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Striatal cholinergic interneurons are required for contending strategy selection while solving spatial navigation problems.

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Abstract

How do animals adopt a given behavioral strategy to solve a recurrent problem when several effective strategies are available to reach the goal? Here we provide evidence that striatal cholinergic interneurons (SCIN) modulate their activity when mice must select between different strategies with similar goal-reaching effectiveness. Using a cell-type specific transgenic murine system, we show that adult SCIN ablation impairs strategy selection in navigational tasks where a goal can be independently achieved by adopting an allocentric or egocentric strategy. SCIN-depleted mice learn to achieve the goal in these tasks, irrespective of their appetitive or aversive nature, similarly to controls. However, they cannot shift away from their initially adopted strategies as control mice do as training progresses. Our results indicate that SCIN are required for shaping the probability function used for strategy selection as experience accumulates throughout training. Thus, SCIN may be critical for resolution of cognitive conflicts emerging when several strategies compete for behavioral control while adapting to environmental demands. Our findings may increase our understanding about the emergence of perseverative/compulsive traits in neuropsychiatric disorders with a reported SCIN reduction, such as Tourette and Williams syndromes.

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Significance statement

Selecting the best-suited strategy to solve a problem is vital. Accordingly, available strategies must be compared across multiple dimensions, such as goal attainment effectiveness, cost-benefit trade-off, and cognitive load. The striatum is involved in strategy selection when strategies clearly diverge in their goal attainment capacity; however, its role whenever several strategies can be used for goal reaching - therefore making selection dependent on additional strategy dimensions- remains poorly understood. Here, we show that striatal cholinergic interneurons can signal strategy competition. Furthermore, they are required to adopt a given strategy whenever strategies with similar goal attainment capacity compete for behavioral control. Our study suggests that striatal cholinergic dysfunction may result in anomalous resolution of problems whenever complex cognitive valuations are required.

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Introduction

Repeatedly recurring to a given strategy constitutes an efficient way to achieve a goal when environmental conditions remain stable. For instance, rodents, as well as humans, can adopt specific goal-directed strategies to navigate to a familiar food source or secure place, either using egocentric or allocentric approaches (Burgess, 2008). When natural conditions do not remain stable and cue-reward contingencies or refuge availability change over time, re-establishing action-outcome relationships or shifting towards an alternative strategy may be sufficient to adapt behavior to the new circumstances, making behavioral flexibility an adaptive response to contextual changes (Yin and Knowlton, 2006; Peak et al., 2019). Behavioral flexibility may be adaptive even when conditions remain stable by allowing the shift from an initially selected strategy that presents an easy implementation towards an alternative one that provides additional benefits in the long run (such as allowing saving energy or time). It is well established that the prefrontal cortex (PFC) controls different forms of reversal learning (Kehagia et al., 2010; Kesner and Churchwell, 2011; Bissonette and Powell, 2012), including strategy switching when an abrupt rule shift occurs (Ragozzino et al., 1999, 2003; Floresco et al., 2008). Thus, PFC inactivation impairs the switch from a spatial to a cue-dependent navigation in goal-directed tasks (de Bruin et al., 1994; Ragozzino et al., 1999) or from a nonmatch-to-sample to a match-to-sample resolution strategy (Joel et al., 1997). The striatum has also been pointed out as a key element in behavioral flexibility (Ragozzino et al., 2002, 2009; Block et al., 2007; Castañé Anna et al., 2010; Gremel and Costa, 2013); however, the nature of its contribution and the role of its cellular players is still under debate. In particular, the role of striatal cholinergic interneurons (SCIN) remains poorly understood, with interpretations differing accordingly to the behavioral paradigm utilized, the nature of the cholinergic manipulation or the striatal sub-regions studied (Tzavos et al., 2004; Bradfield et al., 2013; Aoki et al., 2015 McCool et al., 2008; Okada et al., 2014; Okada et al., 2018). For instance, Bradfield *et al.*, using a variety of technical approaches including local pharmacological manipulations, showed that normal SCIN activity is required to properly encode reversal of action-outcome contingencies (Bradfield et al., 2013). Nevertheless, Okada *et al*. proposed an inhibitory role for SCIN

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on behavioral flexibility based on studies of SCIN ablation and muscarinic M4 receptor downregulation effects on reversal learning (Okada et al., 2014). A subsequent study from this group depicted a more complex scenario by showing that SCIN role on behavioral flexibility depends on timing requirements of the tasks (Okada et al., 2018). The difficulty in appraising SCIN role in behavioral flexibility may indicate a more complex role of SCIN regarding strategy selection. A common aspect of the vast majority of the experimental approaches chosen to assess behavioral flexibility is that action-outcome contingency or rule switch occurs abruptly in a given trial as determined by the researcher (Tait et al., 2014; Izquierdo et al., 2017). Thus, in each phase of the task only one of the many available strategies would allow the animal to efficiently solve the problem. Alternatively, under more natural conditions, several strategies may be available and suitable to achieve the goal in a given setting; and it is under these conditions that the contribution of SCIN has not been explored. Moreover, the role of SCIN during strategy selection when competitive strategies are available has not yet been addressed. We hypothesize that SCIN are required for strategy selection when strategies with similar goal-reaching effectiveness compete for the behavioral control.

We have previously shown that extensive SCIN ablation results in perseverative behaviors directed toward salient features of the environment, leading to compulsive-like social interactions (Martos et al., 2017). We hypothesize that these behaviors may result from a strategy selection deficit emerging from a lack of cholinergic signaling. Therefore, in this work, we evaluate the SCIN response to environmental conditions that promote a conflict between suitable strategies while we also assess the impact of SCIN ablation on strategy selection in navigational tasks where equally effective strategies compete for the behavioral output.

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Materials and methods

Ethics:

All experimental procedures were done in accordance with institutional regulations (Institutional Animal Care and Use Committee of the School of Medicine, University of Buenos Aires, ASP# 21076/15 approval ID: 783/ 15) and government regulations (SENASARS617/2002, Argentina). All efforts were made to minimize the number of mice used and their suffering. Mice were maintained on a 12 h light: 12 h dark cycle in a facility with controlled environmental conditions. Mice received *ad libitum* access to food and water if not stated otherwise.

Animals:

Thirty wild-type C57Bl6 adult (3-8 months-old) male mice were used for p-S6rp immunostaining determinations. For SCIN ablation experiments, seventy adult mice hemizygous for Cre transgene and heterozygous for the loxP-iDTR modified allele (ChAT-Cre$^{+/-}$; DTR$^{LoxP/wt}$, Jackson Laboratories, J06410 and J007900 respectively) were bred as described before (Martos et al., 2017). iDTR line allows the Cre-dependent expression of the diphtheria toxin (DT) receptor rendering the targeted cell susceptible to the toxin (Buch et al., 2005). When food restriction was required, mice were singly housed in a regular mouse cage modified to hold two mice separated by an acrylic barrier in order to decrease social isolation stress. Mice were weighed and monitored daily. Food restriction began at least ten days before training to ensure progressive weight reduction and daily ration was individually adjusted to ensure that mice maintained a relatively constant weight across the experiment (always above a minimum of 1.7 grams per mouse/day). Mouse weight was kept at 85% of initial body weight. Any mice displaying signs of distress were excluded from the food restriction protocol.

SCIN ablation:

To selectively ablate SCIN, we stereotaxically microinjected diphtheria toxin (lesion group, 200pg/ul, D0564 Sigma-Merck, USA) or saline (control group) bilaterally (1.6 ul per hemisphere distributed in 3

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cites, as in Martos *et al.* (2017) under isoflurane anesthesia (Baxter) supplemented with bupivacaine hydrochloride solution as local anesthesia. Ophthalmic ointment was applied in both eyes to prevent corneal desiccation. Briefly, mice received via a 30-gauge stainless steel cannula, three bilateral microinjections in the striatum (Bregma +1.3 mm, lateral +/-1.6 mm at 2.8 and 2.4 mm from dura: 0.44 µl in each injection site and Bregma +0.6 mm, lateral +/- 1.8 mm, at 3 mm from dura: volume: 0.73 µl). The injection flow was set at a constant rate of 0.22 µl/min and the injection cannula was left in place for an additional 1 min before slowly retracting it. Behavioral tasks began at least 2 weeks after surgery, after the lesion had stabilized.

Behavioral tests:

All behavioral tasks were performed during the light phase by an investigator blinded to genotype and treatment. All sessions were video recorded and mouse position was automatically determined by video tracking software (ANY-maze, Stoelting Co). All mice were allowed to habituate in a holding room for at least one hour before testing. Mazes and items used in behavior assays were cleaned with 10% alcohol and dried between animals and were sanitized at the end of the day.

