

Rule learning by zebra finches in an artificial grammar learning task: which rule?

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Abstract A hallmark of the human language faculty is the use of syntactic rules. The natural vocalizations of animals are syntactically simple, but several studies indicate that animals can detect and discriminate more complex structures in acoustic stimuli. However, how they discriminate such structures is often not clear. Using an artificial grammar learning paradigm, zebra finches were tested in a Go/No-go experiment for their ability to distinguish structurally different three-element sound sequences. In Experiment 1, zebra finches learned to discriminate ABA and BAB from ABB, AAB, BBA, and ABB sequences. Tests with probe sounds consisting of four elements suggested that the discrimination was based on attending to the presence or absence of repeated A- and B-elements. One bird generalized the discrimination to a new element type. In Experiment 2, we continued the training by adding four-element songs following a ‘first and last identical versus different’ rule that could not be solved by attending to repetitions. Only two out of five birds learned the overall discrimination. Testing with novel probes demonstrated that discrimination was not based on using the ‘first and last identical’ rule, but on attending to the presence or absence of the individual training stimuli. The two birds differed in the strategies used. Our results thus demonstrate only a limited degree of abstract rule learning but highlight the need for extensive

and critical probe testing to examine the rules that animals (and humans) use to solve artificial grammar learning tasks. They also underline that rule learning strategies may differ between individuals.

Keywords Biolinguistics · Artificial grammar learning · Discrimination learning · Rule learning · Syntax · Songbird

Introduction

A key requirement for language learning is the ability to detect structure and the rules underlying structure when exposed to a stream of speech sounds. Major advances in understanding this ability originate from experiments using the artificial grammar learning (AGL) paradigm, in which subjects are exposed to artificially constructed strings of speech sounds (e.g., syllables) devoid of any meaning, but structured according to a particular rule. Whether subjects detect the underlying rule when exposed to the sound stream or use a different strategy can be traced by examining the responses to novel sounds. This paradigm has proven extremely powerful in analyzing the rule learning abilities of human infants. Among the major findings is the discovery that 8-month-old infants are able to track transitional probabilities in a continuous stream of syllables and use this to distinguish ‘words’ from ‘non-words’ (Saffran et al. 1996), and the discovery that 7-month-old infants can detect an XYY or YXX pattern (X and Y denoting arbitrary sound items) in a sequence of syllables and generalize this to sequences consisting of new syllables (Marcus et al. 1999).

Another major conclusion obtained by artificial grammar learning studies is that mechanisms for learning need not be specific to language alone but might involve more

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domain general ones (reviewed by Gomez and Gerken 2000 and Folia et al. 2010) and show up with non-linguistic stimuli like musical tones and other non-linguistic sounds (Endress 2010; Gebhart et al. 2009; Saffran et al. 1999; but see Creel et al. 2004) and pictures (Saffran et al. 2007). Findings like these raise the question of what aspects of language learning can be explained by general perceptual and computational mechanisms rather than mechanisms that are specific for language acquisition (Newport and Aslin 2004; Saffran et al. 2008; Endress et al. 2009b). In addition to rule learning and statistical learning mechanisms contributing to language learning, it has been demonstrated that early language learning can be guided by so-called perceptual or memory primitives (POMPs), like sensitivity to edges of strings or repeated units (Endress et al. 2005, 2007, 2009b). These primitives are also suggested to provide a basis for the evolution of linguistic learning mechanisms.

While examining rule learning of humans using non-linguistic stimuli is one way to address the questions of domain specificity, another important one is exploring the presence of linguistic rule learning in non-human animals (Hauser et al. 2002a, b; Endress et al. 2009b). This has been done in a handful of species (see ten Cate and Okanoya 2012, for a review) and provides evidence that several species can detect regularities in acoustic input: primates like chimpanzees (Endress et al. 2010), rhesus monkeys (Hauser and Glynn 2009), and tamarins (Hauser et al. 2001; Newport et al. 2004), but also more distantly related mammal species such as rats (Toro and Trobalon 2005; Murphy et al. 2008). Arguably the best, but surprisingly hardly used, model species for examining rule learning abilities in animals are songbirds. In contrast to most mammal species, songbirds have complex, learned vocalizations, and birdsong researchers have discovered remarkable cognitive, neural, and genetic parallels between the processes involved in the production, perception, and development of language and birdsong (Berwick et al. 2011; Bolhuis et al. 2010; Doupe and Kuhl 1999). The first study to address vocal rule learning in a bird species was one in which starlings were examined for their ability to discriminate a 'recursive' A^nB^n structure from $(AB)^n$ sequences (Gentner et al. 2006). In our studies on acoustic stimulus generalization (Verzijden et al. 2007) and rule learning (van Heijningen et al. 2009), we have used zebra finches. Zebra finches, like starlings, could also learn to discriminate AABB from ABAB sequences (van Heijningen et al. 2009). However, further tests suggested that this ability resulted from using mechanisms such as acoustic generalizations and attending to the presence of repeated elements, thus providing an alternative explanation for the results obtained with starlings (Corballis 2007; ten Cate et al. 2010). Nevertheless, these studies have demonstrated

the suitability of birds for addressing question of rule learning in the context of vocal stimuli.

