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This document is part of a collection that serves two purposes. First it is a public archive for data and documents resulting from evolutionary, ecological, and behavioral research conducted by the Ketterson-Nolan research group. The focus of the research is an abundant North American songbird, the dark-eyed junco, *Junco hyemalis*, and the primary sources of support have been the National Science Foundation and Indiana University. The research was conducted in collaboration with numerous colleagues and students, and the objective of this site is to preserve not only the published products of the research, but also to document the organization and people that led to the published findings. Second it is a repository for the works of Val Nolan Jr., who studied songbirds in addition to the junco: in particular the prairie warbler, *Dendroica discolor*. This site was originally compiled and organized by Eric Snajdr, Nicole Gerlach, and Ellen Ketterson.

Context Statement

This document was generated as part of a long-term biological research project on a songbird, the dark-eyed junco, conducted by the Ketterson/Nolan research group at Indiana University. For more information, please see IUScholarWorks (<https://scholarworks.iu.edu/dspace/handle/2022/7911>).

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Behavioral Responses to a GnRH Challenge in Captive Male Dark-eyed Juncos (*Junco hyemalis*)

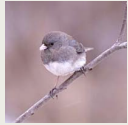


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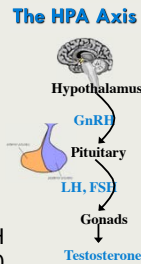
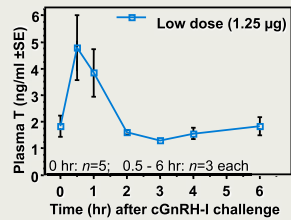
ABSTRACT

This study examined the role of gonadotropin-releasing hormone (GnRH) in eliciting courtship behavior in a male songbird. GnRH is a peptide produced by the hypothalamus that stimulates secretion of gonadotropins from the pituitary. In dark-eyed juncos (*Junco hyemalis*), an intramuscular injection of 50 μ L of cGnRH-I (500 ng/20 μ L PBS, Sigma L-0637) leads to a temporary increase in plasma testosterone (T) ca. 30 minutes post-injection, levels of T return to baseline levels by 60 minutes post-injection. We investigated whether a GnRH-induced peak in T leads to detectable changes in behavior in male juncos in reproductive condition. We observed the behavior of males before and after they received injections of GnRH or an equivalent amount of buffer (PBS). We quantified activity levels, feeding, maintenance (preening), and song for 15 minutes prior to each bird's receiving an injection. We then observed behavior for 15 minute intervals at 30 and 60 minutes post-injection while the males were visually isolated from other juncos and at 90 minutes post-injection in the presence of a female. To assess plasma levels of T, we collected blood samples two days later, prior to and 30 minutes following a second injection of the same type (GnRH or buffer) given previously. Plasma testosterone levels were significantly higher in males given GnRH vs. control injections. However, there were no observable differences in behavior between GnRH- or control-injected birds at any time period, nor was there any correlation within treatment groups between plasma levels of T and rates of behavior. This may be because hormonally-induced behavioral changes occur later than the peak in T-levels, because the brief pulse in T is not sufficient to effect a change in behavior, or because the stimulus environment was not appropriate to elicit a differential change in behavior.



BACKGROUND

- The Challenge Hypothesis suggests that maintaining high T is expensive, and that males elevate T only in response to social encounters (territorial intrusions, e.g.)¹.
- This implies that short-term elevations in T can produce short-term behavioral changes.
- GnRH is ordinarily released from the hypothalamus and acts on the pituitary, leading to a release of gonadal steroids, including T.



- In dark-eyed juncos, exogenous GnRH (1.25 μ g) leads to elevated T ~30 minutes post-injection².

QUESTION

Can a short-term elevation in testosterone cause short-term behavioral effects?