Dual solution cross maze

The test was conducted in a 35 x 35 x 5 cm cross-shaped maze that includes food cups in west and east arms. The maze was located in the center of a dim illuminated room (40 Lux) with salient distal cues as similarly performed by Meirsman *et al.* (2016). Three independent cohorts of transgenic mice (13 previously subjected to the Barnes maze, 15, and 15 naive animals) were pre-exposed to the maze and testing room for 3 days by allowing them to freely explore the maze during five minutes. Training consisted of sixteen 10-trial daily sessions (inter trial interval: 45 seconds). At the beginning of each trial, a mouse was placed in the south arm and was allowed to choose between east or west arms to find a food reward hidden inside a food cup. Reward location was kept constant across days and randomized across animals. The access to the north arm was blocked. The number of correct trials per day and the selected strategy were quantified alongside additional behavioral parameters. Additionally, training sessions 5, 10, 15 and 20 were substituted by probe test sessions to reveal the navigation

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strategy used to reach the goal. Probe test sessions consisted of a single trial in which mice were released from the north arm and were able to choose between east or west arms, while the south arm was blocked.

One cohort of 15 wild type mice was subjected to a modified version of the cross maze test to assess phosphorylation status of S6rp by immunohistochemical staining. These mice received 8 daily training sessions as described above (without intermingled probe tests), On the 9th day, mice were divided into three groups: control (mice were subjected to an additional regular cross maze trial), probe test (mice received a regular probe test trial), and forced (mice were placed into the north arm with only one additional arm available, forcing them to adopt a response-based strategy). All mice were euthanized 30 minutes after testing and transcardially perfused for immunohistological assessment (see Histology section).

Single strategy-solution cross maze task

We subjected SCIN deleted and control mice to a variant of the *dual solution cross maze* that requires performing a response strategy in order to successfully complete the task. Maze and environmental conditions were the same than the ones used for the dual solution version. Before training began, every mouse was pseudorandomly assigned to one “correct egocentric response”: half of the mice received the rule “turn right” and the remaining group, the rule “turn left”. The training consisted of nine days of daily sessions of 10 trials each in which mice were randomly released from the north or south arms. Nevertheless, the reward was always located in accordance to the correct egocentric response for each mouse´s rule. This version of the test does not require probe testing since only one strategy can maximize reward obtention.

Barnes maze

Two independent cohorts of transgenic mice (15 and 20 naive animals respectively) were subjected to Barnes maze, similarly to Patil *et al*. (2009). Briefly, the maze consisted of a white circular 55 cm diameter platform with twenty holes (five centimeters diameter) equally distributed along the edge. One

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of them was connected to a dark escape box hidden below the platform. The maze was located in the center of a highly illuminated (400 Lux) room with distal salient cues. The location of the escape box was kept constant across days. A loud sound (85 dB white noise) was played by hidden speakers once the trial began. The sound stopped when the mouse entered the escape box, where it was allowed to stay for one minute. Training consisted of 12 daily sessions of 4 trials each (inter trial interval: 15 minutes). In each trial, mice had 3 minutes to find the escape hole; otherwise, they were gently guided by the experimenter, assuring that they voluntarily entered the escape box. All trials were video recorded and the animal's path was tracked using ANY-maze. The time to reach the escape hole and the number of errors (incorrect holes checked before locating the correct one) were automatically quantified from video tracking. To prevent mouse guidance by olfactory cues, the maze was cleaned with 10% ethanol between trials and the platform was randomly rotated without altering the position of the escape box in relation to the cues located on the room walls. Strategy analysis consisted in a non-supervised assignment of hole-visiting patterns into one of the following categories: spatial strategy, serial strategy, mixed strategy and random (Kesby et al., 2015). A spatial strategy was defined as finding the target hole directly or after inspecting up to 2 adjacent holes first (maximum 2 errors permitted). Both, serial and mixed strategy required that at least 60% of errors were made in a serial fashion. However, in the serial strategy animals reached the target hole as a part of a series of errors, while in the mixed strategy mice did not reach the target as a part of a series but rather went straightforward to the target or up to 2 adjacent ones. Finally, any pattern that did not fit within the previously described strategies was assigned as a random search, usually characterized by crossing the maze center multiple times to check various holes, re-visiting previously explored ones.

Y maze

Fifteen wild type mice were subjected to eight minute daily sessions into a modified version of the classical Y maze (Braz et al., 2017). In our task, the mice can freely explore two out of the three existing arms of the Y maze located in a dim room with illuminated distal cues. The identity of the restricted arm was pseudorandomized among all mice and days, allowing each mouse to familiarize

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with the entire maze and room perspectives across days. Eight daily training sessions were conducted. On the 9th session mice were divided into 3 groups according to the experimental conditions they were exposed to: control (subjected again to a training session with one arm restricted), Y maze (allowed to freely explore the entire maze), and salient (exposed to a training session with one arm restricted and the addition of salient events including the presence of new texture on the ground in one arm, air puffs delivered every 20-30 seconds between minutes two to four of the task, an intermittent moderate intensity sound played between minutes four to six, and 5-6 novel food pellets dropped randomly during minutes six to eight of the task; all animals received the same protocol). All mice were euthanized 30 minutes after each trial had begun and were transcardially perfused for immunohistological assessment (see Histology section). Alternation behavior was defined as consecutive entries into each of all three arms without repeated entries, as on overlapping triplet sets. Alternation index was calculated as the ratio of actual (= total alternations) to possible (= total arm entries minus 2) number of alternations x 100.

Histology and immunohistochemistry

Mice were anesthetized with an overdose of chloral hydrate 5% in saline and were transcardially perfused with 10 mL of cold saline solution with 0,04% of sodium heparin (Duncan Laboratories, 5000 UI/ml) and 40 mL of cold 4% paraformaldehyde in 0,1 M PBS. Brains were post-fixed overnight in 4% paraformaldehyde at 4ºC, followed by 48 hs of cryoprotection in sucrose 30% in PBS 0,1M at 4ºC. Coronal sections (30 µm) were cut with a sliding microtome equipped with a freezing stage. Mice assigned to p-S6rp immunohistochemistry assay were sacrificed 30 minutes after the start of the corresponding behavioral task.

To verify DT-induced SCINs lesion, immunohistochemical detection of ChAT visualized with 3,3'-diaminobenzidine (DAB; Sigma-Aldrich) was performed on coronal brain sections obtained from all except one of the lesioned and all control mice, as described in Martos *et al.* (2017). Four sections per mice (two slices +0.70mm and two -0.2mm from bregma) were used. Animals with a partial lesion (more than 20% remaining ChAT+ cells compared to control average) or an unbalance within

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hemispheres (one hemisphere with more than 30% ChAT+ cells than the contralateral side) were not taken into consideration for behavioral analysis. For the analysis of the lesion extent along the entire anterior–posterior axis of the striatum, a subset of 54 mice (35 lesioned, 19 control) were studied. 8 coronal brain slices per mouse (bregma +1.2; +0.8; +0.4; 0, in duplicate) were subjected to an immunohistochemical detection of ChAT and visualized with DAB. The lesion extent was determined by counting ChAT positive cell bodies using an automatic and unsupervised FIJI ImageJ macro. Lesions cell-type specificity was assessed by quantifying density of medium spiny neurons in the dorsal striatum after DARPP32 (1:1000, sc-271111 Santa Cruz, USA) immunohistochemical staining.

To measure $Ser^{240-246}$-S6rp phosphorylation levels, we performed a double immunofluorescence staining with anti-ChAT and anti-p-S6rp antibodies following Bertran-Gonzalez et al., (2012). Briefly, free floating sections were incubated in PBS-Triton X-100, blocking was performed in 3% BSA – PBS and primary antibodies incubation was overnight at 4ºC using goat anti-ChAT (1:1000, AB 144P; Millipore) and rabbit anti p-S6rp (1:500, AB #2215; Cell Signaling) antibodies. Sections were then sequentially incubated with anti-goat biotinylated secondary antibody (1:400, BA-9500; Vector Laboratories) and streptavidin-fluorescein isothiocyanate conjugated (1:400, Ref: 434311, Invitrogen). Finally, sections were incubated with goat anti-rabbit CY3-conjugated secondary antibody (1:400, Code: 111-165-144 Jackson ImmunoResearch). All procedures were performed under stirring and three washes with PBS were performed between incubations.

p-S6rp quantitative analysis was conducted by using an automatic and unsupervised FIJI ImageJ macro, which detected ChAT+ interneurons and measured p-S6rp signal in each cell within a predefined region of interest (dorsal striatum). One (bregma +0.9mm, for Y maze) or two (bregma +0.9mm and +0.3mm, for DSCM) coronal planes were independently analyzed. To decrease variability in staining across animals, sections from different mice belonging to all three experimental groups were immunostained together (a distinct hole punch patterning made sections from each animal readily identifiable). Mean gray value for corpus callosum was selected for background subtraction.

Experimental Design and Statistical Analysis

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ANOVAs and Student's t-tests were applied when the data distributions fulfilled parametric assumptions. Fisher's LSD was used as a *post hoc* test in all cases. Statistical significance of nonparametric variables was globally assessed by Pearson chi-square test (2-way contingency tables) or log-linear analysis (3-way contingency table) using an online calculator (http://vassarstats.net/abc.html), followed by *post hoc* comparison using paired chi-square tests if it corresponded. Correlation between variables was assessed by Pearson's correlation test. All findings were confirmed in at least two separate cohorts of mice. Statistical details, including test statistics, degrees of freedom, and exact p value are provided in figure legends to improve readability of main text. Two-tailed parametric statistics were used in all cases, and the threshold for significance was set at p=0.05.

