Behavioral/Cognitive

Striatal Cholinergic Interneurons Are Required for Contending Strategy Selection While Solving Spatial Navigation Problems

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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 (SCINs) 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, regardless of their appetitive or aversive nature, in a similar way as controls. However, they cannot shift away from their initially adopted strategies, as control mice do, as training progresses. Our results indicate that SCINs are required for shaping the probability function used for strategy selection as experience accumulates throughout training. Thus, SCINs may be critical for the 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.

Key words: **allocentric–egocentric navigation; Barnes maze; cell type-specific ablation; cognitive flexibility; problem-solving strategies; striatal cholinergic interneurons**

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.

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, using either 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 toward 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 toward an alternative one that provides additional benefits in the long run (e.g., allowing saving energy or time). It is well established that the prefrontal cortex (PFC) controls different forms

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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 et al., 2010; Gremel and Costa, 2013); however, the nature of its contribution and the role of its cellular players are still under debate. In particular, the role of striatal cholinergic interneurons (SCINs) remains poorly understood, with interpretations differing accordingly to the behavioral paradigm used, the nature of the cholinergic manipulation or the striatal subregions studied (Tzavos et al., 2004; McCool et al., 2008; Bradfield et al., 2013; Okada et al., 2014; Aoki et al., 2015; Okada et al., 2018). For instance, Bradfield et al. (2013), 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. Nevertheless, Okada et al. (2014) proposed an inhibitory role for SCINs on behavioral flexibility based on studies of SCIN ablation and muscarinic M4 receptor downregulation effects on reversal learning. A subsequent study from this group depicted a more complex scenario by showing that the role of SCINs on behavioral flexibility depends on timing requirements of the tasks (Okada et al., 2018). The difficulty in appraising the role of SCINs in behavioral flexibility may indicate a more complex role of SCINs 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 SCINs has not been explored. Moreover, the role of SCINs during strategy selection when competitive strategies are available has not yet been addressed. We hypothesize that SCINs 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.

Materials and Methods

Ethics

All experimental procedures were performed 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/dark cycle in a facility with controlled environmental conditions. Mice had *ad libitum* access to food and water if not stated otherwise.

Animals

Thirty wild-type C57BL6 adult (age, 3–8 months) male mice were used for phosphorylated ribosomal protein S6 (p-S6rp) immunostaining determinations. For SCIN ablation experiments, 70 adult mice hemizygous for Cre transgene and heterozygous for the loxP-iDTR-modified allele (ChAT-Cre$^{+/-}$; DTR$^{LoxP/wt}$; catalog #J06410 and #J007900, respectively, The Jackson Laboratory) were bred as described previously (Martos et al., 2017). The iDTR line allows the Cre-dependent expression of the diphtheria toxin receptor (DTR) 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 to decrease social isolation stress. Mice were weighed and monitored daily. Food restriction began at least 10 d before training to ensure progressive weight reduction, and the daily ration was individually adjusted to ensure that mice maintained a relatively constant weight across the experiment (always above a minimum of 1.7 g/mouse/d). 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 SCINs, we stereotaxically microinjected diphtheria toxin (DT, lesion group, 200 pg/μl; catalog #D0564, Sigma-Merck) or saline (control group) bilaterally (1.6 μl/hemisphere) distributed in three sites, as in the study by 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 (from 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 from 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). All mice were allowed to habituate in a holding room for at least 1 h 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 × 35 × 5 cm cross-shaped maze that includes food cups in the west and east arms. The maze was located in the center of a dimly 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 preexposed to the maze and testing room for 3 d by allowing them to freely explore the maze for 5 min. Training consisted of 16 10-trial daily sessions (intertrial interval, 45 s). At the beginning of each trial, a mouse was placed in the south arm and it 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 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 ninth day, mice were divided into the following 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 killed 30 min 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 (DSCM) that requires performing a response strategy to successfully complete the task. Maze and environmental conditions were the same as 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 9 d 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 with the correct egocentric response for the for each mouse. 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 the Barnes maze, similar to the study by Patil et al. (2009). Briefly, the maze consisted of a white circular 55-cm-diameter platform with 20 holes (diameter, 5 cm) equally distributed along the edge. One 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 1 min. Training consisted of 12 daily sessions of four trials each (intertrial interval, 15 min). In each trial, mice had 3 min 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 path of the animal 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 nonsupervised 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 two adjacent holes first (a maximum of 2 errors were permitted). Both serial and mixed strategies 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 straight to the target or up to two 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, revisiting previously explored ones.

Y maze. Fifteen wild-type mice were subjected to daily 8 min sessions with a modified version of the classical Y maze (Braz et al., 2017). In our task, the mice can freely explore two 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 with the entire maze and room perspectives across days. Eight daily training sessions were conducted. On the ninth session, mice were divided into the following three 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, with air puffs delivered every 20–30 s between minutes 2 to 4 of the task, an intermittent moderate intensity sound played between minutes 4 and 6, and five to six novel food pellets dropped randomly during minutes 6–8 of the task; all animals received the same protocol). All mice were killed 30 min 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 – 2) number of alternations × 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% sodium heparin (5000 IU/ml; Duncan Laboratories) and 40 ml of cold 4% paraformaldehyde in 0.1 M PBS. Brains were postfixed overnight in 4% paraformaldehyde at 4°C, followed by 48 h of cryoprotection in sucrose 30% in PBS 0.1 M at 4°C. Coronal sections (30 µm) were cut with a sliding microtome equipped with a freezing stage. Mice assigned to p-S6rp immunohistochemistry assay were killed 30 min after the start of the corresponding behavioral task.

