1 Training level reveals a dynamic dialogue between stress and

2 memory systems in birds

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25   **Abstract**

26   It is now well-accepted that memory is a dynamic process, and that stress and training level may

27   influence which memory system an individual engages when solving a task. In this work, we

28   investigated whether and how chronic stress impacts spatial and cue-based memories according to

29   training level. To that aim, control and chronically stressed Japanese quail were trained in a task that

30   could be solved using spatial and cue-based memory and tested for their memory performances after 5

31   and 15 training days (initial training and overtraining, respectively) and following an emotional

32   challenge (exposure to an open field). While chronic stress negatively impacted spatial memory in

33   chronically stressed birds after initial training, this impact was lowered after overtraining compared to

34   control quail. Interestingly, the emotional challenge reinstated the differences in performance between

35   the two groups, revealing that chronic stress/overtraining did not eliminate spatial memory. Differences

36   caused by previous stressors can re-emerge depending on the more immediate psychological state of the

37   individual. Contrary to spatial memory, cue-based memory was not impaired in any test occasion,

38   confirming that this form of memory is resistant to chronic stress. Altogether these findings reveal a

39   dynamic dialogue between stress, training, and memory systems in birds.

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41   Keywords: bird cognition; chronic stress; spatial memory; cue-based memory; overtraining; Japanese

42   quail.

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54 **1. Introduction**

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56   It has long been recognized that chronic stress strongly modulates learning and memory

57 performances [1–3]. However, the relationship between chronic stress and memory are still

58 controversial: some studies reported an improving effect of chronic stress on memory performances [4–

59 6], whereas others reported a negative effect [6–8]. Overall, chronic stress generally provokes memory

60 impairment for complex tasks and improves simple tasks [3]. These discrepancies support the fact that

61 memory is not a single unit. Different parts of the brain are responsible for different memory systems

62 that process different types of information and are differently affected by stress [9].

63   Two memory systems have had a considerable amount of scientific interest in the last decades

64 as they present strong parallels between humans and animals. The first one is spatial memory, a form of

65 declarative memory based on the hippocampus and used mainly to establish a relationship between

66 different cues from the environment, which allow the creation of a cognitive mental map [10,11]. As the

67 cues from the environment are treated in relation to each other, the spatial memory is considered to be

68 more complex than the cue-based memory, a non-declarative form of memory that results from a simple

69 association between a salient cue and the target and is based on the striatum [9,12,13]. Studies have

70 shown that chronic stress has a strong and negative impact on declarative memory, such as spatial

71 memory [1,3,6,7,14,15], whereas it spares or even improves forms of non-declarative memory

72 [5,6,15,16].

73   Beyond chronic stress, the training level can also influence the use of multiple memory systems

74 [9,17–20]. Indeed, rats trained to learn the location of a reward in a maze preferentially use their spatial

75 memory after a few days of training and shift to a cue-based dominant response with overtraining [21].

76 Training and stress levels may also interact: the initial differences in memory performances due to stress

77 may disappear with overtraining [3]. Other studies suggest training itself may be a source of stress and

78 reduce hippocampal neurogenesis [22,23], which could explain the shift between memory systems.

79 While much is known on chronic stress effects on declarative spatial or non-declarative cue-based

80 memory performances, the influence of the level of training and its interaction with chronic stress

81 influence remains poorly understood.

82   Using the Japanese quail (*Coturnix coturnix*) as the animal model, in this work, we investigated

83 whether and how chronic stress could differentially impact spatial and cue-based memories according

84 to the level of training. Control and chronically stressed Japanese quail were trained in a dual

85 spatial/cued task, which could be solved either using the spatial or cue-based memories and tested for

86 their performances on each type of memory after five and fifteen training days. Additionally, as an

87 animal's immediate psychological state may interact with chronic stress and impact the relative use of

88 different memory systems and memory performances [24,25], a series of tests were conducted after an

89 emotional challenge that consisted of exposing quail to an unfamiliar environment (open field). Finally,

90   birds were submitted to a test to assess whether they learn to find the target cup by a simple association
91   between the target cup's colour and the reward or by detecting the difference of colours between the
92   target cup and the remaining cups. Based on the literature, we expected chronically stressed quail to
93   show lower performance in the spatial memory test than control ones after initial training (five days of
94   training). These differences were expected to lower or disappear after overtraining (fifteen days of
95   training) and reinstated after the emotional challenge. Concerning the cue-based memory, we expected
96   no differences between chronically stressed and control quail. Since this is a simple form of memory,
97   we expected the emotional challenge would not disrupt it.
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99   **2.   Methods**
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101   **2.1.   Animals**
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103   All Japanese quail were bred and maintained at the Pôle d'Expérimentation Avicole de Tours
104   (UE PEAT, INRAE, 2018. Experimental Poultry Facility, DOI: 10.15454/1.5572326250887292E12)
105   where the experiment took place. On the day of hatching, chicks were transferred to communal floor
106   pens. On the 21st day after hatching, chicks were sexed by feather dimorphism, and males were reared
107   in a single home cage (41×51×25 cm) in a battery under a 12:12h light-dark schedule (light on 08h). A
108   piece of artificial turf was placed on the cage floor to allow animals to exhibit dust bathing behaviour.
109   The ambient temperature was maintained at approximately 20±2°C. Unless otherwise specified, food
110   and water were provided *ad libitum*.
111   Animal care and experimental treatments complied with the French Ministry of Agriculture
112   guidelines for animal experimentation and European regulations on animal experimentation
113   (86/609/EEC). They were performed following the local animal regulation (authorized C37-175-1) of
114   the French Ministry of Agriculture under the EEC directive and under ethics committee approval (Val
115   de Loire, agreement N° 1789 and 1848).
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118   **2.2.   Chronic stress procedure**
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120   At 21th day of age, when quail were transferred in individual cages, they were divided into two
121   groups, a control and a chronic stress group. Quail from the chronic stress group were submitted to
122   chronic unpredictable stress (CUS) for 21 days consisting of five to six negative stimulations or stressors
123   per day. To improve the unpredictability of the stressor delivery and decrease habituation to the stress
124   procedure, each stressor occurred at unpredictable times each day during both night and day. The CUS
125   procedure has been fully described and was shown to induce a state of chronic stress in quail [15,26–

