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Training level reveals a dynamic dialogue between stress and memory systems in birds

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Abstract

Chronic stress profoundly affects forms of declarative memory, such as spatial memory, while it may spare non-declarative memory, such as cue-based memory. It is known, however, that the effects of chronic stress on memory systems may vary according to the level of training of an individual was submitted. Here, we investigated, in birds, how chronic stress impact spatial and cue-based memories according to training level. For that, control and chronically stressed Japanese quail were trained in a task that could be solved using spatial and cue-based memory and tested for their memory performance after 5 and 15 training days (initial training and overtraining, respectively) and following an emotional challenge (exposure to an open field). Our results showed that, compared to control quail, chronic stress impacted negatively spatial memory performances in stressed birds after initial training, but these differences were lowered after overtraining. Control birds seemed to shift from spatial to cue-based memory to solve the task across overtraining. However, an emotional challenge before testing reinstated the negative impact of chronic stress on spatial memory performances between the groups, revealing that chronic stress/overtraining did not eliminate the spatial memory and differences caused by stressors can reemerge depending on the individual's immediate psychological state. Contrary to spatial memory, cue-based memory was not affected in chronically stressed birds compared to control birds in any test occasion, confirming its resistance against the negative effects of chronic stress. Altogether these findings reveal a dynamic dialogue between stress, training level, and memory systems in birds.

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

55

56 **1. Introduction**

57

58 It has long been recognized that chronic stress strongly modulates learning and memory
59 performance [1–3]. However, the relationship between chronic stress and memory are still
60 controversial: some studies reported an improving effect of chronic stress on memory performance [4–
61 6], whereas others reported a negative effect [6–8]. Overall, chronic stress generally provokes memory
62 impairment for complex tasks and improves simple tasks [3]. These discrepancies support the fact that
63 memory is not a single unit. Different parts of the brain are responsible for different memory systems
64 that process different types of information and are differently affected by stress [9].

65 Two memory systems have had a considerable amount of scientific interest in the last decades
66 as they present strong parallels between humans and animals. The first one is spatial memory, a form
67 of declarative memory based on the hippocampus and used mainly to establish a relationship between
68 different cues from the environment, which allow the creation of a cognitive mental map [10,11]. As
69 the cues from the environment are treated in relation to each other, the spatial memory is considered to
70 be more complex than the cue-based memory, a non-declarative form of memory that results from a
71 simple association between a salient cue and the target and is based on the striatum [9,12,13]. From
72 the first week of life, chicks can already be trained to use both their spatial and cue-based memory.
73 When the environmental information allows the use of both memory systems, chicks were seen to rely
74 on their cue-based memory [14]. We recently showed that, in quail, individual differences in
75 behavioral traits, such as fearfulness and stress-coping, alters the way birds use these different
76 systems. Indeed, fearful and easily stressed quail use their spatial memory preferentially, while less
77 fearful/stressed individuals prefer to rely on their cue-based memory [15]. The same occurs for the
78 relationship between individual exploratory level and memory systems in free-range chickens: less
79 exploratory chickens, known to be easily startled, have better spatial memory than more exploratory
80 chickens [16–18].

81 Besides intrinsic factors, external insults, such as hippocampal lesions or chronic stress, may
82 also influence which memory system an individual will preferentially use. Hippocampal lesioned and
83 chronic stressed quail present similar patterns of behavior when different memory systems are
84 requested: while these animals present important impairments on spatial memory, they have their cue-
85 based memory spared and, in some cases, even improved [13,19].

86 Beyond chronic stress, the training level can also influence the use of multiple memory
87 systems [9,20–23]. The majority of the knowledge on this area comes, however, from studies on
88 rodents. Indeed, rats trained to learn the location of a reward in a maze preferentially use their spatial
89 memory after a few days of training and shift to a cue-based dominant response with overtraining [24].
90 Training and stress levels may also interact: the initial differences in memory performance due to

stress may disappear with overtraining [3]. Other studies suggest training itself may be a source of stress and reduce hippocampal neurogenesis [25,26], which could explain the shift between memory systems. While much is known on chronic stress effects on declarative spatial or non-declarative cue-based memory performance, the influence of the level of training and its interaction with chronic stress influence remains poorly understood. Furthermore, the understanding of these interactions and their applicability to other taxa, such as birds, is still limited and merits further investigation.