To verify DT-induced SCIN lesions, immunohistochemical detection of choline acetyltransferase (ChAT) visualized with 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 the study by Martos et al. (2017). Four sections per mice (two slices, +0.70 mm from bregma; two slices, −0.2 mm from bregma) were used. Animals with a partial lesion (>20% remaining $ChAT^+$ cells compared with the control average) or an unbalance within hemispheres (one hemisphere with >30% $ChAT^+$ cells compared with 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. Eight coronal brain slices per mouse (distance from bregma: +1.2, +0.8, +0.4, and 0 mm; in duplicate) were subjected to an immunohistochemical detection of ChAT and visualized with DAB. The lesion extent was determined by counting $ChAT^+$ cell bodies using an automatic and unsupervised FIJI ImageJ macro. The cell type specificity of lesions was assessed by quantifying the density of medium spiny neurons in the dorsal striatum after DARPP32 (1:1000; catalog #sc-271111, Santa Cruz Biotechnology) 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 the study by 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 4°C using goat anti-ChAT (1:1000; catalog #AB 144P, Millipore) and rabbit anti p-S6rp (1:500; catalog #AB 2215; Cell Signaling Technology) antibodies. Sections were then sequentially incubated with anti-goat biotinylated secondary antibody (1:400; catalog #BA-9500, Vector Laboratories) and streptavidin-fluorescein isothiocyanate conjugated (1:400; catalog #434311, Thermo Fisher Scientific). Finally, sections were incubated with goat anti-rabbit CY3-conjugated secondary antibody (1:400; catalog #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 the p-S6rp signal in each cell within a predefined region of interest (dorsal striatum). One (bregma +0.9 mm, for Y maze) or two (bregma +0.9 and +0.3 mm, 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

ANOVAs and Student's *t* tests were applied when the data distributions fulfilled parametric assumptions. Fisher's least significant difference was used as a *post hoc* test in all cases. The statistical significance of nonparametric variables was globally assessed by Pearson χ^2 test (two-way contingency tables) or log-linear analysis (three-way contingency table) using an online calculator (http://vassarstats.net/abc.html), followed by

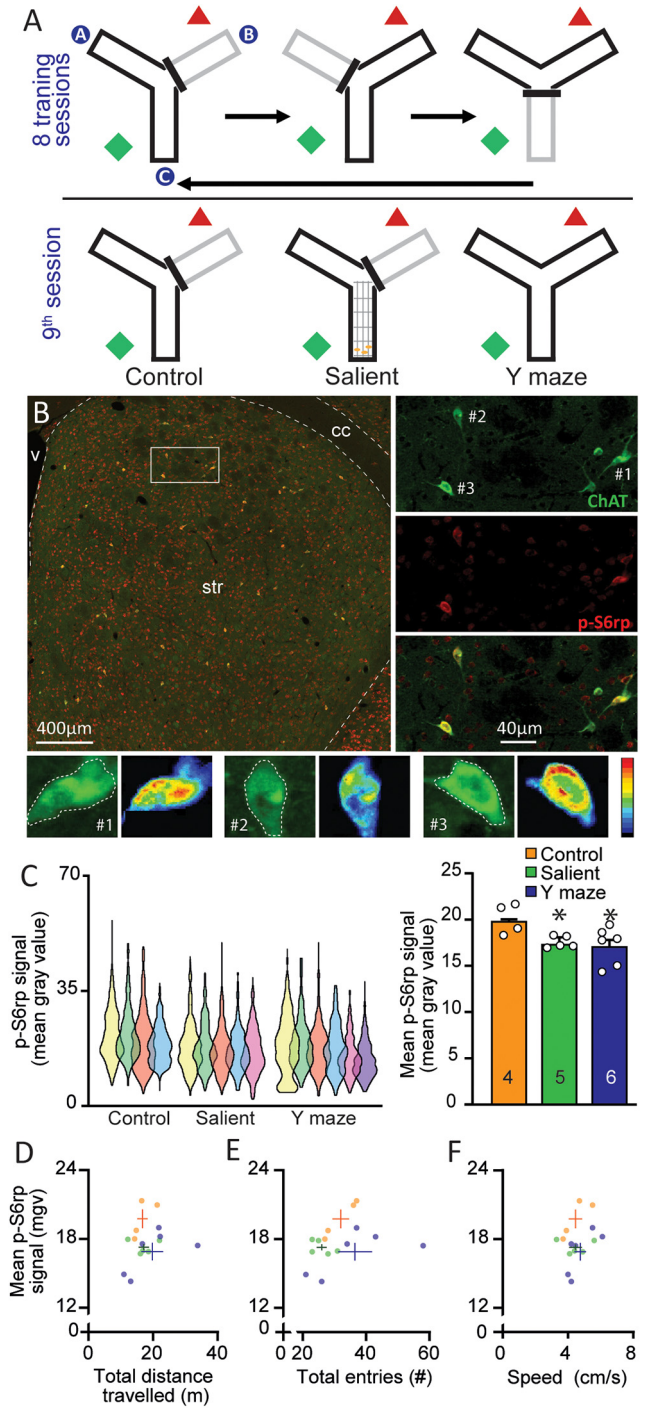

Figure 1. Phosphorylation of S6rp signal in SCINs can be modulated by salient environmental stimuli as well as by the adoption of complex exploratory strategies. ***A***, Schematic representation of the behavioral procedure. Mice were allowed to freely explore two different arms per day of a Y maze during eight daily sessions. On the ninth 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-arm maze but the maze was enriched with salient multisensorial stimuli; and Y maze, mice were allowed to access all three arms simultaneously. ***B***, Representative low-magnification photomicrograph of a coronal brain section (at approximately bregma + 0.9 mm) double immunostained to detect ChAT (green; SCINs) and p-S6rp (red) showing substantial colocalization of high-intensity p-S6rp-labeled cells with ChAT$^+$ cells. Inset corresponds to magnified right panels. Right panels, High-magnification view of boxed area showing ChAT and p-S6rp immunoreactivity in three nearby SCINs. Bottom panels, High-magnification confocal images of the three SCINs identified above (#1, #2, #3). For each cell on the left, ChAT channel with superimposed ROIs defined by automatic ImageJ segmentation (dotted line); for each cell on the right, red channel displayed as a 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 SCINs. Left, Quantification of the p-S6rp signal in individual SCINs in mice subjected to the three different behavioral conditions on day 9. Violin plots representing the distribution of p-S6rp levels in SCINs 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 the 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 the total distance traveled ($F_{(2,12)} = 0.41$, $p = 0.67$; ***D***), the number of arm entries ($F_{(2,12)} = 1.83$, $p = 0.21$; ***E***), or the mean walking speed ($F_{(2,12)} = 0.0001$, $p = 0.99$; ***F***) between treatments. Each cross represents the mean ± SEM of individual mice (color-coded dots). The numbers of mice are the same as in ***C***. No significant correlations were found between motor parameters and p-S6rp signal (Pearson correlation $p = 0.31$, $p = 0.19$, and $p = 0.18$, respectively).

post hoc comparison using paired χ^2 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 the readability of the main text. Two-tailed parametric statistics were used in all cases, and the threshold for significance was set at $p = 0.05$.

