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**The Helico Maze allows testing of early learning and subcategories of long-term memory in mice.**

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**Highlights:**

- A new apparatus for testing learning and memory in mice has been developed
- Olfactory cues are associated with positive or negative rewards
- Procedural long-term memory can be easily evaluated
- Reference long-term memory can be easily evaluated
- Early deficits in reference memory can be observed using this new apparatus

Key words: mouse; learning; memory; procedural memory; reference memory; declarative-like memory; hippocampus

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**Abstract**

Different memory systems operate in parallel to support behaviour. To evaluate procedural and reference subcategories of long-term memory as early as possible in the mouse, the Helico Maze (HM) was developed. BALB/c AnNCr1 (BALB), C57BL/6JRj (C57) and DBA/2JRj (DBA) mice were trained on this new maze. The three strains learned how to use the HM (procedural memory), and they then learned and remembered four odour-reward associations (reference memory). The three strains differed in the number of correct responses. BALB mice showed better performance than C57 and DBA mice. The results of the first block of each session revealed that only the BALB and C57 mice remembered the odour-reward associations. DBA mice needed to relearn the associations each day. With this new apparatus, the number of olfactory cue-reward associations was increased from 2 to 4 in comparison to a previous olfactory tubing maze. Consequently, a supplementary effort of memory was required, and the chance level was decreased from 50% to 25%. Thus, in several important respects, the HM can be considered to measure the hippocampus-dependent behaviour of the mouse, allowing to study, as early as possible in young mice, the different subcategories of long-term memory, such as those observed in humans.

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## 60 **1. Introduction**

61 In humans, cognitive impairments usually result from brain injuries that are due to trauma [1] or  
62 neurodegenerative diseases [2]. Among these cognitive troubles, memory impairment stands out.  
63 Long-term memory is not a monolithic system but is composed of multiple systems [3]. Indeed, since  
64 the famous amnesic patient (Henry Molaison), it has become obvious that only one kind of memory  
65 (declarative memory) can be impaired despite the presence of profound forgetfulness [4]. In this  
66 patient and some other similar patients, memory impairment resulted from bilateral temporal lobe  
67 resection or lesions [5].

68 In fact, different long-term memory systems operate in parallel to support behaviour. An animal model  
69 of human memory impairment was first developed in monkeys and allowed researchers to identify the  
70 anatomical components of memory that support declarative memory [6]. The connectivity between the  
71 hippocampus and the adjacent entorhinal, perirhinal, and parahippocampal cortices constitutes the  
72 memory system that supports declarative or reference (declarative-like) long-term memory and was  
73 characterized in monkeys [7] as well as in rats [5, 6, 8]. Then, with the development of transgenic and  
74 gene-targeting techniques, new knowledge about learning and memory processes was obtained from  
75 studies on genetically modified mice. However, the question arose as to how much mouse models can  
76 reproduce human memory impairment [9]. Indeed, for humans, a key distinction in LTM is between  
77 the capacity for conscious recollection of facts and events (declarative memory) and a heterogeneous  
78 collection of nonconscious learning capacities including procedural memory (nondeclarative memory)  
79 that are expressed through performance and that do not afford access to any conscious memory content  
80 [5]. For mice, procedural memory is the learning of how to operate the task, and reference memory is  
81 the learning of the associations between the cues and the reward/punishment [10]. In humans and

82 mice, dysfunction of the hippocampus impairs declarative or reference LTM while procedural learning  
83 is spared.

84 The response came with the development of the olfactory tubing maze (OTM) for mice, where  
85 selective subcategories of long-term memory could be selectively identified, such as in humans [10].  
86 Indeed, the use of rewarded associations with olfactory cues in macrosmatic species allows one to  
87 access these higher cognitive processes [11]. The OTM was used for fundamental research  
88 demonstrating hippocampal spine sprouting that was related to learning and memory performance and  
89 could be improved by promnesic agents or grafts with adult human stem cells to repair hippocampal  
90 injuries [12-14]. The olfactory tubing maze was also used with hippocampal lesioned mice [15] or  
91 pharmacologically treated mice [13] or in a transgenic Alzheimer mouse model to correlate beta  
92 amyloid plaque accumulation with selective declarative-like memory deficits [9]. These deficits were  
93 substantially decreased by chronic treatment with a promnesic serotonergic agonist [11]. The olfactory  
94 tubing maze was successful at evaluating potent effects, but to go further and to characterise more  
95 subtle effects, such as the time range development of senile plaques and the beginning of memory  
96 deficits in Alzheimer transgenic mice, we decided to develop a new maze, the Helico Maze (HM),  
97 based on the same mouse olfactory ability. With the development of this new apparatus, the number of  
98 olfactory cue-reward associations was increased from 2 to 4 in comparison to the OTM. Consequently,  
99 an effort to learn and to remember two more associations is required. Indeed, with the use of the  
100 olfactory tubing maze at the start of the training, the chance level of success was already 50%, which  
101 usually allowed one improvement of 50%. To be successful at the HM, the starting chance level of the  
102 odour-reward associations was 25%, which allowed a percentage increase of 75%. Nevertheless, with  
103 this increase in learning and memory demands, we questioned whether adult mice could obtain  
104 significant performance differences from the chance level in procedural and reference subcategories of  
105 long-term memory using the HM. To evaluate procedural and reference subcategories of long-term  
106 memory in the HM, three different strains of mice were submitted first to procedural training and then  
107 second to reference training. The first strain choice was BALB/c AnNCrl mice (BALB) because this  
108 albino strain is an excellent learner with olfactory cues [10] but fails to acquire a place-learning

109 response in the Morris water maze, which can be explained by hypersecretion of corticosterone and  
110 marked brain catecholamine alterations following stressor exposure [16, 17]. The second strain was  
111 C57BL/6JRj (C57), which is one of the most common strains used to generate transgenic or gene-  
112 targeting mice. Finally, we trained DBA/2JRj mice (DBA) because they present differences in their  
113 hippocampal anatomy, with fewer pyramidal cells in the dorsal hippocampus [18], fewer mossy fibre  
114 terminals in the inferior region [19] and poor learning performance with spatial [20] or olfactory cues  
115 [12]. Thus, a poorer performance of this strain in the HM with regard to reference memory would  
116 underline the feasibility of the HM for testing hippocampal-dependent cognitive impairments.