126   30]. Briefly, some stressors were delivered directly in the home cage of the quail: confinement of the

127   bird in the corner of its home cage for 30min, food frustration by placing transparent devices on the

128   feeder just before light on for 30min, soft home cage shaking (2×15 min interspaced by 15 min),

129   unexpected sounds (100 dB) composed of different sounds having or not biological signification for

130   quail. Disturbances from outside the home cage were also delivered, such as a rapid passage of a plastic

131   stick on the rods of the home cage twice a day for 2 min, fast sprayings of water or air on feathers (2

132   sprays interspaced by 2 min), waving of a plastic flag in front of the home cage for 2 min. Other stressors

133   were also delivered out of the home cage when birds were transferred individually into a new

134   environment for 30 min. Birds from the same line were also placed together in a transport cage for 30

135   min or placed on a cart and rolled about in the facility.

136   The control group was left undisturbed except for routine husbandry procedures similar to those

137   provided to birds exposed to CUS. The experimenter regularly visited the control quail room to spend

138   the same amount of time with control and chronically stressed birds. Systematically, when stressors

139   prevented animals from having access to food, an opaque device was placed on the feeder of control

140   birds to prevent them from eating food for an equivalent time.

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143   **2.3.   Learning and memory task**

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145   At the end of the CUS procedure, 17 control and 20 chronically stressed birds were submitted

146   to a training procedure followed by a series of memory tests (Figure 1). Briefly, after preliminary phases

147   dedicated to familiarized birds to the food reward (mealworms), the cups and the arena, control and

148   chronically stressed birds were submitted to a dual spatial/cued task during five days that consisted of

149   learning to find food (mealworm) hidden in a single cup (target cup) in an arena that contained eight

150   opaque cups. On each trial, birds were introduced in the arena by a different starting point among three

151   possibilities. The location of the target cup was constant among trials and days of training, allowing

152   animals to solve the task using spatial memory. Moreover, the target cup was black, whereas other non-

153   target cups were white. Thus, birds could also solve this task using their cue-based memory, based on

154   the colour of the cup.

155   The day after the fifth day of training, birds were submitted to a spatial test followed by a

156   displacement test. During the spatial test, all cups were white, and the task could only be solved by

157   remembering the spatial location of the target cup during the training phase. During the displacement

158   test, performed 30 min later, the black target cup was displaced to a new position (different from the

159   position used during training). Thus, both spatial and cue-based memories could provide a solution to

160   the test. Quail could go back either to the spatial location of the target cup during the training phase

161   (spatial memory) or go directly to the displaced black cup (cue-based memory) [15,31,32].

162   After this first test occasion, birds were trained for ten additional days in the dual spatial/cued

163   task before being submitted to the second test occasion of spatial and displacement tests. Following the

164   second test occasion, birds were trained for an additional day (recall training) in the dual spatial/cued

165   task to avoid that lack of food during the second test occasion reduced bird motivation in subsequent

166   tests. After this first recall training day, all birds were challenged by exposure to an open field test and

167   were submitted to the third occasion of spatial and displacement tests.

168   On the following day, quail were submitted to a second additional training day (recall training)

169   to keep birds motivated. The day after, birds were submitted to a new test, called "colour test", to assess

170   whether birds found the target cup by a simple association between its colour (black) and the reward or

171   by learning a more general rule that the target cup was of a different colour compared to the remaining

172   cups.

173   During all the memory tests, behaviours of quail were recorded by a camera placed above the

174   apparatus and computerized by a tracking video system (Ethovision XT; Noldus IT, The Netherlands).

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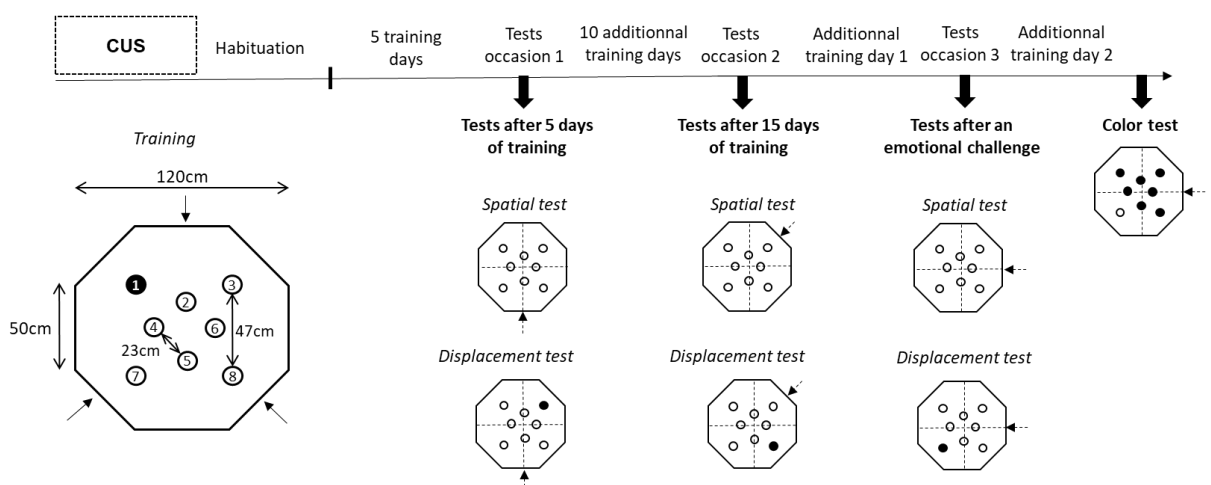
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179   **Figure 1: Schedule of the experiment and schematic representation of the apparatus used**

