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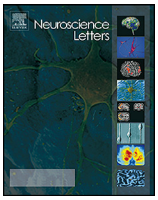

Research paper

Shared neural substrates for song discrimination in parental and parasitic songbirds

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HIGHLIGHTS

- The auditory forebrain is critical for conspecific song recognition in songbirds.
- Unlike most songbirds, brood parasitic pin-tailed whydahs are raised by heterospecifics.
- We tested for differential processing of song in the auditory forebrain of whydahs.
- Whydahs exhibited increased gene expression and BOLD responses to conspecific stimuli.
- Brood parasitism evolution likely involved minor changes to existing neural substrates.

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ABSTRACT

In many social animals, early exposure to conspecific stimuli is critical for the development of accurate species recognition. Obligate brood parasitic songbirds, however, forego parental care and young are raised by heterospecific hosts in the absence of conspecific stimuli. Having evolved from non-parasitic, parental ancestors, how brood parasites recognize their own species remains unclear. In parental songbirds (e.g. zebra finch *Taeniopygia guttata*), the primary and secondary auditory forebrain areas are known to be critical in the differential processing of conspecific vs. heterospecific songs. Here we demonstrate that the same auditory brain regions underlie song discrimination in adult brood parasitic pin-tailed whydahs (*Vidua macroura*), a close relative of the zebra finch lineage. Similar to zebra finches, whydahs showed stronger behavioral responses during conspecific vs. heterospecific song and tone pips as well as increased neural responses within the auditory forebrain, as measured by both functional magnetic resonance imaging (fMRI) and immediate early gene (IEG) expression. Given parallel behavioral and neuroanatomical patterns of song discrimination, our results suggest that the evolutionary transition to brood parasitism from parental songbirds likely involved an "evolutionary tinkering" of existing proximate mechanisms, rather than the wholesale reworking of the neural substrates of species recognition.

1. Introduction

The recognition of conspecifics (species recognition) is essential for diverse functions in animals, including flock formation, foraging, and sexual reproduction. For many social vertebrates, species recognition depends on early exposure to conspecific stimuli [15], where sensory perceptual systems engage in heightened levels of neural plasticity during sensitive periods that subsequently guide both neural response selectivity and behavioral decisions through adulthood [17]. Given the lasting effects of experience-dependent plasticity on species recognition, it is unclear how associated neural circuits evolve to produce dramatically novel phenotypes [26].

For example, many of the vocalizations, behaviors, and mate choice decisions of songbirds are learned from conspecific referents (e.g. parents, siblings) early in ontogeny [2]. Obligate avian brood parasites, however, lay their eggs in the nests of heterospecifics and thus, juvenile parasites are typically raised without exposure to conspecific stimuli. This reproductive strategy has evolved within

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at least seven independent lineages in birds, including twice in songbirds, each time from a non-parasitic, parental ancestor [33]. Yet, whether the transition to a parasitic reproductive strategy was a result of relatively small changes ("evolutionary tinkering" [18]) or substantial physiological shifts remains largely unknown, as previous comparative work on brood parasite neurobiology has focused mostly on gross neuroanatomical differences associated with spatial information processing between parasitic and parental lineages of songbirds [29,30].

In general, neurobiological research using a variety of methodologies including immediate early gene expression [25], electrophysiology [16], and functional magnetic resonance imaging [27], on parental songbirds including the model species zebra finch *Taeniopygia guttata*, finds the primary (field L) and secondary auditory forebrain regions (CMM (caudomedial mesopallium), and NCM (caudomedial nidopallium)) to be critical in the differential processing of auditory input from conspecific vs. heterospecific songs. Furthermore, non-parasitic songbirds raised by heterospecifics exhibit signatures of neural activation within the same telencephalic regions in response to heterospecific songs [12,40], providing evidence for this region's involvement in auditory memory, retrieval, and species recognition [3]. While the auditory forebrain appears anatomically conserved among bird species, studies of some songbirds [1] and non-songbird species [35] were unable to identify signatures of differential neural activation within this region in response to conspecific vs. heterospecific vocalizations. This disparity in observed (lack of) neural responses may reflect functional variability among species.

Here we studied the pin-tailed whydah *Vidua macroura*, an obligate brood parasite and member of the sister family to parental estrildid finches (including zebra finches) to address whether the same auditory brain regions underlie song discrimination in brood parasites and parental songbirds. By comparing our novel data with published information on the zebra finch, we aimed to assess whether behavioral responses [6], patterns of neural activation [27], and immediate early gene expression [25] in response to conspecific vs. heterospecific song and synthetic pure tone stimuli are broadly conserved across parasitic and non-parasitic taxa.

