

Age affects pigeon memory capacity, but not representation of serial order, during a locomotor sequential-learning task

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2 **locomotor sequential-learning task**

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25 **Age affects pigeons' (*Columba livia*) memory capacity, but not representation of serial**
26 **order, during a locomotor sequential-learning task**

27

28 **ABSTRACT**

29 Aging affects individuals of every species, with sometimes detrimental effects on
30 memory and cognition. The simultaneous-chaining task, a sequential-learning task, requires
31 subjects to select items in a predetermined sequence, putting demands on memory and
32 cognitive processing capacity. It is thus a useful tool to investigate age-related differences in
33 these domains. Pigeons of three age groups (young, adult and aged) completed a locomotor
34 adaptation of the task, learning a list of four items. Training began presenting only the first
35 item; additional items were added, one at a time, once previous items were reliably selected
36 in their correct order. Although memory capacity declined noticeably with age, not all aged
37 pigeons showed impairments compared to younger pigeons, suggesting that inter-individual
38 variability emerged with age. During a subsequent free-recall memory test, when all trained
39 items were presented alongside novel distractor items, most pigeons did not reproduce the
40 trained sequence in the absence of reinforcement. During a further forced-choice test, when
41 pigeons were given a choice between only two of the trained items, all three age groups
42 showed evidence of an understanding of the ordinal relationship between items by choosing
43 the earlier item, indicating that complex cognitive processing, unlike memory capacity,
44 remained unaffected by age.

45

46 **KEYWORDS**

47 Aging, locomotor, memory, pigeons, sequential learning, serial order, simultaneous chaining.

48 As we age, so does our brain, and with it many cognitive functions decline. Age-related
49 degeneration of cognitive and memory capacities is well-documented for humans, and is
50 evident even for healthy individuals ^{1,2}. The impact of aging on nonhuman animal cognition
51 is less well explored ³, but this knowledge is important for understanding common effects of
52 non-pathological aging on cognition. The aging process affects every living being regardless
53 of species, but the way in which it manifests in cognitive changes or impairments can
54 sometimes differ greatly among individuals. Establishing how, and to what degree, aging can
55 affect vital cognitive capacities is a first step in assessing the range of normal and abnormal
56 impairments.

57 The simultaneous-chaining task ^{4,5} is cognitively demanding as it requires subjects to
58 reproduce a list of items in a specific sequence, with the only feedback provided regarding
59 the correctness of a choice being the continuation of the trial. This task puts demands on an
60 individual's memory capacity, in terms of both reference memory to learn the sequence, and
61 working memory to update the last choice made in order to determine the next required
62 response. An individual's memory capacity can be measured through the successive chaining
63 of subsequent sequence items ⁶. Each time a subject learns to respond correctly to a sequence
64 of n items, another item is added to the chain ($n+1$). Using this method, pigeons have
65 successfully been trained to reproduce lists of four to five items ^{4,5}.

66 In addition to the task's adoption for evaluating memory capacity, it has also been
67 selected as an assessment tool for cognitive capacity, and as such has been used to study a
68 diverse range of species, from humans and apes to pigeons and chickens ⁴. As suggested by
69 the task name, successful acquisition of the sequence can be achieved through simple
70 associative chaining, with a response to the first item serving as a cue to respond to the
71 second item, and so on. However, a more cognitively complex solution would be to form a
72 mental representation of the item order, whereby each item in the sequence is acquired not

73 only relative to the immediately preceding and following items, but also in terms of its unique
74 ordinal position within the sequence as a whole (e.g., is the item the first, second, third, etc.).
75 One way to assess a subject's ability to form a representation of order is during subsequent
76 pairwise-choice tests, for which only two items of the learned sequence are presented
77 together, and the subject is allowed a single choice. If item order had been represented,
78 subjects would be expected to recognise the ordinal relationship between the two presented
79 items even in the absence of the complete sequence, and accordingly to choose the item that
80 appeared earlier in the training sequence. However, if learning occurred based on associative
81 chaining alone, subjects would be expected to perform well only when the first item is
82 present, but to be unable to distinguish between any later items, as the cue to respond to
83 either – a successful response to the preceding item – would not have occurred.

84 During Terrace's ⁵ studies, pigeons largely responded in line with the predictions for
85 an associative-chaining account, in that for trials in which one of the presented items was the
86 first item of the trained sequence, that item was chosen correctly, and for trials in which the
87 first item was not part of the choice, a choice was made at chance. However, Terrace also
88 found one notable exception to this pattern: when a choice included the last sequence item,
89 the other item was reliably chosen, and the last item was avoided. Terrace ⁵ interpreted these
90 results by assuming that pigeons had learned the special status of the two items to which a
91 response was required first and last, but that they otherwise did not possess a mental
92 representation of the ordinal relationship between two list items. More recently, however,
93 Scarf and Colombo ⁴ argued that pigeons are indeed able to form a mental representation of
94 the sequence order. Proposing that their previous inability to maintain the trained order
95 during two-item tests was due to a contextual change resulting from presenting only two
96 items rather than the full sequence, Scarf and Colombo suggested that this change may have
97 disrupted the pigeons' ability to relate the test to the training and transfer the task

98 requirements accordingly. Taking this critique into consideration, in our current study we
99 presented subjects with a free-recall test, as well as the conventional pairwise-choice test.
100 During the free-recall memory test, all items of the trained sequence were presented, thus
101 preventing the sudden perceptual change that arguably impeded a transfer from training to
102 test. In addition, previously unseen distractor items were shown alongside the familiar
103 training items. Instead of just one choice, subjects were free to make several choices, in order
104 to measure both item recognition and adherence to the trained order.

105 In summary, the simultaneous-chaining task provides an excellent procedure for
106 investigating age-related effects of memory decline, and for comparing cognitive abilities
107 across species. The goals of our study were two-fold. Firstly, to investigate potential age-
108 related decline in memory capacity and secondly, to elucidate the underlying cognitive
109 mechanisms governing task acquisition for pigeons. To achieve the former, we assessed the
110 length of a sequence that a subject could acquire within a reasonable duration of training. For
111 the latter, we investigated whether a subject's age influenced its' ability to create a mental
112 representation of the trained order.

113

114 **METHODS**

115 ***Subjects***

116 Twelve locally sourced racing pigeons (*Columba livia*) completed this experiment.
117 They were grouped into three categories according to their age at test: *young* (1 year of age;
118 $N = 4$, 2 females), *adult* (6-8 years of age; $N = 4$, 2 females) and *aged* (15-17 years of age; $N =$
119 4, 1 female). The pigeons were housed in individual metal (60 x 60 x 38 cm length x width x
120 height) or plastic (75 x 70 x 50 cm) cages in a colony room at the Department of Psychology
121 of the University of Manitoba. The room was kept year-round at a temperature of 21°C and a
122 12-hour light-dark cycle with lights on at 0700 hours. The pigeons' weights were monitored

123 daily and maintained, through controlled feeding of a mixture of yellow and green peas, oat
124 groats, red milo, yellow popping corn, and white millet, at 90% of their free-feeding weight
125 and had *ad lib* access to water and grit inside their cage. The adult and aged pigeons had
126 previous testing experience, but all were naïve to the specific procedures of this experiment.

127 ***Ethical Note***

128 This study was approved by the University of Manitoba's Local Animal Use Committee
129 (protocol number F18-042) in accordance with the Canadian Council on Animal Care and the
130 ARRIVE guidelines.

