Shifting between response and place strategies in maze navigation: effects of training, cue availability and functional inactivation of striatum or hippocampus in rats

Running title: Striatum, hippocampus, and navigation

Julien Gasser[1,2], Anne Pereira de Vasconcelos[1,2], Brigitte Cosquer[1,2], Anne-Laurence Boutillier[1,2], Jean-Christophe Cassel[1,2,3]

[1] Université de Strasbourg, Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA), F-67000 Strasbourg, France

[2] CNRS, LNCA UMR 7364, F-67000 Strasbourg, France

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[3] **Corresponding author:** Dr. Jean-Christophe Cassel, Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA), UMR 7364, Université de Strasbourg-CNRS, 12 rue Goethe, F-67000 Strasbourg, France. E-mail: jcassel@unistra.fr

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Abstract (273 words)

Response and place memory systems have long been considered independent, encoding information in parallel, and involving the striatum and hippocampus, respectively. Most experimental studies supporting this view used simple, repetitive tasks, with unrestrained access to spatial cues. They did not give animals an opportunity to correct a response strategy by shifting to a place one, which would demonstrate dynamic, adaptive interactions between both memory systems in the navigation correction process. In a first experiment, rats were trained in the double-H maze for different durations (1, 6, or 14 days; 4 trials/day) to acquire a repetitive task in darkness (forcing a response memory-based strategy) or normal light (placing response and place memory systems in balance), or to acquire a place memory. All rats were given a misleading shifted-start probe trial 24-hr post-training to test both their strategy and their ability to correct their navigation directly or in response to negative feedback. Additional analyses focused on the dorsal striatum and the dorsal hippocampus using c-Fos gene expression imaging and, in a second experiment, reversible muscimol inactivation. The results indicate that, depending on training protocol and duration, the striatum, which was unexpectedly the first to come into play in the dual strategy task, and the hippocampus are both required when rats have to correct their navigation after having acquired a repetitive task in a cued environment. Partly contradicting the model established by Packard and McGaugh (1996, Neurobiology of Learning and Memory, vol 65), these data point to memory systems that interact in more complex ways than considered so far. To some extent, they also challenge the notion of hippocampus-independent response memory and striatum-independent place memory systems.

1. Introduction

When navigating toward a goal in a familiar environment, animals may use a place strategy based on their cognitive map, pilot themselves toward an explicit cue marking their goal, or repeat a response behavior consisting of a given sequence of actions concatenated in a constant order (e.g., Chersi & Burgess, 2015; Tolman et al., 1946). Research in rodents demonstrated a preferential role of the hippocampus in place memory and of the striatum in response memory (DeCoteau and Kesner, 2000; Gold, 2004; Packard & McGaugh, 1992, 1996; Packard, 1999, Poldrack & Packard, 2003).

Currently it is thought that in the initial stages of learning a repetitive navigation task with double solution (i.e., the goal can be reached either through place or response strategy), the hippocampus memory system quickly integrates information due to its "one-shot" incidental learning capabilities (Chersi and Burgess, 2015). With repetition, the striatal system takes over and starts to guide behavior, as shown in e.g., rodents (e.g., Packard & McGaugh, 1996; Laria et al., 2003; but see Asem & Holland, 2013; Martel et al., 2007). Importantly, even after automation due to sustained practice, subjects may not lose the capacity to shift from response to place strategy upon receiving negative feedback (e.g., when the usual route is unexpectedly compromised or the goal is missed).

Many experiments exploring place and response memories in rodents tested animals with unrestrained access to their environmental landmarks while running on T- or cross-maze devices (McDonald and White, 1994; 1995; 2013; Mizumori et al., 2004; Packard, 1999; Packard & McGaugh, 1992; 1996; Packard and White, 1991; Tolman et al., 1946; White and McDonald, 2002; White et al., 2013). In these experiments, their behavior was usually classified as egocentric (i.e., relying on idiothetic cues) or allocentric (i.e., relying on allothetic cues) based on a single-response probe test (e.g., Packard & McGaugh, 1996; Fouquet et al., 2013). When the response strategy revealed inefficient, the shift to a place strategy was not permitted. Such correction is crucial, however, because it would demonstrate a capability to engage the hippocampal and striatal memory systems in dynamic, interactive and situation-adapted ways (e.g., Berke et al., 2009; Chersi & Burgess, 2015; Eshenko & Mizumori, 2007; Mizumori et al., 2004; Regier et al., 2015).

Past research has suggested that the interaction between response and place

106 memory systems is modulated by saliency of intramaze and/or extramaze cues (e.g.,
107 Packard & Goodman, 2013), task complexity (e.g., Cassel et al., 2012; Ruprecht et
108 al., 2014), practice (e.g., Martel et al., 2007), and a few other factors, including stress
109 (e.g., Gardner et al., 2013; Packard and Goodman, 2013; Quaedflieg and
110 Schwabe, 2018; Schwabe, 2013). However, general conclusions regarding
111 hippocampal-striatal interactions cannot be drawn because the aforementioned
112 factors have most often been tackled by using a diversity of experimental devices,
113 protocols, training levels in different and separate experiments. In order to
114 investigate the dynamic interaction between dorsal striatum and hippocampus in
115 conditions that systematically control modulating factors, we used a repetitive task in
116 a single device with a single type of task motivation (escape from water), and varied
117 the experimental settings across three protocols and durations of training (1, 6 or 14
118 days). The training durations of 6 and 14 days were chosen to parallel Packard and
119 Mc Gaugh's study (1996). The apparatus was the double-H maze described by
120 Cholvin et al. (2013), and our experimental rats had to learn a unique pathway
121 between the same start arm and the same target arm. These experimental rats were
122 trained under normal illumination in a task with a dual response/place strategy (Lt-
123 ON-Dual). One control group was trained in darkness (to minimize perception of
124 allothetic cues) in a task promoting a response strategy (Lt-OFF-Resp). A second
125 control group was trained under normal illumination in a task promoting a place
126 strategy (Lt-ON-Place).

127   One day after the last training session, all rats were given a probe trial (same
128 illumination as for training) for which the start arm was changed in order to identify
129 the initial strategy - place or response - and to subsequently evaluate if an alternative
130 strategy could be adopted upon negative feedback (i.e., platform not found). Based
131 on the current understanding of memory systems and hippocampal-striatal
132 interactions (Chersi and Burgess, 2015; Packard and Goodman, 2013), the
133 predictions were that : **i)** Lt-OFF-Resp rats would favor a response strategy
134 based on the striatum memory system and would not construct a spatial map
135 enabling a strategy shift, whatever the training duration, **ii)** Lt-ON-Dual rats, which
136 would adopt a hippocampal-dependent spatial navigation strategy at the beginning of
137 training and change to a striatum-dependent response strategy with further training,
138 would be able to shift from a response to a place strategy, and **iii)** Lt-ON-Place rats

139 would favor a place strategy based on the hippocampus memory system
140 enabling a strategy shift either directly or after a mistake. We also investigated the
141 memory-based behaviors using c-Fos brain imaging and muscimol inactivation.
142
143 **2. Materials and methods**
144
145 *2.1. Animals*
146 Male Long-Evans rats (Janvier Labs, Le Genest-St-Isle, France) were used.
147 They were aged 6-7 weeks (body weight: 160-170g) at their arrival at the laboratory.
148 They were housed individually with ad libitum food and water in a temperature
149 (22 ± 1 °C) and humidity (55 ± 5%) controlled room under a 12-12 hr light–dark cycle
150 (lights on at 7.00 a.m.). The study respected the rules and guidelines of the
151 European Parliament 2010/63/UE of September 22, 2010, and of the French
152 Department of Agriculture. All experimental protocols used herein have been
153 validated a priori by a local ethical committee (CREMEAS, authorization no.
154 AL/17/24/02/13).
155
156 *2.2. Double-H maze*
157 The double-H testing device has been described in detail in previous articles
158 (e.g., Pol-Bodetto et al., 2011; Cassel et al., 2012; Kirch et al., 2013, 2015) along
159 with the characteristics of the room hosting it (Cholvin et al., 2013). Briefly, the
160 general layout of the apparatus corresponds to the shape of two contiguous capital
161 Hs. It is made of three parallel run arms, 160 cm in length and 18.8 cm wide (internal
162 measure), connected to each other at their center by a perpendicular arm. All side
163 walls, 35 cm high, are made of transparent Plexiglas to favor access to allothetic
164 cues. The two opposite arms in the middle are labeled north (N) and south (S),
165 respectively (see Figure 1). The four other arms are labeled south-east (SE), south-
166 west (SW), north-east (NE), and north-west (NW). For pre-training, training and
167 memory testing, the device was always kept at the same place (on a square table, 80
168 cm from the floor) and all cues in the room (two black disks on one wall, and one
169 large triangle on another wall, two orange-painted heating ducts above the maze,
170 one table, one chair, one computer desk, one boiler…) were left at their original
171 location for the entire duration of the experiments. Most of these landmarks were

172 located close to the maze, i.e., between 1.20 and 1.50 m from the closest maze wall

173 and between 86 and 140 cm above the water surface. To secure the locations of

174 objects, including the maze and its table, their positions were marked on the floor. In

175 the room, there was a small loudspeaker fixed on the wall, playing music at low

176 volume during the 12-h light period.

177

178 ### 2.3. Pre-training and training protocols

179 The double-H was filled with water (20 °C) to a height of 15 cm. A platform,

180 11 cm in diameter, 14 cm high, was ballasted with gravel and used as the escape.

181 For pre-training, the platform protruded 1 cm above the surface of the water at the

182 extremity of the SW arm, the water was left clear and the rats were given four

183 consecutive trials. On each trial, rats had to swim straight from the NW to the SW

184 arm in order to keep the cognitive demand at a low level. A transparent guillotine

185 door blocked the access to the central corridor and thus to all other places in the

186 maze. In all rats, pre-training was performed in a normally lit environment.

187 For the training sessions, the platform was moved to the NE arm and

188 immersed 1 cm under the water surface. Water was made opaque by addition of

189 powdered milk (about 1.5 g/L). Rats were given four daily trials. Each trial lasted a

190 maximum of 60 s. When the rat did not reach the platform within this time, it was

191 gently guided to the platform by the experimenter. Once a rat had climbed on the

192 platform, it was left there for 10 s, after what the next trial was started without delay.

193 Three training protocols were used (Figure 1). In the first protocol (Lt-OFF-

194 Resp), rats were tested in darkness: the only source of light was generated by six red

195 darkroom bulbs, type B22PF712B by Philips, 15 W each, placed near the maze at

196 the extremity of each arm; light intensity in and around the maze was of about 1 lux.

197 The rats were released from the S on all trials and had to swim to the NE, which they

198 reached most directly by a right turn immediately followed by a left turn. The N arm

199 was closed with a transparent guillotine door. The guillotine door prevented the

200 repetition of the straight swimming trajectory rats had to follow during the pre-training

201 phase of the protocol. In the second protocol (Lt-ON-Dual), the same procedure was

202 used except that the room was illuminated by neon lights (180 lux) to make all

203 landmarks easily visible. The N arm was closed with a guillotine door. In the third

204 protocol (Lt-ON-Place), the room was illuminated (180 lux) and rats were released

205 randomly from a different arm on each of the four daily training trials. The N arm was
206 closed.

207   Each trial was videotaped for subsequent off-line scoring. Variables collected
208 were: swim patterns, latencies (s) and distances (cm) to reach the platform, as well
209 as the number of errors. An error was counted each time a rat was swimming in a
210 segment (either in one of the arms with no platform or one of the segments of the
211 central alley to the left or right of the start arm) toward a direction opposite to that
212 leading most directly to the platform (see Pol-Bodetto et al., 2011, for detail). Each
213 time a rat had its head and 4 paws in one of those segments, it was considered to
214 have entered it. For each protocol, three subgroups of rats were constituted. The first
215 one was trained for 1 day (and thus received only four trials), the second one for 6
216 consecutive days (24 trials), and the last one for 14 consecutive days (56 trials).

217   ******************************

218   **Insert Figure 1 about here**

219   ******************************

220

221   ***2.4. Probe trial***

222   The probe trial, for which the platform was removed from the water, was given
223 24 hr after the last trial of the last training day. Probe trial duration was of 60 s.
224 Regardless of their previous training protocol, all rats were released from the SW
225 arm, thus with a 60 cm translation to the left of their usual starting point. In Lt-ON-
226 Dual rats, this translation to the left was previously found to be misleading. Indeed,
227 more than 90% of the rats usually first repeated the right-left (R-L) body turns, ending
228 up in the N arm, instead of using a direct trajectory to the NE arm (Cassel et al.,
229 2012). This high misleading potential can be explained by the fact that the 60-cm
230 translation to the left only slightly alters room perspectives (Cassel et al., 2012). The
231 presence of the guillotine door in front of the arm from which the rat was released
232 possibly added to this misleading potential. Upon negative feedback (i.e., no platform
233 found in N), however, a number of rats leaving the N arm then entered the NE one,
234 where they searched for the platform for a longer time than chance level, further
235 supporting their ability to use a place strategy (Cassel et al., 2012). Variables
236 collected during the probe trial were **i)** type of swim trajectory displayed immediately
237 after the start (R-L turn and thus direct swim to the N, direct swim to the NE, or other)

238 and the capacity to shift to the NE arm after having visited the N one; **ii)** response
239 memory and place memory exploration times over the entire probe trial duration.
240 Response memory exploration time is defined as the cumulated time in any arm that
241 a rat entered after successive R-L turns. Place memory exploration time is defined as
242 the cumulated time in the NE arm. Because a trajectory going from the S to the NE
243 arm could be the result of either a response memory or a place memory, without any
244 possibility for the experimenter to know which strategy a rat had used, the times
245 spent in the NE arm when the rats came directly from the S were discarded from
246 statistical analyses. As a result, only times recorded in the NE arm when rats came
247 from SW, SE or N were considered for analyses. Additional variables analyzed were
248 the swim velocity, the time spent in the first visited arm before leaving it, as well as
249 the time spent in the N or NE arm after the rats had entered it for the first time.
250

251 ### 2.5. Surgery and muscimol (MUSC) infusions

252 For our second experiment, surgery was performed under aseptic conditions.
253 Rats were anesthetized with a ketamine (98 mg/kg)–xylazine (13 mg/kg) mixture
254 injected intraperitoneally. They were secured in a stereotaxic frame (incisor bar at –
255 3.0 mm). Stainless steel guide cannulas (external diameter 0.4 mm) were implanted
256 bilaterally in the dorsal hippocampus (DHip), targeting CA1 (AP –3.6 mm, ML ±2.4
257 mm, DV –2.4 mm from skull), or in the dorsal striatum (DStr; AP +0.72 mm, ML ±2.85
258 mm, DV –4.2 mm from skull). All coordinates are given from Bregma according to
259 Paxinos and Watson (2007). On the basis of the c-Fos expression patterns of our first
260 experiment (see below), we decided to infuse MUSC into a relatively central site of
261 the DStr, rather than separately into the DLS or DMS, where c-Fos expression levels
262 were in most instances not dramatically different between rats trained for 6 as
263 compared to 14 days under each training condition. Each guide cannula was secured
264 to the skull by acrylic dental cement and sterilized stainless steel screws. At the end
265 of surgery, a stainless steel mandrel (external diameter 0.28 mm) was inserted into
266 each guide cannula. Thereafter, rats were allowed to recover under a heating lamp
267 for 20–30 min before being placed back into their home cage. An 8-day
268 rest/manipulation time (for home-cage rats of experiment 1 as well) was given before
269 the start of the behavioral experiment.

270   In contrast to lidocaine or tetrodotoxin, MUSC reportedly induces an
271   inactivation of neurons in the diffusion radius of the drug without changing the
272   excitability of the fibers *en passage* therein (Edeline et al., 2002; Van Duuren et al.,
273   2007). Starting 3 days after surgery, rats were first habituated over 5 consecutive
274   days to being handled and maintained for drug infusions. For infusions, rats were
275   gently restrained by hand in a soft towel, the mandrels were removed, and an
276   infusion needle (external diameter 0.28 mm) was slowly lowered into each guide
277   cannula. The tip of each infusion needle protruded 1.0 mm beyond the tip of the
278   guide cannula into the DHip or the DStr. The other needle tip had been connected to
279   a 10µL Hamilton syringe by polyethylene tubing. Using a microinjection pump
280   (CMA/100), MUSC (Sigma, Saint-Louis, USA; dissolved in artificial cerebrospinal fluid
281   [aCSF]) was infused bilaterally (200 ng/µL in the DStr, and 250 ng/µL in the DHip,
282   each over 60 s; 1µL was infused, whatever the structure, in each hemisphere). In a
283   previous study, such small amounts were found to induce marked cognitive effects
284   when infused in the DHip, the prefrontal cortex, or the ventral midline thalamus
285   (Cholvin et al., 2013). Controls received a bilateral infusion of aCSF (same volume as
286   for inactivation). At the completion of infusion, the needles were left in place for 60 s
287   to allow drug diffusion into the parenchyma. Needles were then slowly retracted and
288   mandrels repositioned into the guide cannulas. Right after the infusion, rats were
289   returned to their home cage until the start of the probe trial, 30 min later. This delay of
290   30 min is within the time window of maximal effect of the drug (i.e., 25 to 90–120
291   min), as shown by electrophysiological (Arikan et al., 2002; Edeline et al., 2002) or
292   autoradiographic studies (using [^{3}H]-MUSC, Edeline et al., 2002; Martin & Ghez,
293   1999). The diffusion radius of MUSC at the time of the probe trial was estimated on
294   brain sections stained for *c*-Fos expression. Possible intergroup differences in the
295   inactivation extent might have induced differences in performance. Therefore, the
296   area covered by the absence of *c*-Fos expression was measured in both
297   hemispheres on coronal sections from both structures. Estimating the extent of a
298   pharmacologic inactivation remains a tricky issue. In previous studies (Cholvin et al.,
299   2013), we used fluorescent MUSC to localize the infusion site and efficiency of
300   diffusion into the parenchyma. The molecular weight of this molecule, however, is 5.3
301   times larger than its natural homologue. Therefore, we chose to measure the extent
302   of reduced *c*-Fos expression around the infusion site. Indeed, this immediate early

303 gene having a very low basal expression level, a region that should be active (as

304 seen in rats infused with aCSF) but would exhibit a very low or no c-Fos expression

305 can be considered efficiently inactivated. A similar approach on zif268 expression in

306 mice was used by Maviel et al. (2004) to assess the effects of lidocaine in the

307 hippocampus and neocortex. All inactivation areas were expressed as a % of the

308 surface of the DStr at about -0.1, 0.1, +0.5, +0.7, +0.8, +1.0, +1.4, and +1.7 mm

309 (ventral limits of the DStr are those shown in Supplementary Figure 1), and of the

310 DHip at -4.4, -4.2, -3.8, -3.6, -3.5, -3.4, -3.1, -2.9, and -2.6 mm, from Bregma

311 (Paxinos and Watson, 2007).

312

313 ### 2.6. Tissue preparation

314 Ninety minutes after the probe trial, rats were injected with an overdose of

315 sodium pentobarbital (200 mg/kg i.p.) and perfused transcardially with 80mL of a 4%

316 phosphate-buffered (0.1 M) paraformaldehyde solution (PFA, 4°C). Brains were

317 removed, post-fixed for 2 hr in 4% PFA (4°C), and placed into a 20% sucrose

318 solution (in 0.1 M PBS) for 48 hr at 4 °C. They were then quickly frozen in isopentane

319 (-40 °C) and stored at -80°C. Floating coronal sections (40 μm) were cut using a

320 cryostat (MICROM HM 500M) in serial sections within a block of tissue extending

321 from +1.90 to -1.90 mm from Bregma for the DStr, and from -2.16 to -4.44 mm from

322 Bregma for the dHip (Paxinos & Watson, 2007).

323

324 ### 2.7. c-Fos immunohistochemistry, imaging and quantification

325 All sections dedicated to c-Fos immunohistochemistry were processed in

326 separate rounds so as to have all between-subject factors equally represented in

327 each round (n = 8 rats for each group). These precautions minimized technical

328 biases. The sections were first rinsed three times during 10 min in PBS and soaked

329 for 1 hr in 5% normal donkey serum in PBS containing 0.5% Triton X-100. All

330 sections were subsequently transferred into the primary anti-Fos rabbit polyclonal

331 antibody solution (1:4,000, Rabbit anti-Fos polyclonal IgG; Santa Cruz, USA), where

332 they were left overnight at room temperature. Then, they were rinsed and soaked in a

333 buffer solution containing biotinylated goat anti-rabbit secondary antibody (1:500,

334 Biotin SP-conjugated affiniPure Goat anti-rabbit IgG; Jackson ImmunoResearch,

335 West Grove, PA, USA).   Staining was revealed with the avidin–biotin peroxidase

336 method (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA) coupled to

337 diaminobenzidine (Lopez et al., 2012). In rats subjected to a functional inactivation by

338 MUSC (see below), additional sections were stained with cresyl violet to make sure

339 that the location of the infusion sites was acceptable.