Using the Japanese quail (*Coturnix coturnix*) as the animal model, in this work, we investigated whether and how chronic stress could differentially impact spatial and cue-based memories according to the level of training. In previous work, we developed reliable protocols to study the effects of chronic stress on different quail behavior and cognitive abilities. In quail, chronic stress is known to increase individuals' emotional reactivity (to both novel objects and humans, for example) and disrupt feeding patterns [27–29]. These negative effects may be long lasting, during up to seven weeks after the termination of the stress procedure [30]. While our results suggested that chronic stress strongly impair quail spatial memory, with lesser impacts on cue-based memory [19], we did not take into consideration the possible effects of training level and their interactions with chronic stress. In order to fill this knowledge gap, here, control and chronically stressed Japanese quail were trained in a dual spatial/cued task, which could be solved using the spatial or cue-based memories and tested for their performances on each type of memory after five and fifteen training days. Additionally, as an animal's immediate psychological state may interact with chronic stress and impact the relative use of different memory systems and memory performance [19,31–33], a series of tests were conducted after an emotional challenge that consisted of exposing quail to an unfamiliar environment (open field). Finally, birds were submitted to a test to assess whether they learn to find the target cup by a simple association between the target cup's color and the reward or by detecting the difference of colors between the target cup and the remaining cups. Based on the literature, we expected chronically stressed quail to show lower performance in the spatial memory test than control ones after initial training (five days of training). These differences were expected to lower or disappear after overtraining (fifteen days of training) and reinstated after the emotional challenge. Concerning the cue-based memory, we expected no differences between chronically stressed and control quail. Since this is a simple form of memory, we expected the emotional challenge would not disrupt it.

2. Methods

2.1. Animals

All Japanese quail were bred and maintained at the Pôle d'Expérimentation Avicole de Tours (UE PEAT, INRAE, 2018. Experimental Poultry Facility, DOI: 10.15454/1.5572326250887292E12)

where the experiment took place. These quail had a controlled and known genetic background, being selected over 60 generations to have intermediate, control-like levels of fear/anxiety responses (intermediate durations of tonic immobility) [34,35].

One-day old chicks were placed on communal floor pens. When chicks reached 21 days old, they were sexed by feather dimorphism, and males were transferred to a single home cage (41×51×25 cm), under a 12:12h light-dark schedule (light was on at 0800h). Artificial turf was available on the cage floor, allowing animals to dust bathe. The ambient temperature was kept at approximately 20±2°C. Food and water were provided *ad libitum*.

Animal care and experimental treatments followed the French Ministry of Agriculture guidelines and European regulations on animal experimentation (86/609/EEC). They also followed local animal regulations (C37-175-1) of the French Ministry of Agriculture under the EEC directive and under ethics committee approval (agreements N° 1789 and 1848, Val de Loire).

2.2. Chronic stress procedure

To stress quail chronically, we followed the same procedures as described in [19]. When quail reached 21 days old, they were transferred to individual cages. They were then divided into two treatment groups: a control and a chronic stress group. Quail from the chronic stress group were submitted to chronic unpredictable stress (CUS) for three weeks consisting of multiple negative stimulations or stressors (from five to six) per day. Each stressor occurred at unpredictable times each day/night to improve the unpredictability of the stressor delivery and decrease habituation to the stress procedure. This CUS procedure has been fully described and was shown to induce a state of chronic stress in quail [19,27,28,30,36,37]. Stressors were delivered directly in the quail's home cage: 30 min of confinement of the bird in the corner of its home cage, 30 min of food frustration by placing transparent devices on the feeder just before light on, 2×15 min of soft home cage shaking, interspaced by 15 min, unpredictable sounds (100 dB) with or without biological significance for quail. Outside disturbances were also delivered, such as a passage of a plastic stick on the home cage rods (twice a day for 2 min), sprayings of air or water on feathers (2 sprays interspaced by 2 min), and 2-min of waving a plastic flag in front of the home cage. Birds were also moved outside their homecage and individually transferred into a new environment for 30 min.

The control group was left undisturbed and underwent similar routine husbandry procedures as CUS birds. The experimenters regularly visited the control quail room to ensure they have spent the same amount of time with control and chronically stressed birds. Systematically, when stressors prevented CUS birds from feeding, an opaque device was also placed on the feeder of control birds to prevent them from feeding for an equivalent amount of time.

2.3. Learning and memory task

The following procedures and the arena used for the task were previously used and validated by our group in chickens and quail [13,15,17,19].

At the end of the CUS procedure, 17 control and 20 chronically stressed birds were submitted to a training procedure followed by a series of memory tests (Figure 1). The CUS procedure was stopped before the start of the training for two main reasons: the first one was to dissociate the effects of chronic stress from those of a possible acute, immediate stress that could occur if animals were stressed just before the training/testing sessions. The second reason is that, since the behavioral and cognitive effects of CUS in quails can be seen long after the procedure stopped [28,30,34], the stopping of CUS allowed us to test for the long-term consequences and implications of the procedure in animals' learning and memory.

After preliminary phases dedicated to familiarizing birds to the food reward (mealworms), the cups and the arena, control and chronically stressed birds were submitted to a dual spatial/cued task during five days that consisted of learning to find food (mealworm) hidden in a single cup (target cup) in an arena that contained eight opaque cups. On each trial, birds were introduced in the arena by a different starting point among three possibilities. The location of the target cup was constant among trials and days of training, allowing animals to solve the task using spatial memory. Moreover, the target cup was black, whereas other non-target cups were white. Thus, birds could also solve this task using their cue-based memory, based on the color of the cup.