Results

SCIN typically display a tonic pattern of firing and are thought to encode salient environmental landmarks and task-related events with complex burst-pause patterns of firing. Recently, the quantification of phosphorylated ribosomal protein S6 (p-S6rp) in individual SCIN has been used as an indirect measure of *in vivo* SCIN activity with lower p-S6rp levels linked to higher, and more regular, firing rate (Bertran-Gonzalez et al., 2012; Matamales et al., 2016). To determine if p-S6rp in SCIN can be modulated by the solving strategy adopted during spatial navigation tasks, wild type mice were exposed to two or three of the arms of a Y maze. The Y maze is commonly used to assess the natural tendency of mice to maximize information gathering from the environment. The usual strategy adopted by rodents in this setting consists in alternating their arm selection to avoid the more recently visited arms. When only two arms are available, the maze can be efficiently patrolled by running back and forth without the need of additional spatial cues to make a choice. Wild type mice were allowed to freely explore two arms out of the three arms of a Y maze located in a room with distal cues during eight days. Each day, mice were exposed to a random pair of arms and allowed to freely explore them for eight minutes, in order to familiarize them with the entire Y maze and its relationship with the distal

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cues (Figure 1A). On the 9th day, during the exploration of the familiar environment, a subset of familiarized mice was presented with unexpected salient stimuli (Figure 1A, Salient group). They were later euthanized and their striata were immunostained to detect p-S6rp and choline acetyltransferase (ChAT, as a SCIN marker, Figure 1B). The salient stimuli significantly reduced the p-S6rp signal in SCIN when compared with control mice that undisturbedly explored two arms of the Y maze (Figure 1C), suggesting that activity changes induced in dorsal striatal cholinergic interneurons by salient environmental stimuli can impact on p-S6rp signal. An additional group of mice was allowed on the 9th day to freely explore the entire labyrinth as in the Y-maze spontaneous alternation task (Figure 1A, Y maze group). As expected, this group exhibited an alternation index significantly different from chance (70.7 ± 2.2 %, t-test versus 50%, t_5=9.49 p = 0.002). Interestingly, when compared with the control condition, they also showed a significant reduction in p-S6rp levels, not significantly different from the one exhibited by the salient group (Figure 1C). The observed changes in p-S6rp levels cannot be attributed to differences in motor activity since total traveled distance, number of visited arms, and mean speed did not differ between groups and were not correlated with p-S6rp signal (Figure 1D-F). These results suggest that SCIN activity can be modulated by the cognitive demands emerging during the exploration of a maze that includes a decision point and prompted the retention of previous paths for its efficient exploration.

To further characterize the response of SCIN during exploratory behavior and strategy selection, we utilized a well characterized navigational task that formally evaluates the strategy utilized by the animals to reach a goal. Accordingly, we trained a group of 15 wild type mice in a dual-solution plus maze (DSPM). During the initial phase (eight consecutive days) food-restricted mice were trained to obtain food pellets, always starting from the same arm (south), from a baited goal cup consistently located in one arm of the maze (east or west, randomized across mice Figure 2A). As learning proceeded, correct choices increased from chance level, reaching 82 % by day five (Figure 2B). During this stage any of two possible solving strategies (i.e. “place”, which uses environmental cues, or “response”, which uses egocentric self-references) have equal goal-reaching effectiveness and can be

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utilized interchangeably without impact on performance. To evaluate the impact on SCIN activity during strategy selection we quantified SCIN p-S6rp levels after a probe test, where mice must select one of the two strategies when only one of them is effective. On the 9th day, control mice were tested as in previous days while probe group mice were released from the north arm (Figure 2C). In the probe test, if the mouse turned toward the same baited arm that was visited during training (e.g. west) indicates the use of a place strategy (based on location of reward with respect to extra-maze cues) while selecting the opposite arm reflects a response strategy (based on body turning direction). Phosphorylation of p-S6rp in SCIN was significantly decreased in probe test group when compared with control mice that have no conflict regarding which strategy to follow (Figure 2D). The reduction was observed along the entire anterior-posterior axis (Figure 2D, right panel) and occurred both in dorsolateral as well as ventromedial regions of the dorsal striatum (Figure 2E). This reduction cannot be due to differences in rewarding properties of the selection made since all arms were baited during the probe test. Moreover, there were no differences in the latency to arm selection, total traveled distance, immobility time or mean walking speed (Figure 2F-I) suggesting that decreased p-S6rp levels cannot be attributable to differences in general motivation or motor function. Given that mice in the probe group were presented with a new perspective of the environment, and SCIN activity may be influenced by environmental stimuli, we subjected a third group of mice to a modified probe test to exclude the possibility that phosphorylation reduction may be a consequence of the exposure to a previously unvisited arm. This group started from the same new arm as the probe test group did (north) but it had no alternative other than turning right at the central area of the maze, therefore adopting a response strategy (Figure 2C, forced group). S6rp phosphorylation levels in the forced group did not differ from control group (Figure2D-E) suggesting that differences observed in probe group may arise during the process of strategy selection required to reach the goal.

To provide a causal link between SCIN function and strategy selection during goal directed behaviors we subjected control and SCIN depleted mice to an extended version of the dual-solution plus maze. To selectively ablate SCIN, we confined the diphtheria toxin receptor (DTR) expression to

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cholinergic cells by using the ChAT-Cre$^{+/-}$ DTR$^{loxP/wt}$ line (hereafter ChAT-DTR) and induced an extensive ablation of SCIN by bilaterally injecting adult ChAT-DTR male mice with diphtheria toxin (lesioned) or solvent (control), following previously reported methods (Martos et al., 2017) (Figure 3A). Lesions spanned the entire anterior-posterior axis of the dorsal striatum and typically comprised more than 95 percent of the SCIN without affecting cholinergic neurons in the nearby septal area (Figure 3B-C). Control and lesioned food-restricted mice were trained daily in the dual-solution plus maze similarly as described above (Figure 4A). Lesioned mice learned the task similarly to control mice although they exhibited a small but significant reduction in the maximal performance attained (Figure 4B). Days to reach criteria (i.e. days required to correctly solve at least 80 percent of the trials) did not differ between groups and performance of lesioned mice did not improve after day six, similarly to controls. To determine the strategy used by each animal to solve this maze, we intercalated probe tests every fifth day of training. Initially, the two possible strategies were equally adopted by control animals (Figure 4C, probe test 1). As training progressed, and consistent with the literature (Packard and McGaugh, 1996), control mice shifted their preference toward a response strategy (egocentric), with 14 out of 16 mice selecting response over spatial strategy by the end of the training. On the first probe test, lesioned mice also adopted either strategy and did not differ from control mice (56% versus 46% mice adopted "response", respectively; Figure 4C). However, SCIN depleted mice did not develop a bias toward either strategy with further training (Figure 4C, right panel) resulting by the end of training in a higher adoption of the spatial strategy in comparison to control mice (cumulative number of probe tests adopting spatial strategy, control: 1.1 ± 0.3 lesion: 2.5 ± 0.2, $t_{42} = 4.05$ $p = 0.002$). The failure of lesioned mice to switch toward a response-based strategy could reflect a perseveration on the initially selected successful strategy. However, this was not the case, since only three out of 28 lesioned mice (10.7%) consistently re-utilized the strategy selected on the first probe test during all subsequent ones. Importantly, no differences were observed between control and lesioned mice among strategies in terms of rewards obtained per day (Figure 4D). It is well accepted that acquisition of a response-based strategy depends on striatum integrity (Packard, 2009). Therefore, lesioned mice may not acquire a

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preference for the egocentric strategy due to a deficit in response-based learning. To test this possibility, an independent cohort of control and lesioned mice were subjected to a single strategy-solution cross maze task. By randomly starting each trial in any arm and reinforcing always the same body turn direction, this experimental setting leads mice to acquire a response-based strategy in order to maximize the rewards obtained. Lesioned and control mice performed equally well in this task (Figure 4E), demonstrating that SCIN ablation does not impair the acquisition of a response strategy but rather the adoption of this strategy over competing ones in the dual-solution cross maze.

