Supplemental method section

The automated H-maze

The automated H-maze (Supp1A) is an H-shaped apparatus made up of two testing chambers (C1 and C2) connected by a straight plastic tube 5 cm in diameter and 23 cm long. Each testing chamber is made of two plastic tubes 5 cm in diameter connected to an empty plastic cube. Cube sides are 6 cm. All tubes are made by joining two half tubes (a top half laid on a bottom half). The top tubes are independent and can be removed easily to clean the maze. On top of the cubes, inverted fans inject neutral or scented air from the outside extremities of the tubes connected to the cubes. At each lateral extremity of the testing chambers, a water port is located above the air and odor port. The activity of a mouse is detected by photoelectric cells located 5 cm from each tube extremity, and 5 cm away from both extremities of the connecting straight tube. In this experiment, the odor was injected through all lateral extremities of the testing chambers at a flow rate of 5 L/min by forcing clean air (0.7 bar) through two Erlenmeyer flasks that contained 200 ml of water mixed with 5 g/L of isoamyl acetate (Sigma-Aldrich). The neutral air (1000 ml Erlenmeyer flask containing 200 ml of water) was injected at the same flow rate on both sides of the two testing chambers. The automated H-maze was set on a square table, 160 cm above the floor. Inside the maze, the mice could move freely and all procedures and recordings were controlled by a computer via a program developed using Lab View (National Instruments, Nanterre, France). Mouse behavior was observed directly in the testing room.

Habituation session

On each daily habituation session, each mouse was weighed and then placed in the automated H-maze for 10 min. On the first day, the mouse could run freely throughout the maze with water and odor delivered ad libitum. The odor is an olfactory cue allowing to keep the environment stable between each sessions, thus decreasing the anxiogenic effect of novelty. Then, the mouse was returned to its cage and was water deprived. On the second day, the odor was injected from each extremity of one randomly selected testing chamber. When the mouse crossed one of the photocells in this testing chamber, 10 µl of water were delivered. The same odor was injected inside the second testing chamber until the mouse crossed one of the photocells to get the same reward. This automated odor injection was maintained for 10 min. On the third day, once the mouse was inside the maze, the same odor was injected randomly from only one of the testing chambers of the maze. When the mouse ran into this chamber, 10 µl of water were distributed in one extremity but not in the other. When the mouse went to the randomly chosen extremity, 10 µl of water were distributed again and so on for 10 min. After 3 days of habituation, it is assumed that the odor was associated with the presence of the reward and contributed, with hydric deprivation, to maintain the necessary motivation to look for water.

Training session

Before the training session, mice were weighed again to verify that they reached 85±5% of their initial weight. At the beginning of the training session, the top half of the connecting straight tube was lifted to introduce the tested mouse into the apparatus, and then replaced. When the mouse was in the middle of the tube, the same odor was injected from

both tubes of one randomly chosen testing chamber. During the whole session, the scented air guided mice to the testing chamber where the reward could be distributed but did not indicate which way to turn. At this time, only one side was associated with the reward. The response was given by crossing one of the photocells at an extremity. The reward was distributed if the mouse chose the correct side and was not distributed on the other side. After that, when the mouse crossed the photocell located at the extremity of the connecting tube closer to the other testing chamber, the same odor was injected at both extremities of the second testing chamber. The mouse had to go to one extremity of the second testing chamber to get the reward from the designated side and not to other end. Between the trials in the first and second testing chambers, neutral air was distributed. Once again, the mouse had to make a choice between the right or left side of the testing chamber to get the reward. This continued until the criterion was reached. In the test, there was no fixed delay between trials. However, the minimal delay between the response in one testing chamber and the entrance to the opposite testing chamber was measured. This minimal inter-trials delay was 4 sec.

