

Is choice bias in delayed matching a consequence of inter-trial interval effects?

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ARTICLE INFO

Dataset link: [Choice biases in delayed matching: is the inter-trial interval always to blame?](#)

Keywords:

Temporal discrimination
Matching to sample
Delay
Inter-trial interval
Pigeons

ABSTRACT

Despite their intended goal of simply separating trials, inter-trial intervals have been found to affect choice behavior in delayed matching-to-sample procedures, leading to choice biases. In the present study, we assessed the effects of delay on choice without the potential influence of inter-trial intervals. Sixteen pigeons learned a symbolic matching-to-sample task with samples differing in duration (3 s vs. 9 s) and no inter-trial intervals. In testing, a delay lasting 2.5, 5, 10 or 20 s was introduced between sample and comparisons. There were two differing delay conditions: for Group Light Off, the delay was spent in darkness and, for Group Light On, the delay was illuminated by a houselight. For Group Light Off, as the delay increased, matching accuracy following both samples decreased towards indifference. For Group Light On, matching accuracy remained relatively high in short-sample trials but decreased abruptly in long-sample trials (choose-short effect). Hence, we found that a choice bias may occur even without inter-trial intervals.

1. Introduction

In a symbolic matching-to-sample task, a sample stimulus is presented followed by a choice between two comparison stimuli. For instance, in an operant chamber, a key might be illuminated with a white hue for a shorter (3 s) or a longer duration (9 s), followed by the presentation of one red key and one green key. A response on the red key is followed by a reinforcer only after a 3-s stimulus, and a response on the green key is followed by a reinforcer only following a 9-s stimulus. Given its association with the short sample, the red key might be designated as the “short” comparison, while the green key might be designated as the “long” comparison. Such a procedure, sometimes with a delay between sample offset and comparison onset (delayed symbolic matching) has been widely used to study memory, choice, stimulus control, or coding strategies (for a review, see [Zentall and Smith, 2016](#)).

Typically, trials are separated by an inter-trial interval (ITI) to prevent proactive interference. Indeed, pigeons’ accuracy in delayed matching-to-sample tasks has been found to improve with ITI duration (e.g., [Edhouse and White, 1988](#); [Grant, 1975](#); [Maki et al., 1977](#); [Roberts, 1980](#); [Roberts and Kraemer, 1982](#)). However, despite their apparent benefits, when temporal samples are used (i.e., samples that differ in

duration, as in the opening example), ITIs may also bring about other, arguably undesirable, effects.

For instance, when ITI and delay intervals are signaled similarly (e.g., both spent in darkness), pigeons have shown a preference for the “short” comparison, irrespective of the sample presented (choose-short effect; e.g., [Kelly and Spetch, 2000](#); [Kraemer et al., 1985](#); [Pinto and Machado, 2011](#); [Spetch, 1987](#); [Spetch and Grant, 1993](#); [Spetch and Wilkie, 1982](#)). Whereas this choose-short effect could be framed as a memory-related bias, some authors suggested it results from confusion between delay and ITI (e.g., [Fetterman and MacEwen, 1989](#); [Sherburne et al., 1998](#); [Zentall, 1997](#); [2006](#)): the delay may be interpreted as a trial-cancelling ITI and thus, when the comparisons become available following a delay, the animals respond as if no sample had been presented. If, by stimulus generalization, the absence of a sample is then considered to be closer to the short than to the long sample, a preference for the “short” comparison is expected. In fact, in some cases, signaling ITIs and delays differently has been found to eliminate choice biases ([Kelly and Spetch, 2000](#); [Sherburne et al., 1998](#); [Spetch and Rusak, 1992](#)); however, in other cases a preference for “short” subsisted ([Kelly and Spetch, 2000](#); [Pinto and Machado, 2011](#); [Pinto and Sousa, 2021](#)). Thus, while similar ITIs and delays consistently lead to a preference for

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the “short” comparison, differentiating these intervals appears to weaken this effect – similarity with delays is an example of how ITIs may affect matching performance.