## 117 **2. Materials and Methods**

### 118 2.1. Animals

119 All of the experimental procedures were conducted on male mice in accordance with national and  
120 European regulations (EU directive N° 2010/63) in agreement with the authorisation for animal  
121 experimentation attributed to the laboratory by the Prefecture des Bouches-du-Rhône (permit number:  
122 D 13 055 08), and all the recommendations of the ARRIVE guidelines [21] were complied. A group of  
123 8 BALB/c AnNCrl mice (BALB) (Société Charles River Laboratoire France CRLF), a group of 8  
124 C57BL/6JRj mice (C57) and a group of 7 DBA/2JRj mice (DBA) (Société Janvier Labs France) that  
125 were 8 weeks old (body weight 20-25 g) were used in all of the experiments. Only males were used in  
126 all the experiments to avoid suspected variability in data, especially since the HM uses olfactory cues.  
127 In total, twenty-three mice were used during the 3 experiments (one per strain). One week before the  
128 beginning of the experiments, the mice were individually housed with free access to food and water in  
129 a room at constant temperature (21°C) that was maintained on a 12 h light-dark cycle (lights on at  
130 07:00 a.m.).

### 131 2.2 Apparatus

132 The HM (Fig. 1A) is composed of two identical testing chambers (TC1 or left testing chamber and  
133 TC2 or right testing chamber) that are joined by one straight plastic tube allowing the connection at the  
134 bottom of each chamber. The straight tube of 100 cm is interrupted in the middle by an automated

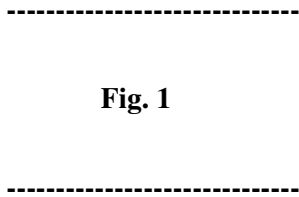
135 door (AD). Each testing chamber comprises an entrance cube that is horizontally divided in the middle  
136 by a platform. The lateral side of the entrance cube is 12 cm. The bottom external side can be raised to  
137 give access to the floor of the cube with a podium of 2 cm diameter and 1.5 cm high to facilitate  
138 access to the platform from above (Fig. 1B). This platform is perforated, with four holes 2 cm in  
139 diameter positioned in front of the entrance of the four tubes. In the centre of this platform, a round  
140 podium 3.5 cm in diameter and 1.5 cm high was added to facilitate access to the tubes. On top of each  
141 testing chamber, an inverted fan exhausts the neutral or scented air that is ejected from the outside  
142 extremities of the tubes that are fastened to the cube of the testing chamber. The four straight plastic  
143 tubes of each testing chamber compose a cross. All of the straight tubes are 5 cm in diameter and 23  
144 cm long and are made by joining half tubes together, where the top half is laid on the bottom half. The  
145 tops are independent and can be easily removed to clean the maze. At each extremity of the four tubes,  
146 one water port (WP) allowed to release the water in a well. It is located above the neutral or scented air  
147 ports (Fig. 1C). A ribbon LED of 10 cm (10 W/meter) that is laid out in a triumphal arc at 10 cm from  
148 the floor surmounts each tube. All of the plastic tubes were especially made for small rodents. The  
149 entrance cubes were homemade.

150 The movements of the mouse are detected in the cubes by photoelectric cells that are located on the  
151 lateral side at 3 cm high and 6 cm laterally (see Fig. 1A, but not shown in B). Additional photoelectric  
152 cells were placed 5 cm before each extremity of the four tubes.

153 The apparatus is set on a table 110 cm long, 60 cm wide and 150 cm high. The four equally angled  
154 tubes that make up the helix were set on the table while the two cubes of the TC and the connecting  
155 straight tube that is interrupted by the AD were below the table top. On a shelf on the table positioned  
156 30 cm above the room floor, 1000-ml Erlenmeyer flasks were set to eject four different odours and  
157 neutral air to the four tubes. The Helico Maze was set in an independent room in total darkness. Four  
158 infrared cameras around the Helico Maze allowed us to follow mouse behaviour on screens in another  
159 room. The electronic device and computer allowing automatic management and recording of the  
160 results were also in this room.

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**Fig. 1**

2.3 Procedure:

2.3.1 Procedural training

Before the training procedure, starting at 08:00 a.m. on the first day, all of the mice were weighed, as every day, to ensure that the weight lost from water deprivation was less than 15%, and they were handled for 5 min (days 1 and 2). On day 3, they were introduced in only the left chamber (TC1) of the HM, where they could find 0.1 ml of water at each extremity of the four tubes (Fig. 2). Access to the second chamber was prevented by keeping the automated door closed. On day 4, the mice were weighed again, as every experimental day, and they were then handled 5 min before being introduced into the right testing chamber (TC2) without access to the left testing chamber (TC1). Again, the mice could find the same amount of water at the extremity of the 4 tubes. On day 5, the same procedure was followed, but this time, the mice were allowed to move freely in all parts of the HM with all the water ports (WPs) loaded with 0.1 ml of water. Usually, after these 15 min of shaping sessions, the mice drank some of the water that was available in the cups at every extremity of the maze. On day 6, at 12:00 a.m., the mice were water deprived until the first training session on day 8.

The mice were introduced to the HM by opening the door in one of the entrance cubes in the testing chamber (TC1) without access to the second one (automated door closed). Once the mice were detected by one photoelectric cell in the entrance box, different odours were immediately ejected from the extremities of the four tubes instead of neutral air. One odour (positive odour) was associated with a positive reward (0.3 ml of water), while the other three (negative odours) were associated with a negative reward (lighting of the ribbon LEDs). Then, the mice climbed on the platform of the cube and randomly chose for this first trial a connected tube and ran to its extremity to be detected by the photoelectric cell that was positioned at the extremity of the tube. If the chosen tube corresponded to the one where the positive odour was ejected, water (positive reward) was released, and the mouse

188 drank the drop of water. This ended the trial, and neutral air was ejected in the tubes. If the mouse  
189 went to the end of the three other tubes, the negative reward (light on) was released. The light was  
190 turned on until the mouse went to the right tube to be positively rewarded, which stopped the trial.  
191 Thus, in this training procedure, a correction procedure was applied since three lights could be turned  
192 on until the mouse reached the end of the tube, where the ejected odour was associated with the  
193 positive reward. After an inter-trial period of 15 seconds, the automated door of the connecting tube  
194 opened, allowing the mouse to enter the other testing chamber (TC2). Once the mouse was detected by  
195 the photo-cell in the entrance cube of this testing chamber, the automated door closed, and the  
196 different odours were differently distributed in the four connected tubes. At this time, the mouse  
197 climbed on the platform and chose a tube for the first trial. Then, if the mouse again ran to the right  
198 tube, the positive reward was released, and the trial stopped. Responses to the three other tubes turned  
199 the light on until the correct response was obtained in the right tube associated with the positive odour  
200 as described above. Then, the automated door opened after 15 seconds, as described above, for the  
201 following trials.