340

341 ### *2.8. Stereological analyses of c-Fos expression*

342 The quantitative analyses of c-Fos-positive nuclei were performed in the DStr

343 and DHip. As the dorsomedial striatum (DMS) contributes to setting up an

344 automatism by its implication in action outcome on a goal-directed navigation task,

345 and the dorsolateral striatum (DLS) to the automatism's storage (e.g., Packard &

346 McGaugh 1996; Thorn et al., 2010), quantifications were made separately in the

347 DMS and DLS. Additional quantifications were also made in CA1, CA3, and dentate

348 gyrus (DG), as c-Fos expression levels accompanying place memory retrieval may

349 differ between these hippocampal subregions (e.g., Lopez et al., 2012). A single

350 investigator, blind to the identity of the rats, analyzed all sections. The overall number

351 of c-Fos immunoreactive cells was estimated with the optical fractionator technique

352 using optical dissectors (45 x 45 µm) allowing unbiased counting (West et al., 1991;

353 West, 2013). For stereological counting we used a Leica DM5500B light microscope

354 coupled with a MicroFire CCD color camera (Optronics) equipped with a motorized

355 x–y stage control. Stereological analyses were performed using the Mercator

356 software (Explora Nova, La Rochelle, France) and all cell counts were processed

357 online on the video image. The same intensity of light in the microscope and the

358 same parameters in the exposure time of the digital camera were used for all

359 sections. Areas of interest in stained sections (see Supplementary Figure 1) were

360 first outlined using a 2.5x objective and c-Fos-positive cells were counted using a

361 100x (1.40 NA) oil-immersion objective. Counting grids (145 x 145 µm for the DStr

362 and 80 x 80 µm for DHip subregions) equidistant from each other were randomly

363 positioned within the area of interest using the Mercator software. The total number

364 of c-Fos positive nuclei/mm^3 of cerebral tissue was estimated from six (for the DHip)

365 or seven (for the DStr) sections per animal (section sampling fraction (ssf) = 1/6 for

366 DHip or 1/12 for DStr, from the total number of nuclei counted in all optical

367 dissectors). Details of stereological parameters were as follows: section interval =

368 200 µm for DHip and 400 µm for DStr; dissector height = 12 µm and guard zone = 2

μm (corresponding to upper and lower border exclusion zone, mean section thickness being at 16 μm). Counting was performed in the DMS, the DLS, and the DHip. Roughly, the DLS region was the one connected to the sensorimotor cortex and the DMS region was the one connected with medial prefrontal regions (see McGeorge & Faull, 1989; Voorn et al., 2004). The error coefficients (see Gundersen et al., 1988) for each estimation and animal ranged from 0.09 to 0.11.

A group of never-tested rats taken from their home cage (HC) was used as a baseline control for c-Fos quantification (see below). Our HC controls were handled daily by the experimenter for the same time as the average duration the rats trained and tested in the double-H maze took to complete four trials. The same was done on the probe trial day. Swimming controls would have been acceptable for a training duration of 1 day (four training trials). However, for 6 and 14 days of training, rats would have received 24 and 56 trials, respectively, without any solution to escape from the water. Typically, this situation is a learned helplessness one, which affects c-Fos expression patterns in the hippocampus (e.g., Huang et al., 2004) or structures innervating the hippocampus (Steciuk et al., 1999). Therefore, swimming-only rats were not included in this study.

2.9. Statistical analyses

For the first experiment, analyses of acquisition scores used a Protocol x Trial (1-day training) or Protocol x Day (6- or 14-day training) ANOVA. For analyses of the corresponding probe trial performance, we considered qualitative and quantitative variables. After having been released in the SW arm of the maze, rats could swim directly to the NE arm, indicating an immediate engagement of place memory, or N arm, indicating a direct engagement of response memory. All other swim patterns (i.e., a first visit of any of the other arms) were considered in an 'Other' category. To compare the number of rats in the response memory category among training protocols (factor called 'Protocol' hereafter) and training durations (factor called 'Duration' hereafter), we used a non-parametric Chi² test. To refine this analysis, we analyzed the latencies to the NE arm as they might provide precious information in rats tested in light about the strategy they used. If this strategy relied on response memory, the latency should be close to that found in rats tested in the absence of light. We also compared the cumulated time spent in the NE arm or in any other arm

402 to which a R-L turn had led for each training protocol and duration. When post-hoc
403 comparisons were required and justified by the ANOVA, we used the Newman-Keuls
404 multiple range test. Exploration times in the R-L and NE arms were compared to
405 chance. As each arm had a surface representing 13.7% of the accessible surface of
406 the maze and as the probe trial duration was of 60 s, chance was computed as 60 x
407 0.137 = 8.22 s. Quantitative c-fos expression data were analyzed using a Protocol X
408 Duration ANOVA for each separate brain region (DMS, DLS, CA1, CA3, DG).

409 For our second experiment, we also used a Chi² test to analyze the first arm
410 choice at the start of the probe trial, and an Inactivation (aCSF, MUSC) x Duration (6,
411 14 days) ANOVA for each protocol to analyze the behavioral consequence of
412 inactivating the DStr or DHip. Multiple comparisons were performed with the more
413 conservative Tuckey test, because, based on graphical observations, they were
414 occasionally run in the absence of significant interactions when a main effect of
415 MUSC was found. Performance was also compared to chance using a Student t-test.
416 To analyze the extent of inactivation (c-Fos imaging) at different anteriority levels, we
417 used a Protocol x Duration x Anteriority (6 or 7 levels)) ANOVA. This was done for
418 each brain region (DStr, DHip).

419

420 **3. Results**

421

422 ***3.1. Experiment 1: Incremental training in the double-H maze and c-fos***
423 ***expression patterns in the dorsal striatum vs. dorsal hippocampus***

424

425 ***3.1.1. Comparable acquisition performance among training protocols***
426 Latencies to reach the platform in the NE arm are shown in Figure 2. Rats trained for
427 one day (four trials) showed performance improvement across trials. (Trial: $F_{(3,63)} =$
428 12,2, $p < 0.001$). This improvement was comparable among the three training
429 protocols (interaction: $F_{(6,63)} = 0.33$). In the rats trained over 6 days (six trial blocks),
430 there was a significant Day effect ($F_{(5/105)} = 46.6$, $p < 0.001$), but no effect of Protocol
431 ($F_{(2/21)} = 0.1$) or of the interaction ($F_{(10/105)} = 0.23$). In the rats trained over 14 days,
432 only the Day effect was significant ($F_{(13,273)} = 43.1$, $p < 0.001$). For the two longest
433 training durations, significant improvements of performance were from day 1 to day 2

434 and then to day 3 (p <0.01). Analyses of the distances (not illustrated) yielded strictly

435 comparable results, as was also the case for errors (see Supplementary Figure 2).

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437 *****************************

438 **Insert Figure 2 about here**

439 *****************************

440

3.1.2. A training-duration-dependent shift from response to place memory in Lt-ON-Dual rats

443 According to our current knowledge, Lt-OFF-Resp rats should form a

444 stimulus-response representation and favor a response strategy without

445 constructing a spatial map. Therefore, they should not shift to spatial navigation,

446 even in response to negative feedback. Conversely, Lt-ON-Dual rats should adopt a

447 hippocampal-dependent spatial navigation strategy after weak training (1 or 6 days),

448 but change to a striatum-dependent response strategy after sustained training (14

449 days). We hypothesized that they should nevertheless be able to shift from a

450 response strategy to a place strategy, either directly or upon negative feedback. In Lt-

451 ON-Place rats, for the three training durations, all behaviors should reflect a place

452 strategy. According to these predictions, our results showed that Lt-OFF-Resp rats

453 did not form a spatial map. However, and unexpectedly, Lt-ON-Dual rats started to

454 acquire the task by response learning, and acquired only later on a place memory

455 enabling a trajectory correction, either directly or in response to negative feedback.

456 Lt-ON-Place rats relied on a spatial map, as soon as after 1 day of training.

457

458 The data are illustrated in Figure 3. Whatever the training duration, almost all

459 Lt-OFF-Resp rats swam directly to the N, a proportion (20/23; 87%) largely above

460 chance (i.e., 25% as there were 4 accessible arms; start arm not considered). Most

461 Lt-ON-Place rats (16/23; 69%) also swam directly to the N. In Lt-ON-Dual rats, most

462 first swim paths ended in the N arm after 1 (100%) or 6 (87%) training days. After 14

463 training days, however, half the rats swam directly to the NE arm. The difference

464 between first choices after 1 and 14 training days was significant (Chi^2 = 4,8, p <

465 0.05), indicating a late emergence of spatial navigation capabilities. Supplementary

466 Table 1 shows the proportion of rats correcting their choice in response to negative

467 feedback, i.e., rats which swam to the NE arm next to having entered the N one.

468   **************************

469   **Insert Figure 3 about here**

470   **************************

471

472   To further analyze probe trial performance, and especially the shift capacity,

473 we considered three additional variables: latencies to the NE arm, cumulated time

474 spent in an arm to which a R-L turn had led (response memory-based behavior), and

475 cumulated time spent in the NE arm (place memory-based behavior). Latencies to

476 the NE arm (Supplementary Figure 3) provided additional information about the

477 nature of the strategy used by the rats. Overall, we found that latencies were

478 significantly reduced in the probe trial of the Lt-ON-Dual rats compared to the Lt-

479 OFF-Resp rats after 14 days of training, thus compatible with the progressive

480 formation of a cognitive map in this group. Swim velocities (see Supplementary Table

481 2) during the probe trial did not differ significantly among groups, allowing us to

482 compare latencies. Regarding the time to exit the first visited arm, there was no

483 significant difference among groups (Supplementary Figure 4). It is noteworthy that

484 when Lt-ON-Dual rats (after 14 days of training) and Lt-ON-Place rats (after 6 and 14

485 days of training) visited the NE or the N arm for the first time, the to exit was longer in

486 the former than in the latter (supplementary Figure 5), a difference not found in Lt-

487 OFF-Resp rats.

488

489   ***3.1.2.1. Cumulated time in arms reached by R-L turns:***

490   This variable shows a response memory-based strategy in Lt-OFF-Resp rats,

491 a strategy abandoned by Lt-ON-Dual rats after the longest training duration, and not

492 existing in Lt-ON-Place rats. Data are shown in Figure 4. In the Lt-OFF-Resp rats, the

493 exploration time was above chance for the two longest durations ($p < 0.01$), and

494 larger after 14 training days than after 1 or 6 days ($p < 0.05$). In the Lt-ON-Place rats,

495 this time was neither significantly different from chance nor affected by training

496 duration. In Lt-ON-Dual rats, the time decayed as a function of training duration,

497 reaching chance level after 14 training days. The Protocol x Duration ANOVA

498 showed significant effects of the Protocol ($F_{(2,58)} = 17.4$, $p < 0.001$), Duration ($F_{(2,58)}$

499   = 0.13, *ns*), and of the interaction between the two factors (F $_{(4,58)}$ = 3,19, *p* < 0.05).

500   The Protocol effect was due to an overall exploration time (collapsed over training

501   durations) that was longest in the Lt-OFF-Resp rats, intermediate in the Lt-ON-Dual

502   rats, and lowest in the Lt-ON-Place ones (all differences were significant: *p* < 0.01).

503   The interaction reflected a time of exploration that increased with training duration in

504   Lt-OFF-Resp rats and decreased in Lt-ON-Dual rats.

505

506   ### 3.1.2.2. Cumulated time in NE arm:

507   This variable shows no place memory-based strategy in Lt-OFF-Resp rats, a

508   place strategy which appeared in L-ON-Dual rats after the longest training duration

509   (14 days) and which was present in Lt-ON-Place rats already after 1 day of training.

510   Statistics for comparisons of performance to chance level are illustrated in Figure 4.

511   ANOVA of exploration time showed significant effects of Protocol (F $_{(2,58)}$ = 25,2, *p* <

512   0.001), Duration (F $_{(2,58)}$ = 8,9, *p* < 0.001), and of the interaction between the two

513   factors (F $_{(4,58)}$ = 2,5, *p* < 0.05). The group effect was due to overall time of

514   exploration that was longest in Lt-ON-Place, intermediate in the Lt-ON-Dual rats, and

515   lowest in Lt-OFF-Resp rats (all differences were significant: *p* < 0.01). The low

516   exploration time in Lt-OFF-Resp rats and the high exploration time in Lt-ON-Place

517   rats were not affected by training duration. Conversely, in Lt-ON-Dual rats, this time

518   increased as a function of training duration, starting at the level of Lt-OFF-Resp rats

519   and ending up at that of Lt-ON-Place ones. This difference explains the interaction

520   between the two factors.

521

522   In summary, rats trained in the response strategy task developed a response

523   strategy and showed a bias towards the response arm during the probe trial. Rats

524   trained in the dual response/place strategy task performed comparably after 1 or 6

525   training days. However, after 14 days, half of them used an initial place strategy and,

526   at the group level, there was a clear cut bias towards the place (NE) arm in the probe

527   trial. Rats trained in the place strategy task did not use a place strategy for their initial

528   swim path, but over the probe trial they showed a clear bias towards the place arm,

529   whatever the training duration. The main result here is that when cues are visible in

530   the dual double-H maze task, rats first approach the task on the basis of response

531   learning and construct a cognitive map later on.

532   *****************************

533   **Insert Figure 4 about here**

534   *****************************

535

536   ***3.1.3. c-fos expression patterns in the dorsal striatum and dorsal***

537 ***hippocampus***

538   c-fos expression was measured by immunohistochemistry in the DMS and

539 DLS and in the dHip (CA1, CA3 and DG). Examples of c-fos expression patterns,

540 including in two control regions (i.e., the primary somatosensory cortex and the

541 primary auditory cortex), are shown in Supplementary Figures 6-10. Quantitative data

542 are illustrated in Figure 5. Given our initial predictions from the literature (e.g.,

543 Packard and McGaugh, 1996), we expected high c-Fos expression levels in the

544 striatum of Lt-OFF-Resp rats and in the hippocampus of Lt-ON-Place regardless of

545 training duration, and a shift over training from high levels in the hippocampus to high

546 levels in the striatum in the Lt-ON-Dual rats. Based on our behavioral data, however,

547 the expectations for Lt-ON-Dual rats should be opposite. Overall, our results show

548 that, during the probe trial, striatal activation was highest in Lt-OFF-Resp rats for all

549 training durations. Striatal activation was weaker in Lt-ON-Dual rats in comparison

550 with Lt-OFF-Resp rats, but yet largely above HC rats. In Lt-ON-Place rats c-Fos

551 levels were higher than in HC rats, but the difference was more pronounced in the

552 DMS than in the DLS. In Lt-OFF-Resp rats, CA1 c-Fos levels were close to those

553 found in HC rats after all training durations. In Lt-ON-Dual rats, CA1 showed the

554 largest evidence for activation, but only after 1 and 6 days of training. In Lt-ON-Place

555 rats, a larger c-Fos expression was found only after 1 day of training.

556

557   ***3.1.3.1. c-Fos quantification in the dorsal striatum:***

558 Analyses of c-Fos positive neurons in the DMS showed significant Protocol ($F_{(3,72)}$ =

559 86.0, $p < 0.001$), Duration ($F_{(2,72)}$ = 3.5, $p < 0.05$), and interaction effects ($F_{(6,72)}$ =

560 2.7, $p < 0.05$). The highest overall c-Fos expression was found in the DMS of Lt-OFF-

561 Resp rats, the second highest one in Lt-ON-Dual rats, then in Lt-ON-Place rats, and

562 finally in HC rats; all intergroup differences were significant ($p < 0.01$, at least).

563 Multiple comparisons showed that the only group in which the number of c-Fos-

564 positive neurons was significantly depending on the duration of training was the Lt-

565 ON-Dual group; this number was reduced significantly after 6 and 14 days compared

566 to 1 day ($p < 0.05$ in each case).

567 Analyses of the DLS data showed significant Protocol ($F_{(3,72)} = 87.2$, $p < 0.001$) and

568 Protocol X Duration interaction effects ($F_{(6,72)} = 2.7$, $p < 0.05$); the overall Duration

569 effect was not significant ($F_{(2,72)} = 1.5$, $p = 0.22$). The highest overall c-Fos

570 expression was found in Lt-OFF-Resp rats, the second highest one in Lt-ON-Dual

571 rats, then in Lt-ON-Place rats, and finally in HC rats; all intergroup differences were

572 significant ($p < 0.01$). The only group in which the number of c-Fos-positive neurons

573 was significantly depending on training duration was the Lt-OFF-Resp group; this

574 number was larger after 6 and 14 days than after 1 day ($p < 0.05$ in each case).

575

576   ***3.1.3.2. c-Fos quantification in the dorsal hippocampus:***

577 Analyses of c-Fos positive neurons in CA1 showed significant Protocol ($F_{(3,72)}$

578 $= 17.9$, $p < 0.001$), Duration ($F_{(2,72)} = 16.1$, $p < 0.001$), and interaction effects ($F_{(6,72)}$

579 $= 11.1$, $p < 0.001$). The same effects were found in CA3 and in the DG, although with

580 less pronounced differences. In the DG, post hoc analyses indicated that overall c-

581 Fos expression was weaker after 14 than after 1 and 6 days of training ($p < 0.05$). In

582 CA1 and CA3, the highest overall c-Fos expression was found in Lt-ON-Dual and Lt-

583 ON-Place rats ($p < 0.001$), which did not differ from each other. The lowest one was

584 found in Lt-OFF-Resp and HC rats, which did not differ from each other. Regarding

585 training durations, the lowest overall c-Fos expression was found for the longest

586 duration in CA1, CA3, and DG ($p < 0.05$). In two groups, the number of c-Fos-positive

587 neurons was dependent on training duration: it was dramatically reduced between 1

588 and 6 days in CA1 of Lt-ON-Place rats ($p < 0.01$), and between 6 and 14 days of Lt-

589 ON-Dual rats ($p < 0.01$). In CA3, it was reduced between 1 and 6 days in Lt-ON-

590 Place rats ($p < 0.05$) and increased after 6 days of training in Lt-ON-Dual rats

591 compared to 1 and 14 days ($p < 0.01$). This was also the case in the DG ($p < 0.05$).

592   ******************************

593   **Insert Figure 5 about here**

594   ******************************

595

596 In summary, there was an increased c-Fos expression in the dorsal striatum of

597 Lt-OFF-Resp rats after all training durations, but after sustained training c-Fos levels

had decreased in the DMS and increased in the DLS. In Lt-ON-Place rats, c-Fos expression was substantially lower and not dependent on training duration. Lt-ON-Dual rats had c-Fos levels comparable to Lt-OFF-Resp rats after the shortest training, and to Light-ON-Place rats after the longest training. In region CA1 of Lt-OFF-Resp rats, c-Fos levels were close to those found in home cage rats. In Lt-ON-Dual and Lt-ON-Place rats these levels were high after one day of training, but had decayed to levels of home cage rats after 6 days of training in Lt-ON-Place rats, and after 14 days of training in Lt-ON-Dual rats, when these were showing evidence for place memory. It is noteworthy that some changes in c-Fos expression did not fit with the literature-based expectations (e.g., Packard and McGaugh, 1996).

3.2. Experiment 2: 6- or 14-day training in the double-H maze and muscimol inactivation before the probe trial

Training conditions were the same as in the first experiment, but durations were of 6 and 14 days only. Thirty minutes before the probe trial, rats were infused with MUSC or aCSF into the DStr or the DHip. Based on the behavioral data of the first experiment, our expectations were that intrastriatal MUSC would disrupt response memory-based behavior, and hence disrupt performance in Lt-OFF-Resp rats after 6 or 14 days of training, and in Lt-ON-Dual rats only after 14 days of training. In Lt-ON-Place rats, striatal inactivation should have no effect. We also expected that intrahippocampal infusions of MUSC would not affect performance in Lt-OFF-Resp rats, but would alter it in Lt-ON-Dual rats after 14 days of training, and in Lt-ON-Place rats after either training duration. Consideration of c-Fos data leave our expectations unchanged for Lt-OFF-Resp rats. For the other groups, it is difficult to make strong predictions because c-Fos expression data were not in line with our behavioral observations.

3.2.1. Muscimol infusion sites

The cannulas/needles reached their intended target in most rats (otherwise, the rats were discarded from analyses). The infusions sites are shown in Figure 6. Briefly, when the cannulas were implanted in the DStr, the between-subject variability of the infusion sites was of about 1.5 mm along the antero-posterior axis, 1.2 mm in laterality, and 1.2 mm ventrally. When the cannulas were implanted in the DHip, the

631 variability of the infusion sites was of about 1.2 mm along the antero-posterior axis,
632 1.0 mm in laterality, and 1.0 mm ventrally.

633 *****************************

634 **Insert Figure 6 about here**

635 *****************************

636

637 ### *3.2.2. Inactivation radius in the dorsal striatum and dorsal hippocampus*
638 ### *as assessed by c-Fos expression*

639 The extent of inactivation was comparable among experimental conditions for each
640 structure. An overview of the diffusion of the MUSC effects as estimated from our c-
641 Fos expression material is shown in Supplementary Figure 11. The inactivation
642 extent was quantified based on c-Fos gene expression staining: Inactivation areas
643 were expressed as a percentage of the surface of the DHip or DStr at different levels
644 of anteriority. Data are shown in Supplementary Figure 12. ANOVA (Protocol X
645 Duration X Anteriority) showed no other significant effect than an effect of Anteriority
646 in the DStr (F $_{(6,252)}$ = 114.7, $p < 0.001$) and DHip (F $_{(6,306)}$ = 136.1, $p < 0.001$). This
647 effect reflected a diffusion decrease as a function of distance from the infusion site.
648 No other single factor effect (Protocol, Duration) and none of the different interactions
649 were significant. Typical examples of MUSC inactivation effects on c-Fos expression
650 are shown in Supplementary Figure 13.

651

652 ### *3.2.3. Drug infusion-free task acquisition regardless of training duration*

653 Acquisition performance was comparable among experimental conditions.
654 Data are shown in Figure 7. In the rats with intrastriatal cannulae and given 6 training
655 days, there was a significant Duration effect (F $_{(5/205)}$ = 124.6, $p < 0.001$), but no effect
656 of Protocol (F$_{(2/41)}$ = 3.00) on latencies; the interaction between the two factors was
657 significant (F $_{(10/205)}$ = 1.9, $p < 0.05$). The Duration effect was due to overall
658 performance that improved significantly over the first three days. In the rats with
659 intrahippocampal cannulae, we only found a significant Duration effect (F $_{(5/300)}$ =
660 155.7, $p < 0.001$) reflecting overall performance improvement over the first three
661 days, not afterwards. For the longest training period (14 days), there were significant
662 Protocol (F $_{(2/47)}$ = 6.7, $p < 0.01$) and Duration (F $_{(13/611)}$ = 152.2, $p < 0.001$) effects in
663 the rats with intrastriatal cannulae. The Duration effect reflected an improvement of

664 overall performance, mainly over the first four days. In the rats with the cannulae

665 implanted in the hippocampus, all factors produced significant effects (Protocol: $F_{(2/46)}$ = 8.1, $p < 0.001$; Duration: $F_{(13/598)}$ = 191.5, $p < 0.001$; Protocol X Duration: $F_{(26/598)}$ = 1.6, $p < 0.05$). The Duration effect was due to an overall improvement during

668 the first three days. The analysis of the distances (not illustrated) and of the number

669 of errors (see Supplementary Figure 14) pointed to **similar** conclusions.

