

Injury and Glial Aromatization Combine to Upregulate Bone Morphogenetic Protein 2 in the Adult Zebra Finch Brain.

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Introduction:

□ “Brain injury is the leading cause of death and disability worldwide.” (International Brain Injury Association).

□ Neuroprotection and neurogenesis may be directed toward the prevention of functional loss and even the repair of brain damage (Alvarez-Buylla *et al.* 2000).

□ In songbirds, brain injury results in a robust upregulation of the estrogen synthesizing enzyme aromatase.

□ This glial aromatization is neuroprotective and likely neurogenic (Saldanha *et al.* 2005, Lee *et al.* 2007)

□ Much like aromatase, BMPs have been shown to mitigate cell death and enhance neurogenesis (Hattori *et al.* 1999, Colak *et al.* 2008).

□ In non-neural tissues, estrogens affect the expression of BMPs (Zhou *et al.* 2003, Zhang *et al.* 2005).

□ BMPs, however, have remained uncharacterized in songbirds...

Methods and Results:

Experiment 1: BMPs are expressed in the adult zebra finch brain

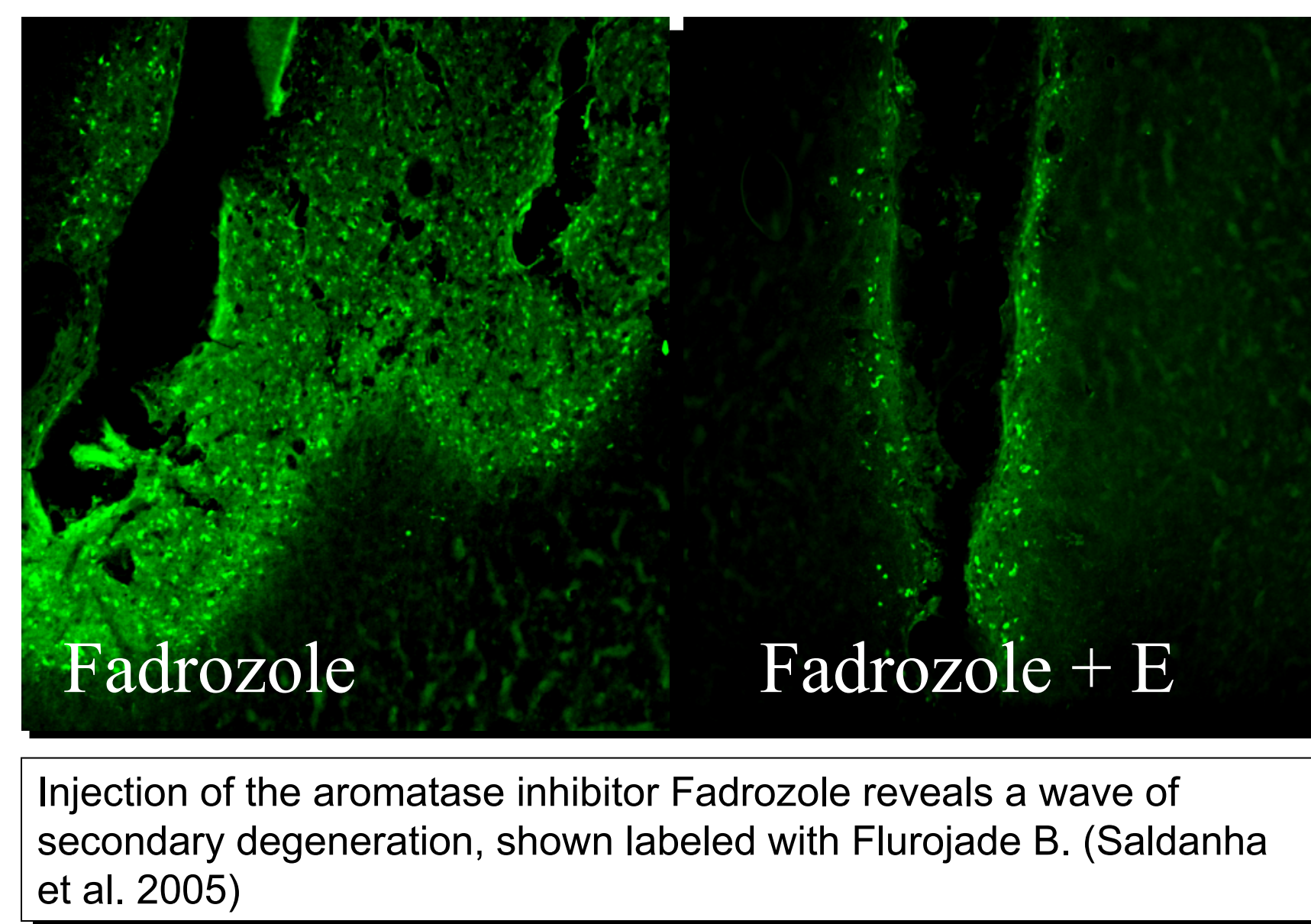
□ The expression of BMPs 2, 4, 6, and 7 in the zebra finch brain was established by...