202 In this procedural training, each mouse performed 8 trials until the session finished for the testing day,  
203 and this procedure was performed on 5 successive days. At the end of each of the four sessions, the  
204 mice were returned to the vivarium with free access to water for 2 minutes. At the end of the last  
205 session (session 5-day 12), deprivation was stopped.

206 On day 13, all mice were again water-deprived at 12:00 a.m. before they were submitted to the  
207 reference training on day 15.

208 Three parameters were examined in the procedural training: (A) the duration in minutes to perform the  
209 8 trials with a maximum session time of 60 minutes, (B) the number of incorrect responses with a  
210 maximum of twenty-four errors by session, and (C) the number of correct responses with 2 out of 8  
211 correct responses reflecting the chance level.

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213 2.3.2 Reference training:

214 The reference training was similar to the procedural training, but this time, a daily session was  
215 composed of 16 trials, and a non-correction method was applied during 5 successive sessions. Once a  
216 correct (drop of water) or incorrect response (the light on for 2 sec. only in the tube where the  
217 incorrect responses were given) was obtained, the odour delivery stopped and replaced by neutral air.  
218 After the 15 sec. minimum inter-trial delay, the mouse had to go on the other testing chamber to  
219 initiate a new trial as described above.

220 In the reference training and using the non-correction method, only the duration (A) and the number of  
221 correct responses (B) expressed as percentages with a chance level of 25% (with 2 out of 8 correct  
222 responses) if all the choices for every trial were made randomly were analysed.

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**Fig. 2**

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#### 228 2.4 Technical details

229 The concentrations of the four odours that were used in these experiments were adjusted in the  
230 preliminary experiments and were ejected in the tubes at a rate of 5 l/min by forcing clean air (0.7 bar)  
231 through dedicated 1000-ml Erlenmeyer flasks that contained only 200 ml of water (neutral air) or 200  
232 ml of mixed water. Randomly, the positive odour that was chosen was strawberry (0.5 ml artificial  
233 extract/200 ml water), and the negative odours were coconut (0.75 ml/200 ml water artificial extract),  
234 jasmine (0.25 ml/200 ml water artificial extract) and violet (0.25 ml/200 ml water artificial extract).  
235 Preliminary tests demonstrated that at these respective concentrations, at the start of the experiments,  
236 all 4 odours had an equal probability of being chosen. The extracts of perfume were purchased from  
237 Céven'Arômes company, France.

238 The solenoid and needle valves, the ribbon LEDs and the automated door were controlled by a  
239 microcomputer (Hewlett Packard, France) from a program developed in our laboratory using  
240 LabVIEW software (National Instruments France). The programmed trials respected a random  
241 distribution of the odours in each of the tubes.

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## 245 2.5 Statistical analysis

246 All of the data were analysed using multivariate analyses of variance (MANOVA) with repeated  
247 measures. Selective analyses of variance (ANOVAs) were then performed followed by Tukey post hoc  
248 comparisons using SPSS/PC Inc., Chicago, IL. In addition, Student's t test allowed the testing of the  
249 eventual difference from the chance level considering the incorrect, correct or percentage of correct  
250 responses. To examine the improved performance in learning and memory from session to session in  
251 reference training, linear regression was attempted from the first block of four trials session1 to the last  
252 block (block 20) on session 5. Another linear regression was performed considering only the first  
253 block of four trials of each session of the reference training.

## 254 3. Results

### 255 3.1 Procedural training

#### 256 3.1.1 *Duration:*

257 From session to session, the time taken to perform the 8 trials decreased for all groups (Fig. 3A) and  
258 reached a similar duration by session 5. Nevertheless, a significant difference over sessions was  
259 observed between the three groups [MANOVA: group x session interaction,  $F(8,80)=4.3$ ,  $p < 0.05$ ]  
260 and between these groups [ $F(2,20)=6.51$ ,  $p < 0.01$ ]. This difference came from the BALB mice, which  
261 started the training at a lower level during all of the sessions. Consequently, a Tukey post hoc  
262 comparison revealed a significant difference between the BALB group and the C57 and DBA groups

263 ( $p < 0.05$ ). Selective ANOVA comparisons on daily sessions revealed a difference except during the  
264 third session [ $F(2,20) \geq 3.95$ ,  $p \leq 0.05$ ], while the post hoc analyses indicated that on sessions four and  
265 five, a difference was only observed between the BALB and C57 groups.

### 266 3.1.2. Incorrect Responses:

267 The number of incorrect responses (Fig. 3B) was not different between the three groups across the five  
268 successive sessions [MANOVA:  $F(8,80) = 0.7$ , NS] without differences between them [ $F(2,20) = 0.88$ ,  
269 NS]. From session to session, only the BALB group significantly decreased its number of incorrect  
270 responses [ANOVAs:  $F(4,35) = 3.13$ ,  $p < 0.05$ ].

271 Compared to the chance level, the BALB mice were different during sessions 3 and 5 [Student's t test:  
272  $t(7) \geq 3.03$ ,  $p \leq 0.05$ ], while the DBA mice were different in sessions 3, 4 and 5 [ $t(6) \geq 3.42$ ,  $p \leq 0.05$ ],  
273 and the C57 mice were different in session 5 [ $t(7) \geq 3.58$ ,  $p < 0.01$ ].