670   *****************************

671   **Insert Figure 7 about here**

672   *****************************

673

674   ***3.2.4. Effects of muscimol inactivation on double-H maze navigation***

675   MUSC altered performance in Lt-OFF-Resp rats, whether infused in the

676 striatum or the hippocampus. In Lt-ON-Dual rats, intrahippocampal MUSC infusion

677 disrupted the response memory after 6 training days and –at least to some extent–

678 the place memory after 14 days. In Lt-ON-Place rats, place memory was altered by

679 MUSC after 6 and 14 days of training. After sustained training, the place memory

680 system has become resistant to intrastriatal MUSC infusion. The data are illustrated

681 in Figures 8 and 9.

682

683   ***3.2.4.1. Initial swimpaths***

684   In the Lt-OFF-Resp condition, almost all rats infused with aCSF in the striatum

685 first swam to the N (i.e., showed an egocentric strategy); their proportion did not differ

686 statistically from chance (i.e., from 25%) and was not influenced by training duration.

687 Very few of them subsequently shifted to the NE arm (see supplementary Table 3).

688 MUSC infusions reduced the number of rats swimming directly to the N arm (Chi² = 4,7, $p < 0.05$ whatever the duration of training). Almost none of these rats shifted to

690 the NE after their visit to the N arm. In the Lt-ON-Dual group, most first-swim paths

691 also consisted in R-L turns after aCSF infusion. The proportion of rats then shifting to

692 the NE was weak after 6 training days, and larger after 14 days (see supplementary

693 Table 3). In Lt-ON-Dual rats given intrastriatal MUSC, the proportion of direct swims

694 to the N was not affected after 6 training days, but it was significantly reduced after

695 14 days of training (Chi² = 4.8, $p < 0.05$). Finally, after intrastriatal aCSF infusion in

696 Lt-ON-Place rats, 3 out of 7 rats swam directly to the N arm (the others swimming to

697 the NE one) after 6 days of training, but after 14 days of training, all of them first
698 swam to the N arm. MUSC did not disrupt this proportion significantly. Under MUSC,
699 however, none of the rats that swam directly to the N shifted to the NE when tested
700 after 6 training days; this shift capability was not affected by MUSC after 14 days of
701 training. Regarding the time to exit the first visited arm, there was no significant
702 difference among groups (supplementary Figure 15).

703 When aCSF was infused in the dorsal hippocampus, a large majority of rats first
704 swam directly to the N arm, whatever the training condition. After intrahippocampal
705 MUSC, this behavior was significantly reduced in Lt-OFF-Resp rats (6 days: Chi² =
706 8.8, $p < 0.01$; 14 days: Chi² = 6.5, $p < 0.05$). The same was observed in Lt-ON-Dual
707 rats (6 days: Chi² = 5.6, $p < 0.05$; 14 days: Chi² = 5.6, $p < 0.05$). Finally, in Lt-ON-
708 Place rats, intrahippocampal MUSC reduced the number of rats swimming directly to
709 the N after 6 days of training (Chi² = 4.7, $p < 0.05$), but not after 14 days of training.

710   Overall, this analysis suggests that MUSC in one or the other memory
711 structure led a proportion of the rats toward the 'Other' category of behaviors (i.e.,
712 neither response nor place strategy), most probably pointing to a general disruption
713 of the memory-based performance, whatever the memory.

714   *****************************

715   **Insert Figure 8 about here**

716   *****************************

717

718   ***3.2.4.2. Cumulated time in R-L turn and NE arms***

719   As in our first experiment, the probe trial performance analyses were refined
720 by considering quantitative variables: **i)** cumulated time spent in arms to which a R-L
721 turn had led regardless of which arm was entered (response memory variable), and
722 **ii)** cumulated time spent in the NE arm (place memory variable). Again, times
723 resulting from an entry in NE when a rat was coming from S were not considered.
724 Data are shown in Figure 9. As shown in Supplementary Table 4, swim velocities
725 during the probe trial did not differ among treatment groups. Time spent in the first
726 arm visited, and time spent in the N arm or in the NE one after the first visit is
727 illustrated in supplementary Figures 15 and 16.

728   Altogether, our data confirm that the possibility to shift to a spatial strategy
729 (and thus a strategy-correction capacity) emerges with increasing training duration

730 (data from aCSF-treated rats). In Lt-OFF-Resp rats, intrastriatal and
731 intrahippocampal MUSC infusions disrupted (or tended to do so) the response
732 memory-based behavior (time in arms after R-L turns); the place memory-based one
733 (time in NE arm) was not different from chance. In the Lt-ON-Dual rats, the
734 intrastriatal infusion of MUSC did not interfere with place memory after 14 days,
735 whereas intrahippocampal MUSC infusion disrupted the response memory after 6
736 and 14 days of training. In Lt-ON-Place rats, intrastriatal infusion of MUSC disrupted
737 the place memory system after 6 days of training, and intrahippocampal infusion did
738 so for both training durations.

739

740   ***3.2.4.3. Cumulated time in arms reached by R-L turns:***
741   MUSC disrupted response memory performance, especially when infused into
742 the dHIP. Data are shown in Figure 9.
743 After intrastriatal infusions, the time spent in the R-L arm was above chance in aCSF-
744 treated Lt-OFF-Resp and Lt-ON-Dual rats after 6 days of training ($p < 0.05$), and only
745 in Lt-OFF-Resp rats after 14 days of training ($p < 0.05$). In rats infused with MUSC, it
746 never exceeded chance significantly. MUSC-induced effects were further analyzed
747 with a MUSC x Duration ANOVA for each training protocol. MUSC infusions into the
748 DStr induced an overall impairment of response-memory-based behavior only in Lt-
749 OFF-Resp rats ($F_{(1,28)} = 8.12$, $p < 0.05$). Multiple comparisons showed that the
750 difference was significant after 6 ($p < 0.05$; Tuckey test) not 14 days of training..

751

752   After intrahippocampal infusions, the time spent in the R-L arm was above
753 chance in aCSF-treated Lt-OFF-Resp and Lt-ON-Dual rats after 6 days of training (p
754 < 0.05), and in Lt-OFF-Resp rats after 14 days of training ($p < 0.05$). In rats infused
755 with MUSC, it never exceeded chance significantly. In Lt-ON-Place rats, MUSC had
756 no significant effect, whatever the training duration. When infused into the
757 hippocampus, MUSC altered the behavior in Lt-OFF-Resp rats ($F_{(1,31)} = 10.74$, $p <$
758 0.01) and in Lt-ON-Dual ones ($F_{(1,26)} = 18.34$, $p < 0.01$); there was no significant
759 effect of Duration and no interaction between both factors. Multiple comparisons
760 showed the MUSC effect to be significant after both training durations in Lt-OFF-
761 Resp rats ($p < 0.05$; Tuckey test). In Lt-ON-Dual rats, the difference was significant

762 after 6 days of training (p < 0.05; Tuckey test), and only tended towards significance

763 after 14 days (p = 0.066; Tuckey test).

764

765   *****************************

766   **Insert Figure 9 about here**

767   *****************************

768

769   *3.2.4.4.Cumulated time in NE arm:*

770   Overall, MUSC disrupted place memory performance. Data are shown in

771 Figure 9.

772   The time in the NE arm exceeded chance in aCSF-treated Lt-ON-Place rats

773 after 6 and 14 days of training (p < 0.05*),* whether subjected to intrastriatal or

774 intrahippocampal infusions*.* Only after 14 days of training did this time also exceed

775 chance in Lt-ON-Dual rats. After MUSC infusion, time in the NE arm did not differ

776 from chance, except when MUSC was infused in the striatum of Lt-ON-Dual and Lt-

777 ON-Place rats after 14 days of training. ANOVA showed that when infused into the

778 striatum, MUSC reduced place-memory-based performance (time in NE arm) in Lt-

779 ON-Place rats, but only after 6 days of training (p < 0.001). When MUSC was infused

780 into the hippocampus, there was a significant overall impairment in Lt-ON-Resp rats

781 ($F_{(1,31)}$ = 7.66, *p* < 0.05) and Lt-ON-Place rats ($F_{(1,30)}$ = 24.5, *p* < 0.001), but in Lt-

782 ON-Dual rats only a tendency was noticed ($F_{(1,26)}$ = 3.56, *p* = 0.07). In Lt-ON-Resp

783 rats, the MUSC effect was significant only after 6 days of training (p < 0.001; Tuckey

784 test). In Lt-ON-Place rats, it was significant after both training durations (p < 0.05;

785 Tuckey test).

786

787 **4. Discussion**

788

789   In tasks with dual place/response solution, the place memory system is the

790 first to find the solution, the response memory system coming into play later on (e.g.,

791 Packard and McGaugh, 1996). We therefore expected Lt-ON-Dual rats to use their

792 place memory system first. Our results point to a different outcome. We also

793 expected that hippocampal c-Fos expression would increase when place memory is

794 used, thus at an early stage of training, and that striatal c-Fos expression would

795 increase later on when response memory is used. Our results only partly support
796 these expectations. We predicted that striatal blockade would affect response
797 memory, whereas hippocampal blockade would affect place memory, as e.g., in
798 Packard and McGaugh (1996). We found that striatal inactivation altered response
799 memory in Lt-OFF-Resp rats. Furthermore, for both training durations, hippocampal
800 inactivation affected performance in Lt-ON-Place and, although to a weaker degree,
801 in Lt-ON-Dual rats, as expected, but also surprisingly in Lt-OFF-Resp rats. These
802 data point to memory systems that interact in more complex ways than considered so
803 far. To some extent, they also challenge the notion of a hippocampus-independent
804 response memory and a striatum-independent place memory.

805

806 **4.1.** **In some conditions, the dorsal striatum memory system may guide**
807 **behavior faster than the hippocampal memory system**

808 Training in darkness promoted the use of response learning, while training in
809 light proposed a dual solution paradigm, in which rats used a response strategy at
810 the early stages of training and then switched to a place strategy. This result
811 contradicted predictions based on Packard and McGaugh (1996), who found in a
812 food-rewarded plus-maze dual solution task that rats tended to use a spatial strategy
813 before shifting to a response one. Our results, however, are in line with a report by
814 Asem and Holland (2013). In an escape-motivated, submerged T maze, rats had to
815 repeatedly swim to the same target. Their first approach of the task was egocentric;
816 spatial skills arose later on. Asem and Holland (2013) proposed that escaping from
817 water is more stressful than approaching food, and that stress-related mechanisms
818 may have disadvantaged hippocampal functions (Kim & Diamond, 2002; see also
819 Vogel et al., 2017 for data in humans). Interestingly, in mice trained in a water maze
820 with a cued platform, Martel et al. (2007) also found that "*the hippocampus was not*
821 *the first to provide solution*". It is noteworthy that with a kind of appetitive and dry
822 variant of our double-H maze (alias the 'Opposing Ts maze'), rats trained to reach the
823 same goal from the same start point first used an allocentric strategy and came to the
824 egocentric one in a second time (Gardner et al., 2013). Taken together, these
825 findings provide support to Asem and Holland's proposal, as other studies also do
826 (e.g., Packard and Goodman, 2013; Schwabe, 2013). Because cues influence the
827 type of strategy adopted (e.g., Packard and Goodman, 2013), an alternative

828 explanation could be that allothetic cues were less salient in the environment of our
829 double-H apparatus than in that of Packard and McGaugh's elevated plus maze,
830 biasing the Lt-ON-Dual rats toward an egocentric solution. However, under exactly
831 the same illumination conditions, Lt-ON-Place rats used allothetic cues already after
832 1 day of training. It could also be that navigation of Lt-ON-Dual rats relied on
833 nonvisual cues such as music playing from the unique loudspeaker in the room (e.g.,
834 Save et al., 1998; Zhang & Manahan-Vaughan, 2015). If so, however, loudspeaker-
835 based guidance should also have been beneficial to Lt-OFF-Resp rats, which was
836 not the case.

837

838 *4.2. c-Fos expression in the dorsomedial striatum is marked regardless of the*
839 *training protocol/duration*

840   In the current study, regardless of training duration, we found enhanced
841 expression of c-Fos in the DMS and DLS of Lt-OFF-Resp and Lt-ON-Dual rats, as
842 well as in the DMS of Lt-ON-Place rats (vs. HC). Using a striatum-dependent cued or
843 hippocampus-dependent spatial version of a water maze task, Teather et al. (2005)
844 found that the c-Fos expression increase in the DMS was undistinguishable between
845 cued and spatial rats, suggesting comparable impact of swimming behavior. In the
846 current study, part of the c-Fos expression in DMS could therefore be due to
847 swimming behavior, whatever the training conditions. In the dark condition, c-Fos
848 expression was highest in DMS and DLS, the absence of visual information from
849 allothetic cues leaving no alternative to the response memory-based strategy. Blind
850 rats (*Spalax ehrenbergi*) can form a primitive map by gradual calibrations over
851 progressive explorations of the perimeter of their testing environment (e.g., Avni et
852 al., 2008). Such rats, however, are blind at birth and develop navigation strategies
853 compensating for their congenital lack of vision, a huge difference with our sighted
854 Long-Evans rats trained and tested in darkness. Interestingly, in Lt-OFF-Resp rats, c-
855 Fos decreased after 14 compared to 6 days in the DMS, and increased after 6
856 compared to 1 training days in the DLS. Devan and White (1999; see also Devan et
857 al., 1999) were the first to show differences between the behavioral functions of the
858 DMS and DLS. Our results are compatible with i) DMS controlling action outcome
859 and participating in goal-directed actions, including navigation corrections and habit
860 formation, and ii) DLS supporting the storage of habits and stimulus-response

861   learning (e.g., Balleine & O'Doherty, 2010; Devan and White, 1999; Hawes et al.,

862   2015; Ito & Doya 2015; Pauli et al., 2012). Indeed, this view predicts a shift of the

863   highest activation from DMS to DLS over training in Lt-OFF-Resp rats. In the DHip of

864   our Lt-ON-Dual rats, a c-Fos increase was found in CA3 and the DG only after 6 days

865   of training. We do not known why, but it is tempting to speculate that these regions

866   could have contributed to the transition from a response memory-based behavior to a

867   place memory based one. CA1 was the only region to show a number of c-Fos-

868   positive neurons largely above controls. This difference, however, was found only

869   after 1 and 6 training days, suggesting that once navigation relied on a place

870   strategy, memory retrieval occurred at a substantially weaker neuronal activation cost

871   in the hippocampus (e.g., Bertaina-Anglade et al., 2000; Shires & Aggleton, 2008).

872   This possibility is compatible with the fact that in Lt-ON-Place rats, c-Fos levels were

873   not different from controls already after 6 days of training. An alternative explanation

874   would be that, over training, the task ceased to depend on the hippocampus, a

875   hypothesis contradicted by our MUSC data. Using a protocol close to that of Packard

876   and McGaugh (1996) in mice, Passino et al. (2002) observed a c-Fos expression that

877   was less pronounced in CA1 (about 45%) after a long training period (18 days; 72

878   trials) compared to a shorter one (9 days; 36 trials). Unfortunately, c-Fos counts were

879   not distinguished according to whether mice were response or place learners. This

880   study nevertheless indicates that well-trained performance is not necessarily

881   correlated with high neuronal activation indexes in the structure presumed to support

882   performance. Another point in Lt-ON-Dual rats was the high c-Fos expression level in

883   the DStr (DMS and DLS) after 1 day of training, which was associated with high c-

884   Fos expression in CA1. The DStr activation most probably reflected the predominant

885   engagement of the R-L turns the rats had learned on the previous day. Upon

886   negative feedback during the probe trial, these rats might have tried to shift to a

887   hippocampal-driven correction, which failed because after 1 day of training, the place

888   memory trace may have been too weak or absent. Indeed, we know that, in the water

889   maze, a 1-day training encompassing 4 consecutive trials does not enable above

890   chance performance in a probe trial given on the next day (e.g., Bousiges et al.,

891   2013, see Fig 1A). Another explanation for the decrease in hippocampal c-Fos

892   expression seen in Lt-ON-Dual rats (between 6 and 14 training days) could be that

893   rats have developed an alternative to the place memory strategy. For instance, they

894   might have learned to reach the NE arm by swimming in the central corridor until

895   facing a wall; once there, they just had to turn on their left. Finally, we cannot exclude

896   that stress inherent to our test has impacted the way rats have tried to solve their

897   respective task, hence their c-Fos activation patterns, and that this factor accounts

898   for differences between expected and observed results. Further experiments,

899   however, perhaps with modified testing device/protocols, are required to explore

900   these issues. Whatever may be, some of our functional imaging results suggest that,

901   in well-trained and well-performing rats, c-Fos expression within the behaviorally-

902   relevant structures may not accurately parallel performance.

903

4.3. Striatal-hippocampal interactions in supporting memory-based behavior

905   Given the literature (e.g., Packard & Goodman, 2013), the disruption of **i)**

906   response memory-based strategy by intrastriatal infusions of MUSC in Lt-OFF-Resp

907   rats, and **ii)** place memory-based strategy by intrahippocampal infusions of MUSC in

908   Lt-ON-Dual and Lt-ON-Place rats was expected. Because after 14 days of training

909   intrahippocampal infusions of MUSC disrupted the place memory-based strategy,

910   which intrastriatal MUSC infusions left unaltered, Lt-ON-Dual rats were relying on

911   hippocampal function after sustained training, as was the case for Lt-ON-Place rats.

912   Not expected – but observed – were **i)** the disruption of the response memory-based,

913   strategy by intrahippocampal MUSC in Lt-OFF-Resp rats after both training

914   durations, and **ii)** given Packard and McGaugh (1996), the absence of an egocentric

915   memory-based deficit in Lt-ON-Dual rats in response to intrastriatal MUSC after 14

916   days of training. The MUSC-induced alteration of performance in Lt-ON-Place after 6

917   days of training was also not expected. Taken together, these results suggest that, in

918   the double-H maze task, the use of a response strategy may depend on both the

919   dorsal striatum and the hippocampus, which do not become functionally independent

920   from each other in Lt-OFF-Resp rats, even after long training. The use of an

921   allocentric strategy also depends on both structures, but transiently. Indeed, following

922   sustained training, the DStr is no longer needed for navigation correction, but it

923   seems important for the acquisition of a spatial approach to a task (Jacobson et al.,

924   2012; Pooters et al., 2015, 2016). In case of a repetitive navigation task with full

925   access to allothetic cues, the DHip appears more crucial than the DStr, first to enable

926   a preferential engagement of the response memory-based system for up to 6 training

days, and later on, between 6 and 14 days, to use the place memory-based system. In Lt-ON-Dual rats, the two systems could disengage from each other after sustained training, but both seem to be needed during the first training days. These observations are compatible with – and therefore reinforce – recent literature showing engagements of the striatum and hippocampus for adapting behavior to navigation task demands (e.g., Berke et al., 2009; Chersi & Burgess, 2015; Eshenko & Mizumori, 2007; Mizumori et al., 2004; Regier et al., 2015). Yet, why the DStr blockade altered spatial memory (even in Lt-ON-Place rats after 6 days of training) and the DHip blockade altered procedural memory cannot be elucidated from our present data. Alterations of spatial memory in a cross maze have been reported after DStr lesions, and alterations of response memory have been observed after hippocampal damage (Jacobson et al., 2012). Along this line, Kathirvelu and Colombo (2013) reported that lentiviral-mediated increase of CREB expression in the DStr enhanced memory for cue learning, but also for context in fear conditioning, and context memory is typically hippocampus-dependent; place learning, however, was impaired. In a recent study, Ferbinteanu (2016) trained rats in a hippocampus-dependent spatial task or a dorsal striatum-dependent cue-response task, as compared with rats that were trained in both. All rats were then subjected to permanent excitotoxic lesions of the DMS or DLS, or of the hippocampus. DMS and hippocampal lesions produced marked retention deficits in rats trained in only the spatial task. In rats trained in only the cue-response task, both types of striatal lesions, but not hippocampal ones, produced marked deficits. Most interestingly, however, when both tasks were acquired concurrently, all lesions induced marked deficits. These observations suggest that when a unique task is learned, the corresponding memory is constructed in the most appropriate system (e.g., place) and does not depend on the other system (e.g., response; see also White et al., 2013). When the two tasks are learned concurrently, however, not only are memories constructed in each memory system, but they also seem to be linked to each other in a way that makes it possible to alter performance by obliterating either memory system. Our results suggest that the same might be true in our Lt-ON-Dual rats when, over learning, they shifted from one to the other memory system. Why, then, could response memory in the double-H task be also depending on the dorsal hippocampus? A possibility would be that the habit was processed by the striatum

960 (e.g., DMS to form it, DLS to store it) and the overall geometry (spatial calibration;

961 see Avni et al., 2008) of the maze by a mechanism partly implicating the

962 hippocampus. Both information aspects may have combined in a modular

963 representation (e.g., Tcheang et al., 2011).