Results

SCINs 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 p-S6rp in individual SCINs 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 whether p-S6rp in SCINs 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 of the three arms of a Y maze located in a room with distal cues over 8 d. Each day, mice were exposed to a random pair of arms and were allowed to freely explore them for 8 min, to familiarize them with the entire Y maze and its relationship with the distal cues (Fig. 1*A*). On the ninth day, during the exploration of the familiar environment, a subset of familiarized mice was presented with unexpected salient stimuli (Fig. 1*A*, salient group). They were later killed, and their striata were immunostained to detect p-S6rp and ChAT (as a SCIN marker; Fig. 1*B*). The salient stimuli significantly reduced the p-S6rp signal in SCINs when compared with control mice that undisturbedly explored two arms of the Y maze (Fig. 1*C*), suggesting that activity changes induced in dorsal striatal cholinergic interneurons by salient environmental stimuli can impact a p-S6rp signal. An additional group of mice was allowed on the ninth day to freely explore the entire labyrinth as in the Y maze spontaneous alternation task (Fig. 1*A*, Y maze group). As expected, this group exhibited an alternation index significantly different from chance (70.7 ± 2.2%, t test vs 50%, $t_{(5)} = 9.49$, $p = 0.002$). Interestingly, when compared with the control condition, they

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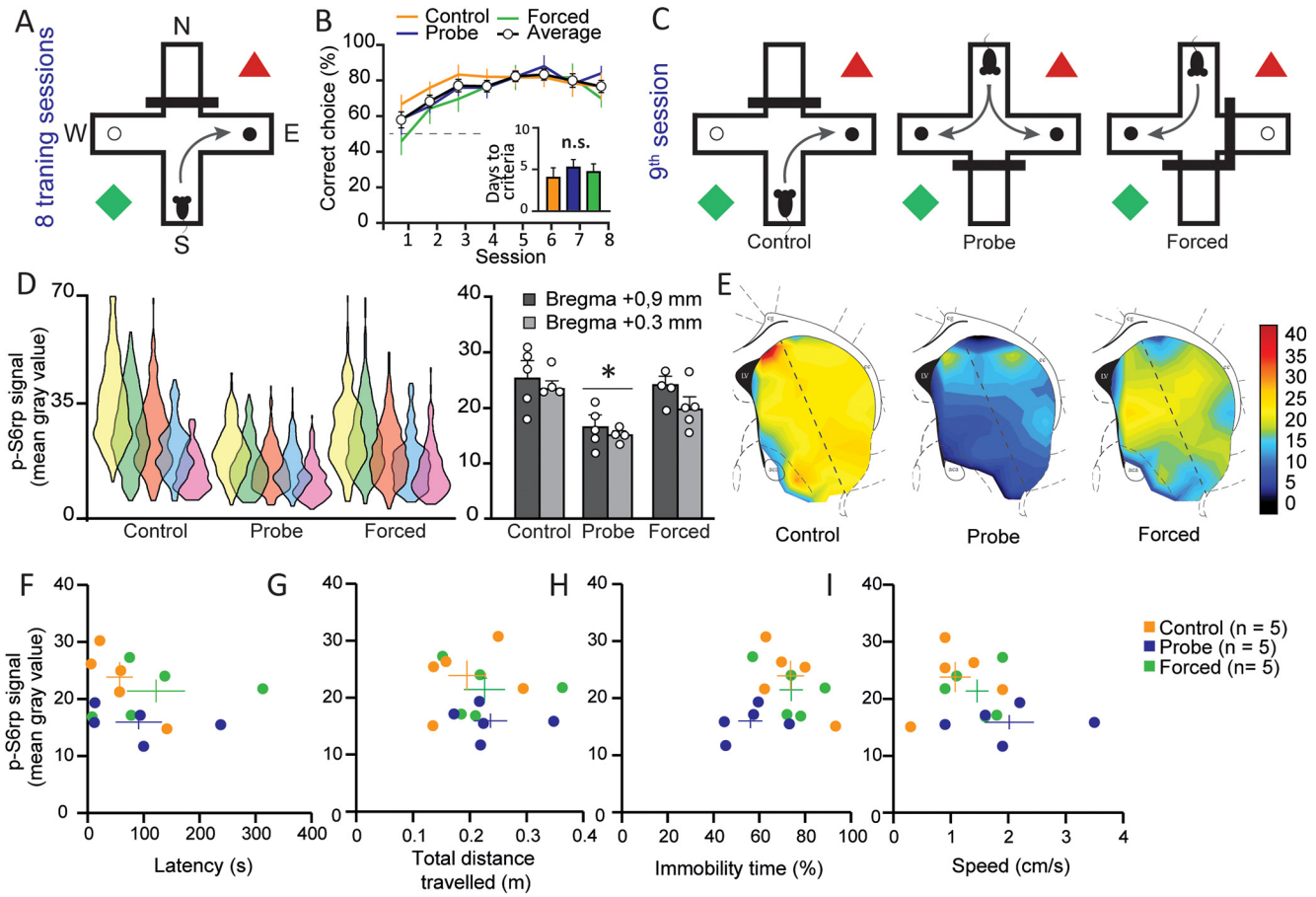

Figure 2. The strategy selection process decreases the phosphorylation levels of S6rp in SCINs. ***A***, Schematic representation of the dual-solution cross-maze task. During training the north (N) arm was closed. The mouse starts training in the south arm (S) and can find a hidden reward pellet in the small cup (black dot) located in the east (E) arm in this example. The training phase consisted of eight daily sessions of 10 trials each. ***B***, Black line depicts the learning curve for all 15 wild-type mice combined during the training phase of the task [one-way repeated-measures (RM) ANOVA: $F_{(7,98)} = 8.3$, $p < 0.001$; day 1 vs 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/group). ***C***, Schematic representation of the experimental conditions for day 9. Mice were divided into 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 the north arm with the 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 the south were blocked). $n = 5$ mice/group. ***D***, Strategy selection during a goal-directed navigational task reduces phosphorylated S6rp levels in SCINs. Left, Quantification of p-S6rp signal in individual SCINs in mice subjected on day 9 to the different behavioral conditions. Violin plots representing the distribution of p-S6rp levels in SCINs 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, two mice adopted a place strategy (number 3, light blue; number 4, pink), and three mice adopted a response strategy. Right, Statistical analysis revealed a significant decrease in p-S6rp levels in SCINs of mice that must opt out between one 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 the mean ± SEM of five individual mice per group. Because of 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: the color scale represents mean gray values in arbitrary units. ***F–I***, No significant differences were observed in the latency to make a decision (one-way ANOVA: $F_{(2,12)} = 0.64$, $p = 0.54$; ***F***), the total distance traveled (one-way ANOVA: $F_{(2,12)} = 0.43$, $p = 0.66$; ***G***), the percentage of time mice spent immobile ($F_{(2,12)} = 3.5$, $p = 0.06$; ***H***), or the mean speed ($F_{(2,12)} = 2.27$, $p = 0.15$; ***I***) 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 $p = 0.37$, respectively). Each cross represents the mean ± SEM of individual mice (color-coded dots). The number of mice is indicated in parentheses.

