Effect of feed-time duration on discrimination of vocalizations in a go/no-go operant paradigm

Prateek K. Sahu [a], Carolina Montenegro [a], Connor T. Lambert [a], Alexandra Oprea [b], Moriah Deimeke [a], Victoria Rennie [a], Sarah Smeltz [a], Thomas J. Benowicz [a], Divya Patel [a], Leslie S. Phillmore [b], Christopher B. Sturdy [a,*]

[a] *Department of Psychology, University of Alberta, Edmonton, T6G 2R3, Canada*

[b] *Department of Psychology and Neuroscience, Dalhousie University, Halifax, B3H 4R2, Canada*

* Correspondence to: Department of Psychology, University of Alberta, Edmonton, T6G 2R3, Canada

Email address: csturdy@ualberta.ca (C.B. Sturdy)

Abstract

Refining and modifying experimental procedures play a vital role in improving methodology while also reducing animal distress. In this study, we asked if an increase in feed time duration affects discrimination in an operant go/no-go task. Specifically, we used zebra finches' sexually dimorphic distance calls as acoustic stimuli to test whether there were any significant differences in performance on an operant discrimination task requiring zebra finches to classify calls according to the sex of the producer when a key experimental parameter, feed time duration, was increased from 1 second to 2 seconds. We found no differences in learning speed (trials to criterion) between birds that were given 1 sec or 2 sec of food access following a correct go response. Our results indicate doubling food access duration did not impact the speed of acquisition of distance call discrimination in zebra finches. These findings suggest that we can provide twice as much time for zebra finches to access food, potentially improving animal welfare, with no impact on experimental outcomes.

Keywords: Auditory discrimination; Distance calls; Experimental methodologies; Operant conditioning; Refinement of procedures; Zebra finch

1. Introduction

Experimental methodologies often focus on optimizing behavioural responses and minimizing distress in animals, whether for a newly developed paradigm, or to refine existing paradigms (Klump et al., 1995). Operant conditioning procedures are often optimized for high rates of engagement response by balancing high levels of motivation and optimal food access (Gess et al., 2011; Goltstein et al., 2018; Kim et al., 2017; Phillips et al., 2017). Many laboratories have developed operant paradigms to test auditory perception in songbirds (Gentner et al., 2000; Gess et al., 2011; Houx & ten Cate, 1999; Nagel et al., 2010; Njegovan et al., 1994; Park et al., 1985; Scharff et al., 1998; Sturdy & Weisman, 2006). Refinement and improvement of an extensively used operant paradigm (Njegovan et al., 1994; Sturdy and Weisman, 2006) can involve manipulation of reinforcement factors such as magnitude, intensity, and schedule of a reinforcer, coupled with investigating the effect of these variations on performance in the operant task (Scheiner et al., 2004; Stebbins et al., 1959; Trosclair-Lasserre et al., 2008). Reinforcer magnitude, including concentration, volume, and duration can impact response during operant conditioning (Bonem and Crossman, 1988; Doughty and Richards, 2002; Reed and Wright, 1988). Duration of food access during operant conditioning trials may impact motivation, task performance, and even the wellbeing of an animal (Ferster and Skinner, 1957; Patterson-Kane et al., 2008).

In the current study, we investigated whether changing the duration of food access after a correct response in an operant go/no-go discrimination task impacted the discrimination performance of zebra finches (*Taeniopygia guttata*), a widely used model species for neurobiological and behavioural studies of song development and auditory perception (e.g., Adret, 1993; Brainard and Doupe, 2002; Geberzahn and Derégnaucourt, 2020). In previous research, each bird received 1 sec of food access after rewarded responses during discrimination trials (e.g., Campbell et al., 2020; Congdon et al., 2021; Guillette et al., 2013; Hahn et al., 2015; Hoeschele et al., 2013, 2012; Montenegro et al., 2021; Scully et al., 2020). In an attempt to refine our procedures, and potentially increase welfare of our experimental animals, we increased the feed duration from 1 sec to 2 sec which allowed us to directly compare discrimination performance between conditions. If birds perform similarly in both 1 sec and 2 sec conditions, we could then refine our experimental procedures by increasing the feed duration without compromising the comparability of future studies with past results. Two groups of birds were trained to discriminate zebra finch distance calls (Elie and Theunissen, 2016; Gess et al., 2011; Zann, 1996) with either 1 sec and 2 sec of reward; both groups of birds discriminated female distance calls (go or S+) from male distance calls (no-go or S-).

