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Research report

An interspecific comparison using immunofluorescence reveals that synapse density in the avian song system is related to sex but not to male song repertoire size

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Abstract

Immunofluorescent labeling of synaptic vesicle protein 2 (SV2) and confocal microscopy were employed to assess the role of synapse density in the functioning of the avian song system. Synapse density in premotor nuclei HVC and RA was measured, in both sexes of two species characterized by male-only singing behavior: the zebra finch *Taeniopygia guttata*, which sings a single, stereotyped song, and the Carolina wren *Thryothorus ludovicianus*, which sings a large repertoire of different songs. Multiple levels of analyses demonstrate overall similarity of synapse density between nuclei HVC and RA, suggesting that synapse density is regulated uniformly across these regions within individuals. Male zebra finches and male Carolina wrens have equivalent synapse densities, suggesting a common pattern of masculinized development despite dramatic behavioral differences. Female Carolina wrens have synaptic density similar to that of males of both species, while female zebra finches exhibit greater synaptic densities in both regions than do male zebra finches or both sexes of wrens. Prior reports implicate testosterone as a regulator of synapse density in this system; sex differences in circulating or neural testosterone may contribute to the sexual dimorphism of synapse density observed here. Interspecific comparison of song system synapse density in nonsinging females suggests that synapse density in female songbirds may be a particularly labile trait.

Theme: Neural basis of behavior
Topic: Neuroethology

Keywords: Synapse density; Song system; Immunohistochemistry; SV2; Sexual dimorphism

1. Introduction

Comparative studies of neuroanatomy often attempt to relate neural characteristics to behavior. For example, early studies of avian olfaction suggested that the size of the olfactory bulb was related to a species' nominal habitat [22]. Similarly, Nol [50] suggested that the size of the avian tectum was related to foraging method in shorebirds. In this type of comparison, a correlation between the physical volume of a neural structure and the type or amount of behavior it supports suggests that neural investment in a behavior is an indicator of behavioral capability. Intriguingly, such correlations also suggest that the amount of neural investment in a sensory or behavioral system may actually determine the degree of functional elaboration of the system.

The avian song system, a series of discrete, interconnected brain regions which underlie passerine song learning and performance [9,53,57], remains a prominent model for comparative study of the relationship between neural investment and behavioral capability. Most comparisons have focused on neural investment in song premotor areas, principally nucleus HVC (used here as the proper name) [56] and the robust nucleus of the arcopallium (RA). Interspecific comparisons have suggested differential investment in song-related neural regions among passerine birds

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and have demonstrated positive relationships between the size of song premotor areas and the size of the syllable or song repertoire performed [12,27] (but see Ref. [43]). Similarly, intersexual comparisons suggest that, in species exhibiting male-only song, males have larger song premotor brain regions than do females [6,45,49,52], while both sexes of duetting songbirds have song premotor areas of sizes proportionate to their song repertoire [12,13] (but see Gahr et al. [31]).

Interspecies and intersexual comparisons generally suggest that the amount of neural investment in these passerine brain regions (measured by neuron number or brain region volume) contributes to behavioral function. However, intrasexual tests among males within species have met with mixed results. For example, Nottebohm et al. [54] demonstrated that the size of nuclei HVC in male canaries *Serinus canarius* was positively correlated with the size of the song repertoire performed, while Leitner and Catchpole [43] found no such relationship. Canady et al. [19] and Airey et al. [1] have demonstrated similar relationships for nucleus HVC in marsh wrens *Cistothorus palustris* and sedge warblers *Acrocephalus schoenobaenus*, respectively, yet other comparisons have failed to similarly relate overall brain region characteristics and song repertoire size within species (red-winged blackbirds *Agelaius phoeniceus* [40]; rufous-sided towhee *Pipilo erythrophthalmus* [14]; marsh wrens [15]; European starling *Sturnus vulgaris* [7]). In these studies, the volumes of song-related brain regions were not correlated with the size of the song repertoire performed, despite considerable behavioral and neuroanatomical variation among individuals or study groups. These results suggest that the factors which regulate brain/behavior scaling among species or between the sexes may be distinct from those which operate among males within species.

How then might neural investment within a brain region be related to behavioral diversity within species? One hypothesis suggests that individuals within a species follow a common development scheme, and then differ in the degree to which they utilize their full behavioral capacity [54]. By this model, no gross anatomical marker (e.g., neuron number or brain region volume) for versatility of singing behavior will be apparent: males of a species might have similar (but not necessarily identical) amounts of neural investment in song-related brain regions, but some learn and use more song than do others. Another alternative is that cytoarchitectural elaboration of song-related brain regions bears more strongly on aspects of behavior other than song repertoire size, such as the ability to accurately copy tutored material [60] or the sexual quality of song components [43]. Neural elaboration could also be driven by perceptual, rather than premotor, needs [42]. However, the possibility remains that we have not yet identified or measured that aspect of song system cytoarchitecture which is most directly related to within-species singing diversity.

1.1. Behavior-related variation in song nuclei dendritic fields

Dramatic changes in the size and morphology of song-related brain regions occur during normal development in passerines, over a time course parallel with critical stages in song learning. Sexual dimorphism in the size and number of neurons within both nuclei HVC and RA have been observed during zebra finch *Taeniopygia guttata* development [10,11,32,41], and adult sexual dimorphism in the cytoarchitecture of behaviorally dimorphic species can be dramatic [49,52]. Given that sexual differences in the size/number of neurons in these song-related brain regions can be marked, most studies of the relationship between neural investment and singing versatility have focused upon the overall volume of brain regions within the song premotor pathway, and neuron size/number within these regions. Importantly, several studies have reported concomitant variation in the density of synapses and/or dendritic spines within these regions, in both developmental and experimental preparations. For example, nucleus RA in adult female canaries (which do not normally sing) is less than 40% of adult male nucleus RA size [52], and sex differences in the dendritic characteristics of canary RA neurons are pronounced [25]. Experimental administration of testosterone to juvenile female canaries results in adult females which have a large nucleus RA and sing male-like song [24]; the administration of androgens in this species is known to effect a marked increase in the number of RA synapses, suggesting that new synapses are formed as the new behavior (singing) is acquired [20,26]. Similarly, the number and source of RA synapses in intact zebra finch males changes markedly during the periods of development thought to involve the most crucial stages of song learning in this species [35], while hormonal treatment of female zebra finches can induce male-like song in association with increased nucleus RA volume and increased dendritic arbors of nucleus RA neurons [33]. These studies demonstrate that dendritic and/or synaptic features in the avian song system can be related to behavioral capability. Given the presumed role of synapses in this and other systems as sites of information storage and transmission [2,21], it is possible that rearrangements of, or increases in, synapse number facilitate song learning. Potentially, intrasexual, intersexual, or interspecific differences in behavioral versatility may be represented by observable differences in synapse number/density within the relevant brain regions.

1.2. Focus of current study

Prior studies have utilized electron microscopy to investigate synapse characteristics in female canary [20,26] and male zebra finch [35] nucleus RA and have suggested that changes in synapse number occur in conjunction with song learning. Here, confocal microscopy and an unbiased sampling method [47] are used to measure