131 ***Apparatus***

132 Training and testing sessions were conducted in an enclosed rectangular arena made
133 of Styrofoam®. The walls (60 cm tall) were lined with white Con-Tact® paper to permit
134 cleaning. The base (200 x 100 cm length x width) was covered with black rubber flooring.
135 White shower curtains enclosed the arena to block access to external visual cues. Six feeders
136 were placed in a circular array inside the arena and fixed to the floor with Velcro (see Figure
137 1). Each feeder consisted of a wooden base (10 x 10 x 10 cm length x width x height) and a
138 wooden ramp covered with sandpaper (10 x 20 cm base with a 26.6° incline) leading up to
139 the base. A plastic cup (6 cm diameter) was attached to the inside of the base. To make the
140 inside of the feeder only visible from the top of the ramp, the three sides of the base facing
141 away from the ramp were lined with white paper (30 cm height), and a white foam cone was
142 attached to the top of the paper barrier. The white cones could be replaced by equally-sized
143 cones of different colours to make each feeder visually distinct (herein these coloured cones
144 are referred to as *features*). A centrally-mounted Logitech HD Webcam C270, connected to a
145 Dell desktop computer (running 64-bit Windows 10 Enterprise) in the adjacent room, was
146 suspended from the ceiling for recording of trials. Two Conair® white noise generators were
147 placed external to the arena, at opposing corners to mask sounds.

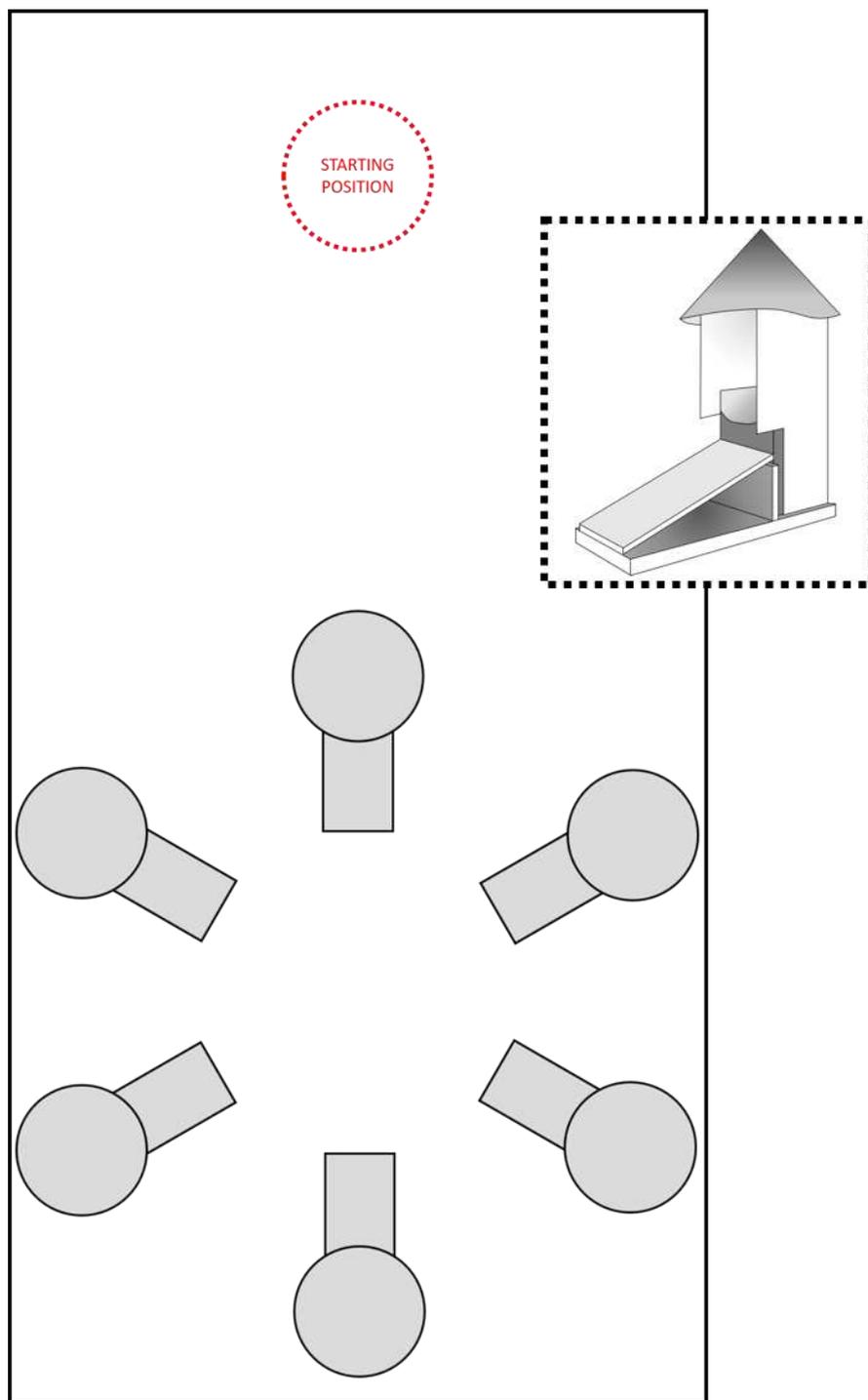


Figure 1. Top-down view of the inside of the experimental arena. The six feeders (in grey) were placed in a circular array in one half of the arena. The pigeon entered at the opposite side of the arena (the approximate location is marked in red). The insert in the broken-line box shows a 3D model of a feeder.

150

151 ***Procedure***

152 *General Procedure*

153 A pigeon was transported from the colony room to the procedure room in an opaque
154 white transport container, which also served as a holding container between trials. At the
155 beginning of each trial, the lights in the procedure room and arena were extinguished, and
156 the pigeon was removed from the holding container and placed into the arena at the starting
157 position (see Figure 1). The experimenter exited the procedure room and remotely
158 illuminated the procedure room lights, which delimited the start of a trial. Once the pigeon
159 completed the choice requirement (see below), or ten minutes elapsed, whichever occurred
160 first, the lights were extinguished, and the pigeon was removed from the darkened arena and
161 returned to the holding container where it remained while the arena was prepared for the
162 next trial.

163 *Feeder Training Phase*

164 To train the pigeons to retrieve maple peas from the feeders, a single feeder with a
165 white cone (herein referred to as a *white feeder*) was located in one of the six possible array
166 locations (with location counterbalanced across trials). Five maple peas were placed at each
167 of the following locations: in the plastic cup inside the feeder, on the ramp, and on the floor
168 of the arena at the base of the ramp. With training, the number of maple peas was gradually
169 reduced from fifteen to two, and only placed inside the feeder. Pigeons were given a
170 maximum of 60 minutes to consume the maple peas. A trial ended 30 seconds after the last
171 maple pea was consumed, after which the arena was re-set and a new trial started. As each
172 daily session lasted a maximum of 60 minutes, a pigeon could receive between one and twelve
173 trials per daily session. Pigeons experienced trials with a single feeder until all maple peas
174 were consumed within 5 minutes of trial start, for a minimum of 12 trials in total. Next,

175 pigeons were presented with all six identical feeders arranged in the circular array, with one
176 maple pea inside each feeder. Pigeons progressed to the Memory Training phase once every
177 feeder was visited within the first 5 minutes of a trial for 5 consecutive trials.

178 *Memory Training Phase*

179 A daily training session consisted of 10 trials, with the six feeders positioned in the
180 circular array formation. Across four distinct stages, the pigeons were trained to visit four of
181 the six feeders in a specific sequence, as indicated by distinctly coloured features (red, green,
182 blue, yellow, orange and purple; herein referred to as a *sequence item*). The order in which
183 the feeders had to be visited was consistent for each pigeon, but pseudo-counterbalanced
184 among the pigeons such that one of the six colours was assigned to be the first item in the
185 sequence for two pigeons. The location of each sequence item within the array was
186 randomised across trials so that only the colours on the feeders, but not the feeder locations
187 within the arena or relative to each other, indicated the correct sequence.