In the current study, we address the ability of zebra finches to distinguish an XYX structure, where the first element is identical to the last one in the sequence, from XXY and XYY. Marcus et al. (1999) showed that 7-month-old infants can distinguish XYX from XYY speech stimuli. As regards the abilities of non-human animals to detect such a structure, the results are ambiguous. The first study to address this, using a habituation paradigm in tamarin monkeys (Hauser et al. 2002a, b), suggested that tamarins could resolve the task, but this paper was subsequently withdrawn (Hauser et al. 2010). No evidence for discriminating XXY from XYY in human speech stimuli by rule learning was obtained in a study in rats using a habituation paradigm (Toro and Trobalon 2005). However, Hauser and Glynn (2009) showed that rhesus monkeys could discriminate XYY from XXY when X and Y were rhesus monkey calls. Recently, using an operant paradigm, Murphy et al. (2008) also showed that rats could distinguish an XYX (i.e., ABA, BAB–A being a bright light, B being dim light), from an XYY (i.e., ABB, BAA) and a XXY (i.e., AAB and BBA) configuration, in which one of these configurations signaled a food reward (see also Fountain and Rowan 1995). A second experiment in which A and B were tones demonstrated that the rats could transfer the discrimination from one set of tones to a novel one. Fascinating as this finding might be, in this study, as well as in earlier experiments (Hauser and Glynn 2009; Toro and Trobalon 2005) the emphasis was on showing that the animals could transfer the discrimination from one training set of specific Xs and Ys to a novel set. However, these studies leave open another question, which is *how* the animals make the distinction between the sequences (see also Corballis 2009). Murphy et al. (2008) suggested that the rats learned an overall rule, rather than the individual strings, but neither the presence nor the nature of this rule can be directly deduced from the results. The rats might, for instance, have learned to respond only to a structure without immediate repetition, without noticing the similarity of the first and last items. Studies examining these rules in any detail are still lacking (ten Cate and Okanoya 2012). The current study addresses this issue.

In Experiment 1, we trained zebra finches to distinguish XYX from XXY and YXX. Our prime aim was not to address whether this distinction, once made, could be transferred to similar sequences of novel sounds, but to examine how the birds made the distinction. To this end, we tested the birds with probe stimuli to examine various alternative rules that might be used for discrimination. In a subsequent experiment, we added an additional set of training stimuli in order to make the birds switch to a

different rule and again used probe tests to explore the outcome.

Experiment 1: can zebra finches learn to discriminate XYX from XXY and YXX , and if so: how?

Introduction

This experiment examined whether zebra finches are able to discriminate three-element stimuli with an XYX structure (ABA and BAB–Go stimuli) from stimuli with an XXY or YXX structure (BAA, AAB, BAA, and ABB–No-go stimuli) and, in particular, addressed the question *how* the birds solved the task. Note that the composition of the stimulus sets precludes some rules or some simple cues from being used. For instance, using the presence or absence of an AB (or BA) bigram does not allow discrimination of the two sets, nor does learning about the absolute position of an A or B-element, attending to the first or last two elements of the sequences, or the number of A's and B's present in a stimulus. Nevertheless, there are still multiple ways of discriminating between the sets. One rule would be an abstract algebraic rule like 'If the first and last element of a sequence are the same (independent of sequence length and types of element used), Go, and if they are different, No-go'. A variant might be to learn the structure rule either for the Go or for the No-go stimuli and to treat all other stimuli as belonging to the other category. Other rules might be 'If the stimulus contains no repeated element, Go, and if it does, treat it as an No-go,' or 'If the stimulus contains two transitions between different element types (i.e., both AB and BA), Go; if it has one transition between types, No-go' (again, variants can be to learn a structure rule for one set of stimuli and ignore the structure of the others). Apart from using a rule, however, the discrimination can also be done by memorizing the individual sequences, either all six, or alternatively just the ones connected to the S^+ or those connected to the S^- , without recognizing the structural similarities among S^+ or S^- stimuli. To examine how the zebra finches distinguished the training sounds, they were tested with various probe stimuli (lacking reinforcement for responding).

Subjects and housing

Ten adult zebra finches (at least 120 days old, five females and five males) from our own breeding colony were trained and tested in individual operant conditioning chambers using a Go/No-go procedure. The birds were naïve to the setup and training. In the breeding colony, adult birds are housed in same-sex aviaries on a 13.5 L: 10.5 D schedule at 20–22 °C. Cuttlebone, drinking water, and commercial tropical seed mix

(Tijssen, Hazerswoude) enriched with minerals were available ad libitum. The birds received a limited amount of egg food and fresh seedlings twice a week.

Apparatus

During the whole sequence of experiments and tests, the birds were individually housed in operant conditioning cages [70 (l) × 30 (d) × 45 (h) cm] made of wire mesh with a plywood back wall. Each cage was in a separate sound attenuated room, so the birds could not hear or see each other. The floor was covered with sand and grit. A fluorescent tube on top of the cage emitted daylight spectrum light (Lumilux DeLuxe Daylight, Osram) on a light/dark schedule identical to the breeding colony except for negative reinforcement periods (see Go/No-go procedure) during which the light was switched off temporarily. The back wall contained a food hatch and two red pecking keys, each containing a red LED (see Go/No-go procedure). The pecking keys and food hatch could be reached from wooden perches, with four additional perches to enable hopping behavior. A small mirror was placed on a side wall as cage enrichment.

