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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

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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.

Keywords: hippocampus; spatial navigation; place memory; response memory; rat; striatum

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

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

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

would favor a place strategy based on the hippocampus memory system enabling a strategy shift either directly or after a mistake. We also investigated the memory-based behaviors using c-Fos brain imaging and muscimol inactivation.

2. Materials and methods

2.1. Animals

Male Long-Evans rats (Janvier Labs, Le Genest-St-Isle, France) were used. They were aged 6-7 weeks (body weight: 160-170g) at their arrival at the laboratory. They were housed individually with ad libitum food and water in a temperature (22 ± 1 °C) and humidity (55 ± 5%) controlled room under a 12-12 hr light–dark cycle (lights on at 7.00 a.m.). The study respected the rules and guidelines of the European Parliament 2010/63/UE of September 22, 2010, and of the French Department of Agriculture. All experimental protocols used herein have been validated a priori by a local ethical committee (CREMEAS, authorization no. AL/17/24/02/13).

2.2. Double-H maze

The double-H testing device has been described in detail in previous articles (e.g., Pol-Bodetto et al., 2011; Cassel et al., 2012; Kirch et al., 2013, 2015) along with the characteristics of the room hosting it (Cholvin et al., 2013). Briefly, the general layout of the apparatus corresponds to the shape of two contiguous capital Hs. It is made of three parallel run arms, 160 cm in length and 18.8 cm wide (internal measure), connected to each other at their center by a perpendicular arm. All side walls, 35 cm high, are made of transparent Plexiglas to favor access to allothetic cues. The two opposite arms in the middle are labeled north (N) and south (S), respectively (see Figure 1). The four other arms are labeled south-east (SE), south-west (SW), north-east (NE), and north-west (NW). For pre-training, training and memory testing, the device was always kept at the same place (on a square table, 80 cm from the floor) and all cues in the room (two black disks on one wall, and one large triangle on another wall, two orange-painted heating ducts above the maze, one table, one chair, one computer desk, one boiler…) were left at their original location for the entire duration of the experiments. Most of these landmarks were

located close to the maze, i.e., between 1.20 and 1.50 m from the closest maze wall and between 86 and 140 cm above the water surface. To secure the locations of objects**,** including the maze and its table, their positions were marked on the floor. In the room, there was a small loudspeaker fixed on the wall, playing music at low volume during the 12-h light period.

2.3. Pre-training and training protocols

The double-H was filled with water (20 °C) to a height of 15 cm. A platform, 11 cm in diameter, 14 cm high, was ballasted with gravel and used as the escape. For pre-training, the platform protruded 1 cm above the surface of the water at the extremity of the SW arm, the water was left clear and the rats were given four consecutive trials. On each trial, rats had to swim straight from the NW to the SW arm in order to keep the cognitive demand at a low level. A transparent guillotine door blocked the access to the central corridor and thus to all other places in the maze. In all rats, pre-training was performed in a normally lit environment.

For the training sessions, the platform was moved to the NE arm and immersed 1 cm under the water surface. Water was made opaque by addition of powdered milk (about 1.5 g/L). Rats were given four daily trials. Each trial lasted a maximum of 60 s. When the rat did not reach the platform within this time, it was gently guided to the platform by the experimenter. Once a rat had climbed on the platform, it was left there for 10 s, after what the next trial was started without delay.

Three training protocols were used (Figure 1). In the first protocol (Lt-OFF-Resp), rats were tested in darkness: the only source of light was generated by six red darkroom bulbs, type B22PF712B by Philips, 15 W each, placed near the maze at the extremity of each arm; light intensity in and around the maze was of about 1 lux. The rats were released from the S on all trials and had to swim to the NE, which they reached most directly by a right turn immediately followed by a left turn. The N arm was closed with a transparent guillotine door. The guillotine door prevented the repetition of the straight swimming trajectory rats had to follow during the pre-training phase of the protocol. In the second protocol (Lt-ON-Dual), the same procedure was used except that the room was illuminated by neon lights (180 lux) to make all landmarks easily visible. The N arm was closed with a guillotine door. In the third protocol (Lt-ON-Place), the room was illuminated (180 lux) and rats were released

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

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

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Insert Figure 1 about here

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2.4. Probe trial

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

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

2.5. Surgery and muscimol (MUSC) infusions

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

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

gene having a very low basal expression level, a region that should be active (as seen in rats infused with aCSF) but would exhibit a very low or no c-Fos expression can be considered efficiently inactivated. A similar approach on zif268 expression in mice was used by Maviel et al. (2004) to assess the effects of lidocaine in the hippocampus and neocortex. All inactivation areas were expressed as a % of the surface of the DStr at about -0.1, 0.1, +0.5, +0.7, +0.8, +1.0, +1.4, and +1.7 mm (ventral limits of the DStr are those shown in Supplementary Figure 1), and of the DHip at -4.4, -4.2, -3.8, -3.6, -3.5, -3.4, -3.1, -2.9, and -2.6 mm, from Bregma (Paxinos and Watson, 2007).

2.6. Tissue preparation

Ninety minutes after the probe trial, rats were injected with an overdose of sodium pentobarbital (200 mg/kg i.p.) and perfused transcardially with 80mL of a 4% phosphate-buffered (0.1 M) paraformaldehyde solution (PFA, 4°C). Brains were removed, post-fixed for 2 hr in 4% PFA (4°C), and placed into a 20% sucrose solution (in 0.1 M PBS) for 48 hr at 4 °C. They were then quickly frozen in isopentane (-40 °C) and stored at -80°C. Floating coronal sections (40 µm) were cut using a cryostat (MICROM HM 500M) in serial sections within a block of tissue extending from +1.90 to -1.90 mm from Bregma for the DStr, and from -2.16 to -4.44 mm from Bregma for the dHip (Paxinos & Watson, 2007).

2.7. c-Fos immunohistochemistry, imaging and quantification

All sections dedicated to c-Fos immunohistochemistry were processed in separate rounds so as to have all between-subject factors equally represented in each round (n = 8 rats for each group). These precautions minimized technical biases. The sections were first rinsed three times during 10 min in PBS and soaked for 1 hr in 5% normal donkey serum in PBS containing 0.5% Triton X-100. All sections were subsequently transferred into the primary anti-Fos rabbit polyclonal antibody solution (1:4,000, Rabbit anti-Fos polyclonal IgG; Santa Cruz, USA), where they were left overnight at room temperature. Then, they were rinsed and soaked in a buffer solution containing biotinylated goat anti-rabbit secondary antibody (1:500, Biotin SP-conjugated affiniPure Goat anti-rabbit IgG; Jackson ImmunoResearch, West Grove, PA, USA). Staining was revealed with the avidin–biotin peroxidase

method (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA) coupled to diaminobenzidine (Lopez et al., 2012). In rats subjected to a functional inactivation by MUSC (see below), additional sections were stained with cresyl violet to make sure that the location of the infusion sites was acceptable.

2.8. Stereological analyses of c-Fos expression

The quantitative analyses of c-Fos-positive nuclei were performed in the DStr and DHip. As the dorsomedial striatum (DMS) contributes to setting up an automatism by its implication in action outcome on a goal-directed navigation task, and the dorsolateral striatum (DLS) to the automatism's storage (e.g., Packard & McGaugh 1996; Thorn et al., 2010), quantifications were made separately in the DMS and DLS. Additional quantifications were also made in CA1, CA3, and dentate gyrus (DG), as c-Fos expression levels accompanying place memory retrieval may differ between these hippocampal subregions (e.g., Lopez et al., 2012). A single investigator, blind to the identity of the rats, analyzed all sections. The overall number of c-Fos immunoreactive cells was estimated with the optical fractionator technique using optical dissectors (45 x 45 µm) allowing unbiased counting (West et al., 1991; West, 2013). For stereological counting we used a Leica DM5500B light microscope coupled with a MicroFire CCD color camera (Optronics) equipped with a motorized x–y stage control. Stereological analyses were performed using the Mercator software (Explora Nova, La Rochelle, France) and all cell counts were processed online on the video image. The same intensity of light in the microscope and the same parameters in the exposure time of the digital camera were used for all sections. Areas of interest in stained sections (see Supplementary Figure 1) were first outlined using a 2.5x objective and c-Fos-positive cells were counted using a 100x (1.40 NA) oil-immersion objective. Counting grids (145 x 145 µm for the DStr and 80 x 80 µm for DHip subregions) equidistant from each other were randomly positioned within the area of interest using the Mercator software. The total number of c-Fos positive nuclei/mm^3 of cerebral tissue was estimated from six (for the DHip) or seven (for the DStr) sections per animal (section sampling fraction (ssf) = 1/6 for DHip or 1/12 for DStr, from the total number of nuclei counted in all optical dissectors). Details of stereological parameters were as follows: section interval = 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 Chi^2 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

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

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

3. Results

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

3.1.1. Comparable acquisition performance among training protocols

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

and then to day 3 (p <0.01). Analyses of the distances (not illustrated) yielded strictly comparable results, as was also the case for errors (see Supplementary Figure 2).

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Insert Figure 2 about here

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

According to our current knowledge, Lt-OFF-Resp rats should form a stimulus-response representation and favor a response strategy without constructing a spatial map. Therefore, they should not shift to spatial navigation, even in response to negative feedback. Conversely, Lt-ON-Dual rats should adopt a hippocampal-dependent spatial navigation strategy after weak training (1 or 6 days), but change to a striatum-dependent response strategy after sustained training (14 days). We hypothesized that they should nevertheless be able to shift from a response strategy to a place strategy, either directly or upon negative feedback. In Lt-ON-Place rats, for the three training durations, all behaviors should reflect a place strategy. According to these predictions, our results showed that Lt-OFF-Resp rats did not form a spatial map. However, and unexpectedly, Lt-ON-Dual rats started to acquire the task by response learning, and acquired only later on a place memory enabling a trajectory correction, either directly or in response to negative feedback. Lt-ON-Place rats relied on a spatial map, as soon as after 1 day of training.

The data are illustrated in Figure 3. Whatever the training duration, almost all Lt-OFF-Resp rats swam directly to the N, a proportion (20/23; 87%) largely above chance (i.e., 25% as there were 4 accessible arms; start arm not considered). Most Lt-ON-Place rats (16/23; 69%) also swam directly to the N. In Lt-ON-Dual rats, most first swim paths ended in the N arm after 1 (100%) or 6 (87%) training days. After 14 training days, however, half the rats swam directly to the NE arm. The difference between first choices after 1 and 14 training days was significant (Chi^2 = 4,8, p < 0.05), indicating a late emergence of spatial navigation capabilities. Supplementary

Table 1 shows the proportion of rats correcting their choice in response to negative feedback, i.e., rats which swam to the NE arm next to having entered the N one.

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Insert Figure 3 about here

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To further analyze probe trial performance, and especially the shift capacity, we considered three additional variables: latencies to the NE arm, cumulated time spent in an arm to which a R-L turn had led (response memory-based behavior), and cumulated time spent in the NE arm (place memory-based behavior). Latencies to the NE arm (Supplementary Figure 3) provided additional information about the nature of the strategy used by the rats. Overall, we found that latencies were significantly reduced in the probe trial of the Lt-ON-Dual rats compared to the Lt-OFF-Resp rats after 14 days of training, thus compatible with the progressive formation of a cognitive map in this group. Swim velocities (see Supplementary Table 2) during the probe trial did not differ significantly among groups, allowing us to compare latencies. Regarding the time to exit the first visited arm, there was no significant difference among groups (Supplementary Figure 4). It is noteworthy that when Lt-ON-Dual rats (after 14 days of training) and Lt-ON-Place rats (after 6 and 14 days of training) visited the NE or the N arm for the first time, the to exit was longer in the former than in the latter (supplementary Figure 5), a difference not found in Lt-OFF-Resp rats.

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

This variable shows a response memory-based strategy in Lt-OFF-Resp rats, a strategy abandoned by Lt-ON-Dual rats after the longest training duration, and not existing in Lt-ON-Place rats. Data are shown in Figure 4. In the Lt-OFF-Resp rats, the exploration time was above chance for the two longest durations ($p < 0.01$), and larger after 14 training days than after 1 or 6 days ($p < 0.05$). In the Lt-ON-Place rats, this time was neither significantly different from chance nor affected by training duration. In Lt-ON-Dual rats, the time decayed as a function of training duration, reaching chance level after 14 training days. The Protocol x Duration ANOVA showed significant effects of the Protocol ($F_{(2,58)} = 17.4$, $p < 0.001$), Duration ($F_{(2,58)}$

= 0.13, *ns*), and of the interaction between the two factors ($F_{(4,58)} = 3{,}19$, $p < 0.05$). The Protocol effect was due to an overall exploration time (collapsed over training durations) that was longest in the Lt-OFF-Resp rats, intermediate in the Lt-ON-Dual rats, and lowest in the Lt-ON-Place ones (all differences were significant: $p < 0.01$). The interaction reflected a time of exploration that increased with training duration in Lt-OFF-Resp rats and decreased in Lt-ON-Dual rats.

3.1.2.2. Cumulated time in NE arm:

This variable shows no place memory-based strategy in Lt-OFF-Resp rats, a place strategy which appeared in L-ON-Dual rats after the longest training duration (14 days) and which was present in Lt-ON-Place rats already after 1 day of training. Statistics for comparisons of performance to chance level are illustrated in Figure 4. ANOVA of exploration time showed significant effects of Protocol ($F_{(2,58)} = 25{,}2$, $p < 0.001$), Duration ($F_{(2,58)} = 8{,}9$, $p < 0.001$), and of the interaction between the two factors ($F_{(4,58)} = 2{,}5$, $p < 0.05$). The group effect was due to overall time of exploration that was longest in Lt-ON-Place, intermediate in the Lt-ON-Dual rats, and lowest in Lt-OFF-Resp rats (all differences were significant: $p < 0.01$). The low exploration time in Lt-OFF-Resp rats and the high exploration time in Lt-ON-Place rats were not affected by training duration. Conversely, in Lt-ON-Dual rats, this time increased as a function of training duration, starting at the level of Lt-OFF-Resp rats and ending up at that of Lt-ON-Place ones. This difference explains the interaction between the two factors.

In summary, rats trained in the response strategy task developed a response strategy and showed a bias towards the response arm during the probe trial. Rats trained in the dual response/place strategy task performed comparably after 1 or 6 training days. However, after 14 days, half of them used an initial place strategy and, at the group level, there was a clear cut bias towards the place (NE) arm in the probe trial. Rats trained in the place strategy task did not use a place strategy for their initial swim path, but over the probe trial they showed a clear bias towards the place arm, whatever the training duration. The main result here is that when cues are visible in the dual double-H maze task, rats first approach the task on the basis of response learning and construct a cognitive map later on.

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Insert Figure 4 about here

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

c-fos expression was measured by immunohistochemistry in the DMS and DLS and in the dHip (CA1, CA3 and DG). Examples of c-fos expression patterns, including in two control regions (i.e., the primary somatosensory cortex and the primary auditory cortex), are shown in Supplementary Figures 6-10. Quantitative data are illustrated in Figure 5. Given our initial predictions from the literature (e.g., Packard and McGaugh, 1996), we expected high c-Fos expression levels in the striatum of Lt-OFF-Resp rats and in the hippocampus of Lt-ON-Place regardless of training duration, and a shift over training from high levels in the hippocampus to high levels in the striatum in the Lt-ON-Dual rats. Based on our behavioral data, however, the expectations for Lt-ON-Dual rats should be opposite. Overall, our results show that, during the probe trial, striatal activation was highest in Lt-OFF-Resp rats for all training durations. Striatal activation was weaker in Lt-ON-Dual rats in comparison with Lt-OFF-Resp rats, but yet largely above HC rats. In Lt-ON-Place rats c-Fos levels were higher than in HC rats, but the difference was more pronounced in the DMS than in the DLS. In Lt-OFF-Resp rats, CA1 c-Fos levels were close to those found in HC rats after all training durations. In Lt-ON-Dual rats, CA1 showed the largest evidence for activation, but only after 1 and 6 days of training. In Lt-ON-Place rats, a larger c-Fos expression was found only after 1 day of training.

3.1.3.1. c-Fos quantification in the dorsal striatum:

Analyses of c-Fos positive neurons in the DMS showed significant Protocol ($F_{(3,72)} = 86.0$, $p < 0.001$), Duration ($F_{(2,72)} = 3.5$, $p < 0.05$), and interaction effects ($F_{(6,72)} = 2.7$, $p < 0.05$). The highest overall c-Fos expression was found in the DMS of Lt-OFF-Resp rats, the second highest one in Lt-ON-Dual rats, then in Lt-ON-Place rats, and finally in HC rats; all intergroup differences were significant ($p < 0.01$, at least). Multiple comparisons showed that the only group in which the number of c-Fos-positive neurons was significantly depending on the duration of training was the Lt-

ON-Dual group; this number was reduced significantly after 6 and 14 days compared to 1 day ($p < 0.05$ in each case).