Spetch and Rusak (1989) trained pigeons to discriminate between 2 and 10-s samples in a delayed symbolic matching-to-sample task with 45-s ITIs and 10-s delays. In testing, they varied the ITI duration: In some sessions, ITIs were 5 or 90-s long and, in other sessions, 15 or 75-s long. Regardless of the sample duration, when the ITI was shorter than in training (i.e., 5 or 15 s), pigeons showed a preference for the “long” comparison (choose-long effect). When the ITI was longer than in training (i.e., 75 or 90 s), there was a preference for the “short” comparison (choose-short effect). Thus, pigeons’ performance was affected by ITI duration. Spetch and Rusak (1989, 1992) suggested that the animals judged the sample duration relative to a temporal background composed by the ITI and the delay – a sample would seem shorter in the context of a lengthy ITI and longer in the context of a short ITI (relative duration hypothesis). Once more, the ITI reveals itself to be more than a simple trial separator.

Besides influencing performance under delayed choice, the ITI itself may be used to learn a task, at the expense of other stimuli. For instance, Pinto and Machado (2015, 2017) trained pigeons in a matching-to-sample task with three sample durations and two comparisons; one comparison was correct following one sample and the other comparison was correct following the other two samples. A 30-s ITI illuminated by a houselight separated trials. The houselight offset signaled the end of the ITI and coincided with sample onset. Thus, pigeons could have ignored the sample and used the houselight as a time marker to learn the task. When delays were introduced (which lengthened the interval between ITI offset and comparison onset), pigeons showed a preference for the comparison associated with the long sample. In tests where no sample was presented, preference for the comparison associated with the long sample increased with time elapsed since the ITI. Taken together, these results suggest that pigeons were using ITI offset (signaled by the houselight) as a time marker.

All aforementioned instances of choice biases occurred in tasks with ITIs. Not only that, but ITIs have been frequently invoked to explain these biases. In the present study we ask whether choice biases occur only because of ITIs. So, pigeons learned a temporal matching-to-sample task without ITIs. Our goal was to assess the effect of delays on matching performance without the potential interference of ITI effects. Additionally, in tasks with ITIs, delay illumination appears to affect pigeons’ choices (e.g., Dorrance et al., 2000; Spetch and Rusak, 1989; Kelly and Spetch, 2000). To explore the potential effect of delay illumination in a task without ITIs, we included two delay conditions: For half of the pigeons, the delays were spent in darkness, and for the other half the delays were houselight-illuminated.

2. Method

2.1. Subjects

Sixteen pigeons (*Columba livia*), maintained at 85 % of their free-feeding body weight, participated in the experiment. The birds were housed individually in a colony room with a light/dark cycle of 13:11 h (lights on at 8 AM) and controlled temperature (between 20 and 22°C). All pigeons had water and grit always available. The experiment was conducted once a day, at approximately the same time for each pigeon, six days a week. All pigeons had experience with matching-to-sample, choice, and temporal discrimination tasks.

2.2. Apparatus

Four operant chambers were used: three Lehigh Valley Electronics (LVE) chambers and a homemade chamber. The LVE chambers measured 340 mm high by 350 mm long by 310 mm wide and were equipped with an exhaust fan that circulated air and masked outside

noise. The front panel contained three circular response keys (25 mm in diameter), horizontally aligned, 90 mm apart from each other (center-to-center), located 225 mm above the wire mesh floor. The keys were equipped with an Industrial Electronics Engineers (IEE) 12-stimulus in-line projector and each stimulus was illuminated with a 28-V, 0.1-A light bulb. On the back panel, 300 mm above the floor, a houselight (28 V, 0.1 A) provided general illumination to the chamber. Mixed grain served as reinforcement and was delivered through a LVE feeder through an opening measuring 60 mm wide by 50 mm high, centered horizontally on the response panel, 85 mm above the floor. When the feeder was raised to allow access to reinforcement, a 28-V, 0.04-A light illuminated the opening.