Sound stimuli were played via a loudspeaker (Vifa MG10SD109-08) placed 1 m above the operant conditioning chamber and calibrated to an output of 70 dB (SPL meter, RION NL 15, RION) at the food hatch. A custom-made control unit (ELD, Leiden University) was connected to the fluorescent tube, loudspeaker, pecking keys, and food hatch, to control the Go/No-go procedure and registered the birds' key pecking behavior during this procedure.

Food intake was monitored daily, and before and after the experiment, the birds were weighed to monitor their condition.

Sound stimuli

Stimuli were constructed from zebra finch song elements originating from our zebra finch song database, consisting of undirected song (see Holveck et al. 2008 for recording specifications). From this database, we selected six element types ('flat,' 'trill,' 'downslide,' 'high,' 'curve,' and 'noisy'), based on optimal discriminability between the elements (see Fig. 1); See van Heijningen et al. (2009) for a description of these elements (except for the 'curve' type of element). All elements were ramped (3 ms) and rms (0.1) equalized in PRAAT (version 5.1.15, freeware available at www.praat.org). We inserted 40 ms of silence between consecutive elements in the various element sequences and added 50 ms of silence at the start and end of each song to avoid acoustic distortions during playback.

To avoid pseudo-replication and order effects, each bird was exposed to an individual combination of element

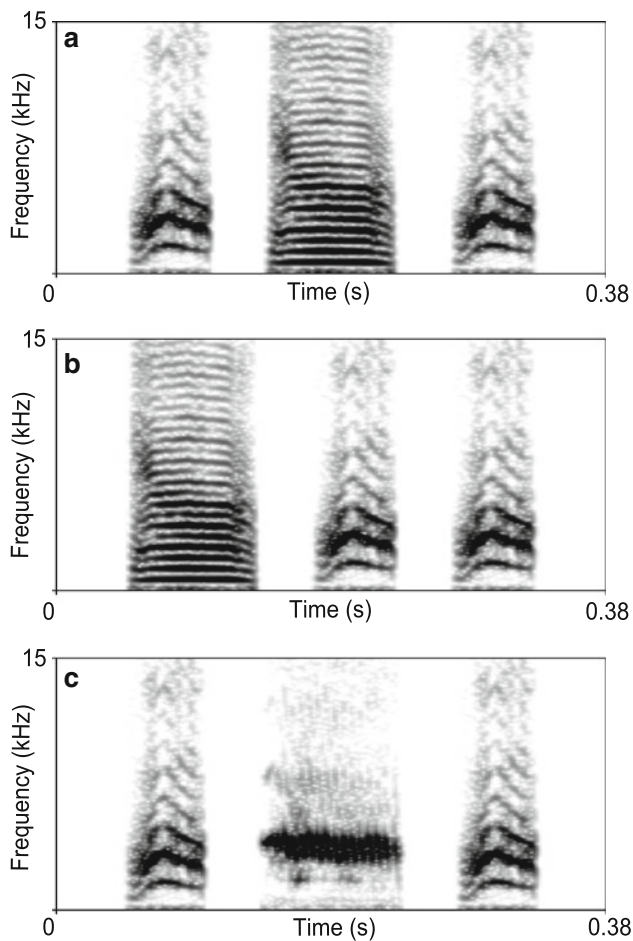


Fig. 1 Spectrograms examples of constructed song stimuli. **a** Curves and stacks in ABA order. **b** Stacks and curves in BAA order. **c** Curves and trills in ABA order

types. So, the song structure *XYX* for one bird, for instance, consisted of ‘flat-downslide-flat,’ while for another bird, this was ‘high-trill-high’ and so on (see Fig. 1 for examples).

Go/No-go training and probe tests

The zebra finches were trained in a Go/No-go operant procedure with food as a reward. In the operant cage, the left sensor was illuminated with a red LED. A peck on this sensor resulted in a sound stimulus and also activated the right sensor, indicated by switching on the LED of this sensor. In 50 % of all cases, the sound was a ‘Go’ stimulus (S^+) after which the bird had to peck the right sensor within 6 s. Subsequently, the food hatch opened for 10 s, and the bird was able to eat. In the other 50 % of the cases (in randomized order) that the bird pecked the left sensor, a ‘No-go’ sound (S^-) was played. If the bird subsequently pecked the right sensor within 6 s, the cage light was switched off for 15 s. For three birds, this period was temporarily prolonged with a maximum of 15 s to promote

discrimination learning. For a more detailed description of the Go/No-go procedure, see van Heijningen et al. (2009).

To learn the Go/No-go procedure, birds were pre-trained with a natural song from our database as S^+ and a 2 kHz tone as S^- (equalized on rms 0.1 and ramped with 3 ms) of equal duration (0.58 s) constructed in PRAAT. After reaching criterion performance (>75 % Go-response after hearing S^+ and <25 % Go-response after hearing S^- , on at least two consecutive days), the pre-training stimuli were replaced by the training stimuli of Experiment 1 (transfer on the third day). A DR (discrimination ratio, calculated as $S^+/(S^+ + S^-)$) larger than 0.75 was taken as an alternative learning criterion if a positive or negative response bias prevented some birds from reaching the separate criteria for S^+ or S^- .