Three different rules had to be discovered: an alternation rule (delayed alternation task), a non-alternation rule (delayed non-alternation task (N-ALT) and a reversal rule (REV). The three tasks implementing the three rules were performed in succession. The rule switched automatically once the criterion (four consecutive successful trials) was met, 1 hour had passed, or 80 trials had been completed, whichever came first. From a previous study (Belhaoues et al., 2005; Del'Guidice et al., 2009), the success criterion was set at 4 consecutive trials, which reduced to 1/16 the probability of success due to chance.

Test session

In the first task (ALT), mice had to learn to alternately move between both chambers of the H-maze to get reinforcement (turn left in C1, turn right in C2 in alternation). A mouse responded correctly by going to the side where the reinforcement was distributed. On the next trial, the reinforcement was distributed on the opposite side, and so on (Supp1A blue arrows).

Once the mouse had succeeded in the first task, one of the sides (right or left) was randomly chosen to be the one to get the reinforcement on the N-ALT (Supp1A violet arrows). Mice had to inhibit the response learned in the first task (ALT) in order to learn the second task (N-ALT). The N-ALT task consists in always turning to the same side to enter each chamber. Maintenance of the ALT strategy under N-ALT conditions resulted in 50% reward while full reward could only be achieved by extinguishing the ALT strategy and learning the N-ALT rule.

Once the criterion of the second task was met, the third task (REV) started. The mouse had to perform the opposite strategy to that learned in the N-ALT task and received the reward on the opposite side to that assigned in the N-ALT (Supp1A red arrows). This paradigm requires mental flexibility and attentional set.

The Cross maze

The cross maze is an X-shaped maze made of black opaque acrylic walls and floor (30 x 10 x 20 cm for all four arms and 10 x 10 x 20 cm for the central area) in which four independent gates can be added or removed individually to modify spatial configuration, in order to prevent access to one or some arms and to isolate mice in a chosen compartment.

Small plastic cups were left at the distal end of each arm to hide food pellets. Experiments were administered on a table elevated 90 cm above ground.

Habituation session

Mice were first familiarized with the cross maze. They were food deprived for one day. On the day prior to familiarization, 45 mg sucrose pellets (Noyes Precision Pellets, Test Diet, Richmond, IN) were scattered in their home cage in order to prevent food neophobia. Familiarization lasted 7 days and consisted of 3 separate trials per day during which a mouse was free to explore the maze and eat pellets for 5 min. On the first day, pellets were scattered on the arms and on the central area, as well as in containers at the end of each arm. On the three subsequent days, pellets were gradually removed from the maze, until only two were left in each container at the start of a trial. When mice were able to eat 80% of pellets or more within 5 min, the cross maze task began.

Training and testing session

The cross maze task was administered upon 2 consecutive days. On the first day, 15 consecutive training trials were administered: the first five were forced-choice trials. The mouse was placed at the end of one arm (start arm) and there was only one open arm, the other two being blocked by sliding doors. A sucrose pellet was placed in the container at the end of the open arm (for a given mouse, the open reinforced arm was always the arm on the immediate left or the right of the start arm, 50% of mice having been randomly assigned to one or the other condition). Upon the next 10 trials, the mouse was placed on the start arm and had to choose between 3 arms; only the arm visited on the forced-choice trials was

reinforced. Between trials, the mouse was returned to its home cage for 30 sec and the maze's surfaces were cleaned with a 30% alcohol solution in order to avoid guidance by olfactory cues on the subsequent trial.

On the next day, the first phase of testing (P1) was administered: Mice were placed on the same start arm as on the previous day and the same arm was reinforced. When they chose the reinforced arm on 6 consecutive trials, they started the second phase (P2). On that phase, mice were placed on a new start arm, located directly across the original one. The previous start arm became the new reinforced arm, requiring mice to emit a new behavioral response: choosing the arm facing forward to obtain reinforcement. In order to complete the second phase, mice had to once again reach the criterion of 6 consecutive reinforced choices. The number of trials required to reach each criterion, as well as the number of perseverative errors made (choosing the same non reinforced arm on six consecutive trials or more) were recorded. Also, after 100 trials without reaching the criterion (no inter-trial delay), mice were considered to have failed learning the task. The number of mice in each group learning and not learning the task was recorded.