964   Another possibility could be related to mechanisms of navigation in darkness,

965 which, regarding self-motion information in goal-directed behavior, involves the

966 cerebellum and its functional connection with the hippocampus (e.g., Rochefort et al.,

967 2011). This functional connection might have been disrupted by dorsal hippocampus

968 inactivation. Why could spatial memory be depending on the dorsal striatum after 6

969 (and not 14) days of training? Given its location, our MUSC inactivation affected

970 partly the DMS and partly the DLS (see supplementary figure 11). Furthermore,

971 correcting a response memory-based strategy by using a place memory-based one

972 requires behavioral flexibility driven by the prefrontal cortex (e.g., Cholvin et al.,

973 2013). Because the DMS is involved in action outcome and behavioral flexibility,

974 notably through its connections with the (pre)frontal cortex (e.g., Baker and

975 Ragozzino, 2014; Ragozzino et al., 2002), it is well possible that MUSC has affected

976 one or both of these functions, perhaps even without affecting hippocampus-

977 dependent spatial memory processes per se. Interestingly, Ragozzino (2003) found

978 that DMS cholinergic interneurons contributed to behavioral flexibility, which is further

979 supported in the studies by e.g., Aoki et al. (2013; but see Okada et al., 2014, or

980 Braun and Hauber, 2011; Braun et al., 2012). Still along these lines, we reported that

981 reversible inactivation of the prefrontal cortex, which did not alter spatial memory

982 retrieval in a water maze task, profoundly disrupted strategy adaptation in the double-

983 H maze (Cholvin et al., 2013). Indeed, after MUSC inactivation of the prefrontal

984 cortex, rats were unable to shift from the response-based to the place-based

985 strategy. A functional alteration of the prefrontal cortex being a potential

986 consequence of dorsal striatum inactivation, it is possible that something similar

987 occurred in our Lt-ON-Dual rats. This possibility is in line with a report showing that

988 the DMS plays a role in adapting a habitual strategy to a sudden modification of the

989 contingency in a spatial task (Regier et al., 2015).

990   Based on our findings, it is not possible to provide clear and solid arguments

991 to explain the discrepancy between our expectations and the observed results. It is

992 noteworthy that striatal inactivation affected the DMS and DLS, when previous

993 studies used inactivation of one or the other of these regions. Alterations of both the
994 DMS and DLS should conjointly disrupt control of action outcome, goal-directed
995 actions, habit retrieval, and expression of stimulus-response learning. These
996 modifications might have weakened the expression of place memory. Furthermore,
997 as many of the experiments leading to the view positing a functional dichotomy
998 between response and place memory systems have been carried out in appetitive
999 tasks, it cannot be excluded that stress linked to the aversive motivation in the
1000 double-H maze has been a major actor of this discrepancy. Addressing this
1001 possibility requires experiments in which, using a same device (e.g., double-H or
1002 cross maze), rats would be compared for c-Fos expression and inactivation effects
1003 according to whether training motivation is aversive or appetitive.

1004 In environments more complex than a T- or cross-maze, spatial calibration
1005 may remain necessary over repetitive tasks, even in the absence of visual cues (e.g.,
1006 based on perimeter exploration as in Avni et al., 2008). If so, this calibration might
1007 require, in addition to a contribution of the striatum, some mechanisms orchestrated
1008 by the hippocampus. From our inactivation approach, it is possible to speculate about
1009 some mechanisms compatible with our observations. When rats have to reach a goal
1010 in a maze like the double-H, their constrained navigation may be supported by a
1011 multimodal representation resulting from both allothetic visual inputs (when cues are
1012 visible) and idiothetic motion cues (of e.g., proprioceptive or kinesthetic nature),
1013 including experience of the borders of the maze (e.g., Tcheang et al., 2011). It is
1014 noteworthy that idiothetic cues can be used for path integration (Cheung et al., 2012).
1015 That the hippocampus contributes to the processing of allothetic visual cues and the
1016 striatum to that of idiothetic motion cues is not nonsense and, as such, both
1017 structures may conjointly participate in the construction of this multimodal
1018 representation. It is known that path integration and border information, when
1019 combined, can support hippocampus-dependent spatial representations in darkness
1020 (Zhang et al., 2014). This could be a reason why, in darkness, the inactivation of the
1021 hippocampus altered performance (time in R-L arm) similarly to striatal inactivation.
1022 Lt-ON-Dual rats roughly behaved as Lt-OFF-Resp rats after 6 days of training. At this
1023 time point, their navigation system may have been on the way to rely on the
1024 multimodal representation, from which it later on shifted to the visual representation,
1025 as there was no effect of intrastriatal MUSC after 14 days of training.

1026

1027 ### *5. Conclusion*

1028   In this study, predictions based on our current understanding of response and
1029 place memory systems have not been verified in extenso. Indeed, when cues were
1030 visible, the rats first acquired a response memory-based behavior and only later on
1031 could navigate by using place memory. Our results suggest that the striatum and the
1032 hippocampus are both required when rats have to retrieve a repetitive maze-
1033 navigation task in the double-H maze, be allothetic cues visible or not. In case of
1034 visible cues, however, with extensive training (14 days), retrieving the task is entirely
1035 hippocampus-dependent. Thus, the degree to which the striatum and the
1036 hippocampus contribute to navigation behavior, including navigation correction in
1037 response to negative feedback, depends on previous training level and cue
1038 availability. Our data point to related systems, in line with recent findings in both
1039 animals (Delcasso et al., 2014; Jacobson et al., 2012; Rice et al., 2015) and humans
1040 (e.g., Brown & Stern, 2014; Woolley et al., 2015). These systems may operate in a
1041 baton-passing way under some task constraints (task repetitiveness, cue availability,
1042 and training duration), and in a different way under other constraints. Disruption of
1043 the DStr affects place memory retrieval, and thus navigation correction capacities,
1044 following moderate training (6 days) in a spatial task, in line with a role of this
1045 structure in allocentric navigation. Inactivating the DHip affects retrieval of response
1046 memory following both moderate (6 days) and extensive (14 days) training in an
1047 egocentric task. These findings qualify our current knowledge and call for further
1048 research on the implication of striatal and hippocampal mechanisms in goal
1049 navigation and navigation correction; there might also be a need to consider the
1050 communication of these structures with other brain regions (e.g., prefrontal cortex;
1051 Cholvin et al., 2013; Dahmani & Bohbot, 2015).

1052

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Figure captions

<u>Figure 1</u>: Summary of the experimental protocol used in Experiment 1. (a) Rats were trained for 1, 6 or 14 days in the double-H water maze. The escape platform was hidden at the extremity of the NE arm. Each trial lasted for a maximum of 60 s. **(b)** Three training protocols were used. In the first protocol (Lt-OFF-Resp; left), rats serving as controls were trained in a darkened room lit by red light (1 lux) to prevent the use of landmarks. On all trials, they were released from the S arm and had to swim to the NE arm. This protocol promoted a response memory-based body-turn strategy. In the second protocol (Lt-ON-Resp; middle), rats were trained in the same room but with normal neon light (180 lux) to enable the use of landmarks. This protocol proposed a task with a dual body-turn/spatial strategy. In the third protocol, which promoted a spatial strategy, another group of control rats were trained in the same lit environment but for each daily trial they were released from a different arm (Lt-ON-Place; right). In all training protocols, access to the N was blocked by a guillotine door. **(c)** Twenty-four hours after the last training trial (i.e., on day 2, 7 or 15), all rats were given a probe trial which lasted 60 s. Light conditions were the same as for the training but there was no platform in the maze. All rats were released from the SW arm and the NW arm was blocked. Ninety minutes after the probe trial, the rats were killed and their brain processed for subsequent c-Fos immunostaining and stereological quantifications.

<u>Figure 2</u>: Acquisition performance: similar performance in Lt-OFF-Resp, Lt-ON-Dual and Lt-ON-Place rats. Whatever the training protocol, average latencies to platform are represented for the first training day (trials 1 to 4) and over the 6- and 14-day training sessions (daily blocks of four trials). Statistical analyses showed no significant difference among training protocols. Note that the Y axis corresponding to the first training day indicates latencies for each trial, whereas in the other two panels on the right it indicates latencies for each 4-trial block, hence the different scale. The number of animals in each condition can be found in Figure 3 underneath each pie chart.

Figure 3: **Swim paths adopted by rats in the probe trial: response memory-based behavior in Lt-OFF-Resp rats for all training durations, place memory-based behavior in Lt-ON-Place rats for all training durations vs. a shift from response memory- to place memory-based behavior in Lt-ON-Dual rats.** In white: proportion of rats that swam directly to the NE arm. Percentages of rats that swam to the NE arm after having left the N one are shown in supplementary Table 1. Statistics: * significant modification of the proportion of rats that swam directly to the N arm as shown by a Chi² analysis, $p < 0.05$. The total number of rats tested with each protocol / training duration is indicated under the corresponding pie chart.

Figure 4: **Exploration time in R-L or NE arm during the probe trial: shift from response memory- to place memory-based is confirmed in Lt-ON-Dual rats.** Average cumulated times (+s.e.m.) in the arms rats had reached by successive right (R) and left (L) turns (white bars), or in the NE arm (greyish bars) for each training protocol and each training duration (1, 6, 14 days; indicated in the white bars). The probe trial was given with a 24-h delay after the last training trial. Times in the NE arm when coming from the S arm were discarded from the analysis (see Methods and Supplementary material for an explanation). The stippled lines indicate chance level (see Methods for precisions on its computation). Statistical analyses: * significantly different from chance, $p < 0.05$; # significantly different from Lt-ON-Place, $p < 0.05$; § significantly different from the corresponding 1-d training group, $p < 0.05$. The number of animals in each condition can be found in Figure 3.

Figure 5: **Quantification of c-Fos expression: increased c-Fos expression in the striatum of Lt-OFF-Resp and Lt-ON-Dual rats, and a transient increase in the dorsal hippocampus of Lt-ON-Dual and Lt-ON-Place, but delayed decay in Lt-ON-Dual rats as compared to Lt-ON-Place rats.** Number of c-Fos positive neurons quantified stereologically in the dorsomedial (DMS) and dorsolateral (DLS) striatum, as well as in regions CA1, CA3, and dentate gyrus (DG) of the dorsal hippocampus after a probe trial for the different training durations (1, 6, and 14 days) and protocols. Statistical analyses: # significantly different from Lt-ON-Place, $p < 0.05$; § significantly different from the corresponding 1-d training group, $p < 0.05$.

1383 <u>**Figure 6:**</u> **Location of the infusion sites ; the sites were located where expected**
1384 **(rats with misplaced sites were discarded from analyses and are not illustrated**
1385 **here).** The infusion sites are indicated on coronal sections through the striatum (left)
1386 and the dorsal hippocampus (right) at various levels of anteriority according to
1387 Bregma, for each training duration (6 and 14 days). Each site corresponds to the tip
1388 of the infusion needle as identified in Lt-OFF-Resp (open circles for aCSF, black
1389 circles for MUSC), Lt-ON-Dual (open squares for aCSF, black squares for MUSC),
1390 and Lt-ON-Place (open triangles for aCSF, black triangles for MUSC) rats.
1391 Coordinates are given in mm from Bregma according to Paxinos and Watson (2007).
1392

1393 <u>**Figure 7:**</u> **Acquisition performance: very similar performance in Lt-OFF-Resp,**
1394 **Lt-ON-Dual and Lt-ON-Place rats.** In each of the three training protocols (**Lt-OFF-**
1395 **Resp, Lt-ON-Dual, Lt-ON-Place**), average latencies over the 6- and 14-day training
1396 sessions (daily blocks of four trials are presented). These rats had been implanted
1397 with intrastriatal (STRIATUM) or intrahippocampal (HIPPOCAMPUS) cannulas to be
1398 used for subsequent MUSC inactivation or control aCSF infusion. Statistical analyses
1399 did not show any significant difference among training protocols, nor among the
1400 different groups. The number of animals in each condition can be found in Figure 8.
1401

1402 <u>**Figure 8:**</u> **Initial swim paths adopted by rats at the start of the probe trial**
1403 **indicate that memory system-based behavior, whether response or place, is**
1404 **disrupted by MUSC.** In each of the three training protocols (**Lt-OFF-Resp, Lt-ON-**
1405 **Dual, Lt-ON-Place**), training lasted for 6 or 14 days and the probe trial was given 24
1406 hr after the last training day. Rats were subjected to bilateral intrastriatal
1407 (STRIATUM) or intrahippocampal (HIPPOCAMPUS) infusions of muscimol (MUSC)
1408 or artificial cerebrospinal fluid (aCSF) as control, 30 min before the probe trial. In
1409 white: proportion of rats that swam directly to the NE arm. Percentages of rats that
1410 swam to the NE arm after having left the N one are shown in supplementary Table 2.
1411 Statistics: * significant modification of the proportion of rats that swam directly to the
1412 N arm as shown by a Chi² analysis, $p < 0.05$. The total number of rats tested under
1413 each condition is indicated under the corresponding pie chart.
1414

1415 **Figure 9: Response memory is disrupted by intrastriatal (STRIATUM) and**
1416 **intrahippocampal (HIPPOCAMPUS) MUSC in Lt-OFF-Resp rats; however, the**
1417 **shift to place memory-based behavior is resistant to intrastriatal MUSC after 14**
1418 **days of training in Lt-ON-Dual and Lt-ON-Place rats.** Average cumulated times
1419 (+s.e.m.) in the arms rats had reached by successive right (R) and left (L) turns
1420 (white bars), or in the NE arm (greyish bars) for each of the three training protocols
1421 (Lt-OFF-Resp, Lt-ON-Dual, Lt-ON-Place) and each training duration (6, 14 days).
1422 The probe trial was given with a 24-h delay after the last training trial. Times
1423 consecutive to swim paths ending in the NE arm when coming from the S arm were
1424 discarded from the analysis (see Methods). The stippled lines indicate chance level
1425 (see Methods for precisions on its computation). Statistical analyses: * significantly
1426 different from chance, $p < 0.05$; # significantly different from aCSF, $p < 0.05$. The total
1427 number of rats tested under each condition is indicated in Figure 8.
1428

Figure 1

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Training duration
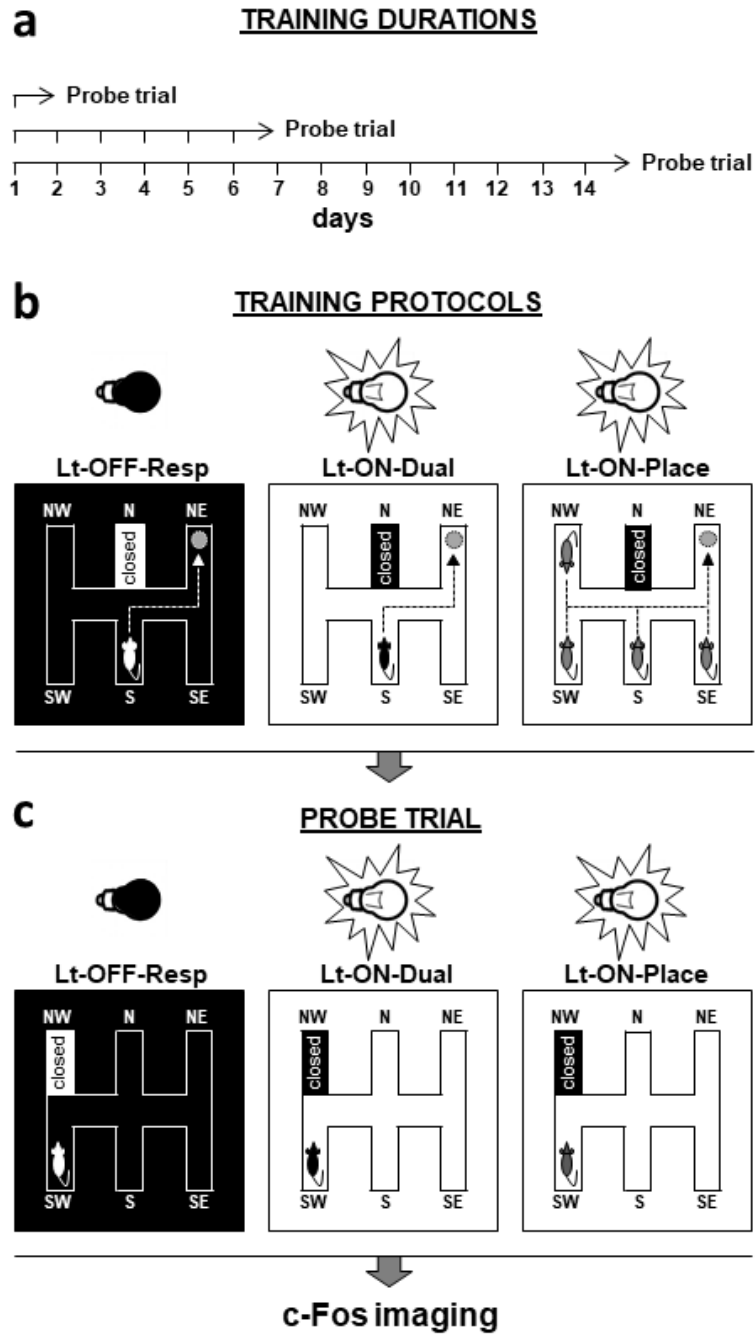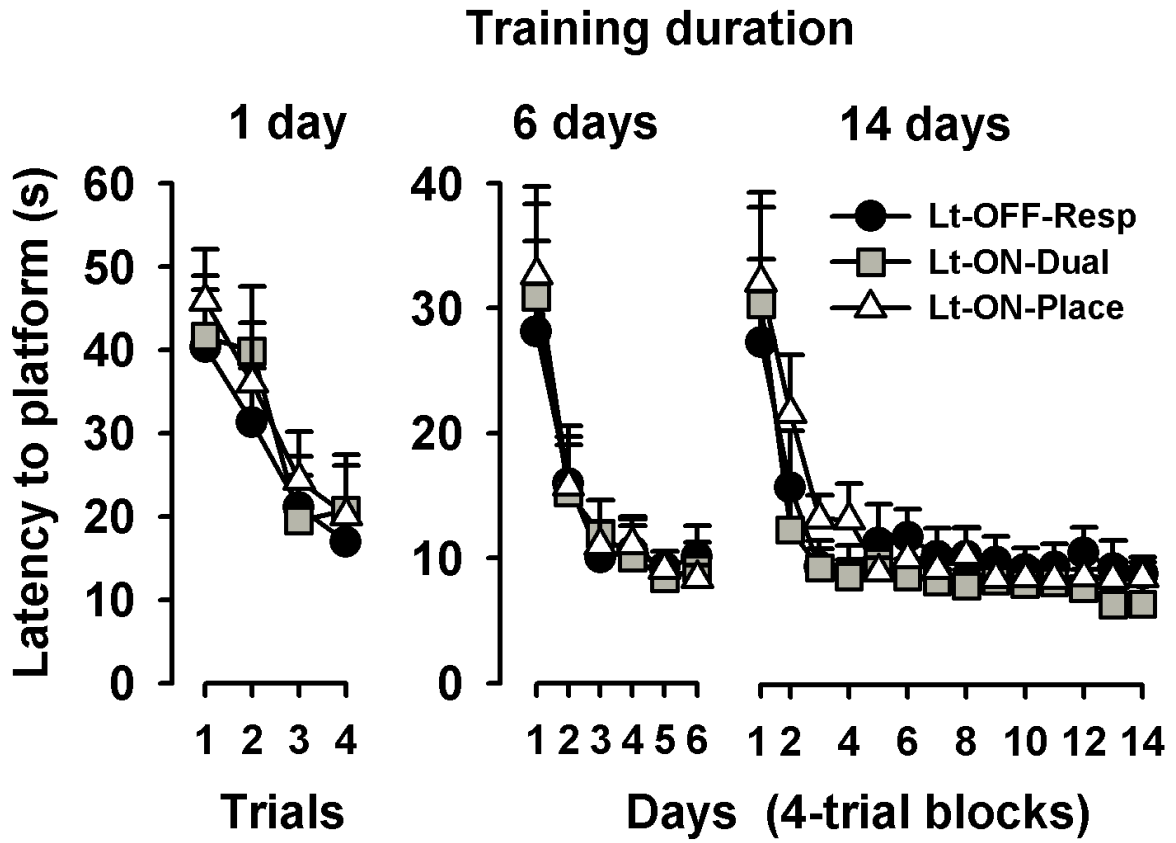
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1434   Figure 2

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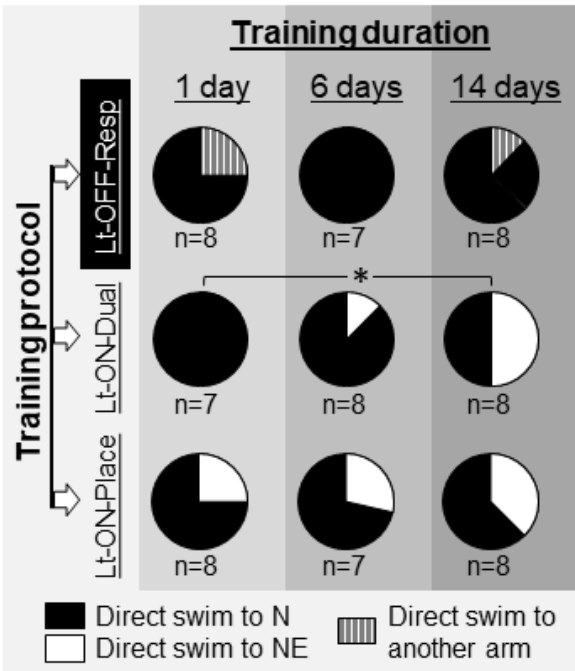
Figure 3