To verify and extend our findings, we next utilized the Barnes maze, a task that explicitly assesses which navigational strategy is selected to reach a goal (Harrison et al., 2006). In this task, a mouse is released in the center of an aversive circular arena that has 20 holes equally spaced along the border of the platform. At the beginning, it learns to locate the only hole that is connected to an escape box by randomly exploring the arena (random search). Further training results in the emergence of a structured search of the escape hole by using either spatial environmental cues (spatial strategy), or by sequentially exploring the holes (serial strategy). In this paradigm, the reinforcer is not a palatable food, as is the case in the previous task, but the innate tendency to avoid a potentially risky environment. Consistent with the dual-solution cross maze results, both experimental groups showed significant learning, reaching a plateau after day five (Figure 5A). Except for day one, no significant differences in the number of errors (incorrect holes checked before locating the correct one) were detected between control and lesioned mice. Moreover, both groups similarly reduced the time to reach the target hole with training (Figure 5B), suggesting comparable motivation to escape from the maze. It has been shown that maze geometry, number of holes and environmental conditions can heavily bias the selection of one strategy to find the goal (O'Leary and Brown, 2012). Therefore, we balanced experimental conditions to promote different strategies in the control group by including extra-maze visual cues placed on the walls, selecting a maze without wall and with an appropriated number of holes in relation to the perimeter length (Figure 5C). When training began, mice of both groups randomly searched for the escape hole in near 50% of the trials (Figure 5D left). By day four, the

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385 random search was superseded by a well-defined strategy-based exploration of the arena in more than
386 95% of the trials, with the serial strategy prevailing in both experimental groups (70.8% and 64.1% of
387 total trials in control and lesion groups respectively). In the few trials when random search was still
388 adopted after day 4, no statistical differences were found regarding search efficiency between groups
389 (path efficiency: 0.37 ± 0.07 vs. 0.31 ± 0.02, t_{83}=0.94 p = 0.35, control n=9 and lesion n=76
390 respectively). From day 4, control mice slowly but persistently switched towards a new strategy profile
391 increasingly adopting the spatial strategy. By day 9, the distribution of adopted strategies significantly
392 differed from the one of day 4 (Figure 5D upper right panel), with the spatial strategy becoming the
393 predominant one by the end of testing (Figure 5D, right). In striking contrast, lesioned mice did not shift
394 away from their initial distribution of selected strategies (Figure 5D, right). So, by day 12, SCIN
395 depleted mice still chose the initially favored serial strategy in most trails, differing significantly from the
396 control group (Figure 5E). Strategy classification using a stricter or a more relaxed criterion did not
397 modify the overall conclusion presented here (data not shown, stricter criteria: only one error in spatial
398 strategy, global chi-square test p=0.013; relaxed criteria: up to three errors in spatial strategy, global
399 chi-square test p<0.001). Selection of a given strategy may depend on the proficiency to execute it;
400 however, no significant differences were observed between control and lesioned mice in their
401 performance with either strategy (Figure 5F). These results suggest that inability of SCIN lesioned mice
402 to switch toward the spatial strategy is not due to a deficit in spatial navigation or to a higher proficiency
403 in serial learning. To investigate the possibility that lesioned mice took a win-stay approach and
404 persevered in a given successful strategy, we visually inspected strategy selection at individual level
405 throughout training (Figure 6A). Then, we calculated the fraction of mice displaying first trial
406 consistency (i.e., how many mice repeated on first trial of day 12 the strategy used in the first trial of
407 day 11), consecutive trial consistency (i.e., used the same strategy in the last trial of day 11 and the
408 first trial of day 12), and global strategy consistency (preferred strategy on day 11 vs preferred strategy
409 on day 12). We found no evidence that individual mice consistently adopted a given strategy across
410 days in neither group, even when strategy distribution was stable at the population level and was highly

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effective in terms of reaching the goal. For instance, no significant differences were observed between groups in first trial consistency (Figure 6B), consecutive trial consistency (Figure 6C), or global strategy consistency (Figure 6D). As another index of perseverance, we also calculated the number of consecutive days each mouse had displayed a bias toward the strategy finally adopted on day 12; no significant differences were observed between control and lesioned mice (0.91 ± 0.26 vs 1.78 ± 0.66 days respectively, t-test, t_{33}=0.92, p=0.36). Overall, regardless of the measure used to determine adoption of a given strategy, each mouse only showed preference for one particular strategy during one or two consecutive days.

In summary, these behavioral results confirm that SCIN are necessary to update the probability function used for strategy selection. Thus, SCIN may be essential for selecting which strategy will dominate the behavioral response when strategies with similar goal-reaching effectiveness compete during the resolution of navigational problems.

Discussion

Here we provide evidence showing that a global change in SCIN activity occurs concomitantly with the strategy selection process that takes place during resolution of navigational problems. Moreover, we found that SCIN are not needed to learn goal location in two different navigational tasks, nor to learn the advantages of a navigational strategy over random meandering to solve a spatial task. Furthermore, when only one strategy is effective in reaching a goal, SCIN do not appear to be required for the adoption of a given navigational, either egocentric or allocentric, strategy. On the contrary, SCIN are necessary to switch from the initially preferred task-solving strategy toward an alternative, contending one, possessing a similar goal-reaching effectiveness but which may present additional adaptive advantages in the long run. Notably, the deficit in strategy switching occurs after SCIN lesion regardless of the nature (egocentric or allocentric) of the finally adopted strategy. This may reflect the impossibility of SCIN depleted mice to update the probability function used for strategy selection, which remains unaltered even after new information is acquired by experience.

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436 Measuring global SCIN activity in rodents *in vivo* with electrophysiological methods is challenging
437 due to their sparseness. Even with contemporary imaging techniques only a few SCIN can be
438 measured simultaneously during behavioral studies (Rehani et al., 2019).It was proposed that
439 decreased p-S6rp levels reflected lower SCIN firing (Bertran-Gonzalez et al., 2012). However, the
440 relationship between SCIN p-S6rp levels and firing rate could be more complex when differences in
441 firing pattern are taken into consideration (Matamales et al., 2016). By measuring p-S6rp in SCIN, we
442 have shown that selecting between alternative problem-solving strategies triggers a change in SCIN
443 activity equivalent to the one seen after animal exposure to novel, salient environmental stimuli
444 previously reported to impact on SCIN firing *in vivo* (Kimura et al., 1984; Apicella et al., 1991; Aosaki et
445 al., 1994, 1995). Our results are in line with previous studies showing that transition from place to turn
446 strategy in a T maze was accompanied by changes in extracellular acetylcholine levels in the striatum
447 (Chang and Gold, 2003). Still, p-S6rp assessment only provides an indirect reflection of SCIN activity,
448 which integrates modulations of SCIN function throughout the duration of the study, and therefore
449 these results must be cautiously interpreted.

450 To fully address SCIN participation on strategy selection, we selectively ablated SCIN in dorsal
451 striatum while assessing the behavioral impact of the manipulation. SCIN depleted mice displayed, in
452 coincidence with recent studies (Okada et al., 2014; Matamales et al., 2016), normal or quasi-normal
453 learning curves in all of our behavioral paradigms and were equally effective in obtaining rewards or
454 finding an escape box than control mice. Independently of the task, control and SCIN depleted mice
455 initially displayed a similar preference for the available strategies. But, as training progressed, control
456 mice slowly but consistently adopted a new dominant solving strategy. In striking contrast, SCIN
457 depleted mice remained invariant in their initial profile of strategy usage. Interestingly, this impossibility
458 of SCIN lesioned mice to update their strategy selection as training evolved is manifested
459 independently of the nature of the goal (obtain a reward or avoid a risk). More remarkably, this
460 impairment occurs irrespective of whether an egocentric (i.e. response strategy in the dual solution
461 cross maze) or an allocentric strategy (i.e. spatial strategy in Barnes maze) prevails after training in

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462 control mice and results in the preferred strategy at a population level. Furthermore, our results
463 demonstrate that SCIN are not required to execute any of these individual strategies per se.

464 In control mice, we observed that migration toward the finally adopted strategy continues even when
465 goal-achievement has reached an asymptotic value under invariant task conditions. Thus, when two
466 strategies with similar goal-reaching effectiveness compete for behavioral control, other factors,
467 including energy preservation or cognitive effort, may govern strategy adoption. As mentioned before,
468 in the initial stages of both tasks, control and lesioned mice showed similar preferences for the
469 available strategies. Thus, under conditions where limited information is available, SCIN are not critical
470 to determine the chance of a given strategy to gain control of the behavioral response. Instead, the
471 SCIN are pivotal to bias the selection of one strategy over the other as training progresses and the
472 available information, apart from the goal-reaching effectiveness, presumably increases.

473 Previous studies have shown a critical role of PFC in different forms of strategy switching. In these
474 studies cue/action contingency to obtain reward, secure shelter, or acquire environmental information is
475 abruptly reversed and transferred from one strategy to the opposite one (Izquierdo et al., 2017). PFC
476 manipulations consistently impair this form of strategy switch and, together with experiments involving
477 rule reversal, provide a solid argument for implicating PFC in behavioral flexibility. Similar approaches
478 have been used to evaluate SCIN participation in behavioral flexibility although with mixed results
479 (Bradfield et al., 2013; Okada et al., 2014). Our results prompt further studies on behavioral flexibility
480 utilizing sudden rule reversal to take into consideration the strategy adopted by animals along training
481 and considering that SCIN manipulation may affect strategy selection without impacting on goal-
482 achievement performance.