Olfactory perception in a novelty-related test

The apparatus was a clean, unused cover grid resting on top of the cage and from which two Pasteur pipettes were hanging. The first pipette contained a saturated wathman paper with non-odorant mineral oil whereas the second one contained the odor, with different concentrations of isoamyl acetate or octanol. Time spent sniffing odors was measured for four concentrations (10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} µl/l), tested in four different 5 min sessions from the least to the most concentrated. We considered mice to have normal olfactory perception

when time spent sniffing the odor was significantly greater than time spent investigating the mineral oil-containing pipette .

Sucrose preference and quinine aversion tests

Individually housed mice had ad libitum access to food during this test. Bottles were weighed every 24 h. On the first day of testing, two bottles were placed on top of mice's cages: One filled with distilled water, the other filled with either a 1% (weight/volume; w/v) sucrose solution or 0.025% (w/v) quinine solution. The starting solution was randomly assigned between mice, 50% beginning the test with either solution. Bottles were weighed every 24 h and their left-right position was alternated every day to avoid a spatial preference effect. After 48 h, the bottle containing a tasting solution was rinsed and filled with the other solution. The procedure was repeated with the new solution for another 48 h. The weight of liquid consumed served to calculate a preference ratio (ie, sucrose / [sucrose + water]), which was used for analyses.