also showed a significant reduction in p-S6rp levels that was not significantly different from the one exhibited by the salient group (Fig. 1*C*). The observed changes in p-S6rp levels cannot be attributed to differences in motor activity since the total traveled distance, number of visited arms, and mean speed did not differ between groups and were not correlated with p-S6rp signal (Fig. 1*D–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 SCINs during exploratory behavior and strategy selection, we used a well characterized navigational task that formally evaluates the strategy used by the animals to reach a goal. Accordingly, we trained a group of 15 wild-type mice in a dual-solution plus maze. During the initial phase (8 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; Fig. 2*A*). As learning proceeded, correct choices increased from chance level, reaching 82% by day 5 (Fig. 2*B*). 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 used 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 ninth day, control mice were tested as on previous days while the probe group mice were released from the north arm (Fig. 2*C*). In the probe test, if the mouse turned toward the same baited arm that was visited during training (e.g., west), that indicates the use of a place strategy (based on the location of reward with respect to extramaze cues), while selecting the opposite arm reflects a response strategy (based on the body-turning direction). Phosphorylation of p-S6rp in SCINs was significantly decreased in the probe test group when compared with control mice that have no conflict regarding which strategy to follow (Fig. 2*D*). The reduction was observed along the entire anterior–posterior axis (Fig. 2*D*, right) and occurred both in dorsolateral as well as ventromedial regions of the dorsal striatum (Fig. 2*E*). This reduction cannot be because of differences in the 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 distance traveled, immobility time, or mean walking speed (Fig. 2*F–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 on the environment and that 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 (Fig. 2*C*, forced group). S6rp phosphorylation levels in the forced group did not differ from those of the control group (Fig. 2*D–E*), suggesting that differences observed in the 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 DTR expression to cholinergic cells by using the ChAT-Cre$^{+/-}$ DTR$^{loxP/wt}$ line (hereafter called ChAT-DTR) and induced an extensive ablation of SCINs by bilaterally injecting adult ChAT-DTR male mice with diphtheria toxin (lesioned) or solvent (control), following previously reported methods (Martos et al., 2017; Fig. 3*A*). Lesions spanned the entire anterior–posterior axis of the dorsal striatum and typically comprised >95% of the SCINs without affecting cholinergic neurons in the nearby septal area (Fig. 3*B*,*C*). Control and lesioned food-restricted mice were trained daily in the dual-solution plus maze similar to the one described above (Fig. 4*A*). Lesioned mice learned the task in a manner similar to control mice, although they exhibited a small but significant reduction in the maximal performance attained (Fig. 4*B*). Days to reach criteria (i.e., days required to correctly solve at least 80% of the trials) did not differ between groups, and the performance of lesioned mice did not improve after day 6, similar 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 (Fig. 4*C*, 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 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% vs 46% mice adopted "response," respectively; Fig. 4*C*). However, SCIN-depleted mice did not develop a bias toward either strategy with further training (Fig. 4*C*, right), 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 3 of 28 lesioned mice (10.7%) consistently reused 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 (Fig. 4*D*). It is well accepted that the acquisition of a response-based strategy depends on striatum integrity (Packard, 2009). Therefore, lesioned mice may not acquire a preference for the egocentric strategy because of 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 always reinforcing the same body turn direction, this experimental setting leads mice to acquire a response-based strategy to maximize the rewards obtained. Lesioned and control mice performed equally well in this task (Fig. 4*E*), 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 used 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 either by using 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 5 (Fig. 5*A*). Except for day 1, 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 (Fig. 5*B*), suggesting comparable motivation to escape from the maze. It has been shown that maze geometry, the 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 extramaze visual cues placed on the walls, selecting a maze without a wall and with an appropriated number of holes in relation to the perimeter length (Fig. 5*C*). When training began, mice of both groups randomly searched for the escape hole in nearly 50% of the trials (Fig. 5*D*, left). By day 4, the random search was superseded by a well defined strategy-based exploration of the arena in >95% of the trials, with the serial strategy prevailing in