274

### 275 3.1.3. Correct Responses:

276 For the correct responses, a slight increase was observed across sessions (Fig. 3C), mainly between the  
277 first and fifth sessions, but it did not reach the significance level [MANOVA:  $F(8,80) = 0.48$ , NS], and  
278 no significant difference between the three groups was observed [ $F(2,20) = 0.35$ , NS].

279 A significant difference from the chance level was reached only by the BALB mice in sessions 3 and 5  
280 [ $t(7) \geq 3.05$ ,  $p \leq 0.05$ ] and the C57 mice in session 5 [ $t(7) > 2.72$ ,  $p < 0.05$ ].

281 -----

282 **Fig. 3**

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## 285 3.2. Reference training

### 286 3.2.1. Duration:

287 No significant difference in performing the 16 daily trials was observed throughout the five training  
288 sessions (Fig. 4A) between the three groups [MANOVA: group x session interaction  $F(8,80)=1.01$ ,  
289 NS]; however, a significant mean difference was observed between the groups [ $F(2,20)=16.25$ ,  $p<$   
290  $0.001$ ]. The BALB mice took 8 to 10 minutes to process the 16 trials, the C57 required 15 to 17  
291 minutes and the DBA mice needed 12 to 14 minutes. Tukey post hoc comparisons revealed a  
292 significant difference between the BALB, D, and C57 mouse groups ( $p<0.05$ ). Selective session  
293 ANOVAs confirmed the observations with a significant difference between each day [ $F(2,20)\geq 6.24$ ,  
294  $p\leq 0.01$ ], except on session 1 [ $F(2,20)=2.16$ , NS]. The post hoc analyses showed a significant  
295 difference between the BALB and DBA groups only in session 4 ( $p<0.05$ ), between the BALB and  
296 C57 groups on all days ( $p\leq 0.01$ ) and between the DBA and C57 groups only in session 5 ( $p<0.01$ ).

297

### 298 *3.2.2. Percentage of Correct Responses:*

299 All of the groups improved their percentage of correct responses [MANOVA:  $F(2, 20) = 34.14$   
300  $p<0.001$ ] from session 1 to session 5 (Fig. 4B). Selective ANOVAs revealed that it was only during  
301 the fifth session that a significant difference appeared between the three groups [ $F(2,20)=4.38$ ,  
302  $p<0.05$ ]. The post hoc analysis discriminated the performance between the BALB and DBA groups  
303 ( $p<0.05$ ). From the chance level, the percentages of the three groups of mice were different during all  
304 of the sessions [Student's t test:  $t(7)$  or  $t(6)\geq 2.39$ ,  $p\leq 0.05$ ]. To examine this improving performance  
305 session by session, blocks of 4 trials were analysed (Fig. 5). A highly significant correlation was found  
306 from the first block on session 1 to the last block (block 20) on session 5 in the increase of the  
307 percentage of correct responses for the BALB ( $r=0.65$ ;  $p<0.001$ ) as well as for the DBA ( $r=0.30$ ;  
308  $p<0.05$ ) and C57 groups of mice ( $r=0.27$ ;  $p<0.05$ ). Nevertheless, considering only the first blocks of  
309 each session (Fig. 6), a significant correlation was only observed for the C57 group ( $r= 0.92$ ;  $p<0.01$ )  
310 and not for the BALB or DBA groups ( $r=0.08$  and  $r=0.24$ ; NS). Indeed, the C57 group improved its  
311 performance at the start from session to session, while the BALB group was already at a high  
312 percentage level at the start of each session. Conversely, the DBA group performed at almost the same  
313 level (close to the chance level) during the first 4 trials of the 5 sessions. To confirm these

314 observations, we compared these data to chance. The performances of the DBA mice were never  
315 different from the chance level [Student's t test:  $t(6) \leq 1.54$ ; NS], while except for the fourth block for  
316 the BALB mice, and the first, second and fourth blocks for the C57 mice, all of the percentages of  
317 correct responses were significantly different from the chance level [respectively:  $t(7) \geq 3.42$ ,  $p \leq 0.05$   
318 and  $t(7) \geq 2.64$ ,  $p \leq 0.05$ ].

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**Fig. 4**

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**Fig. 5**

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**Fig. 6**

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#### 331 **4. Discussion**

332 As the results demonstrated, the three strains mastered the procedure in the use of the Helico Maze  
333 after the five training sessions. Indeed, in session five, all of the mice performed the 8 trials in  $10 \pm 2$   
334 minutes. The albino BALB/c mice probably paid less attention to the intra- and extravisual cues and  
335 were the fastest in session one, needing twenty minutes to achieve the 8 trials. From the second  
336 session, only  $10 \pm 2$  minutes were necessary for each session. For the C57 and DBA strains, 35 minutes

337 were required for session one, and then a progressive decrease in session time was observed, reaching  
338 the same level as the BALB mice in session 5. The incorrect responses during this corrected method  
339 decreased slightly from session to session in a similar way for the three strains. Starting at  
340 approximately 12 incorrect responses in session 1, the observed decrease was close to only 8 errors in  
341 session 5. As expected, the three strains gave only 2 correct responses among the 8 trials, which  
342 corresponded with the chance level. A slight increase in the correct responses was mainly observed at  
343 session 5. Of note, only the BALB mice in sessions 3 and 5 and the C57 mice in session 5 obtained a  
344 number of correct responses that were significantly different from the chance level. Surprisingly, once  
345 they mastered the use of the HM, all of the mice, when submitted to the reference training with 16  
346 trials and a non-corrected method, required a different amount of time to perform session one of the  
347 reference training. The session duration was 10 minutes for the BALB mice, while the C57 and DBA  
348 mice started with a significantly higher duration. Then, a split was observed between the BALB and  
349 DBA mice starting at session two, and it decreased the amount of time to achieve the session, while  
350 the C57 mice inversely increased this duration. This dichotomy between the BALB and C57 mice  
351 increased until the last session, while the duration for the DBA mice was intermediate between the two  
352 other strains. We hypothesize that while the BALB mice paid attention only to the incoming olfactory  
353 cues, the C57 mice could not ignore the visual environment and tried to obtain information clues to  
354 obtain the positive reward, which added some time on to each trial. Nevertheless, a difference in  
355 performance between these three strains coming from differences in their locomotor activity cannot be  
356 excluded.