180   **for training and testing.** At three-weeks-old, quail were submitted to chronic unpredictable stress

181   (CUS) for three weeks. At the end of the CUS procedure, stressed and control birds were trained in a

182   dual spatial/cued learning task and submitted to spatial and displacement memory tests after 5 and 15

183   days of training. After an additional training day, quail were submitted to an emotional challenge

184   (exposure to an open field) followed by a new series of tests. After the last training day, birds were

185   submitted to the colour test. Quail could enter the arena from three different starting points (dark arrows),

186   three starting points during training, and one during testing. The dotted lines represent the division of

187   apparatus into four different quadrants.

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191   **2.4.**   **The dual spatial/cued task**

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193   The following procedures and the arena used for the task were previously used and validated by

194 our group in chickens and quails [13,15,32,33].

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196   2.4.1.   *Mealworms familiarization*

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198   One week before the beginning of training, a brown ceramic cup (6x7cm) was placed in each

199 quail's home cage. For seven days, three times per day (spaced about 3h), 3 or 4 mealworms were

200 deposited in the cup to familiarize birds with the cups and mealworms.

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202   *2.4.2.   Habituation*

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204   The day following the end of the familiarization phase, birds were submitted to the habituation

205 phase. One hour before each habituation session, food was removed from the home cage of the animals.

206 During five days, once a day, each quail was introduced into the centre of a beige octagonal arena

207 (120cm long; 50cm high) surrounded by a blue curtain (1.90m high) and lighted by a bulb at the ceiling

208 (18 Lux). Four black spatial cues were placed on the walls of the arena and four others on the curtain.

209 Eight ceramic cups similar to those used for the familiarization were placed in the arena and contained

210 mealworms. Four cups were covered with white paper, whereas black paper covered another four cups.

211 Each bird was allowed to explore the arena and the cups in each habituation session until it found and

212 ate all mealworms or after a maximum time of 600 s. Between each quail and day of habituation, the

213 position of black and white cups was randomly moved. On each day, the number of cups visited was

214 recorded to estimate the level of exploration and habituation of the birds (Figure 1).

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216   *2.4.3.   Training*

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218   On each day of training, birds were submitted to 2 training trials per day with an inter-trial

219 interval of 1 hour. On each trial, birds were introduced in the arena by a different starting point among

220 three possibilities (Figure 1). Only one cup was rewarded and contained three mealworms (target cup).

221 The target cup was always black and at a fixed position on each trial, whereas the seven other cups were

222 non-rewarded and white. The trial ended when the bird reached the target cup and ate the mealworms,

223 or after a maximum time of 300 s. If the bird did not find the target cup, it was gently guided to it and

224 allowed to eat the mealworms before being removed from the arena. Between each trial, the bird returned

225 to its home cage. The latency to find and eat the food in the rewarded black cup was recorded on each

226 trial.

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2.4.4. The spatial test

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230   This test was used to assess the impact of chronic stress on spatial memory. Each bird was
231   allowed to freely explore the arena and the 8 cups for 1 minute. All cups were empty and, more
232   importantly, of similar colour (white). The number of cups visited (number of errors) before reaching
233   the target cup was scored. Moreover, the arena was virtually divided into four equal quadrants, and the
234   time spent in each quadrant (spatial quadrant and three other quadrants) during the test was measured
235   using the tracking video system (Figure 1). The time spent on the three other quadrants was averaged.
236   The spatial test was conducted after 5 and 15 days of training (initial training and overtraining,
237   henceforth) and after an emotional challenge by exposing birds to an open field (emotional challenge,
238   henceforth).

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2.4.5. The displacement test

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242   The displacement test was used to assess the impact of chronic stress on the use of spatial and
243   cue-based memory systems. It was systematically conducted 30 min after the spatial test. During this
244   test, similarly to the training phase, the arena was equipped with one black cup and seven white cups,
245   but all cups were empty of food. The black cup was displaced to a new position (Figure 1). The new
246   position was changed for each test conducted after initial training, overtraining, and emotional challenge.
247   All birds were allowed to explore the arena and the cups for 1 min freely. The number of errors before
248   reaching the spatial and displaced cup was scored. Similar to the spatial test, the arena was virtually
249   divided into four equal quadrants (spatial quadrant, displaced cup quadrant, and two other quadrants),
250   and the time spent in each quadrant during the test was measured using the tracking video system. The
251   time spent on the two other quadrants was averaged.

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2.4.6. Tests after an emotional challenge

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255   The third occasion of both tests (spatial and displacement tests) was conducted after an
256   emotional challenge. This emotional challenge consisted of exposing the birds to an open field test,
257   which is known to provoke anxiety-like quail behaviors [15,34,35]. Each quail was individually placed
258   in the centre of a square arena ($80 \times 80 \times 80$ cm) of white wood with a beige linoleum floor. The arena
259   was placed into a new experimental room, surrounded by a green curtain and strongly lighted (50 lux).
260   Each quail was allowed to explore the arena for 5 min before being withdrawn from the arena. Thirty
261   minutes after the open field test, quail were submitted to a spatial test followed by a displacement test.