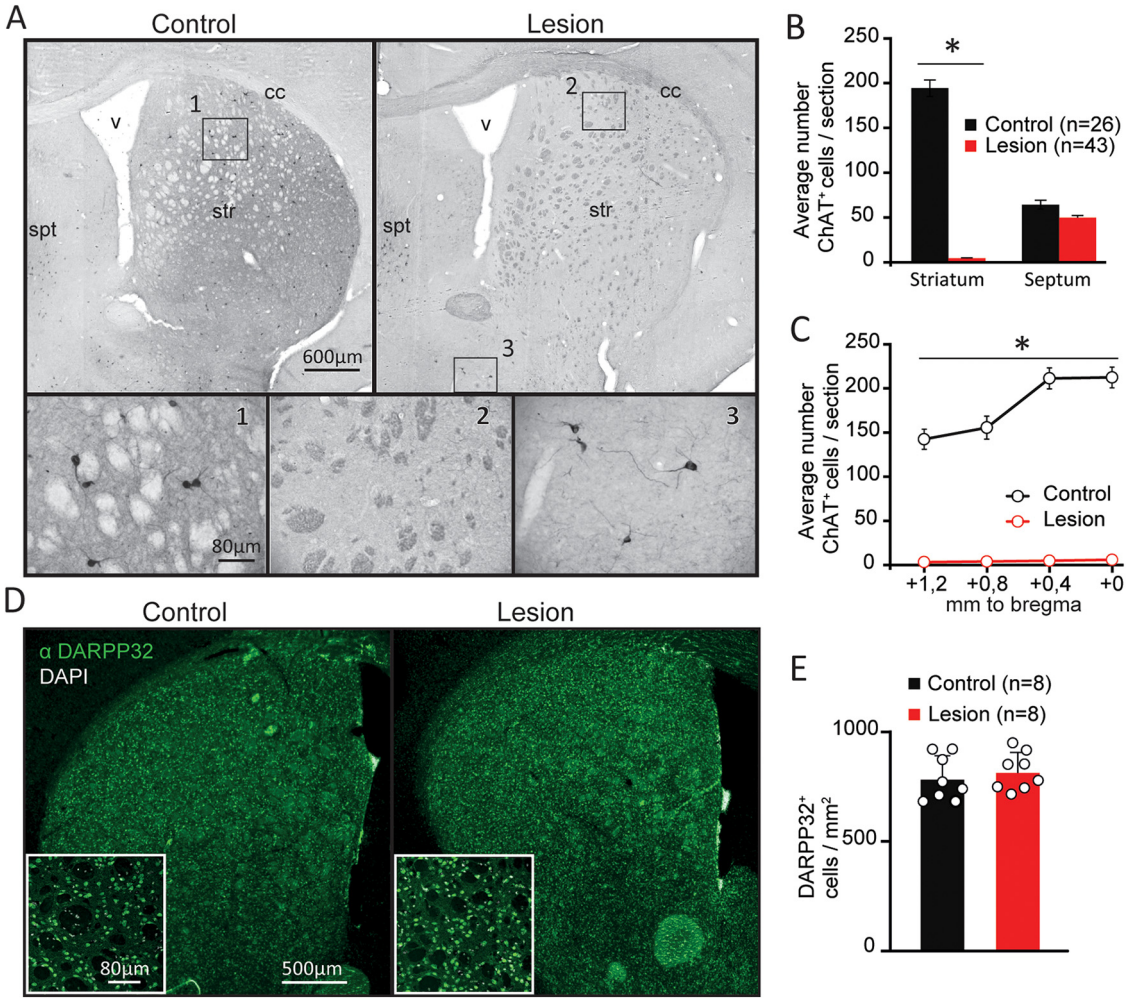

Figure 3. Conditional bilateral ablation of SCINs *in vivo* using the diphtheria toxin system. ***A***, Representative photographs of coronal brain sections immunostained against ChAT (DAB) from ChAT-Cre:iDTR transgenic mice treated with solvent (control) or DT (lesion) depicting the extent of the lesion. High magnification images of boxed areas are shown below; large ChAT$^+$ 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 [repeated-measures (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 the mean ± SEM of 26–43 mice. ***C***, DT treatment significantly reduced SCINs 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 the 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 the 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 the mean ± SEM of eight control and eight lesioned mice subjected to behavioral testing.

both experimental groups (70.8% and 64.1% of total trials in control and lesion groups, respectively). In the few trials when random search was still adopted after day 4, no statistical differences were found regarding search efficiency between groups (path efficiency: 0.37 ± 0.07 vs 0.31 ± 0.02, $t_{(83)} = 0.94$, $p = 0.35$; control, $n = 9$; lesion, $n = 76$, respectively). From day 4, control mice slowly but persistently switched toward a new strategy profile increasingly adopting the spatial strategy. By day 9, the distribution of adopted strategies significantly differed from the strategy of day 4 (Fig. 5*D*, top right), with the spatial strategy becoming the predominant one by the end of testing (Fig. 5*D*, right). In striking contrast, lesioned mice did not shift away from their initial distribution of selected strategies (Fig. 5*D*, right). So, by day 12, SCIN-depleted mice still chose the initially favored serial strategy in most trials, differing significantly from the control group (Fig. 5*E*). Strategy classification using a stricter or a more relaxed criterion did not modify the overall conclusion presented here (data not shown; stricter criteria: only one error in spatial strategy; global χ^2 test, $p = 0.013$; relaxed criteria: up to three errors in spatial strategy; global χ^2 test, $p < 0.001$). Selection of a given strategy may depend on the proficiency to execute it; however, no significant differences were observed between control and lesioned mice in their performance with either strategy (Fig. 5*F*). These results suggest that the inability of SCIN-lesioned mice to switch toward the spatial strategy is not because of a deficit in spatial navigation or to a higher proficiency in serial learning. To investigate the possibility that lesioned mice took a win–stay approach and persevered in a given successful strategy, we visually inspected strategy selection at the individual level throughout training (Fig. 6*A*). Then, we calculated the fraction of mice displaying first-trial consistency (i.e., how many mice repeated in first trial of day 12 the strategy used in the first trial of day 11), consecutive trial consistency (i.e., used the same strategy in the last trial of day 11 and the first trial of day 12), and global strategy consistency (i.e., the preferred strategy on day 11 vs the preferred strategy on day 12). We found no evidence that

individual mice consistently adopted a given strategy across days in either group, even when strategy distribution was stable at the population level and was highly effective in terms of reaching the goal. For instance, no significant differences were observed between groups in first trial consistency (Fig. 6*B*), consecutive trial consistency (Fig. 6*C*), or global strategy consistency (Fig. 6*D*). As another index of perseverance, we also calculated the number of consecutive days that 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 d, respectively; *t* test: $t_{(33)} = 0.92$, $p = 0.36$). Overall, regardless of the measure used to determine the adoption of a given strategy, each mouse only showed preference for one particular strategy during 1 or 2 consecutive days.

In summary, these behavioral results confirm that SCINs are necessary to update the probability function used for strategy selection. Thus, SCINs 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.