188 Originally, the stages described below were administered with all six coloured
189 features placed on the feeders. However, although all subjects quickly passed the first
190 training stage, during which only the first item in the sequence was reinforced, acquisition of
191 any following memory items appeared hindered by this procedure. In particular, following a
192 successful visit to the first item, the subjects stopped exploring the arena and were reluctant
193 to visit any additional feeders, as visiting them had previously not been reinforced. For the
194 majority of subjects, the transition to training stage 2 resulted in reduced attention to the
195 previously learned first item. Following ten sessions of continued decline in performance by
196 all subjects, we revised the training procedures to encourage exploration of the area after the
197 first item had been visited. Thus, the entire training phase was restarted from stage 1 for all
198 subjects as follows.

199 During the adjusted memory training stage 1 (TS1; Figure 2 panel A), pigeons were
200 presented with five white feeders and the first sequence item. Each successive training stage
201 differed from the previous in that the next new sequence item replaced one of the white
202 feeders in the array. Whereas the white feeders were always non-reinforced, during TS1, four
203 maple peas were placed into the first sequence item; the pigeon was allowed to inspect any
204 feeder. During training stage 2 (TS2; Figure 2B), three maple peas each were placed into the
205 first and second sequence items. If the pigeon approached any feeder before visiting the first
206 sequence item, the lights were extinguished immediately to indicate an incorrect response
207 and the trial ended. If the pigeon successfully visited the first sequence item, it could inspect
208 any other feeder. During training stage 3 (TS3; Figure 2C), two maple peas each were placed
209 into the three sequence items. Only after the pigeon had approached the first and second
210 sequence items, it was allowed to inspect any feeder. During training stage 4 (TS4; Figure
211 2D), two maple peas each were placed into the four presented sequence items. Only after
212 visiting the first, second and third items in sequence, the pigeon was allowed to freely inspect
213 any feeder. During each stage, a trial ended with the extinction of the lights after the pigeon
214 consumed all available peas and exited the final feeder, or if the pigeon visited the feeders in
215 any other order than described, or after ten minutes of inactivity. Training progressed to the
216 next stage once a pigeon consistently visited all presented sequence items in the correct order
217 without visiting any other feeders in at least seven out of ten trials of a session, for two
218 consecutive sessions. If a pigeon failed to advance from one training stage to the next within
219 60 sessions, it was considered to have reached the maximum number of sequence items it
220 could memorise, and proceeded to the Testing phase.

221 *Testing Phase*

222 Two tests were administered in alternating blocks of three sessions: a choice test,
223 consisting of nine sessions, and a memory test, consisting of a minimum of six sessions. The

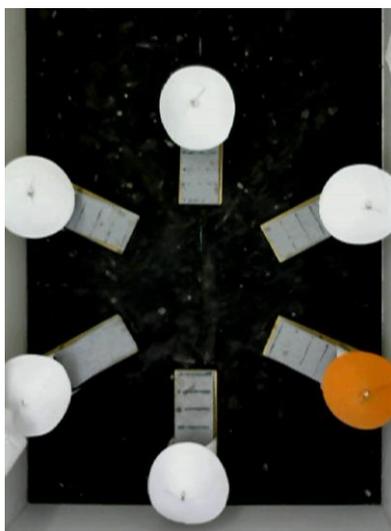
224 tests served to examine the successful encoding of the trained sequence items, both regarding
225 their identity (colour) and their position within the sequence. To this effect, the previously
226 trained items were presented alongside novel items of the colours not used during the
227 subject's training.

228 *Choice Test.* This test recreated the pairwise forced-choice tests conducted in
229 previous studies on sequence learning to determine whether there was evidence of a mental
230 representation of the trained order. Each session consisted of five reinforced trials, which
231 were identical to training trials during the final stage a pigeon had reached, and five non-
232 reinforced test trials, presented in alternating fashion. During test trials, two feeders had
233 coloured features. All 15 possible combinations of the coloured features were presented once
234 per three-session block, resulting in a total of 45 test trials. Test trials ended after the first
235 visit to any feeder.

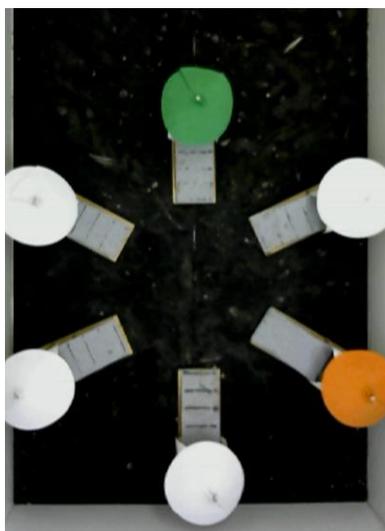
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a: Training Stage 1



b: Training Stage 2



c: Training Stage 3

d: Training Stage 4

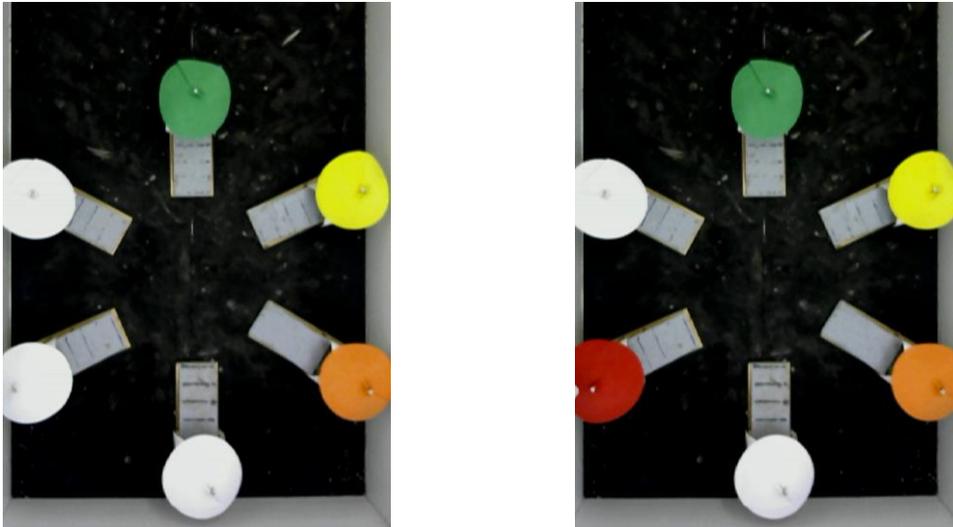


Figure 2. Examples of a trial in each memory training stage. The location of each sequence item (coloured feeder) changed between trials as to not systematically reinforce a certain location. The sequence shown here is for illustrative purposes.

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240 *Memory Test.* Accounting for the critique by Scarf and Colombo ⁴ regarding the
241 disruptive effect of pairwise tests on performance potentially being due to the change in
242 presentation of the sequence items, we additionally incorporated an open memory test
243 presenting all sequence items and novel items together. Each session started with at least two
244 reinforced trials, which were identical to training trials during the final stage a pigeon had
245 reached. Following two correct baseline trials, a non-reinforced test trial was administered,
246 during which all six (sequence and novel) items in a predetermined, randomised order. Test
247 trials ended after the pigeon made four visits, including repeat visits to the same feeder.
248 Memory test sessions were continued until a pigeon had completed ten test trials. Due to the
249 criterion of two correct baseline trials preceding any test trial, each session could contain
250 between zero and three test trials.

251

252 **RESULTS**

253 *Data Collection*

254 Data collection was prematurely terminated due to restrictions put in place by the
255 University of Manitoba in response to the Covid-19 pandemic. Two young subjects had
256 completed 30 and 44 sessions of TS4, respectively, and one aged subject had completed 4
257 sessions of TS4 when the experiment was ceased; only their data from the completed TS3
258 were included in the analysis to provide an accurate image of learning. One adult subject
259 (Orange 11) was in the middle of the test phase when data collection was suspended; for this
260 subject, all completed tests sessions were included in the data analysis (providing data for 35
261 of the 45 Choice Test trials and 3 of 10 Memory Test trials).