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264   *2.4.7.   The colour test*

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266   This test aimed to assess whether birds learn to find the target cup by a simple association
267   between its colour (black) and the reward or by detecting the difference of colours between the target
268   cup and the remaining cups (Figure 1). Each bird was allowed to freely explore the arena and the eight
269   cups for 1 minute. All cups were empty and black, except for one white cup and not located in the spatial
270   position used for training. The number of visits before reaching the white cup was scored. Moreover,
271   the number of visits before reaching the black cup located in the spatial location used during training
272   was scored and compared to those to reach the white cup and other black cups. As for previous tests, the
273   time spent in the four equal quadrants (white cup quadrant, spatial black cup quadrant, and two other
274   black cup quadrants) of the arena was measured. This test was performed after a second recall training
275   day conducted the day after the third test occasion.

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277   **2.5.   Statistical analysis**

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279   Data from habituation (number of cups visited) and from training (latency to reach the target
280   cup) were analysed by parametric analyses of variance (ANOVA repeated measures) with stress group
281   (control and chronically stressed quail) as between-subject factors and days (mean values for both
282   training trials within days) as within-subject factors.

283   For spatial, displacement, and colour tests, the number of errors (or visits) before reaching the
284   target cup(s) and the time spent in the different quadrant of the arena were analysed by ANOVA with
285   groups (control and stressed) as between-subject factors and test occasions (test after initial training, test
286   after overtraining and test after the emotional challenge) as within-subject factors. Additional within-
287   subject factors (e.g., quadrants or cups considered) were also included, dependent on the variables under
288   consideration. When main effects or interactions were significant, analyses were followed by multiple
289   comparisons corrected by Tukey HSD.

290   When quail did not find a target cup and visited less than 4 cups, they were considered non-
291   explorer. For these birds, a number of 8 errors and an equivalent time of 15 sec in each quadrant were
292   attributed. All statistical analyses were performed using IBM SPSS 21 and R version 3.6.1. Statistical
293   significance was set at $p < 0.05$.

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3. RESULTS

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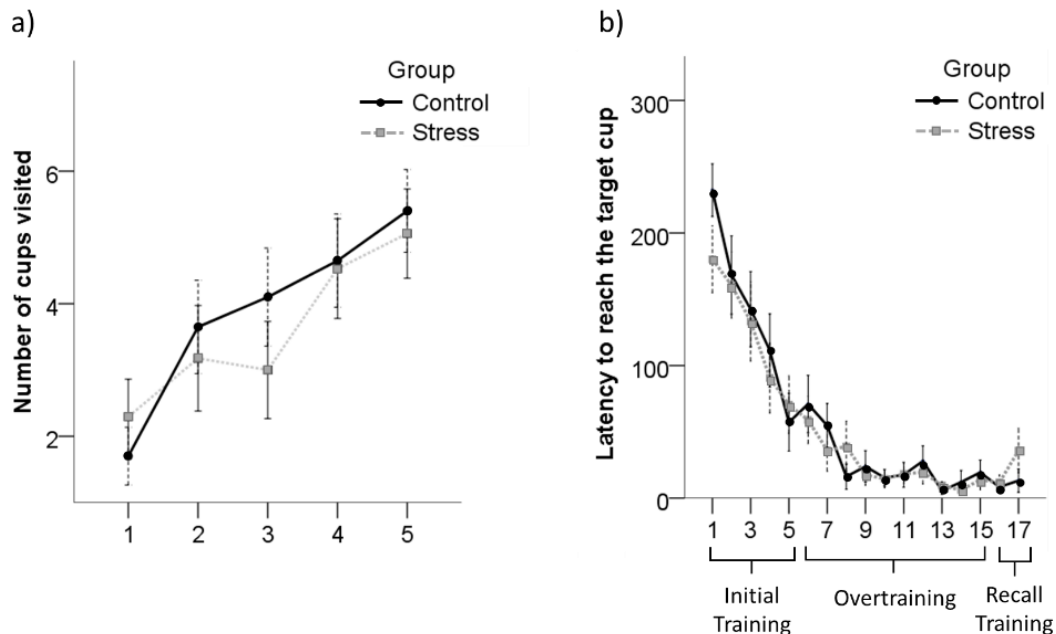### 3.1. Effect of chronic stress on habituation and training in the dual spatial/cued task

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During habituation, both groups visited more cups and ate significantly more mealworms over days without any differences between control and chronically stress quail (effect of days: $F_{4,140}=18.15$, $p<0.001$; effect of stress: $F_{1,35}=0.13$, $p=0.72$; interaction days x stress: $F_{4,140}=1.11$, $p=0.35$; Figure 2a).

During the initial training period, both groups similarly learned the location of the target cup since the latency to find the target cup significantly decreased over days (effect of days: $F_{4,140}=19.60$, $p<0.001$; effect of stress: $F_{1,35}=0.28$, $p=0.60$; interaction days x stress: $F_{4,140}=0.93$, $p=0.45$; Figure 2). Similarly, during the overtraining period, the latency to reach the target cup significantly decreased without any effect of chronic stress (effect of days: $F_{9,315}=5.89$, $p<0.001$; stress effect: $F_{1,35}=0.13$, $p=0.73$; interaction days x stress: $F_{9,315}=0.58$, $p=0.81$; Figure 2b).

The latency between the last day of overtraining (the fifteenth day of training) and the recall period between tests (last two additional days) was not different between days, nor between control and chronically stressed quail (effect of days: $F_{2,70}=1.12$, $p=0.33$; effect of stress: $F_{1,35}=0.65$, $p=0.42$; interaction days x stress: $F_{2,70}=1.10$, $p=0.33$), indicating that animals reached a learning plateau.