483 Although speculatively, we would like to propose a model for the strategy selection processes in
484 which, when previous information indicates that only one strategy is available to achieve a goal, SCIN
485 are not engaged in the strategy selection process. Alternatively, when competing strategies are
486 available to solve a problem, SCIN will be necessary to bias strategy selection based on long term
487 valuation of outcome. Outcome assessment could take into account not only goal achievement

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effectiveness but also a trade-off between alternative strategies that maximizes the benefit/cost ratio in the long run. Under this model, neuronal activity coding for multiple strategies is feed forwarded to the striatum and passed through a probabilistic filter governed by SCIN. In our view, strategies may compete within the striatum in a similar way as it has been previously proposed for motor commands (Mink, 1996), but with SCIN playing a central role in biasing the selection toward one ensemble representing the selected strategy. SCIN-dependent corticostriatal plasticity (Ding et al., 2010) may be responsible for tuning the probabilistic filter by integrating the goal reaching effectiveness of each strategy to additional information, including energy balance or cognitive demand throughout training.

Our present study has unveiled a critical role of SCIN in strategy selection, specifically in the context of spatial problems. However, we would like to speculate that this process may exemplify a more general function of SCIN. Competition between problem-solving strategies may also occur during motor learning (Taylor and Ivry, 2012), social decision-making (van Baar et al., 2019), or abstract reasoning (Siegler, 1988). Even more, emotion regulatory strategies are non-consciously selected in response to stressful events and may be compromised in mental disorders (Aldao et al., 2010). In this regard, we have previously demonstrated that SCIN ablation results in a compulsive-like increase in social investigation of novel conspecifics and perseverative behaviors directed toward salient features of the environment (Martos et al., 2017). Comparable symptoms are observed in Williams syndrome (Pober, 2010), a neuropsychiatric disorder characterized by an unusually high drive to engage in social interactions, especially with strangers (Järvinen-Pasley et al., 2010; Järvinen et al., 2013). Moreover, 86 percent of Williams syndrome patients display repetitive behaviors and compulsive needs (Davies et al., 1998; Janes et al., 2014; Huston et al., 2016; Royston et al., 2018). Strengthening the parallelism between SCIN depleted mice and Williams syndrome patient, Hanson *et al.* have recently reported a selective decrease of SCIN in postmortem caudate of these patients (Hanson et al., 2018). Moreover, Williams syndrome children showed abnormal usage of egocentric and allocentric strategies to navigate in a virtual environment when compared with typically developing children (Broadbent et al., 2015; Purser et al., 2015). Whether a reduction in cholinergic signal results in strategy selection

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514 impairment in Williams syndrome patients remains to be determined. A SCIN reduction has been also
515 reported in Tourette syndrome (Kataoka et al., 2010; Lennington et al., 2014). Although the reduced
516 cholinergic signal may not be the sole cause of the characteristic tics, it has been proposed to
517 contribute to the altered social behavior displayed by some Tourette syndrome patients by affecting the
518 function of the social decision-making network (Albin, 2018). Furthermore, SCIN dysfunction has not
519 only been connected to abnormal social engagement. Recent experimental evidence showed that
520 interruption of cholinergic transmission in the dorsolateral striatum results in setting-specific compulsive
521 eating (Favier et al., 2020) a phenotype that may reflect an imbalance during normal selection of
522 exploration/exploitation strategies.

523 Overall, our results suggest that SCIN may be critical for normal resolution of cognitive conflicts
524 emerging when similarly effective, but unequally efficient, strategies compete for the behavioral control
525 in a given setting. Then, failure to optimize behavior by shifting to the more economical strategy to
526 solve a problem may lead to perseverative/compulsive phenotypes, as the ones reported after
527 experimental interference of the striatal cholinergic signaling and characteristic symptoms of disorders
528 with affected SCIN, including Tourette and Williams syndromes.

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Figure legends:

FIGURE 1) Phosphorylation of S6rp signal in SCIN can be modulated by salient environmental stimuli as well as by adoption of complex exploratory strategies.

A) Schematic representation of the behavioral procedure. Mice were allowed to freely explore two different arms per day of a Y maze during 8 daily sessions. On the 9th day, mice were assigned to one of the following conditions, *control:* animals were subjected to a ninth training session; *salient*: animals explored the familiar two-arms maze but maze was enriched with salient multi-sensorial stimuli, and *Y maze*: mice were allowed to access all three arms simultaneously.

B) Representative low-magnification photomicrograph of a coronal brain section (around bregma + 0.9 mm) double-immunostained to detect ChAT (green, SCIN) and p-S6rp (red) showing substantial co-localization of high intensity p-S6rp labeled cells with ChAT positive ones. Inset corresponds to magnified right panels. Right panels: high magnified view of boxed area showing ChAT and p-S6rp immunoreactivity in three nearby SCIN. Lower panels: High-magnification confocal images of the three SCIN identified above (#1 to 3). For each cell on the left: ChAT channel with superimposed ROIs defined by automatic ImageJ segmentation (dotted line); on the right: red channel displayed as 16 pseudocolor palette highlighting the intensity of p-S6rp fluorescence within the ROI.

C) Exposure to salient environmental stimuli or Y maze exploration decrease of p-S6rp levels in SCIN.

Left: quantification of the p-S6rp signal in individual SCIN in mice subjected to the three different behavioral conditions on day 9. Violin plots representing the distribution of p-S6rp levels in SCIN for each mouse (each color indicates one independent animal), 1522, 1733, and 2289 neurons from 4, 5, and 6 mice subjected to control, salient and Y maze conditions respectively.

Right: statistical analysis revealed a significant difference between salient and control groups, one-way ANOVA $F_{2,12}$= 4.81 p= 0.030, *p<0.05 versus control group. Each bar represents mean ± SEM of individual mice (number of mice are indicated inside bars).

D-F) No significant differences were observed after one-way ANOVAs in either total distance traveled (**D**, $F_{2,12}$= 0.41 p= 0.67) or number of arms entries (**E**, $F_{2,12}$ = 1.83 p= 0.21) or mean walking speed (**F** $F_{2,12}$ = 0.0001 p = 0.99) between treatments. Each cross represents mean ± SEM of individual mice (color-coded dots). Numbers of mice are the same than in panel C. No significant correlations were found between motor parameters and p-S6rp signal (Pearson correlation p=0.31, p=0.19, and 0.18 respectively).

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FIGURE 2) Strategy selection process decreases phosphorylation levels of S6rp in SCIN.

A) Schematic representation of the dual-solution cross-maze task. During training the north (N) arm was closed. The mouse starts training in the south arm (S) and can find a hidden reward pellet in the small cup (black dot) located in the east (E) arm in this example. Training phase consisted of 8 daily sessions of 10 trials each.

B) Black line depicts the learning curve for all 15 wild type mice combined together during the training phase of the task (one-way repeated measures ANOVA $F_{7,98}$= 8.3 p<0.001, day 1 versus any other day p<0.05). Color curves indicate the performance of mice according to their assignment *a posteriori* to one of the three possible behavioral conditions evaluated on day 9. No significant differences were observed in the learning curve between these groups (RM ANOVA groups $F_{2,84}$ = 0.39 p = 0.69, interaction $F_{14,84}$= 0.71 p= 0.76, n= 5 mice per group).

C) Schematic representation of the experimental conditions for day nine. Mice were divided in three groups and subjected to different procedures. *Control*: regular training trial identical to a single trial of previous days; *Probe*: a regular probe test (mice were released from north arm with south arm blocked and must decide between the east or west arm); *Forced*; mice were released from north arm but no decision was allowed (two arms including south were blocked). n = 5 mice per group.

D) Strategy selection during a goal-directed navigational task reduces phosphorylated S6rp levels in SCIN.

Left: quantification of p-S6rp signal in individual SCIN in mice subjected on day 9 to the different behavioral conditions. Violin plots representing the distribution of p-S6rp levels in SCIN pooled for each mouse (each color indicates one independent animal), 1002, 1008, and 1178 neurons from five mice per group subjected to control, probe and forced conditions respectively. In the probe group, 2 mice adopted a place strategy (number 3 -light blue- and number 4 -pink-) and 3 a response strategy.

Right: statistical analysis revealed a significant decrease in p-S6rp levels in SCIN of mice that must opt out between one out of the two possible competing strategies to reach the reward (probe group) when compared with control mice or against mice forced to adopt a given strategy (forced). Two-way ANOVA, treatment factor $F_{2,21}$ = 8.02 p= 0.002, *p<0.05 versus other groups. Each bar represents mean + SEM of five individual mice per group. Due to technical issues, p-S6rp levels could not be accurately measured in one anterior and two posterior sections from different mice.

E) Heat map of p-S6rp signals overlaid on schematic coronal striatal sections. Phosphorylation levels are homogenously decreased in all striatal regions in the probe test group: color scale represent mean grey values in arbitrary units.

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F-I) No significant differences were observed in latency to make a decision (**F**, one-way ANOVA, $F_{2,12}$ = 0.64 p = 0.54), total distance travelled (**G**, one-way ANOVA $F_{2,12}$ = 0.43 p = 0.66), percentage of time mice spent immobile (**H**, $F_{2,12}$ = 3.5 p = 0.06), or mean speed (**I**, $F_{2,12}$ = 2.27 p = 0.15) in dual-solution cross-maze task. No significant correlations were found between motor parameters and p-S6rp signal (Pearson correlation p=0.47, p=0.74, p=0.77, and 0.37 respectively). Each cross represents mean ± SEM of individual mice (color-coded dots). Number of mice are indicated in parentheses.