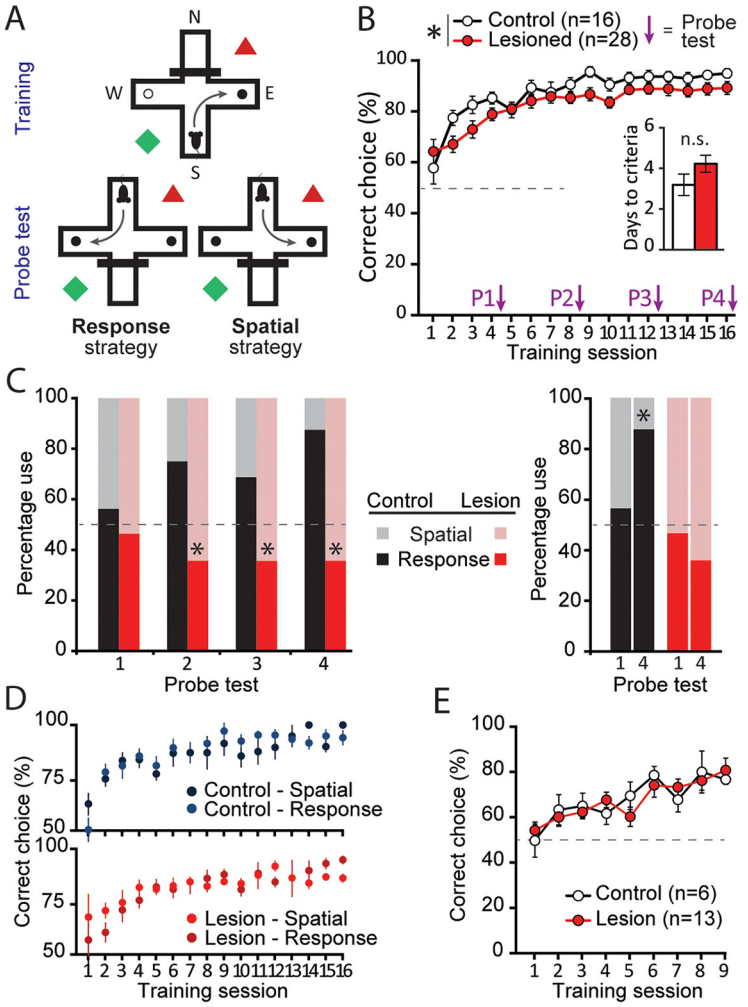

Figure 4. SCIN ablation impairs normal strategy selection during a goal-directed navigational task. ***A***, Schematic representation of experimental design used in the dual-solution cross-maze task. Control and lesioned mice were trained daily (10 trials/d), and probe tests were intercalated every 5 d. ***B***, SCIN-ablated mice were capable of learning goal locations across days [repeated-measures (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 the 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 legend. ***C***, Lesioned and control groups presented different goal-reaching strategy profiles from probe test 2. Left, Strategy selection probabilistic profiles for control and lesion groups throughout all probe tests. The graph displays the percentage of mice adopting a given strategy for each probe session. Significant interaction (treatment × time × strategy) was observed by global log-linear three-way contingency table analysis ($G^2_{(10)} = 24.16$, $p = 0.0072$). *$p < 0.05$, χ^2 test versus control (probe test 1: $\chi^2 = 0.39$, $p = 0.53$; P2: $\chi^2 = 6.29$, $p = 0.012$; P3: $\chi^2 = 4.45$, $p = 0.035$; P4: $\chi^2 = 11.0$, $p = 0.0009$; df = 1 in all cases). Right, Control group, but not the lesioned group, showed a significant change in their profile of selected strategies after training. *$p < 0.05$, χ^2 test versus probe test 1 (control: $\chi^2 = 3.86$, $p = 0.04$; lesion: $\chi^2 = 0.30$, $p = 0.58$; 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 mice (two-way ANOVA; strategy factor: $F_{(1,224)} = 0.92$, $p = 0.34$) or 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. The number of mice is indicated in graph references.

Discussion

Here we provide evidence showing that a global change in SCIN activity occurs concomitantly with the strategy selection process that takes place during the resolution of navigational problems. Moreover, we found that SCINs are not needed to learn goal location in two different navigational tasks or 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, SCINs do not appear to be required for the adoption of a given navigational, either egocentric or allocentric, strategy. On the contrary, SCINs are necessary to switch from the initially preferred task-solving strategy toward an alternative, contending one possessing a similar goal-reaching effectiveness but that may present additional adaptive advantages in the long run. Notably, the deficit in strategy switching occurs after an 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.

Measuring global SCIN activity in rodents *in vivo* with electrophysiological methods is challenging because of their sparseness. Even with contemporary imaging techniques, only a few SCINs can be measured simultaneously during behavioral studies (Rehani et al., 2019). It was proposed that decreased p-S6rp levels reflected lower SCIN firing (Bertran-Gonzalez et al., 2012). However, the relationship between SCIN p-S6rp levels and firing rate could be more complex when differences in firing pattern are taken into consideration (Matamales et al., 2016). By measuring p-S6rp in SCINs, we have shown that selecting between alternative problem-solving strategies triggers a change in SCIN activity equivalent to the one seen after animal exposure to novel, salient environmental stimuli previously reported to impact

