Song discrimination by nestling collared flycatchers during early development

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Pre-zygotic isolation is often maintained by species-specific signals and preferences. However, in species where signals are learnt, as in songbirds, learning errors can lead to costly hybridization. Song discrimination expressed during early developmental stages may ensure selective learning later in life but can be difficult to demonstrate before behavioural responses are obvious. Here, we use a novel method, measuring changes in metabolic rate, to detect song perception and discrimination in collared flycatcher embryos and nestlings. We found that nestlings as early as 7 days old respond to song with increased metabolic rate, and, by 9 days old, have increased metabolic rate when listening to conspecific when compared with heterospecific song. This early discrimination between songs probably leads to fewer heterospecific matings, and thus higher fitness of collared flycatchers living in sympatry with closely related species.

1. Introduction

When males produce signals that are only preferred by conspecific females, costly heterospecific matings can be avoided. The association between male signals and female preferences can break down if both traits are learned prior to sexual maturity, and there is a risk of learning or preferring a heterospecific signal [1]. For example, both male and female songbirds imprint on the songs of adult male tutors as juveniles, which guides subsequent song learning [2]. The ability of juvenile songbirds to discriminate between conspecific and heterospecific songs is thought to ensure that songbirds learn to produce and prefer conspecific songs [3] and therefore form conspecific pairs.

The auditory brain regions underlying song discrimination differentiate even before hatching [12], suggesting that neural responses to song may be present well before behavioural responses. The brain is a metabolically costly organ, suggesting that physiological responses to songs, such as increased heart rate, could be used to evaluate discrimination of acoustic cues at very young and even embryonic developmental stages, as suggested by Shizuka [10]. For example, heart rate responses have been used to evaluate discrimination in fledgling songbirds [8] and even in embryos of precocial species [13]. Here, we evaluate song discrimination ability in embryonic and nestling songbirds, before individuals...
are capable of producing visible or audible behavioural responses, using metabolic rate as a proxy for physiological and neural responses to different song types.

Collared flycatchers (Ficedula albicollis) are cavity-nesting passerines that co-occur with closely related pied flycatchers (F. hypoleuca) in central Europe and on the Baltic island of Öland [14]. While pied flycatcher males often learn and incorporate song elements from collared flycatchers, leading to mixed song, collared flycatcher males produce only pure collared flycatcher songs [15,16]. This difference suggests that collared flycatchers have particularly selective song discrimination that arises before song learning. Collared flycatchers express strong behavioural discrimination of conspecific songs as 12-day-old nestlings, but it is unknown when collared flycatchers first express song discrimination [17]. Here, we used metabolic rate to determine whether collared flycatcher nestlings express physiological responses related to discrimination of songs prior to behavioural responses. We assumed that an increase in metabolic rate when exposed to song following a silence treatment was indicative of neural responses, and we therefore predicted that nestlings that were discriminating between songs would have higher metabolic responses to conspecific when compared with heterospecific songs.

2. Material and methods

We have been monitoring populations of collared and pied flycatchers breeding in nest boxes on the Swedish island of Öland (57°10’ N, 16°56’ E) since 2002. By monitoring nest boxes at regular intervals, we determined the precise laying date, hatching date, and thus age of all nestlings. Both parents were ringed and measured, females during incubation and males while feeding nestlings. Collared and pied flycatchers sometimes hybridize [14], but we did not include any nestling that had mixed-species parents in our experiment. All nestlings were weighed with unique alphanumeric rings, and blood sampled at 6 days post-hatching and were re-weighed at 12 days post-hatching.

To test whether collared flycatcher embryos and hatched nestlings had different metabolic responses to conspecific and heterospecific songs, we measured the change in respiration during the period prior to and during song playbacks, hereafter referred to as ‘metabolic response’. We used a respirometer, where embryos and nestlings were kept at a constant temperature, and air parameters were measured once per second, although we used the mean oxygen estimate over each period as our response (see the electronic supplementary material for more details). In 2015, we collected eggs 2 days prior to the expected hatching date, as well as 4-, 7-, 9- and 12-day-old nestlings. Eggs and nestlings were exposed to 5 min of silence followed by 5 min of song from collared flycatchers or from conspecific when compared with heterospecific songs.

Figure 1. Spectrograms (frequency by time plots) of typical songs from (a) great tit, (b) collared flycatcher and (c) pied flycatcher.