After the birds had reached the performance criterion on the pre-training, the pre-training stimuli were abruptly replaced by the training stimuli (S^+ : ABA, BAB; S^- : AAB, ABB, BAA, BBA). The overall ratio of positive and negative sounds played was 50/50. At the end of this training phase (when the criterion for reaching discrimination had been reached again), probe sounds were used to assess which of several potential strategies the birds might have used to discriminate the training sets. Responses to these probe songs were not reinforced to avoid any additional learning. Probe stimuli were intermixed with the already familiar training stimuli, constituting 20 % of stimulus presentations. Each probe stimulus was presented at least 40 times in a pseudo-random order without replacement. Also, ‘control’ probes that were identical to the training stimuli but not followed by reinforcement were added to control for the effect of non-reinforcement.

Two consecutive probe tests were done. In the first one, we presented the probe stimuli ACA, CAC, ABBA, and BAAB (C being a novel element type), together with probe (not followed by reinforcement) versions of the positive training stimuli. If the birds had learned the ‘first and last identical’ rule, a response to ACA and CAC would indicate that the rule was generalized to new element types, as ‘C’ was unknown to them. A more restricted version of the rule would be ‘first and last identical, but only for stimuli consisting of A’s and B’s’. Applying this rule would result in treating ACA as familiar and CAC as a novel, and initially neutral stimulus. Probing with ABBA and BAAB would be predicted to give the same probability of responding as to ABA and BAB (as the length of the sequence is irrelevant). Alternatively, if the birds were responding to the presence of repetitions, ABBA and BAAB should be treated as S^- stimuli.

The second probe test contained ABAB, BABA, AABB, and BBAA, together with (control) probe versions of the negative training stimuli. If the birds had learned the ‘first and last different’ rule for the S^- , they should not respond

to these probes. Sensitivity to transitions between subsequent elements (that is, absence of repeats) or to the presence of repeats should make them treat the ABAB and BABA stimuli as positive, and AABB and BBAA as negative.

Results

Eight out of ten birds learned to distinguish ABA and BAB from ABB, BAA, BBA, and AAB (average 24,975 trials, $\pm 5,185$ SEM/range, 6,956–47,513, $n = 8$). The number of trials per day varied among the birds but was on average 363, SEM 31/day. There was no indication of a sex difference in responding.

The response probabilities for the two probe tests are given in Fig. 2. For statistical testing, we took the average scores to training- and test-stimuli with a similar structure. We applied simultaneous testing procedures (STP) based on *G*-tests of independence to assess which stimuli were responded to similarly (a Williams correction was calculated when numbers were lower than 5 but this never changed the outcome; Sokal and Rohlf 1995). Levels of response to the probe version of ABA and BAB during the first probe test and to the probe versions of BBA, ABB, BAA, and AAB during the second probe test were homogeneous compared with the training (reinforced) version of these structures, with the exception of the response to ABA for one bird. We therefore compared the probability of response to all test probes with the probability of response to the training stimuli.

The level of response to the ACA and CAC probes did not, at group level, differ from those to the S^- stimuli, showing that the birds did not generalize from their training to new element types arranged in a similar structure as the positive stimuli. However, at the individual level, one bird (822) responded to both ACA and CAC in the same way as to ABA and BAB. Three birds treated ACA and CAC as

neutral, as their probability of response was intermediate compared with both sets.

At group level, responding to the ABBA and BAAB probes was not similar to ABA and BAB. Instead, it was similar to the S^- training set. Five individual birds showed this pattern, while three birds differed from both S^- and S^+ .

In the second probe test, ABAB and BABA were treated similarly to the positive training set ABA and BAB at group level (see Fig. 2b). On the individual level, however, these probes were treated as neutral by four birds, as the level of response to them was in between those to the positive and negative training stimuli.

At group level, AABB and BBAA were treated differently from both positive and negative training sets, in this case by even fewer responses to these probes than to the S^- stimuli. This pattern was present in five individuals, of which three never responded to these probes.

While most birds followed the general patterns described above, one (bird 822) deviated notably in its probability of response to the probe stimuli. As mentioned above, he was the only bird to respond with a high probability to the ACA and CAC songs and could therefore have learned a rule not linked to the identity of A and B. He also had an intermediate probability of response to ABBA and BAAB. We therefore added a third test to explore what rule this bird might be using. This set consisted of ADA, ADD, ABBBA, ACBCA, CADC, and ACDC and thus contained stimuli with a novel element type (D), as well as probes with multiple transitions. The bird responded to the new probes CADC and ACDC in a similar way as to the positive training set ABA and BAB. He responded at an intermediate level to ADA, ADD, and ACBCA, and his response level to ABBBA was more similar to that shown to the negative training set. These results suggest that 822 responded with a high probability to stimuli with new element types, in particular when these had transitions between element types. ABBBA seemed to be perceived as