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Figure 2: Effects of chronic stress on habituation and training performances in the dual spatial/cued task. a) Number of visited cups over days of habituation in control and chronically stressed quail. During habituation, all eight cups were rewarded with mealworms. **b)** Latency to reach the

324   location of the target cup over days of training in control and chronically stressed quail. Mean ± SEM

325   are given.

3.2. Effect of chronic stress during the spatial test

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328   The number of errors made before reaching the spatial cup was significantly increased in

329   chronically stressed quail (effect of stress: $F_{1,35}=5.62$, p=0.023, Figure 3a), independently of the test

330   occasion and the interaction with stress (effect of test occasion: $F_{2,70}=1.45$, p=0.24; interaction test

331   occasion x stress: $F_{2,70}=2.72$, p=0.072).

332   The time spent in the spatial quadrant compared to other quadrants was affected by the quadrant

333   considered, stress, and the interaction quadrant, test occasion and stress (effect of quadrant: $F_{1,35}=7.58$,

334   p=0.009; effect of stress: $F_{1,35}=6.13$, p=0.018; interaction quadrant x test occasion x stress: $F_{2,70}=3.36$,

335   p=0.040). Post-hoc analyses revealed that, during the tests after the initial training and the emotional

336   challenge, control birds spent significantly more time in the spatial quadrant compared to other

337   quadrants (p<0.001 and p=0.001, for the tests after the initial training and the emotional challenge,

338   respectively). In contrast, chronically stressed birds spent an equivalent time in the spatial quadrant

339   compared to other quadrants (p=1 and p=1 for the tests after the initial training and the emotional

340   challenge, respectively, Figure 3b). No differences were found for the test after overtraining: both

341   control and chronically stressed quail spent an equivalent time in the spatial quadrant compared with

342   other quadrants (p=1 and p=1, for control and chronically stressed quail, respectively, Figure 3b).
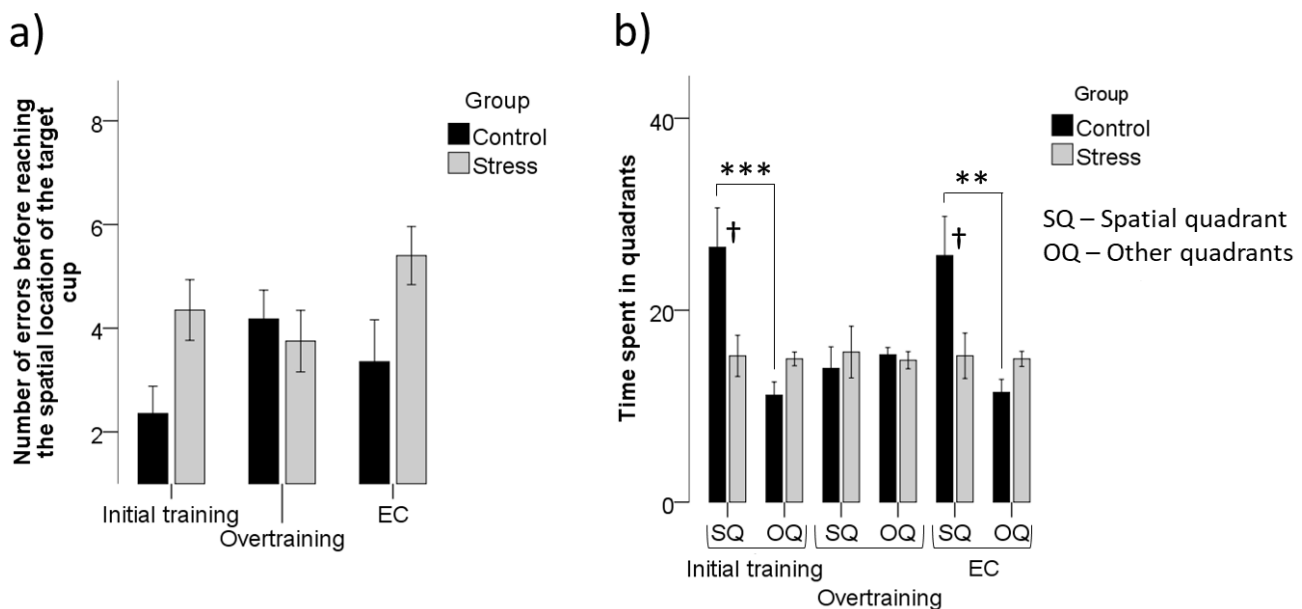
343   **Figure 3. Effects of chronic stress in spatial memory tests performed after initial training (5 days),**

344   **overtraining (15 days), and after an emotional challenge (EC, exposure to an open field). a)**

345   Number of errors before reaching the spatial location of the target cup, and **b)** Time (in seconds) spent

346   in different quadrants of the arena (spatial and other quadrants). † p≤0.05, significant difference between

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347   control and chronically stressed quail. ** $p \leq 0.01$; *** $p \leq 0.001$, significant difference between the spatial

348   quadrant and other quadrants. Mean ± SEM are given.