The homemade chamber measured 319 mm high by 330 mm long by 330 mm wide and was placed inside a PVC sound attenuation cubicle (Med Associates, ENV-018 V) equipped with an exhaust fan. In the front panel, 210 mm above the wire mesh floor, there were three response keys, 25 mm in diameter, horizontally aligned, 90 mm apart (center-to-center). Stimuli were presented through a 12-stimulus IEE in-line projector and illuminated by a 28-V, 0.1-A lamp. Centered horizontally on the response panel, 65 mm above the floor, an opening (60-mm wide x 45-mm high) provided access to reinforcement. When food was available, the feeder opening was illuminated by a 28-V, 0.04-A light. On the opposite wall, 275 mm above the floor, there was a 28-V, 0.1-A light fixture. A computer with the ABET II software (Lafayette Instrument Company) controlled the experiment and recorded the data.

2.3. Procedure

2.3.1. Training

All pigeons were trained in a symbolic matching-to-sample task that featured two samples and two comparisons. A trial started with the illumination of the center key with a white hue for either 3 s (short sample) or 9 s (long sample). After the sample duration elapsed, the central key was turned off and the two side keys were illuminated, one with a red hue and the other with a green hue (comparisons). One comparison was correct following the 3-s sample and the other comparison was correct following the 9-s sample. A peck on either key, turned both comparisons off. When a response was correct, a reinforcer (mixed grain) was delivered. A correction procedure was in effect: When a response was incorrect, the trial repeated until a response was correct, up to a maximum of three repetitions. After three consecutive incorrect responses, only the correct comparison was presented. Following a correct response, a reinforcer was delivered and the session progressed to a new trial.

Excluding correction trials, each session consisted of 60 trials divided in three 20-trial blocks. Each block contained ten trials for each sample duration, randomly distributed. The location of the comparisons was counterbalanced within each block to ensure that each comparison was presented the same number of times in each side key. The division of the session in blocks ensured a more balanced distribution of trials throughout the session. The color associated with each comparison was counterbalanced across pigeons: for half of the pigeons, red was correct following a short sample and green following a long sample, whereas for the other half it was the other way around.

Training lasted a minimum of 15 sessions and continued until the percentage of correct responses following each sample was at least 80 % within a session (excluding correction trials), for three consecutive sessions. To minimize feeding outside the experimental sessions, the duration of the reinforcement was adjusted individually, ranging from 1.5 to 4 s.

2.3.2. Delay test

Each session consisted of 64 trials divided in two 32-trial blocks. Each block comprised 24 training trials (12 short-sample trials and 12 long-sample trials) and eight delay-test trials (four for each sample). Training trials were similar to those of the training phase and the

correction procedure was also maintained for these trials. On delay-test trials, a delay was introduced between sample offset and comparisons onset. The delay could last 2.5, 5, 10 or 20 s. For each sample, there were eight delay-test trials – two trials per delay duration – and reinforcement was delivered on half of those, non-differentially. There was no correction procedure on test trials. Reinforcement duration was individually adjusted and varied between 1.5 and 4 s. Testing lasted for five sessions.

Pigeons were divided in two groups: For half of the pigeons (Group Light Off), the delays were spent in the darkness, and for the other half (Group Light On), the delays were illuminated by the houselight.

2.4. Data analysis

We analyzed matching accuracy to each sample in the last three sessions of training and in the five sessions of delay testing. Statistical analyses were conducted using Microsoft Excel and jamovi for Windows (Version 2.3). Type-1 error rate was set at 0.05. For the delay tests, we conducted Friedman tests to assess the effect of delays on accuracy in each condition. Conditions were compared via an adjusted rank transform test (Leys and Schumann, 2010). A Kruskal-Wallis test compared speed of acquisition between the present study and an experiment that employed inter-trial intervals. For each pigeon, to compare choices following delays with chance levels, we calculated 95 % confidence intervals (Normal approximation to the binomial) for choices of the “short” comparison.