The day after the fifth day of training, birds were submitted to a spatial test followed by a displacement test. During the spatial test, all cups were white, and the task could only be solved by remembering the spatial location of the target cup during the training phase. During the displacement test, performed 30 min later, the black target cup was displaced to a new position (different from the position used during training). Thus, both spatial and cue-based memories could provide a solution to the test. Quail could go back either to the spatial location of the target cup during the training phase (spatial memory) or go directly to the displaced black cup (cue-based memory) [16,17,19].

After this first test occasion, birds were trained for ten additional days in the dual spatial/cued task before being submitted to the second test occasion of spatial and displacement tests. Following the second test occasion, birds were trained for an additional day (recall training) in the dual spatial/cued task to avoid that lack of food during the second test occasion reduced bird motivation in subsequent tests. After this first recall training day, all birds were challenged by exposure to an open field test and were submitted to the third occasion of spatial and displacement tests.

On the following day, quail were submitted to a second additional training day (recall training) to keep birds motivated. The day after, birds were submitted to a new test, called the "color test", to assess whether birds found the target cup by a simple association between its color (black) and the

reward or by learning a more general rule that the target cup was of a different color compared to the remaining cups.

During all the memory tests, behaviors of quail were recorded by a camera placed above the apparatus and computerized by a tracking video system (Ethovision XT; Noldus IT, The Netherlands).

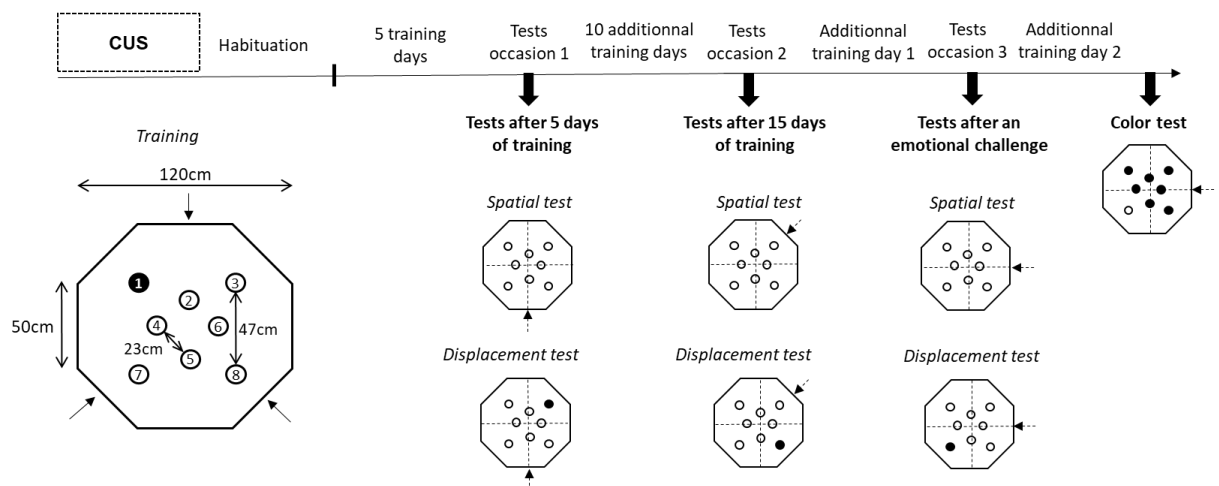


Figure 1: Schedule of the experiment and schematic representation of the apparatus used for training and testing. At three-weeks-old, quail were submitted to chronic unpredictable stress (CUS) for three weeks. At the end of the CUS procedure, stressed and control birds were trained in a dual spatial/cued learning task and submitted to spatial and displacement memory tests after 5 and 15 days of training. After an additional training day, quail were submitted to an emotional challenge (exposure to an open field) followed by a new series of tests. After the last training day, birds were submitted to the color test. Quail could enter the arena from three different starting points (dark arrows), three starting points during training, and one during testing. The dotted lines represent the division of apparatus into four different quadrants.

2.4. The dual spatial/cued task

2.4.1. Mealworms familiarization

One week before the beginning of training, a brown ceramic cup (6x7cm) was placed in each quail's home cage. For seven days, three times per day (spaced about 3h), 3 or 4 mealworms were deposited in the cup to familiarize birds with the cups and mealworms.

2.4.2. *Habituation*

After familiarization, birds were submitted to the habituation phase. Birds were food-deprived one hour before each habituation session. During five days, once a day, each quail was introduced into the center of a beige octagonal arena (120cm long; 50cm high) surrounded by a blue curtain (1.90m high) and lighted by a bulb at the ceiling (18 Lux). Eight black cues were disposed of in the arena, four on the walls and four on the curtain. Eight ceramic cups similar to those used for the familiarization were placed in the arena and contained mealworms. Four cups were wrapped with white paper, whereas black paper wrapped another four cups. In each habituation session, exploration of the arena and the cups was allowed until the bird found and ate all mealworms or after a maximum time of 600 s. Between each quail and day of habituation, the position of black and white cups was randomly moved. The number of cups visited was recorded on each day to estimate the level of exploration and habituation of the birds (Figure 1).

