

Adrenal steroidogenesis in territorial female tree swallows

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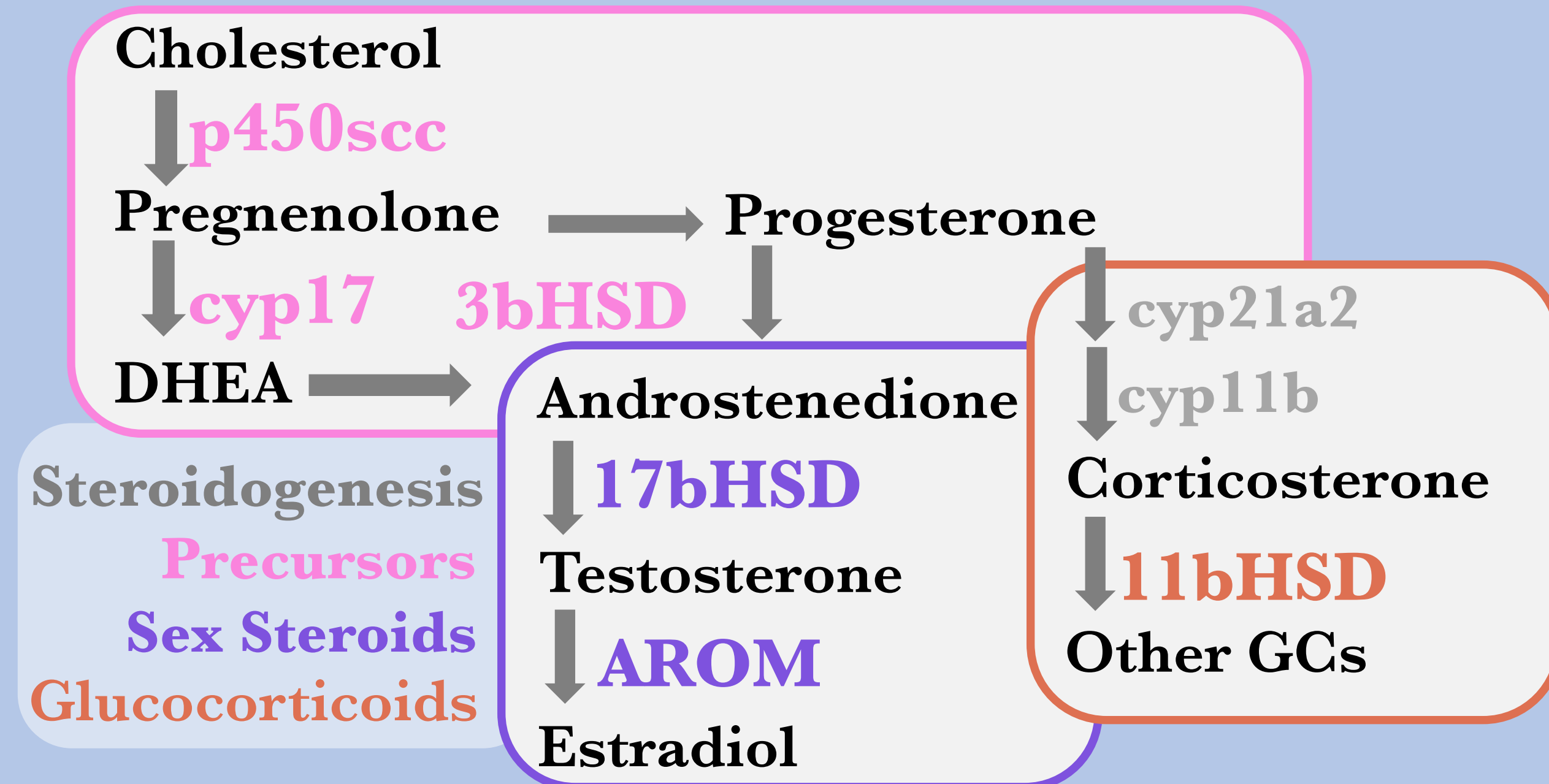
Steroid production beyond the gonads

- Challenge hypothesis posits that breeding males increase testosterone (T) to facilitate aggression¹
- But high T in circulation can have physiological costs²
- In winter, some animals make hormone 'precursors' in adrenals,³ keeping T low in circulation and converting to T in other tissues
- Adrenals make glucocorticoids, which have many functions, including restoring homeostasis after metabolic challenges

Study species

- Female tree swallows (*Tachycineta bicolor*) are obligate cavity nesters who must be aggressive in territorial establishment to secure a nest⁴
- Social challenges are common during territorial establishment, but they do not lead to elevated T in circulation⁵