3. Results

3.1. Training

On average, pigeons from Group Light Off took 27 (range: 15–42) sessions to complete training. On the last three sessions of training, matching accuracy was 89 % for 3-s samples (range: 80–100 %) and 90 % for 9-s samples (range: 80–100 %). Pigeons from Group Light On took, on average, 23 (range: 15–39) sessions to complete training. One pigeon failed to reach the learning criteria after 60 sessions and did not progress to testing. For the seven pigeons that did advance to testing, matching accuracy on the last three sessions of training was 92 % for 3-s samples (range: 83–100 %) and 90 % for 9-s samples (range: 80–100 %).

3.2. Delay test

The upper panel of Fig. 1 depicts matching accuracy to each sample as a function of delay duration for Group Light Off. A Friedman test revealed no significant difference in matching accuracy following 3-s and 9-s samples, $\chi^2(1) = 0.11$, $p = .739$. That is, the percentage of correct responses significantly decreased with delay for both samples, towards chance level.

To characterize the data at the individual level, a 95 % Confidence Interval (CI) for choices of the “short” comparison on delayed trials was calculated for each pigeon. Half of the pigeons are well described by the average function: Matching accuracy decreased with the delays, with no clear preference for any comparison, and choice approached chance levels – the CIs included 50 %; [35.3 %, 57.2 %], [37.8 %, 59.7 %], [40.3 %, 62.2 %], and [46.7 %, 68.3 %]. For the remaining pigeons, two showed a preference for the “short” comparison, significantly above chance, 95 % CI [61.3 %, 81.2 %] and [72.7 %, 89.8 %]. The last two pigeons showed the opposite bias, and preferred the “long” comparison, 95 % CI [28.1 %, 49.4 %] and [15.5 %, 34.5 %]. As a group, there was no clear preference for one of the options, and the trend towards indifference shown by the average function reflects that.

The bottom panel of Fig. 1 shows, for Group Light On, the percentage of correct responses following each sample in the delay test. A Friedman test revealed a significant difference in matching accuracy following 3-s and 9-s samples, $\chi^2(1) = 8.00$, $p = .005$, showing that the percentage of

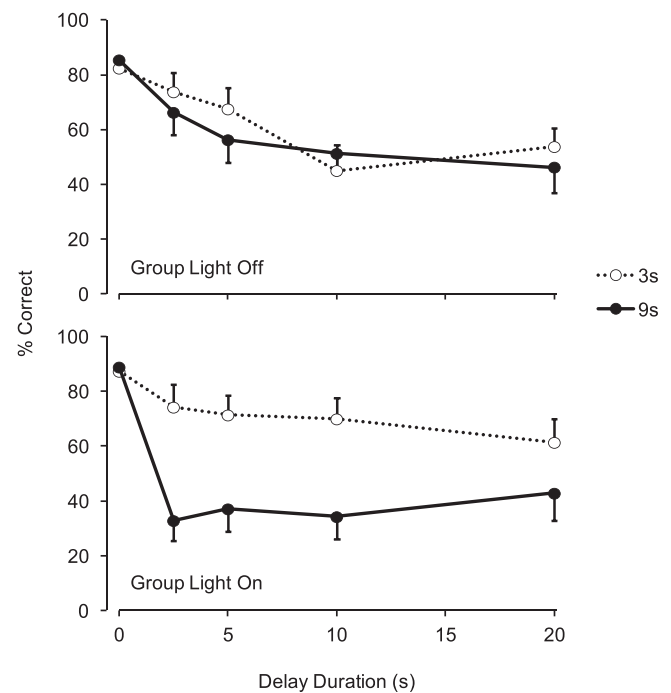


Fig. 1. Mean percentage correct responses (with standard error of the mean) as a function of delay duration, following 3-s (empty data points) and 9-s (filled data points) samples, for Group Light Off (upper panel) and Group Light On (bottom panel).

correct responses changed differently for the two samples – for 3-s samples accuracy decreased very slowly, but for 9-s samples accuracy decreased steeply when a trial had a delay.