2. Methods

2.1. Subjects

In total, 25 adult zebra finches were tested (14 male, 11 female) between February and August 2021. One female bird died during the training stage due to natural causes. Six birds (two males and four females) failed to learn to use the perch and feeder to obtain food during the training stage so were removed from the experiment and any further analyses. Birds were bred and raised at the University of Alberta, Canada. The housing rooms were maintained on a 14:10 light:dark cycle (lights on 0700, full spectrum lights - Standard, 32W, T8 Daylight) at ~ 21°C temperature and humidity ~ 40%. Birds were fed mixed seed (Hagen Canada, Quebec, Canada) while in colony housing rooms and during the experiment. Birds were provided with spray millet once per week as well as spinach and Prime Vitamin Supplement (Hagen) three times a week. The birds (25 in total) were naïve to the experimental procedures, including the acoustic operant conditioning task, and call stimuli, but had experience with other cognitive tasks (Camacho-Alpízar et al., 2021; Lambert et al., 2022). For detailed housing information refer to Lambert et al. (2022).

2.2. Apparatus

During the experiment, each bird was housed in a modified cage (30 × 40 × 40 cm) placed inside a ventilated, sound-attenuating operant chamber, and maintained with a 14:10 hour light:dark cycle. Birds had ad libitum access to grit, cuttlebone, and water. The request perch with an infrared sensor was located near the entrance of the feeder. A personal computer connected to a single-board computer (Palya & Walter, 2001) scheduled trials and recorded responses to stimuli. Stimuli were played from a personal computer hard drive through an amplifier to a Fostex full-range speaker located inside the operant chamber beside the feeder. For all other details about apparatus refer to Lambert et al. (2022). For a diagram and detailed description of the apparatus, see supplementary material Fig. S1 and Sturdy and Weisman (2006).

2.3. Acoustic stimuli

A total of 60 zebra finch distance calls were used in the experiment: 30 male and 30 female calls produced by 18 males and 18 females with 1-2 calls used per individual (Lambert et al. 2022). The calls were obtained from the datasets of D'Amelio et al. (2017) and Elie and Theunissen (2016) and from adult zebra finches recorded by members of the Phillmore lab at Dalhousie University, Halifax, NS, Canada. All the calls were resampled at 44,000 Hz and normalized for root mean square amplitude.

2.4. Procedure

2.4.1. Initial shaping and training

The training sessions were continuous and lasted the entire duration of the daylight hours while the houselights were illuminated. Trials were discreet and occurred throughout the day when the bird requested trial. Once each bird learned to use the request perch and feeder to obtain food, training to discriminate a tone (1,000 Hz; to receive food access) from no tone began. At this point in tone/no tone training, each bird was randomly assigned to one of two treatment groups (1s group: 1 sec food access and 2s group: 2 sec food access). A trial was initiated, and a stimulus was played once a bird landed and remained on the request perch for at least 10 ms. Birds had to fly inside the feeder to obtain food within one second of the stimuli played. Birds entering the feeder counted as a response. After successfully learning to discriminate (DR $\geq$ 0.80 for three 500 trial bins; see definition of DR in section 2.5) tone from no tone, each bird moved to Non-differential training where they were exposed to and reinforced for responding to each of the 60 stimuli. The goal of this phase was to ensure each bird responded to all stimuli equivalently prior to discrimination training. For Non-differential training, a bird could initiate a trial by landing and remaining at the request perch for a random interval between 900-100 ms. Following this, a call was randomly selected without replacement and played once. If the bird left the request perch before the entire duration of the call was played, the trial was considered interrupted. Interrupted trials (I) were not used in the calculation of the DR. Birds obtained food by entering the feeder within one second of stimulus played followed by a 30-sec inter-trial interval. Birds performed trials daily throughout 14-hour light on inside the operant chamber. The response measures for all the stages were automated. For a detailed training criteria for the initial shaping and training, refer to Lambert et al. (2022).