357 The main difference comes from the number of correct responses devoted to each strain. Starting at  
358 chance, the BALB mice showed significantly more correct responses than the C57 and DBA mice.  
359 Nevertheless, when analysing the first bloc of each session, it appears that only the BALB and C57  
360 mice remembered from session to session the odour–reward associations, while the DBA mice learned  
361 throughout the sessions but forgot the right associations and consequently restarted every day from the  
362 chance level.

363 Taken as a whole, our results demonstrate that the three strains at the age of three months were able to  
364 learn the long-term memory procedure requested to obtain their reward in the HM, but only the BALB  
365 and C57 mice consolidated the right associations in their long-term reference memory, while the DBA  
366 mice started every session from chance. This is not surprising, considering that DBA/2J mice are  
367 known for a deficiency in synaptic plasticity in the hippocampus. Consequently, behavioural  
368 hippocampal-dependent learning and memory impairments most likely result from this physiological  
369 deficit [22-24].

370 The use of the HM allows us to observe performance on learning and on different sub-categories of  
371 long-term memory at the age of three months, which can be impaired by hippocampal dysfunction.  
372 This is of primary interest in the development of neurodegenerative disease in a transgenic mouse  
373 model. Indeed, in humans, it is clear that most clinical trials of potential disease-modifying therapeutic  
374 agents have been tested too late in the pathophysiological course of Alzheimer's disease (AD) [25].  
375 Thus, the next strategies to achieve success should focus on earlier interventions correlated with the  
376 early diagnosis of AD. The same problem arises using mouse models for AD. In a recent review [26],  
377 the use of several different mouse models for AD in preclinical studies reported cognitive deficits  
378 often after at least 7 months and rarely from 4 months of age, but never before. However, in these  
379 models, neurodegenerative markers of the disease were present several months before learning and  
380 memory impairments were observed. In this view, HM could significantly improve the adequacy  
381 between the expression of biomarkers and cognitive deficits.

382 The HM was used only with male mice to exclude data variability, which was due to sexual olfactory  
383 cues, but could also be used with female mice. Now, several other training parameters are tested to  
384 decrease the deprivation period and duration of the test. To this end, handling and habituation duration  
385 to run inside the apparatus will be significantly increased every day. The limitations of the use of the  
386 HM, because of the structure and training procedure, are that the experimenter has no access to the  
387 mouse (e.g., with cannulae, laser probes, and miniscopes) and that water deprivation may introduce a  
388 confounding factor to assess the effects of novel drugs on memory impairment.

389

390 In conclusion, the use of the Helico Maze allowed clear early discrimination between the different  
391 sub-categories of long-term memory, such as those in humans. Indeed, as in humans and in DBA  
392 mice, dysfunction of the hippocampus impairs reference or declarative LTM while procedural learning  
393 is spared.

394 The use of olfactory cues in the Helico Maze is more ethologically relevant for mice, providing better  
395 access to the underlying cognitive processes, and its automation in the absence of human intervention  
396 reduces the stress component that affects many behavioural mouse assays. These two major  
397 advantages allow an increase in mnesic requests in comparison to several other behavioural tasks and  
398 may enable earlier detection of the progressive decline in cognitive function in Alzheimer's mouse  
399 models, for example. Indeed, preliminary results with 5XFAD mice indicated a deficit starting at 3  
400 months of age. This is of interest from the perspective of testing therapeutic drugs at a time when  
401 neuropathological hallmarks, including extracellular deposits of amyloid  $\beta$  ( $A\beta$ ) peptides (amyloid  
402 plaques) and intracellular aggregates of hyper-phosphorylated Tau protein (neurofibrillary tangles)  
403 [27, 28], appear.

404

#### 405 Conflicts of Interest

406 The authors declare no conflicts of interest regarding the publication of this paper.

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483 **Figure legends**

484 **Figure 1:** The Helico Maze (HM).

485 A: Overview of the HM, which consists of two testing chambers (TC1 and TC2). Odours O1,  
486 O2, O3, and O4 are the injected odours instead of the air during a trial, knowing that only one odour is  
487 specified for each tube and is different for each trial.

488 B: A detail of one testing chamber. As indicated by the curved arrow, the front door can be  
489 raised to introduce the mouse to or remove it from the HM.

490 C: Details of one tube extremity, which contains air, odours and water ports (WPs) that are  
491 surmounted by one ribbon LED.

492 **Figure 2:** Timeline depicting the protocol (i.e., days of handling, water deprivation ON/OFF,  
493 procedural, and reference training).

494 **Figure 3:** Learning and memory performance in procedural learning using the HM apparatus with 8  
495 trials by session.

496 A: Mean performance ( $\pm$ SEM) of the session duration in minutes. \*  $p < 0.05$ , between BALB/c and  
497 C57BL, DBA/2JRj.

498 B: Mean performance ( $\pm$ SEM) of the number of incorrect responses. \*  $p < 0.05$ , difference from the  
499 chance level

500 C: Mean performance ( $\pm$ SEM) of correct responses. \*  $p < 0.05$ , difference from the chance level

501 Dark lines indicate the chance level.

502

503

504 **Figure 4:** Learning and memory performance in reference learning using the HM apparatus with 16  
505 trials by session.

506 A: Mean performance ( $\pm$ SEM) of the session duration in minutes. \*\*  $p < 0.01$ , between BALB/c and  
507 C57BL.  $\delta$   $p < 0.05$ , between BALB/c and DBA/2JRj.  $\alpha$   $p < 0.01$ , between C57BL and DBA/2JRj.

508

509 B: Mean performance ( $\pm$ SEM) of correct responses in percentage by session.\*  $p < 0.05$ , all the  
510 groups in all sessions were different from the chance level.

511 Dark line indicates the chance level.

512 **Figure 5:** Percentage of correct responses (Mean  $\pm$  SEM) in reference learning by blocks of 4 trials.

513 **Figure 6:** Mean performance ( $\pm$ SEM) of correct responses in percentage in reference to learning  
514 obtained with the first block of 4 trials at the beginning of each daily session. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ;  
515 \*\*\* $p < 0.001$  BALB/c difference from the chance level.  $\alpha$   $p < 0.05$  and  $\alpha\alpha\alpha$   $p < 0.001$ , C57BL difference  
516 from the chance level.