The asymmetry between samples was confirmed at the individual level, with four out of the seven pigeons showing a statistically significant preference for the “short” comparison, 95 % CI [53.2 %, 74.3 %], [71.2 %, 88.8 %], [74.2 %, 90.8 %], and [86.7 %, 98.3 %]. The remaining three pigeons did not clearly prefer any comparison, and choices did not differ significantly from chance levels, 95 % CI [34.1 %, 55.9 %], [35.3 %, 57.2 %], and [42.8 %, 64.7 %].

The difference between Group Light Off and Group Light On was confirmed by an adjusted rank transform test that revealed a significant interaction between delay illumination and accuracy following each sample, $F(1, 146) = 7.41$, $p = .007$, $\eta^2 = 0.05$. In other words, the impact of delays on matching accuracy differed when delays were illuminated or spent in darkness.

4. Discussion

Inter-trial intervals (ITI) are commonly employed to prevent inter-trial interference. However, the impact of ITIs can go beyond that simple purpose, affecting performance in unexpected ways. For instance, how an ITI is signaled, or its duration, can lead to choice biases. As such, ITIs can complicate our understanding of performance in standard tasks. Given the innumerable ways an ITI may affect delayed matching, we investigated whether choice biases were exclusively a by-product of ITIs, by assessing choices in delayed temporal matching without the interference of ITI effects. To do so, pigeons were trained in a temporal symbolic matching-to-sample task (3 s vs. 9 s) without ITIs. Then, a delay was introduced between sample offset and comparisons onset. This delay could be either spent in darkness or houselight-illuminated.

Given that inter-trial interference is more likely without ITIs, the present task could be more difficult to learn. To have a sense of how the absence of ITIs might have affected acquisition – and given that in this study there was no condition with ITIs – we compared the present results

with another experiment from our laboratory (Pinto and Castanheira Dinis, 2024). That experiment used the same stimuli to signal the sample and comparisons, and even though sample durations were not the same (2 and 6 s versus 3 and 9 s in the present study), they had the same 1:3 ratio, so the discriminations should be equivalent (Weber's law; see, e.g., Gibbon, 1977). Trials were separated by a 30-s, houselight-illuminated ITI. Session length was similar between the two studies (64 trials versus 60 trials in the present study). In the present experiment, training could be completed after a minimum of 15 sessions, so Fig. 2 compares how overall matching accuracy evolved in the first 15 sessions of training in both experiments.

The task with ITIs had consistently higher accuracy, and also seemingly faster acquisition. To complement the figure, we compared the number of sessions needed for a pigeon to first reach 80 % correct to both samples simultaneously. The task with ITIs required an average of 9.8 sessions (range: 5–18), approximately half the number of sessions required for the task without ITIs, 18.4 (range: 6–40). This difference was statistically significant, $\chi^2(1) = 5.30$, $p = .021$. Despite that, by the end of 15 sessions the two tasks showed similar matching accuracies. It is interesting to note that in the two experiments there were pigeons learning the task similarly fast, with the difference between experiments stemming from how slow the slower birds were. In conclusion, pigeons are able to learn the discrimination in the absence of ITIs, albeit taking longer than if the task had included ITIs.

Regarding the delay tests, the two illumination conditions yielded different results. When the delays were spent in darkness, matching accuracy to both samples decreased gradually with delay, approaching indifference. That is, choices followed a typical retention curve – the longer the delay, the less likely an animal was to remember the sample and thus, random choices became more likely. A similar pattern is found in non-temporal discriminations, namely featuring visual stimuli, such as colors or shapes (e.g., Nelson and Wasserman, 1978; Roberts, 1972; Roberts and Grant, 1978). Therefore, without the interference of the ITI, delay testing for temporal discriminations appears similar to other stimulus modalities.

However, when the delays were illuminated, results were quite different. While accuracy remained relatively high for 3-s samples, it decreased abruptly for 9-s samples: there was an overall preference for the comparison associated with the short sample (choose-short effect). In addition, the fact that matching accuracy following 9-s samples was similarly low for all delays (Fig. 1, bottom panel, filled data points) suggests that the introduction of an illuminated delay caused a generalized disruption in performance. That is, the choice bias is likely due to a process unrelated to forgetting (in case of forgetting, the effect of the delay would be expected to become more pronounced as delay increased).