2.4.2. Discrimination training

In Discrimination training, 40 of the 60 calls were randomly selected and presented as training stimuli. Responses to half of the stimuli (S+ = 20 female distance calls) were reinforced with 1 sec or 2 sec access to food, according to the treatment group, and responses to the other half (S- = 20 male distance calls) were not followed by food with a 30 sec intertrial interval with lights off as punishment. Discrimination training continued until each bird completed six 320-trial blocks with a discrimination ratio (DR, see definition in section 2.5) ≥ 0.80 with the last two blocks out of six blocks being consecutive with DR ≥ 0.80.

2.5. Response measures

For tone/no tone training, the discrimination ratio, or DR, is calculated as the number of responses to tone trials (S+) divided by the sum of the number of responses to tone and no tone trials (S-) in a 500 trial block. For discrimination training, the DR is a measure of how accurately a bird discriminates rewarded calls (S+) from unrewarded calls (S-). A DR was calculated using the formula: ((R+S+) - I)/sum(R+S+ and R+S-), where R+S+ is the mean proportion of responses for block of 320 trials when rewarded calls (S+) were played, R+S- is the mean proportion response when unrewarded calls (S-) were played and I is the interrupted trials. A DR of 0.50 indicates equal response to rewarded (S+) and unrewarded (S-) stimuli, and a DR of 1.00 indicates a bird only responded to S+, thus a perfect discrimination. Average number of trials per day was measured as a proxy to compare motivation and total trial blocks required to reach the criterion in the Discrimination training stage was measured to compare the speed of acquisition between birds in the treatment groups.

2.6. Statistical analysis

All statistical analyses were conducted in R v4.2.1 (R Core Team, 2022). Below, data are represented as mean ± SD. We conducted a mixed-model Analysis of Variance (ANOVA) on average number of trials per day as a proxy for motivation to compare across feed time groups and sex in different experimental stages. If there are significant differences in average trial numbers for each stage, it would mean a change in feed time duration affecting completion time of an experiment. The Greenhouse-Geisser sphericity correction was applied due to a violation of sphericity the assumptions. The assumption of normality was violated (from the Shapiro–Wilks test) for the distribution of a few groups below. Therefore, we used a robust two-way ANOVA with the WRS2 package (Mair & Wilcox, 2020) using trimmed means (20%) to examine the main effect of feed time groups (1s and 2s) and sex (female and male), and the interaction between feed time group and sex based on the total number of trial blocks required to reach criterion. An alpha of p =.05 was used as the cutoff for significance. We constructed Bayesian models and calculated Bayes factors (BFs) with the package rstanarm (Goodrich et al., 2022) and bayestestR (Makowski et al., 2019) to interpret any potential null findings of null hypothesis significance testing (NHST).

3. Results

3.1. Trials per day

We used the average number of trials completed per day for different stages (Non-differential and Discrimination training) as a proxy to compare motivation for speed of task completion for feed time groups and for females and males. We conducted a Group × Experiment Stage × Sex, mixed model ANOVA on average number of trials per day with experiment stage as the within-subject factor and feed time groups and subject sex were between-subject factors. There was a significant main effect of the experimental stage ($F(1,40) = 10.6$, $p = 0.002$, $\eta^2=0.21$). There were no other significant main effects or interactions (all $ps > 0.07$, $\eta^2_{Group}= 0.04$, $\eta^2_{Sex}= 0.08$, $\eta^2_{Group\times Sex} = 0.006$, $\eta^2_{Sex\times Stage} = 0.08$; Fig.1). Birds performed significantly more trials per day in Discrimination (1391.7 ± 374.5) than Non-differential training (883.65 ± 167.1).

We conducted pairwise comparisons using *t*-tests with Bonferroni corrections to determine if 1s and 2s groups and females and males differed in the average trials per day in each experiment stage. There were no significant differences in the average number of trials completed per day for 1s vs 2s group and female vs male, in each individual experimental stage (all $ps > 0.42$, Non-differential stage: $\eta^2_{Group}= 0.06$, $\eta^2_{Sex}= 0.03$; Discrimination: $\eta^2_{Group}= 0.02$, $\eta^2_{Sex}= 0.07$).