Analyses of the DLS data showed significant Protocol ($F_{(3,72)} = 87.2$, $p < 0.001$) and Protocol X Duration interaction effects ($F_{(6,72)} = 2.7$, $p < 0.05$); the overall Duration effect was not significant ($F_{(2,72)} = 1.5$, $p = 0.22$). The highest overall c-Fos expression was found in Lt-OFF-Resp rats, the second highest one in Lt-ON-Dual rats, then in Lt-ON-Place rats, and finally in HC rats; all intergroup differences were significant ($p < 0.01$). The only group in which the number of c-Fos-positive neurons was significantly depending on training duration was the Lt-OFF-Resp group; this number was larger after 6 and 14 days than after 1 day ($p < 0.05$ in each case).

3.1.3.2. c-Fos quantification in the dorsal hippocampus:

Analyses of c-Fos positive neurons in CA1 showed significant Protocol ($F_{(3,72)} = 17.9$, $p < 0.001$), Duration ($F_{(2,72)} = 16.1$, $p < 0.001$), and interaction effects ($F_{(6,72)} = 11.1$, $p < 0.001$). The same effects were found in CA3 and in the DG, although with less pronounced differences. In the DG, post hoc analyses indicated that overall c-Fos expression was weaker after 14 than after 1 and 6 days of training ($p < 0.05$). In CA1 and CA3, the highest overall c-Fos expression was found in Lt-ON-Dual and Lt-ON-Place rats ($p < 0.001$), which did not differ from each other. The lowest one was found in Lt-OFF-Resp and HC rats, which did not differ from each other. Regarding training durations, the lowest overall c-Fos expression was found for the longest duration in CA1, CA3, and DG ($p < 0.05$). In two groups, the number of c-Fos-positive neurons was dependent on training duration: it was dramatically reduced between 1 and 6 days in CA1 of Lt-ON-Place rats ($p < 0.01$), and between 6 and 14 days of Lt-ON-Dual rats ($p < 0.01$). In CA3, it was reduced between 1 and 6 days in Lt-ON-Place rats ($p < 0.05$) and increased after 6 days of training in Lt-ON-Dual rats compared to 1 and 14 days ($p < 0.01$). This was also the case in the DG ($p < 0.05$).

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Insert Figure 5 about here

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

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

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Insert Figure 6 about here

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

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

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

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

overall performance, mainly over the first four days. In the rats with the cannulae 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 the first three days. The analysis of the distances (not illustrated) and of the number of errors (see Supplementary Figure 14) pointed to **similar** conclusions.

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Insert Figure 7 about here

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

MUSC altered performance in Lt-OFF-Resp rats, whether infused in the striatum or the hippocampus. In Lt-ON-Dual rats, intrahippocampal MUSC infusion disrupted the response memory after 6 training days and –at least to some extent– the place memory after 14 days. In Lt-ON-Place rats, place memory was altered by MUSC after 6 and 14 days of training. After sustained training, the place memory system has become resistant to intrastriatal MUSC infusion. The data are illustrated in Figures 8 and 9.

3.2.4.1. Initial swimpaths

In the Lt-OFF-Resp condition, almost all rats infused with aCSF in the striatum first swam to the N (i.e., showed an egocentric strategy); their proportion did not differ statistically from chance (i.e., from 25%) and was not influenced by training duration. Very few of them subsequently shifted to the NE arm (see supplementary Table 3). MUSC infusions reduced the number of rats swimming directly to the N arm ($Chi^2 = 4{,}7$, $p < 0.05$ whatever the duration of training). Almost none of these rats shifted to the NE after their visit to the N arm. In the Lt-ON-Dual group, most first-swim paths also consisted in R-L turns after aCSF infusion. The proportion of rats then shifting to the NE was weak after 6 training days, and larger after 14 days (see supplementary Table 3). In Lt-ON-Dual rats given intrastriatal MUSC, the proportion of direct swims to the N was not affected after 6 training days, but it was significantly reduced after 14 days of training ($Chi^2 = 4.8$, $p < 0.05$). Finally, after intrastriatal aCSF infusion in Lt-ON-Place rats, 3 out of 7 rats swam directly to the N arm (the others swimming to

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

When aCSF was infused in the dorsal hippocampus, a large majority of rats first swam directly to the N arm, whatever the training condition. After intrahippocampal MUSC, this behavior was significantly reduced in Lt-OFF-Resp rats (6 days: $Chi^2 = 8.8$, $p < 0.01$; 14 days: $Chi^2 = 6.5$, $p < 0.05$). The same was observed in Lt-ON-Dual rats (6 days: $Chi^2 = 5.6$, $p < 0.05$; 14 days: $Chi^2 = 5.6$, $p < 0.05$). Finally, in Lt-ON-Place rats, intrahippocampal MUSC reduced the number of rats swimming directly to the N after 6 days of training ($Chi^2 = 4.7$, $p < 0.05$), but not after 14 days of training.

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

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Insert Figure 8 about here

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

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

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

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

3.2.4.3. Cumulated time in arms reached by R-L turns:

MUSC disrupted response memory performance, especially when infused into the dHIP. Data are shown in Figure 9.

After intrastriatal infusions, the time spent in the R-L arm was above chance in aCSF-treated Lt-OFF-Resp and Lt-ON-Dual rats after 6 days of training ($p < 0.05$), and only in Lt-OFF-Resp rats after 14 days of training ($p < 0.05$). In rats infused with MUSC, it never exceeded chance significantly. MUSC-induced effects were further analyzed with a MUSC x Duration ANOVA for each training protocol. MUSC infusions into the DStr induced an overall impairment of response-memory-based behavior only in Lt-OFF-Resp rats ($F_{(1,28)} = 8.12$, $p < 0.05$). Multiple comparisons showed that the difference was significant after 6 ($p < 0.05$; Tuckey test) not 14 days of training..

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

after 6 days of training ($p < 0.05$; Tuckey test), and only tended towards significance after 14 days ($p = 0.066$; Tuckey test).

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Insert Figure 9 about here

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3.2.4.4.Cumulated time in NE arm:

Overall, MUSC disrupted place memory performance. Data are shown in Figure 9.

The time in the NE arm exceeded chance in aCSF-treated Lt-ON-Place rats after 6 and 14 days of training ($p < 0.05$), whether subjected to intrastriatal or intrahippocampal infusions. Only after 14 days of training did this time also exceed chance in Lt-ON-Dual rats. After MUSC infusion, time in the NE arm did not differ from chance, except when MUSC was infused in the striatum of Lt-ON-Dual and Lt-ON-Place rats after 14 days of training. ANOVA showed that when infused into the striatum, MUSC reduced place-memory-based performance (time in NE arm) in Lt-ON-Place rats, but only after 6 days of training ($p < 0.001$). When MUSC was infused into the hippocampus, there was a significant overall impairment in Lt-ON-Resp rats ($F_{(1,31)} = 7.66$, $p < 0.05$) and Lt-ON-Place rats ($F_{(1,30)} = 24.5$, $p < 0.001$), but in Lt-ON-Dual rats only a tendency was noticed ($F_{(1,26)} = 3.56$, $p = 0.07$). In Lt-ON-Resp rats, the MUSC effect was significant only after 6 days of training ($p < 0.001$; Tuckey test). In Lt-ON-Place rats, it was significant after both training durations ($p < 0.05$; Tuckey test).

4. Discussion

In tasks with dual place/response solution, the place memory system is the first to find the solution, the response memory system coming into play later on (e.g., Packard and McGaugh, 1996). We therefore expected Lt-ON-Dual rats to use their place memory system first. Our results point to a different outcome. We also expected that hippocampal c-Fos expression would increase when place memory is used, thus at an early stage of training, and that striatal c-Fos expression would

increase later on when response memory is used. Our results only partly support these expectations. We predicted that striatal blockade would affect response memory, whereas hippocampal blockade would affect place memory, as e.g., in Packard and McGaugh (1996). We found that striatal inactivation altered response memory in Lt-OFF-Resp rats. Furthermore, for both training durations, hippocampal inactivation affected performance in Lt-ON-Place and, although to a weaker degree, in Lt-ON-Dual rats, as expected, but also surprisingly in Lt-OFF-Resp rats. 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 a hippocampus-independent response memory and a striatum-independent place memory.

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

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

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

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

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

learning (e.g., Balleine & O'Doherty, 2010; Devan and White, 1999; Hawes et al., 2015; Ito & Doya 2015; Pauli et al., 2012). Indeed, this view predicts a shift of the highest activation from DMS to DLS over training in Lt-OFF-Resp rats. In the DHip of our Lt-ON-Dual rats, a c-Fos increase was found in CA3 and the DG only after 6 days of training. We do not known why, but it is tempting to speculate that these regions could have contributed to the transition from a response memory-based behavior to a place memory based one. CA1 was the only region to show a number of c-Fos-positive neurons largely above controls. This difference, however, was found only after 1 and 6 training days, suggesting that once navigation relied on a place strategy, memory retrieval occurred at a substantially weaker neuronal activation cost in the hippocampus (e.g., Bertaina-Anglade et al., 2000; Shires & Aggleton, 2008). This possibility is compatible with the fact that in Lt-ON-Place rats, c-Fos levels were not different from controls already after 6 days of training. An alternative explanation would be that, over training, the task ceased to depend on the hippocampus, a hypothesis contradicted by our MUSC data. Using a protocol close to that of Packard and McGaugh (1996) in mice, Passino et al. (2002) observed a c-Fos expression that was less pronounced in CA1 (about 45%) after a long training period (18 days; 72 trials) compared to a shorter one (9 days; 36 trials). Unfortunately, c-Fos counts were not distinguished according to whether mice were response or place learners. This study nevertheless indicates that well-trained performance is not necessarily correlated with high neuronal activation indexes in the structure presumed to support performance. Another point in Lt-ON-Dual rats was the high c-Fos expression level in the DStr (DMS and DLS) after 1 day of training, which was associated with high c-Fos expression in CA1. The DStr activation most probably reflected the predominant engagement of the R-L turns the rats had learned on the previous day. Upon negative feedback during the probe trial, these rats might have tried to shift to a hippocampal-driven correction, which failed because after 1 day of training, the place memory trace may have been too weak or absent. Indeed, we know that, in the water maze, a 1-day training encompassing 4 consecutive trials does not enable above chance performance in a probe trial given on the next day (e.g., Bousiges et al., 2013, see Fig 1A). Another explanation for the decrease in hippocampal c-Fos expression seen in Lt-ON-Dual rats (between 6 and 14 training days) could be that rats have developed an alternative to the place memory strategy. For instance, they

might have learned to reach the NE arm by swimming in the central corridor until facing a wall; once there, they just had to turn on their left. Finally, we cannot exclude that stress inherent to our test has impacted the way rats have tried to solve their respective task, hence their c-Fos activation patterns, and that this factor accounts for differences between expected and observed results. Further experiments, however, perhaps with modified testing device/protocols, are required to explore these issues. Whatever may be, some of our functional imaging results suggest that, in well-trained and well-performing rats, c-Fos expression within the behaviorally-relevant structures may not accurately parallel performance.

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

Given the literature (e.g., Packard & Goodman, 2013), the disruption of **i)** response memory-based strategy by intrastriatal infusions of MUSC in Lt-OFF-Resp rats, and **ii)** place memory-based strategy by intrahippocampal infusions of MUSC in Lt-ON-Dual and Lt-ON-Place rats was expected. Because after 14 days of training intrahippocampal infusions of MUSC disrupted the place memory-based strategy, which intrastriatal MUSC infusions left unaltered, Lt-ON-Dual rats were relying on hippocampal function after sustained training, as was the case for Lt-ON-Place rats. Not expected – but observed – were **i)** the disruption of the response memory-based, strategy by intrahippocampal MUSC in Lt-OFF-Resp rats after both training durations, and **ii)** given Packard and McGaugh (1996), the absence of an egocentric memory-based deficit in Lt-ON-Dual rats in response to intrastriatal MUSC after 14 days of training. The MUSC-induced alteration of performance in Lt-ON-Place after 6 days of training was also not expected. Taken together, these results suggest that, in the double-H maze task, the use of a response strategy may depend on both the dorsal striatum and the hippocampus, which do not become functionally independent from each other in Lt-OFF-Resp rats, even after long training. The use of an allocentric strategy also depends on both structures, but transiently. Indeed, following sustained training, the DStr is no longer needed for navigation correction, but it seems important for the acquisition of a spatial approach to a task (Jacobson et al., 2012; Pooters et al., 2015, 2016). In case of a repetitive navigation task with full access to allothetic cues, the DHip appears more crucial than the DStr, first to enable 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

(e.g., DMS to form it, DLS to store it) and the overall geometry (spatial calibration; see Avni et al., 2008) of the maze by a mechanism partly implicating the hippocampus. Both information aspects may have combined in a modular representation (e.g., Tcheang et al., 2011).

Another possibility could be related to mechanisms of navigation in darkness, which, regarding self-motion information in goal-directed behavior, involves the cerebellum and its functional connection with the hippocampus (e.g., Rochefort et al., 2011). This functional connection might have been disrupted by dorsal hippocampus inactivation. Why could spatial memory be depending on the dorsal striatum after 6 (and not 14) days of training? Given its location, our MUSC inactivation affected partly the DMS and partly the DLS (see supplementary figure 11). Furthermore, correcting a response memory-based strategy by using a place memory-based one requires behavioral flexibility driven by the prefrontal cortex (e.g., Cholvin et al., 2013). Because the DMS is involved in action outcome and behavioral flexibility, notably through its connections with the (pre)frontal cortex (e.g., Baker and Ragozzino, 2014; Ragozzino et al., 2002), it is well possible that MUSC has affected one or both of these functions, perhaps even without affecting hippocampus-dependent spatial memory processes per se. Interestingly, Ragozzino (2003) found that DMS cholinergic interneurons contributed to behavioral flexibility, which is further supported in the studies by e.g., Aoki et al. (2013; but see Okada et al., 2014, or Braun and Hauber, 2011; Braun et al., 2012). Still along these lines, we reported that reversible inactivation of the prefrontal cortex, which did not alter spatial memory retrieval in a water maze task, profoundly disrupted strategy adaptation in the double-H maze (Cholvin et al., 2013). Indeed, after MUSC inactivation of the prefrontal cortex, rats were unable to shift from the response-based to the place-based strategy. A functional alteration of the prefrontal cortex being a potential consequence of dorsal striatum inactivation, it is possible that something similar occurred in our Lt-ON-Dual rats. This possibility is in line with a report showing that the DMS plays a role in adapting a habitual strategy to a sudden modification of the contingency in a spatial task (Regier et al., 2015).

Based on our findings, it is not possible to provide clear and solid arguments to explain the discrepancy between our expectations and the observed results. It is noteworthy that striatal inactivation affected the DMS and DLS, when previous

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

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

5. Conclusion

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

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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.

<u>Figure 3:</u> 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.

<u>Figure 4:</u> 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.

<u>Figure 5:</u> 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, $p < 0.05$.

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

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

Figure 8: Initial swim paths adopted by rats at the start of the probe trial indicate that memory system-based behavior, whether response or place, is disrupted by MUSC. In each of the three training protocols (**Lt-OFF-Resp, Lt-ON-Dual, Lt-ON-Place**), training lasted for 6 or 14 days and the probe trial was given 24 hr after the last training day. Rats were subjected to bilateral intrastriatal (STRIATUM) or intrahippocampal (HIPPOCAMPUS) infusions of muscimol (MUSC) or artificial cerebrospinal fluid (aCSF) as control, 30 min before the probe trial. 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 2. Statistics: * significant modification of the proportion of rats that swam directly to the N arm as shown by a Chi^2 analysis, $p < 0.05$. The total number of rats tested under each condition is indicated under the corresponding pie chart.

<u>Figure 9:</u> Response memory is disrupted by intrastriatal (STRIATUM) and intrahippocampal (HIPPOCAMPUS) MUSC in Lt-OFF-Resp rats; however, the shift to place memory-based behavior is resistant to intrastriatal MUSC after 14 days of training in Lt-ON-Dual and Lt-ON-Place 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 of the three training protocols (Lt-OFF-Resp, Lt-ON-Dual, Lt-ON-Place) and each training duration (6, 14 days). The probe trial was given with a 24-h delay after the last training trial. Times consecutive to swim paths ending in the NE arm when coming from the S arm were discarded from the analysis (see Methods). 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 aCSF, $p < 0.05$. The total number of rats tested under each condition is indicated in Figure 8.