FIGURE 3) Conditional bilateral ablation of SCIN in vivo using diphtheria toxin system.

A) Representative photographs of coronal brain sections immunostained against ChAT (DAB) from ChAT-Cre:iDTR transgenic mice treated with solvent (control) or diphtheria toxin (DT, lesion) depicting the extent of the lesion. High magnifications of boxed areas are shown below, large ChAT positive cells can be observed in the control mouse and areas not affected by DT in the lesioned mouse. cc: corpus callosum, str: striatum, spt: septum, v: lateral ventricle.

B) Quantitative *post hoc* verification of SCIN ablation of behaviorally tested mice. A significant decrease in the number of ChAT-IR cells was observed in the striatum of lesioned mice but not in the nearby septal area (RM ANOVA, significant interaction $F_{1,67}$ = 607.1 p<0.0001, *p<0.001 post hoc test, post hoc test for septum, p=0.062, statistical power = 0.925). Data represent mean ± SEM of 26-43 mice as indicated in figure reference.

C) DT treatment significantly reduced SCIN along the entire anterior–posterior axis of the striatum (RM ANOVA; *p < 0.001 post hoc test. A subset of mice displayed in B was analyzed to quantify the lesion extent in the anterior–posterior axis. Data represent mean ± SEM of 19 control and 35 lesioned mice.

D) Representative photographs of coronal brain sections immunostained against DARPP32 (green) and counterstained with DAPI (white) from control and lesioned mice depicting normal density of medium spiny neurons in the striatum. Corresponding high magnification images are shown as insets.

E) SCIN ablation did not reduce the density of medium spiny neurons verifying the cell-type specificity of the ablation. Data represent mean + SEM of 8 control and 8 lesioned mice subjected to behavioral testing.

FIGURE 4) SCIN ablation impairs normal strategy selection during a goal-directed navigational task.

A) Schematic representation of experimental design used in the dual-solution cross-maze task. Control

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and lesioned mice were trained daily (10 trials per day) and probe tests were intercalated every 5 days.

B) SCIN ablated mice were capable of learning goal locations across days (RM ANOVA, time factor $F_{15,630}$=19.0, p<0.001), although their performance was slightly inferior to that of control mice (treatment factor, $F_{1,630}$ = 8.22, p<0.01). No significant differences were observed regarding mean number of sessions required to reach criterion (inset, t-test t_{41} = 1.4 p = 0.08). Mice numbers are indicated in the graph references.

C) Lesioned and control groups presented different goal-reaching strategy profiles from probe test two.

Left: strategy selection probabilistic profiles for control and lesion groups throughout all probe tests. The graph displays the percent of mice adopting a given strategy for each probe session. Significant interaction (treatment x time x strategy) was observed by global log-linear 3-way contingency table analysis ($G^2_{(10)}$ = 24.16 p= 0.0072). * p<0.05 chi-square test versus control (Probe test 1: χ^2 = 0.39 p=0.53, P2: χ^2= 6.29 p=0.012, P3: χ^2= 4.45 p=0.035, P4: χ^2= 11.0 p=0.0009; df =1 in all cases).

Right: Control group, but not the lesioned one, showed a significant change in their profile of selected strategies after training. * p<0.05 chi-square test versus probe test 1 (control: χ^2= 3.86 p=0.04, lesion: χ^2= 0.30 p=0.58, df =1)

D) Dual-solution cross-maze performance of control and SCIN lesioned mice segregated according to the strategy adopted in the nearest probe test. No significant differences in learning were observed either in control (two way ANOVA strategy factor $F_{1,224}$= 0.92 p= 0.34) nor in SCIN ablated mice (two-way ANOVA strategy factor $F_{1,436}$= 0.01 p= 0.92). Each dot represents the mean ± SEM value of the corresponding strategy adopted in each trial.

E) SCIN ablation did not impair the acquisition of a goal-directed task that depends on a response strategy. No significant differences were observed between control and lesion groups in the performance of a single-solution cross-maze task. (RM ANOVA, time factor $F_{8,136}$ = 7.17 p < 0.001; treatment factor, $F_{1,136}$ = 0.009, p = 0.92. No significant interaction). Number of mice is indicated in graph references.

FIGURE 5) SCIN are required to update probability function used for strategy selection throughout training in a goal-directed navigational task.

Control and SCIN ablated mice significantly improved their performance over the course of training reaching the same final performance in the Barnes maze. Analysis of errors (**A**, number of incorrect holes checked prior to locating the escape box) and latency (**B**, time to locate the escape box). No

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852 significant differences were found other than in session 1 between control and SCIN ablated mice (RM
853 ANOVA, interaction: for errors $F_{11,363}$ = 2.97 p< 0.001, for latency; $F_{11,363}$ = 3.09 p<0.001, * p<0.001
854 versus same day control group). Number of mice is indicated in graph references.

855 **C)** Representative examples of individual mouse trajectories depicting random search and the different
856 strategies. Track plots exemplify random search (top left) or serial (top right), spatial (bottom left), and
857 mixed (bottom right) strategies. Blue dot: starting tracking point; red dot: final mouse position; purple
858 line: mouse path, green zone: goal location.

859 **D)** Left: graphs depict the percentage of trials solved using a well-defined strategy throughout training.
860 Control and lesion groups significantly increased the use of a goal-reaching strategy during initial days
861 of training (control: global chi-square test $\chi^2_{(11)}$=132.1 p<0.0001, lesion: global chi-square test
862 $\chi^2_{(11)}$=131.4 p<0.0001, 48 trials from 12 control mice and 92 trials from 23 lesioned mice per session).

863 Right: probability profiles of strategy selection for control (top) and SCIN depleted mice (bottom)
864 throughout training. After random search is superseded (session 4), control mice progressively
865 changed their profile of strategy selection shifting from a predominant serial strategy on session 4 to a
866 profile biased toward a spatial strategy after session 8 (global chi-square test $\chi^2_{(22)}$= 52.5 p=0.0003,
867 *p<0.05 chi-square test versus session 4). Instead, lesion group displayed the same probability profile
868 of strategy selection throughout the entire experiment (global chi-square test $\chi^2_{(22)}$= 19.3 p=0.63).

869 **E)** Between-groups comparison of the probability profiles of strategy selection. At session 4 the
870 distribution of selected strategies was not significantly different between control and lesion groups
871 (global chi-square test $\chi^2_{(2)}$= 0.21 p=0.90). Instead, at the end of training (session 12) the profile of
872 SCIN lesioned mice was significantly different from the one displayed by the control group (global chi-
873 square test $\chi^2_{(2)}$= 18.9 p<0.0001).

874 **F)** Control and SCIN ablated mice executed their strategies in a similar way. Top: latency to reach the
875 escape box in trials when an spatial strategy was adopted (two-ways ANOVA, treatment factor $F_{1,405}$=
876 0.98 p= 0.32.) Bottom: maximal number of errors made during a sequential search of the escape box in
877 trials when a serial strategy was adopted (two-way ANOVA, treatment factor $F_{1,683}$ = 0.05 p = 0.82).
878 Each dot represents the mean ± SEM value of the corresponding strategy adopted in each trial.

879

880 **FIGURE 6) Control group shifted their preference throughout training toward a spatial strategy**
881 **without adopting a win-stay strategy at individual level.**

882 **A)** Graphical representation of the individual strategies adopted by control and lesioned mice in each

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trial of the Barnes maze throughout the 12 daily sessions. Each square represents one trial of one session for one mouse. Each line contains trials of one individual mouse. Four trials (t1-t4) were conducted per day. Adopted strategies are color-coded as indicated in figure reference. Mouse C2, strategy adoption on day 1 (trials 2 and 3) could not be determined due to a technical problem.

B-D) No significant differences could be observed between control and lesioned mice for first trial consistency (**B**, 6/12 vs 7/23 respectively, Chi-squared test $\chi^2(1) = 1.29$, $p = 0.25$), consecutive trial consistency (**C** 6/12 vs 11/23, Chi-squared test $\chi^2(1) = 0.015$, $p = 0.90$), or global strategy consistency (**D** 3/12 vs 3/23, Chi-squared test $\chi^2(1) = 0.79$, $p = 0.37$).