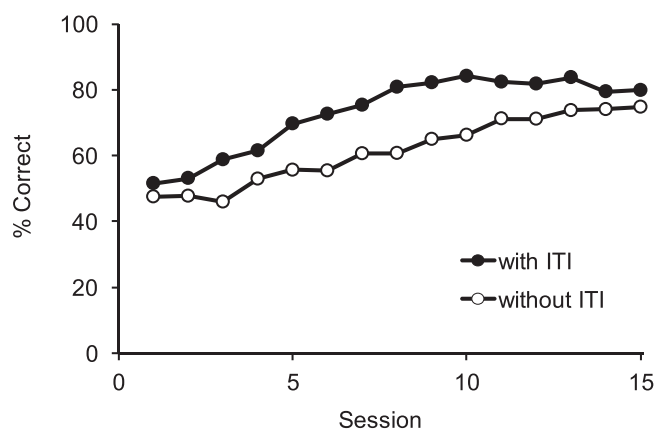


Fig. 2. Average matching accuracy for the first 15 sessions of training, in the present experiment (empty data points) and in a similar procedure that featured inter-trial intervals (filled data points) (Pinto and Castanheira Dinis, 2024).

The possibility that delay illumination is disruptive has been corroborated by studies featuring visual discriminations, which found an abrupt decrease in matching accuracy when a delay was illuminated compared to a gradual and smaller decrease when a delay was spent in darkness (e.g., Calder and White, 2014; Case et al., 2015; Grant and Robert, 1976; Harper and White, 1997; Maki et al., 1977; Roberts and Grant, 1978; White, 1985). To illustrate, Roberts and Grant (1978, Exp. 1) found that, when delays were spent in darkness, matching accuracy progressively decreased with delay and remained above 75 % even at the longest delay (12 s). Yet, when delays were illuminated, accuracy decreased abruptly (to around 75 %) with a delay as short as 0.5 s, and approached indifference at the longest delay. The impact of illumination was clearly confirmed by Grant and Robert (1976), who, by varying the intensity of the delay illumination, found that matching accuracy progressively decreased as light intensity increased. A possible explanation for these results is that stimuli presented during a delay (such as turning on a light to illuminate the chamber) may compete for the animals' attention, increasing the likelihood that they disengage from the task, thus reducing accuracy.

Taking these studies into account, our findings suggest that, when there are no ITIs, the degree of disruption caused by the introduction of a delay may lead to qualitatively different outcomes. A less disruptive delay (spent in darkness) yields a typical forgetting function, while a more disruptive delay (illuminated) results in a marked preference for one of the options. Interestingly, illuminated delays do not always lead to a "short" bias. As described in the Introduction, similarity between ITI and delay may affect delayed choices; when that potential confound was controlled for by having dark ITIs and illuminated delays, a tendency towards indifference has been observed for both samples (Kelly and Spetch, 2000; Sherburne et al., 1998; Spetch and Rusak, 1992). The discrepancy between those studies and the present data may suggest that, even when ITI-delay confusion is controlled for, the ITI may still be affecting choices.

Despite overall group differences, there were some similarities at the individual level between the two illumination conditions. In both dark and illuminated delay tests, some pigeons showed no bias and some showed a bias for "short". Differences in delay-test performance may be due to differences in stimulus control, or in the way the task was learned. These differences could also be reflected in the speed of acquisition. For instance, instead of establishing two response rules – "if 3 s, choose red" and "if 9 s, choose green" – pigeons may create a specific response rule for one sample and a general response for all other cases – "if 3 s, choose red" and "if not 3 s, choose red" (see single-code/default; e.g., Pinto and Machado, 2015; Singer, et al., 2006). The latter coding strategy could result in faster learning and in a choice bias in delay testing. Across both conditions, pigeons with a "short" bias in testing took an average of 18 (range: 10–31) sessions to reach the learning criterion. Pigeons that showed no bias were slightly slower to learn the task, taking on average 26.7 (range: 16–41) sessions. This is a post-hoc hypothesis, focused on a subset of our animals, so it should be taken conservatively. However, it may warrant further exploration.