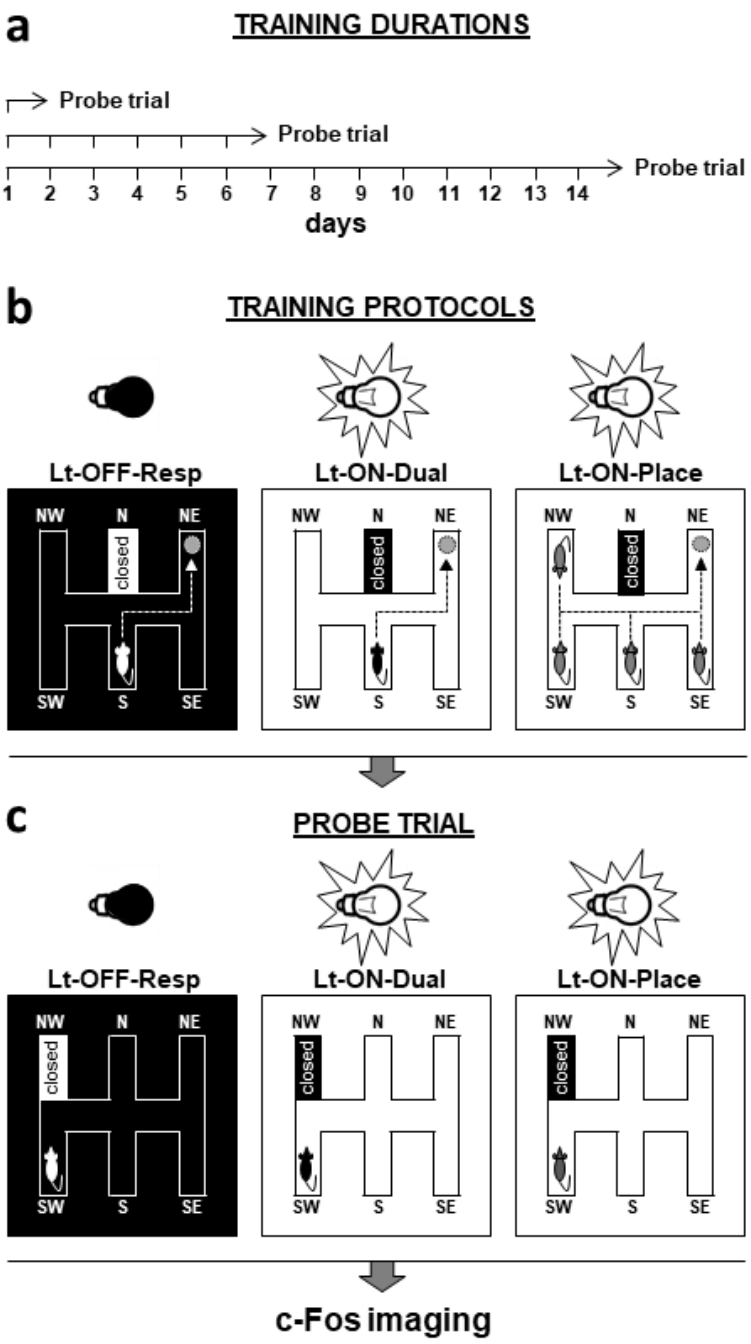
a
TRAINING DURATIONS
Probe trial
Probe trial
Probe trial
1 2 3 4 5 6 7 8 9 10 11 12 13 14
days
b
TRAINING PROTOCOLS
Lt-OFF-Resp
Lt-ON-Dual
Lt-ON-Place
NW N NE
closed
SW S SE
c
PROBE TRIAL
Lt-OFF-Resp
Lt-ON-Dual
Lt-ON-Place
NW N NE
closed
SW S SE
c-Fos imaging

Figure 1

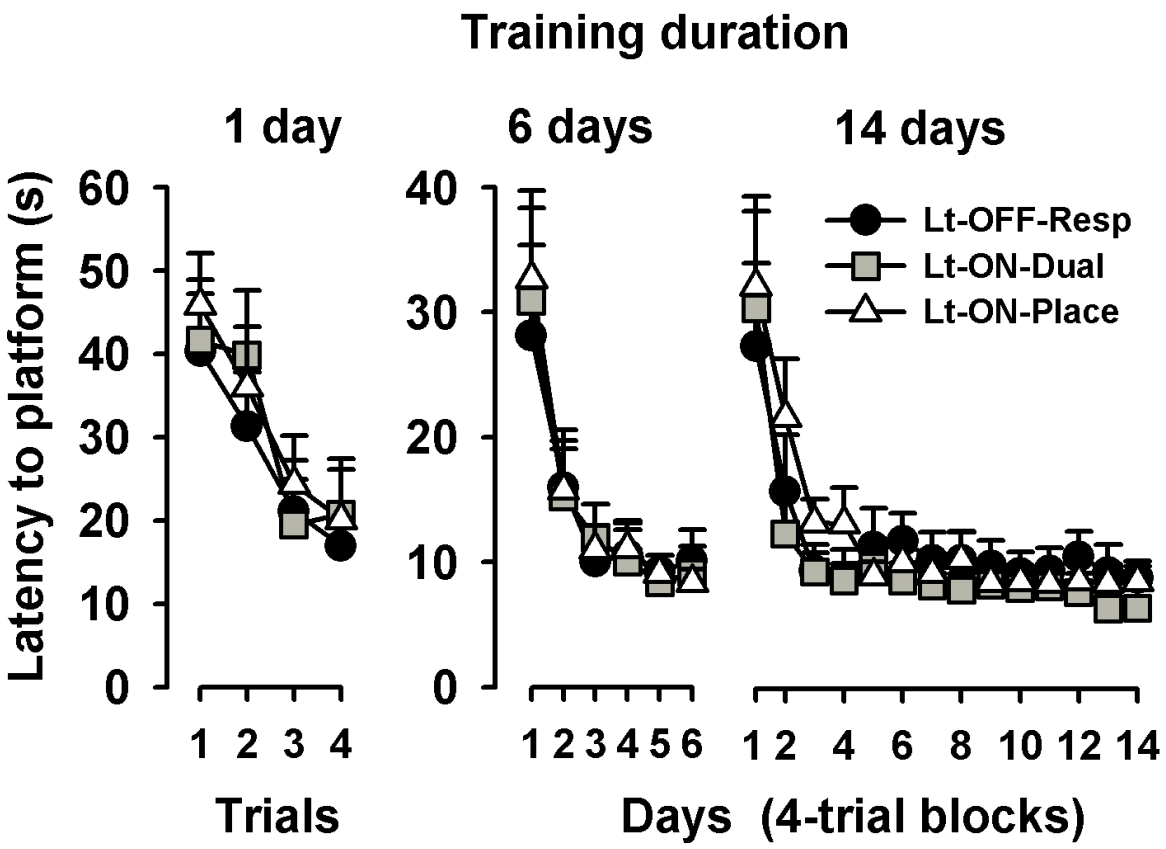
Training duration
1 day
6 days
14 days
Latency to platform (s)
60
50
40
30
20
10
0
40
30
20
10
0
Lt-OFF-Resp
Lt-ON-Dual
Lt-ON-Place
1 2 3 4
1 2 3 4 5 6
1 2 4 6 8 10 12 14
Trials
Days (4-trial blocks)

Figure 2

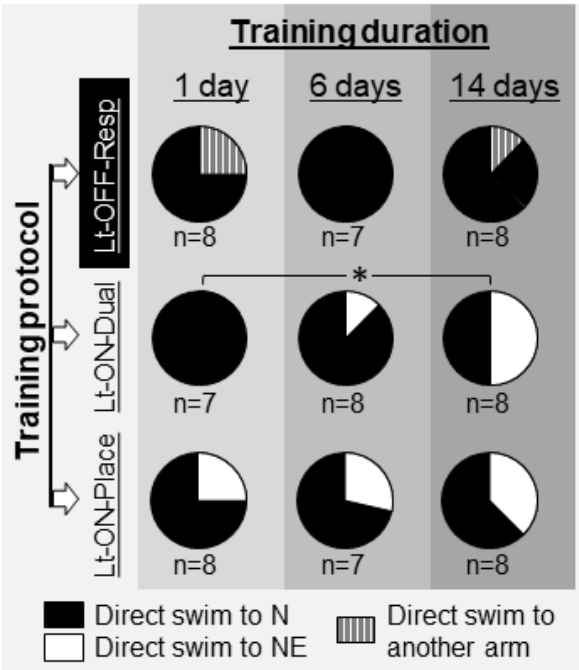

Figure 3

1436

1437

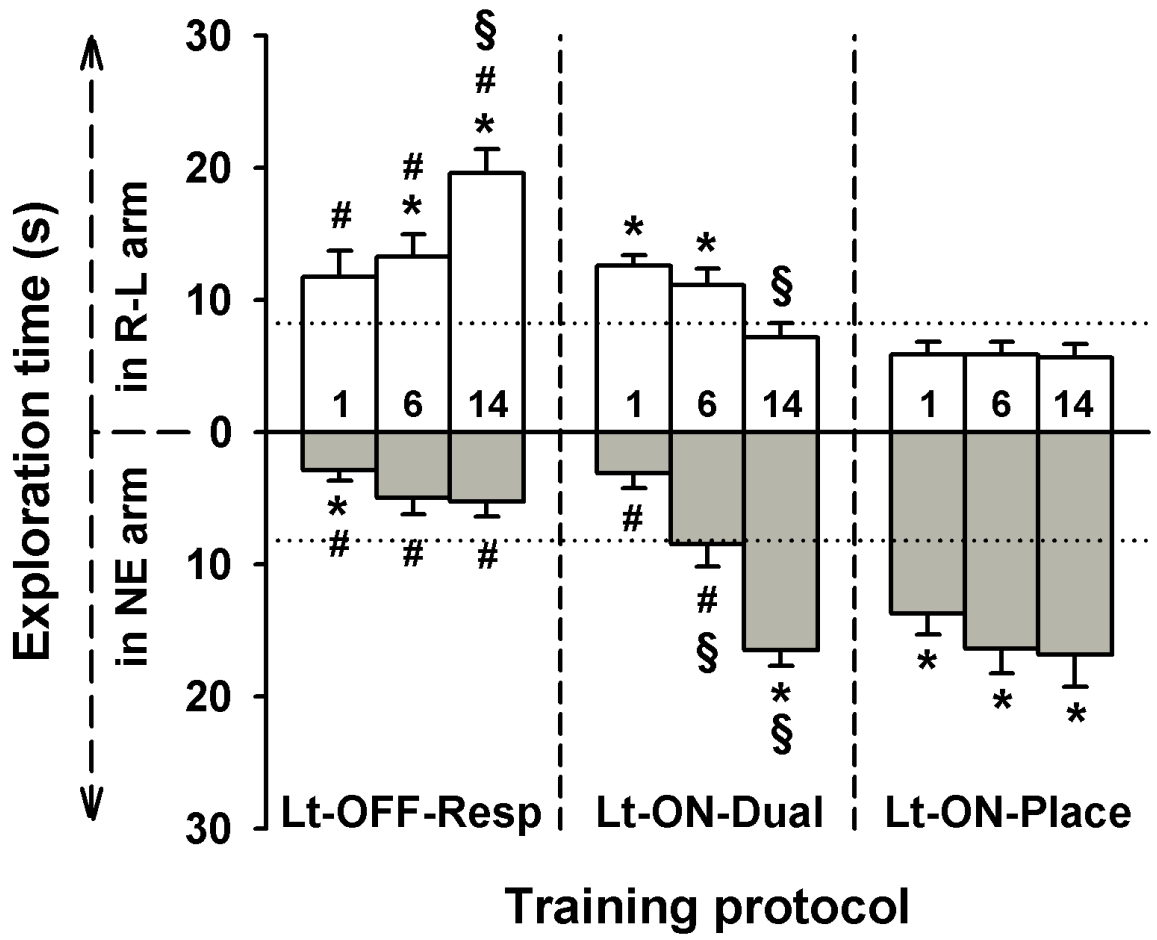
Exploration time (s)
in R-L arm
in NE arm
30
20
10
0
10
20
30
1
6
14
Lt-OFF-Resp
Lt-ON-Dual
Lt-ON-Place
Training protocol

Figure 4

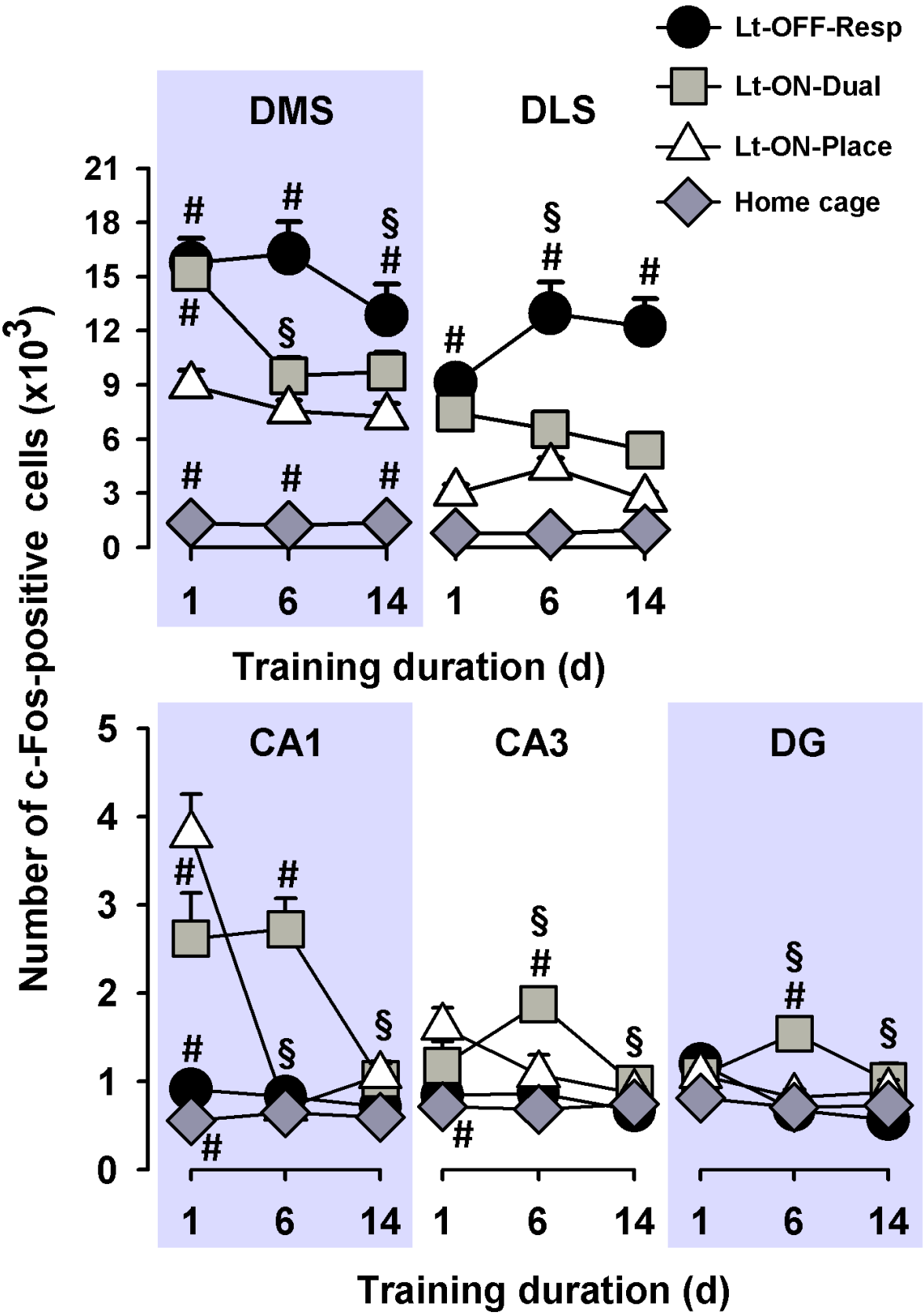
Lt-OFF-Resp
Lt-ON-Dual
Lt-ON-Place
Home cage
DMS
DLS
CA1
CA3
DG
Number of c-Fos-positive cells (x10^3)
Training duration (d)
Training duration (d)