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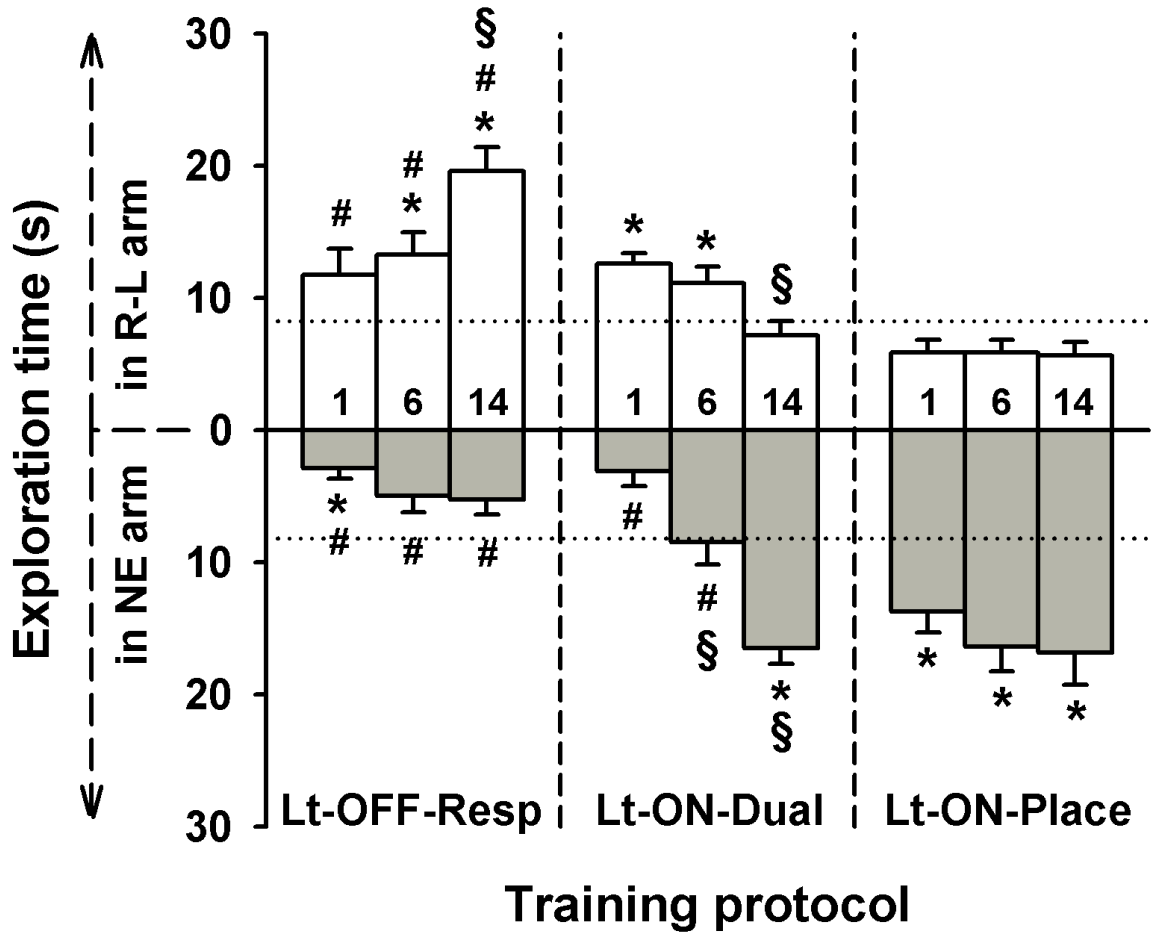
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1441   Figure 4

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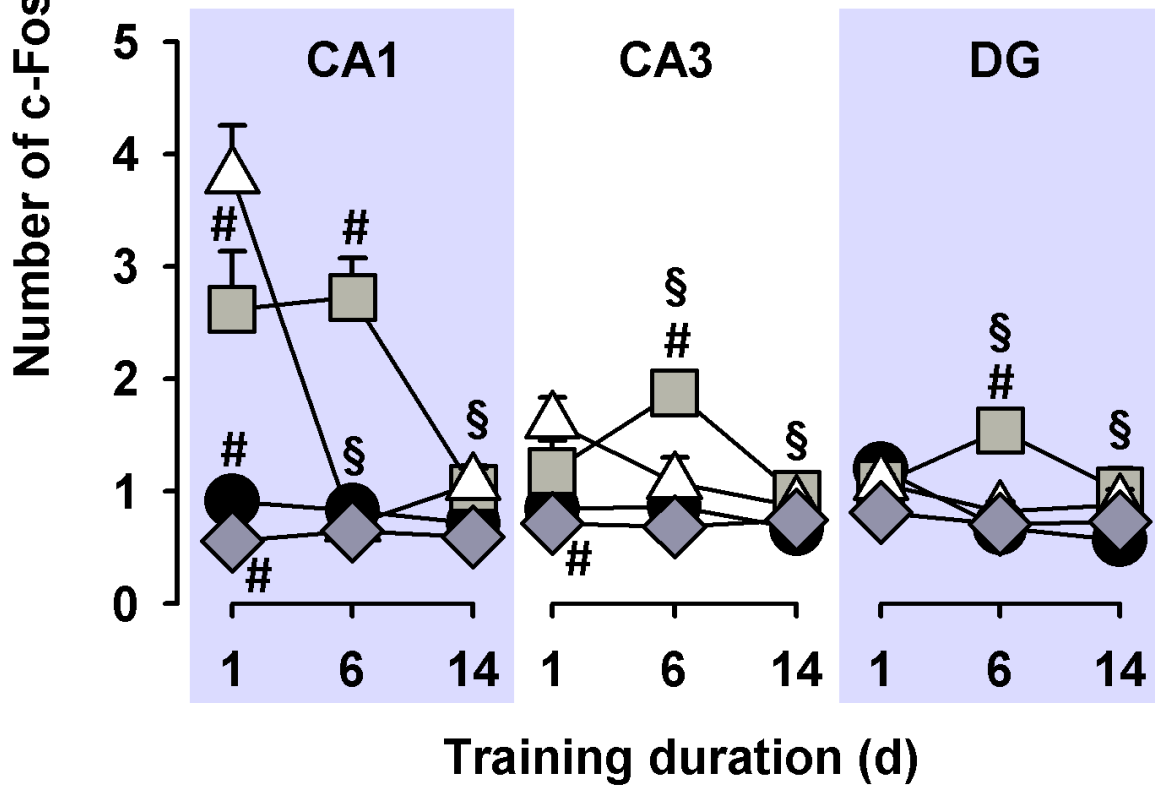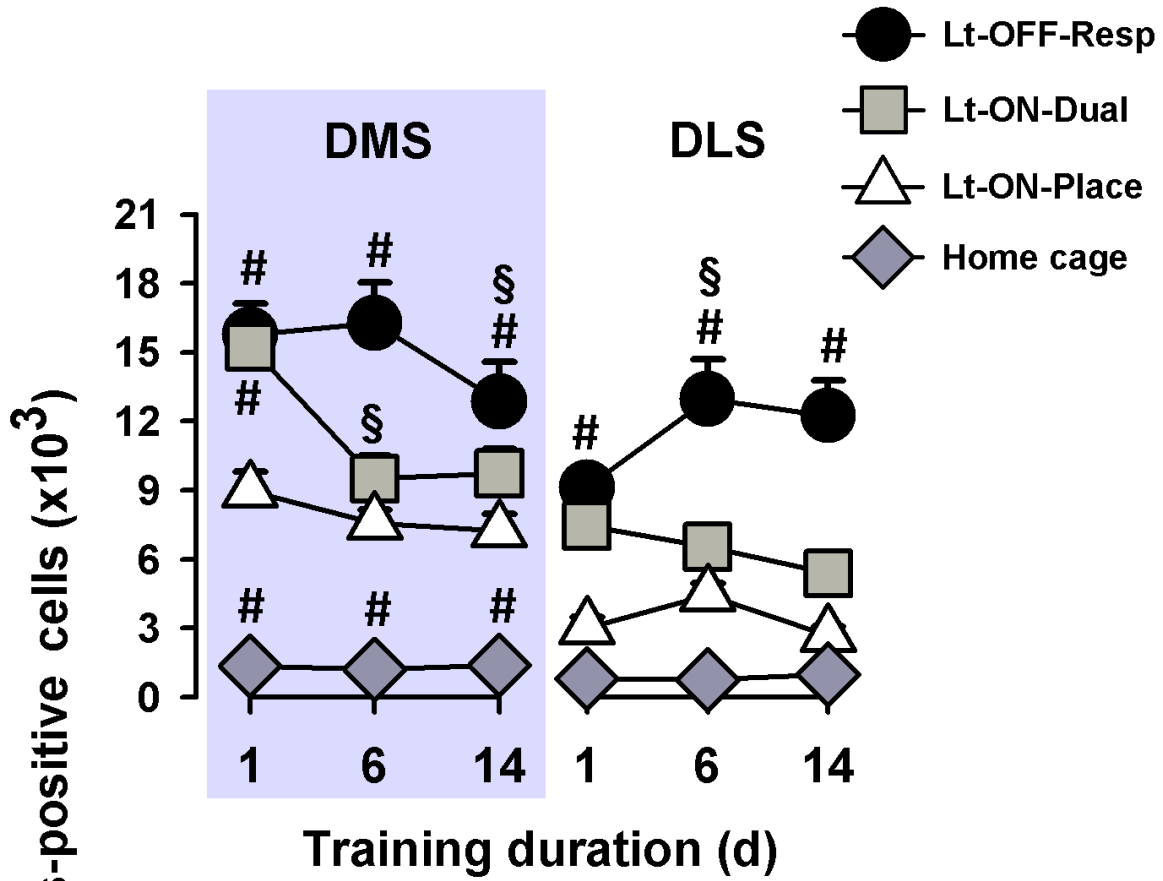
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1444

1445   Figure 5

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Figure 6

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1448

STRIATUM

Legend:
- ● Lt-OFF-Resp
- ▢ Lt-ON-Dual
- △ Lt-ON-Place

HIPPOCAMPUS

Y-axis: **Latency to platform (s)**

X-axis: **Days (4-trial blocks)**

1449

1450

1451

1452   Figure 7

1453

Figure 8

1454

1455

STRIATUM

6 days of training

14 days of training

HIPPOCAMPUS

6 days of training

14 days of training

1456

1457   Figure 9

1458

Shifting between response and place strategies in maze navigation: effects of training, cue availability and functional inactivation of striatum or hippocampus in rats

Running title: Striatum, hippocampus and navigation

Julien Gasser[1,2], Anne Pereira de Vasconcelos[1,2], Brigitte Cosquer[1,2], Anne-Laurence Boutillier[1,2], Jean-Christophe Cassel[1,2,3]

[1] Université de Strasbourg, Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA), F-67000 Strasbourg, France

[2] CNRS, LNCA UMR 7364, F-67000 Strasbourg, France

[3] **Corresponding author:** Dr. Jean-Christophe Cassel, Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA), UMR 7364, Université de Strasbourg-CNRS, 12 rue Goethe, F-67000 Strasbourg, France. E-mail: jcassel@unistra.fr

Supplementary material (25 pages)

Number of supplementary text   :   2 (Methods and References)

Number of supplementary figures   :   16

Number of supplementary tables   :   4

1483   **<u>Supplementary Methods:</u>**

1484   A trajectory going from the S to the NE arm can reflect a response (successive R-L

1485   turns) memory or a place memory. In such case, it is not possible for the

1486   experimenter to know which strategy a rat has actually used. Therefore, the times

1487   spent in the NE arm when a rat was coming from the S were not considered. In fact,

1488   only times recorded in the NE arm when rats came from SW, SE or N were

1489   considered. This correction represented in average a subtraction of 2.34 ± 0.51 s

1490   (range 0-5.5 s) in experiment 1, and of 2.22 ± 0.56 (range 0-5.6) and 1.2 ± 0,32

1491   (range 0-3.8) in rats with intrastriatal and intrahippocampal cannulas, respectively, in

1492   experiment 2. The correction was applied whatever the training protocol and duration.

1493   It was computed for each rat and subtracted from its probe trial performance (i.e.,

1494   time in target after R-L turns, and time in NE) before statistical analyses of individual

1495   scores were performed.

1496

1497 **Supplementary Figure 1:** **Subregions of the dorsal striatum and of the dorsal**

1498 **hippocampus in which the number of c-Fos positive cells was quantified.**

1499 Abbreviations: DLS: dorsolateral striatum; DMS: dorsomedial striatum; CA1: region

1500 CA1 of the cornu Ammonis; CA3: region CA3 of the cornu Ammonis; DG: dentate

1501 gyrus. Anteriority levels under each plate are indicated in mm from Bregma (Paxinos

1502 and Watson, 2007). The distinction between DLS and DMS is based on the article by

1503 Voorn et al. (2004), with the DLS corresponding essentially to the afferents from the

1504 sensorimotor cortex, and the DMS to afferents from other cortical structures (e.g.,

1505 medial prefrontal cortex, visual cortex, auditory cortex, perirhinal cortex, and

1506 entorhinal cortex).

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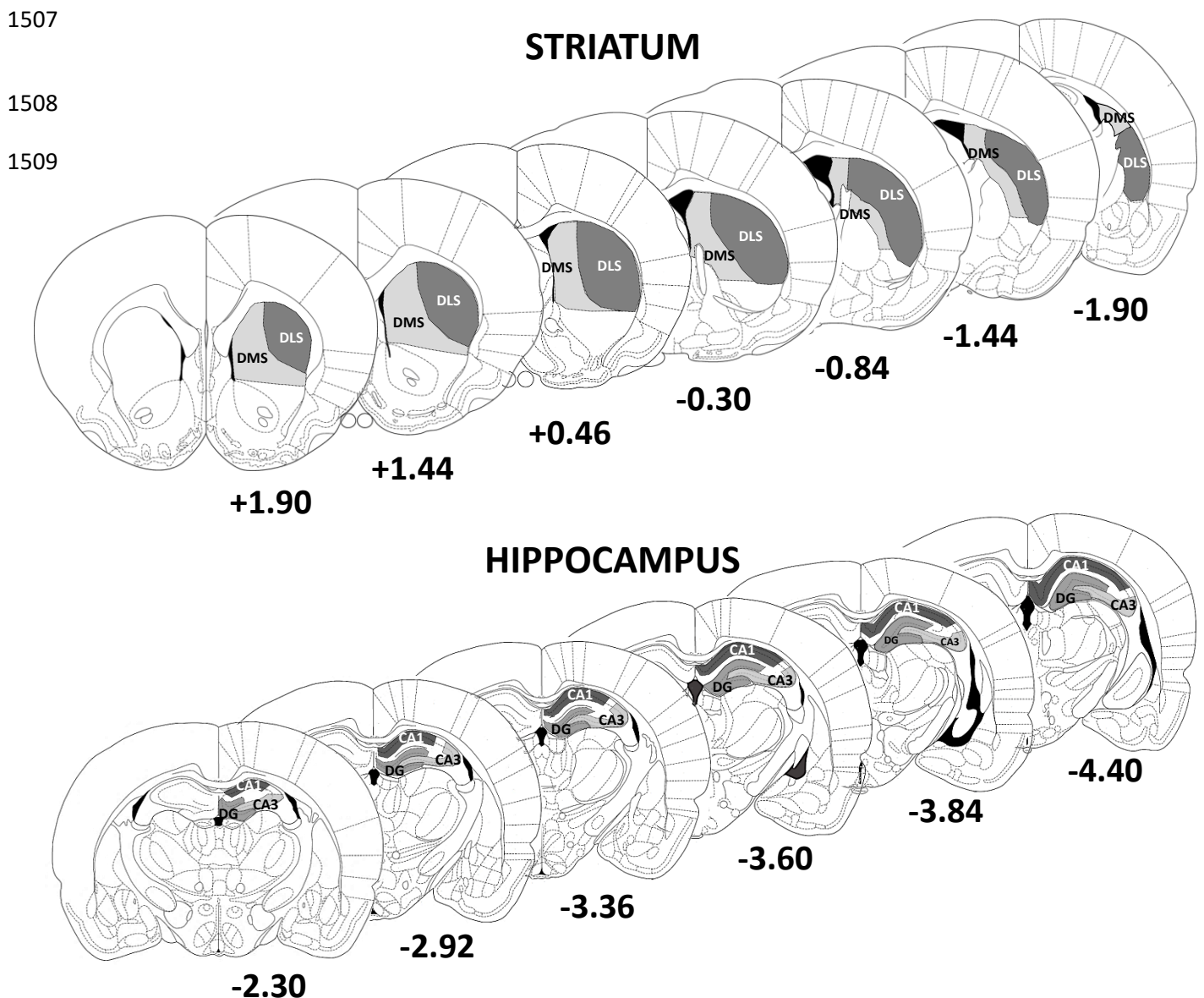
1510 **Supplementary Figure 2: Number of errors showed comparable acquisition**

1511 **curves in Lt-OFF-Resp, Lt-ON-Dual and Lt-ON-Place rats.** In each training

1512 protocol of the first experiment (Lt-OFF-Resp, Lt-ON-Dual and Lt-ON-Place), mean

1513 numbers of errors (+ sem) are represented for the first training day (trial by trial) and

1514 over the 6- and 14-day training sessions (in daily blocks of four trials). Statistical

1515 analyses showed no significant difference between the training conditions. Notice

1516 that the Y axis corresponding to the first training day indicates a number of errors for

1517 each trial whereas in the two other panels (middle, right), it indicates the mean

1518 number of errors for each 4-trial block, hence the different scales of the Y-axis. The

1519 number of animals in each condition can be found in Figure 3 of the article. Statistical

1520 analyses showed the same effect (Trial or Day) as for the latencies to reach the

1521 platform. The same was true for distances.

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Training duration
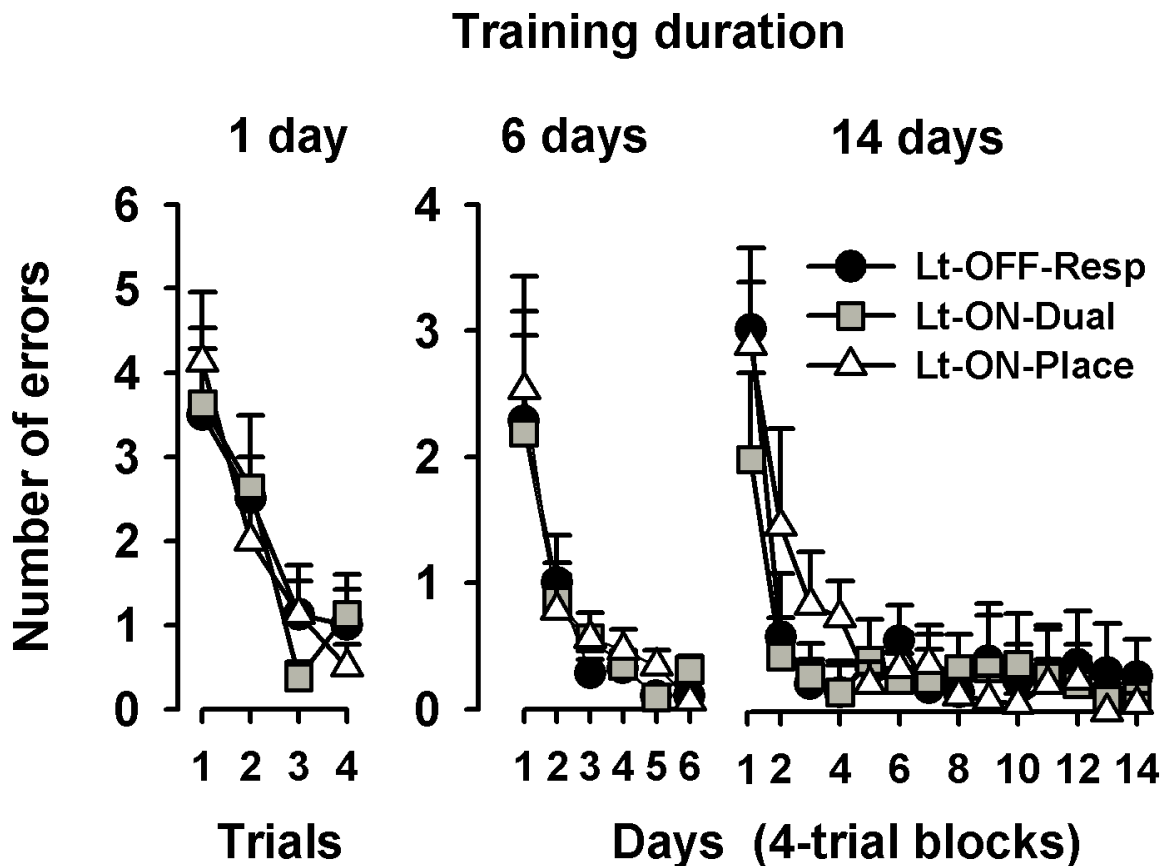
1525

1526 **Supplementary Table 1: Percent of rats shifting to the NE arm right after having**

1527 **first visited the N arm was not (significantly) different among groups.** Number of

1528 rats showing a direct swim to the N arm after having been released in the maze are

1529 indicated between brackets as "(n/ N)", the "N" indicating the group size. The table

1530 reads as follows : "of the N tested rats, % of the n rats first entering the N arm and

1531 which then shifted to the NE one". For instance, regarding Lt-OFF-Resp rats after

1532 one day of training : one should read 33% of the 6 rats out of the 8 which first entered

1533 the N arm then shifted to the NE arm.

Training protocol	Training duration		
	1 day	**6 days**	**14 days**
Lt-OFF-Resp	33 % (6/8)	14 % (7/7)	29 % (7/8)
Lt-ON-Dual	14 % (7/7)	38 % (8/8)	50 % (4/8)
Lt-ON-Place	<u>83 %</u> (6/8)	60 % (5/7)	60 % (5/8)

1534 In Lt-ON-Place rats, this proportion was significantly higher than chance after 1 day

1535 of training (Chi² = 10.89, p < 0.01), and tended to exceed chance after 6 or 14 days

1536 of training (Chi² = 3.27, p = 0.071). The significant percentage is underlined. In the

1537 other cells, there was no significant difference from chance.