2.4.3. *Training*

On each day of training, birds were submitted to 2 training trials per day (1 hour between each trial). On each trial, birds were introduced in the arena by a different starting point among three possibilities (Figure 1). Only one cup was rewarded and contained three mealworms (target cup). The target cup was always black and at a fixed position on each trial, whereas the seven other cups were non-rewarded and white. When the bird reached the target cup and ate the mealworms, the trial was ended, or after a maximum time of 300 s. If the bird did not find the target cup, the experimenter gently guided it and allowed it to eat the mealworms. The bird returned to its home cage between each trial. The latency to find and eat the food in the rewarded black cup was recorded on each trial.

2.4.4. *The spatial test*

This test was used to assess the impact of chronic stress on spatial memory. Each bird was allowed to freely explore the arena and the 8 cups for 1 minute. All cups were empty and, more importantly, of similar color (white). The number of cups visited (number of errors) before reaching the target cup was scored. Moreover, the arena was virtually divided into four equal quadrants, and the time spent in each quadrant (spatial quadrant and three other quadrants) during the test was measured using the tracking video system (Figure 1).

The spatial test was conducted after 5 and 15 days of training (initial training and overtraining, henceforth) and after an emotional challenge by exposing birds to an open field (emotional challenge, henceforth).

2.4.5. *The displacement test*

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

2.4.6. *Tests after an emotional challenge*

The third occasion of both tests (spatial and displacement tests) was conducted after an emotional challenge. This emotional challenge consisted of exposing the birds to an open field test, which is known to provoke anxiety-like quail behaviors [19,38,39]. Each quail was individually placed in the center of a square arena (80 × 80 × 80 cm) of white wood with a beige linoleum floor. The arena was placed into a new experimental room, surrounded by a green curtain and strongly lighted (50 lux). Each quail was allowed to explore the arena for 5 min before being withdrawn from the arena. Thirty minutes after the open field test, quail were submitted to a spatial test followed by a displacement test.

2.4.7. *The color test*

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

2.5. Statistical analysis

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

For spatial, displacement, and color tests, the number of errors (or visits) before reaching the target cup(s) and the time spent in the different quadrant of the arena were analyzed by ANOVA with groups (control and stressed) as between-subject factors and test occasions (test after initial training, test after overtraining and test after the emotional challenge) as within-subject factors. Additional within-subject factors (e.g., quadrants or cups considered) were also included, dependent on the variables under consideration. Exploratory analyses showed that when the time spent on quadrants was averaged (the three “other quadrants” during the spatial test and the two “other quadrants” during the displacement and color tests), the results were similar than when quadrants were analyzed separately. To facilitate data comprehension and visualization, we therefore kept and included only the averaged time in other quadrants in our analyses. When main effects or interactions were significant, analyses were followed by multiple comparisons corrected by Tukey HSD.

When an individual did not find a target cup and visited less than 4 cups, it was attributed a number of 8 errors and an equivalent time of 15 sec in each quadrant. All statistical analyses were performed using IBM SPSS 21 and R version 3.6.1. Statistical significance was set at $p < 0.05$.

3. RESULTS

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

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.