262 ***Data Analysis***

263 Given the format of the training data, we ran a linear mixed model (LMM) with the
264 simple factor Age Group (young, adult, aged) and the polynomial factor Training Stage (TS1,
265 TS2, TS3, TS4), the hatch year of subjects as an unscaled covariate and pigeon identity as the
266 cluster variable assuming correlated effects. Planned pairwise comparisons of performance
267 were conducted as *t*-tests. A simple-effect analysis was conducted to investigate the presence
268 of age-group differences within training phases. Lastly, a Kruskal-Wallis non-parametric one-
269 way ANOVA was performed to assess potential age-group differences in the highest daily
270 performance score achieved during the first half of training in TS4.

271 Analyses of test performance were carried out separately for the two groups of
272 pigeons for which sequence item 3 or 4 had been the last trained item. For the Choice Test,
273 choices between two previously trained items were assessed by conducting Friedman non-
274 parametric repeated-measures ANOVAs on the percentage of trials during which a subject
275 chose the item that had appeared earlier in the sequence, for each combination of trained
276 sequence items. Choices between a trained and a novel item were assessed by conducting
277 Friedman non-parametric repeated-measures ANOVAs on the percentage of trials during
278 which a subject chose a previously trained item over a novel item. To meaningfully analyse

279 performance in the Memory Test, we considered the correct execution of smaller “chunks”
280 within the trained sequence, specifically, a subject’s tendency to choose sequence item 1 first
281 before visiting any other item, to choose at least two sequence items in their correct order
282 (i.e., 1 followed by 2, 2 followed by 3, or 3 followed by 4, regardless of which item was chosen
283 before or after this isolated combination of two items), or to choose the final trained item last.
284 Friedman non-parametric repeated-measures ANOVAs were performance on the percentage
285 of trials during which a subject completed each of these chunks. For any of these ANOVAs,
286 pairwise post-hoc Durbin-Conover comparisons between individual factor levels were
287 performed if an analysis indicated a significant main effect. All analyses were carried out in
288 jamovi version 1.2 ⁷.

289 ***Memory Training***

290 All 12 subjects successfully passed TS1 and TS2, with the exception of one aged
291 subject that failed to continuously search the feeders during TS2 and was removed from the
292 experiment. First performance differences emerged in TS3, as only two subjects each in the
293 adult and aged group reached the training criterion, in contrast to all four subjects in the
294 young group that passed this stage. Finally, none of the subjects that entered TS4 were able
295 to pass the criterion within the number of training sessions they received.

296 The analysis revealed a significant effect of the factor Training Stage (LMM: $F_{3,18.78} =$
297 130.14 , $P < 0.001$; Figure 3), as the number of training sessions required to pass a stage
298 increased as more sequence items were added during consecutive stages, assuming a
299 significant cubic function (LMM: $t_{17.72} = 5.67$, $P < 0.001$). Pairwise comparisons confirmed
300 that, although training performance in TS1 and TS2 did not differ significantly (t -test: $t_{19.1} =$
301 2.60 , $P = 0.11$), the number of training days required to pass TS3 was significantly higher
302 compared to the former two stages (t -tests: TS3 vs. TS1: $t_{19.1} = 15.37$, $P < 0.001$; TS3 vs. TS2:

303 $t_{18.4} = 12.54, P < 0.001$), and higher yet again in TS4 (t -tests: TS4 vs. TS3: $t_{21.2} = 2.92, P = 0.049$;
304 comparisons to TS1 and TS2: both $P \leq 0.001$).

305 The factor Age Group had no relevant influence on the number of training sessions
306 required to pass a training stage across all four stages, as no age group showed a consistently
307 lower or higher number of sessions than the other groups (LMM: $F_{2,6.65} = 0.87, P = 0.462$).
308 However, the emerging interaction effect of the two factors (LMM: $F_{6,18.76} = 2.62, P = 0.051$)
309 pointed towards differences between age groups within single training stages, confirmed by
310 the subsequent simple-effect analysis; although subjects within the three age groups were
311 equally successful in TS1 (ANOVA: $F_{2,10.5} = 0.03, P = 0.97$) and TS2 (ANOVA: $F_{2,10.6} = 0.18, P =$
312 0.84), this was not the case for TS3 (ANOVA: $F_{2,10.6} = 5.46, P = 0.024$). For TS3, young subjects
313 performed significantly better than their adult counterparts (t -test: $t_{9,08} = 2.27, P = 0.049$; the
314 high variance within the aged group makes comparisons to this age group inconclusive, both
315 $P \geq 0.346$). In contrast, all three age groups were equally unable in completing TS4 within the
316 60 training sessions provided (ANOVA: $F_{2,12.1} = 0.03, P = 0.97$).

317 To obtain a measure of progress for TS4, we evaluated the highest daily performance
318 score (correctly completed trials out of the ten daily trials) obtained halfway through the
319 training stage, after 30 sessions of TS4 had been completed. This allowed us to include the
320 data from the two young subjects that had to cease training before completing all 60 sessions
321 of TS4. The analysis revealed that there were no statistically significant differences between
322 the three age groups in this early performance level (Kruskal-Wallis test: $\chi^2_2 = 1.56, P =$
323 0.459).

324 In summary, increasing the number of sequence items with each training stage led to
325 an increase in the number of sessions required to acquire the sequence. Age differences were
326 only notable for TS3, as young subjects were able to successfully complete this stage within
327 fewer sessions than adult or aged subjects.

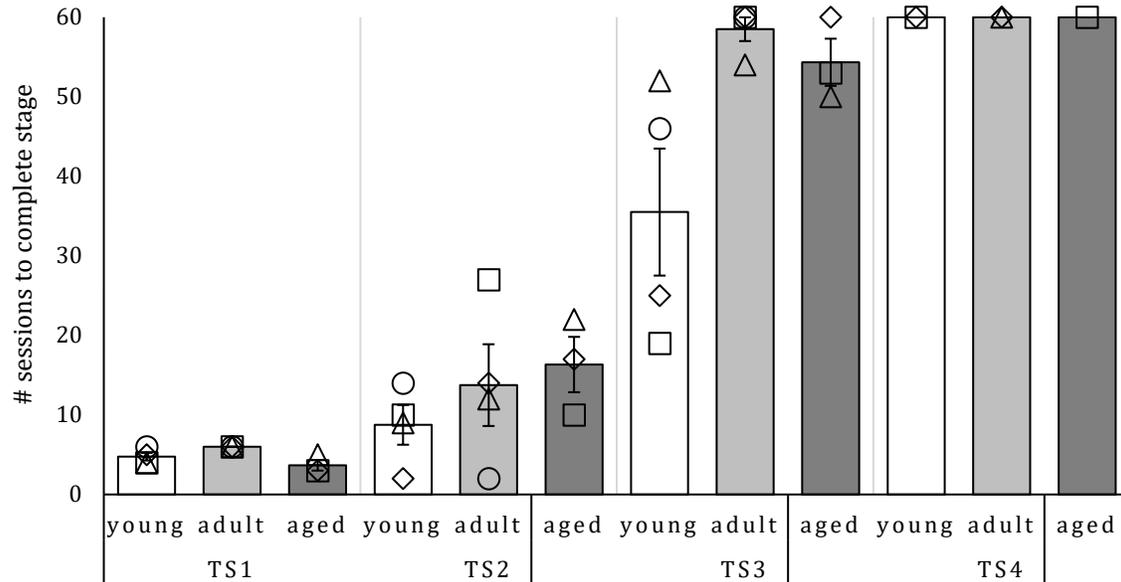


Figure 3. Mean number of sessions required to reach completion criterion in each training stage (TS) for each age group. Individual subject data are plotted as point values. Error bars represent standard errors.

