

Seasonal variation in energy balance and the distribution of orexigenic neuropeptides within the social behavior network of a free-living songbird.

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ABSTRACT

Animal reproduction in seasonal breeders is typically timed to periods of high food availability. During this time territorial behavior may be used to monopolize food resources, and this aggression is largely maintained by the neural conversion of testosterone to estrogen, by the enzyme P₄₅₀ aromatase (AROM). In birds, that are territorial year-round, non-breeding aggression may be related to accessing food resources. Neuropeptide Y (NPY) and orexin (OX) are neural proteins that stimulate food intake and maintain energy balance and immunoreactive cell and fiber populations have been observed within the avian social behavior network, yet their specific role has not been identified. We tested the hypothesis that NPY and OX immunoreactivity differs between breeding and nonbreeding year-round territorial song sparrows (*Melospiza melodia*) in concordance with seasonal changes in energy balance. We also explored regional colocalization of these neuropeptides with the AROM to gain support for a possible interaction between energy balance and AROM activity.

INTRODUCTION

Previous research on song sparrows has demonstrated that territorial behavior occurs year-round and is dependent on P₄₅₀ aromatase (AROM) within the brain to convert circulating androgens to estrogens. Although breeding territoriality secures mating opportunities, nonbreeding territorial behavior may be related to accessing food resources, as nonbreeding birds in the temperate zone may have increased metabolic costs due to colder weather, and decreased overall food abundance thus requiring the need to defend patchy food areas (i.e. territories). Despite this reasoning, few studies have tried to link energy-regulating neural systems with the social behavior brain regions that regulate aggressive territoriality. Neuropeptide Y (NPY) is a 36-amino acid neuropeptide serving many functions within the hypothalamus including the regulation of food intake in both mammals and birds, and the storage of fat in mammals. Orexin (OX) is a neuropeptide found in two isoforms (OX-A; OX-B) that regulates both arousal and appetite in mammals, and particularly may assist in establishing long-term patterns of energy balance. *Here, we compare the distribution and abundance of NPY and OX between breeding and nonbreeding song sparrows particularly with respect to AROM distribution within the avian social behavior network.*

HYPOTHESIS

Nonbreeding song sparrows have higher metabolic costs and thus actively defend territories to gain access to food. Thus, we predict **higher** neural immunoreactivity for both NPY and OX in nonbreeding sparrows compared to breeding birds within the social behavior regions containing AROM.

METHODS

Bird Sampling

Breeding (June 2012; N=8) and nonbreeding (Jan 2012, N=10) adult male song sparrows were wild-caught in the Point Grey area of Vancouver, BC, Canada using mist nets coupled to song playback. Upon capture, birds were euthanized and the brain was dissected and immersion fixed in 4% paraformaldehyde.

Tissue Processing

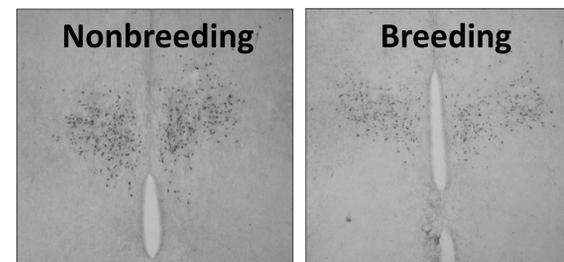
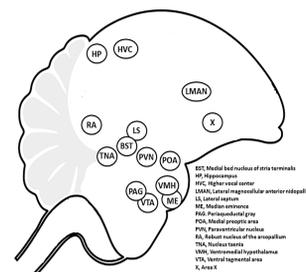
Brains were then sectioned on a cryostat at 30µm, and then free-floating sections were alternately immunostained for either NPY, OX, or aromatase, using indirect immunohistochemistry using specific polyclonal antibodies.