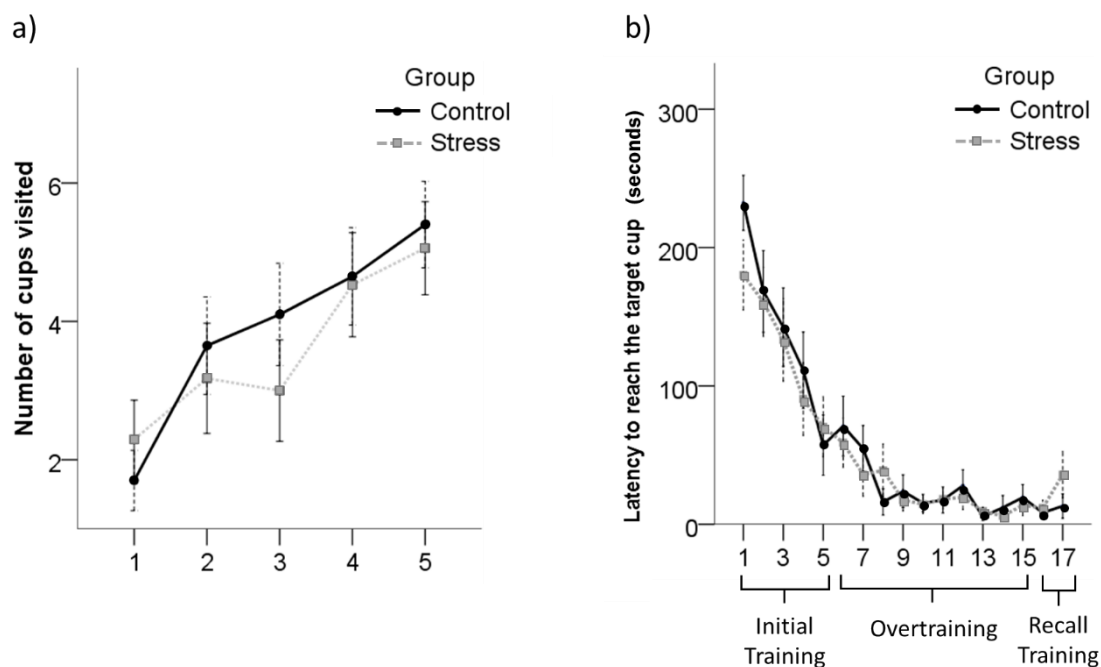


Figure 2: Effects of chronic stress on habituation and training performances in the dual spatial/cued task. a) Number of visited cups over days of habituation in control and chronically stressed quail. During habituation, all eight cups were rewarded with mealworms. b) Latency to reach the location of the target cup over days of training in control and chronically stressed quail. Mean \pm SEM are given. ($n_{\text{control quail}} = 17$, $n_{\text{chronically stressed quail}} = 20$)

3.2. Effect of chronic stress during the spatial test

The number of errors made before reaching the spatial cup was significantly increased in chronically stressed quail (effect of stress: $F_{1,35}=5.62$, $p=0.023$, Figure 3a), independently of the test occasion and the interaction with stress (effect of test occasion: $F_{2,70}=1.45$, $p=0.24$; interaction test occasion x stress: $F_{2,70}=2.72$, $p=0.072$).

The time spent in the spatial quadrant compared to other quadrants was affected by the quadrant considered, stress, and the interaction quadrant, test occasion, and stress (effect of quadrant: $F_{1,35}=7.58$, $p=0.009$; effect of stress: $F_{1,35}=6.13$, $p=0.018$; interaction quadrant x test occasion x stress: $F_{2,70}=3.36$, $p=0.040$). Post-hoc analyses revealed that, during the tests after the initial training and the emotional challenge, control birds spent significantly more time in the spatial quadrant compared to other quadrants ($p<0.001$ and $p=0.001$, for the tests after the initial training and the emotional challenge, respectively). In contrast, chronically stressed birds spent an equivalent time in the spatial quadrant compared to other quadrants ($p=1$ and $p=1$ for the tests after the initial training and the

emotional challenge, respectively, Figure 3b). No differences were found for the test after overtraining: both control and chronically stressed quail spent an equivalent time in the spatial quadrant compared with other quadrants ($p=1$ and $p=1$, for control and chronically stressed quail, respectively, Figure 3b).

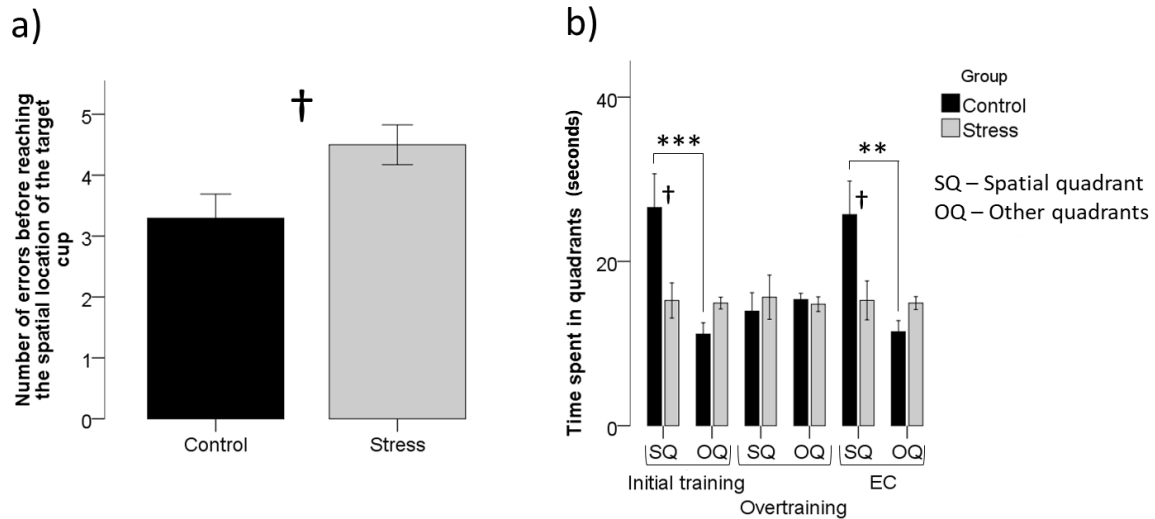


Figure 3. Effects of chronic stress in spatial memory tests performed after initial training (5 days), overtraining (15 days), and after an emotional challenge (EC, exposure to an open field). a) Number of errors before reaching the spatial location of the target cup, and b) Time (in seconds) spent in different quadrants of the arena (spatial and other quadrants). \dagger $p \leq 0.05$, significant difference between control and chronically stressed quail. $**$ $p \leq 0.01$; $***$ $p \leq 0.001$, significant difference between the spatial quadrant and other quadrants. Mean \pm SEM are given. ($n_{\text{control quail}} = 17$, $n_{\text{chronically stressed quail}} = 20$)

3.3. Effect of chronic stress during the displacement test

Analyses on the number of errors made before reaching the target cups (either the spatial cup or the displaced cup) showed that animals were more performant to reach the displaced cup, independent of their treatment group. There was also a significant interaction between the test occasion and the target 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 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 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-hoc analyses revealed animals did significantly fewer errors before reaching the displaced cup on all test occasions, compared to the errors before reaching the spatial cup ($p=0.001$, $p<0.001$ and $p<0.001$, for the tests performed after initial training, overtraining, and just after the emotional challenge, respectively, Figure 4a). Between test occasions and within-target cup,

post-hoc comparisons revealed that animals made significantly fewer errors before reaching the displaced cup during the tests after overtraining and after the emotional challenge, compared to the test after initial training ($p=0.001$ and $p=0.005$, respectively, Figure 4a).

