Neuroscience Letters 578 (2014) 61–65

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Expression of androgen receptor in the brain of a sub-oscine bird with an elaborate courtship display

Leonida Fusani[a,*], Zoe Donaldson[b], Sarah E. London[c], Matthew J. Fuxjager[d], Barney A. Schlinger[d]

[a] Department of Life Science and Biotechnology, University of Ferrara, 44121 Ferrara, Italy
[b] Division of Integrative Neuroscience, Department of Psychiatry, Columbia University, New York, NY 10023, USA
[c] Department of Psychology, Institute for Mind and Biology, University of Chicago, Chicago, IL, USA
[d] Departments of Integrative Biology and Physiology, Ecology and Evolutionary Biology and the Laboratory of Neuroendocrinology, Brain Research Institute, University of California, Los Angeles, CA 90095, USA

HIGHLIGHTS

- Androgen controls elaborate courtship of male golden-collared manakins.
- Brain androgen receptor expression is unusual and includes the entire arcopallium.
- Brain androgen sensitivity might be linked to elaborate motor patterns of courtship.

ARTICLE INFO

Article history:
Received 13 May 2014
Received in revised form 4 June 2014
Accepted 11 June 2014
Available online 20 June 2014

Keywords:
Androgen receptor
Estrogen receptor
Bird
Avian
Courtship

ABSTRACT

Sex steroids control vertebrate behavior by modulating neural circuits specialized for sex steroid sensitivity. In birds, receptors for androgens (AR) and estrogens (ERα) show conserved expression in neural circuits controlling copulatory and vocal behaviors. Male golden-collared manakins have become a model for evaluating hormonal control of complex physical courtship displays. These birds perform visually and acoustically elaborate displays involving considerable neuromuscular coordination. Androgens activate manakin courtship and AR are expressed widely in spinal circuits and peripheral muscles utilized in courtship. Using in situ hybridization, we report here the distributions of AR and ERα mRNA in the brains of golden-collared manakins. Overall patterns of AR and ERα mRNA expression resemble what has been observed in non-vocal learning species. Notably, however, we detected a large area of AR expression in the arcopallium, a forebrain region that contains a crucial premotor song nucleus in vocal learning species. These results support the idea that AR signaling both centrally and peripherally is responsible for the activation of male manakin courtship, and the arcopallium is likely a premotor site for AR-mediated displays

1. Introduction

The evolution of neuronal steroid sensitivity enables sex steroids to exert control over vertebrate behavior. Initially, studies exploring brain–steroid interactions focused on conserved hypothalamic circuits that control copulatory behaviors, with studies of birds playing a prominent role [3]. It is now recognized that sex steroids have diverse neural functions, so brain–steroid studies have expanded considerably. In birds, for example, steroids impact learning and memory [45], sensory processing [43,53], parental care [49], aggression [36], and socio-sexual behaviors involving vocal and visual communication [19].

Many animals also perform impressive physically complex courtship displays, but little is known about the extent to which steroids control these diverse behaviors. Among birds, the Manakins (Family *Pipridae*) stand out. Most manakins are polygynic; males breed in leks where they perform physically and acoustically elaborate courtship displays with unique mechanical sonations produced by rapid and/or powerful movements of the wings [41].

Our laboratories explore neuroendocrine control of courtship behavior in male golden-collared manakins (*Manacus vitellinus*;

* Corresponding author. Tel.: +39 0532 455478; fax: +39 0532 455478.
E-mail addresses: leofusani@gmail.com, leonida.fusani@unife.it (L. Fusani), schlinge@lifesci.ucla.edu (B.A. Schlinger).

http://dx.doi.org/10.1016/j.neulet.2014.06.028
0304-3940/

GC-manakins). In mid-January, adult male GC-manakins arrive on their traditional leks and remain on or near their courtship arenas for the duration of their 6–8 month-long breeding season. On the lek, adjacent males interact by producing loud roll-snaps and performing courtship displays to females. Courtship involves rapid movements within the arena punctuated by single wing-snaps produced in mid-air jumps between saplings [9,21]. Our studies indicate that androgens activate courtship behaviors, including wing- and roll-snaps [47]. Compared to females and non-breeding males, testosterone (T) is elevated in displaying males [12,18]. Exogenous T treatments activate courtship in non-breeding males and females [12] whereas treatment with AR antagonists [18,22] blunts performance of courtship in wild adult breeding males. Elevated expression of AR in spinal cord and peripheral muscles [17,23] might have evolved in manakins to facilitate androgenic control of courtship. Given the numerous coordinated neuromuscular systems required for male courtship, we were curious to examine AR expression in manakin brain.

Steroid receptor expression in the manakin brain is also of interest in an evolutionary contex. The Order Passeriformes includes two taxa: oscines (also called songbirds) and suboscines. The latter do not learn song and accordingly lack a neural song system. Across many species of oscines, nuclei comprising the well characterized neural circuitry subserving the learning and performance of song can express AR and ERα at high levels [8,25,38,39]. To date, AR and ERα have never been detected in the forebrain of avian species that do not belong to the oscine group such as suboscines, budgerigars, doves, owls, gulls, quails, and fowls (often referred to as non-oscines) [24]. The GC-manakin, like all other studied non-oscines, shows no anatomical evidence for a neural song system [46]. Thus, a second focus of this study was to compare distributions of AR and ERα we observe in manakins to those of other bird species.