3. Results

We measured 15 embryos and 45 nestlings (17 four-day-old nestlings, 11 seven-day-old nestlings, 9 nine-day-old nestlings and 8 twelve-day-old nestlings) in 2015 (the specific sample sizes are in electronic supplementary material, table S1). We found that nestlings have an increased metabolic rate response to sound (i.e. either great tit, collared or pied flycatcher song) compared with a silence treatment as they age, where 7-day-old (est = 0.153 ± 0.04, t54 = 4.03, p = 0.0002), 9-day-old (est = 0.173 ± 0.04, t54 = 4.29, p = 0.0002) and 12-day-old (est = 0.136 ± 0.04, t54 = 3.25, p = 0.002) nestlings responded more than embryos in eggs did, while 4-day-old nestlings responded similarly to embryos (electronic supplementary material, table S2).

We further investigated whether nestlings had differential responses to conspecific and heterospecific songs by comparing the increase in metabolic rate to different song treatments. We found that nestling responses depended on the song type they were exposed to. Seven-day-old nestlings did not respond differently to the various song types (figure 2; electronic supplementary material, table S3). However, 9-day-old nestlings had a higher metabolic response to conspecific collared song than to the heterospecific song playbacks, while 12-day-old nestlings responded more strongly to heterospecific pied flycatcher song than to collared song (figure 2; electronic supplementary material, table S3).

4. Discussion

We demonstrate that collared flycatcher nestlings had increased metabolic rates in response to song playbacks 7 days after hatching, more than 10 days before fledging. As we did not use a non-song playback as a control, we...
9-day-old, small sample size of pied flycatcher song playback (nestlings responded greatest to pied flycatcher song, the higher metabolic response to collared flycatcher song than that as young as 9-day-old collared flycatcher nestlings had (and not specifically to song). More importantly, we found displayed as a Tukey’s boxplot. The asterisks denote a significant interaction.

Figure 2. The differences in metabolic response of collared flycatchers at five different ages when played conspecific and two different heterospecific songs, displayed as a Tukey’s boxplot. The asterisks denote a significant interaction.

can only conclude that they can respond to sound at this age (and not specifically to song). More importantly, we found that as young as 9-day-old collared flycatcher nestlings had a higher metabolic response to collared flycatcher song than to either pied flycatcher or great tit song. While 12-day-old nestlings responded greatest to pied flycatcher song, the small sample size of pied flycatcher song playback \((n = 1 \text{ at } 9 \text{ days, } n = 1 \text{ at } 12 \text{ days}; \text{electronic supplementary material, table S1}), \) limits our ability to compare different heterospecific song types specifically.

Our results suggest that collared flycatchers begin to respond to sounds as early as 7 days post-hatching, and are able to discriminate between conspecific and heterospecific songs as early as 9 days post-hatching. The regions in the brain involved in song processing, discrimination and learning differentiate and become interconnected at different time periods in development [12]. For example, the thalamic auditory regions, involved in sound discrimination, begin to develop before hatching; the auditory cortex, which selectively responds to conspecific song (reviewed in [21]), differentiates only after hatching; and connections between the auditory cortex and song system, important for song learning and production, arise closer to the date of fledging [12]. This staggered, but rapid development of the songbird auditory system could explain why nestlings first demonstrate metabolic rate responses to all songs at day 7, but then begin to discriminate at day 9 (figure 2).

Passerine nestlings develop consistently exposed to a cacophony of sounds that they must ignore in order to learn their conspecific song adequately. By developing song discrimination well before song learning, as appears to be the case in collared flycatchers and other species [10], nestlings may be at less risk for mistaken mate choice when they become sexually mature [25]. Establishing the timing of song recognition and discrimination allows us to identify the stages in nestling brain development that are likely to be under strong selection to prevent hybridization later in life, and give further insight into pre-zygotic isolation in wild systems.

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**Ethics.** All procedures on flycatchers were approved by the Linköping Animal Care Board, permission number Dnr 10–13.

**Data accessibility.** The data for this project are available in Dryad http://dx.doi.org/10.5061/dryad.8n45g [26].

**Authors’ contributions.** D.W., S.E.M. and A.Q. designed the study. A.S. and S.E.M. carried out the experiment, and the statistical analyses. All authors contributed to writing the manuscript and have given final approval for publication. All the authors agree to be held accountable for the content.

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