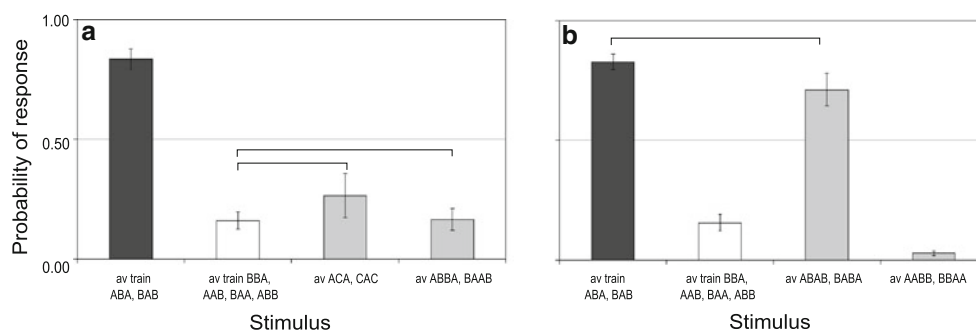


Fig. 2 Averaged response probabilities to the stimuli ($n = 8$) during the first (a) and second (b) probe test (\pm SEM) of Experiment 1. *Black bars* indicate positive training stimuli; *white bars* negative training stimuli and *gray bars* probe stimuli. *Lines* indicate homogenous

response following *G*-tests of independence between training stimuli and between training and probe stimuli (Sokal and Rohlf 1995) with $\chi^2 = 9.49$ ($P < 0.05$)

a compound negative stimulus (ABB and BBA). However, there was no clear indication of the use of a particular rule.

Discussion

Our results confirm earlier findings (Verzijden et al. 2007; van Heijningen et al. 2009) that zebra finches are sensitive to element sequence in acoustic stimuli. The stimulus set used in the current training demonstrates that zebra finches can distinguish stimuli in which single element position or number of A- or B-elements in a stimulus cannot be used as a cue for discriminating the stimuli. The results of the probe tests also exclude the use of some alternative rules. First, if the birds learned a rule, it was linked to the presence of A- and B-elements and did not transfer to novel elements (with the possible exception of bird 822). Secondly, the high probability of response to ABAB and BABA and the low probability of response to ABBA, BAAB, AABB, and BBAA show that the birds did not use the ‘first and last identical’ or ‘first and last different’ rule.

Most birds did not treat the probes as neutral, but differentiated among them, classifying some of them with S^+ and some with S^- stimuli. One explanation might be that the birds (excluding 822) responded to the probes according to whether these contained the exact three-element sequences from the training instead of using an abstract rule (S^+ stimuli present in: ABAB, BABA; S^- present in: BAAB, ABBA, AABB, BBAA). An alternative hypothesis is that they were sensitive to the presence/absence of adjacent repeats (bound to A's and B's). This latter hypothesis is supported by the observation that AABB and BBAA, each containing two repeated elements, were treated as ‘super negative’ stimuli as if there was a ‘peak shift’ (Verzijden et al. 2007).

Experiment 2: can zebra finches switch rules?

Introduction

Experiment 1 suggested that most zebra finches discriminated ABA and BAB from AAB, ABB, BAA, and BBA by attending to the presence or absence of adjacent AA or BB repeats. To further explore the rules that the birds used to distinguish the training sets and also to see whether these rules can be altered if training sounds cannot be classified correctly with these rules, all birds were subjected to a further training procedure. In this training procedure, we added ABAB, BABA, AABB, and BBAA to the S^- stimuli of Experiment 1, and ABBA and BAAB to the S^+ stimuli of Experiment 1. Compared with Experiment 1, the current task could not be solved by attending to repeated elements,

as both training sets contained repeats, and hence, these were no longer diagnostic. The same held for attending to transitions. As ABAB and BABA in the new S^- set consisted of a series of transitions, these also could not be used to indicate a positive stimulus. However, the training stimuli could still be discriminated in a number of ways. One of them was still to compare the first and last element of the sequences (first and last identical). Alternatively, the stimulus sets could be discriminated by rote memorization of the individual stimuli. For those birds that succeeded in the training, we again used probe tests to examine how they achieved the discrimination.

Training and probe tests

The experiment was done with the eight birds that succeeded in the training of Experiment 1. After the probe test of Experiment 1, the training sets were extended to include ABBA and BAAB as S^+ stimuli in addition to ABA and BAB, and ABAB, BABA, AABB, and BBAA as S^- stimuli in addition to AAB, ABB, BAA, and BBA.

The birds that reached the learning criterion received two consecutive probe tests to uncover how they discriminated between the extended stimulus sets. The first test included ACA, CAC, ABAA, AABA, AABBA, ABBAB, and control probes ABA, BAAB, AAB, and ABAB. ACA and CAC were used (again) as a control for whether the birds would now generalize to new element types. ABAA and AABA both contained one positive (ABA) and one negative sequence (BAA and AAB, respectively). Responding positively could indicate the use of the ‘first and last identical’ rule. However, a high probability of response might also indicate that the bird paid attention to the presence of ABA in the full sequence and ignored the fact that the sequence also contained a negative stimulus (BAA or AAB). A low probability of response might be due to a focus on the presence of an S^- stimulus in the novel stimulus. AABBA includes more negative than positive stimuli (positive: ABBA, negative: AABB, AAB, ABB, and BBA) but has an overall ‘first and last identical’ structure. If the birds learned the ‘first and last identical’ rule, they should have a high probability of response to AABBA, but a low one to ABBAB. However, if the birds paid attention to the presence of ABBA and/or BAB instead of a more general rule, they would be expected to have a high probability of responding to ABBAB.

In the second probe test, we tested the probability of response to AAA, ACCA, CAAC, ACAC, CACA, DCD, DDC, and CDD (ABA and AAB were added as control probe sequences). AAA is an example of a non-transition song, but the first element is the same as the last one. The responses to the other probe songs should indicate whether the birds could generalize what they had learned to new

element types, as the birds had not been trained before with Cs and Ds.