1538

1539 **Supplementary Table 2: Average swim velocity were comparable during the**

1540 **probe trial among protocols and training durations.** Data indicated in cm/s are

1541 means (sem). Statistical analyses showed no significant difference among groups,

1542 indicating that other variables were not biased by changes in swim velocity.

Training protocol	Training duration		
	1 day	**6 days**	**14 days**
Lt-OFF-Resp	24.6 (1.1)	25.6 (1.0)	24.7 (1.1)
Lt-ON-Dual	25.3 (1.6)	26.1 (1.2)	24.6 (1.4)
Lt-ON-Place	25.8 (1.2)	27.2 (1.0)	25.5 (1.6)

1543 An ANOVA of the average swim velocities showed that Protocol ($F_{(2,60)}$ = 0.7, *ns*),

1544 Duration ($F_{(2,60)}$ = 0.9, *ns*) and their interaction ($F_{(4,60)}$ = 0.0, *ns*) had no significant

1545 effect.

1546

1547

1548   <u>**Supplementary Figure 3:**</u> **Latencies to the place target (i.e., the NE arm) in Lt-**

1549   **OFF-Resp, Lt-ON-Dual and Lt-ON-Place rats during the probe trial are**

1550   **compatible with the progressive formation of a cognitive map in Lt-ON-Dual**

1551   **rats.** All means are given in seconds (+ sem). This variable was computed to refine

1552   the analysis of the training-dependent evolution of strategies. We reasoned that if Lt-

1553   ON-Dual rats behaved according to an egocentric strategy incompatible with

1554   navigation correction, their latencies should be close to those of Lt-OFF-Resp rats.

1555   On the contrary, if they behaved according to an allocentric strategy enabling

1556   correction, whether direct or indirect, their latencies should be close to that of Lt-ON-

1557   Place rats. The idea behind this reasoning is that if a Lt-ON-Dual rat entered the N

1558   arm by R-L turns, it would expect to find the platform here and, consequently, spend

1559   some time looking for it in this arm. If, however, it already had some capability for a

1560   spatial approach of the task, and thus for navigation correction, but entered the N

1561   arm due to repetition of the R-L turns after starting, it would immediately leave the

1562   arm and swim to the NE one, whereby its latency to enter the NE arm should be

1563   shorter than in Lt-OFF-Resp rats. A Protocol x Duration ANOVA showed significant

1564   overall Protocol ($F_{(2,60)} = 15.5$, $p < 0.001$) and Duration ($F_{(2,60)} = 3.9$, $p < 0.05$)

1565   effects, but no significant interaction between both factors ($F_{(4,60)} = 1.5$, $p = 0.22$). A

1566   Newman-Keuls multiple comparisons test showed that Lt-ON-Dual rats did not differ

1567   significantly from Lt-OFF-Resp rats after 1 or 6 training days, but their latencies were

1568   significantly below those of their Lt-OFF-Resp counterparts after 14 training days ($p <$

1569   0.05). Conversely, latencies of Lt-ON-Dual rats were significantly larger than those of

1570   Lt-ON-Place rats after 1 or 6 training days ($p < 0.05$), not after 14 days. Statistical

1571   analysis: # different from Lt-ON-Dual rats, $p < 0.05$.

1572   (see next page)

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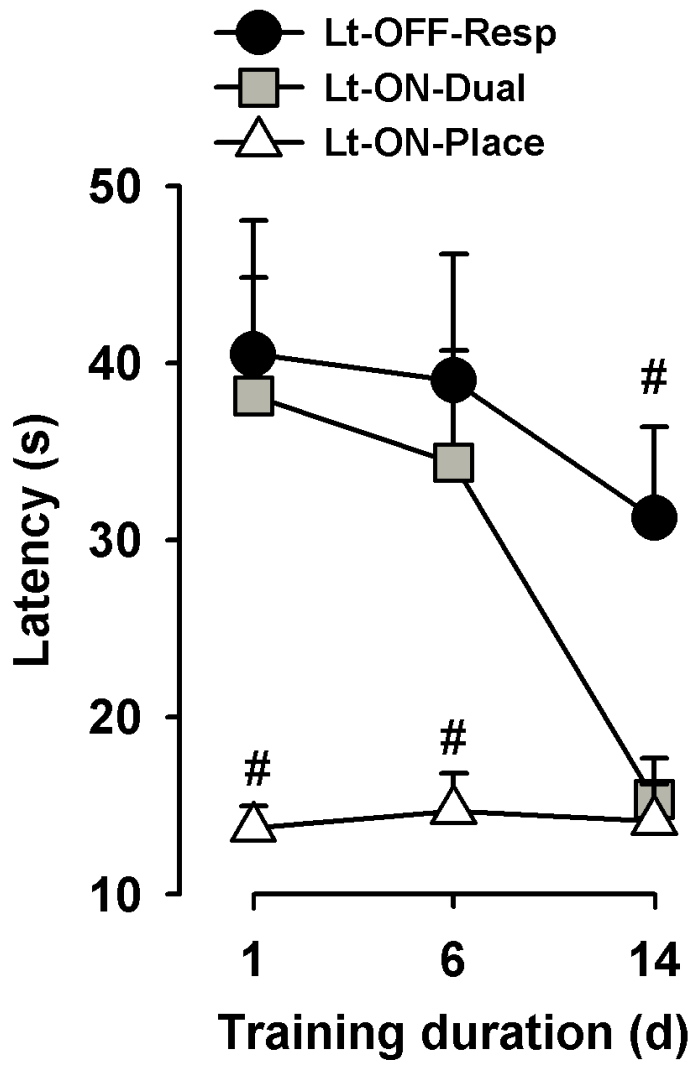
1581 <u>**Supplementary Figure 4 :**</u> **Average time to exit the arm which the rats from the**

1582 **different training protocols and durations have visited first, be it N, NE or any**

1583 **other arm.** Once having entered their first arm, the time spent therein was

1584 comparable among training protocols and duration conditions. The data illustrated

1585 are given in seconds (+ sem). Analysis of variance showed no significant Protocol ($F_{(2,60)}$

1586 = 2.32, p = 0.11) or Duration ($F_{(2,60)}$ = 1.21, p = 0.31) effects, and no significant

1587 interaction between the two factors (($F_{(4,60)}$ = 0,37, p = 0.83).

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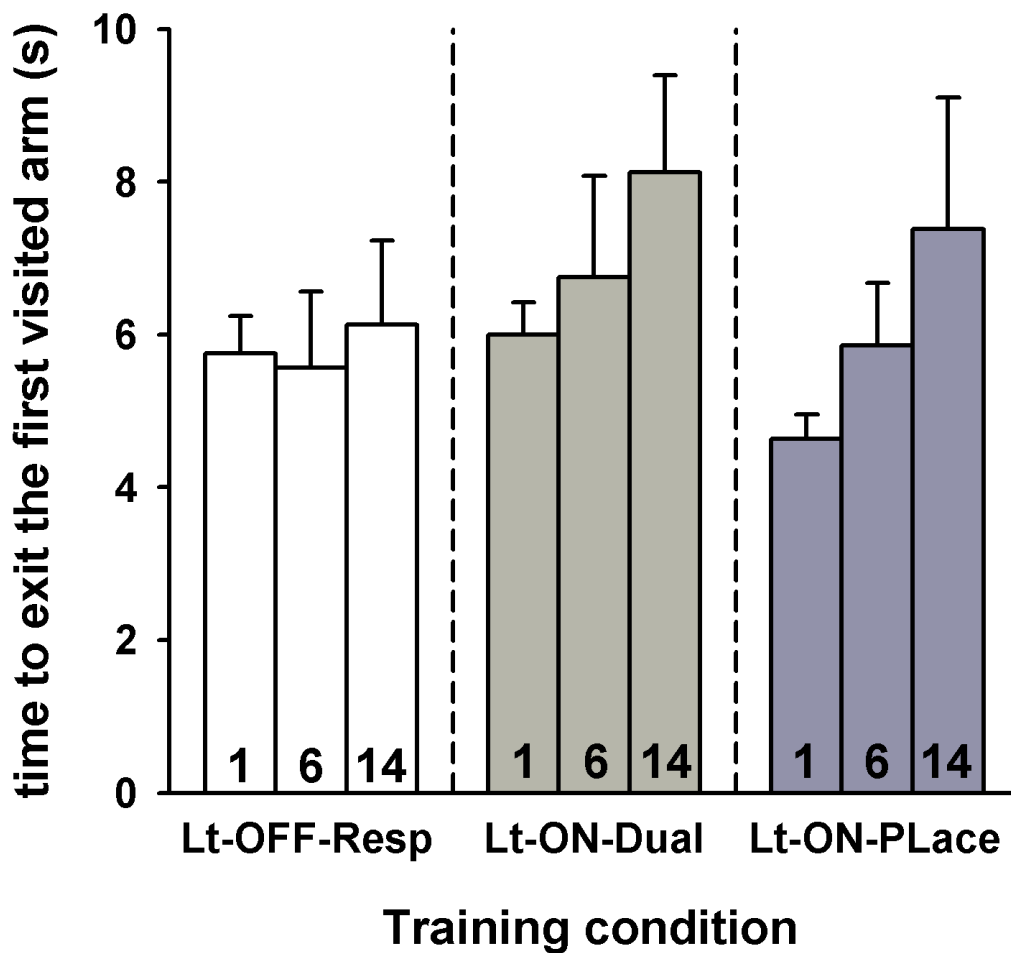
1590

1591   <u>**Supplementary Figure 5 :**</u> **Time to exit the N or NE arm after the rats had**

1592   **entered it for the first time is compatible with the progressive formation of a**

1593   **cognitive map in Lt-ON-Dual rats.** All means are given in seconds (+ sem). White

1594   bars correspond to the N arm, grey ones to the NE arm. For each training protocol

1595   and duration (indicated by the numbers at the bottom of the white bars), time to exit

1596   the N arm was compared to time to exit the NE one using a Student's t-test for paired

1597   samples. Statistical analyses : * indicates a significant difference between time to exit
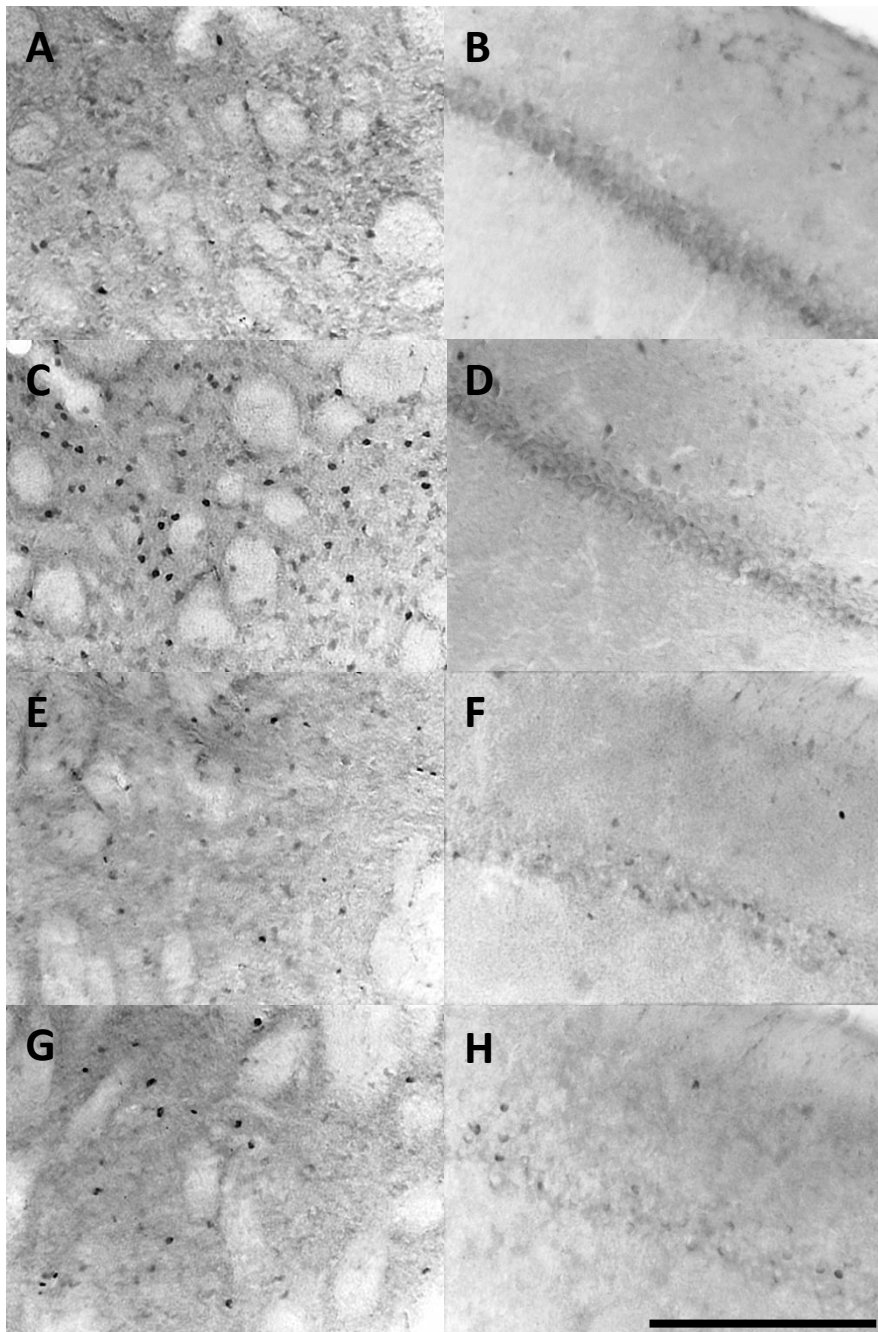
1598   N as compared to time to exit NE ; $p < 0.05$.

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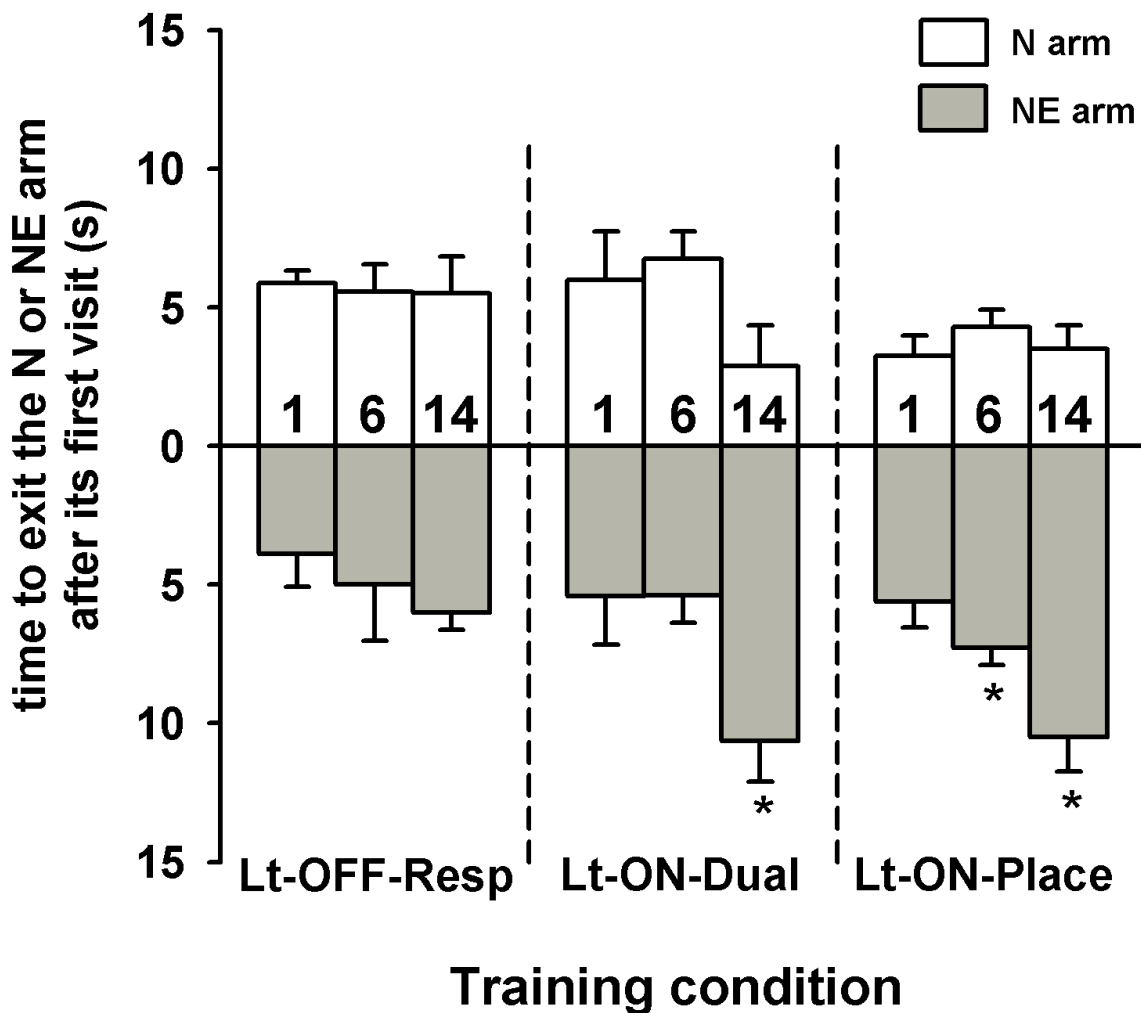
1600

1601   **Supplementary Figure 6: c-Fos staining, 1 day of training.** Typical examples of

1602   c-Fos staining observed in the medial part of the dorsal striatum (A, C, E, G) and in

1603   region CA1 (B, D, F, H) of the dorsal hippocampus from rats tested in a probe trial 24

1604   hours after a 1-day training duration (4 trials/day) or taken from their home cage (A,