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330

331 *Testing*

332 Of the 12 pigeons that started the experiment, six completed all nine sessions of the
 333 Choice Test and sufficient sessions of the Memory Test to accumulate at least ten Memory
 334 Test trials. Given the low number of subjects, we analysed individual performance (see Tables
 335 1 through 3) and grouped subjects based on the number of sequence item they had been
 336 trained on instead of age groups. To facilitate the description and analysis of the tests, in
 337 addition to the sequence items used during training (i.e., sequence items 1 through 4), the
 338 novel items presented during the tests were numbered consecutively (i.e., sequence items 5
 339 and 6). However, there was no qualitative difference to the latter two items.

340 *Choice Test*

341 The Choice Test recreated the pairwise forced-choice tests conducted in previous
342 studies on sequence learning to determine whether subjects had encoded the trained order.
343 Table 1 shows the percentage of trials during which a subject chose the item that had
344 appeared earlier in the sequence for each combination of trained sequence items. For the
345 subjects experiencing up to TS3, although performance was numerically worse for choices
346 between items 2 and 3, there was no statistically significant difference in performance
347 between choices (Friedman test: $\chi^2_2 = 2.6, P = 0.273$). However, for the four subjects that had
348 reached TS4, whether or not the earlier item was chosen significantly depended on the
349 presented options (Friedman test: $\chi^2_5 = 13.5, P = 0.019$). The pigeons consistently chose item
350 1 when it was presented against any other item, and preferred item 2 in choices between
351 items 2 and 4, but were significantly less likely to choose the earlier item for choices between
352 items 3 and 4 and for choices between items 2 and 3, as confirmed by pairwise post-hoc
353 comparisons (Durbin-Conover tests: comparing choices 2 vs. 3 and 3 vs. 4 to all other choices:
354 all $P = 0.004$; all other comparisons: all $P = 1.0$). These results confirm that sequence item 1
355 was reliably recognised as the first item to attend to, whereas choices between other
356 sequence items, including the final item, were less accurate.
357
358

Table 1. Percentage of trials (out of three trials) during which a subject chose the item that had appeared earlier in the sequence. Individuals are listed in order of the number of items they received during training; those that only completed up to TS3 were not presented with choices including sequence item 4.

Subject	choice between sequence items					
	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4
Yellow 23	100%	67%	-	100%	-	-
Green 1076	100%	67%	-	0%	-	-
DV 312	100%	100%	-	33%	-	-
Average	100%	78%	-	44%	-	-
Yellow 2	100%	100%	100%	33%	100%	67%

White 8174	100%	100%	100%	0%	100%	100%
Orange 11	100%*	100%	100%*	50%*	100%	0%
DV 573	100%	100%	100%	100%	100%	67%
Average	100%	100%	100%	46%	100%	58%

* - based on two trials instead of three.

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In addition to the above, we examined pigeons' overall encoding of the identity of trained sequence items by examining their choices when presented with a trained sequence item and a novel item. Table 2 shows the percentage of trials during which a subject chose the trained item for such choices. For those subjects that only completed TS3, the percentage of choice for the known item did not significantly change depending on which trained sequence item was presented (Friedman test: $\chi^2_2 = 1.64, P = 0.44$). For subjects experiencing TS4, the percentage of choice for the known item also did not significantly change with the presented items (Friedman test: $\chi^2_3 = 6.26, P = 0.099$), although the pigeons tended to choose trained items that appeared earlier in the sequence. Pairwise post-hoc comparisons (Durbin-Conover) confirmed that the choice of item 1 was significantly higher than choices of items 3 or 4 (comparisons of "known option was item 1" to either other option: both $P \leq 0.029$; comparison of "known option was item 1" to "known option was item 2": $P = 0.071$; all other comparisons: $P \geq 0.48$). This result indicates that, when trained with four items, the preference for a known item over a novel item decreased the later the item appeared in the trained sequence.

Table 2. Percentage of Choice Test trials during which a subject chose the known item when the choice was between a known (sequence items 1, 2, 3, or 4, as applicable) and an unknown item (sequence items 5, 6, or 4, as applicable), or between two unknown items. Individuals are listed in order of the number of items they were trained on; those that only completed up to TS3 were not presented with sequence item 4 as a known option.

Subject	known option was			
	Item 1	Item 2	Item 3	Item 4
Yellow 23	100%	67%	70% ^{\$}	-
Green 1076	89%	89%	100%	-
DV 312	89%	56%	50% ^{\$}	-
Average	93%	71%	74%	-
Yellow 2	100%	100%	67%	100%
White 8174	100%	83%	83%	67%
Orange 11	80% [*]	75% [^]	60% [*]	25% [^]
DV 573	100%	50%	67%	67%
Average	95%	77%	69%	65%

Note: Percentages are based on nine trials for subjects Yellow 23, Green 1076 and DV 312, and on six trials for Yellow 2, White 8174 and DV 753.

^{\$} - based on ten trials. ^{*} - based on five trials. [^] - based on four trials.

378

379

380 *Memory Test*

381 As seen in Table 3, the overall tendency to visit items in the correct sequence was low
382 (final column “correct sequence”), with the highest success rate of 45% shown by the
383 youngest subject, Yellow 2. To analyse performance, the following chunks within the
384 sequence were considered: choosing sequence item 1 first, choosing item 2 directly after item
385 1, item 3 directly after 2, item 4 directly after 3 (if applicable), and choosing the final trained
386 item last. For the group experiencing up to TS3, the percentage of trials during which a subject
387 completed a chunk of one or two items in their correct order significantly decreased the later
388 the chunk appeared in the sequence (Friedman test: $\chi^2_3 = 9.0$, $P = 0.029$). Pairwise post-hoc
389 comparisons (Durbin-Conover) further confirmed that the percentage of choosing sequence
390 item 1 first was significantly higher than choosing later chunks (comparisons of “1 chosen
391 first” to any other option: $P \leq 0.001$), and lowest for choosing sequence item 3 after item 2
392 and for avoiding the last item until the ultimate choice (comparison of “2 chosen after 1” to
393 “3 chosen after 2” and to “3 chosen last”: both $P < 0.001$; comparison of “3 chosen after 2” to
394 “3 chosen last”: $P = 1.0$). Similarly, performance was significantly affected by the position of

395 the chunk within the sequence for the subjects experiencing TS4 (Friedman test: $\chi^2_4 = 12.2$, P
 396 = 0.016). The percentage of choosing sequence item 1 first was significantly higher than
 397 choosing later chunks (comparisons of “1 chosen first” to any other option: Durbin-Conover
 398 tests: all $P \leq 0.016$). Pigeons also completed the chunk “2 chosen after 1” significantly more
 399 often than “3 chosen after 2” and “4 chosen last” (Durbin-Conover tests: both $P \leq 0.036$; any
 400 other comparisons: $P \geq 0.16$). Taken together, all pigeons were decreasingly likely to
 401 complete a chunk the later it appeared within the sequence. This decrease was visible as early
 402 as the second sequence item, as predominantly only the first item was visited correctly.

403

404

Table 3. Percentage of Memory Test trials (out of ten trials) during which a subject chose sequence item 1 before any other items (“1 first”), chose sequence item 2 directly after item 1 (“1 → 2”), chose sequence item 3 directly after item 2 (“2 → 3”), chose sequence item 4 directly after item 3 (“3 → 4”; only for those pigeons that had experienced training stage 4), chose the final trained items as its last choice (“3/4 last”), and when a subject completed the entire sequence of either three or four trained items in its correct order (“1 → 2 → 3 (→ 4)”). Individuals are listed in order of the number of items they were trained on; those that only completed up to TS3 were not expected to visit sequence item 4.