Results

In the first days after switching to the new training sets, all birds changed their probability of responding to the original training sounds (ABA, BAB, ABB, BBA, AAB, and BAA) even though their responses continued to be reinforced in the same way as before. Table 1 shows the individual probability of response to the original training stimuli just before and over the first 9 days after introducing the additional training sets. On day one after transfer, the averaged probability of response to the familiar negative three-element stimuli (ABB, ABB, BAA and BBA) was under 0.25, as it was during the training. However, over time this value increased to, on average, 0.55 on day nine. The average probability of response to the positive three-element stimuli, ABA and BAB, remained above 0.75.

After day nine, for logistic reasons, we were only able to continue the training for five birds (825, 779, 796, 813, and 820). Over time, only birds 825 and 779 reached the overall criterion performance (after 36112 and 52474 trials, in 151 and 107 days, respectively). Two birds did not improve their level of discrimination over several weeks of training and were withdrawn from the experiment. One bird was withdrawn for health reasons.

Interestingly, both bird 825 and bird 779 were relatively quick in improving their DR for the three-element stimuli immediately after the start of the new training phase (0.65 and 0.72 on day 9), compared with the other birds (0.55, 0.57, and 0.59), suggesting that the speed of learning predicts later success. Figure 3 shows the response probabilities for birds 825 and 779 to the various probes. As in Experiment 1, G-tests confirmed that each control probe was homogeneous with its reinforced version, and we

therefore compared the responses to the unfamiliar probes with those to the training stimuli.

For each bird, we tested whether the response probabilities to specific stimulus subsets within the training sets (ABA and BAB; ABBA and BAAB; etcetera) were homogenous. This was the case for bird 825 only (Fig. 3b, d). This bird also treated all new probes of both series as homogenous with the negative training set (BBA, ABB, AAB, BAA, ABAB, BABA, AABB, and BBAA). For bird 779, the within-training sets comparisons were not homogeneous (see Fig. 3a, c). The score for ABBA and BAAB was high, but not as high as for ABA and BAB (0.74 compared with 0.93, respectively). Of the negative training stimuli, AABB and BBAA were homogeneous with AAB, ABB, BAA, and BBA during the second probe test, but otherwise the three negative training sets gave rise to different levels of responses. The bird responded at a low level to any probe including novel element types (C or D) and to the AAA probe, but showed an intermediate response level to AABA, AABBA and ABBAB.

Discussion

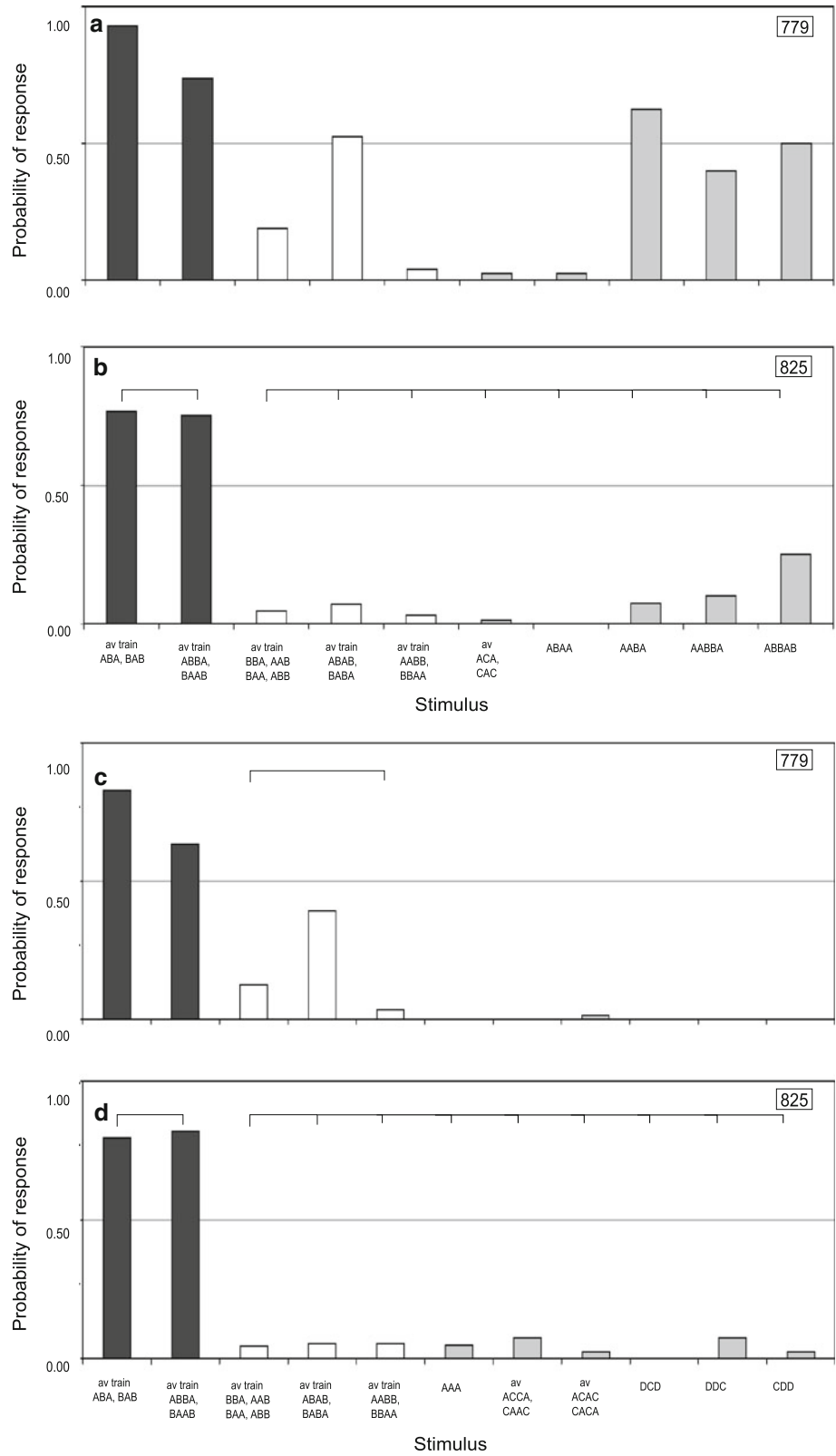
All birds showed marked changes in their probability of responding to the original three-element training sounds after adding the novel training sounds to this set, even though the responses remained to be reinforced in the same way as before. This adds to the evidence that the discrimination between the S^+ and S^- shown in Experiment 1 may have been based on using a more general ‘rule,’ such as ‘presence (or absence) of repeats’ rather than on memorizing each individual stimulus separately. In Experiment 2, such a rule was no longer applicable. Therefore, the change in probability of responding might indicate that the birds stopped using (‘unlearned’) that rule after transfer to the larger training sets.