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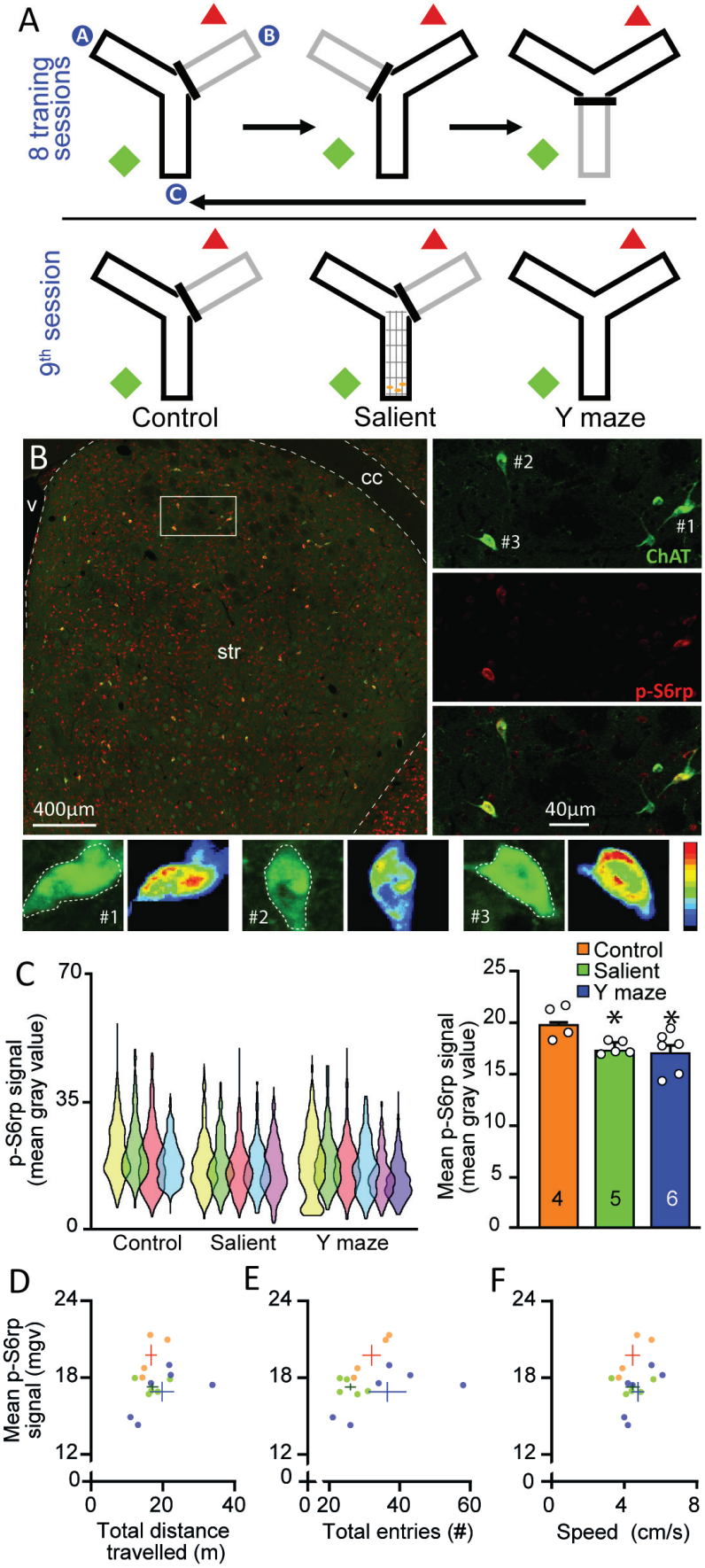

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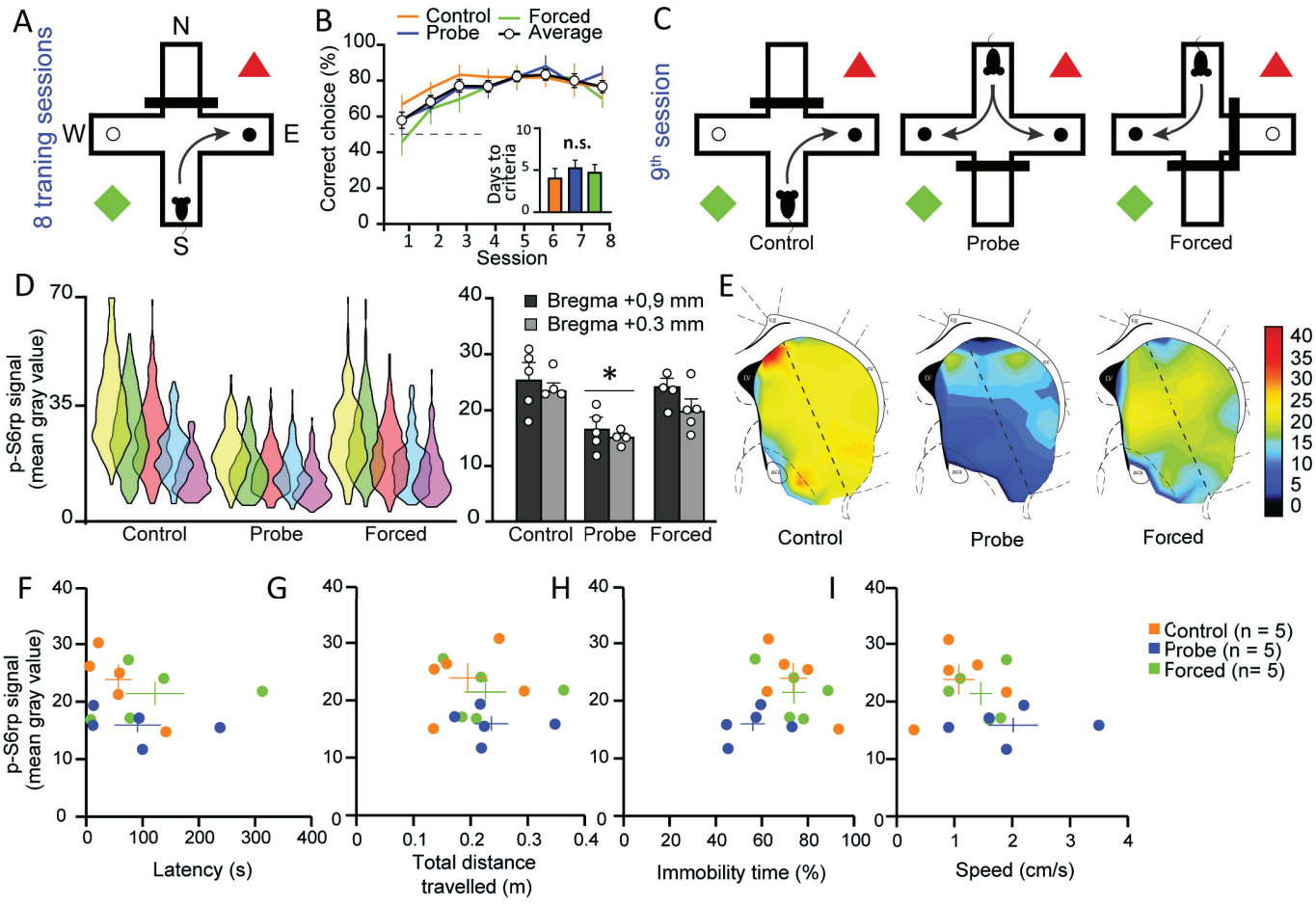

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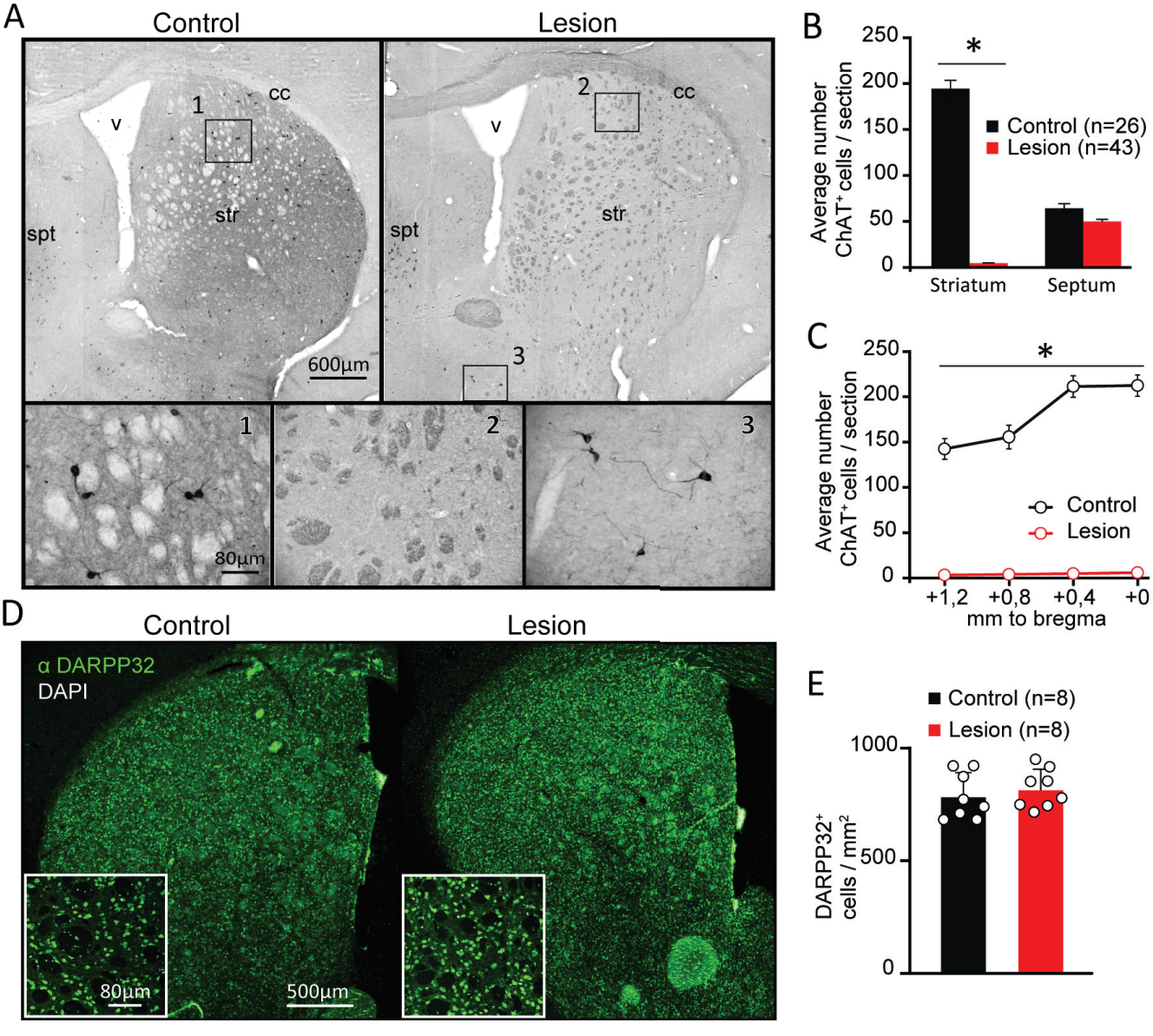