1605   B). C and D are from a Lt-OFF-Resp rat, E and F from a Lt-ON-Dual one, and G and

1606   H from a Lt-ON-Place one. Scale bar = 250 µm.

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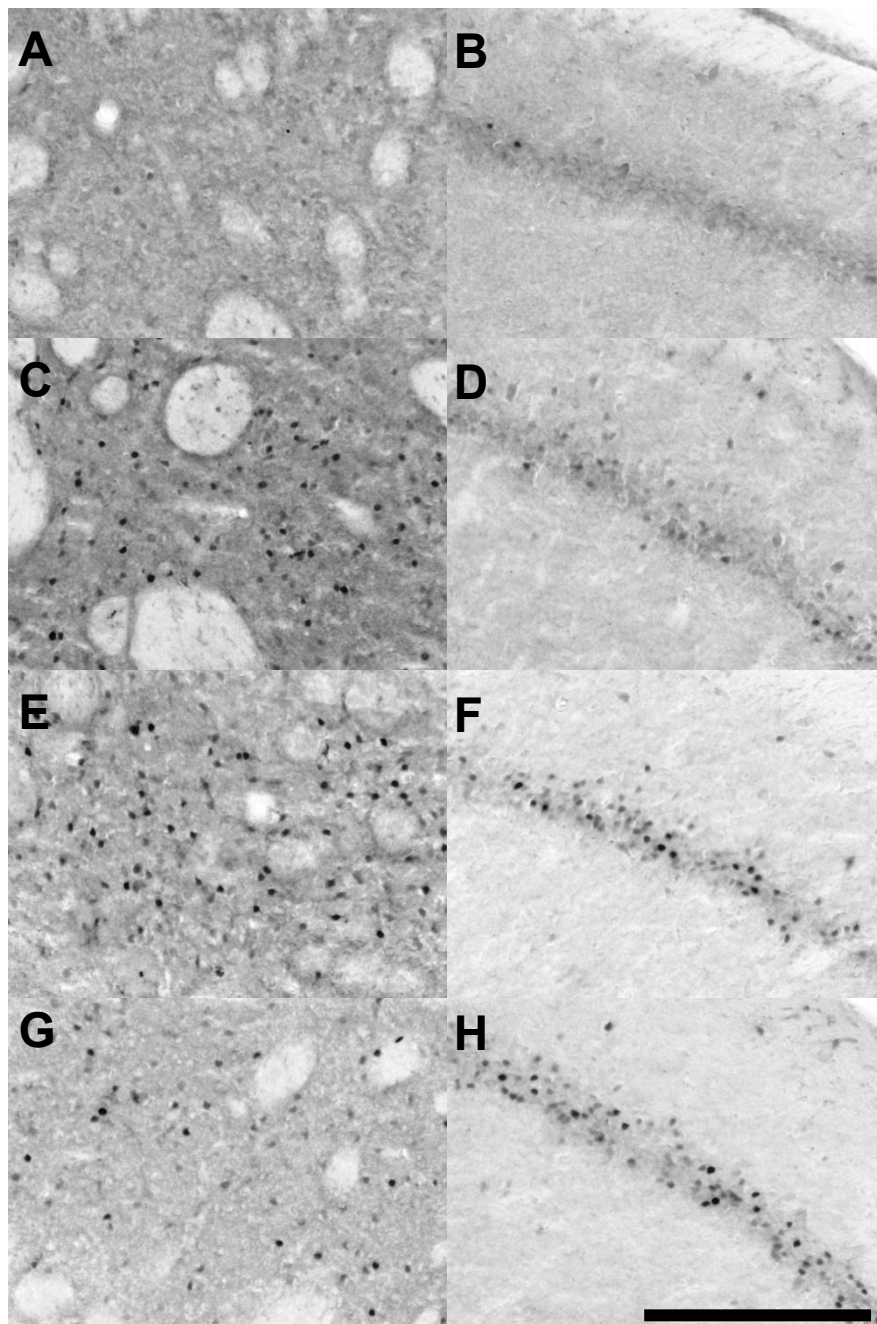
Supplementary Figure 7: c-Fos staining, 6 days of training. Typical examples of c-Fos staining observed in the medial part of the dorsal striatum (A, C, E, G) and in region CA1 (B, D, F, H) of the dorsal hippocampus from rats tested in a probe trial 24 hours after a 6-day training duration (4 trials/day) or taken from their home cage (A, B). C and D are from a Lt-OFF-Resp rat, E and F from a Lt-ON-Dual one, and G and H from a Lt-ON-Place one. Scale bar = 250 μm.
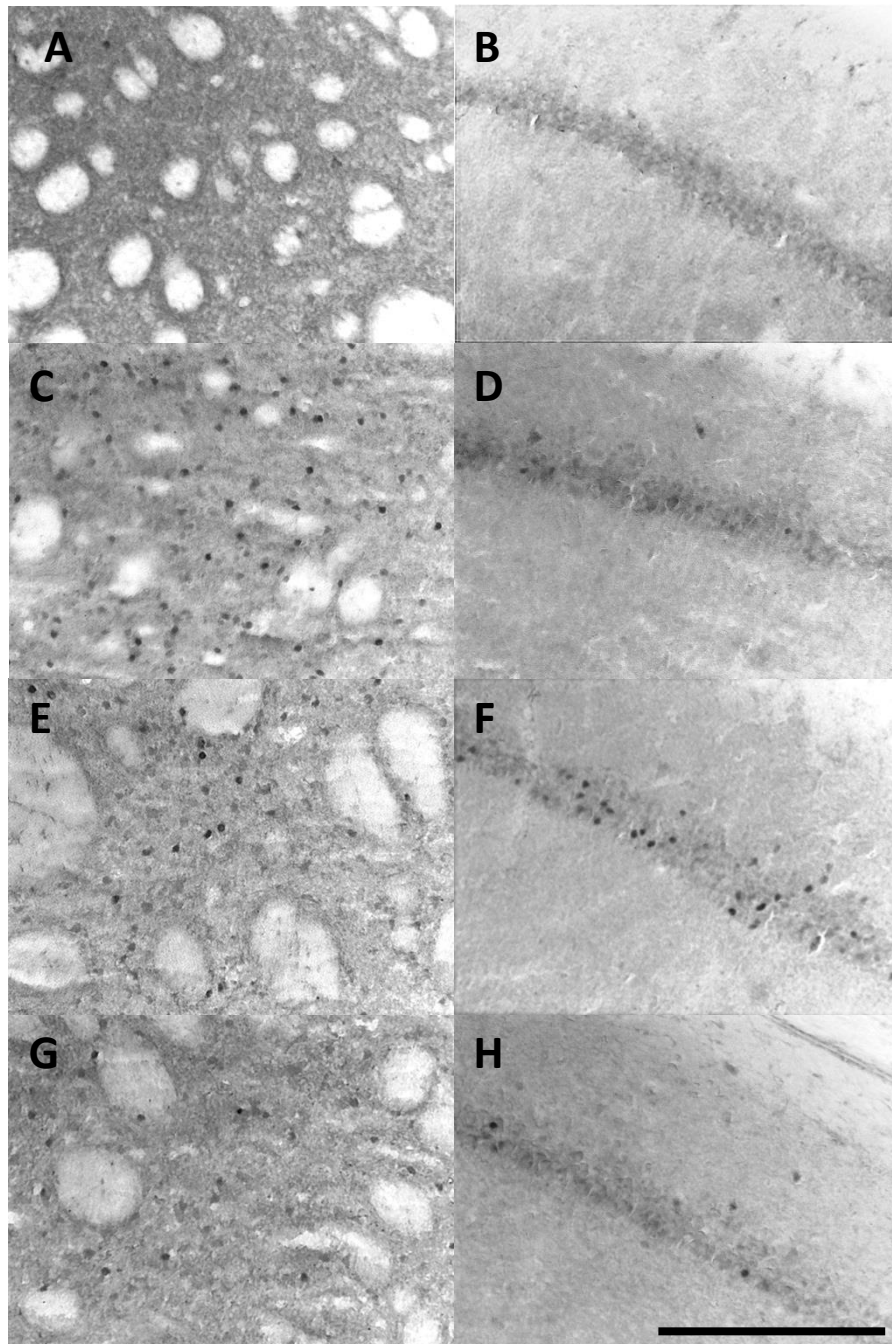
Supplementary Figure 8: c-Fos staining, 14 days of training. Typical examples of c-Fos staining observed in the medial part of the dorsal striatum (A, C, E, G) and in region CA1 (B, D, F, H) of the dorsal hippocampus from rats tested in a probe trial 24 hours after a 14-day training duration (4 trials/day) or taken from their home cage (A, B). C and D are from a Lt-OFF-Resp rat, E and F from a Lt-ON-Dual one, and G and H from a Lt-ON-Place one. Scale bar = 250 µm.

1664

1665 **Supplementary Figure 9: c-Fos staining control (auditory cortex).** Typical

1666 examples of c-Fos staining observed in the primary auditory cortex of rats tested in a

1667 probe trial 24 hours after a 1- (left), 6- (middle) or 14-day (right) training duration (4

1668 trials/day) or taken from their home cage (A, B, C). D, E, F are from a Lt-OFF-Resp

1669 rat, G, H, I from a Lt-ON-Dual one, and J, K, L from a Lt-ON-Place one. Scale bar =

1670 250 µm.

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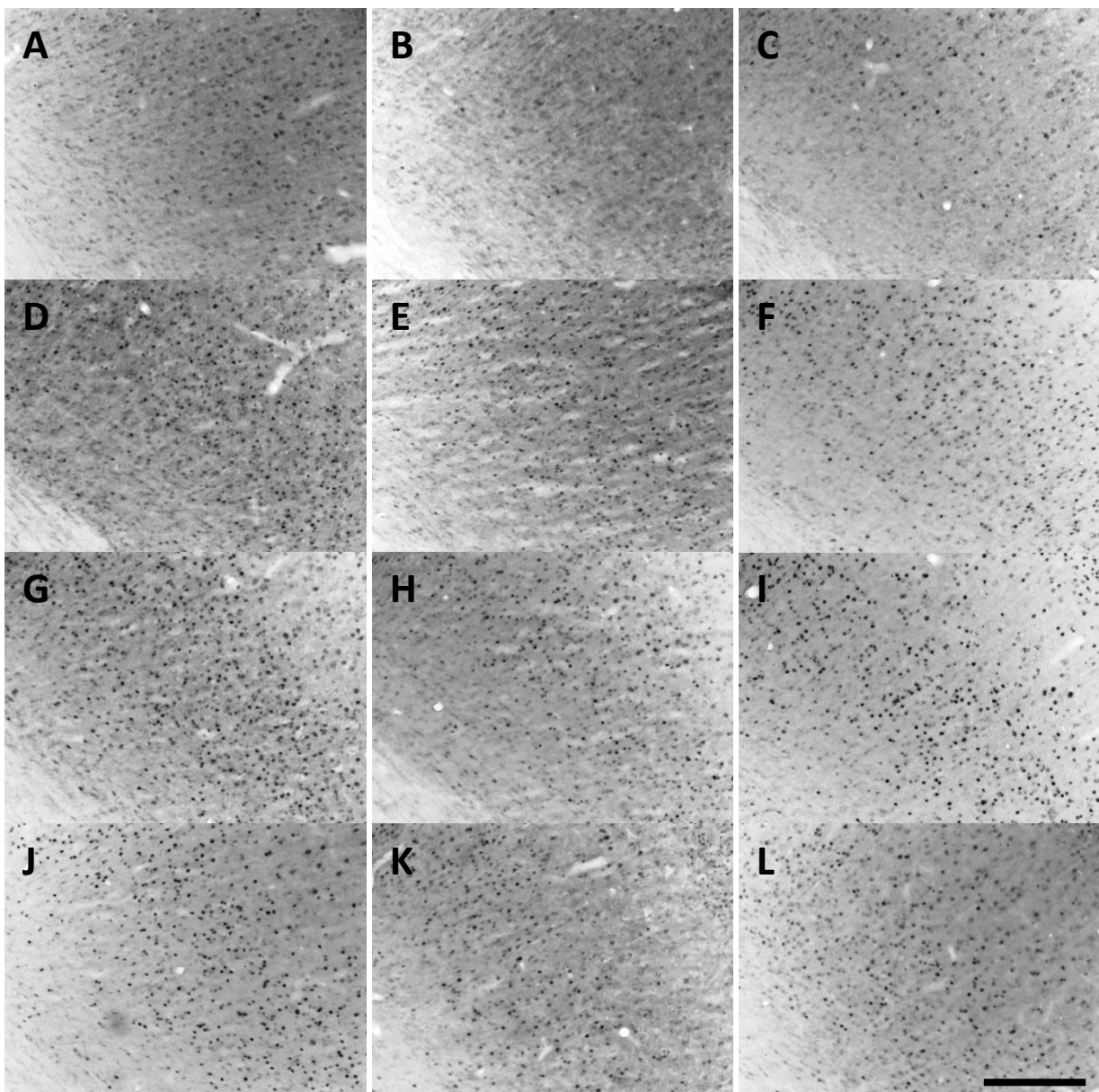
Supplementary Figure 10: c-Fos staining control (primary somatosensory cortex). Typical examples of c-Fos staining observed in the primary somatosensory cortex of rats tested in a probe trial 24 hours after a 1- (left), 6- (middle) or 14-day (right) training duration (4 trials/day) or taken from their home cage (A, B, C). D, E, F are from a Lt-OFF-Resp rat, G, H, I from a Lt-ON-Dual one, and J, K, L from a Lt-ON-Place one. Scale bar = 250 μm.
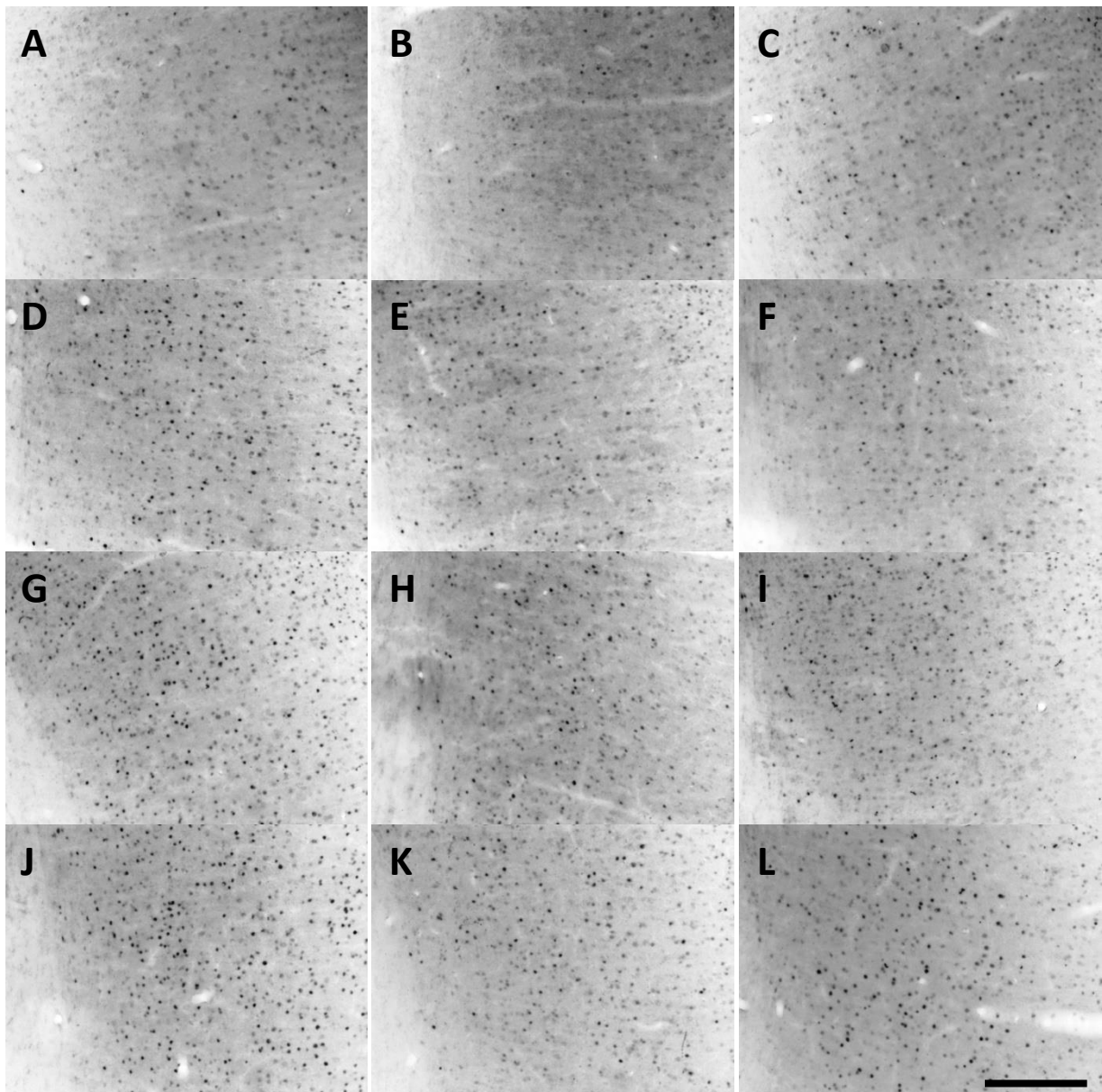
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1732   **<u>Supplementary Figure 11:</u> Diffusion of MUSC-induced inactivation around the**

1733   **infusion site was comparable among training protocols and durations.** Grey

1734   and black areas indicate the largest and smallest diffusion of the inactivation,

1735   respectively, in either the dorsal striatum (left panel) or the dorsal hippocampus (right

1736   panel). This diffusion radius of MUSC effects was estimated on coronal sections

1737   stained for c-Fos expression in Lt-OFF-Resp (left), Lt-ON-Dual (middle) and Lt-ON-

1738   Place rats (right). The rats were killed 90 min after the end of their 1-min probe trial.

1739   Before the probe trial, the rats had been trained for 6 (top) or 14 (bottom) days.

1740   Muscimol was infused 30 min before the probe trial. Coordinates are given in mm

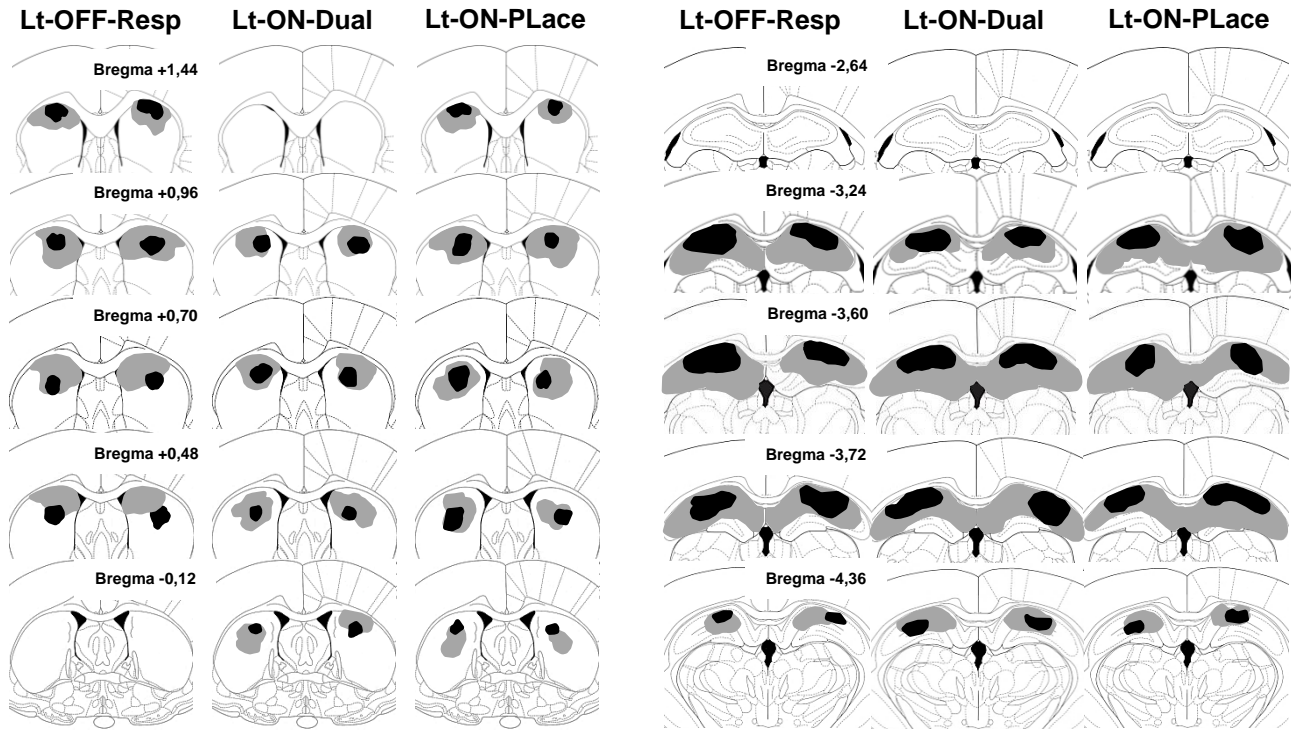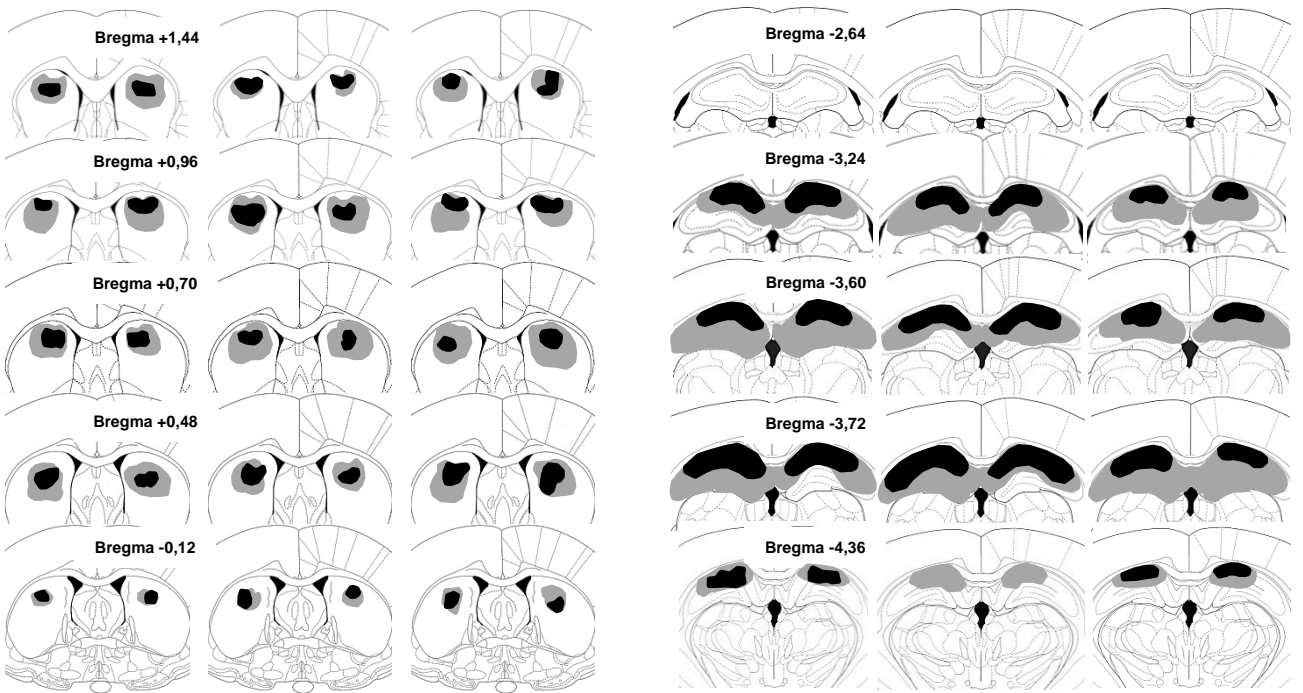1741   from Bregma (Paxinos and Watson, 2007).

1742

1743

1744

STRIATUM HIPPOCAMPUS

Training : 6 days

1745

Lt-OFF-Resp **Lt-ON-Dual** **Lt-ON-PLace** **Lt-OFF-Resp** **Lt-ON-Dual** **Lt-ON-PLace**

Bregma +1,44   Bregma -2,64

Bregma +0,96   Bregma -3,24

Bregma +0,70   Bregma -3,60

Bregma +0,48   Bregma -3,72

Bregma -0,12   Bregma -4,36

Training : 14 days

Bregma +1,44   Bregma -2,64

Bregma +0,96   Bregma -3,24

Bregma +0,70   Bregma -3,60

Bregma +0,48   Bregma -3,72

Bregma -0,12   Bregma -4,36

1746 <u>**Supplementary figure 12:**</u> **Quantification on coronal sections of the diffusion**

1747 **radius of the MUSC-induced inactivation around the infusion sites in the dorsal**

1748 **striatum and dorsal hippocampus provides evidence for comparable diffusion**

1749 **areas among experimental conditions.** All inactivation areas were expressed as a

1750 percentage of the surface of the region of interest at -0.1, +0.1, +0.5, +0.7, +0.8,

1751 +1.0, +1.4 and +1.7 mm (STRIATUM; dorso-ventral limits of the DStr are those

1752 shown in Supplementary Figure 1), and at -4.4, -4.2, -3.8, -3.6, -3.5, -3.4, -3.1, -2.9

1753 and -2.6 mm (HIPPOCAMPUS); all coordinates are given in mm from Bregma

1754 according to Paxinos and Watson (2007). Data illustrated are means (+ sem). The

1755 ANOVA only showed a significant Anteriority effect. All other effects (Protocol,

1756 Duration, or any of the second or third order interactions) were not significant. Notice

1757 that for the statistical analyses, anteriority levels where zero values were found in all

1758 rats have not been included in the analyses. This was the case for levels of -0.1 mm

1759 in the DStr, and -2.6 and -4.4 mm in the DHip.