2. Methods

2.1. Behavioral responses to auditory stimuli

Commercially sourced adult pin-tailed whydahs (n = 4 males, 4 females) were housed in the Hunter College Animal Facility in groups containing a male and female whydah with a pair (male and female) of zebra finches in each cage on a 12:12 L:D light cycle with food and water provided ad libitum.

For experimental playback sessions, two whydah subjects previously housed together (one male, one female) were moved to an observation cage (65″ × 21″ × 34″ cage soundproofed with studio foam) in a separate room from the colony, and left to acclimate overnight. Cardboard visors as well as a curtain were installed on and around the cage to minimize potentially confounding visual input. Placement of food, water, and perches was symmetrical across the lengthwise midline to minimize side bias. The following morning, the two birds were presented with playback stimuli, comprising 4-s clips played every 15 s over a three minute period. The playback stimuli consisted of eight songs of different conspecifics, 8 songs of zebra finches, sourced from Macaulay Library (Cornell Lab of Ornithology, Ithaca, NY, USA), and 8 sets of synthetic pure tones designed to mimic the power-spectrum, amplitude and spectral modulation in zebra finch songs (tone pips, sourced directly from Ref. [13]). To eliminate background noise, songs were processed through a high-pass filter set to 500 Hz using Raven software (Cornell University, Ithaca, NY, USA), and the peak amplitude was standardized using Audacity. The playback volume was adjusted to be 74 dB SPL at 1.5 m distance from the speaker, which was the same as the sound pressure measured in our captive colony at the same distance. The order of playback stimuli was randomly selected prior to experimental trials. Alternative playback trials were continued following a ten-minute silent period between presentations. Each three minute presentation was recorded with a wide-angle webcam for analysis. A behavior was defined by us based on an a priori criterion to be a "response" to the stimulus if it occurred during the four-second playback clip or within three seconds of its end. Response types recorded from both subjects were aerial turns (defined as an oppositional change of direction midflight), crosses (defined as crossing the length-wise midline of the cage), and vocalizations. Immediately following the conclusion of the experimental session, the pair was returned to their permanent housing and the experimental cage was cleaned and food and water replenished for the next male/female whydah pair. For statistical analyses, we performed repeated measures ANOVA for the vocalizations and movements (turns and crosses combined) of each individual and included song stimuli type and sex as explanatory variables. We used a Tukey adjustment for comparisons of responses among stimuli.

2.2. Functional magnetic resonance imaging (fMRI)

Whydahs of either sex (n = 5 males, 4 females; the same individuals and housed as above) were placed in a 7.0 T Avance III Biospec 70/30 USR small animal MRI system (Bruker Biospin MRI, Inc., Billerica, MA) equipped with a 12 cm diameter, 450 mT/m amplitude and 4500 T/m/s slew rate actively shielded gradient subsystem with integrated shim capability. A customized 20 mm diameter litzcage coil with holes at the position of the ears (Doty Scientific) was used for transmission and reception of the MR signal, as well as for head fixation. Birds were lightly anesthetized with Diazepam (0.05 mL) injected into the leg muscles, targeting a concentration of 7.5 mg/kg body weight [37,38]. Birds were immobilized by wrapping them in soft tissue paper and placing them within plastic holders within the radiofrequency coil and equipped with custom headphones to deliver song playback. The RF coil was placed in an RF shield (Doty Scientific) and the RF shield into a layer of acoustical rubber for sound isolation. To further reduce ambient scanner noise, the scanner's helium compressor was switched off during the auditory fMRI runs. A heated water circulated warming blanket was used to keep the bird's temperature as measured under the wing at approximately 39 °C. Respiration was monitored with a pneumatic pillow sensor under the bird. Both temperature and respiration trends were visualized during the experiments (Small Animal Instruments, Inc.).

Each run corresponded to eight repetitions of each of the three stimulus blocks (including eight different individuals' whydah songs and zebra finch songs, respectively, sourced as above), with each block containing eight samples of a 4 s stimulus each followed by six samples of 4 s of silence each. Therefore, each of the 24 blocks lasted 56 s. The order of the stimuli was quasi-randomized for each bird, ensuring that all transitions between stimuli occurred the same number of times.

After initial calibration and localizer scans for functional imaging, seven gradient echo EPI image slices of 1.1 mm thickness (including a gap of 0.1 mm between slices) were acquired sagittally with the fourth slice centered on the sagittal brain mid-plane. We used gradient-echo [4,36] rather than spin-echo methods [27] due to its higher BOLD contrast-to-noise ratio [28]. The echo time was TE = 16 ms, the repeat time TR = 4 s, and the matrix size = 64 × 64, defining an in-plane spatial resolution of 0.22 × 0.27 mm (overall voxel size = 0.0594 mm^3). We obtained 336 vols over the course of