Figure 5

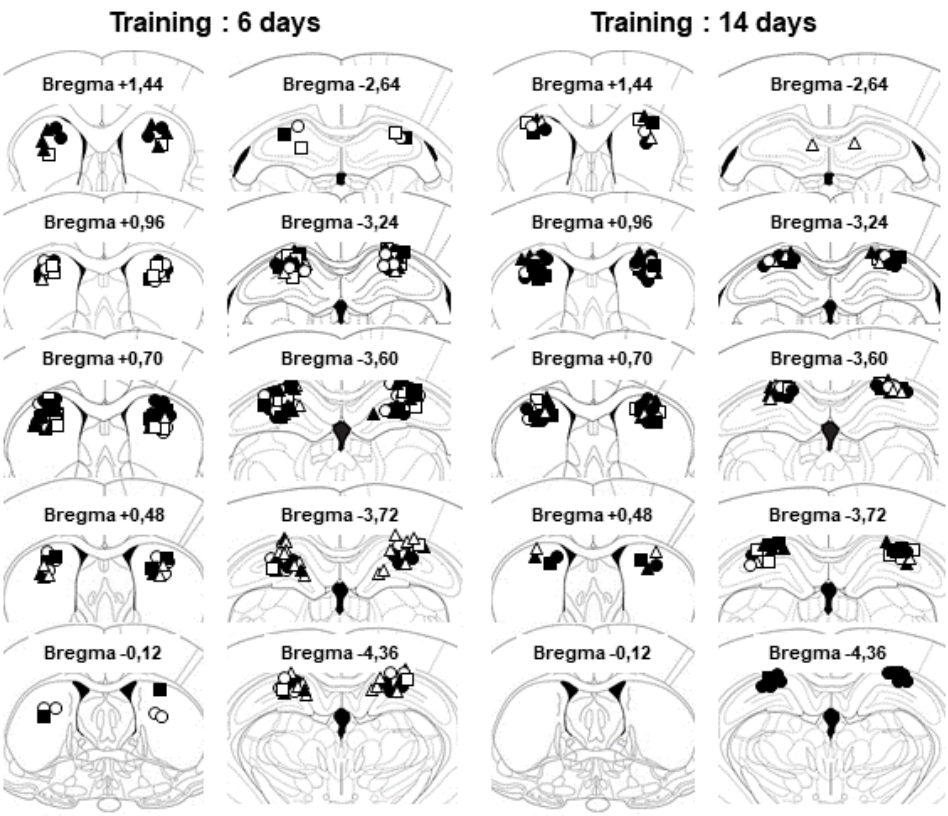
Training : 6 days
Training : 14 days
Bregma +1,44
Bregma -2,64
Bregma +1,44
Bregma -2,64
Bregma +0,96
Bregma -3,24
Bregma +0,96
Bregma -3,24
Bregma +0,70
Bregma -3,60
Bregma +0,70
Bregma -3,60
Bregma +0,48
Bregma -3,72
Bregma +0,48
Bregma -3,72
Bregma -0,12
Bregma -4,36
Bregma -0,12
Bregma -4,36

Figure 6

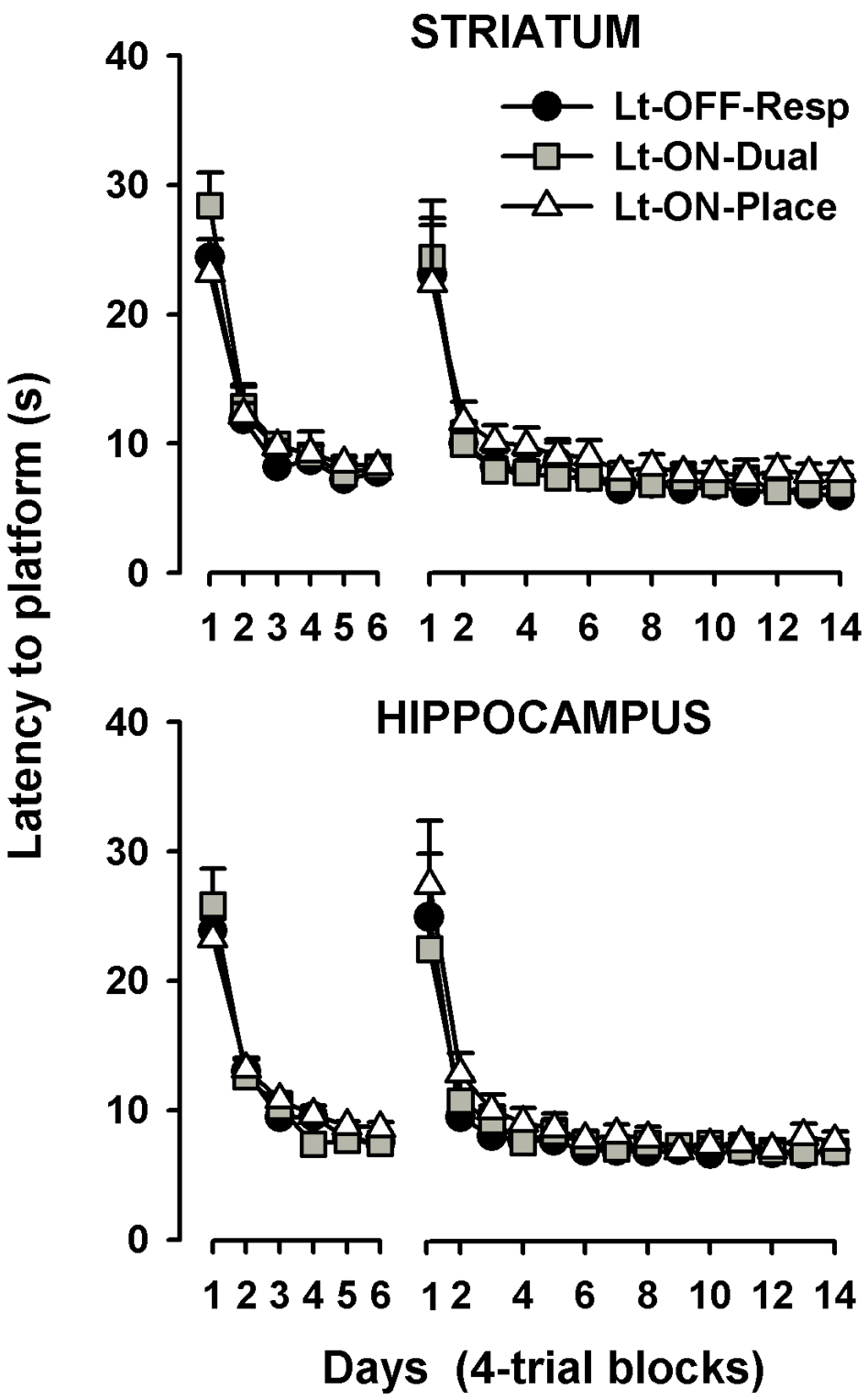
STRIATUM
Lt-OFF-Resp
Lt-ON-Dual
Lt-ON-Place
HIPPOCAMPUS
Latency to platform (s)
0
10
20
30
40
1 2 3 4 5 6 1 2 4 6 8 10 12 14
Days (4-trial blocks)

Figure 7

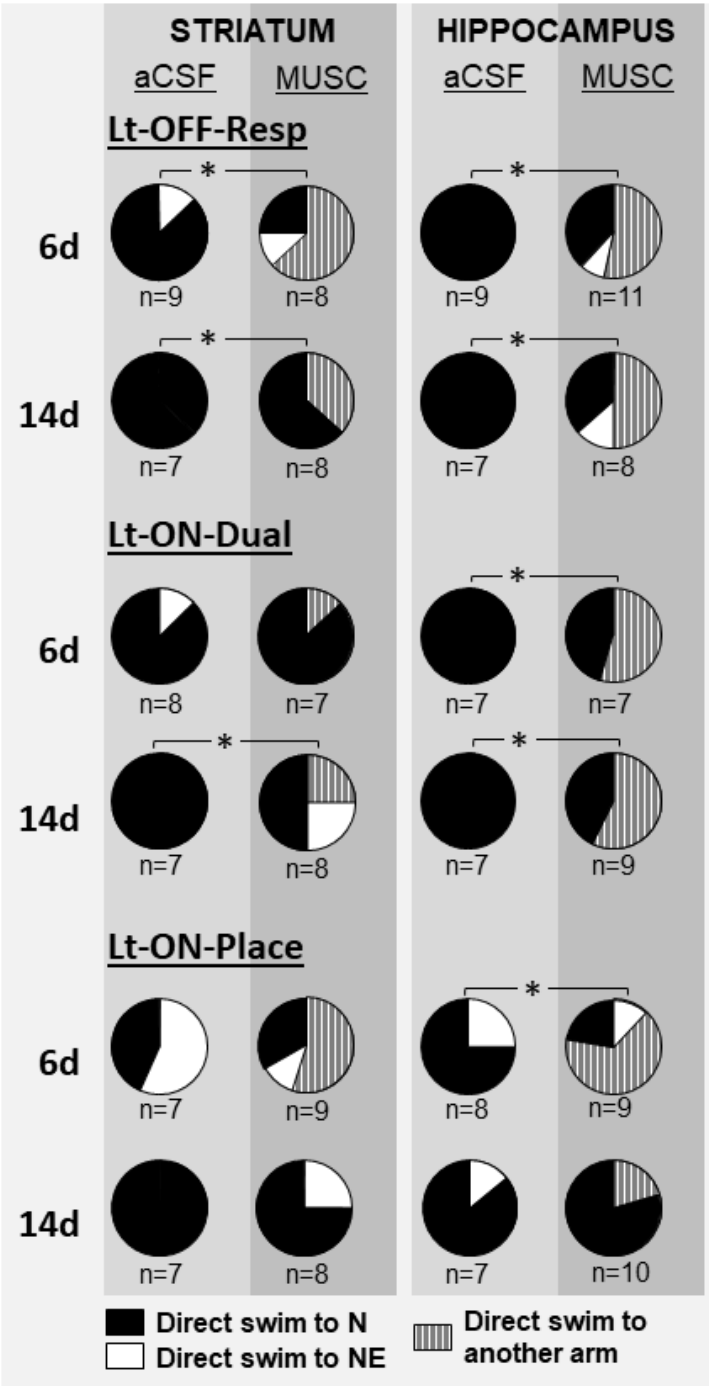

Figure 8

1454

1455

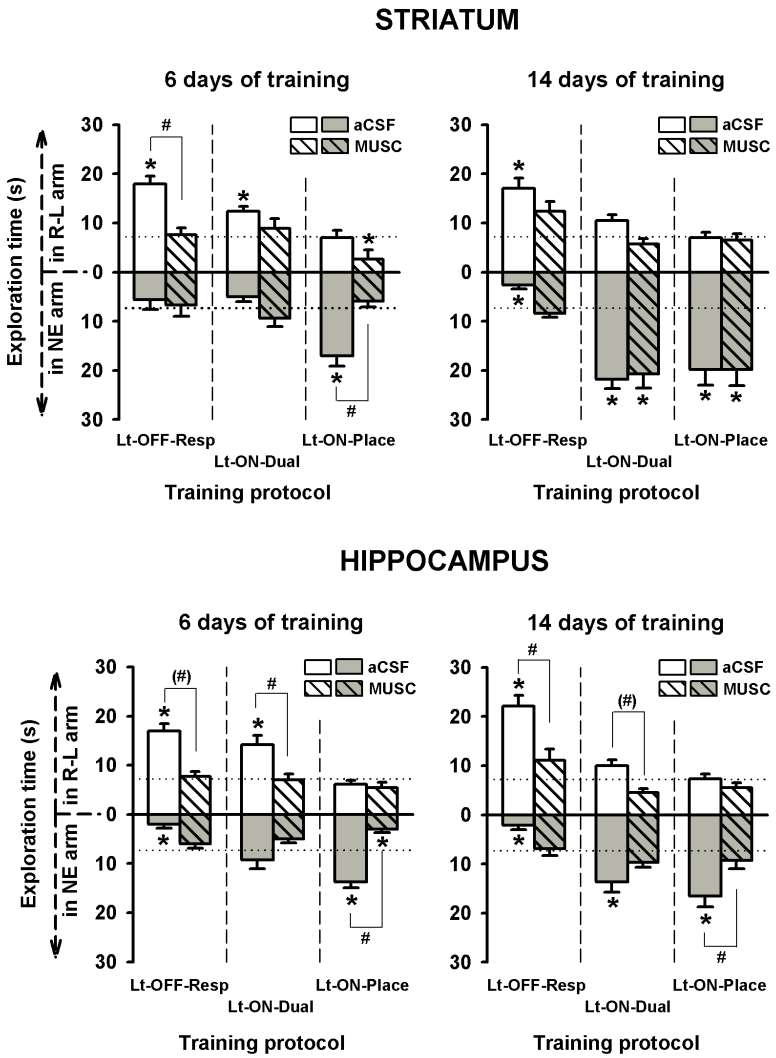
STRIATUM
6 days of training
14 days of training
aCSF
MUSC
Exploration time (s)
in R-L arm
in NE arm
Lt-OFF-Resp
Lt-ON-Place
Lt-ON-Dual
Training protocol
HIPPOCAMPUS
6 days of training
14 days of training
aCSF
MUSC
Exploration time (s)
in R-L arm
in NE arm
Lt-OFF-Resp
Lt-ON-Place
Lt-ON-Dual
Training protocol

Figure 9

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

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Supplementary material (25 pages)

Number of supplementary text : **2 (Methods and References)**

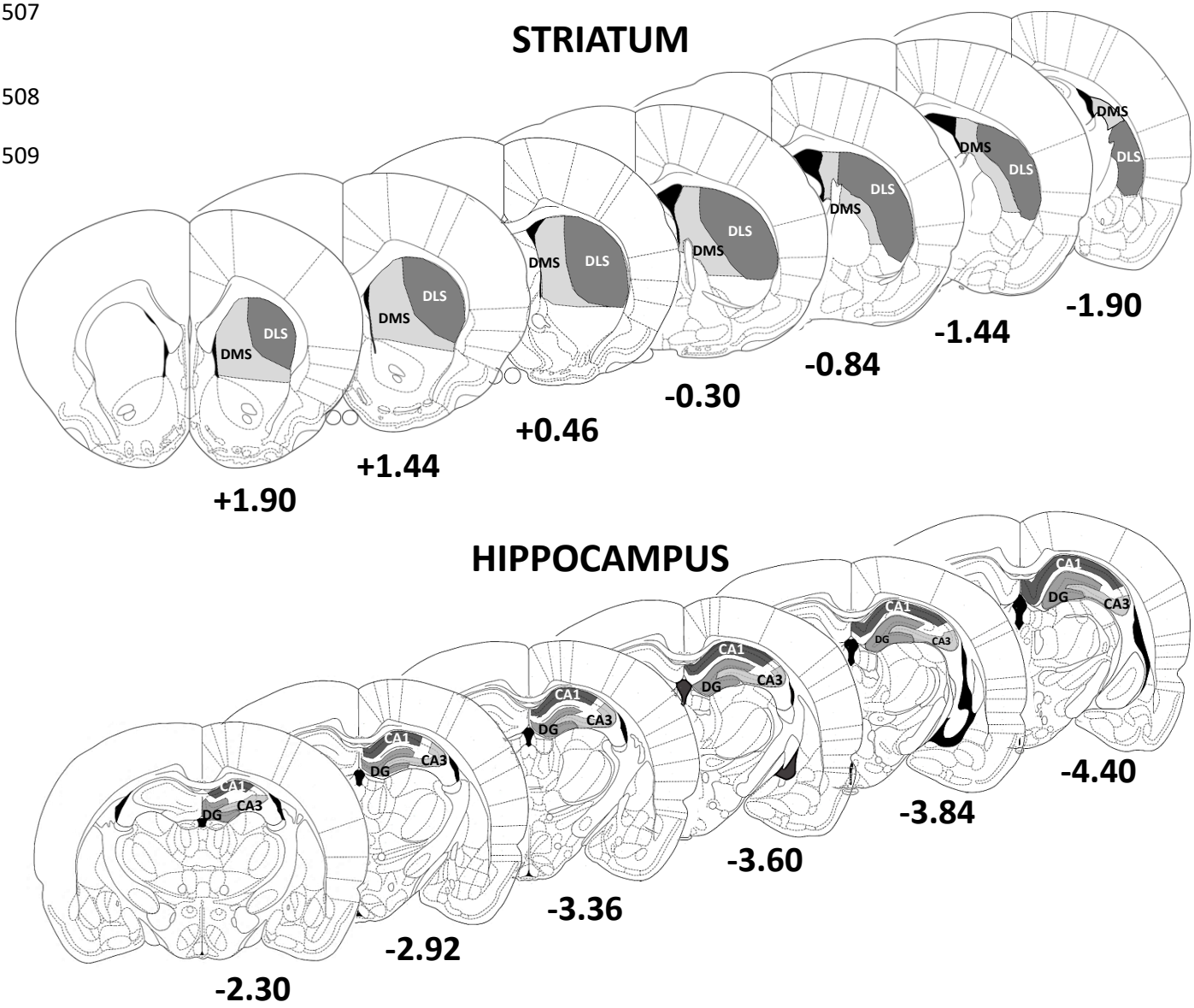
Number of supplementary figures : **16**