We constructed a Bayesian mixed model with the same set-up and parameters as above and found similar results. There was strong evidence in favor of absence of effect of feed time groups on average number of trials completed per day for Non-differential stage (BF= 0.049) and Discrimination stage (BF= 0.09). There was strong evidence in favor of absence of effect of subject sex for Nondifferential stage (BF= 0.049) and moderate evidence in favor of absence of effect of subject sex for Discrimination stage (BF=0.224). See Supplementary materials for additional information.

3.2. Trials to criterion in Discrimination phase

We used the total number of 320-trial blocks required to reach the criterion (DR ≥ 0.80 for six blocks, last two blocks consecutive) to compare the speed of acquisition for feed time groups and for females and males. We conducted a two-way ANOVA with trial blocks required to reach the criterion as the dependent variable with feed time group and subject sex as factors. There were no significant main effects of the feed time group (adj. critical value = 0.49, $p = 0.5$, $\eta^2_{Group} = 0.04$) or subject sex (adj. critical value = 3.3, $p = 0.1$, $\eta^2_{Sex} = 0.08$), nor was there a significant interaction between feed time group and subject sex (adj. critical value = 0.15, $p = 0.71$, $\eta^2_{Group \times Sex} < 0.001$), see Fig. 2.

We constructed a Bayesian generalized linear model with the same set-up and parameters as above and found similar results. There was moderate evidence in favor of absence of effect of feed time groups (BF=0.119) and subject sex (BF=0.180). See Supplementary materials for additional information.

4. Discussion

In the current study, we asked whether modifying feed time duration from 1 sec to 2 sec influences the performance of zebra finches learning to discriminate between female and male distance calls. Birds given either 1 or 2 seconds to obtain food after responding to a rewarded stimulus did not differ in the number of trials completed per day, nor did the groups differ in the speed of acquisition of the discrimination task.

We used the number of trials per day as a proxy measure for motivation which was important to measure since the duration of food access might be expected to impact motivation. However, the increase in feed duration did not significantly impact the number of trials per day performed in either Non-differential or Discrimination stage. However, birds differed in the number of trials per day during Non-differential vs Discrimination stage. This is likely due to differing degrees of difficulty of the experimental stages, as there is punishment (light-off for 30 sec) for responding to S- stimuli in the Discrimination stage, compared to responding in the Non-differential stage where responses to all the stimuli are rewarded. Nevertheless, this means we can double the feed duration without significantly affecting the number of operant trials performed per day, which can impact the completion of an experimental stage. The operant experiments with the new feed duration (i.e., 2 seconds) can take a similar number of trials for completion as previous work with 1 second feed access, aiding in comparisons across studies. Birds can have more access to food reward during an experiment, potentially reducing the stress to the demands of having to eat quickly during a trial. These conclusions should be taken with caution as we did not measure and compare body weights or food consumed by the birds.

Our results show that at a minimum, we can successfully double the access time to food for birds without a significant impact on desired outcomes for sex-based discrimination of distance calls, at least in terms of the speed of learning. Doubling the feed access duration ensures birds get more time to eat food, which can potentially impact the well-being of the animals. Studies should consider examining the effect of an increase in feed duration on the well-being of the animals with comparisons of welfare measurements like weight, fat content, etc. before and after an experiment. These studies can help guide decisions about food access for long-term experiments where animals have limited food access for a longer period. Further studies with different species and more challenging discriminations for longer periods are required to get a complete picture of the effect of feed duration on operant-based discrimination tasks (Sturdy and Weisman, 2006). Nevertheless, our study takes an important step towards improving and refining experimental operant procedures without sacrificing discrimination performance.

Our study used calls as discriminative stimuli to look at the effect of feed time duration. Overall, it illustrates the effect of changing feed duration in an operant discrimination task. In the future, similar studies on the manipulation of other experimental factors can be conducted with other passerine bird species, or with more complex song discrimination in order to further improve the operant paradigm. Here, we have taken a step forward in improving experimental methodology and possibly animal-wellbeing while providing information about sex-based discrimination.

CRediT authorship contribution statement

Prateek K. Sahu: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Carolina Montenegro:** Investigation, Writing- review and editing. **Connor T. Lambert**: Writing- review and editing. **Alexandra Oprea:** Resources, Writing- review and editing. **Moriah Deimeke**: Investigation, Writing-review and editing. **Victoria Rennie:** Investigation, Writing-review and editing. **Sarah Smelt:** Investigation, Writing-review and editing. **Thomas J. Benowicz:** Investigation, Writing-review and editing. **Divya Patel:** Investigation **Leslie S. Phillmore:** Conceptualization, Writing - review & editing. **Christopher B. Sturdy:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition, Supervision.