- extracting RNA from the forebrains of adult birds,
- reverse transcribing and amplifying the mRNA
- sequencing the resultant cDNA

□ Sequences were obtained for all 4 transcripts and demonstrated a remarkable degree of similarity to known BMP sequences in other vertebrates.

ZebraFinch	123
Chicken	123
ZebraFinch	243
Chicken	243
ZebraFinch	363
Chicken	363
ZebraFinch	403
Chicken	403

Partial BMP2 sequence obtained from zebra finch RNA is 95% similar to that in the chicken. Pictured here, similarities in the sequences are indicated in red, differences in white. Comparable results were obtained for BMPs 4, 6, and 7, which were 93, 80, and 92% similar to chicken sequence, respectively.

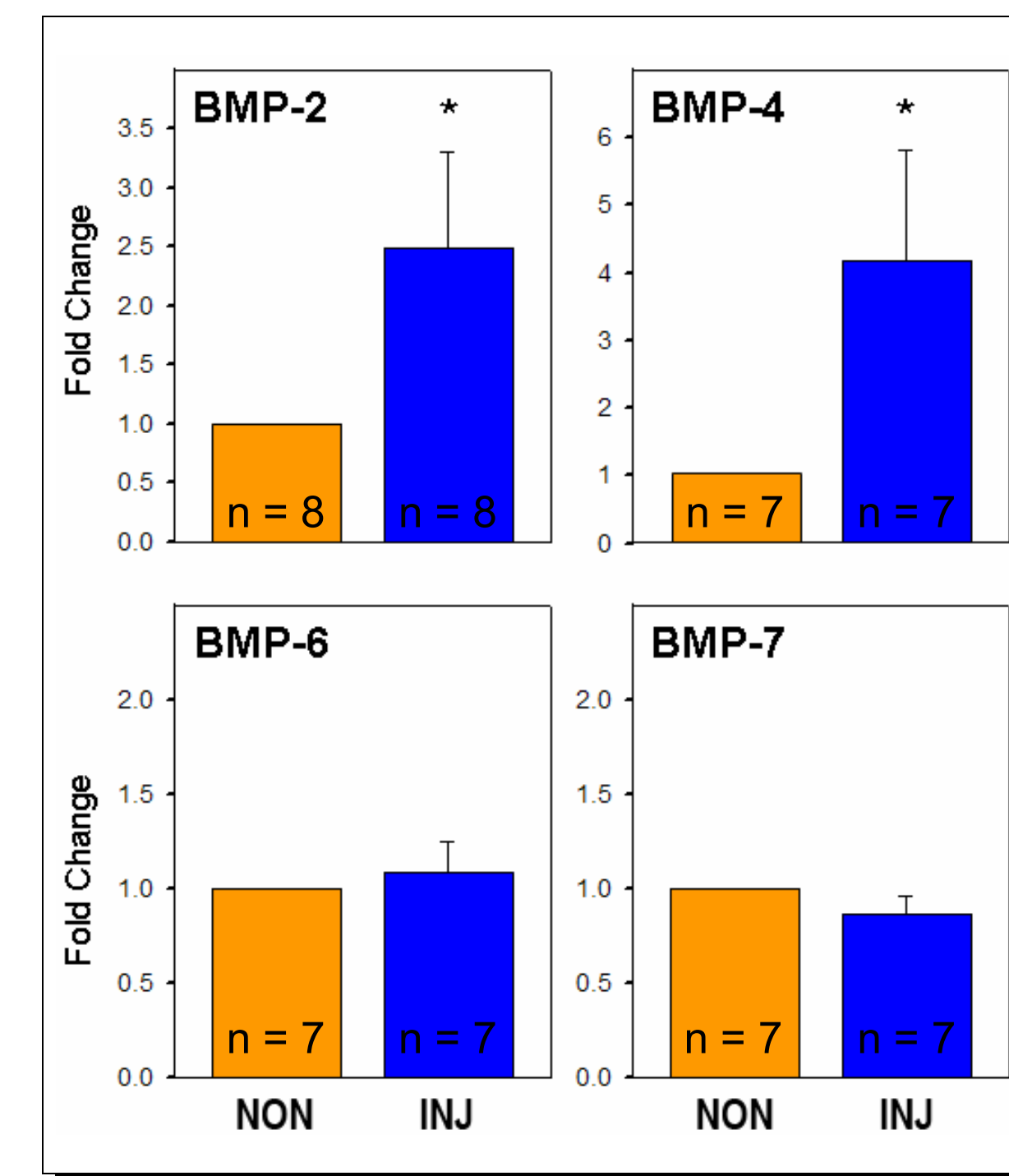


Experiment 2: Brain injury affects BMP expression.

□ BMP receptors are upregulated after injury (Charytoniuk *et al.* 2000).

□ We therefore determined the effect of neural insult on BMP expression by...
 ➢ injuring one side of the brain
 ➢ extracting and amplifying the Transcripts (Real-time PCR)
 ➢ comparing the abundance across Injured and uninjured hemispheres (Walters and Saldanha, 2008).

Expression of BMPs 2 and 4, but not 6 and 7, was increased 24 hours post-injury. Asterisks indicate $p < 0.05$ main effect of injury on delta-Ct values in a 2-way repeated measures ANOVA. Graphs represent fold change of mRNA expression based on the delta-delta Ct method, assuming 100% primer efficiency.