2. Experimental procedures

2.1. Animals and tissue preparation

We collected seven male GC-manakins in February and March using mistnets in the canal zone of Panama. Manakins were killed by an overdose of isofluorane and immediately perfused through the heart with 30 ml 0.9% saline followed by ice cold 4% neutral buffered formaldehyde using a peristaltic pump. All procedures were approved by the UCLA Chancellor's Animal Care Committee and the AALAC committee of the Smithsonian Tropical Research Institute.

Brains were postfixed for 2 h in 4% formaldehyde, and then cryoprotected with 30% sucrose in PB. The brains were frozen and stored on dry ice, transported to our lab at UCLA, and stored at - 80° until processed.

2.2. Subcloning of the manakin AR

Total RNA was isolated from adult male manakin brain (Trizol; Invitrogen). Two microgram of DNase *I*-treated RNA was reverse transcribed with random primers. A 741 bp cDNA was amplified HotStarTaq (Qiagen) with PCR primers based on the canary AR sequence (S: TGA CGT GTG GGA GCT GCA AA and AS: GGC CAT CCA CTG GAA TAA TAC TGA). The amplification proceeded at 95 °C for 15 min, then 40 cycles of 95 °C for 30 s, 58 °C for 45 s, and 72 °C for 1 min, with a 5 min final extension at 72 °C. Gel-purified amplicons were ligated into the *SrfI* site of PCRScript per manual (Stratagene) and clones were sequenced to confirm identity. We selected two clones, pman200B and pman50B, as templates for downstream in vitro transcription reactions (below) because they were identical except that the cDNA had ligated into the plasmid in opposite orientations.

2.3. Synthesis of riboprobe

Antisense- and negative control sense-configured AR riboprobes were transcribed from two pmanAR clones after linearization with NotI (T7 RNA polymerase; Promega, Madison, WI). Riboprobes for ERα were synthesized by linearizing the plasmid containing the 2792-bp zebra finch ERα sequence (EJZER1, [30]) with MluI or EcoRI to obtain the antisense (T7) and sense (SP6 RNA polymerase) probes, respectively. ^{33}P labeled probes were prepared by in vitro transcription from ~100 ng of linearized plasmid, 10 μl ^{33}P-UTP (2000Ci/mmol; New England Nuclear, Boston, MA), and 1 μl of the appropriate RNA polymerase. Unincorporated nucleotides were removed with G-50 sephadex columns (Boehringer Manneheim, Indianapolis, IN).

For in situ hybridization, 20 μm tissue sections were processed as described in [34] with modifications: we did not include proteinase K digestion, and hybridization was performed at 55 °C with 60 °C high-stringency post-hybridization washes. Dried sections were exposed to film (Kodak BioMax) for 2–3 days to estimate the length of exposure needed for subsequent emulsion autoradiography. The slides were then dipped in emulsion (Kodak NTB-2, Eastman Kodak, Rochester, NY) at 42 °C, stored in light proof, desiccated boxes at 4 °C, and developed after 3–4 weeks (Eastman Kodak D-19; Fixer). Slides were examined under light and darkfield microscopy to determine the presence and distribution of labeled cells.

3. Results

The neural distribution of AR, ERα, and their mRNAs in the brain has previously been described in details for several avian species [8,24,25,30,38]. Thus, here we focus only on the differences in AR and ERα mRNA expression between the GC-manakin and other studied bird species. In particular, we found novel expression of AR mRNA in a large field of labeled cells in the arcopallium, a pallial region that is the main output of the avian telencephalon and that in oscines contains the n. robustus arcopallii (RA) of the song system. To our knowledge, this is the first report of substantial AR mRNA expression in the forebrain of a non-oscine bird [24]. In the following description, we adhere to the revised avian brain nomenclature [42]. Although we studied only the distribution of the AR and ERα mRNAs, hereafter we will speak of AR and ER expression for brevity. Previous studies have shown that the mRNA localization matches well the distribution of the receptor protein [8,24,25,30,38].

3.1. Androgen receptors (AR)

The distribution of AR in the GC-manakin forebrain resembled that previously described for non-oscine species, in that no vocal control nuclei containing AR mRNA were found in the forebrain [5,24,38]. However, there was one noticeable exception: we found intense AR expression in the nucleus taeniae amygdalae (TnA) and in the arcopallium (Fig. 1D, E, G and H). Rostrally, the field of AR expression extended from TnA to the arcopallium mediale and then laterally into the arcopallium dorsale, following the structure previously called lamina archistriatilis dorsalis (LAD, Fig. 1E). This field of AR expression extends caudally to occupy virtually the whole arcopallium intermedium (AI), extending from the medial end of the forebrain laterally to the LAD, and from the caudal portion of TnA to the caudal end of the forebrain. This same region in oscines contains the AR-sensitive nucleus robustus arcopallii (RA), but a distinct RA is not recognizable in the GC-manakin (Fig. 1G and H).