The moment of introduction of the delays also appears to affect how disruptive they can be. When delays are introduced only in testing, abrupt decreases in matching accuracy are commonly found, with retention functions approaching a step-like pattern (e.g., Grant and Talarico, 2004; Pinto and Machado, 2015; 2017; Pinto and Sousa, 2021; Sherburne et al., 1998; Spetch, 1987; Spetch and Rusak, 1992). Conversely, when delays are present since the beginning of training (and other delay durations are introduced in testing), retention functions show a gradual decrease as a function of delay duration (e.g. Dorrance et al., 2000; Kelly and Spetch, 2000; Spetch and Rusak, 1989). This may happen because introducing delays only during testing may induce greater generalization decrement and disruption of stimulus control (ambiguity hypothesis; Zentall, 1997, 2006). In that vein, it would be interesting to conduct a variation of the present experiment in which pigeons are initially trained with a delay and then tested with other

delay durations. That experiment would control for two important aspects that seem to affect pigeons' performance on delay tasks, the influence of ITIs and the novelty of the delays.

Our results suggest that choice biases occur when testing induces significant disruption to the discrimination learned. A similar result has been described by Ward and Odum (2007), who trained pigeons in a temporal bisection task that featured eight sample durations (ranging from 2 to 8 s); one comparison was correct following the four shorter durations, and the other comparison was correct for the four longer durations. There were no delays between sample and comparisons. The impact of several disruptors was then assessed; in one manipulation, food was delivered during the ITI, and pigeons' accuracy decreased mainly following long samples. Thus, even in the absence of delays, choice biases can emerge as a result of task disruptions.

Some explanations of the choose-short effect have suggested that a delay, either through a gradual (Spetch and Wilkie, 1983) or sudden (Kraemer et al., 1985) process, leaves the animal in a state similar to as if no sample was presented to begin with. The absence of a sample (a 0-s sample) would lead, by stimulus generalization, to a preference for the comparison associated with the shortest sample. Other accounts (Gaitan and Wixted, 2000; Wixted and Gaitan, 2004) suggested that animals learn the discrimination on the basis of the presence or absence of the most salient stimulus (in this case, the long sample). Then, the disruption brought by a delay would mean that long samples would be perceived as absent – leading to choices of the “short” comparison. These explanations do not seem able to accommodate the results of the present study, where delays led to a choice bias in only one of our two delay-test conditions.

In closing, the fact that we found a choice bias in a task with no ITIs reveals that other mechanisms besides how ITI and delay are signaled, or their relative durations, can lead animals to prefer one comparison over another. Additionally, the present results suggest that, even though there may be many ways that stimulus control by the sample can be weakened – such as due to memory-related processes (such as following a long span of time), or due to the introduction of a disruptive event – they do not necessarily lead to the same outcomes. Different types of disruption may yield different responses.

Even though the absence of ITIs may make a task more difficult to learn, it does not do so to an insurmountable degree. Therefore, given the unintended influence ITIs have on performance, further exploration of tasks without ITIs may prove fruitful, as the present results suggest.

Compliance with ethical standards

The research was carried out in agreement with the European (Directive 2010/63/EU) and Portuguese law (Ordinance 1005/92 of October 23), being approved by the Directorate-General for Food and Veterinary, the Portuguese national authority for animal health (Authorization #024946).

CRedit authorship contribution statement

Soares Catarina: Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. **Santos Mairiele:** Writing – original draft, Investigation. **Pinto Carlos:** Writing – review & editing, Visualization, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

Funding: The present work was conducted at the Psychology

Research Centre (PSI/01662), School of Psychology, University of Minho, supported by the Foundation for Science and Technology (FCT) through the Portuguese State Budget (Ref.: UIDB/PSI/01662/2020).

Data availability

Data and code are available in the DataRepositoriUM repository, <https://doi.org/10.34622/datarepositorium/U01CBM>

Choice biases in delayed matching: is the inter-trial interval always to blame? (DataRepositoriUM)

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