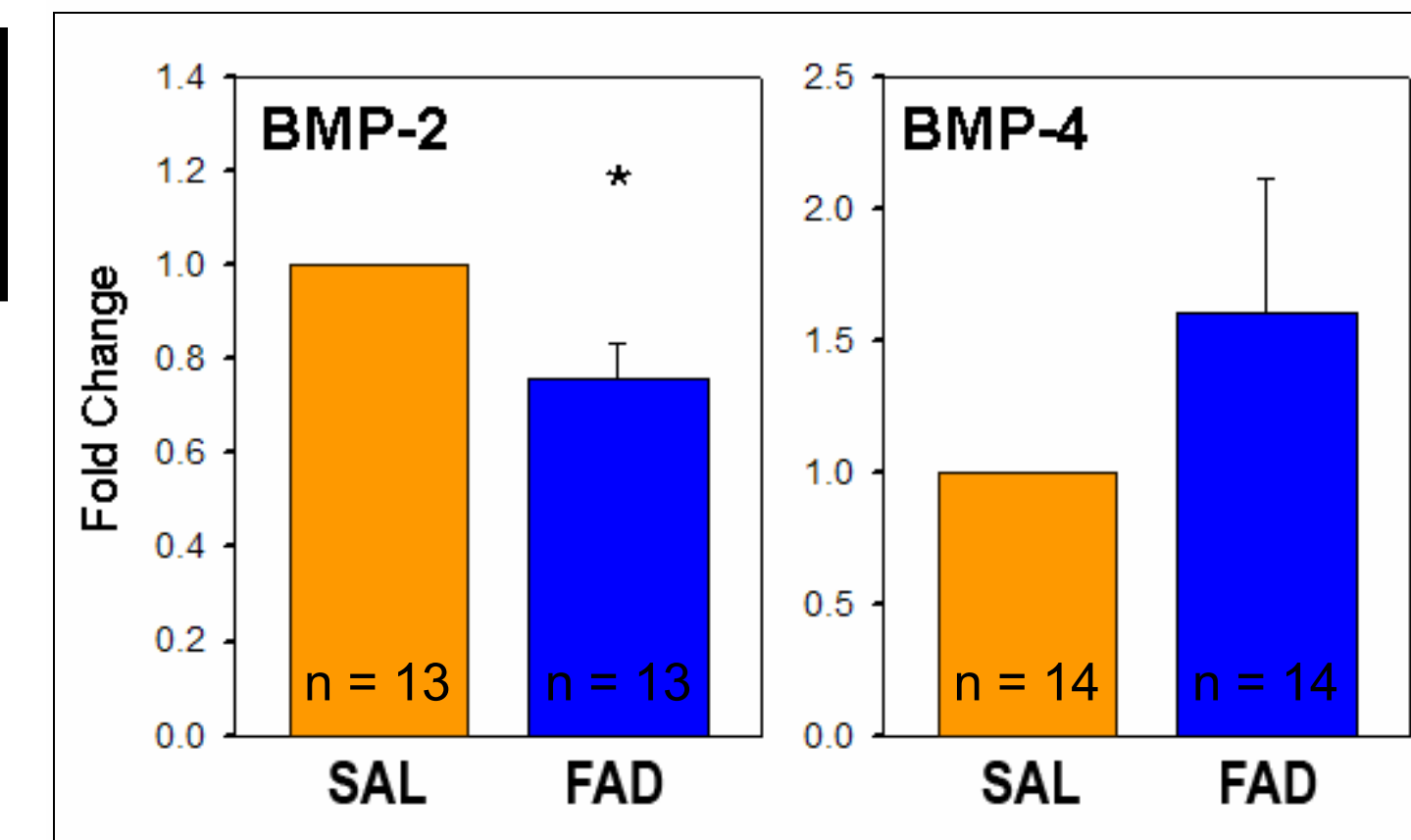
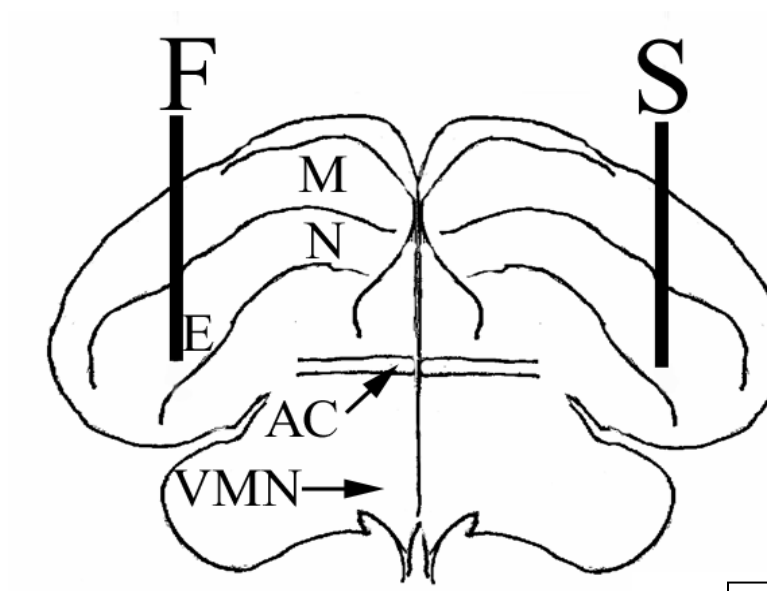


Experiment 3: Glial aromatization affects BMP expression.

□ Estrogens have been implicated in the expression of BMPs 2 and 4 (Zhou *et al.* 2003, Zhang *et al.* 2005).