1760   .

1761   (see next page)

1762
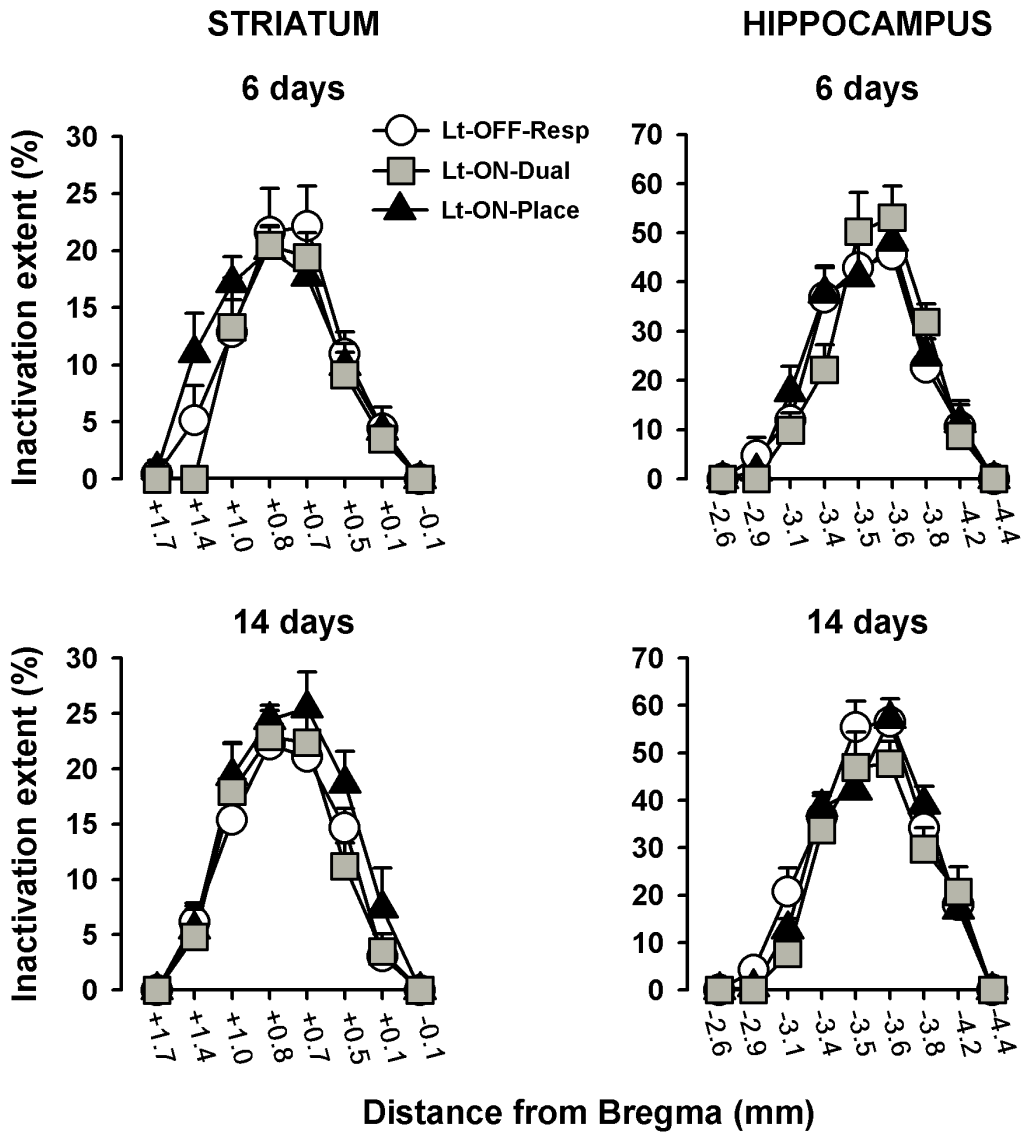
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1764

Supplementary Figure 13: Effect of MUSC infusion on c-Fos staining. Typical examples of c-Fos staining observed in the medial part of the dorsal striatum (A, B) and in region CA1 (C, D) of the dorsal hippocampus from rats tested in a probe trial 30 min after an intrastriatal or an intrahippocampal infusion of aCSF (A, C) or muscimol (B, D). Scale bar = 250 μm.
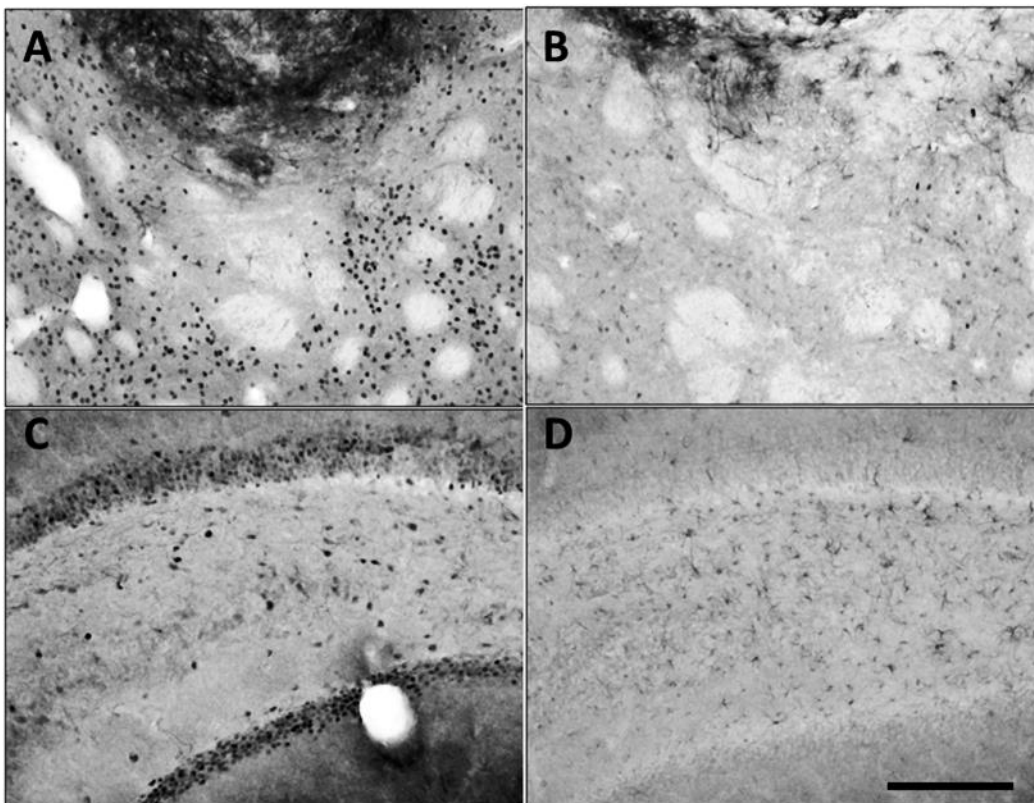
1773 **<u>Supplementary Figure 14:</u> Number of errors was comparable among**

1774 **experimental conditions, indicating similar performance in all groups of rats.** In

1775 each of the four training conditions of our second experiment (Protocol X Duration),

1776 mean numbers of errors (+ sem) are represented over the 6- and 14-day training

1777 sessions (daily blocks of four trials). Statistical analyses showed no significant

1778 difference between the training conditions. The same was true for distances. The

1779 number of animals in each condition can be found in Figure 8 of the article.

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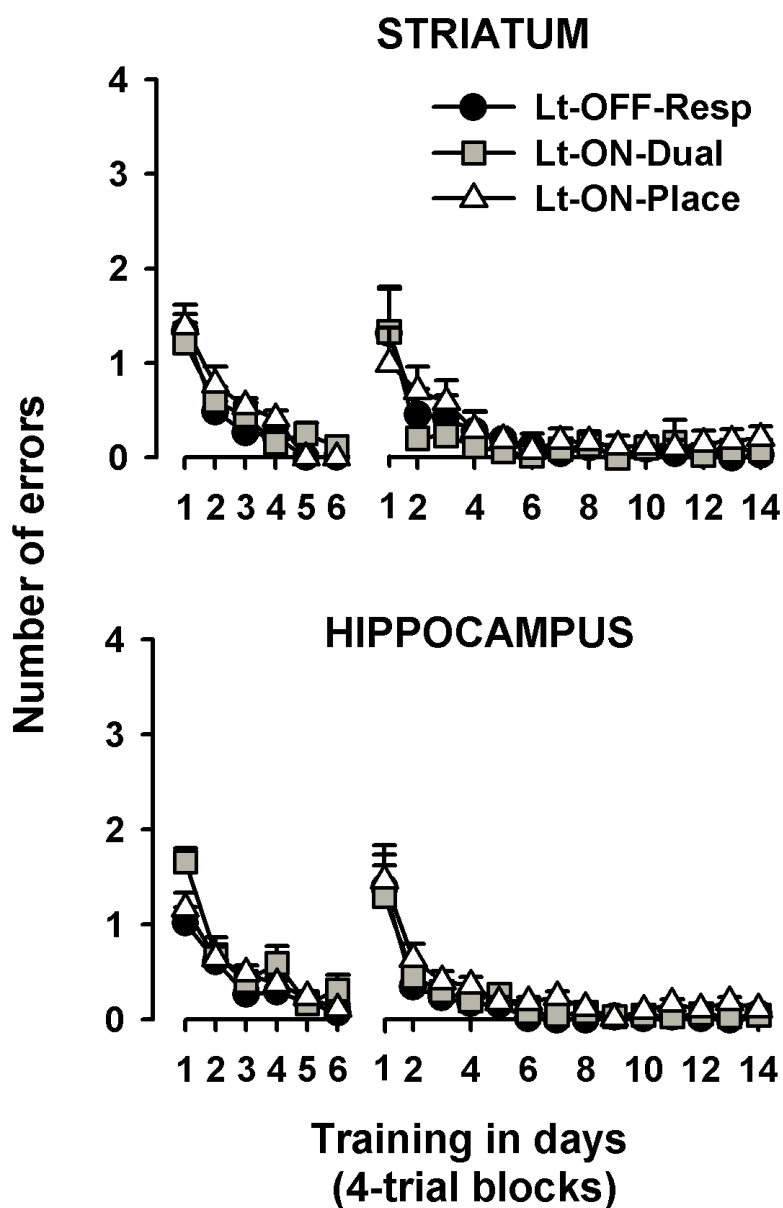
1781

1782 <u>**Supplementary Table 3**</u> **: Percent of rats shifting to the NE arm right after**

1783 **having first visited the N arm showed MUSC-induced disruption of**

1784 **performance, except when infused in the striatum after 14 days of training in** Lt-

1785 ON-Dual and Lt-ON-Place rats. Number of rats showing a direct swim to the N arm

1786 after having been released in the maze are indicated between brackets as "(n/ N)".

1787 aCSF stands for artificial cerebrospinal fluid, MUSC for muscimol. The table reads as

1788 follows : "of the N tested rats, % of the n rats that first entered the N arm and which

1789 then shifted to the NE one".

Training condition	Training duration	Infusion structure			
		Striatum		Hippocampus	
		aCSF	MUSC	aCSF	MUSC
Lt-OFF-Resp	6 days	38 % (8/9)	0 % (2/8)	0 % (9/9)	25 % (4/11)
	14 days	14 % (7/7)	20 % (5/8)	0 % (7/7)	33 % (3/8)
Lt-ON-Dual	6 days	14 % (7/8)	50 % (6/7)	43 % (7/7)	0 % (3/7)
	14 days	<u>71%</u> (7/7)	<u>100 %</u> (4/8)	<u>86 %</u> (7/7)	25 % (4/9)
Lt-ON-Place	6 days	66 % (3/7)	0 % (3/9)	<u>66 %</u> (6/8)	50 % (2/9)
	14 days	<u>71 %</u> (7/7)	<u>100 %</u> (6/8)	<u>100 %</u> (6/7)	38 % (8/10)

1790 The proportion of rats having first swum to the N arm and then shifted to the NE was

1791 significantly higher than chance in the following groups : after 6 days of training, in Lt-

1792 ON-Place rats subjected to intrahippocampal infusion of aCSF ; after 14 days of

1793 training, in Lt-ON-Dual rats subjected to intrastriatal aCSF or MUSC infusions (Chi^2 =

1794 8.05, p < 0.01 and Chi^2 = 18.0, p < 0.001, respectively) or to intrahippocampal aCSF

1795 infusions (Chi^2 = 13,76, p < 0.001), and in Lt-ON-Dual rats subjected to intrastriatal

1796 aCSF or MUSC infusions (Chi^2 = 8.05, p < 0.01 and Chi^2 = 12, p < 0,001) or

1797 intrahippocampal infusions of aCSF(Chi^2 = 18.00, p < 0.001). The corresponding

1798 percentages are underlined. In the other cells, there was no significant difference

1799 from chance.

1800

1801 **Supplementary Table 4:** Average swim velocity during the probe trial was similar,

1802 regardless of training protocol, duration and inactivation conditions. aCSF stands for

1803 artificial cerebrospinal fluid, MUSC for muscimol. Data are indicated in cm/s as

1804 means (sem). Statistical analyses showed no significant difference among groups.

Training protocol	Training duration	Infusion structure			
		Striatum		Hippocampus	
		aCSF	MUSC	aCSF	MUSC
Lt-OFF-Resp	6 days	26.3 (0.9)	27.2 (1.7)	26.4 (0.9)	24.7 (1.2)
	14 days	26.1 (1.6)	26.8 (1.6)	26.2 (1.3)	26.3 (1.7)
Lt-ON-Dual	6 days	24.0 (0.9)	23.6 (1.4)	26.0 (1.2)	26.3 (1.6)
	14 days	25.7 (1.2)	26.0 (1.0)	25.7 (1.4)	25.0 (0.5)
Lt-ON-Place	6 days	26.9 (0.7)	26.3 (0.5)	27.2 (1.0)	26.2 (1.0)
	14 days	26.4 (0.9)	26.5 (1.2)	25.6 (1.4)	26.8 (1.1)

1805 The 3 (Protocol) X 2 (Duration) X 2 (Inactivation) ANOVAs were performed

1806 separately for rats infused into the striatum or the hippocampus. ANOVA of the

1807 average swim velocities in rats subjected to intrastriatal infusions showed that

1808 Protocol (F $_{(2,87)}$ = 0.4, *ns*), Duration (F $_{(1,87)}$ = 0.1, *ns*), Inactivation (F $_{(1,87)}$ = 0.2, *ns*),

1809 and the second or third order interactions (F $_{(2,87)}$ or $_{(1,87)}$ < 1.0, *ns*) had no significant

1810 effect on swim velocities. ANOVA of the swim velocities in rats subjected to

1811 intrahippocampal infusions supported similar conclusions.

1812

1813   **Supplementary Figure 15:** **Time to exit the first visited arm, be it N, NE or any**

1814   **other arm, did not show any difference among experimental conditions (light,**

1815   **training, inactivation).** All data illustrated are given in seconds (+ sem). Analysis

1816   (ANOVA) of the data from rats subjected to intrastriatal infusions showed no

1817   significant Protocol ($F_{(2,81)} = 2.61$, $p = 0.08$), Duration ($F_{(1,81)} = 0.08$, $p = 0.77$) or

1818   Inactivation effects ($F_{(1,87)} = 1.74$, $p = 0.19$), and none of the second or third order

1819   interactions was significant. Analysis of the data from rats subjected to

1820   intrahippocampal infusions showed no significant Protocol ($F_{(2,87)} = 0.91$, $p = 0.40$),

1821   Duration ($F_{(1,87)} = 1.17$, $p = 0.28$) or Inactivation effects ($F_{(1,87)} = 0.04$, $p = 0.83$), and

1822   none of the second or third order interactions was significant.

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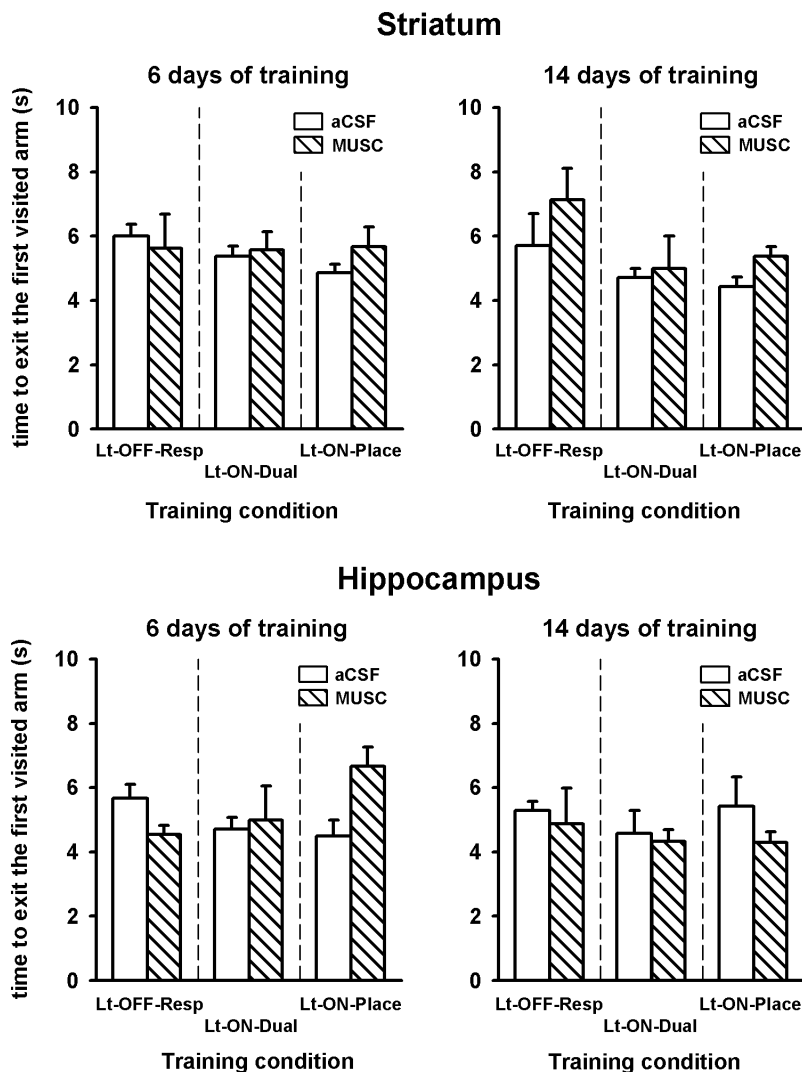
1824

1825   **<u>Supplementary Figure 16:</u> Average time to exit the N or NE arm confirmed**

1826   **MUSC-induced disruptions, although they were less marked than in Figure 9 of**

1827   **the article.** Data are shown according to the different protocol, duration and

1828   inactivation conditions. All means are given in seconds (+ sem). White bars

1829   correspond to the N arm, grey ones to the NE arm. For each condition, time in the N

1830   arm was compared to time in the NE arms using a Student's t-test for paired

1831   samples. Statistical analysis : * indicates a significant difference between time in N

1832   and time in NE ; $p < 0.05$.

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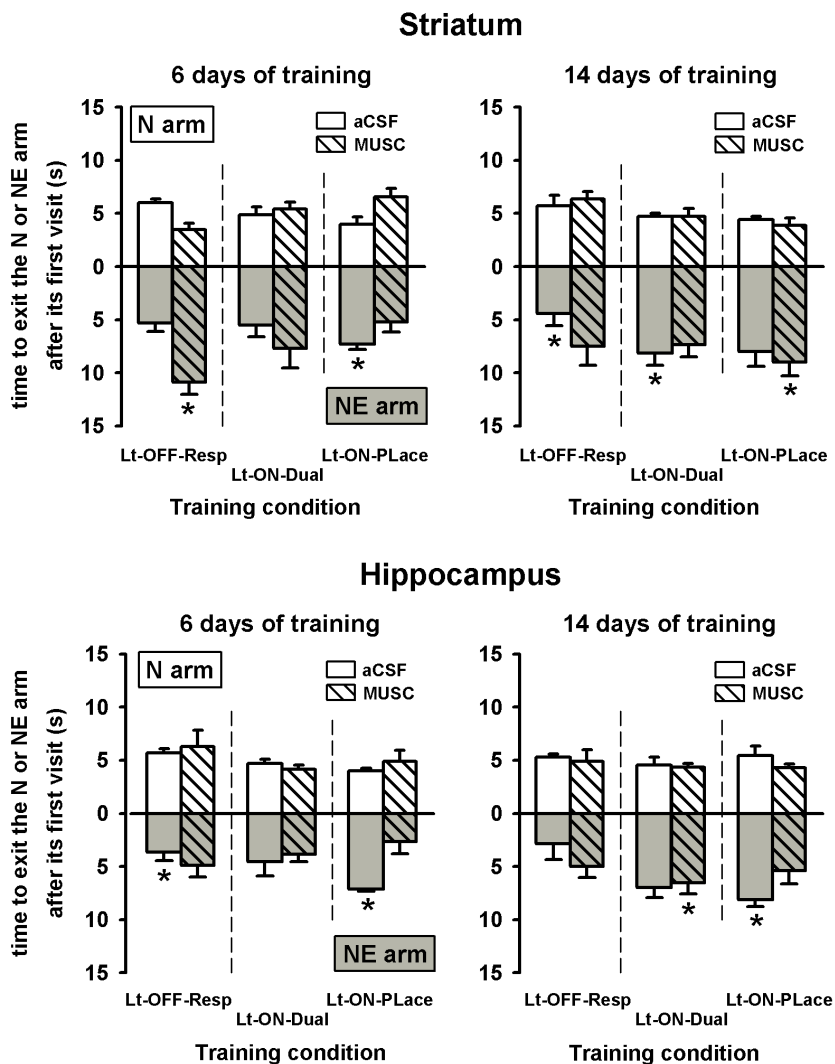
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1835 **Supplementary list of references**

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