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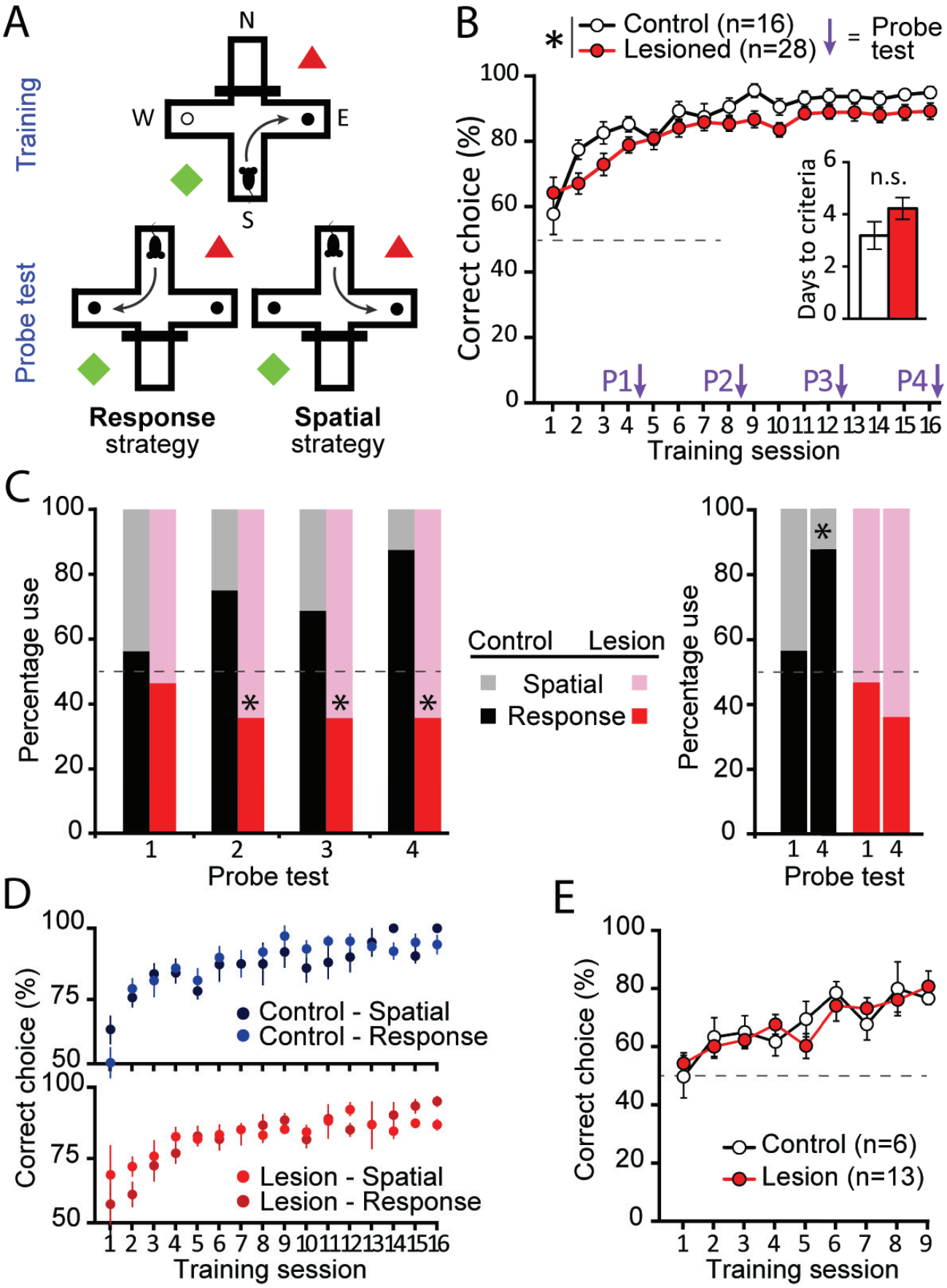

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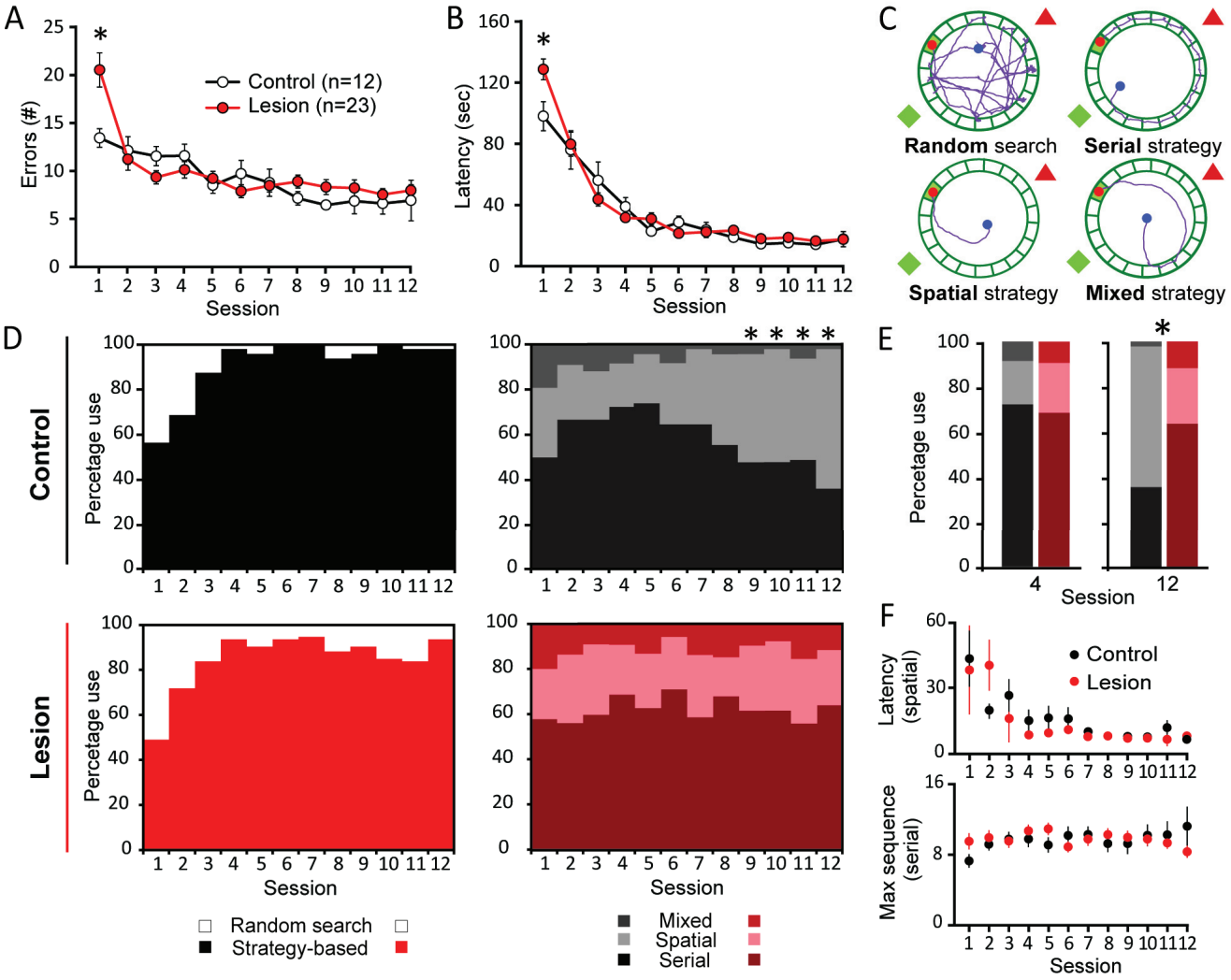

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