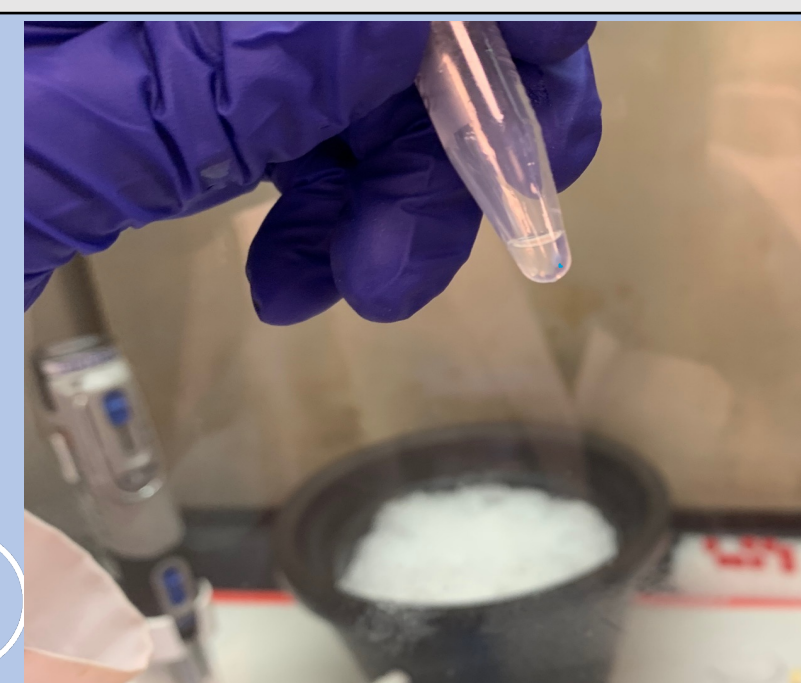
Does adrenal steroidogenesis respond when females aggressively defend their nesting territory?



Methods: From field to lab



10 females: social challenge with conspecific decoy for 25 min, caught within 90 min (27.3 ± 19.8 min)
10 unchallenged controls (2 later omitted for low quality/yield)

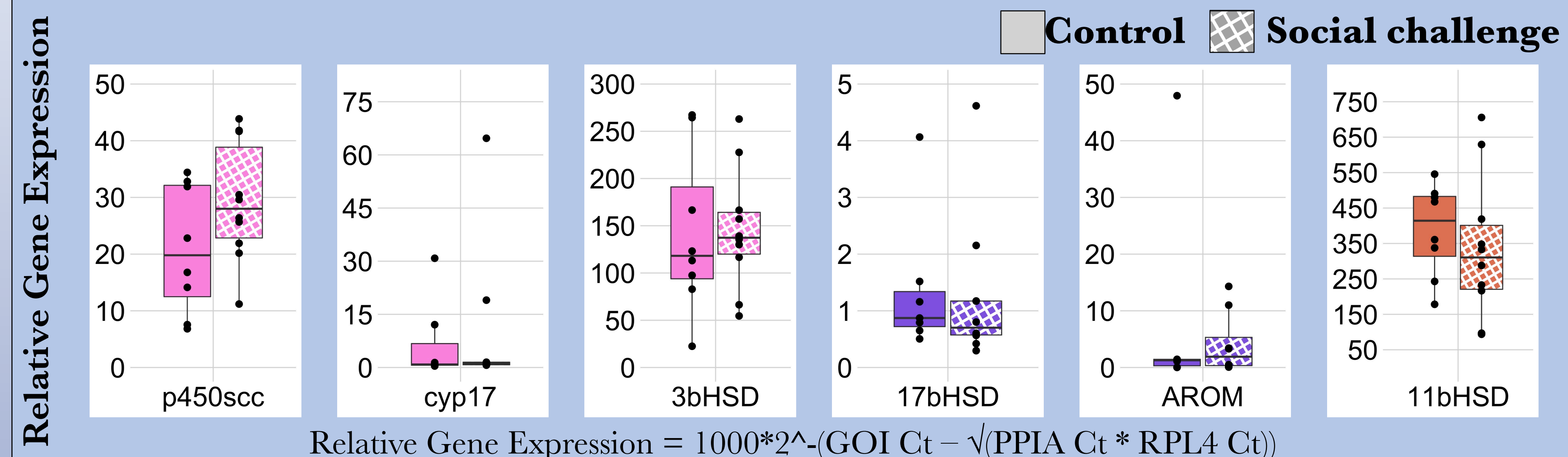


Extracted RNA from adrenals (Trizol)
Synthesized cDNA from 166ng RNA



qPCR: 6 GOI & 2 reference genes (PPIA and RPL4)
Triplicates, SYBR green, QuantStudio5

Social challenge does not affect gene expression



$$\text{Relative Gene Expression} = 1000 \cdot 2^{-(\text{GOI Ct} - \sqrt{\text{PPIA Ct} \cdot \text{RPL4 Ct}})}$$

