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Sex Differences in Hormone-modulated Neuroplasticity in the Songbird *(Serinus canarius)*

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Perineuronal net expression in adult-hormone modulated neuroplasticity *(Serinus canaria)*

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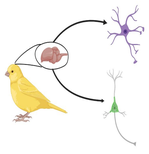
Introduction: Hormone-modulated plasticity in seasonal songbirds

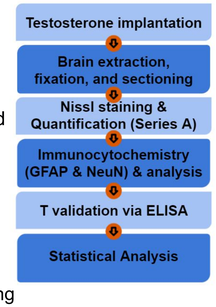
- In temperate zone species such as canaries (*Serinus canaria*) the neural circuitry modulating song behavior undergoes well-defined changes in neuroplasticity across the seasons.
- In males, increased daylengths in spring initiates an increase in gonadal volumes and circulating testosterone driving marked changes in brain morphology and song frequency.
- Females given exogenous testosterone in adulthood, also demonstrate male-like changes in brain morphology and song behavior.
- Testosterone-induced changes in female HVC volumes only had limited amplitude and these volumes never reached male-typical levels, suggesting that there are sex differences in the neural substrate that responds to testosterone.



Purpose

We sought to investigate the role of perineuronal nets in adult hormone modulated neuroplasticity in male and female canaries.

MATERIALS AND METHODS



Animals

22 male and female American Singer canaries were housed on short days (8L:16D) for at least 6 weeks. Males were castrated and females were photoregressed and later surgically implanted with either a 10 mm testosterone packed silastic implant or an empty implant as a control for 7 days.

Immunohistochemistry

Brains were sectioned through the rostral-caudal extent at 30 µm using a cryostat and free-floating sections were stored at -20C n cryoprotectant. Brain sections were double labeled for glial fibrillary acidic protein (GFAP) and neuronal nuclear protein (NeuN).

Nissl staining

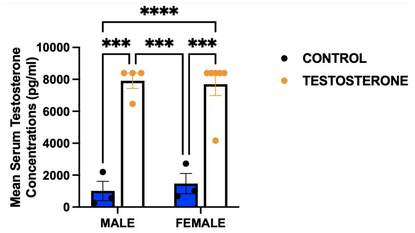
Area X, RA, and HVC volumes were quantified in Nissl stained sections.

Enzyme Immunoassay

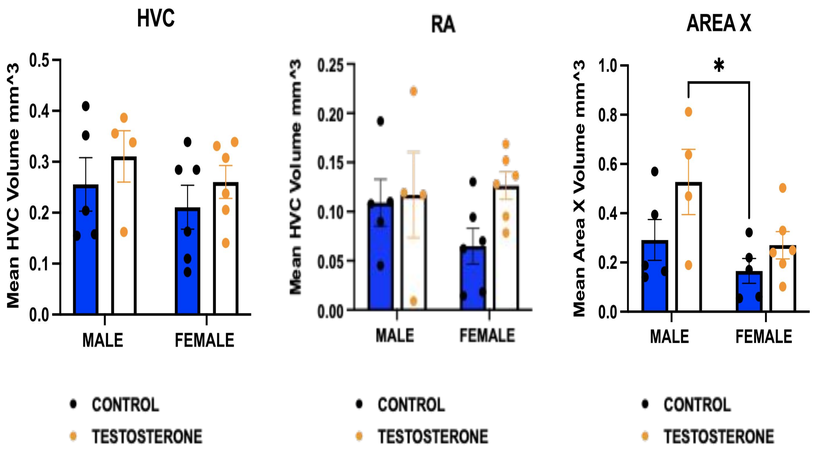
Serum testosterone concentration was validated with an enzyme immunoassay (EIA).

RESULTS

Testosterone concentrations were significantly higher in male and female canaries treated with testosterone.

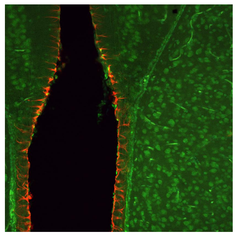


Testosterone treated canaries did not demonstrate significantly larger HVC, RA, and Area X volumes compared to controls.



GFAP and NeuN immuno- reactivity in the subventricular zone of the canary

A)



B)

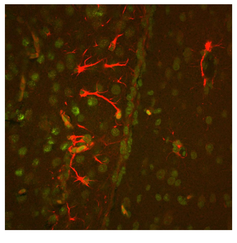


Image A and B: GFAP, glial fibrillary acidic protein, labels glial bodies such as astrocytes (red fluorescence), and NeuN labels neuronal bodies (green fluorescence). A) GFAP immunoreactive cells are seen predominantly along the ventricles of the brain. B) In a closer view, astrocytes are seen interacting with neurons, potentially playing a role in neural migration.

Discussion

Conclusions

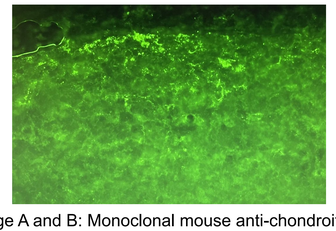
- Although testosterone concentrations were significantly higher in treated males and females, there was not a significant difference in the telencephalic nuclei volumes. This is likely due to the short duration of the treatment (7 days).
  - A previous study found that male and female canaries treated with exogenous testosterone for 3 weeks had a significantly larger nuclei volumes, while those treated for 1 week had insignificant differences (Madison et al., 2015).
  - GFAP and NeuN immuno-reactivity in the subventricular zone of the canary suggests that astrocytes may play a role in the guidance and survival of new neurons migrating to the HVC in both males and females.

Ongoing Work-Perineuronal Nets (PNN)

- Due to the complicated nature of immunocytochemistry to stain for astrocytes, focus is shifting towards perineuronal nets (PNN).
- PNNs play a key role in determining the plasticity that is associated seasonal shifts in song and sensorimotor learning in songbirds (Balmer et al., 2009; Cornez et al., 2017)
  - Differential expression of PNN in different songbird species indicates that PNN plays a role in open ended and close ended learning
- Previous studies have reported an increase in PNN and parvalbumin neurons in the HVC, RA, and Area X and increased PNN localization in both T-treated males and females. In females, there were no differences in T-induced PNN and PV changes until day 21 of treatment (Cornez et al., 2020).
- Currently, our lab is labeling GABAergic parvalbumin-expressing interneurons to elucidate differences in perineuronal net (PNN) densities between male and females across treatment groups.
- We aim to elucidate statistical differences between sexes after one week of T treatment

Perineuronal Nets in HVC region of the canary

A)



B)

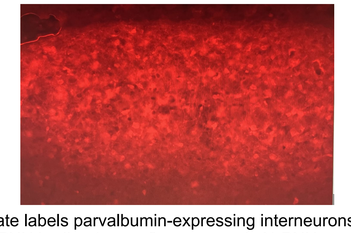


Image A and B: Monoclonal mouse anti-chondroitin sulfate labels parvalbumin-expressing interneurons (red fluorescence), and polyclonal rabbit anti-PV labels PNN bodies (green fluorescence). A) PNNs are seen predominantly within the HVC region along a ventricle. B) With a different channel, parvalbumin-expressing interneurons are seen in the same location.