Table 1 Response probabilities and discrimination ratios (DR) to the original training stimuli before and after transfer to Experiment 2

| Days after transfer | −2 ^a | | | 1 | | | 4 | | | 9 | | |
|---------------------|-----------------|----------------|------|----------------|----------------|------|----------------|----------------|------|----------------|----------------|------|
| | S ⁺ | S [−] | DR | S ⁺ | S [−] | DR | S ⁺ | S [−] | DR | S ⁺ | S [−] | DR |
| 778 | 0.75 | 0.10 | 0.88 | 0.57 | 0.03 | 0.95 | 0.68 | 0.16 | 0.81 | 0.71 | 0.19 | 0.79 |
| 779 | 0.89 | 0.11 | 0.89 | 0.88 | 0.19 | 0.83 | 0.87 | 0.50 | 0.63 | 0.93 | 0.51 | 0.65 |
| 796 | 0.65 | 0.12 | 0.84 | 0.73 | 0.10 | 0.88 | 0.85 | 0.51 | 0.62 | 0.95 | 0.79 | 0.55 |
| 813 | 0.90 | 0.16 | 0.85 | 0.93 | 0.54 | 0.63 | 0.97 | 0.86 | 0.53 | 0.98 | 0.74 | 0.57 |
| 825 | 0.76 | 0.02 | 0.98 | 0.50 | 0.04 | 0.93 | 0.70 | 0.31 | 0.70 | 0.60 | 0.23 | 0.72 |
| 820 | 0.82 | 0.14 | 0.85 | 0.80 | 0.18 | 0.81 | 0.72 | 0.35 | 0.67 | 0.84 | 0.59 | 0.59 |
| 822 | 0.90 | 0.17 | 0.84 | 0.81 | 0.14 | 0.85 | 0.95 | 0.42 | 0.69 | 0.89 | 0.64 | 0.58 |
| 844 | 0.78 | 0.09 | 0.90 | 0.95 | 0.56 | 0.63 | 0.85 | 0.63 | 0.57 | 0.86 | 0.72 | 0.54 |
| Average | 0.81 | 0.12 | 0.88 | 0.77 | 0.22 | 0.81 | 0.82 | 0.47 | 0.65 | 0.84 | 0.55 | 0.62 |

^a Data of day −2 contain the averaged data over the last 2 days before transfer

Fig. 3 Response probabilities to the stimuli of the first (a, b) and second (c, d) probe test of Experiment 2 of birds 779 and 825, respectively. *Black bars* indicate positive training stimuli; *white bars* negative training stimuli, and *gray bars* probe stimuli. *Lines* indicate homogenous response following G-tests of independence between training stimuli and between training and probe stimuli ($\chi^2 = 22.36, P < 0.05$)



The probe tests for the two birds that managed to discriminate the novel training sets show that, as in Experiment 1, both birds did not generalize to new element types:

the probability of response to all songs with C's or C's and D's were low, as were those to songs resembling positive songs in overall structure (ACA and ACCA). Also, neither

bird used the comparison between the first and last element (similar for S^+ , dissimilar for S^-) to distinguish the training sets, as they responded with a high probability or ambiguously to probes that should be classified as negative according to this rule.

For bird 825, the most likely interpretation of his response pattern to the new probes (all with low probability) is that he memorized each positive training sequence separately and rejected all other sequences, and hence also novel ones.

The interpretation for 779 is slightly different. She responded more ambiguously to AABA, AABBA, and ABBAB. She also showed an intermediate probability of response to the training stimuli ABAB, BABA, ABBA, and BAAB. An explanation might be that these training stimuli and probes all contained three-element sequences for which the birds were reinforced in the opposite direction compared with the full sequence (e.g., ABAB was a negative stimulus, containing the positive sequences ABA and BAB). Taken together, 779 most likely did not acquire a general rule based on a shared structure of the stimuli but paid attention to both the full sequences as well as to the presence of positive and negative three-element training stimuli in the probe sets.