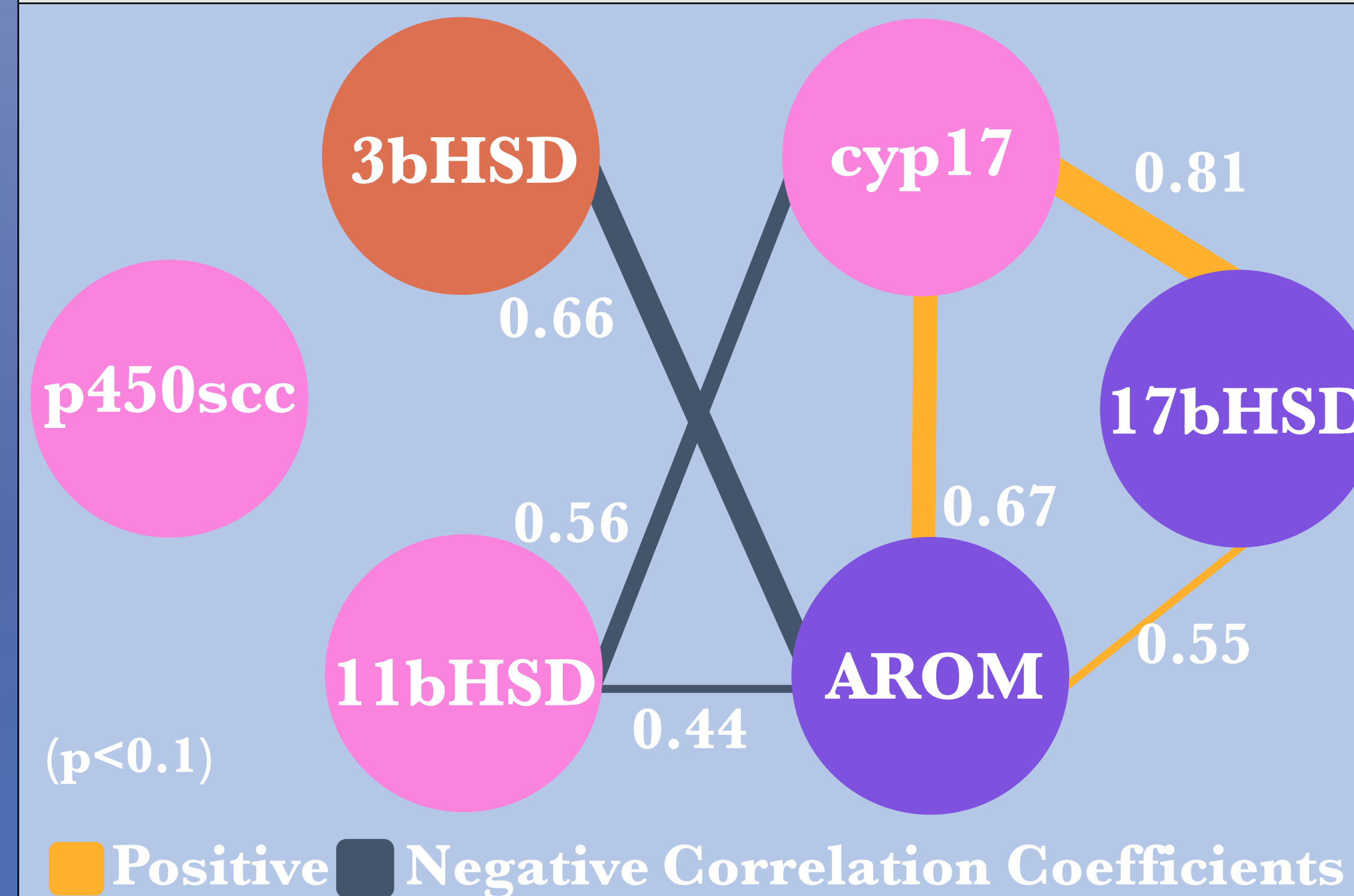
- Within each gene: no significant difference between control and challenge groups
 - All p-values ≥ 0.13 (t-tests and Wilcoxon rank sum test)
 - For analysis, we used log₂ of cyp17, 17bHSD, AROM

Gene expression relative to 17bHSD

p450scc = 21x cyp17 = 6x 3bHSD = 116x
17bHSD = 1x AROM = 4x 11bHSD = 289x

- Relative expression was highest along glucocorticoid pathway
 - Values standardized to a fold-change, compared to the least abundant transcript (17bHSD)

Correlations among genes reveal flux through steroidogenic pathways



Across groups

- Genes along pathway to sex steroids are positively correlated with each other
- 3bHSD and 11bHSD are negatively correlated with genes unique to the sex steroid pathway

Between groups

- In the control group, p450scc and 3bHSD are positively correlated (p=0.02); in the challenge group, there is no correlation (p=0.92)
- In the control group, 3bHSD is not correlated with cyp17 or 17bHSD (p>0.66); in the challenge group, 3bHSD is negatively correlated with cyp17 (p=0.06) and 17bHSD (p=0.09)

Conclusions

- Adrenal steroidogenic gene expression does not significantly respond to social challenge, at least not at this timescale of sampling
- All the birds are making a lot of glucocorticoids; sex steroid synthesis may be a secondary function to glucocorticoid synthesis
- These functions may trade off: birds with higher expression along glucocorticoid pathway show less expression along the sex steroid pathway, and vice versa
- Correlations among some genes differ between control and challenge groups, suggesting a potentially subtle effect on flux through steroidogenic pathways

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References:

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- Rendon et al 2015
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- George et al 2021



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