Ethical note

All the procedures were conducted in accordance with the Canadian Council on Animal Care (CCAC) Guidelines and Policies with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (AUP 1937 and AUP 2923), which is consistent with the Animal Care Committee Guidelines for the Use of Animals in Research.

Declaration of Competing Interest

None.

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Figures

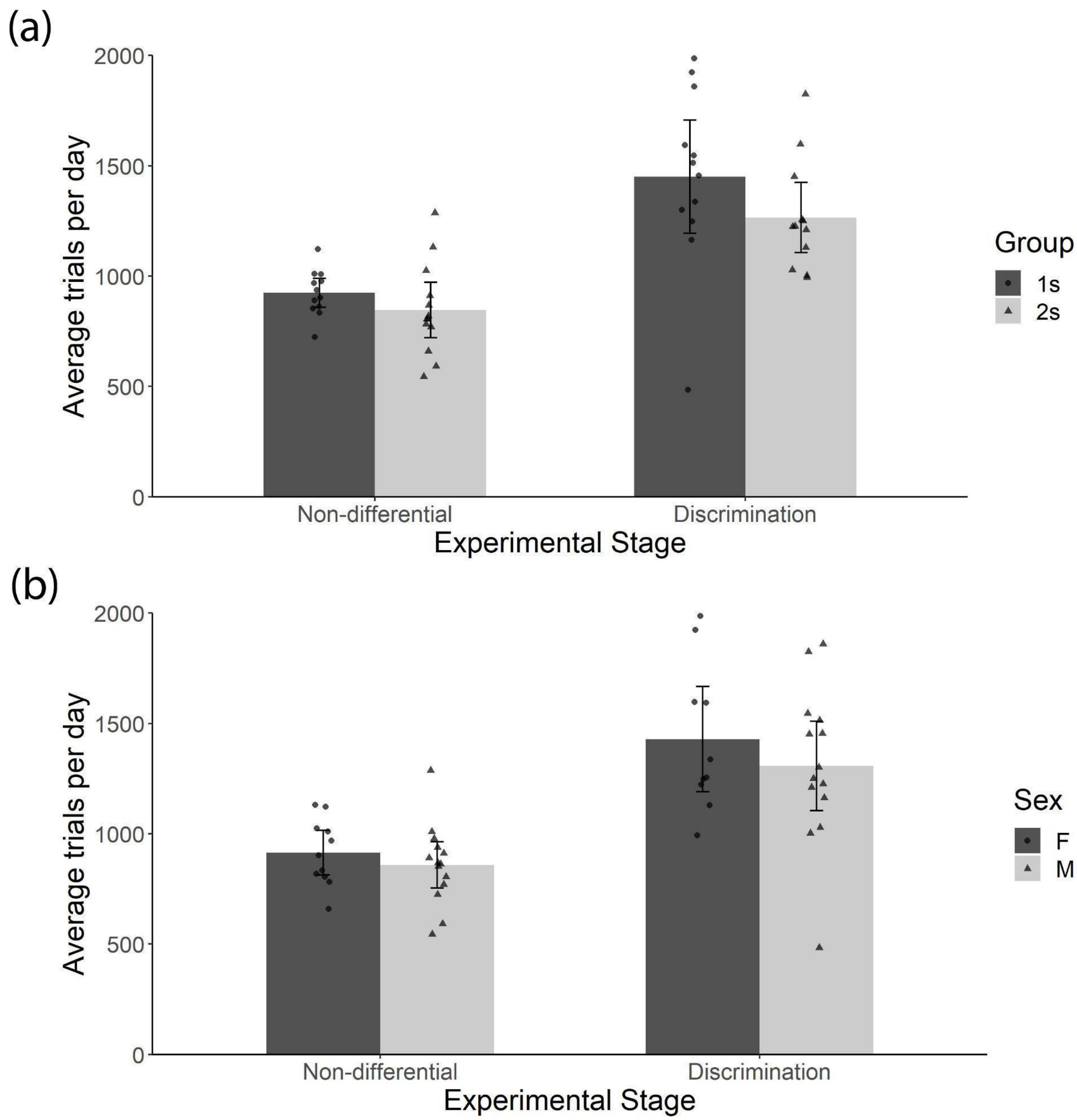

Fig.1 Bar and scatter (jittered) plot showing average trials per day for feed time groups (a), and for females and males (b). The bars represent average number of trials completed per day for experimental stages (Non-differential and Discrimination) for feed time groups and sex (dark gray: 1s and female and light gray: 2s and males). No significant difference across feed time