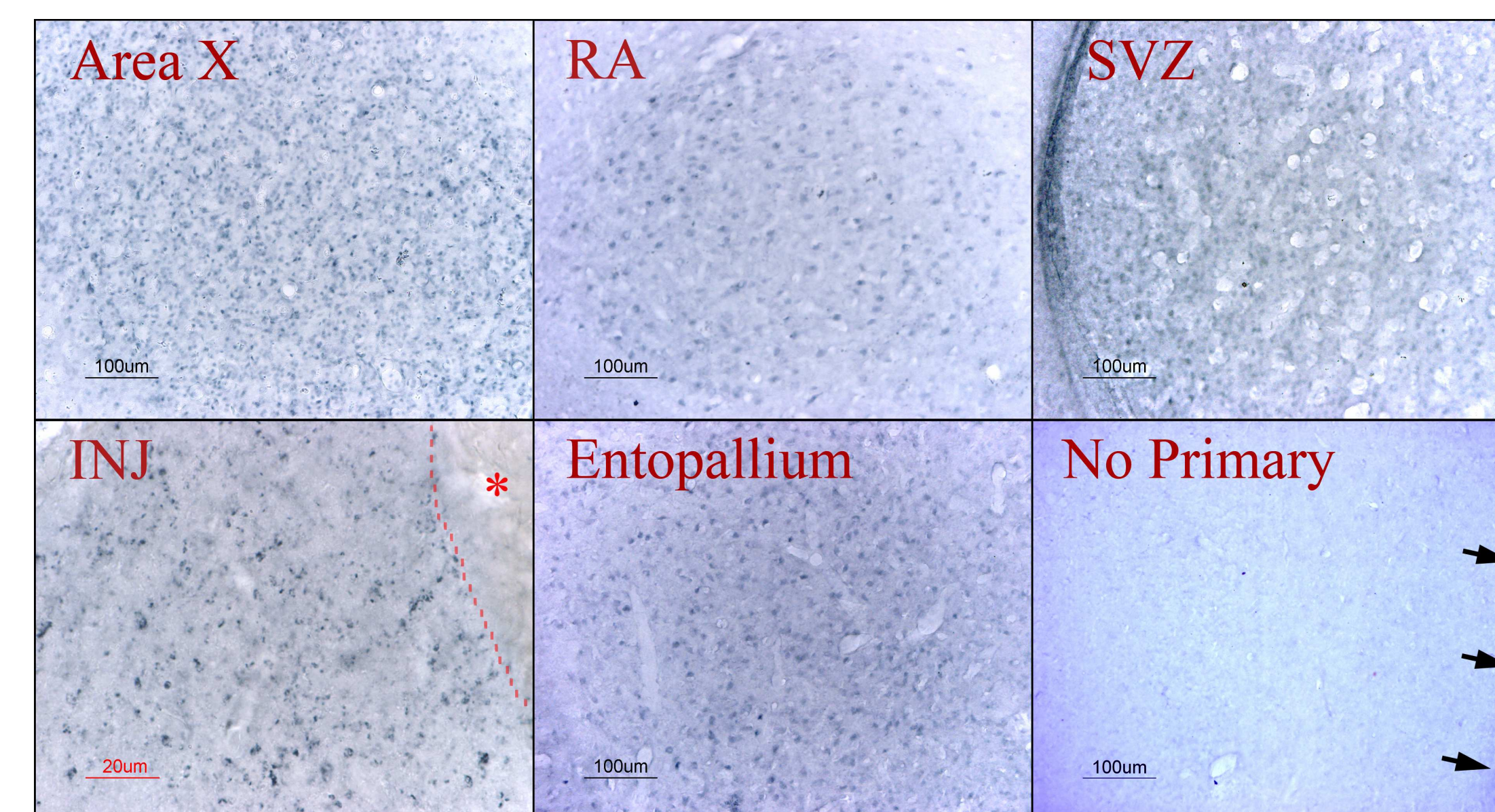
□ To determine the influence of injury induced aromatase on the expression of these BMPs, we compared mRNA levels across fadrozole or vehicle treated hemispheres.

Diagram of a coronal section of the zebra finch brain demonstrating the experimental design. Fadrozole (F, 5µL at 20mg/mL) was injected into one hemisphere, while vehicle (S, saline) was injected contralaterally.



Aromatase inhibition resulted in a significant ($p < 0.05$, 2-way repeated measures ANOVA of delta Ct values) decrease in BMP-2 mRNA expression. Fadrozole administration did not significantly affect BMP-4 expression. Fold change is based on the delta-delta Ct method, assuming 100% efficiency.

Experiment 4: BMP2 is expressed in several forebrain areas.