Number of supplementary tables : **4**

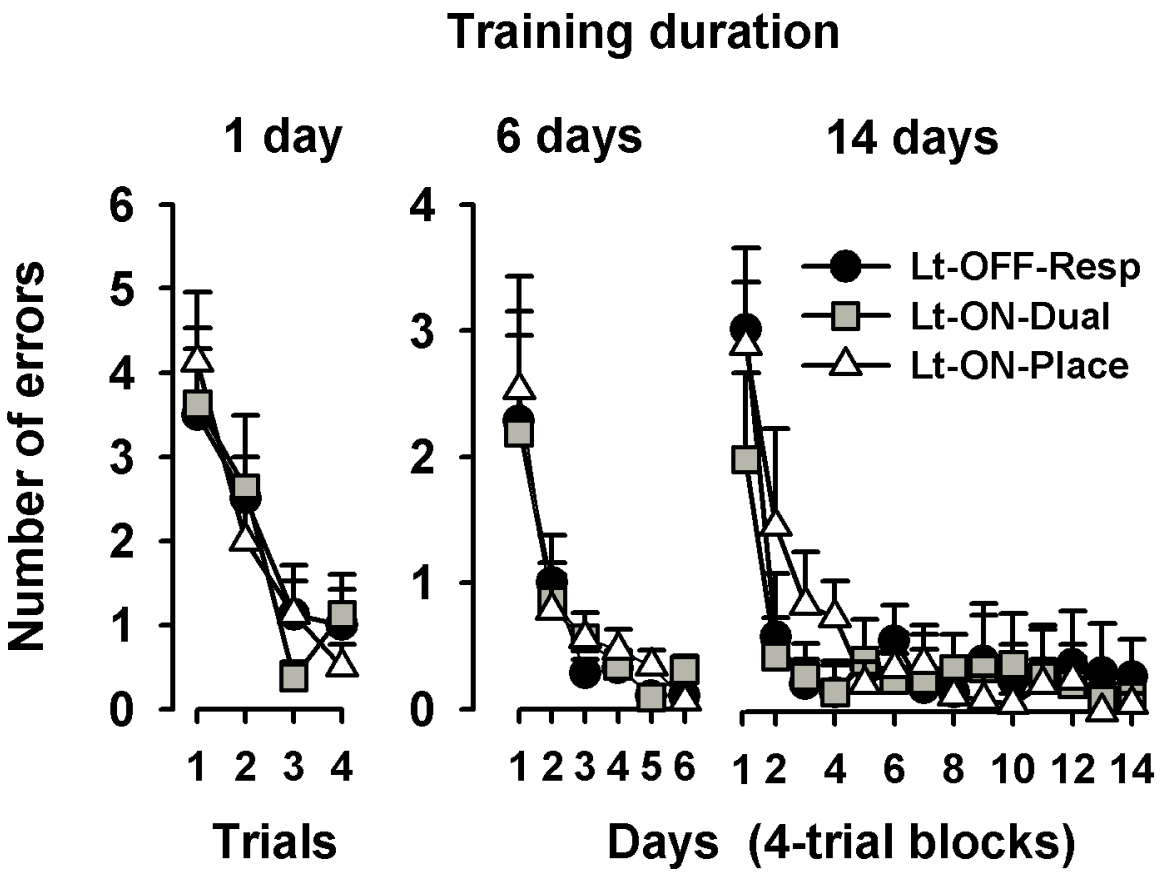
Supplementary Methods:

A trajectory going from the S to the NE arm can reflect a response (successive R-L turns) memory or a place memory. In such case, it is not possible for the experimenter to know which strategy a rat has actually used. Therefore, the times spent in the NE arm when a rat was coming from the S were not considered. In fact, only times recorded in the NE arm when rats came from SW, SE or N were considered. This correction represented in average a subtraction of 2.34 ± 0.51 s (range 0-5.5 s) in experiment 1, and of 2.22 ± 0.56 (range 0-5.6) and 1.2 ± 0,32 (range 0-3.8) in rats with intrastriatal and intrahippocampal cannulas, respectively, in experiment 2. The correction was applied whatever the training protocol and duration. It was computed for each rat and subtracted from its probe trial performance (i.e., time in target after R-L turns, and time in NE) before statistical analyses of individual scores were performed.

<u>Supplementary Figure 1:</u> Subregions of the dorsal striatum and of the dorsal hippocampus in which the number of c-Fos positive cells was quantified. Abbreviations: <u>DLS:</u> dorsolateral striatum; <u>DMS:</u> dorsomedial striatum; <u>CA1:</u> region CA1 of the cornu Ammonis; <u>CA3:</u> region CA3 of the cornu Ammonis; <u>DG:</u> dentate gyrus. Anteriority levels under each plate are indicated in mm from Bregma (Paxinos and Watson, 2007). The distinction between DLS and DMS is based on the article by Voorn et al. (2004), with the DLS corresponding essentially to the afferents from the sensorimotor cortex, and the DMS to afferents from other cortical structures (e.g., medial prefrontal cortex, visual cortex, auditory cortex, perirhinal cortex, and entorhinal cortex).



<u>Supplementary Figure 2:</u> Number of errors showed comparable acquisition curves in Lt-OFF-Resp, Lt-ON-Dual and Lt-ON-Place rats. In each training protocol of the first experiment (Lt-OFF-Resp, Lt-ON-Dual and Lt-ON-Place), mean numbers of errors (+ sem) are represented for the first training day (trial by trial) and over the 6- and 14-day training sessions (in daily blocks of four trials). Statistical analyses showed no significant difference between the training conditions. Notice that the Y axis corresponding to the first training day indicates a number of errors for each trial whereas in the two other panels (middle, right), it indicates the mean number of errors for each 4-trial block, hence the different scales of the Y-axis. The number of animals in each condition can be found in Figure 3 of the article. Statistical analyses showed the same effect (Trial or Day) as for the latencies to reach the platform. The same was true for distances.



<u>Supplementary Table 1:</u> Percent of rats shifting to the NE arm right after having first visited the N arm was not (significantly) different among groups. Number of rats showing a direct swim to the N arm after having been released in the maze are indicated between brackets as "(n/ N)", the "N" indicating the group size. The table reads as follows : "of the N tested rats, % of the n rats first entering the N arm and which then shifted to the NE one". For instance, regarding Lt-OFF-Resp rats after one day of training : one should read 33% of the 6 rats out of the 8 which first entered 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)

In Lt-ON-Place rats, this proportion was significantly higher than chance after 1 day of training ($Chi^2 = 10.89$, $p < 0.01$), and tended to exceed chance after 6 or 14 days of training ($Chi^2 = 3.27$, $p = 0.071$). The significant percentage is underlined. In the other cells, there was no significant difference from chance.

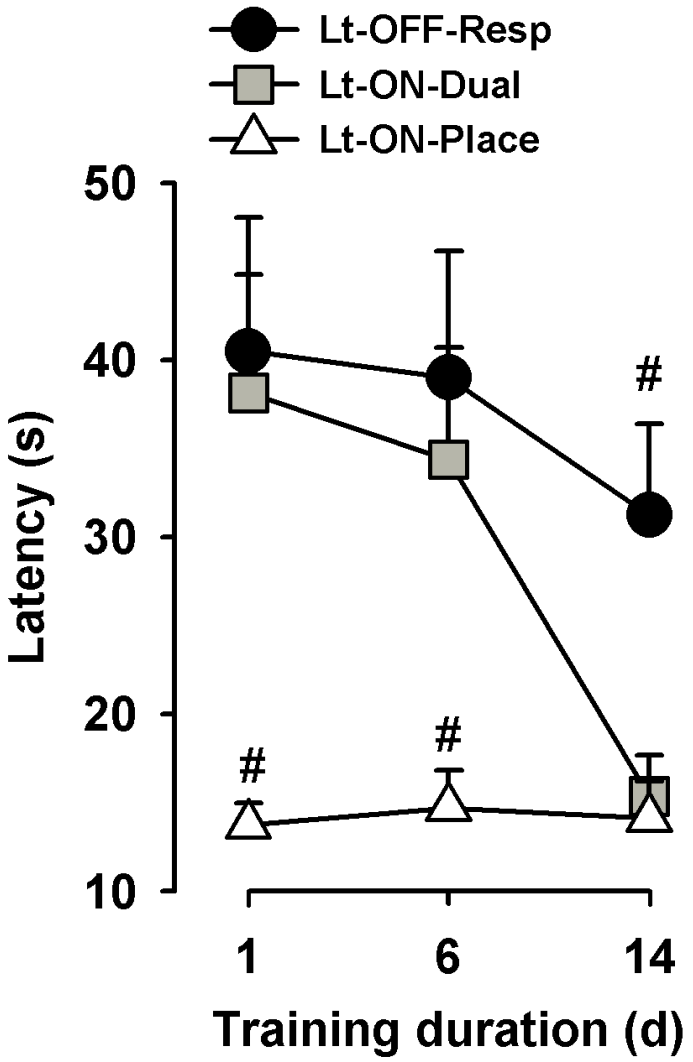
<u>Supplementary Table 2:</u> Average swim velocity were comparable during the probe trial among protocols and training durations. Data indicated in cm/s are means (sem). Statistical analyses showed no significant difference among groups, indicating that other variables were not biased by changes in 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)

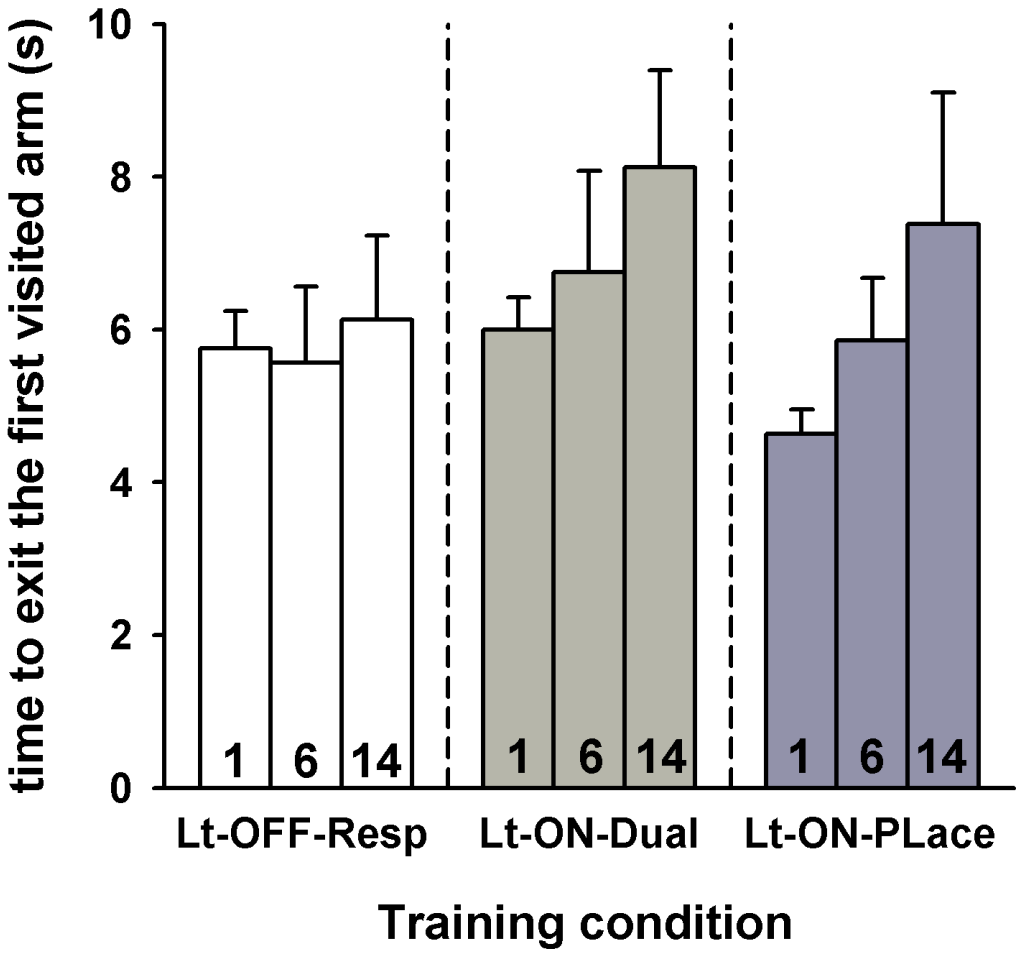
An ANOVA of the average swim velocities showed that Protocol ($F_{(2,60)} = 0.7$, *ns*), Duration ($F_{(2,60)} = 0.9$, *ns*) and their interaction ($F_{(4,60)} = 0.0$, *ns*) had no significant effect.

<u>Supplementary Figure 3:</u> Latencies to the place target (i.e., the NE arm) in Lt-OFF-Resp, Lt-ON-Dual and Lt-ON-Place rats during the probe trial are compatible with the progressive formation of a cognitive map in Lt-ON-Dual rats. All means are given in seconds (+ sem). This variable was computed to refine the analysis of the training-dependent evolution of strategies. We reasoned that if Lt-ON-Dual rats behaved according to an egocentric strategy incompatible with navigation correction, their latencies should be close to those of Lt-OFF-Resp rats. On the contrary, if they behaved according to an allocentric strategy enabling correction, whether direct or indirect, their latencies should be close to that of Lt-ON-Place rats. The idea behind this reasoning is that if a Lt-ON-Dual rat entered the N arm by R-L turns, it would expect to find the platform here and, consequently, spend some time looking for it in this arm. If, however, it already had some capability for a spatial approach of the task, and thus for navigation correction, but entered the N arm due to repetition of the R-L turns after starting, it would immediately leave the arm and swim to the NE one, whereby its latency to enter the NE arm should be shorter than in Lt-OFF-Resp rats. A Protocol x Duration ANOVA showed significant overall Protocol ($F_{(2,60)} = 15.5$, $p < 0.001$) and Duration ($F_{(2,60)} = 3.9$, $p < 0.05$) effects, but no significant interaction between both factors ($F_{(4,60)} = 1.5$, $p = 0.22$). A Newman-Keuls multiple comparisons test showed that Lt-ON-Dual rats did not differ significantly from Lt-OFF-Resp rats after 1 or 6 training days, but their latencies were significantly below those of their Lt-OFF-Resp counterparts after 14 training days ($p < 0.05$). Conversely, latencies of Lt-ON-Dual rats were significantly larger than those of Lt-ON-Place rats after 1 or 6 training days ($p < 0.05$), not after 14 days. Statistical analysis: # different from Lt-ON-Dual rats, $p < 0.05$.