METHODS

- Twenty-four captive male dark-eyed juncos were housed individually prior to the beginning of the study.
- Prior to testing, birds were individually housed overnight in one half of the testing aviary, visually isolated from other birds.
- The next day, birds were given an injection of 50 μ L (500 ng/20 μ L) cGnRH-I or PBS in the pectoral muscle, and returned to the testing aviary for behavioral observations.
- Two days later, birds received a second injection of the same type received previously. Blood samples were taken pre-injection and at 30 minutes post-injection to assess testosterone response.

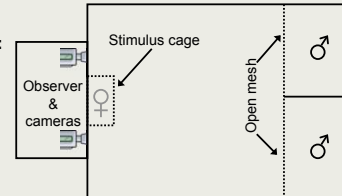
BEHAVIORAL OBSERVATION

Behavior was observed in 15 minute sessions prior to injection, and at 30, 60, and 90 minutes post-injection. A random, unrelated female was placed in the stimulus cage in the testing apparatus prior to the 90-minute observation.

ETHOGRAM

Presence/Absence Sampling:

- Hopping
- Flying
- Eating
- Preening
- Bill wiping
- Displaying
- Carrying nest material



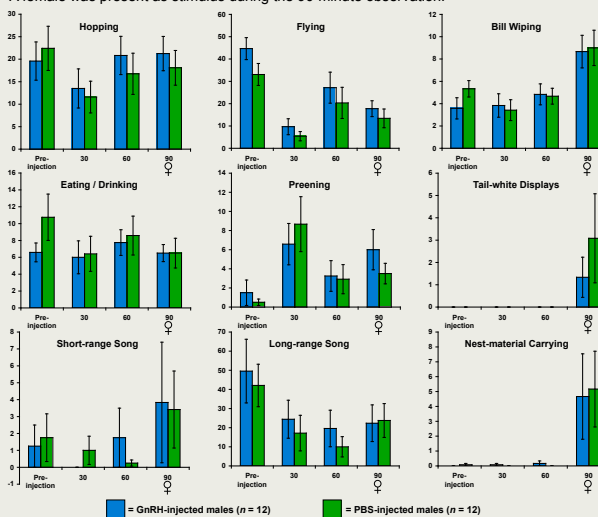
All-occurrence Sampling:

- Short-range song
- Long-range song

Both males could be observed and videotaped from behind one-way reflective film. Male compartments were 86x119x172 cm, and 2.49 m from the observer.

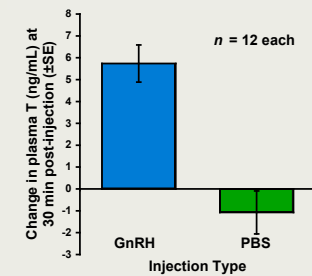
BEHAVIORAL RESULTS

Graphs indicate the mean number (\pm SE) of 15s intervals with behavior present (or the absolute number of songs) during 15 min trials before, 30, 60, and 90 minutes post-injection. A female was present as stimulus during the 90-minute observation.



- Behavior did differ significantly between time periods.
 - Increase in preening and decrease in activity immediately after injection
 - Increase in courtship-typical activities when female was present

HORMONE RESULTS



- GnRH injections increased plasma T relative to control injections ($t = 5.246$, $df = 22$, $p < 0.001$).

- GnRH- and PBS-injected males did not differ significantly in any behavior in any time period.
- GnRH- and PBS-injected males also did not differ in the change from baseline behavior during any time period.
- The change in plasma testosterone in response to injection was not correlated with any behavior, either across all birds or within treatment groups.

DISCUSSION

- Behavior did not differ between birds that received a GnRH or control injection. This may be because:
 - Timing of observations was inappropriate.
 - T response to GnRH was not sufficient to elicit behavior.
 - Stimulus was not appropriate to elicit differential response between the groups.
 - T is not causal to behavior changes during normal social interactions.
- Current/future work includes looking at the relationship between behavior during territorial intrusions and response to GnRH in wild male juncos.

ACKNOWLEDGEMENTS

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REFERENCES

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2. Jawor, J., et al. submitted.