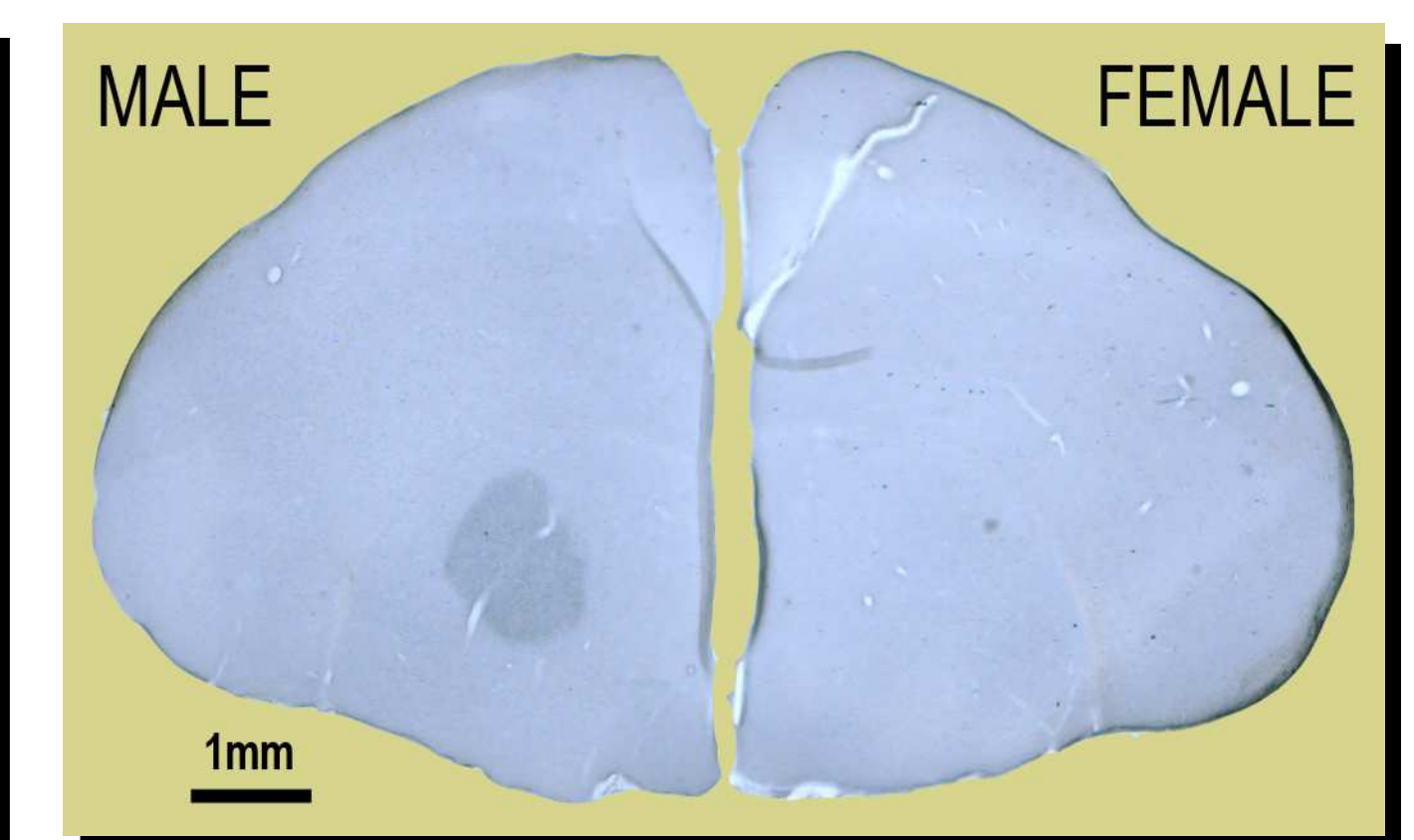
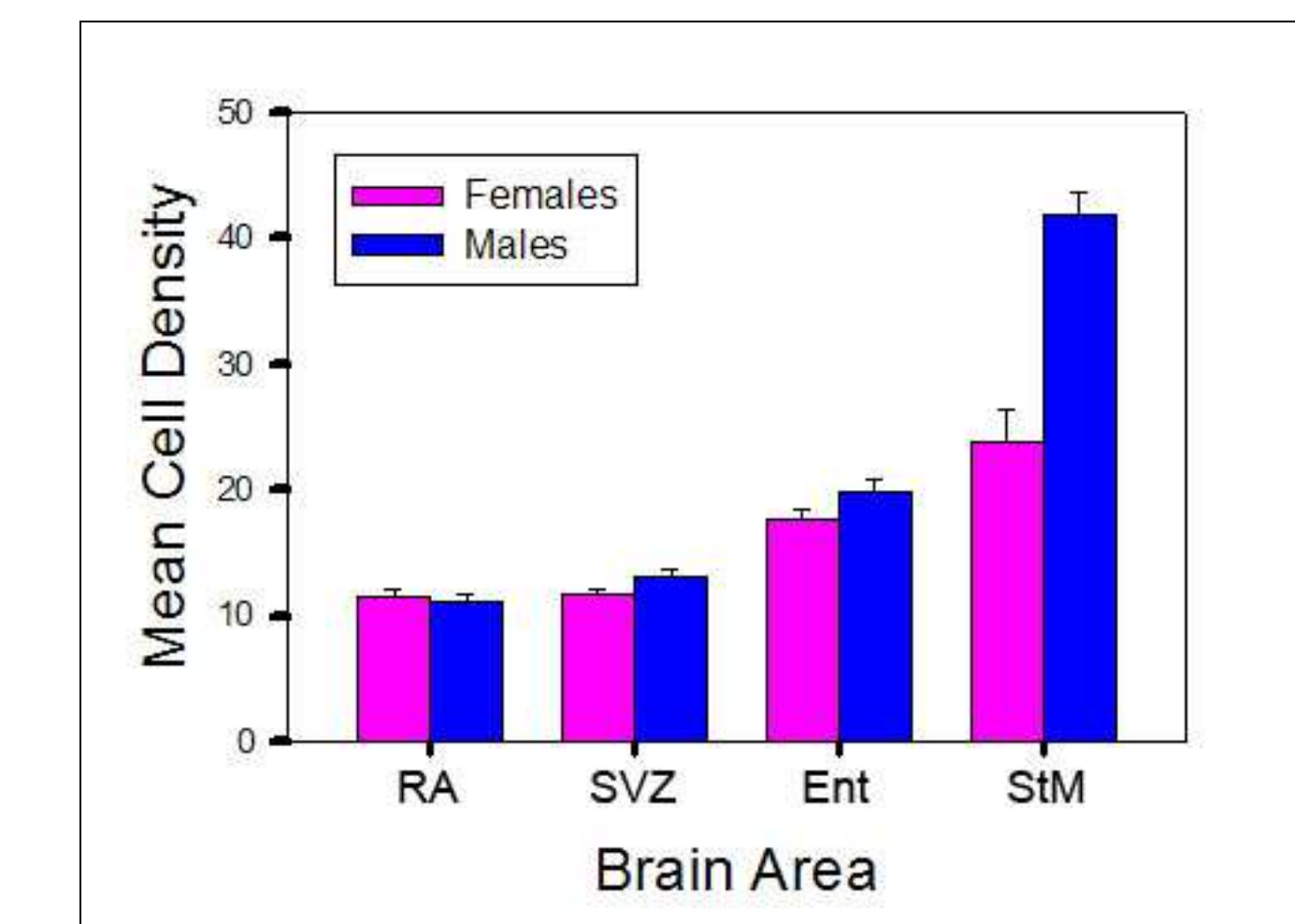
Immunocytochemistry against BMP2 revealed prominent protein expression in the song nuclei Area X and RA (robust nucleus of the archistriatum), as well as in the neurogenic subventricular zone (SVZ). BMP2 immunoreactivity was also detected in the entopallium and in tissue adjacent to the injury. Arrowheads indicate the midline.