Subject	1 first	1 → 2	2 → 3	3 → 4	3/4 last	1 → 2 → 3 (→ 4)
Yellow 23	80%	40%	30%	-	30%	30%
Green 1076	70%	30%	10%	-	10%	10%
DV 312	80%	30%	20%	-	20%	20%
Average	77%	33%	20%	-	20%	20%
Yellow 2	100%^	64%^	45%^	73%^	45%^	45%^
White 8174	100%	70%	50%	30%	10%	10%
Orange 11	67%*	33%*	33%*	33%*	33%*	33%*
DV 573	82%^	36%^	9%^	18%^	18%^	0%^
Average	87%	51%	34%	39%	27%	22%

* - based on three trials. ^ - based on 11 trials.

405

406

407 DISCUSSION

408 Increasing age can have detrimental effects on memory and cognition ^{1,2}. Here, we
 409 investigated potential age effects on pigeons’ memory and the encoding of serial order during
 410 sequence learning. Memory capacity declined noticeably with age, although not all aged

411 pigeons suffered impairments when compared to their younger conspecifics, indicating some
412 interindividual variability that emerges with age. The cognitive processes underlying
413 sequence learning, however, appeared to be independent of age, as all pigeons performed in
414 a way that indicated a mental encoding of sequence order.

415 The training data provided insight into an individual's ability to remember sequences
416 of increasing length. Lists of one and two items were acquired with relative ease by the
417 pigeons regardless of age. However, as early as the three-item sequence, pronounced
418 differences in acquisition rates emerged between the three age groups. Young pigeons
419 learned the list within significantly fewer sessions than adult and aged pigeons, and half of
420 the adult and aged individuals were unable to reproduce the sequence sufficiently within the
421 session limit. No subject reached the learning criterion for the four-item sequence. Although
422 the criterion was deliberately set relatively high to avoid ceiling effects, it was expected that
423 at least the younger pigeons would be capable of reaching this standard, as the one-year-old
424 pigeons in Terrace's ^{5,6} experiment did achieve a comparable level of performance not only
425 for four-item lists but also for five-item lists. Considering that the current study employed an
426 open-field paradigm, whereas previous studies used computerised tasks, it is difficult to
427 pinpoint the cause of this discrepancy, which could range from visual aspects and differences
428 in the mental representation of 2D and 3D stimuli (cf. ⁸⁻¹⁰) to motoric aspects when pigeons
429 have to move only their heads compared to their entire body from one stimulus to the next.

430 To acquire a measure of progress with the four-item list, we compared the highest
431 reached daily score (correctly completed trials out of the ten daily trials) after the pigeons
432 had received 30 of the 60 TS4 sessions. Although the highest scores were achieved by the
433 youngest age group, older subjects did not fall far behind, and all subjects that had reached
434 this training stage reached the threshold set for success in other related studies (i.e., 30%
435 correct in a single session ⁴). The lack of clear age differences in TS4 despite observable

436 differences in TS3 suggests that age does not affect pigeons' memory performance uniformly.
437 As is the case for humans ¹¹, there appear to be well-aging individual pigeons that preserve a
438 high level of memory capacity (comparable to the performance of the least successful young
439 subjects) and less well-aging individuals that show noticeable declines in memory. During a
440 highly complex memory task such as the simultaneous-chaining task presented here, the
441 decline is already noticeable at a relatively early age.

442 We incorporated two tests, a Choice Test and a Memory Test, to assess pigeons'
443 encoding of sequential order and infer potential differences regarding the cognitive
444 mechanisms underlying sequence learning across the age groups. There was no indication in
445 the obtained data to suggest performance differed between young, adult and aged pigeons in
446 the two administered tests.

447 The Choice Test allowed for a comparison of the observed behaviour to previous
448 studies. When presented with a forced choice between two list items, pigeons chose the first
449 list item over items presented later in the sequence, and chose earlier items over the final list
450 item, but they showed no preference for earlier sequence items when the choice was between
451 two items presented in the middle of the list. Terrace ⁵ interpreted these findings as evidence
452 that pigeons chained responses to neighbouring items within a sequence, learning that a
453 response to item n was only correct when preceded by a response to item $n-1$, but with no
454 further reasoning about sequential order among the items. He further asserted that the
455 special positions of the first and the last sequence items were encoded separately, resulting
456 in correctly ordered responses when the choices included one of these items, but disrupted
457 performance when the choice did not include the first or the last item. The results from our
458 Choice Test only replicate Terrace's findings in part – our pigeons also showed their weakest
459 choice performance during trials that included internal items of the sequence (2 vs 3 for those
460 subjects that had trained with four items). However, performance was just as impaired when

461 the choice included the two final items (3 vs 4, or 2 vs 3 for those subjects that had trained
462 with three items), an observation that is directly contrary to Terrace's argument of the final
463 item assuming a "special role". Indeed, reduced performance occurred primarily when the
464 choice was between sequentially neighbouring items, regardless of whether the item pair
465 occurred in the middle or at the end of the sequence. Item pairs that did not consist of
466 immediately neighbouring items within the sequence reliably led to good performance (2 vs
467 4), which was at the same level of accuracy as any choices including item 1. The special role
468 of the first item also mentioned by Terrace persisted in the current study as well, however,
469 this was most likely due to overlearning. Taken together, these results suggest that, instead
470 of associatively chaining responses to neighbouring items, during the current experiment the
471 pigeons encoded the order of items to some degree, although it might have been more in
472 terms of items that appear "earlier" or "later" within the sequence than in terms of individual
473 ordinal positions. The salience of the last item, which was so prominent in Terrace's studies,
474 was not confirmed here. However, Terrace's pigeons were trained on five-item lists, which
475 might have posed an additional level of cognitive difficulty to his subjects, resulting in good
476 memory only of the first and last item but with little representation of the order of internal
477 items. Instead, as noted by Scarf and Colombo ⁴, Terrace's subjects relied on the display of all
478 five list items together to be able to reproduce the order. With shorter lists of four or three
479 items, as used in this experiment, the cognitive load may have been reduced sufficiently to
480 allow an encoding of the relative position of items as appearing earlier or later within the list.

481 The Memory Test allowed a further assessment of whether the observed behaviour
482 supported the hypothesis proposed by Terrace ⁵ regarding chaining of responses without an
483 inherent concept of order, or the more cognitively complex process proposed by Scarf and
484 Colombo ⁴. The most obvious result of this test was that item pairs further down the list were
485 increasingly less likely to be chosen in their correct order. It is unlikely that this failure to

486 adhere to the trained sequence stemmed from a lack of memory of the later items themselves
487 – although the subjects had received less training sessions with later sequence items overall,
488 all items were equally preferred over novel items in the pairwise-choice test. Instead, we
489 observed that subjects abandoned the sequence as soon as their visit to the first item resulted
490 in an absence of reinforcement. This observation is difficult to combine with Terrace's
491 account of chained responses, by which responding to one item automatically cues the
492 response to the next. As the pigeon had to complete two baseline trials correctly before
493 entering a Memory Test trial, it is unlikely that the sudden stop was due to a lack of motivation
494 or an inability to remember the sequence that the pigeon had completed successfully in the
495 immediately preceding trial. Although unexpected, the observed behaviour indicates that
496 pigeons had formed a concept of the goal of completing the sequence (to maximise reward)
497 and acted in a planned way to achieve it. The pigeons did not perceive each item as an
498 individual stimulus-outcome event, but indeed as part of a connected sequence. When the
499 expected reward was not encountered in sequence item 1 during the non-reinforced test
500 trials, this disruption was likely extrapolated to the entire sequence and the subjects changed
501 their behaviour from the formerly goal-directed completion of the sequence to an
502 unstructured search.