General discussion

Our data demonstrate that zebra finches can learn to discriminate ABA, BAB from BBA, BAA, ABB, and AAB. At first sight, such discrimination may suggest that the birds obtained some insight in the abstract rules differentiating the stimulus sets (e.g., learning a ‘first and last similar/different’ rule). Such an interpretation has been given for rats differentiating stimuli structured in a similar way to those in our experiment (Murphy et al. 2008). However, demonstrating such an ability requires two types of tests. The first, and the one most commonly applied in artificial language experiments, is whether training with one set of tokens (in this case A- and B-elements) induces generalization to a novel set (C and D-elements), analogous to a ‘new vocabulary’ in human language (Marcus et al. 1999; Marcus 2006; Frank et al. 2009). Such a generalization test has been done for instance in tamarins (Endress et al. 2009a), but most experiments have focused on generalization to new tokens of familiar types (i.e., replacing a specific ‘A’ element by another ‘A’) or new combinations of familiar types (i.e., Abe and Watanabe 2011; Gentner et al. 2006; Hauser and Glynn 2009; Herbranson and Shimp 2008; Newport et al. 2004—see ten Cate and Okanoya (2012) for a review). Only this study, van Heijningen et al. (2009) and Murphy et al. (2008) tested generalization with sequences of new element types, although in this last

study, the novelty of these stimuli was questioned by Corballis (2009). In our experiment, only one out of eight birds seemed to generalize to novel elements and treated ACA and CAC as similar to ABA and BAB. This result is comparable to that we obtained in our previous study (van Heijningen et al. 2009) in which we showed that zebra finches had no difficulty in generalizing to novel exemplars of the same element type, but only one bird transferred a learned discrimination to a novel element type. It shows that zebra finches have the potential for such a generalization but do not use this spontaneously. The current experiment was, however, not aiming to see whether this ability could be specifically trained. This would require an extension of the training by providing many stimulus sequences of identical structure but with novel items.

Our experiment focused on a second type of test, addressing the question how the ABA, BAB versus AAB, ABB, BBA, BAA distinction is made once it has been learned. Probe tests with novel sequences of A’s and B’s are essential for this, as has also been demonstrated by Gentner et al. (2006) and van Heijningen et al. (2009). Van Heijningen et al. (2009) showed that the birds relied on simple strategies when possible, such as the presence of repeats of familiar elements. This also is the outcome of the present study. The results of Experiment 1 show no evidence of learning a rule like ‘first and last elements are similar versus dissimilar’. Instead, the probe tests of Experiment 1 and the response probabilities to the new training stimuli in Experiment 2 suggest that most birds learned to attend to the presence or absence of element repeats (AA or BB). Interestingly, as mentioned in the introduction, repeats have previously been suggested to be important perceptual or memory primitives in humans (Endress et al. 2009a). Such primitives may serve an important role in early learning and are suggested to provide a basis for the evolution of linguistic learning mechanisms (Endress et al. 2009b). Our study suggests that not just humans and chimpanzees, but also other animal species might be sensitive to such primitives.

In Experiment 2, we extended the training set and the presence or absence of repetitions could no longer be used to discriminate between the sets. The rapid loss of discrimination among the 3-element stimuli and difficulties in mastering the new discrimination demonstrates that the birds abandoned one type of rule (‘repeats’) but struggled to replace it by a new one. Neither of the two birds succeeding in the discrimination solved it by attending to the regularity of ‘first-last identical versus different,’ and both most likely used a strategy (albeit a different one) based on rote memorization of the individual training stimuli.

Both experiments demonstrated individual variation in strategies. Such variation has also been observed in our earlier study (van Heijningen et al. 2009) and has also been

shown for humans in artificial grammar learning studies (Zimmerer et al. 2011). This may reflect the fact that what might seem to be a simple task, discriminating ABA from AAB and BAA, is a problem that can be solved in different ways. As also noted for infant studies on the same paradigm, any set of experiences potentially supports an infinite number of possible generalizations (Gerken 2006) and which particular one is used by an individual may depend on individual characteristics. Also, the low level cognitive strategies used in the current experiments do not prove that zebra finches (or any other species) are unable to learn and apply a more abstract rule. The strategies currently employed may be bound to the type of training stimuli used and the sequence in which they are presented, as such parameters may affect what is learned (Gerken 2006). Using a different paradigm, a study of Bengalese finches, a relative of the zebra finch, suggests they could acquire a more complex rule (Abe and Watanabe 2011), although this finding has been challenged (Beckers et al. 2012). Hence, it may be that a different training paradigm might also result in more abstract rule learning in zebra finches. Our results also highlight that regularities of a stimulus set are noticed and used for generalization to novel stimuli. However, given that the zebra finches readily mastered the basic training by using simple regularities rather than more abstract rules, our study demonstrates the need for extensive and critical probe testing to detect what these regularities might be and also to examine whether these differ among individuals (see also ten Cate and Okanoya 2012). Finally, we suggest that our results might also indicate that human experiments may benefit from a wider use of probe stimuli to analyze the rules humans use to solve similar problems.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards This study was conducted according to the Association for the Study of Animal Behavior guidelines on animal experimentation as well as to the Dutch law on animal experimentation. The Leiden committee for animal experimentation (DEC) approved the experiment under number 09228.

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