Experiment 5: BMP 2 expression in the zebra finch brain is sexually dimorphic.

□ Songbirds provided the first conclusive model for structural sex differences in the brain (Nottebohm and Arnold, 1976).

□ These differences were observed in areas involved in song learning and production (in species where males sing and females do not).

□ BMP2 expression differs across brain areas and across sex in adult zebra finches.



Left: Comparison of cell densities across sex and brain area. A four-way, repeated measures ANOVA revealed significant differences ($p < 0.005$) across sex and brain area, but not across injury or time after injury ($p > 0.05$). The main effect across sex was driven by the mean cell density values in the medial striatum (StM). Error bars represent one standard error. (SVZ = subventricular zone, Ent = entopallium) Right: BMP2 staining distinguishes Area X from the surrounding medial striatum (StM) in males, but not females.

Discussion/Future Directions:



□ BMP2 can enhance gliogenesis, neurogenesis, and cell survival (Zhang *et al.* 2008, Hattori *et al.* 1999).

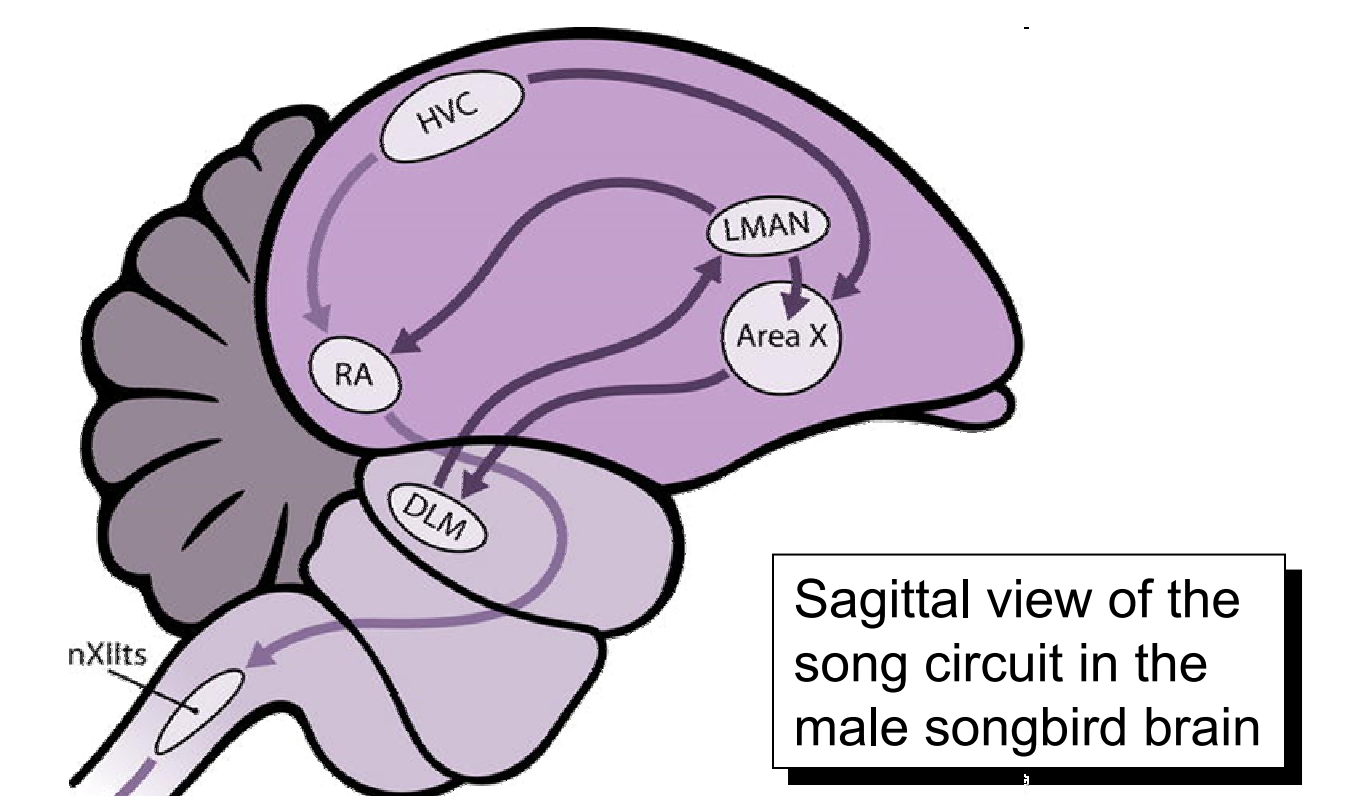
□ Future studies will test whether (or not) the neuroprotective or neurogenic effects of aromatase upregulation are mediated by BMP2.

□ Additionally, BMP signaling enhances synaptic plasticity (Sun *et al.* 2007) and deletions in the human BMP2 gene are correlated to cognitive dysfunction (Lalani *et al.* 2008).

□ The presence of BMP2 in Area X and RA suggests that it may be involved in memory and learning in the songbird brain as well.

□ Songbirds possess a discrete, well-mapped neural circuit for song learning and production.

□ Zebra finches are a powerful model for elucidating the role of BMP2 in neural repair and in learning and memory.



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