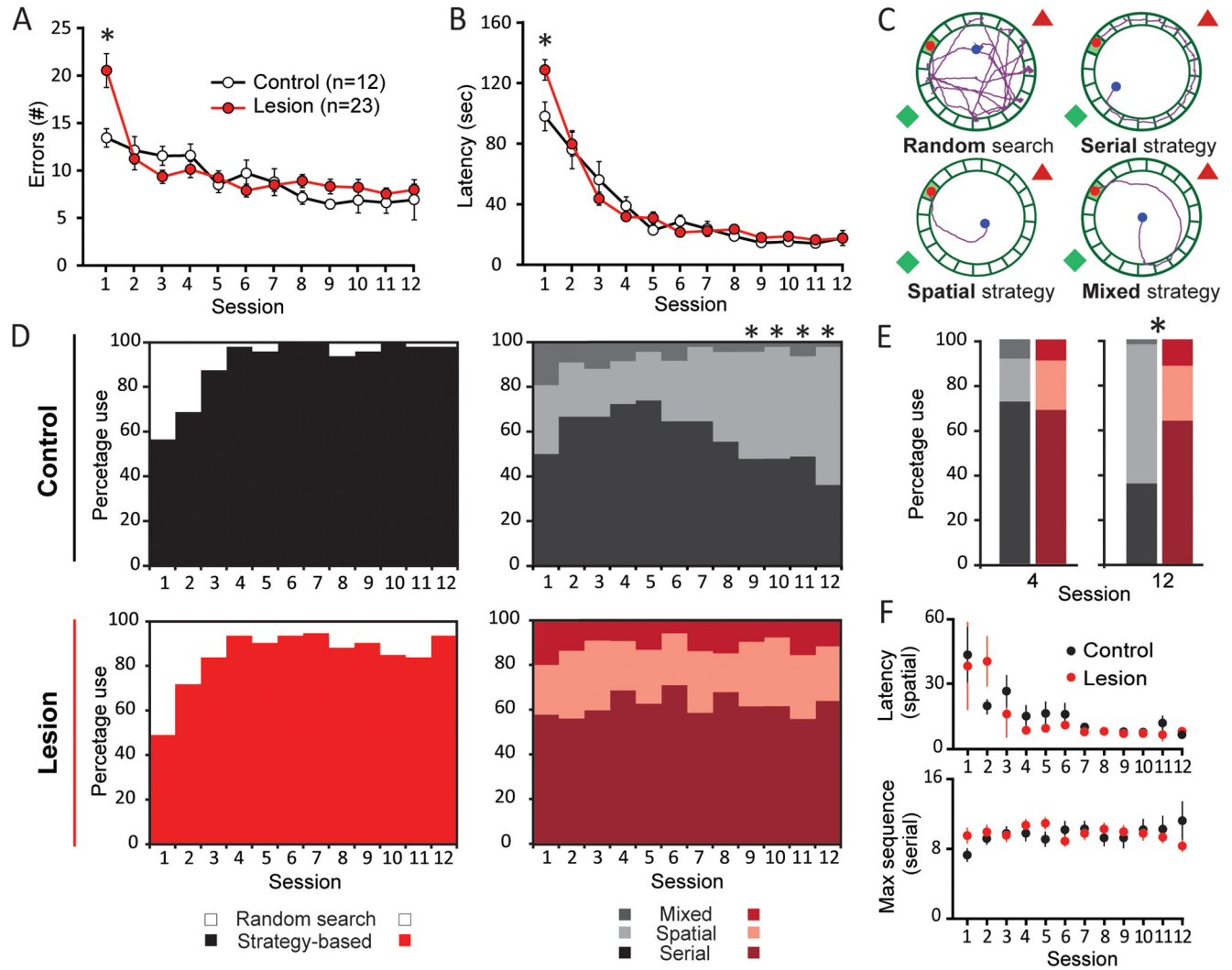

Figure 5. SCIN are required to update probability function used for strategy selection throughout training in a goal-directed navigational task. Control and SCIN-ablated mice significantly improved their performance over the course of training, reaching the same final performance in the Barnes maze. ***A***, ***B***, Analysis of errors (number of incorrect holes checked before locating the escape box; ***A***) and latency (time to locate the escape box; ***B***). No significant differences were found other than in session 1 between control and SCIN-ablated mice (repeated-measures ANOVA interaction: for errors: $F_{(11,363)} = 2.97$, $p < 0.001$; for latency: $F_{(11,363)} = 3.09$, $p < 0.001$; $^*p < 0.001$ vs same-day control group). The number of mice is indicated in graph legend. ***C***, Representative examples of individual mouse trajectories depicting random search and the different strategies. Track plots exemplify random search (top left) or serial (top right), spatial (bottom left), and mixed (bottom right) strategies. Blue dot, Starting tracking point; red dot, final mouse position; purple line, mouse path; green zone, goal location. ***D***, Left, Graphs depict the percentage of trials solved using a well defined strategy throughout training. Control and lesion groups significantly increased the use of a goal-reaching strategy during initial days of training (control, global χ^2 test: $\chi^2_{(11)} = 132.1$, $p < 0.0001$; lesion, global χ^2 test: $\chi^2_{(11)} = 131.4$, $p < 0.0001$; 48 trials from 12 control mice and 92 trials from 23 lesioned mice per session). Right, Probability profiles of strategy selection for control (top) and SCIN-depleted mice (bottom) throughout training. After random search is superseded (session 4), control mice progressively changed their profile of strategy selection shifting from a predominant serial strategy on session 4 to a profile biased toward a spatial strategy after session 8 (global χ^2 test: $\chi^2_{(22)} = 52.5$, $p = 0.0003$; $^*p < 0.05$, χ^2 test vs session 4). Instead, the lesion group displayed the same probability profile of strategy selection throughout the entire experiment (global χ^2 test: $\chi^2_{(22)} = 19.3$, $p = 0.63$). ***E***, Between-group comparison of the probability profiles of strategy selection. At session 4, the distribution of selected strategies was not significantly different between the control and lesion groups (global χ^2 test: $\chi^2_{(2)} = 0.21$, $p = 0.90$). Instead, at the end of training (session 12) the profile of SCIN-lesioned mice was significantly different from the one displayed by the control group (global χ^2 test: $\chi^2_{(2)} = 18.9$, $p < 0.0001$). ***F***, Control and SCIN-ablated mice executed their strategies in a similar way. Top, Latency to reach the escape box in trials when a spatial strategy was adopted (two-way ANOVA; treatment factor: $F_{(1,405)} = 0.98$, $p = 0.32$). Bottom, Maximal number of errors made during a sequential search of the escape box in trials when a serial strategy was adopted (two-way ANOVA; treatment factor: $F_{(1,683)} = 0.05$, $p = 0.82$). Each dot represents the mean ± SEM value of the corresponding strategy adopted in each trial.

SCIN firing *in vivo* (Kimura et al., 1984; Apicella et al., 1991; Aosaki et al., 1994, 1995). Our results are in line with those of previous studies showing that transition from place to turn strategy in a T maze was accompanied by changes in extracellular acetylcholine levels in the striatum (Chang and Gold, 2003). Still, p-S6rp assessment only provides an indirect reflection of SCIN activity, which integrates modulations of SCIN function throughout the duration of the study, and therefore these results must be cautiously interpreted.

To fully address SCIN participation on strategy selection, we selectively ablated SCINs in dorsal striatum while assessing the behavioral impact of the manipulation. SCIN-depleted mice displayed, consistent with recent studies (Okada et al., 2014; Matamales et al., 2016), normal or quasi-normal learning curves in all of our behavioral paradigms and were equally effective in obtaining rewards or finding an escape box as control mice. Independent of the task, control and SCIN-depleted mice initially displayed a similar preference for the available strategies.

