



# Limitations of a habituation task to demonstrate discrimination of natural signals in songbirds



Satoko Ono<sup>a</sup>, Hiroko Kagawa<sup>a</sup>, Miki Takahashi<sup>a,1</sup>, Yoshimasa Seki<sup>b,2</sup>, Kazuo Okanoya<sup>a,b,\*</sup>

<sup>a</sup> Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan

<sup>b</sup> Japan Science and Technology Agency, ERATO, Okanoya Emotional Information Project, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

## ARTICLE INFO

### Article history:

Received 6 November 2014

Received in revised form 3 March 2015

Accepted 3 March 2015

Available online 17 March 2015

### Keywords:

Animal cognition

Bengalese finch

Discrimination task

Habituation

Songbird

## ABSTRACT

The habituation–dishabituation (HDH) paradigm is a common method used to examine animal cognition. Recent studies reported that spontaneous vocalizations could be used as an index of song familiarity and novelty in songbirds. However, these studies assigned only a few stimulus sets for all subjects, which might cause pseudoreplication. Therefore, we examined universality and general applicability of this method in Bengalese finches using a variety of stimulus sets. Seven unfamiliar conspecific songs were collected as a stimulus pool and a habituation song was randomly chosen for each subject. The subject was exposed to the habituation song repeatedly over 2 h. During the test phase, the habituation song and a novel song randomly chosen from the stimulus pool were presented. We compared the degree of increase in call production during playback of those two songs. Although the degree was greater for the novel song compared with the habituation song in some birds, the trend was not consistent across all stimulus sets tested in these birds. Our results show that the HDH paradigm is not suitable to test song discrimination in songbirds unless precautions are taken to increase external validity by utilizing a variety of stimulus sets for each subject.

© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

The habituation–dishabituation (HDH) paradigm is one of the most common methods used in cognitive and behavioral studies in infants and animals. The method takes advantage of the process of habituation, which is the response decrement to a stimulus after repeated exposure (Rankin et al., 2009), and dishabituation, the response recovery to a novel stimulus. Although a comprehensive review of habituation (Peeke and Petrinovich, 1984; Rankin et al., 2009) defines the latter phenomenon as “stimulus specificity”, we term it “dishabituation” in the present context. The experimental design is as follows. In the familiarization phase, a subject is exposed to a stimulus X repeatedly. Next, in the test phase, the subject is exposed to the familiarized stimulus X and a novel stimulus Y. Finally, the response levels to stimulus X and Y are compared. The

HDH paradigm is a standard procedure to test infants' abilities such as facial recognition (Turati et al., 2006), speech perception (Kaplan et al., 1995), and auditory statistical learning (Saffran et al., 1996). In addition, the HDH method has been well utilized to explore animal cognition in a wide range of species (e.g., nonhuman primates, Saffran et al., 2008; frogs, Wang and Ewert, 1992; songbirds, Zucchi and Bergmann, 1975) and modalities (e.g., odor stimuli, Chaudhury et al., 2010; auditory stimuli, Saffran et al., 2008; visual stimuli, Wang and Ewert, 1992).

Compared to an operant conditioning paradigm, another common method in studies of animal cognition, the HDH paradigm has at least three advantages. First, the method can be very easily applied. Generally, with the HDH paradigm we can get the results in a shorter time, more economically, and with less effort than an operant task. Second, care of animal subjects is more humane than in operant conditioning. In general, to facilitate the reinforcement salience (i.e., increase the value of food rewards) in operant conditioning, experimenters keep animals under restricted food access, which is not necessary with HDH. Third, if animals respond significantly different between stimulus X and Y, we can interpret the results in a biological context. The different responses between stimulus X and Y without reinforcement may suggest an adaptive value to distinguish stimulus X and Y in nature. In an operant task, the results only show what the animals can do but do not reveal

\* Corresponding author at: Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan. Tel.: +81 3 5454 6266; fax: +81 3 5454 6725.

E-mail address: [cokanoya@mail.ecc.u-tokyo.ac.jp](mailto:cokanoya@mail.ecc.u-tokyo.ac.jp) (K. Okanoya).

<sup>1</sup> Current address: Laboratory for Language Development, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan.

<sup>2</sup> Current address: Faculty of Letters, Aichi University, 1-1 Machihata-cho, Toyohashi, Aichi 441-8522, Japan.

the biological relevance of these discrimination abilities (Weary, 1992).

On the other hand, a major disadvantage of the HDH paradigm is the increased risk of false negatives. In an operant task, reward and a punishment ensure that the animals respond to physical differences between stimuli (ten Cate and Okanoya, 2012). We can conclude that the animals cannot discriminate between stimuli when there are no significant differences in response, provided that the stimuli are well controlled. However, in the HDH paradigm, there is a possibility that the animals may not respond to a novel stimulus even if they have the ability to detect physical differences in the stimuli. This may be due to various reasons; stimulus-specific preferences may interfere with discrimination (Oakes, 2010), or response to the change in stimuli may lack adaptive values. Furthermore, the HDH paradigm is not suitable for a test with many stimulus sets (Weary, 1992) because we cannot distinguish between the animal being unable to discriminate between stimuli and the animal being unwilling to (due to habituation to the overall experimental setting). If one can minimize these problems and optimize the behavioral index and the experimental design to cause habituation and dishabituation regardless of stimulus properties, the HDH paradigm would be a strong method for the cognitive and ethological study of many species.