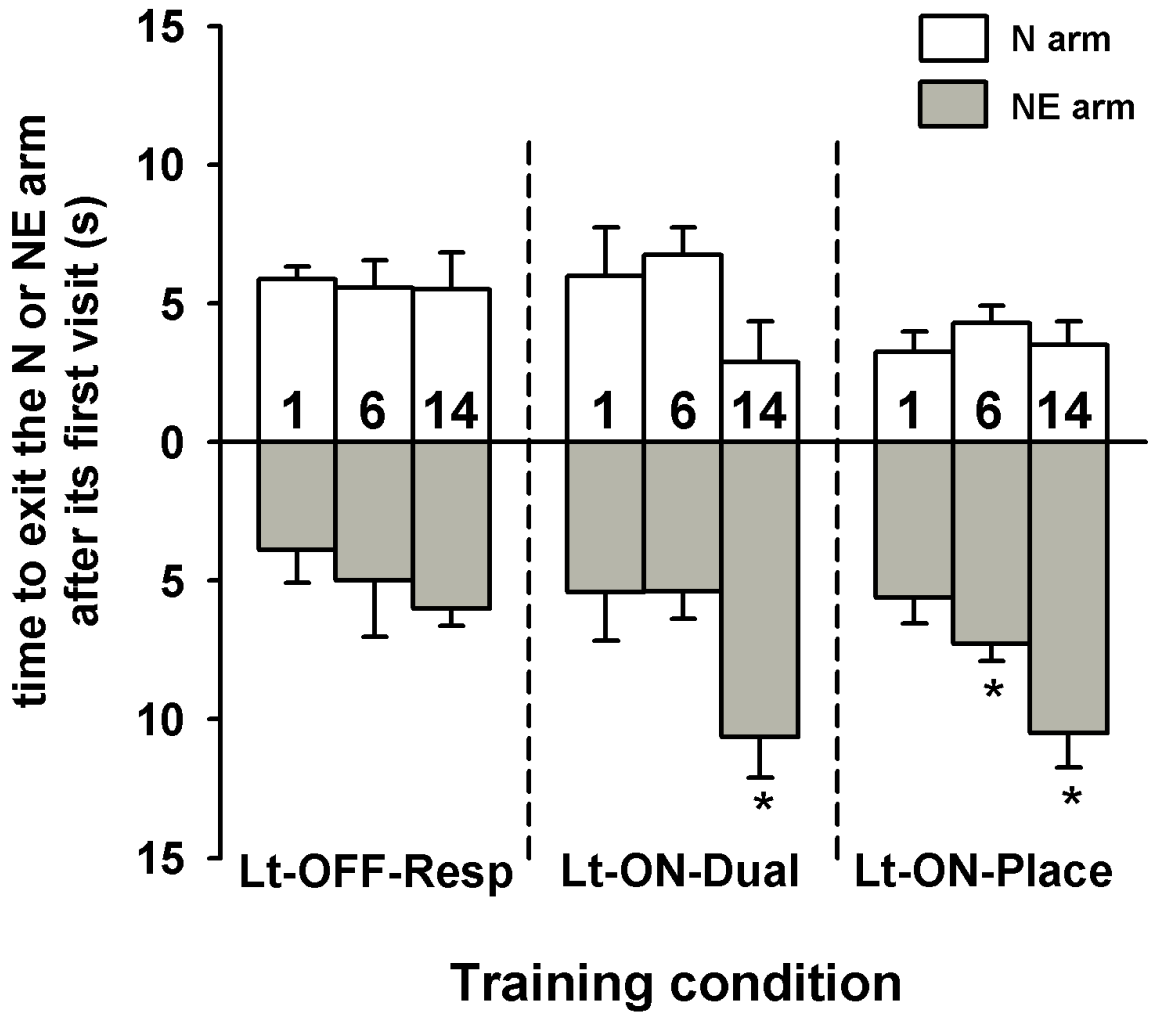
(see next page)


Lt-OFF-Resp
Lt-ON-Dual
Lt-ON-Place
50
40
30
20
10
Latency (s)
#
#
#
1
6
14
Training duration (d)

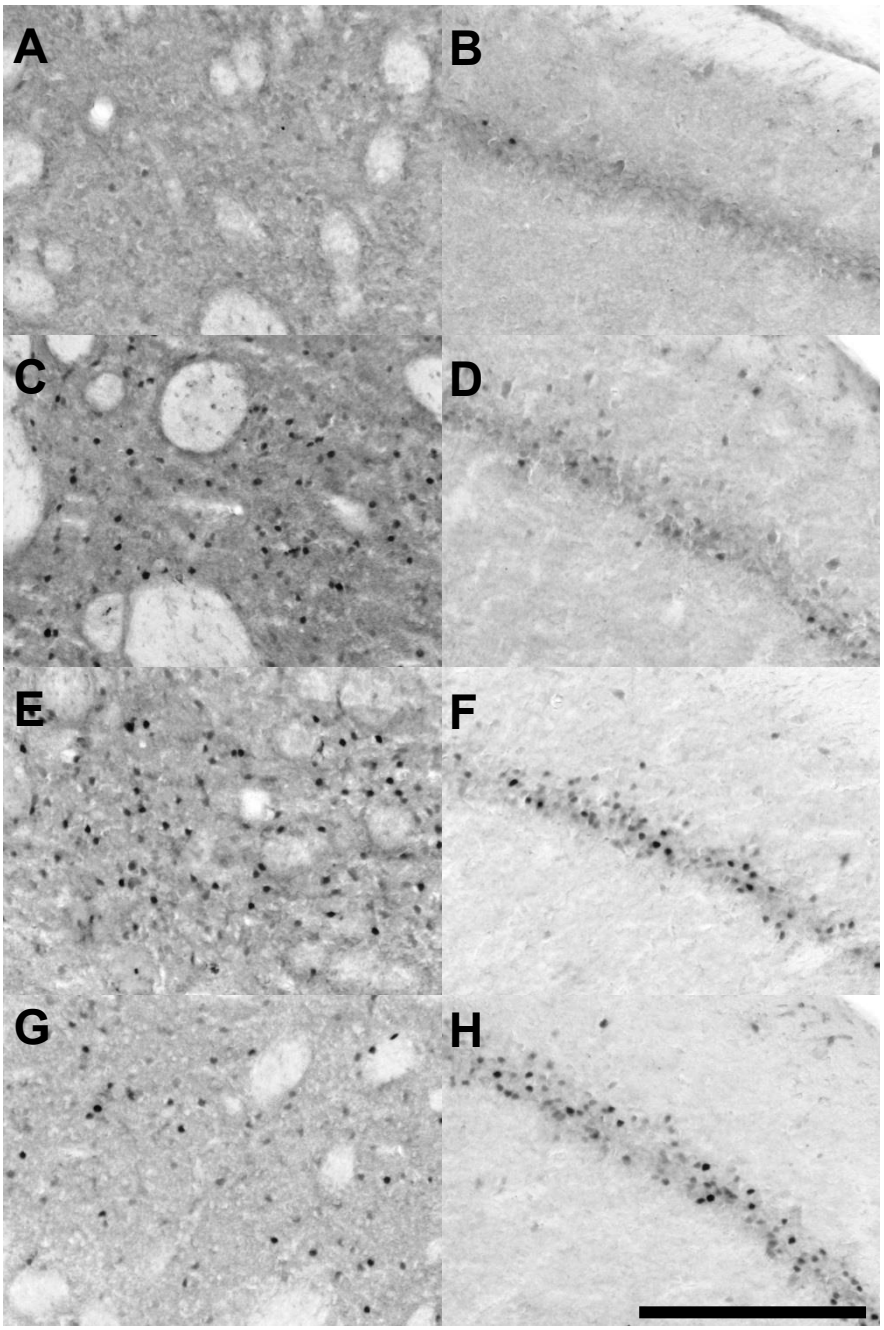
<u>Supplementary Figure 4 :</u> Average time to exit the arm which the rats from the different training protocols and durations have visited first, be it N, NE or any other arm. Once having entered their first arm, the time spent therein was comparable among training protocols and duration conditions. The data illustrated are given in seconds (+ sem). Analysis of variance showed no significant Protocol ($F_{(2,60)} = 2.32$, $p = 0.11$) or Duration ($F_{(2,60)} = 1.21$, $p = 0.31$) effects, and no significant interaction between the two factors (($F_{(4,60)} = 0,37$, $p = 0.83$).



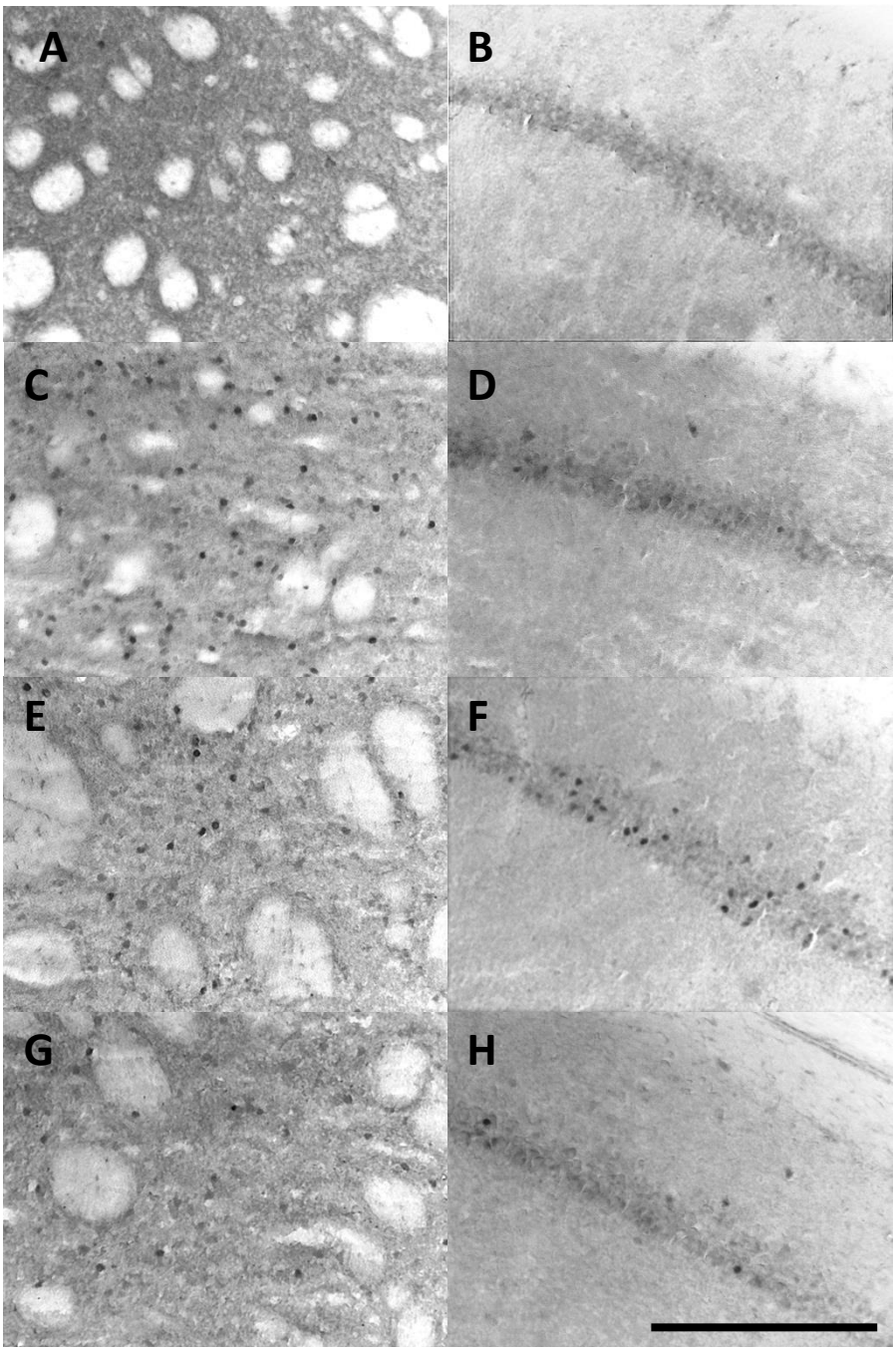
<u>Supplementary Figure 5 :</u> Time to exit the N or NE arm after the rats had entered it for the first time is compatible with the progressive formation of a cognitive map in Lt-ON-Dual rats. All means are given in seconds (+ sem). White bars correspond to the N arm, grey ones to the NE arm. For each training protocol and duration (indicated by the numbers at the bottom of the white bars), time to exit the N arm was compared to time to exit the NE one using a Student's t-test for paired samples. Statistical analyses : * indicates a significant difference between time to exit N as compared to time to exit NE ; $p < 0.05$.



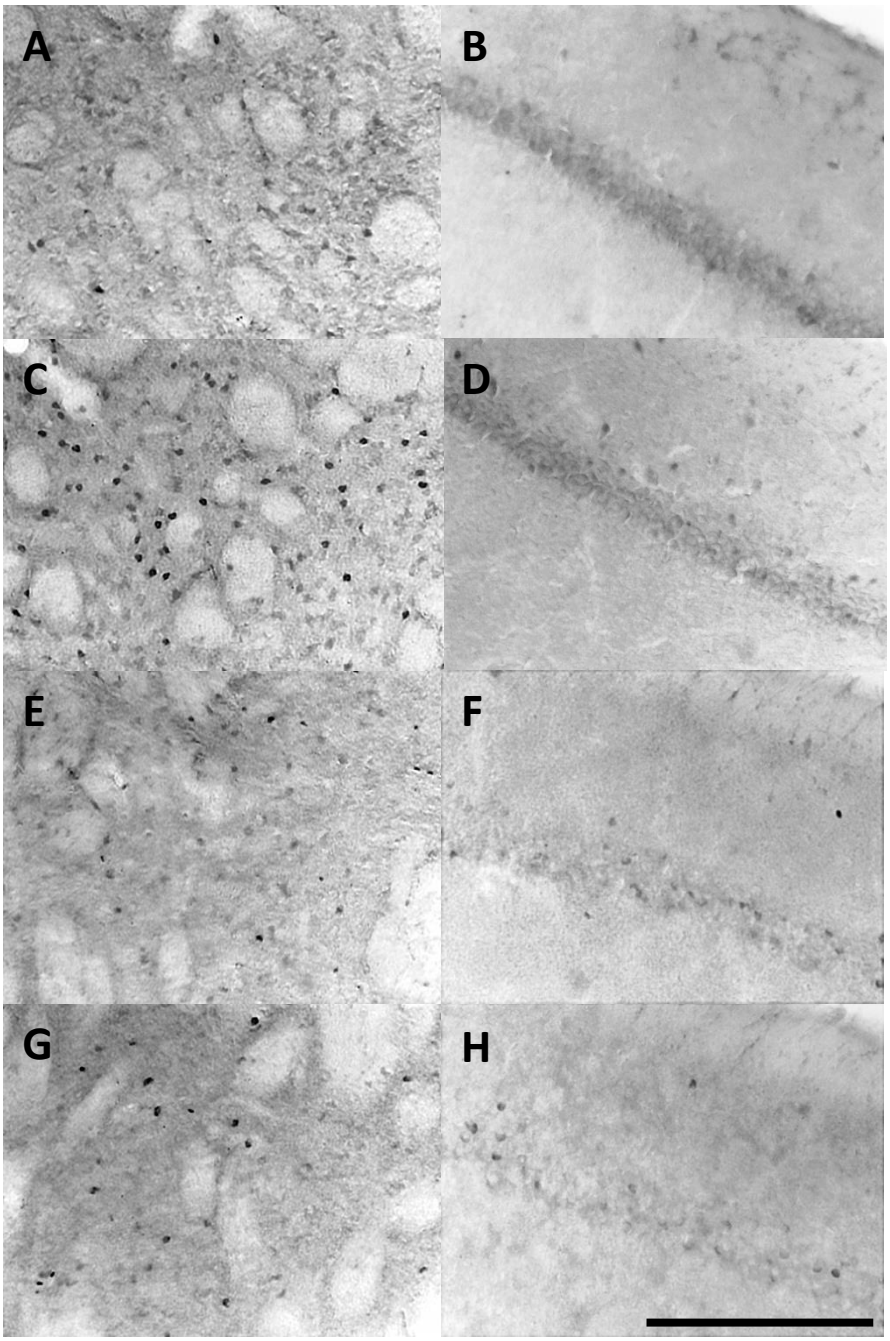
Supplementary Figure 6: c-Fos staining, 1 day of training. Typical examples of c-Fos staining observed in the medial part of the dorsal striatum (A, C, E, G) and in region CA1 (B, D, F, H) of the dorsal hippocampus from rats tested in a probe trial 24 hours after a 1-day training duration (4 trials/day) 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.