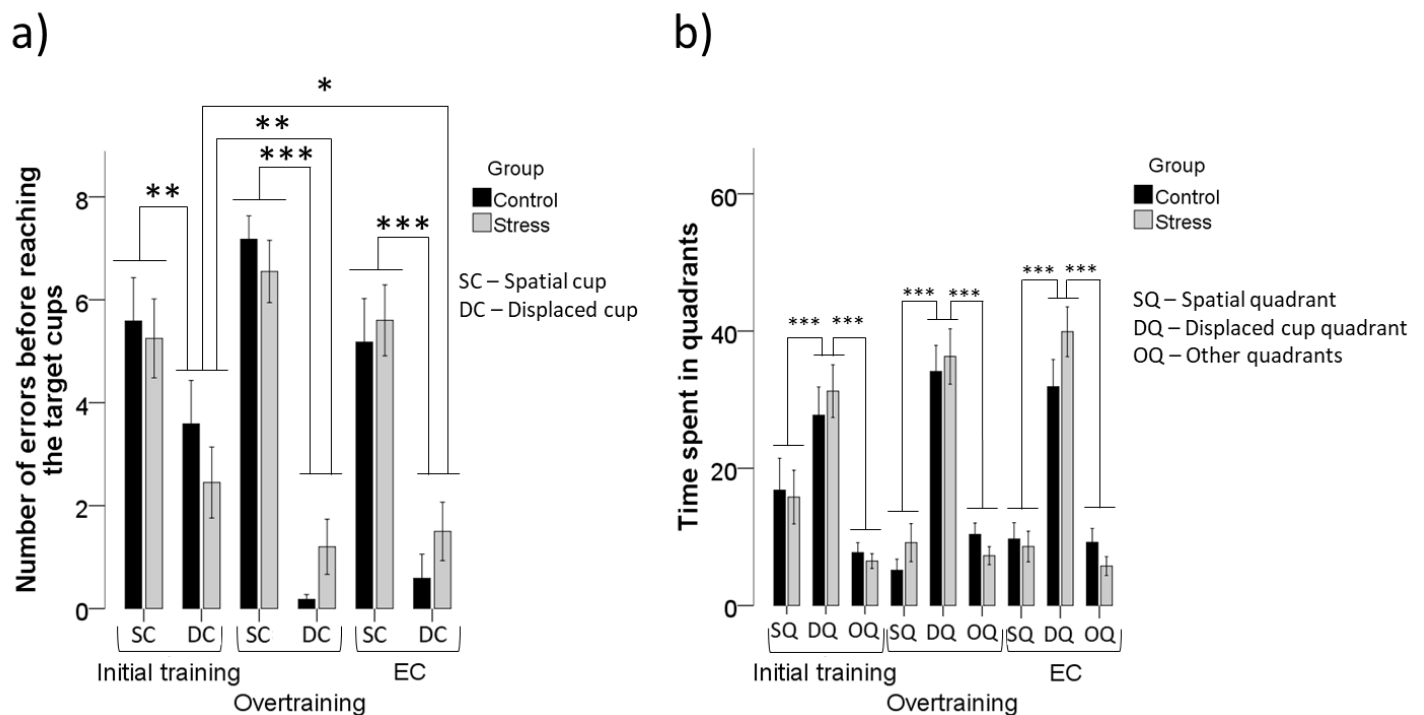349   **3.3. Effect of chronic stress during the displacement test**

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351   Analyses on the number of errors made before reaching the target cups (either the spatial cup or

352   the displaced cup) showed that animals were more performant to reach the displaced cup, independent

353   of their treatment group. There was also a significant interaction between the test occasion and the target

354   cups (effect of target cup: $F_{1,35}=100.71$, $p<0.001$; effect of test occasion: $F_{2,70}=3.22$, $p=0.046$; effect of

355   stress: $F_{1,35}=0.010$, $p=0.92$; interaction target cup x stress: $F_{1,35}=0.27$, $p=0.6$; interaction target cup x test

356   occasion: $F_{2,70}=8.26$, $p=0.001$; interaction target cup x test occasion x stress: $F_{2,70}=0.87$, $p=0.42$). Post-

357   hoc analyses revealed animals did significantly fewer errors before reaching the displaced cup in all test

358   occasions, compared to the errors before reaching the spatial cup ($p=0.001$, $p<0.001$ and $p<0.001$, for

359   the tests performed after initial training, overtraining, and just after the emotional challenge,

360   respectively, Figure 4a). Between test occasions and within-target cup, post-hoc comparisons revealed

361   that animals made significantly fewer errors before reaching the displaced cup during the tests after

362   overtraining and after the emotional challenge, compared to the test after initial training ($p=0.001$ and

363    $p=0.005$, respectively, Figure 4a).

364   Analyses on time spent in different quadrants showed that animals did not spend their time

365   evenly between quadrants. They spent significantly more time than the chance level in the displaced cup

366   quadrant independent of their treatment group. There was also significant a significant interaction

367   between the test occasion and the time spent in different quadrants (effect of quadrants: $F_{2,70}=67.19$,

368    $p<0.001$; effect of test occasion: $F_{2,70}=0.82$, $p=0.44$; effect of stress: $F_{1,35}=3.81$, $p=0.059$; interaction

369   quadrants x stress: $F_{2,70}=1.09$, $p=0.34$; interaction quadrants x test occasion: $F_{4,140}=3.25$, $p=0.014$.

370   interaction quadrants x test occasion x stress: $F_{4,140}=0.42$, $p=0.78$). Post-hoc analyses revealed that the

371   time spent in the displaced cup quadrant was higher for all test occasions compared to those spent on

372   the spatial quadrant and other quadrants ($p<0.05$, for the tests performed after initial training,

373   overtraining, and just after the emotional challenge, respectively, Figure 4b).

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Figure 4. Effects of chronic stress in the displacement tests performed after initial training (5 days), overtraining (15 days), and after an emotional challenge (EC, exposure to an open field). a) Number of errors before reaching the target cups (spatial cup or displaced cup), and **b)** Time (in seconds) spent in different quadrants of the arena (either the spatial quadrant, SQ, the displaced cup quadrant, DQ, or the other quadrants, OQ). * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$, significant difference between the spatial cup and displaced cup or between spatial quadrant, displaced cup quadrant, and other quadrants. Mean ± SEM are given.