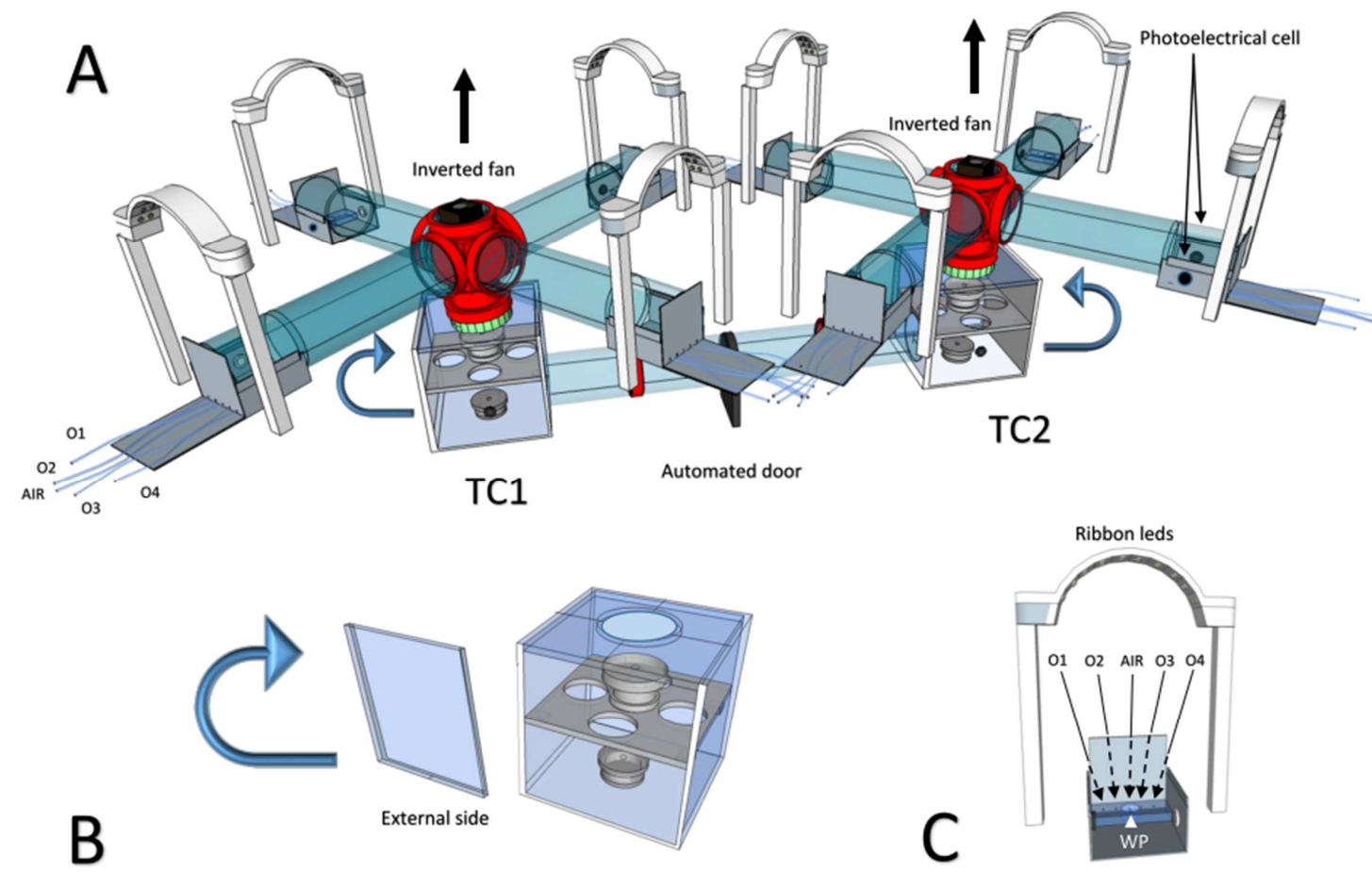


Figure 1: Escoffier et al. 2020

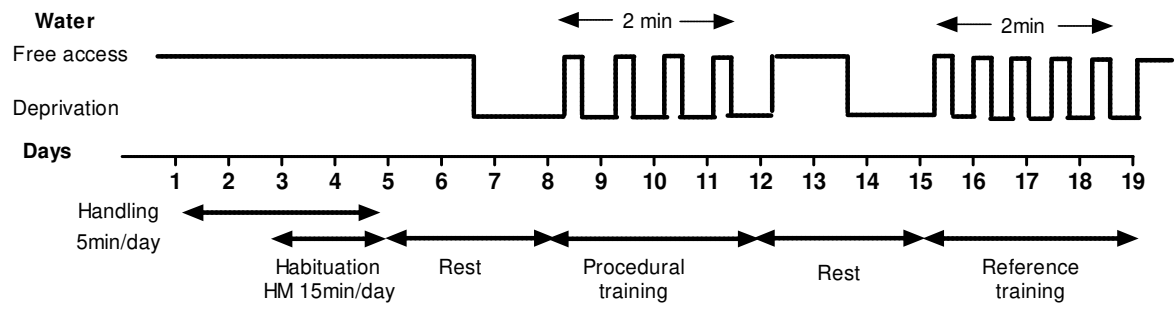


Figure 2: Escoffier et al. 2020

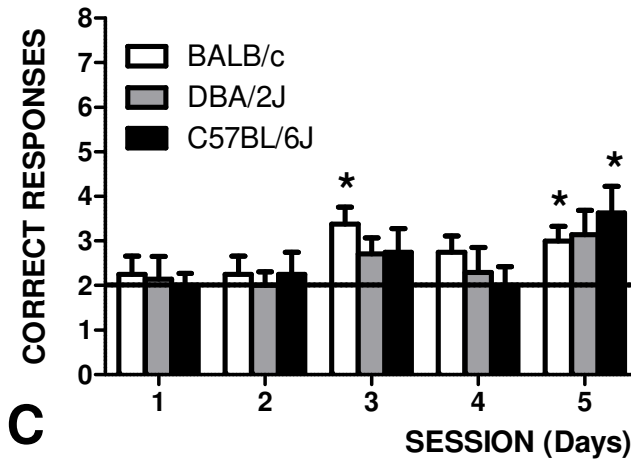