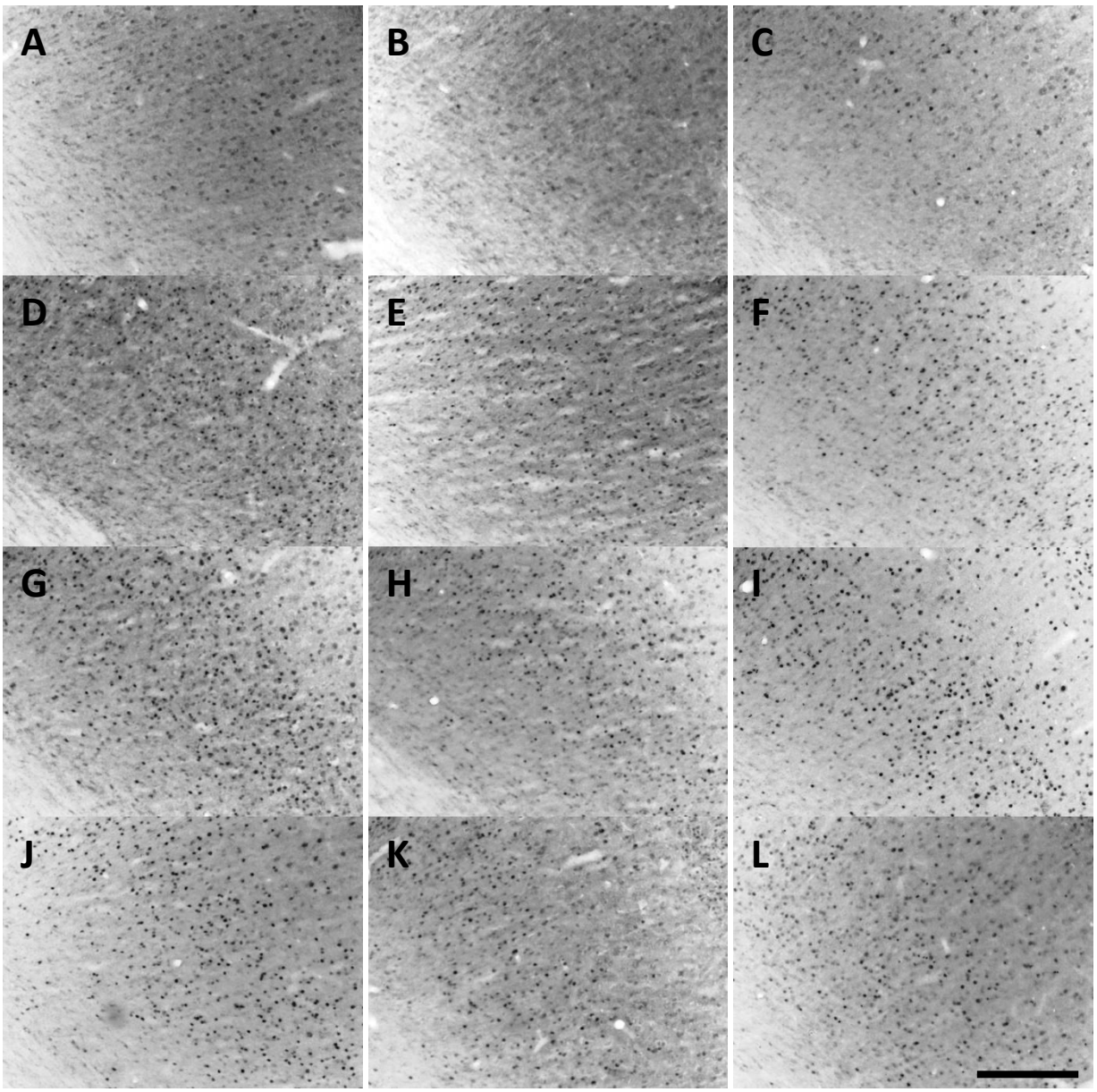
<u>Supplementary Figure 7:</u> c-Fos staining, 6 days of training. Typical examples of c-Fos staining observed in the medial part of the dorsal striatum (A, C, E, G) and in region CA1 (B, D, F, H) of the dorsal hippocampus from rats tested in a probe trial 24 hours after a 6-day training duration (4 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.



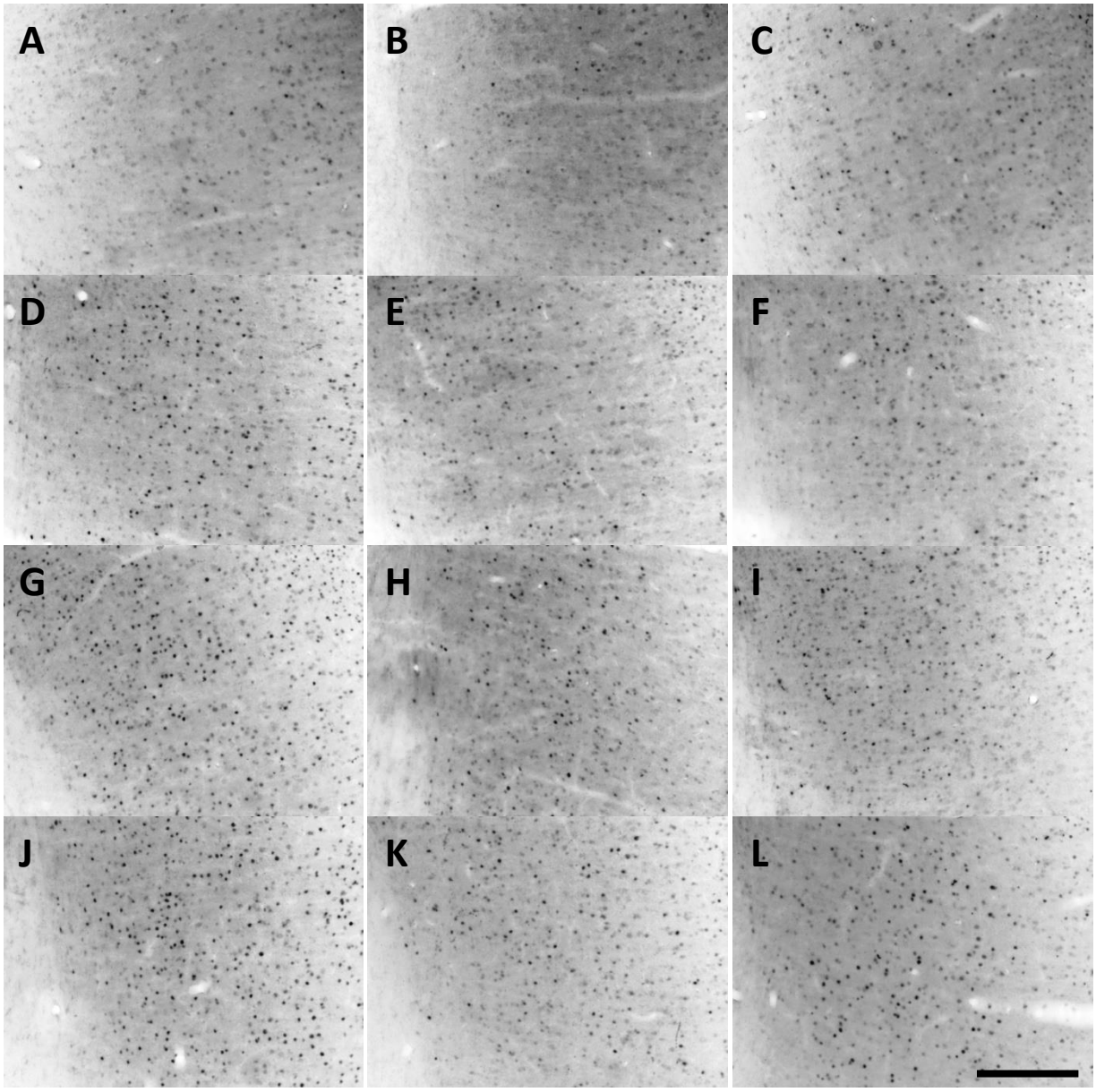
<u>Supplementary Figure 8:</u> c-Fos staining, 14 days of training. Typical examples of c-Fos staining observed in the medial part of the dorsal striatum (A, C, E, G) and in region CA1 (B, D, F, H) of the dorsal hippocampus from rats tested in a probe trial 24 hours after a 14-day training duration (4 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.



<u>Supplementary Figure 9:</u> c-Fos staining control (auditory cortex). Typical examples of c-Fos staining observed in the primary auditory 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.



<u>Supplementary Figure 10:</u> 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.



<u>Supplementary Figure 11:</u> Diffusion of MUSC-induced inactivation around the infusion site was comparable among training protocols and durations. Grey and black areas indicate the largest and smallest diffusion of the inactivation, respectively, in either the dorsal striatum (left panel) or the dorsal hippocampus (right panel). This diffusion radius of MUSC effects was estimated on coronal sections stained for c-Fos expression in Lt-OFF-Resp (left), Lt-ON-Dual (middle) and Lt-ON-Place rats (right). The rats were killed 90 min after the end of their 1-min probe trial. Before the probe trial, the rats had been trained for 6 (top) or 14 (bottom) days. Muscimol was infused 30 min before the probe trial. Coordinates are given in mm from Bregma (Paxinos and Watson, 2007).

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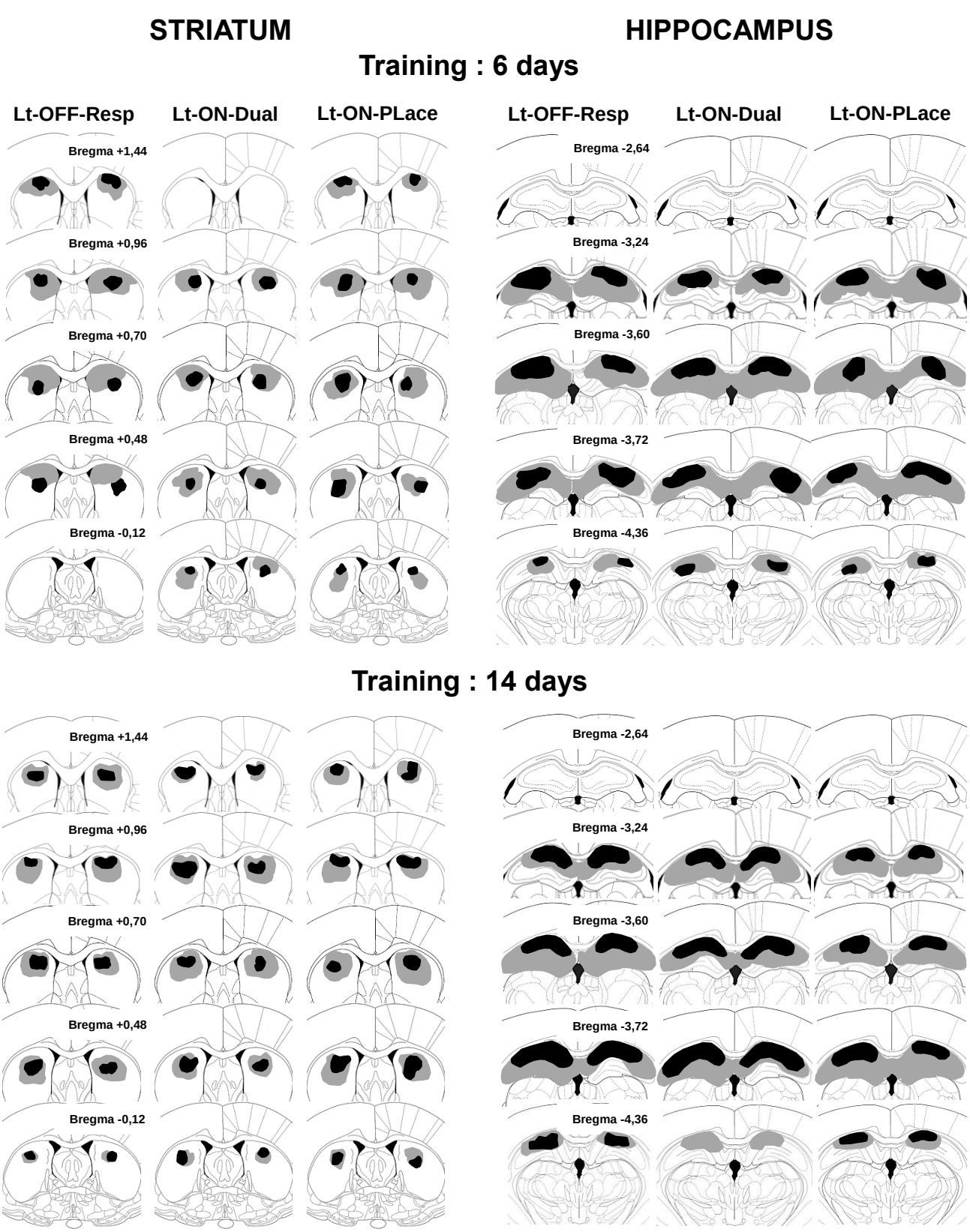
STRIATUM
HIPPOCAMPUS
Training : 6 days
Lt-OFF-Resp
Lt-ON-Dual
Lt-ON-PLace
Lt-OFF-Resp
Lt-ON-Dual
Lt-ON-PLace
Bregma +1,44
Bregma +0,96
Bregma +0,70
Bregma +0,48
Bregma -0,12
Bregma -2,64
Bregma -3,24
Bregma -3,60
Bregma -3,72
Bregma -4,36
Training : 14 days
Bregma +1,44
Bregma +0,96
Bregma +0,70
Bregma +0,48
Bregma -0,12
Bregma -2,64
Bregma -3,24
Bregma -3,60
Bregma -3,72
Bregma -4,36

<u>Supplementary figure 12:</u> Quantification on coronal sections of the diffusion radius of the MUSC-induced inactivation around the infusion sites in the dorsal striatum and dorsal hippocampus provides evidence for comparable diffusion areas among experimental conditions. All inactivation areas were expressed as a percentage of the surface of the region of interest at -0.1, +0.1, +0.5, +0.7, +0.8, +1.0, +1.4 and +1.7 mm (STRIATUM; dorso-ventral limits of the DStr are those shown in Supplementary Figure 1), and at -4.4, -4.2, -3.8, -3.6, -3.5, -3.4, -3.1, -2.9 and -2.6 mm (HIPPOCAMPUS); all coordinates are given in mm from Bregma according to Paxinos and Watson (2007). Data illustrated are means (+ sem). The ANOVA only showed a significant Anteriority effect. All other effects (Protocol, Duration, or any of the second or third order interactions) were not significant. Notice that for the statistical analyses, anteriority levels where zero values were found in all rats have not been included in the analyses. This was the case for levels of -0.1 mm in the DStr, and -2.6 and -4.4 mm in the DHip.