Imaging Analysis

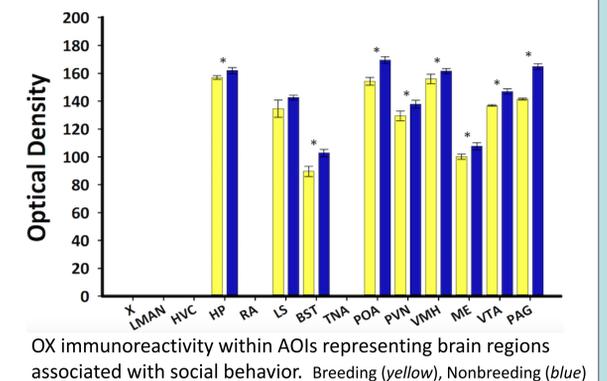
Images of behavior regions were taken at 40x objective magnification, and converted to a grayscale with background corrected across the images to be compared. Optical densities (relative units) were calculated within randomly positioned areas of interest (AOIs) using ImageJ 1.48v software (National Institutes of Health).

RESULTS

1. OX immunoreactivity is higher in nonbreeding birds across the social behavior network

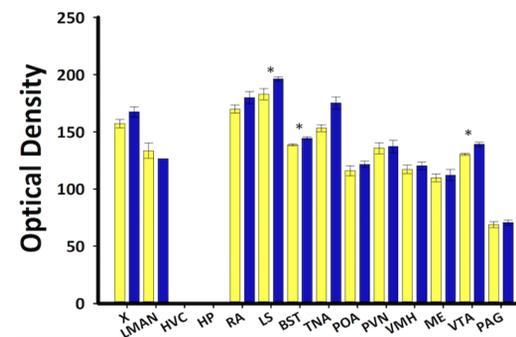


Photos indicating seasonal difference in OX immunoreactive cells within the PVN.

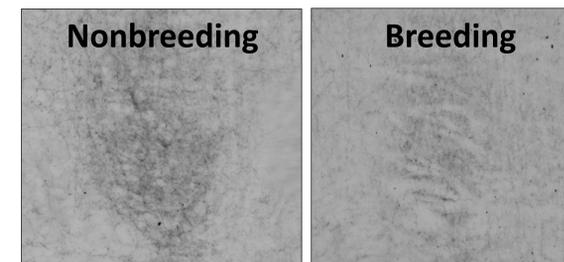


OX immunoreactivity within AOIs representing brain regions associated with social behavior. Breeding (yellow), Nonbreeding (blue)

2. NPY immunoreactivity is higher within the LS, BST, and VTA of nonbreeding birds



NPY immunoreactivity within AOIs representing brain regions associated with social behavior. Breeding (yellow), Nonbreeding (blue)

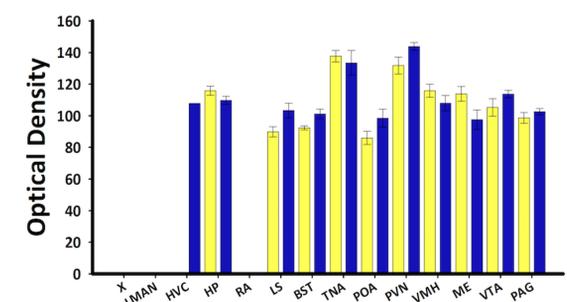


Photos indicating seasonal difference in NPY immunoreactive fibers within the VTA.

3. Extensive regional overlap in NPY, OX and AROM within the social behavior network

	POA	VMH	PVN	BST	LS	PAG	VTA
OX	+	+	C	+	+	+	C, ++
NPY	+++	C, +++	+	++	+	++	C, +++
AROM	C	C	C	C	C	C	C

Distribution of immunoreactive cells (C) and intensity of fiber staining (+, ++, +++) demonstrates substantial neuroanatomical colocalization between AROM, NPY and OX.



AROM immunoreactivity within AOIs representing brain regions associated with social behavior. Breeding (yellow), Nonbreeding (blue)

CONCLUSIONS

- As predicted, OX and NPY levels were higher in nonbreeding male song sparrows compared to breeding birds.
- This seasonal variation may reflect a number of factors including changes in food abundance in a manner suggestive of increased pressure on nonbreeding birds to find food.
- Extensive overlap between AROM, NPY and OX provides opportunity for neural steroid signaling to be impacted by changes in energetic status.

ACKNOWLEDGMENTS

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