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3.4. Effect of chronic stress during the colour test

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During the colour test, independently of chronic stress, the number of visits made before reaching a cup was different depending on the cup considered (effect of cup: $F_{2,70}=18.60$, $p<0.0001$; effect of stress: $F_{1,35}=0.03$, $p=0.87$; interaction cup x stress: $F_{2,70}=0.06$, $p=0.95$; Figure 5). Post-hoc analyses showed that the number of visits before reaching the spatial black cup was significantly lower than those performed before reaching other black cups or the white cup ($p=0.02$ and $p<0.001$, respectively, Figure 5a).

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393   Finally, the time spent in the different quadrants was also significantly dependent on the
394   quadrant considered (effect of quadrants: $F_{2,70}=3.62$, p=0.032). Post-hoc analyses revealed a
395   significantly larger amount of time spent in the black spatial cup quadrant compared to other black cups
396   and white cup quadrants (p=0.01). No differences were found between the treatment groups, nor for the
397   interaction quadrants and treatment groups (effect of stress: $F_{1,35}=1.02$, p=0.31; interaction quadrants x
398   stress: $F_{2,70}=0.59$, p=0.55, Figure 5b).
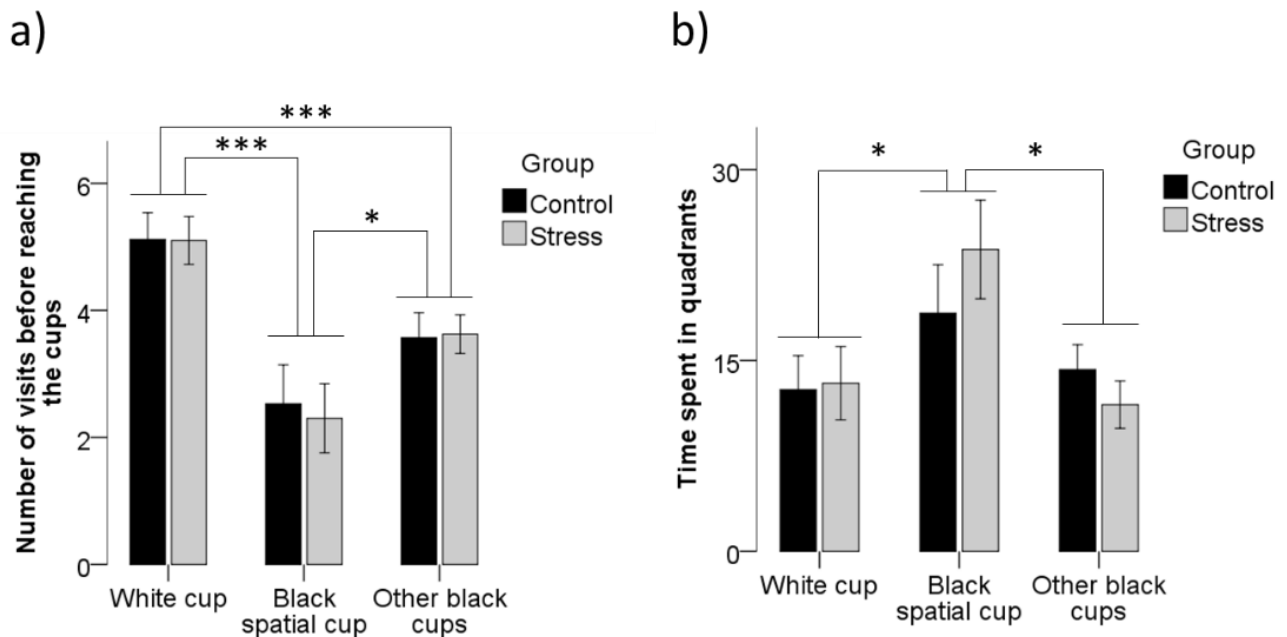
399   **Figure 5. Effects of chronic stress on the colour test. a)** Number of visits before reaching the white
400   cup, the black spatial cup, or other black cups in chronically stressed and control birds and **b)** Time (in
401   seconds) spent in different quadrants of the arena containing the white cup, the spatial black cup, other
402   black cups in chronically stressed and control birds. * p≤0.05; ** p≤0.01; *** p≤0.001, significant
403   difference between the white cup, black spatial cup, and other black cups. Mean ± SEM are given.

404

405   **4.   DISCUSSION**

406

407   The main objective of the present study was to address, in birds, whether chronic stress can
408   differentially impact two forms of long-term memory, the declarative spatial and the non-declarative
409   cue-based memories, according to the level of training. To this end, control and chronically stressed
410   Japanese quail were trained in a task that could be solved using spatial and cue-based memory, the dual
411   spatial/cued task, and tested for their performances on each type of memory after 5 and 15 days of
412   training (initial training and overtraining, respectively). Our results showed that the spatial memory of
413   quail was sensitive to the deleterious effect of chronic stress. Performances of chronically stressed birds
414   during the spatial test after initial training were impaired compared to that of control birds. During the
415   test after overtraining, however, these differences were lowered. Interestingly, during the test after an

14

416   emotional challenge, when birds were exposed to an open field, the negative impact of chronic stress on

417   spatial memory performances was fully reinstated. These findings indicate that the relationships between

418   chronic stress and spatial memory are complex and dynamic. By contrast, cue-based memory was not

419   affected in chronically stressed birds compared to control birds on any test occasion. Thus, this form of

420   memory is relatively resistant to the negative effect of chronic stress. Finally, a test conducted to better

421   qualify birds' cue-based memory performances, the colour test, provided support that chronically

422   stressed birds were still able to efficiently remember the spatial location of the target cup when this

423   location was signalled by a colour similar to that used during training. This suggests that chronically

424   stressed birds cannot efficiently recall stored spatial information in a context different from the one

425   experienced during training, which highlights that chronic stress reduces the ability to use spatial

426   memory flexibly.