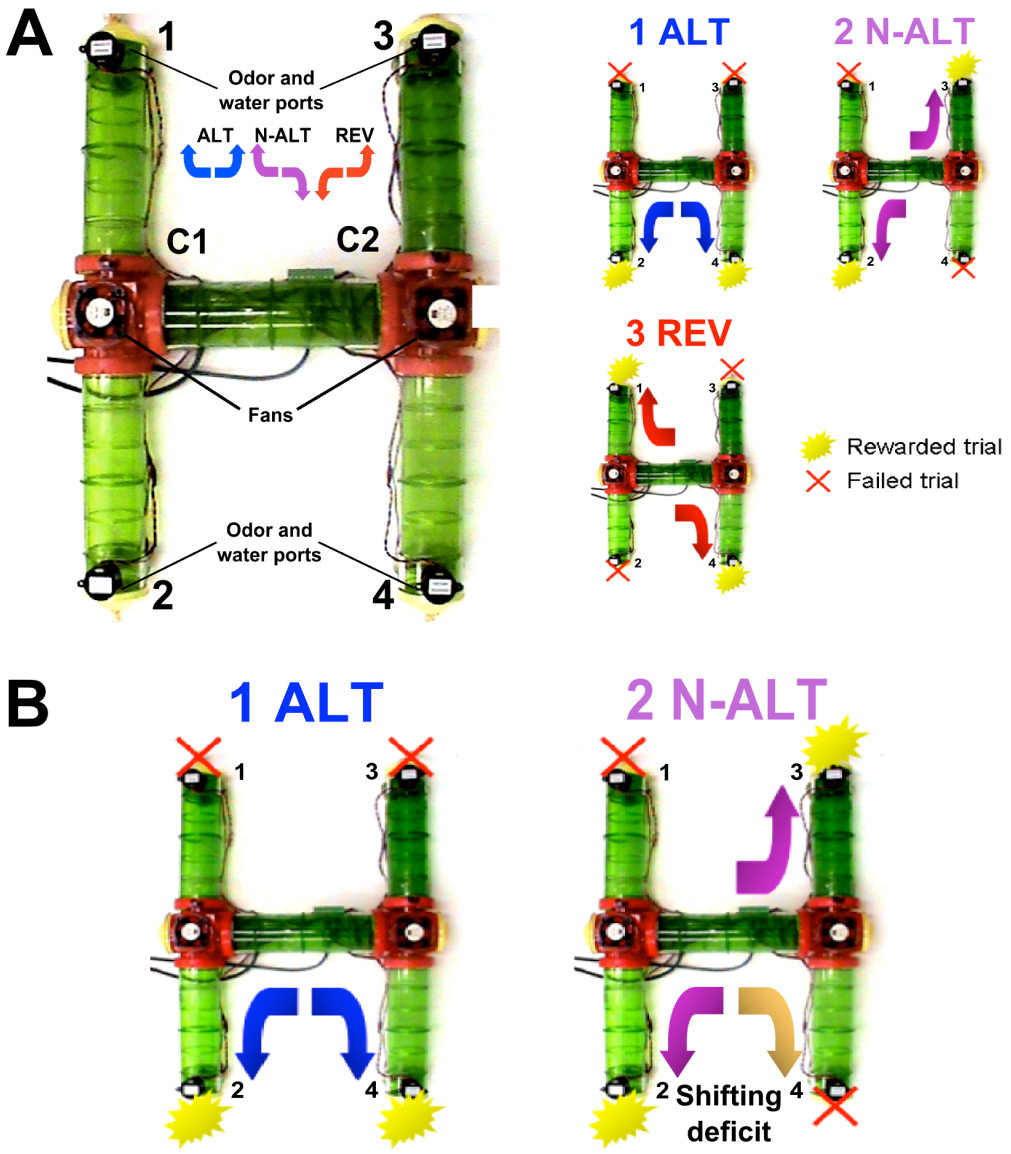

Figure S1. (A) Picture of the automated H-maze with its two testing chambers (C1 and C2) and the three sequential rewarded learning rules. (B) Shifting deficit (orange arrow) occurring at the beginning of the N-ALT task and showing the persistence of the ALT strategy during the N-ALT task.

