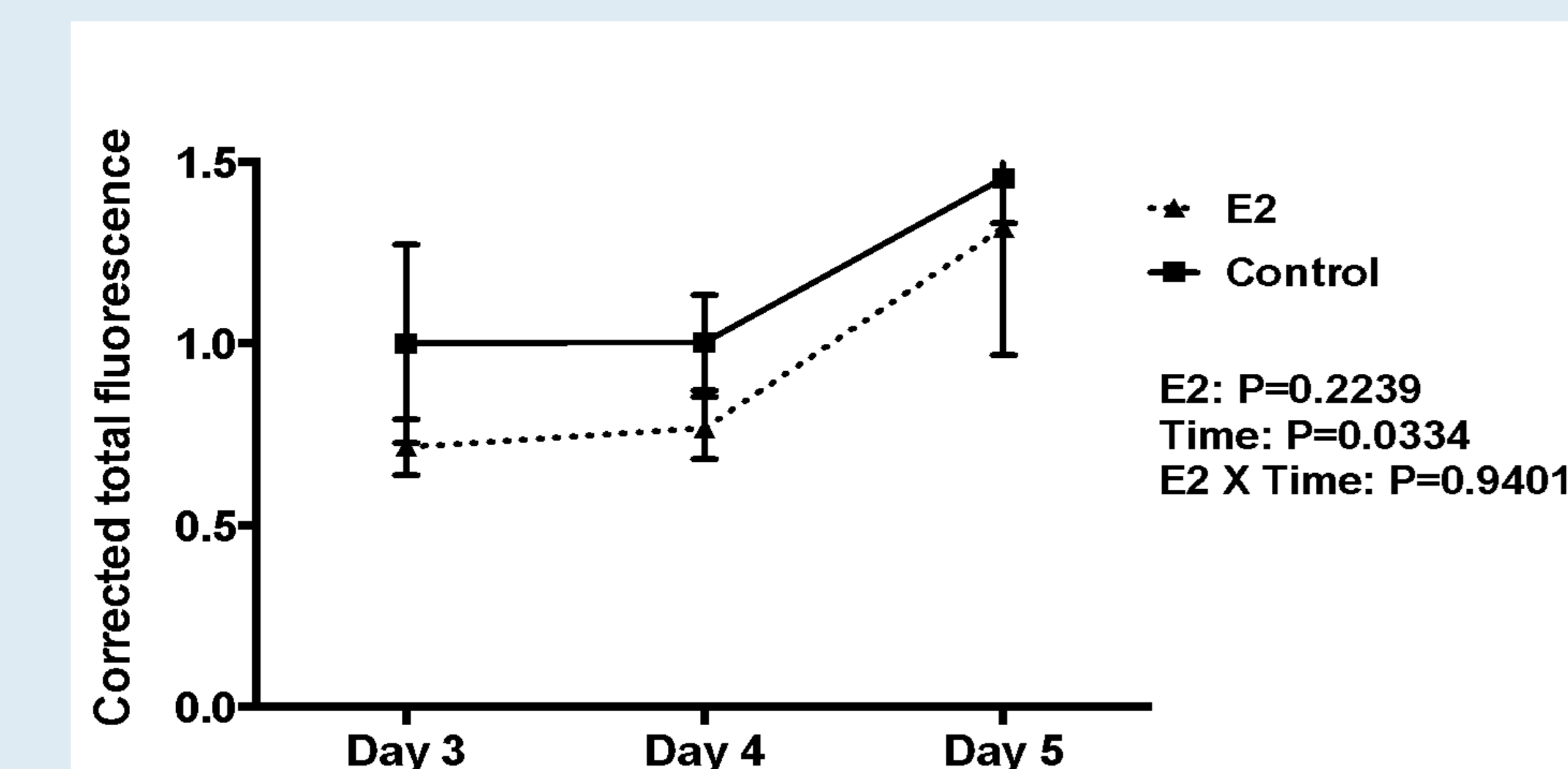


Figure 4. Effect of estradiol on fluorescent intensity of nestin in the brain of nestin-GFP transgenic zebrafish larvae. Zebrafish exposed to estradiol had not significant increase in fluorescence. A two-way ANOVA test was performed to determine the effects of estradiol on nestin expression. Error bars represent SEM. CTF was calculated based off the fluorescent images from the second nestin:GFP experiment. Day 3 n=6 and n=4 treated; Day 4-5 n=6.

Discussion & Conclusions

- The results of this study demonstrate that estradiol leads to an increase of fluorescence in the cyp19a1b-GFP transgenic fish indicating that E2 increases aromatase B-positive radial glial cells in the larval zebrafish brain.
- However, the same estradiol treatment did not increase fluorescence in the nestin-GFP fish and thus, E2 does not affect nestin expression in the brain of zebrafish larvae.
- These results do not support the hypothesis of this study that estradiol would increase nestin.

Future studies

- To continue this research it may be informative to quantify aromatase B and nestin expression in zebrafish larvae using other protein quantification techniques, such as Western blot this would ensure that the observed results are not due to limitations of image acquisition.
- In addition, it would be interesting to perform a similar experiment with adult zebrafish and observe the effects of injected estradiol on neurogenesis using nestin as a marker for progenitor cells.

References

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- 3 Xing L, Esau C and Trudeau VL (2016) Direct Regulation of Aromatase B Expression by 17 β -Estradiol and Dopamine D1 Receptor Agonist in Adult Radial Glial Cells. *Front. Neurosci.* 9:504.
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