427   Our results from habituation showed that the number of mealworms eaten increased over days

428   without any significant effect of chronic stress. Moreover, the latency to reach the target cup decreased

429   over days of training in both groups. These findings indicate that both control and chronically stressed

430   birds similarly habituated to the arena and the cups and similarly learned the dual spatial/cued task. They

431   also indicate differences between groups observed during the different tests performed could not be

432   attributed to evident motor or motivational differences and instead reflect stress-related changes in

433   memory performances.

434   During the spatial test, the number of errors before reaching the spatial location of the target cup

435   was higher in all test occasions (initial training, overtraining, after an emotional challenge) for

436   chronically stressed birds compared to control birds, evidencing the negative impact of chronic stress

437   on spatial memory performances. Although not significant (p=0.07), the interaction between test

438   occasion and stress suggests that the performance of both groups during overtraining tests was similar,

439   compared to initial training and after emotional challenge tests, when their performances differed more.

440   Confirming this tendency, the time spent in different quadrants revealed some specificities of the

441   animals' performance over the different test occasions. During spatial tests performed after initial

442   training, chronically stressed birds spent their time evenly between the quadrants, while control birds

443   spent significantly more time in the spatial quadrant. The same did not occur after overtraining. Indeed,

444   performances of both control and chronically stressed birds were relatively low during the spatial test

445   performed after overtraining. Contrasting to the spatial tests, chronic stress did not affect cue-based

446   memory performances in all displacement tests performed. In all test occasions, chronically stressed

447   birds and control had the same performance and followed the displaced black cup preferentially. These

448   findings indicate that chronic stress differentially impacts memory depending on the memory system,

449   with the spatial memory being much more sensitive to the adverse effects of chronic stress than the cue-

450   based memory [1,3,6,36].

451   Our results suggest that chronic stress has a stronger detrimental effect on spatial memory at

452   early stages compared to the late stages of training. They confirm previous studies in birds and mammals

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reporting chronic stress that can negatively impact this memory system [1,3,6,7,15]. Moreover, the lowering of chronic stress effects on spatial memory performances after overtraining may be explained by a progressive acquisition of a predominant cue-based strategy to solve the spatial test over days of training. In mammals, it is well documented that the involvement of memory systems is a dynamic process that can vary over time of training. In particular, overtraining in mammals favours cue-based memory [17,18,20,21]. In line with this, the number of errors to reach the black target cup during the displacement tests decreased with training, evidencing an improvement of this type of memory over time. These results confirm the idea that the protective effect of an extended period of training on the negative consequences of chronic stress on memory may be due to a progressive shift toward a non-declarative, cue-based memory system.

An alternative explanation for these results could be that extensive exposure to the task was perceived, by the control animals, as stressful on its own, worsening their performance. For rats, while a 4-day training did not impact brain plasticity, a 14-day training schedule drastically reduced hippocampal neurogenesis [23,37]. If that is the case, it is noteworthy to state that the stress from overtraining compared to the CUS procedure was perceived differently by the animals since the negative effect of chronic stress on spatial memory performances were specifically restored after an emotional challenge. Previous studies highlight that acute stress or stress hormone injections experienced just before testing can induce the re-emergence of an extinguished memory [24,25]. These results suggest that the progressive shift toward a cue-based memory system by control quail after an extending period of training is not due to the elimination of the spatial memory, which can re-emerge depending on the more immediate psychological state of the quail. Similarly, studies on mammals have shown that the shift from spatial memory to cue-based memory with increased training is not due to the elimination of the spatial memory [18,21]. Moreover, even after overtraining, the reappearance of chronic stress adverse effects on the spatial memory of chronically stressed quail could indicate a long-lasting sensibility of this memory system.

Finally, our study highlighted that chronic stress affected specific characteristics of spatial memory but did not erase this form of memory. Indeed, in the last test, the colour test, control, and chronically stressed birds made fewer visits before reaching the black cup located in the same spatial location used during training. Chronically stressed birds seemed thus able to remember the location of the target cup when this cup was black, as during training. It suggests that chronic stress specifically impaired the capacity of birds to remember a previously learned location when this location was no more signalled by the cue (black colour). This capacity is crucial to perform the spatial test efficiently. Numerous studies conducted in birds and mammals evidenced that spatial memory critically requires the hippocampus [11,38–42]. In mammals, the hippocampus was shown to be critically involved in forming distinct memories from events with a high level of similarities (pattern separation) and to re-establish previously acquired memory by using incomplete information as recall cues (pattern completion) [43,44]. During spatial tests, the spatial location was no more signalled by the cue (all cups

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490 were white). Birds should re-establish the complete spatial-cued information learned during training

491 (one rewarded black cup among seven non-rewarded white cups) from this incomplete information.

492 Under this framework, chronic stress, which negatively affects the hippocampus's functioning in quail

493 [15], may impair hippocampus-dependent processing of pattern completion.

494 In conclusion, the present study presents evidence that chronic stress can negatively impact the

495 birds' declarative spatial memory. It highlights that chronic stress specifically alters the ability to use

496 spatial memory flexibly. Also, we show for the first time in birds that an extended period of training can

497 lower the spatial memory differences between control and chronically stressed individuals, either by a

498 progressive shift to cue-based memory or by the fact that overtraining can be in itself perceived as

499 stressful by control animals, hindering their performance. These hypotheses should be tested further. An

500 emotional challenge, before testing, was capable of reinstating the differences in performance between

501 chronically stressed and control quail showing the long-lasting sensibility of the spatial memory system.

502 Altogether these findings reveal an original and dynamic dialogue between stress and memory systems

503 in birds.

504

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512

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