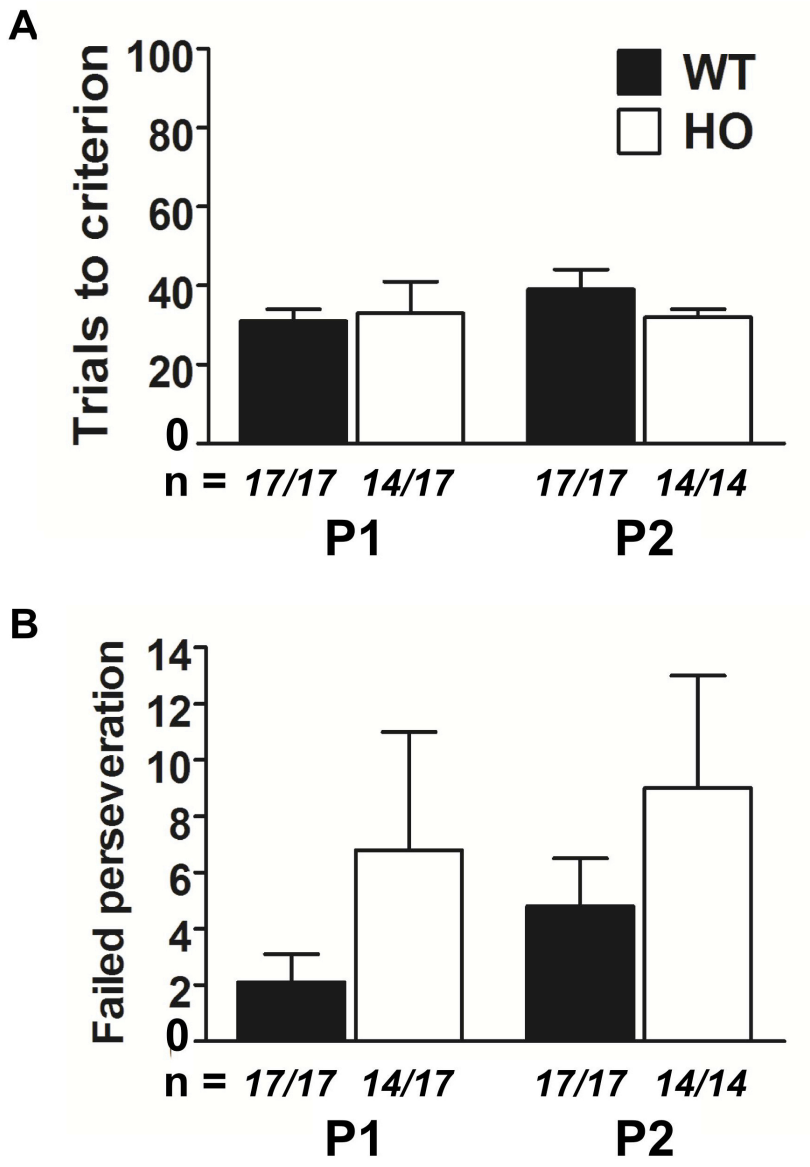

Figure S2. (A) Tph2-KI mice do not show cognitive deficits in the cross maze through two spatial tasks (P1 and P2). Average number (± SEM) of perseveration errors for HO Tph2-KI mice during the P1 and the P2 tasks. We considered six consecutive errors as a perseverative behavior when performed in the same non-rewarded arm because random alternation between arms can be considered as an attempt to learn a new strategy. Data are means ± SEM. Two way ANOVA test with Bonferroni post-hoc tests (n = 17).