groups and sex was found. Each filled circle (1s group and females) and filled triangle (2s group and males) represents individual birds. Error bars represent 95% confidence intervals.

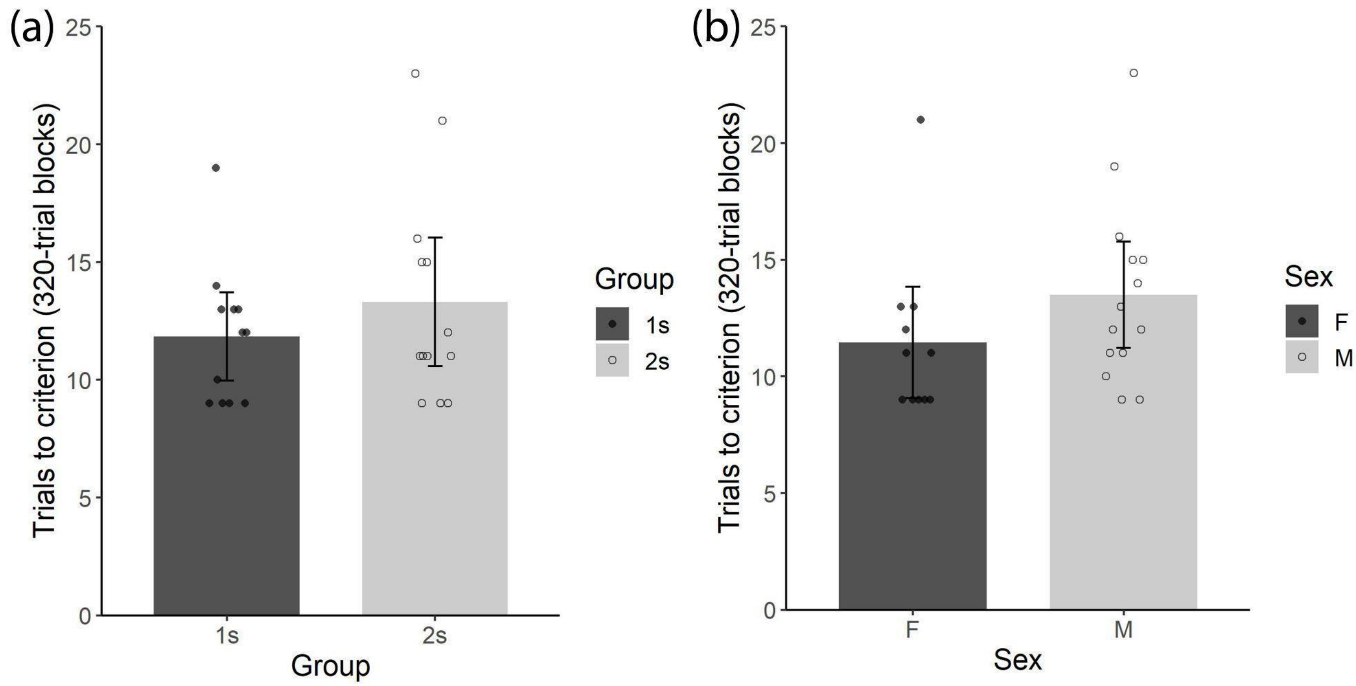

Fig. 2. Bar and scatter (jittered) plot showing trial blocks required to reach criterion for Discrimination training for feed time groups (a), and for females and males (b). The bars represent the average number of trial blocks required for each group (dark gray: 1s and female and light gray: 2s and males). Each filled circle (1s group and female) and open circle (2s group and male) represents individual birds. No significant difference across feed time groups and sex was found. Error bars represent 95% confidence intervals.