Analyses on time spent in different quadrants showed that animals did not spend their time evenly between quadrants. They spent significantly more time than the chance level in the displaced cup quadrant independent of their treatment group. There was also a significant interaction between the test occasion and the time spent in different quadrants (effect of quadrants: $F_{2,70}=67.19$, $p<0.001$; effect of test occasion: $F_{2,70}=0.82$, $p=0.44$; effect of stress: $F_{1,35}=3.81$, $p=0.059$; interaction quadrants x stress: $F_{2,70}=1.09$, $p=0.34$; interaction quadrants x test occasion: $F_{4,140}=3.25$, $p=0.014$. interaction quadrants x test occasion x stress: $F_{4,140}=0.42$, $p=0.78$). Post-hoc analyses revealed that the time spent in the displaced cup quadrant was higher for all test occasions compared to those spent on the spatial quadrant and other quadrants ($p<0.05$, for the tests performed after initial training, overtraining, and just after the emotional challenge, respectively, Figure 4b).

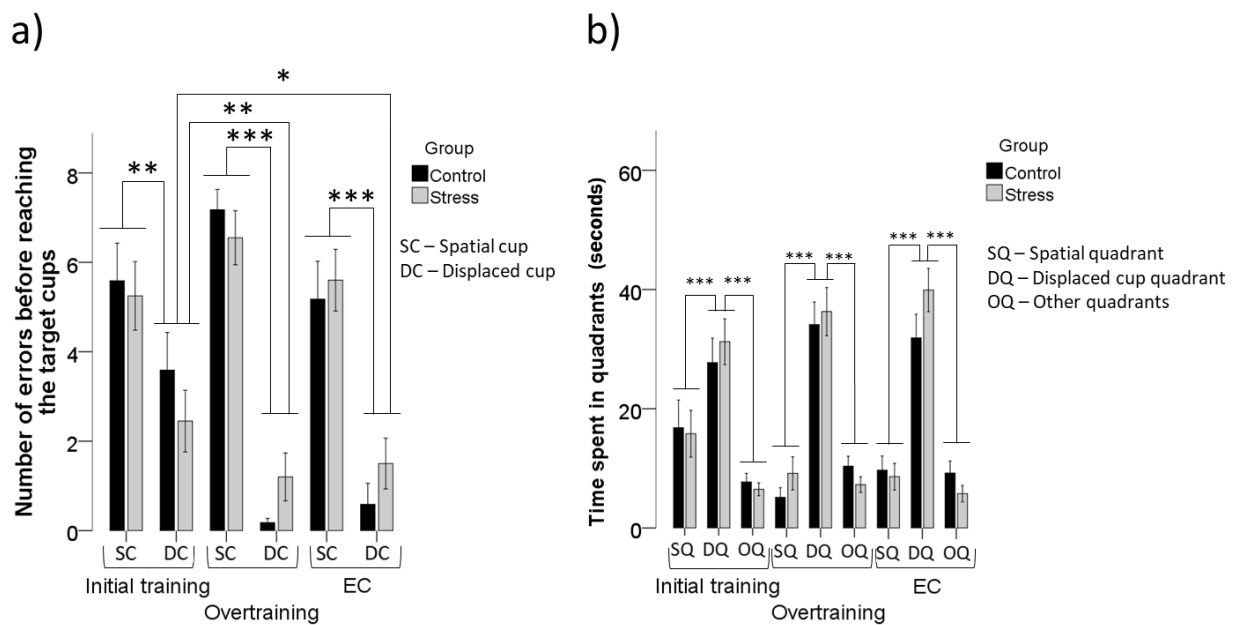


Figure 4. Effects of chronic stress in the displacement tests performed after initial training (5 days), overtraining (15 days), and after an emotional challenge (EC, exposure to an open field). a) Number of errors before reaching the target cups (spatial cup or displaced cup), and b) Time (in seconds) spent in different quadrants of the arena (either the spatial quadrant, SQ, the displaced cup quadrant, DQ, or the other quadrants, OQ). * $p\leq0.05$; ** $p\leq0.01$; *** $p\leq0.001$, significant difference between the spatial cup and displaced cup or between spatial quadrant, displaced cup quadrant, and other quadrants. Mean \pm SEM are given. ($n_{\text{control quail}} = 17$, $n_{\text{chronically stressed quail}} = 20$)

3.4. Effect of chronic stress during the color test

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).