503 The combination of results of both tests makes it evident that the arguments put
504 forward by Terrace ⁵ are not compatible with the behaviour shown by our pigeons. Instead
505 of acquiring simple response-response associations, the pigeons showed evidence of forming
506 a representation of the order of list items, although this representation might be less
507 sophisticated than proposed by Scarf and Colombo ⁴, and instead limited to a concept of items
508 that appear earlier and later in the sequence. As Terrace pointed out correctly, the first item
509 takes a special role, likely due to excessive overtraining. Furthermore, although the absolute
510 length of the sequence that could effectively be memorised was reduced for some older

511 pigeons, the ability to form a mental representation of order was preserved in all pigeons.
512 Regardless of age, pigeons were able to perform in a way that was consistent with the task
513 goal of maximising reward.

514 Thus, the current study showed that age can have a noticeable effect on the cognition
515 of pigeons. As previously shown for humans ^{12,13}, aging does not impact all cognitive abilities
516 uniformly, nor are all individuals affected to the same degree. It is possible to identify
517 cognitive capacities that are highly susceptible to age for many species, such as memory, as
518 shown in this study. Our study further supports that aging is an individual process,
519 manifesting itself differently in “healthy-aging” or “poorly-aging” individuals. Although aging
520 research is currently still limited to a few species, it is evident that age plays a crucial role in
521 many aspects of animal cognition and behaviour. Identifying suitable paradigms, like the one
522 presented in this study, to investigate such age effects in a wide range of species is the first
523 step to closing the gap.

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560

561 **AUTHOR CONTRIBUTIONS**

562 CM designed the study, CM and PS created the material and conducted the
563 experiment, CM analysed and interpreted the data and wrote the manuscript, PS and DK
564 edited the manuscript. All authors approve the submitted version and agree to be personally
565 accountable for their own contributions and to ensure that questions related to the accuracy
566 or integrity of any part of the work, even ones in which the author was not personally
567 involved, are appropriately investigated, resolved, and the resolution documented in the
568 literature.

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570

571 **ADDITIONAL INFORMATION**

572 The authors declare no competing interests.

573 **FIGURE AND TABLE LEDENDS**

574 **Figure 1. Top-down view of the inside of the experimental arena.** The six feeders
575 (in grey) were placed in a circular array in one half of the arena. The pigeon entered at the
576 opposite side of the arena (the approximate location is marked in red). The insert in the
577 broken-line box shows a 3D model of a feeder.

578 **Figure 2. Examples of a trial in each memory training stage.** The location of each
579 sequence item (coloured feeder) changed between trials as to not systematically reinforce a
580 certain location. The sequence shown here is for illustrative purposes.

581 **Figure 3. Mean number of sessions required to reach completion criterion in**
582 **each training stage (TS) for each age group.** Individual subject data are plotted as point
583 values. Error bars represent standard errors.

584

585 **Table 1.** Percentage of trials (out of three trials) during which a subject chose the
586 item that had appeared earlier in the sequence. Individuals are listed in order of the number
587 of items they received during training; those that only completed up to TS3 were not
588 presented with choices including sequence item 4.

589 **Table 2.** Percentage of Choice Test trials during which a subject chose the known item
590 when the choice was between a known (sequence items 1, 2, 3, or 4, as applicable) and an
591 unknown item (sequence items 5, 6, or 4, as applicable), or between two unknown items.
592 Individuals are listed in order of the number of items they were trained on; those that only
593 completed up to TS3 were not presented with sequence item 4 as a known option.

594 **Table 3.** Percentage of Memory Test trials (out of ten trials) during which a subject
595 chose sequence item 1 before any other items ("1 first"), chose sequence item 2 directly after
596 item 1 ("1 → 2"), chose sequence item 3 directly after item 2 ("2 → 3"), chose sequence item 4
597 directly after item 3 ("3 → 4"; only for those pigeons that had experienced training stage 4),

598 chose the final trained items as its last choice (“3/4 last”), and when a subject completed the
599 entire sequence of either three or four trained items in its correct order (“1 → 2 → 3 (→ 4)”).
600 Individuals are listed in order of the number of items they were trained on; those that only
601 completed up to TS3 were not expected to visit sequence item 4.

Figures

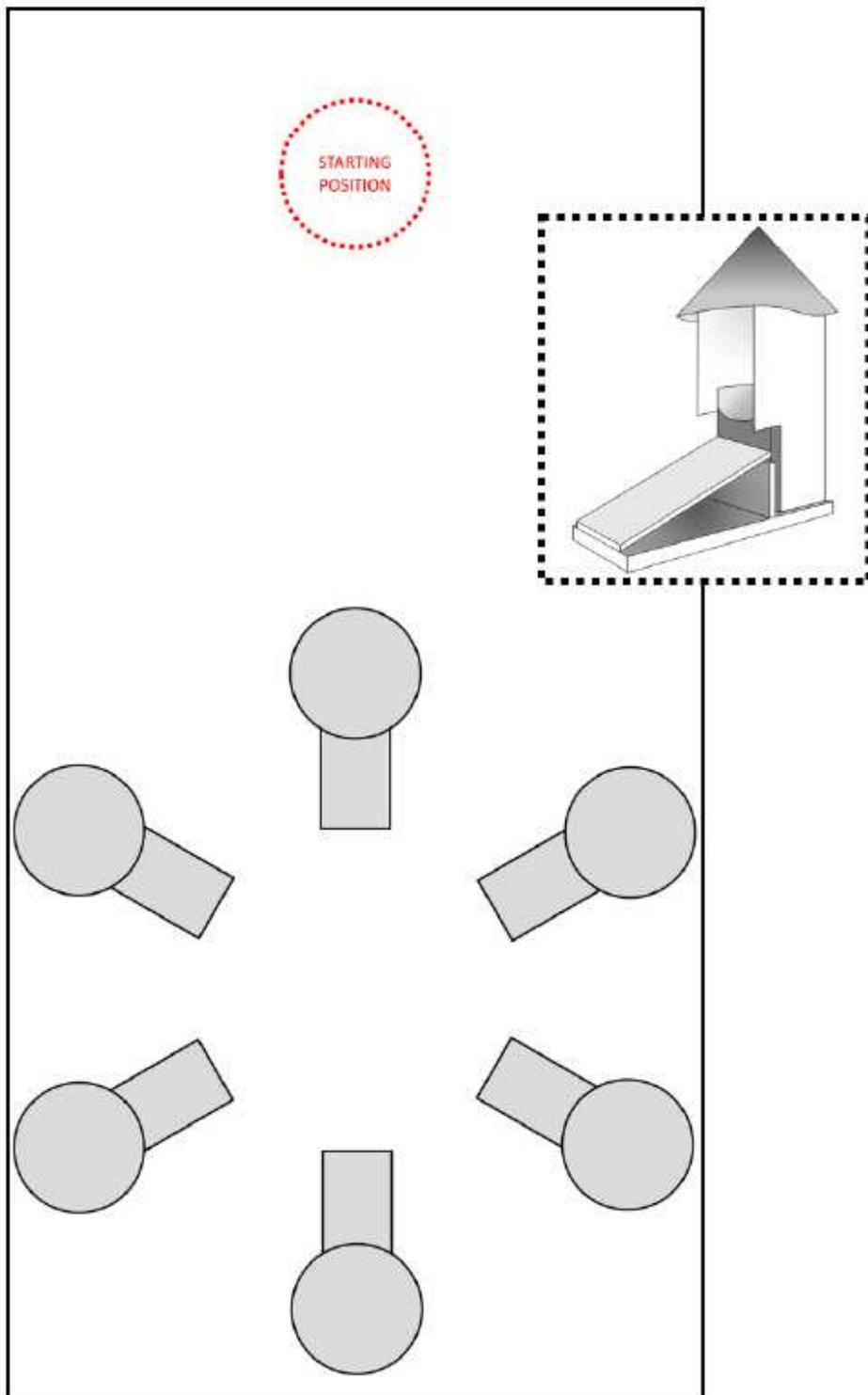
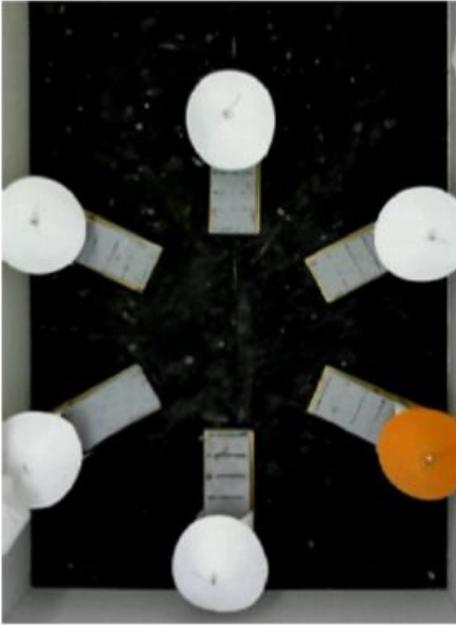