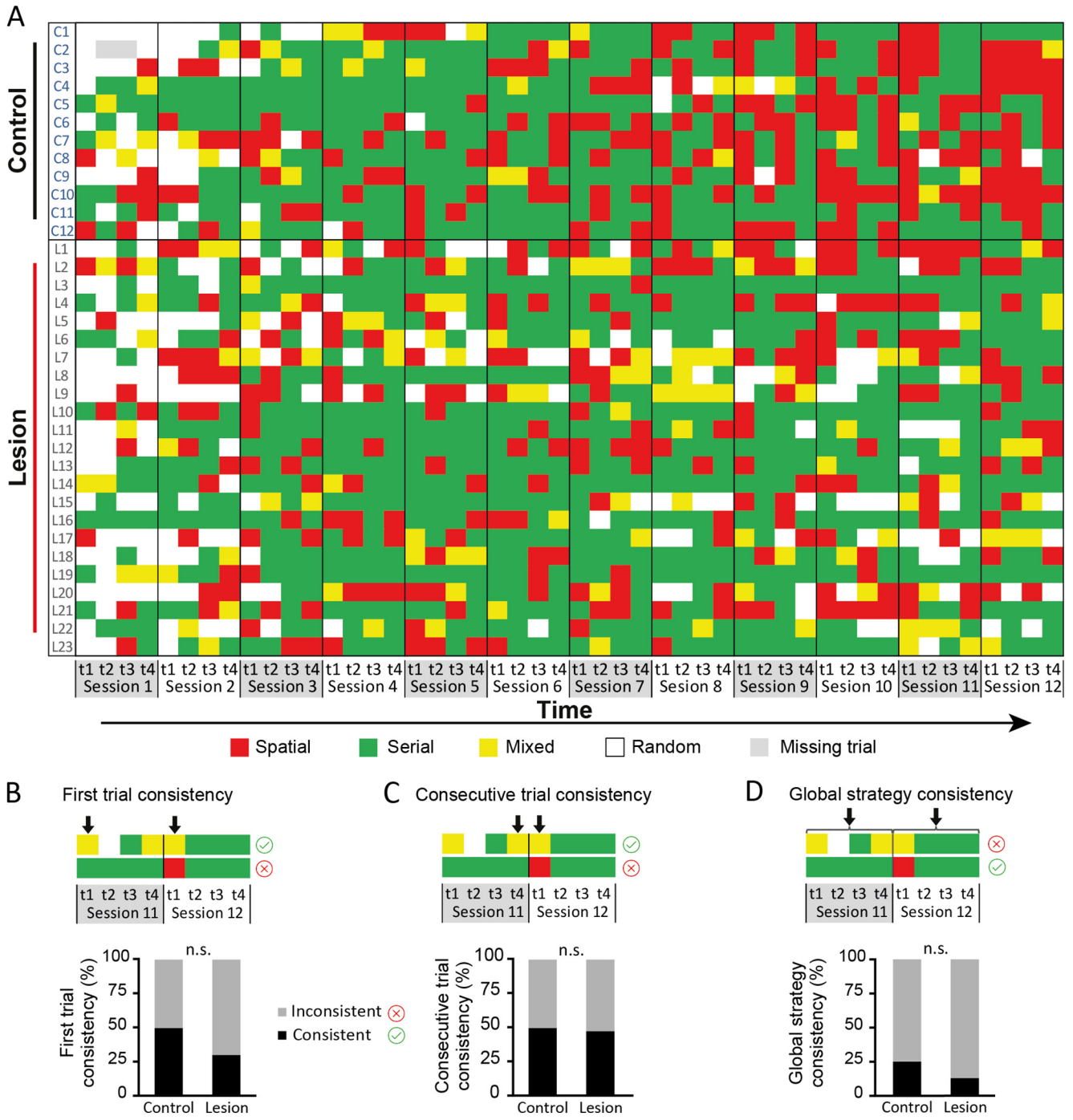

Figure 6. Control group shifted of their preference throughout training toward a spatial strategy without adopting a win–stay strategy at the individual level. ***A***, Graphical representation of the individual strategies adopted by control and lesioned mice in each trial of the Barnes maze throughout 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 to 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 because of a technical problem. ***B–D***, No significant differences could be observed between control and lesioned mice for first trial consistency (6 of 12 vs 7 of 23, respectively; χ^2 test: $\chi^2(1) = 1.29$, $p = 0.25$; ***B***), consecutive trial consistency (6 of 12 vs 11 of 23; χ^2 test: $\chi^2(1) = 0.015$, $p = 0.90$; ***C***), or global strategy consistency (3 of 12 vs 3 of 23; χ^2 test: $\chi^2(1) = 0.79$, $p = 0.37$; ***D***).

But, as training progressed, control mice slowly but consistently adopted a new dominant solving strategy. In striking contrast, SCIN-depleted mice remained invariant in their initial profile of strategy usage. Interestingly, this impossibility of SCIN-lesioned mice to update their strategy selection as training evolved is manifested independently of the nature of the goal (obtain a reward or avoid a risk). More remarkably, this impairment occurs regardless of whether an egocentric strategy (i.e., response strategy in the dual-solution cross-maze) or an allocentric strategy (i.e., spatial strategy in Barnes maze) prevails after training in control mice and results in the preferred strategy at a population level. Furthermore, our results demonstrate that SCINs are not required to execute any of these individual strategies per se.

In control mice, we observed that migration toward the finally adopted strategy continues even when goal achievement

has reached an asymptotic value under invariant task conditions. Thus, when two strategies with similar goal-reaching effectiveness compete for behavioral control, other factors, including energy preservation or cognitive effort, may govern strategy adoption. As mentioned before, in the initial stages of both tasks, control and lesioned mice showed similar preferences for the available strategies. Thus, under conditions where limited information is available, SCINs are not critical to determine the chance of a given strategy to gain control of the behavioral response. Instead, the SCINs are pivotal to bias the selection of one strategy over the other as training progresses and the available information, apart from the goal-reaching effectiveness, presumably increases.

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

Although speculative, we would like to propose a model for the strategy selection processes in which, when previous information indicates that only one strategy is available to achieve a goal, SCINs are not engaged in the strategy selection process. Alternatively, when competing strategies are available to solve a problem, SCINs will be necessary to bias strategy selection based on long-term valuation of outcome. Outcome assessment could take into account not only goal achievement effectiveness but also a trade-off between alternative strategies that maximize the benefit/cost ratio in the long run. Under this model, neuronal activity coding for multiple strategies is fed forward to the striatum and passed through a probabilistic filter governed by SCINs. 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 SCINs 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 with additional information, including energy balance or cognitive demand throughout training.

Our present study has unveiled a critical role of SCINs in strategy selection, specifically in the context of spatial problems. However, we speculate that this process may exemplify a more general function of SCINs. 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 nonconsciously 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% 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 patients, Hanson et al. (2018) have recently reported a selective decrease of SCINs in postmortem caudate of these patients. 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 impairment in Williams syndrome patients remains to be determined. A SCIN reduction also has been reported in Tourette syndrome (Kataoka et al., 2010; Lennington et al., 2014). Although the reduced cholinergic signal may not be the sole cause of the characteristic tics, it has been proposed to contribute to the altered social behavior displayed by some Tourette syndrome patients by affecting the function of the social decision-making network (Albin, 2018). Furthermore, SCIN dysfunction has not only been connected to abnormal social engagement. Recent experimental evidence showed that the interruption of cholinergic transmission in the dorsolateral striatum results in setting-specific compulsive eating (Favier et al., 2020), a phenotype that may reflect an imbalance during normal selection of exploration/exploitation strategies.

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

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