Finally, the time spent in the different quadrants was also significantly dependent on the quadrant considered (effect of quadrants: $F_{2,70}=3.62$, $p=0.032$). Post-hoc analyses revealed a significantly larger amount of time spent in the black spatial cup quadrant compared to other black cups and white cup quadrants ($p=0.01$). No differences were found between the treatment groups, nor for the interaction quadrants and treatment groups (effect of stress: $F_{1,35}=1.02$, $p=0.31$; interaction quadrants x stress: $F_{2,70}=0.59$, $p=0.55$, Figure 5b).

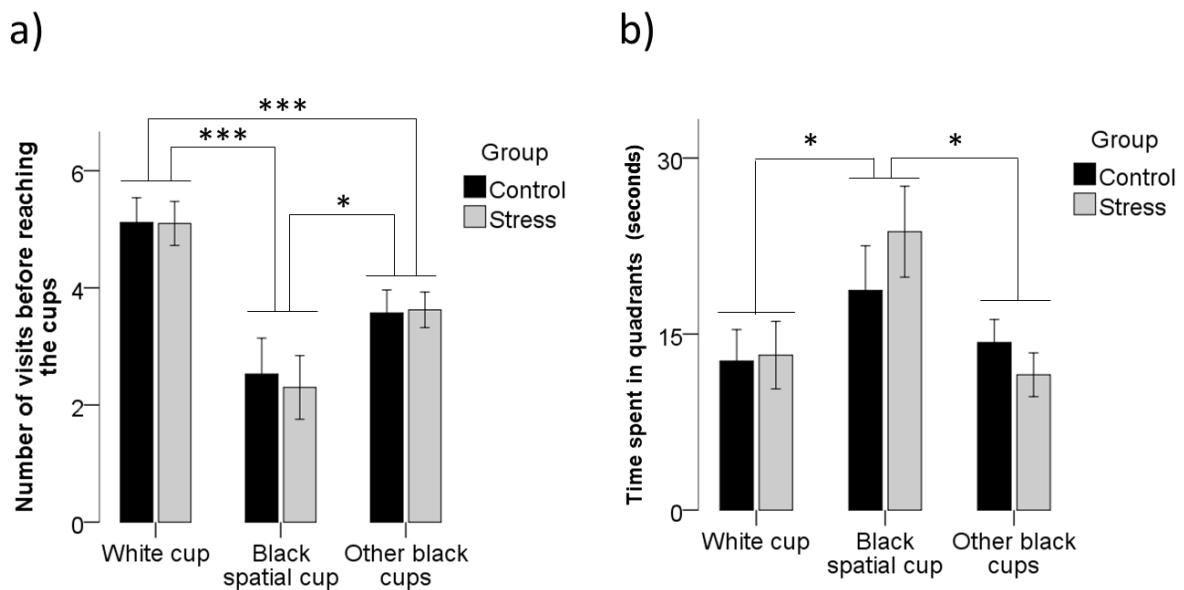


Figure 5. Effects of chronic stress on the color test. **a)** Number of visits before reaching the white cup, the black spatial cup, or other black cups in chronically stressed and control birds and **b)** Time (in seconds) spent in different quadrants of the arena containing the white cup, the spatial black cup, other black cups in chronically stressed and control birds. * $p\leq0.05$; ** $p\leq0.01$; *** $p\leq0.001$, significant difference between the white cup, black spatial cup, and other black cups. Mean \pm SEM are given. ($n_{\text{control quail}} = 17$, $n_{\text{chronically stressed quail}} = 20$)

4. DISCUSSION

Since the majority of the studies investigating the relationship between chronic stress and training level comes from studies on rodents, until now, we have had little understanding on how these interactions apply to other taxa, such as birds. To fill this gap, using Japanese quail as the animal model, the main objective of the present study was to address whether chronic stress can differentially impact two forms of long-term memory, the declarative spatial and the non-declarative cue-based memories, according to the level of training. To this end, control and chronically stressed Japanese quail were trained in a task that could be solved using spatial and cue-based memory, the dual spatial/cued task, and tested for their performances on each type of memory after 5 and 15 days of training (initial training and overtraining, respectively). Our results showed that the spatial memory of quail was sensitive to the deleterious effect of chronic stress. Performances of chronically stressed birds during the spatial test after initial training were impaired compared to that of control birds. During the test after overtraining, however, these differences were lowered. Interestingly, during the test after an emotional challenge, when birds were exposed to an open field, the negative impact of chronic stress on spatial memory performance seemed to be fully reinstated. These findings indicate that the relationships between chronic stress, training level, and spatial memory are complex and dynamic. By contrast, cue-based memory was not affected in chronically stressed birds and control birds on any test occasion, which indicates that this form of memory is relatively resistant to the negative effect of chronic stress. Finally, a test conducted to better qualify birds' cue-based memory performance, the color test, provided support that chronically stressed birds were still able to efficiently remember the spatial location of the target cup when this location was signaled by a color similar to that used during training. This suggests that chronically stressed birds cannot efficiently recall stored spatial information in a context different from the one experienced during training, which highlights that chronic stress reduces the ability to use spatial memory flexibly.