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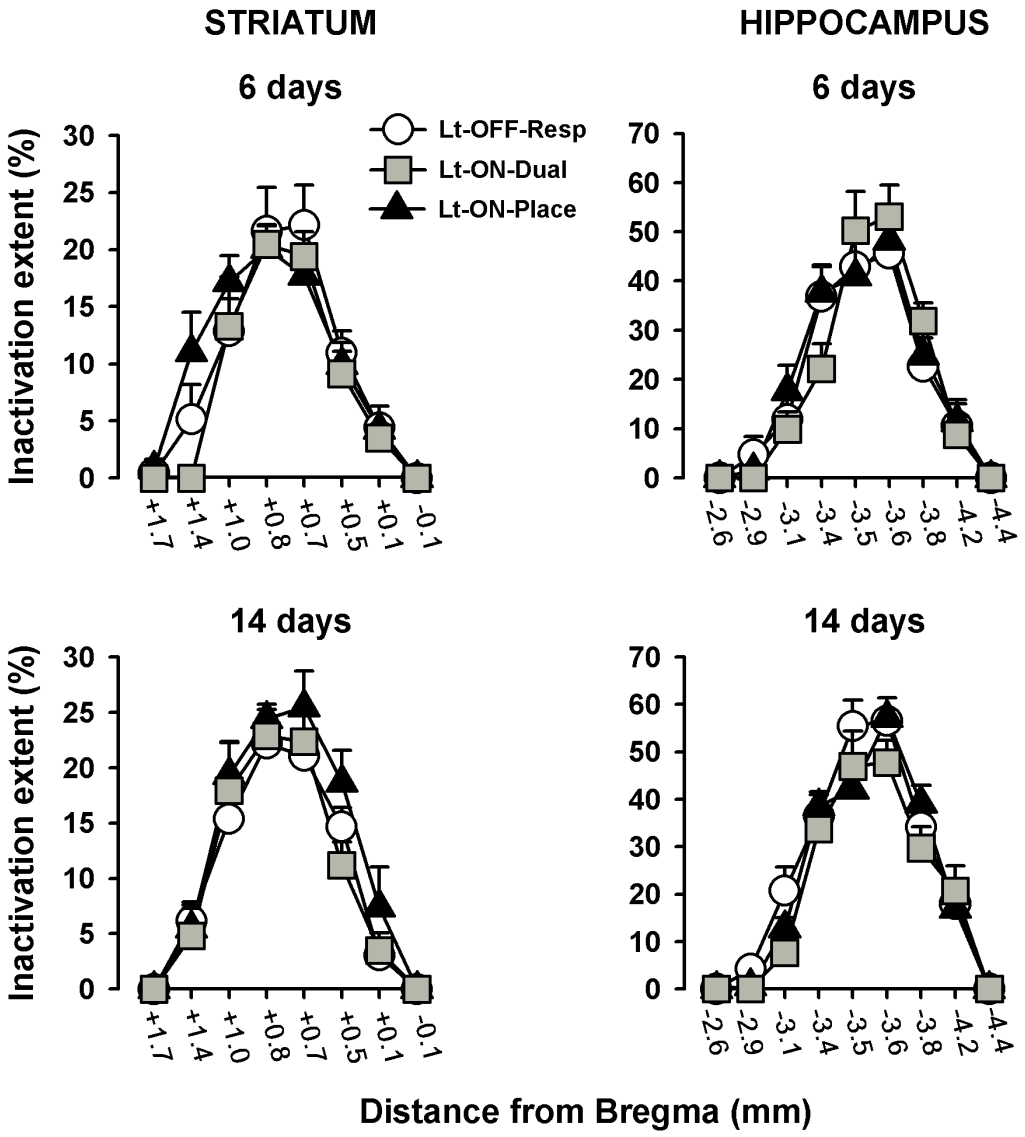
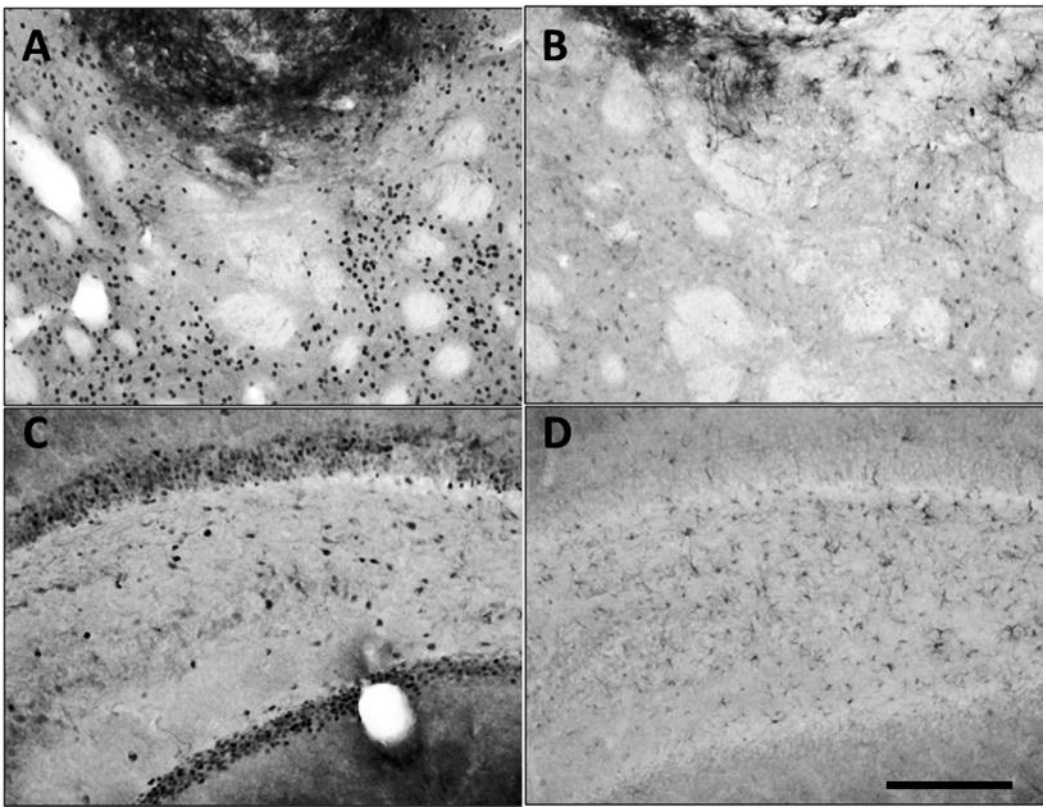
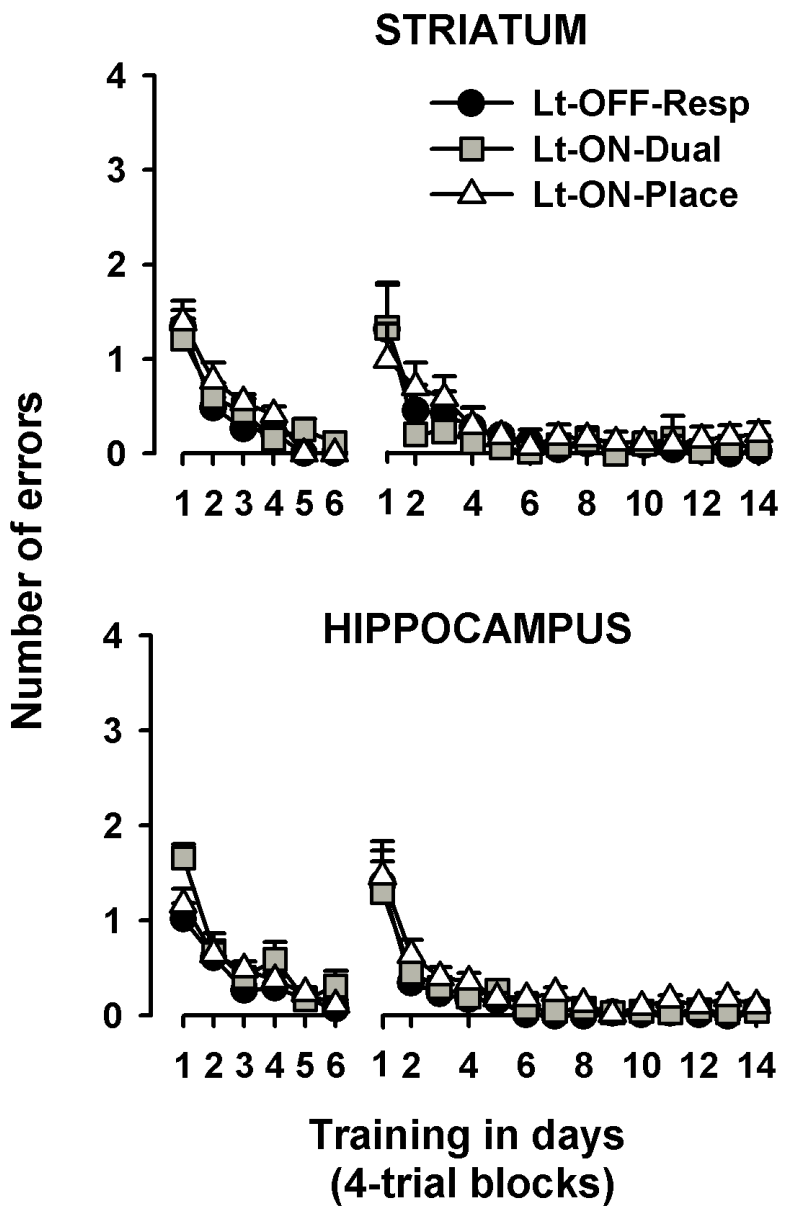

STRIATUM
HIPPOCAMPUS
6 days
6 days
14 days
14 days
Lt-OFF-Resp
Lt-ON-Dual
Lt-ON-Place
Inactivation extent (%)
Inactivation extent (%)
+1.7
+1.4
+1.0
+0.8
+0.7
+0.5
+0.1
-0.1
-2.6
-2.9
-3.1
-3.4
-3.5
-3.6
-3.8
-4.2
-4.4
Distance from Bregma (mm)

<u>Supplementary Figure 13:</u> 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.



<u>Supplementary Figure 14:</u> Number of errors was comparable among experimental conditions, indicating similar performance in all groups of rats. In each of the four training conditions of our second experiment (Protocol X Duration), mean numbers of errors (+ sem) are represented over the 6- and 14-day training sessions (daily blocks of four trials). Statistical analyses showed no significant difference between the training conditions. The same was true for distances. The number of animals in each condition can be found in Figure 8 of the article.



<u>Supplementary Table 3 :</u> Percent of rats shifting to the NE arm right after having first visited the N arm showed MUSC-induced disruption of performance, except when infused in the striatum after 14 days of training in Lt-ON-Dual and Lt-ON-Place rats. Number of rats showing a direct swim to the N arm after having been released in the maze are indicated between brackets as "(n/ N)". aCSF stands for artificial cerebrospinal fluid, MUSC for muscimol. The table reads as follows : "of the N tested rats, % of the n rats that first entered the N arm and which 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)

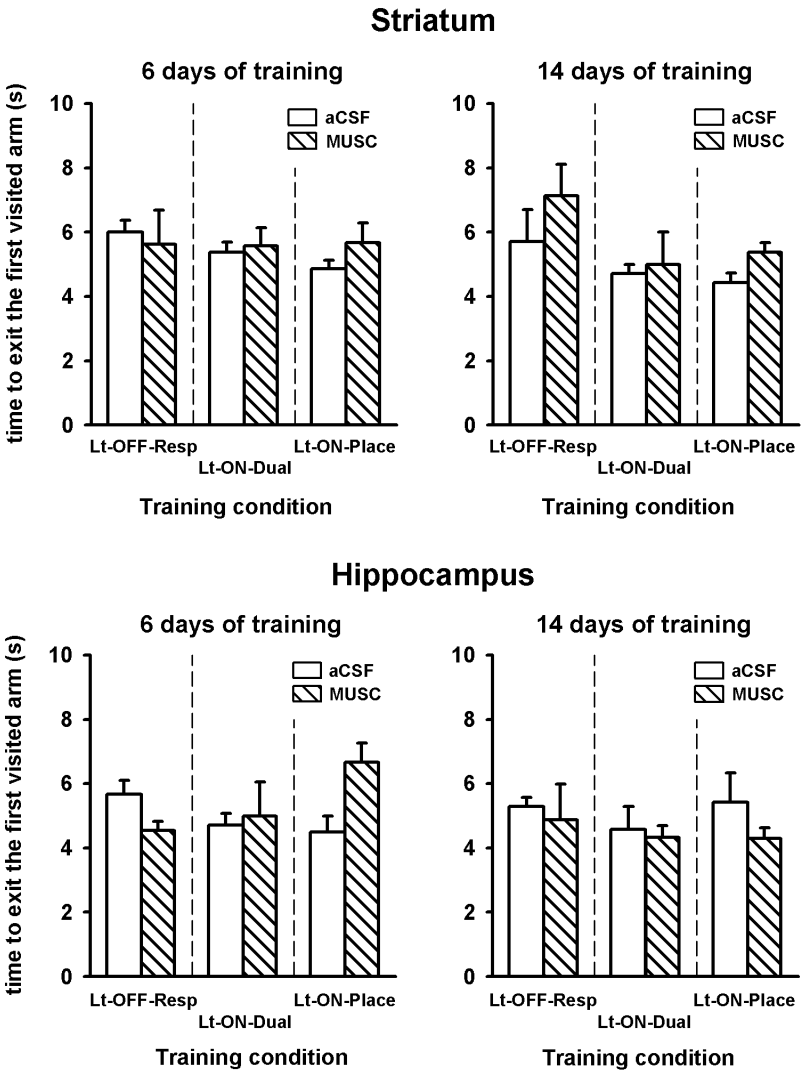
The proportion of rats having first swum to the N arm and then shifted to the NE was significantly higher than chance in the following groups : after 6 days of training, in Lt-ON-Place rats subjected to intrahippocampal infusion of aCSF ; after 14 days of training, in Lt-ON-Dual rats subjected to intrastriatal aCSF or MUSC infusions ($Chi^2 = 8.05$, $p < 0.01$ and $Chi^2 = 18.0$, $p < 0.001$, respectively) or to intrahippocampal aCSF infusions ($Chi^2 = 13,76$, $p < 0.001$), and in Lt-ON-Dual rats subjected to intrastriatal aCSF or MUSC infusions ($Chi^2 = 8.05$, $p < 0.01$ and $Chi^2 = 12$, $p < 0,001$) or intrahippocampal infusions of aCSF($Chi^2 = 18.00$, $p < 0.001$). The corresponding percentages are underlined. In the other cells, there was no significant difference from chance.

Supplementary Table 4: Average swim velocity during the probe trial was similar, regardless of training protocol, duration and inactivation conditions. aCSF stands for artificial cerebrospinal fluid, MUSC for muscimol. Data are indicated in cm/s as means (sem). Statistical analyses showed no significant difference among groups.

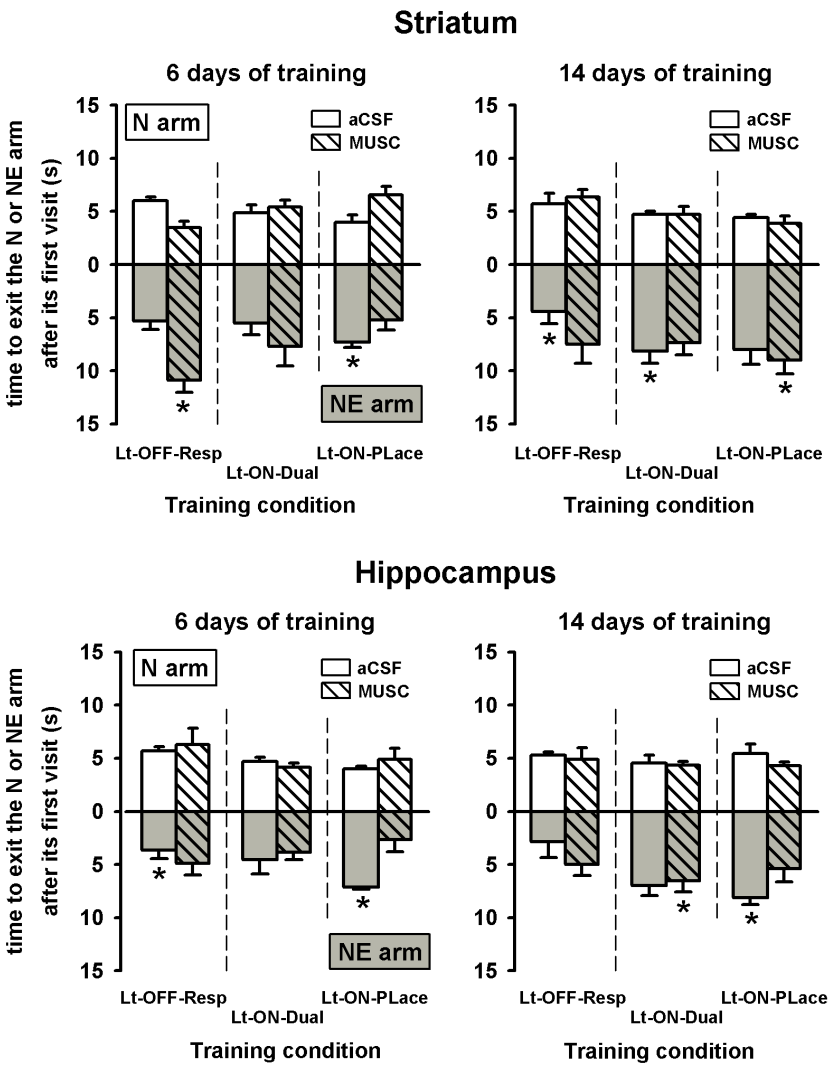
Training protocol	Training duration	Infusion structure			
		Striatum		Hippocampus	
		aCSF	MUSC	aCSF	MUSC
Lt-OFF-Resp	6 days	26.3 (0.9)	27.2 (1.7)	26.4 (0.9)	24.7 (1.2)
	14 days	26.1 (1.6)	26.8 (1.6)	26.2 (1.3)	26.3 (1.7)
Lt-ON-Dual	6 days	24.0 (0.9)	23.6 (1.4)	26.0 (1.2)	26.3 (1.6)
	14 days	25.7 (1.2)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

The 3 (Protocol) X 2 (Duration) X 2 (Inactivation) ANOVAs were performed separately for rats infused into the striatum or the hippocampus. ANOVA of the average swim velocities in rats subjected to intrastriatal infusions showed that Protocol ($F_{(2,87)} = 0.4$, *ns*), Duration ($F_{(1,87)} = 0.1$, *ns*), Inactivation ($F_{(1,87)} = 0.2$, *ns*), and the second or third order interactions ($F_{(2,87)}$ or $_{(1,87)} < 1.0$, *ns*) had no significant effect on swim velocities. ANOVA of the swim velocities in rats subjected to intrahippocampal infusions supported similar conclusions.

<u>Supplementary Figure 15:</u> Time to exit the first visited arm, be it N, NE or any other arm, did not show any difference among experimental conditions (light, training, inactivation). All data illustrated are given in seconds (+ sem). Analysis (ANOVA) of the data from rats subjected to intrastriatal infusions showed no significant Protocol ($F_{(2,81)} = 2.61$, $p = 0.08$), Duration ($F_{(1,81)} = 0.08$, $p = 0.77$) or Inactivation effects ($F_{(1,87)} = 1.74$, $p = 0.19$), and none of the second or third order interactions was significant. Analysis of the data from rats subjected to intrahippocampal infusions showed no significant Protocol ($F_{(2,87)} = 0.91$, $p = 0.40$), Duration ($F_{(1,87)} = 1.17$, $p = 0.28$) or Inactivation effects ($F_{(1,87)} = 0.04$, $p = 0.83$), and none of the second or third order interactions was significant.



<u>Supplementary Figure 16:</u> Average time to exit the N or NE arm confirmed MUSC-induced disruptions, although they were less marked than in Figure 9 of the article. Data are shown according to the different protocol, duration and inactivation conditions. All means are given in seconds (+ sem). White bars correspond to the N arm, grey ones to the NE arm. For each condition, time in the N arm was compared to time in the NE arms using a Student's t-test for paired samples. Statistical analysis : * indicates a significant difference between time in N and time in NE ; $p < 0.05$.



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