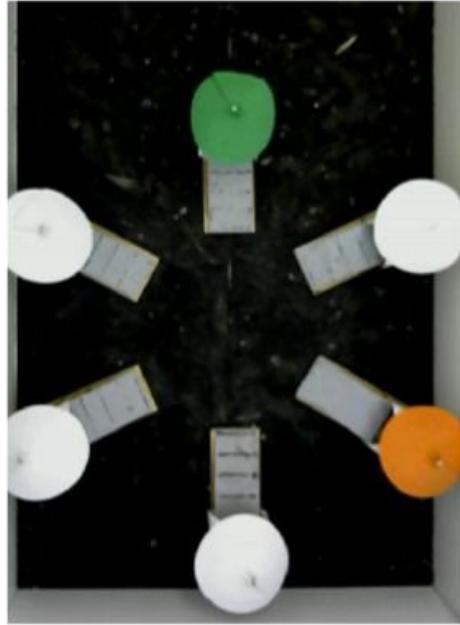
Figure 1

Top-down view of the inside of the experimental arena. The six feeders (in grey) were placed in a circular array in one half of the arena. The pigeon entered at the opposite side of the arena (the approximate location is marked in red). The insert in the broken-line box shows a 3D model of a feeder.

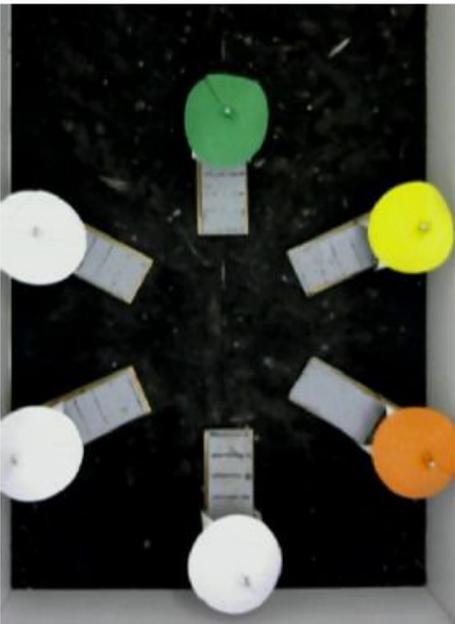
a: Training Stage 1



b: Training Stage 2



c: Training Stage 3



d: Training Stage 4

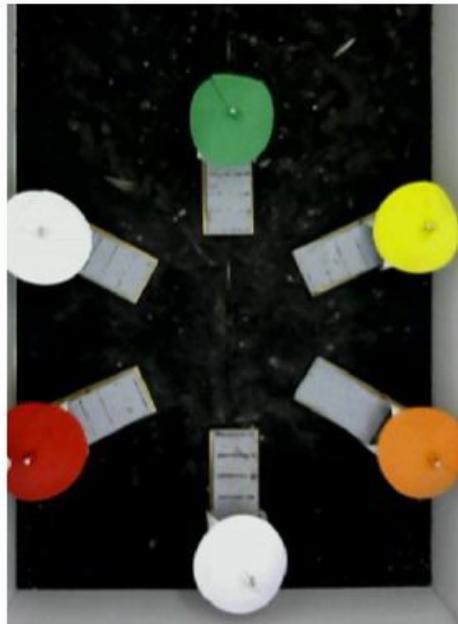


Figure 2

Examples of a trial in each memory training stage. The location of each sequence item (coloured feeder) changed between trials as to not systematically reinforce a certain location. The sequence shown here is for illustrative purposes.

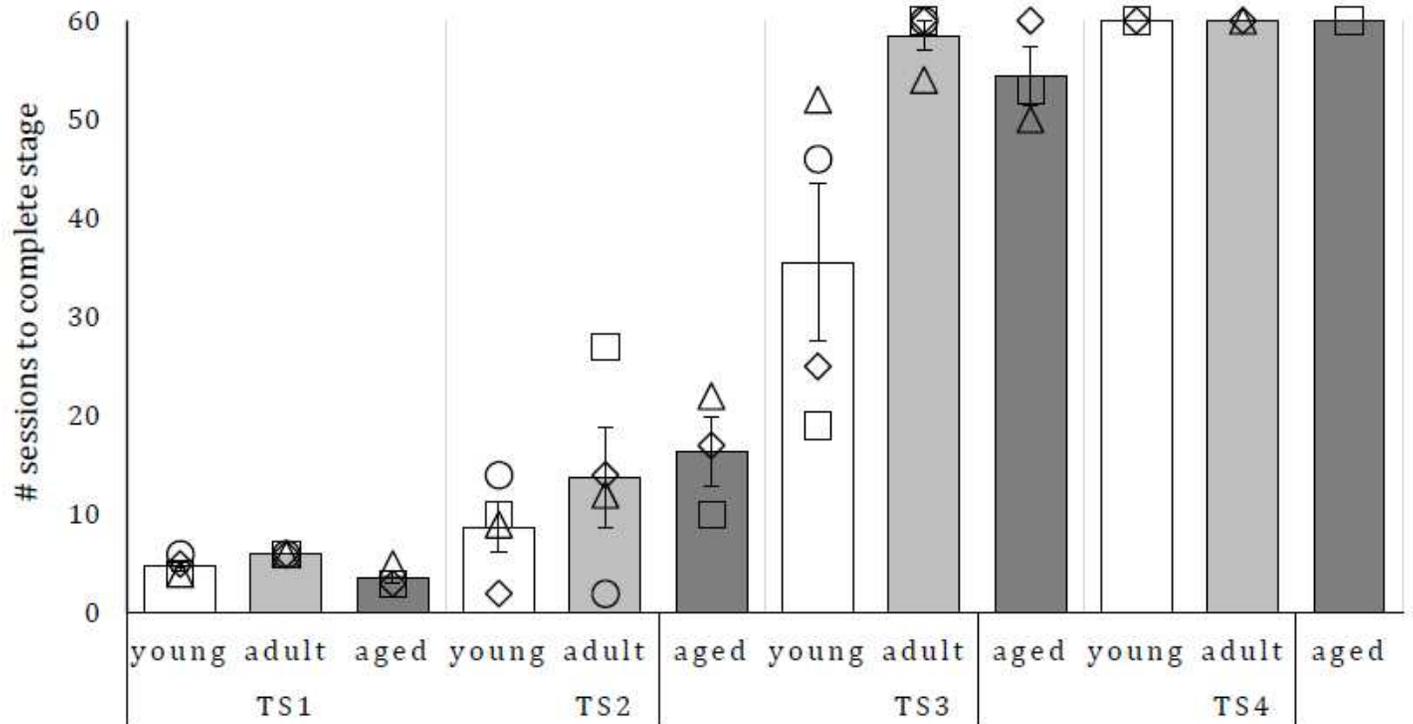


Figure 3

Mean number of sessions required to reach completion criterion in each training stage (TS) for each age group. Individual subject data are plotted as point values. Error bars represent standard errors.