Our results from habituation showed that the number of mealworms eaten increased over days without any significant effect of chronic stress. Moreover, the latency to reach the target cup decreased over days of training in both groups. These findings indicate that both control and chronically stressed birds similarly habituated to the arena and the cups and similarly learned the dual spatial/cued task. They also indicate differences between groups observed during the different tests performed could not be attributed to evident motor or motivational differences and instead reflect stress-related changes in memory performance.

During the spatial test, the number of errors before reaching the spatial location of the target cup was higher in all test occasions (initial training, overtraining, after an emotional challenge) for chronically stressed birds compared to control birds, evidencing the negative impact of chronic stress

on spatial memory performance. Although not significant ($p=0.07$), the interaction between test occasion and stress suggests that the performance of both groups during overtraining tests was similar, compared to initial training and after emotional challenge tests, when their performances differed more. Confirming this tendency, the time spent in different quadrants revealed some specificities of the animals' performance over the different test occasions. During spatial tests performed after initial training, chronically stressed birds spent their time evenly between the quadrants, while control birds spent significantly more time in the spatial quadrant. The same did not occur after overtraining. Indeed, performances of both control and chronically stressed birds were relatively low during the spatial test performed after overtraining. Contrasting the spatial tests, chronic stress did not affect cue-based memory performance in all displacement tests performed. On all test occasions, chronically stressed birds and control had the same performance and followed the displaced black cup preferentially. These findings show that, similar to what is already known in rodents, chronic stress in birds differentially impacts memory depending on the memory system, with the spatial memory being much more sensitive to the adverse effects of chronic stress than cue-based memory [1,6,40]. They also confirm that more complicated tasks are more impacted by chronic stress than simple tasks [3], corroborating our previous work on chickens and quail where we found different level of cognitive performance between animals from different groups (hippocampal lesioned vs non-lesioned, stressed vs non-stressed, fearful vs non-fearful, exploratory vs non-exploratory) only during complex tasks, which are more cognitively demanding, rather than during simple tasks, where animals may use stimulus-response strategies [13,16,17,19,41,42].

The low spatial memory performance after overtraining for control individuals 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 [20,21,23,24]. 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 highlight the importance of taking training level into account when testing memory in birds, since, similar to mammals a progressive shift from declarative, spatial memory use to a non-declarative, cue-based memory use may occur.

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, in other words, control quail may have become stressed with overtraining which caused a worsening in their performance. In previous work we confirmed that stressed quail have poor spatial memory, but improved cue-based memory [19], which gives support to this alternative hypothesis. If indeed stress from overtraining is behind the worsening in spatial memory for control individuals, it is noteworthy to state that the different stressors (overtraining vs chronic stress) were probably perceived differently by the birds, since the differences between control and chronically stressed quail on spatial memory performance were

restored after an emotional challenge. Once more, our results give us further understanding of the relationship between chronic stress and memory in birds and reveal it is not that different from that of mammals: previous studies in rodents highlighted that acute stress or stress hormone injections experienced just before testing can induce the reemergence of an extinguished memory [31,32]. Another possibility for the suddenly use of spatial information in control birds is that, exposure to an open field is also an exposure to a novel spatial environment, that induces hippocampus dependent mechanisms. Such process may contribute to reactivate hippocampus dependent spatial memory in control birds. More studies need to be done to disentangle these two hypotheses. In any case, the worsening in performance of control birds after an extending period of training seems to not be due to an elimination of the spatial memory, which can re-emerge depending on the more immediate psychological state of the quail. Moreover, the reappearance, even after overtraining, of chronic stress adverse effects on the spatial memory of 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 make it impossible for stressed animals to have this form of memory. Indeed, in the last test, the color 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 signaled by the cue (black color). This capacity is crucial to perform the spatial test efficiently. Numerous studies conducted in chicks and quail evidenced that spatial memory critically requires the hippocampus [13,19,43]. 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) [44,45]. During spatial tests, the spatial location was no more signaled by the cue (all cups were white). Birds should re-establish the complete spatial-cued information learned during training (one rewarded black cup among seven non-rewarded white cups) from this incomplete information. Under this framework, chronic stress, which negatively affects the hippocampus's functioning in quail [19], may impair hippocampus-dependent processing of pattern completion.

In conclusion, the present study presents evidence that chronic stress can negatively impact the birds' declarative spatial memory. It highlights that chronic stress specifically alters the ability to use spatial memory flexibly. Also, we show for the first time in birds that an extended period of training can lower the spatial memory differences between control and chronically stressed individuals, either by a progressive shift to cue-based memory or by the fact that overtraining can be in itself perceived as stressful by control animals, hindering their performance. These hypotheses should be tested further. Before testing, an emotional challenge was capable of reinstating the differences in performance

between chronically stressed and control quail, showing the spatial memory system's long-lasting sensibility. To our knowledge, this is the first work that highlights the dynamic dialogue between stress, training level, and memory systems in birds, increasing the scientific understanding of these interactions and their applicability to other taxa than mammals.

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