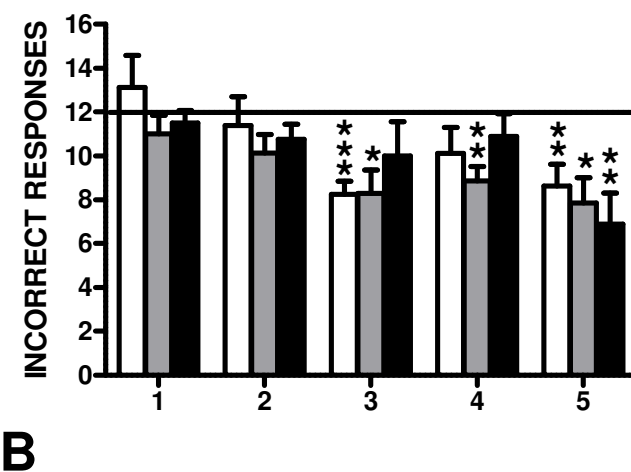
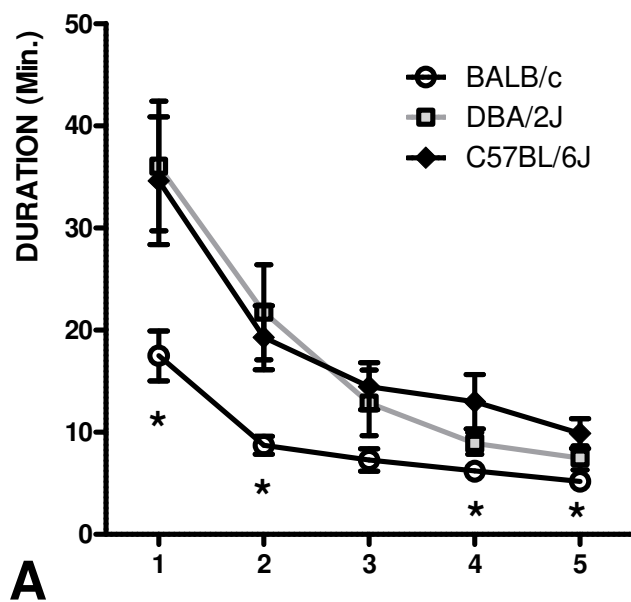


Figure 3: Escoffier et al. 2020

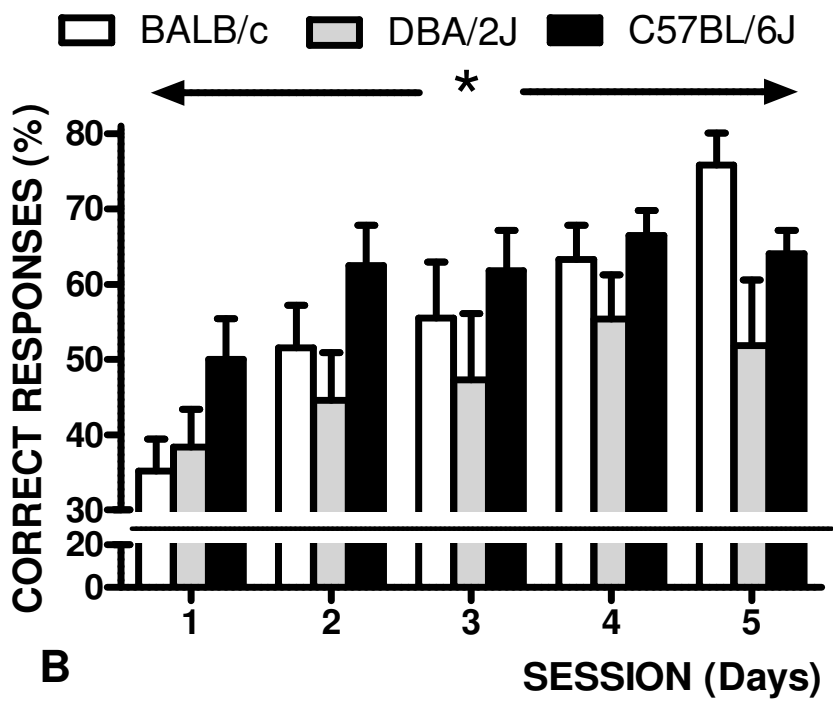
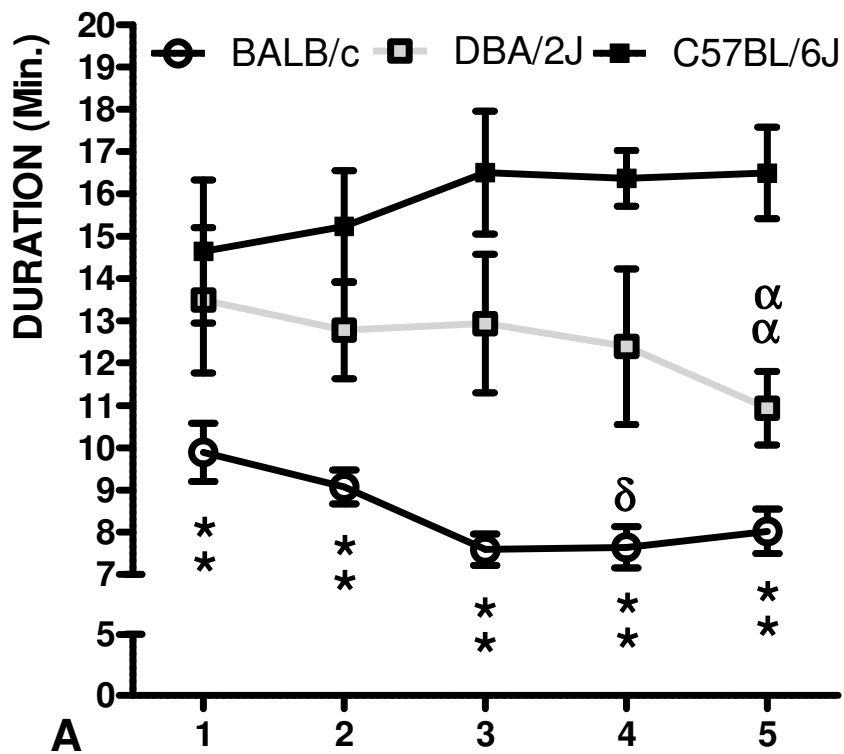