Songbirds are one of the species in which the HDH paradigm allows us to investigate a variety of ethological and comparative psychological themes. In most species, male songbirds sing for territorial defense and courtship (Catchpole and Slater, 2008). Birdsong consists of sound elements arranged in a particular temporal order, and some species sing structurally complex songs with repetition or branching transitions (Berwick et al., 2011). The acoustic features of birdsong are both species-specific and individual-specific and a receiver uses them for conspecific recognition (Clayton and Pröve, 1989), individual recognition (Stoddard et al., 1991), and mate value assessment (Searcy and Marler, 1981). By studying song recognition in songbirds, we can explore various themes such as what components are relevant to birds in discriminating between songs and the limits of what acoustic sequences birds can perceive.

Recent songbird studies reported that spontaneous behaviors such as vocalization could be used as an index of song familiarity and novelty (Abe and Watanabe, 2011; Stripling et al., 2003). Stripling et al. (2003) used zebra finches (*Taeniopygia guttata*) and Abe and Watanabe (2011) used Bengalese finches (*Lonchura striata* var. *domestica*) to demonstrate the utility of this method. Subjects were exposed to the song of a bird (song X) repeatedly for familiarization and then exposed to song X again or the song of another bird (song Y) for the test. Zebra finches significantly increased the latencies to calls, hops, and beak swipes in response to the novel song, but not the familiar song. In Bengalese finches, the number of calls produced increased in response to novelty. The authors also applied the HDH method in other experiments. Dong and Clayton (2008) investigated the relationship between behavioral habituation and immediate early gene expression in the zebra finch brain. Abe and Watanabe (2011) examined the ability of Bengalese finches to discriminate sound sequences using the HDH method. If these behavioral indices and experimental designs are applicable to a variety of stimuli, it is expected that these methods could be applied to various cognitive studies in songbirds.

However, these previous experiments suffer from a fundamental methodological problem: the small size of the stimulus sets. One should assign a different stimulus set of the familiarized song X and the novel song Y for each subject in order to avoid pseudoreplication (Kroodsma, 1989; McGregor et al., 1992). The previous studies (Abe and Watanabe, 2011; Stripling et al., 2003), however, used only two songs. In the zebra finch study (Stripling et al., 2003), they used two stimulus sets: the familiar song and the novel song

were randomly assigned for subjects. In the first experiment of the Bengalese finch study (Abe and Watanabe, 2011), they used only one stimulus set: song A was always used for the familiar song and song B for the novel song. Under such conditions one cannot exclude the possibility of a stimulus-specific effect such that subjects responded differently to song A and B, and the external validity of these results cannot be assumed.

In the current study, we tried to expand upon the method of Abe and Watanabe (2011) to create a more universal condition using a variety of stimulus sets. In Experiment 1, we investigated the replicability of the previous study with assignment of different stimulus sets for each subject. In Experiment 2, we retested the subjects in Experiment 1 with another novel stimulus set in order to explore the robustness of habituation tendency within subjects. Our main goal was to investigate whether the results obtained through the previous HDH method can be replicated in more universal conditions; in other words, whether the previous HDH method is suitable for discrimination of a variety of songs in Bengalese finches. Our sub goal was to explore the influence of stimulus-specific factors and subject-specific factors.

## 2. Experiment 1

### 2.1. Methods

The main goal of our study is to examine whether the method in Abe and Watanabe (2011) can measure habituation and dishabituation to a variety of song stimuli in Bengalese finches. We replicated the methods used in their study as closely as possible and kept the experimental environment, stimulus playback schedule, behavioral measurements, and analysis procedures the same. The only difference was the assignment of different stimulus sets for each subject.

#### 2.1.1. Subjects

Sixteen adult Bengalese finches (eight males) at least 193 days of age were picked from different families for this study. Birds were kept in our laboratory at a temperature of 25–30 °C and a humidity of 60%, under a 14:10 h light:dark cycle, with food and water *ad libitum*. The experimental procedures and housing conditions were approved by the Institutional Animal Care and Use Committee at the University of Tokyo.

#### 2.1.2. Stimuli

Stimuli were conspecific songs, which originated from three birds. The subjects had never heard the songs because the singers had passed away before the subjects were hatched. The songs were digitized at 44.1 kHz or 48 kHz sampling rate. Sixteen sound sequences (6–9 s each), including a typical rendition, were isolated from songs of each individual singer. Eight of the 16 sound sequences from each bird were temporally reversed. Finally, 3 forward song sets (each set includes 16 forward sequences of one individual bird) and 3 reversed song sets (each set includes 8 reversed sequences) were prepared. See Fig. 1 for sound spectrograms. The sound amplitude was adjusted to around 70 dBA at the center of the experimental cage by a sound level meter (NA-27, RION, Tokyo, Japan). Song modification was performed using sound analysis software (SASLab Pro version 5.2, Avisoft Bioacoustics, Berlin, Germany). For each subject, one habituation song and one novel song were randomly chosen to balance the stimulus assignment.

Download English Version:

<https://daneshyari.com/en/article/2426597>

Download Persian Version:

<https://daneshyari.com/article/2426597>

[Daneshyari.com](https://daneshyari.com)