Figure 4: Escoffier et al. 2020

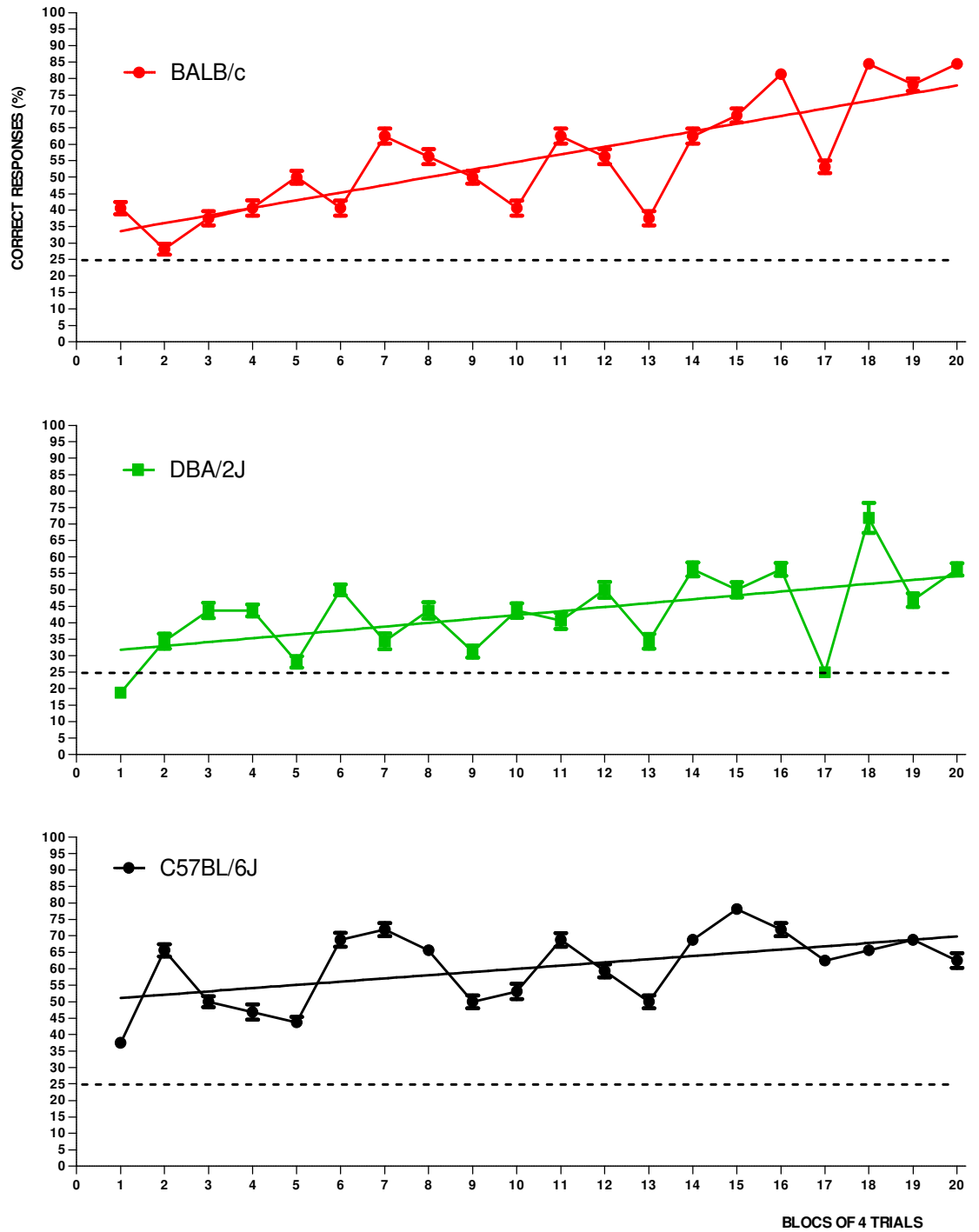


Figure 5: Escoffier et al. 2020

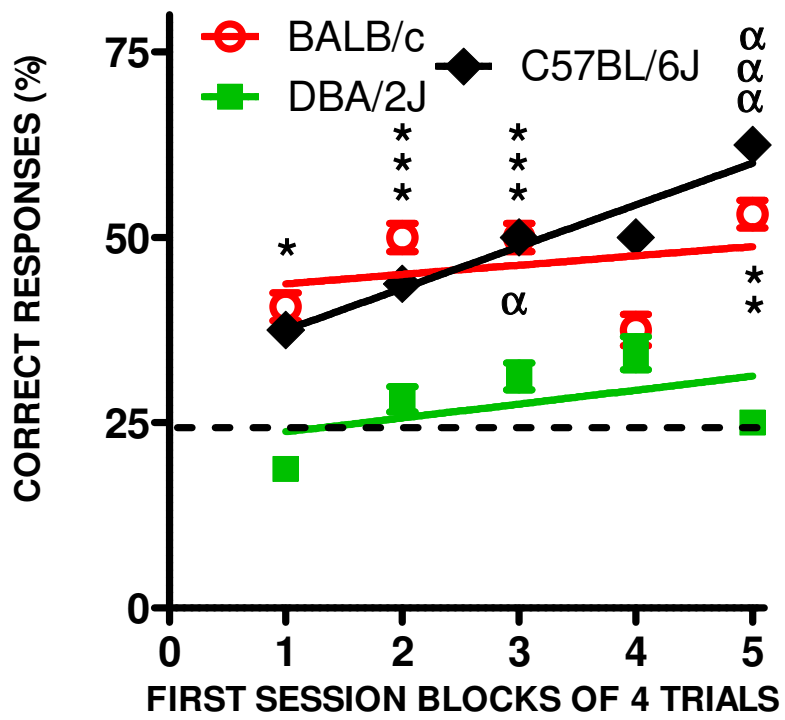


Figure 6: Escoffier et al. 2020