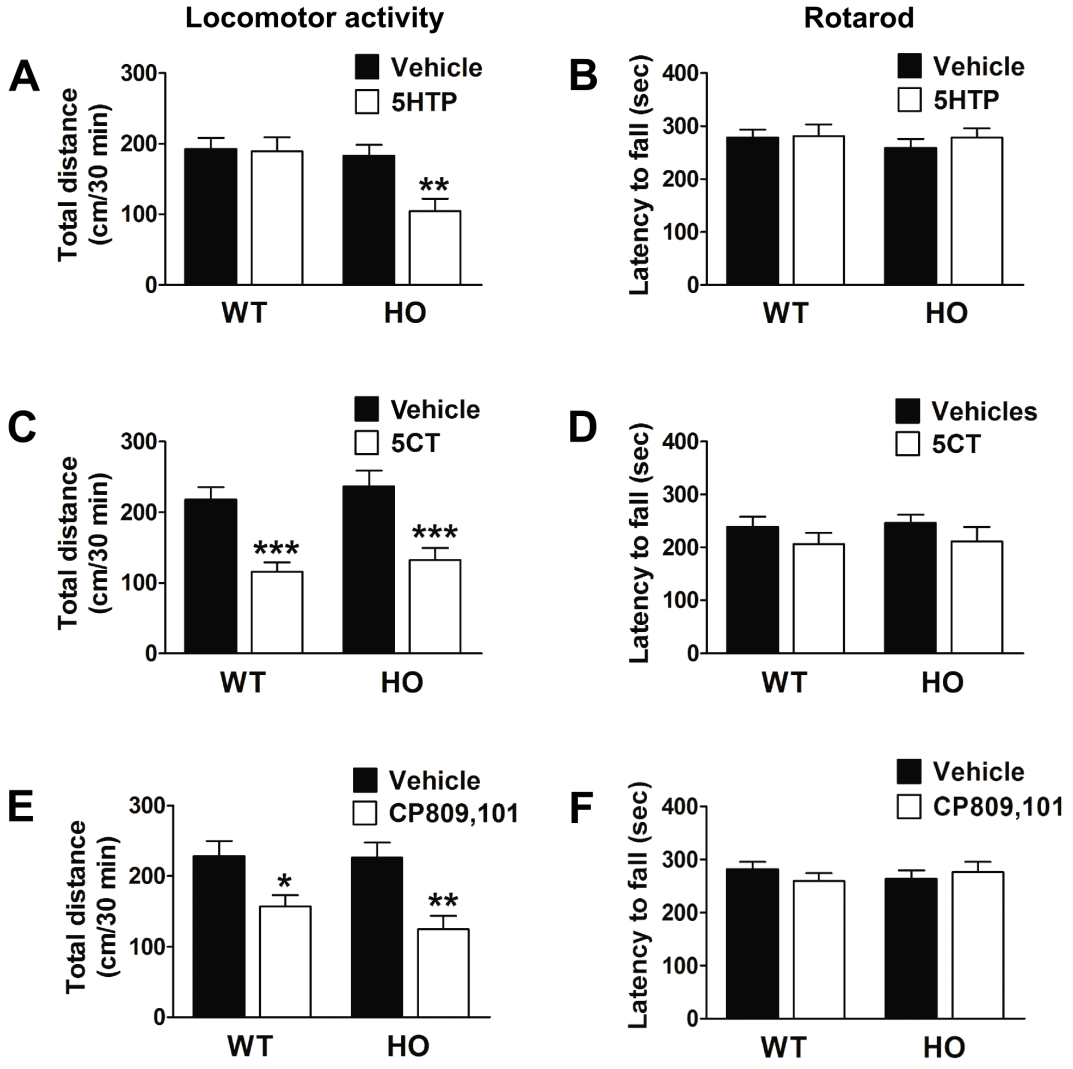

Figure S3. Serotonergic drugs differently regulate locomotor activity (A, C, E), but not motor coordination (B, D, F) (rotarod), in Tph2-KI genotypes. Data are means ± SEM. Two way ANOVA test with Bonferroni post-hoc tests. *, ** and *** $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ (n = 10).

Table S1: Behavioral effects of drug treatments on cognitive performances of WT and Tph2-KI mice in the automated H-Maze

	Vehicle	Methylphenidate	5HTP	5CT	CP809,101
WT	Successful spatial learning, cognitive flexibility and reversal learning	- Spatial learning, cognitive flexibility and reversal learning deficits - Hyperlocomotion	- Successful spatial learning, cognitive flexibility and reversal learning - Improved reversal learning in REV task	- Successful spatial learning, cognitive flexibility and reversal learning - Reduced locomotion	- Successful spatial learning, cognitive flexibility and reversal learning - Improved reversal learning in REV task - Reduced locomotion
HO	- Spatial learning, cognitive flexibility and reversal learning deficits - Two perseverative behaviors	- Spatial learning, cognitive flexibility and reversal learning deficits - Non rewarded perseverative behavior exacerbated - Hyperlocomotion	- Improvement in spatial learning, in cognitive flexibility and in reversal learning - Perseverative behaviors abolished - Reduced locomotion	- Spatial learning, cognitive flexibility and reversal learning deficits - Non rewarded perseverative behaviors - Reduced locomotion	- Improvement in spatial learning, in cognitive flexibility and in reversal learning - Perseverative behaviors abolished - Reduced locomotion

Table S2: Gender repartition of mice used for the different automated H-Maze experiments

Condition	Male	Female	Total
WT, non injected	5	5	10
HET, non injected	5	5	10
HO, non injected	5	5	10
WT, vehicle	4	4	8
HO, vehicle	4	4	8
WT, MPH	4	4	8
HO, MPH	4	4	8
WT, 5HTP	4	4	8
HO, 5HTP	4	4	8
WT, 5CT	4	4	8
HO, 5CT	4	4	8
WT, CP809	4	4	8
HO, CP809	4	4	8

Table S3: Affinity (Ki) of 5-HT receptor agonists for 5-HT receptors

Receptors	5-CT	CP809,101
5-HT2A	>1 μM	6.0 nM
5-HT2B	>1 μM	64 nM
5-HT2C	>1 μM	1.6 nM
5-HT1A	0.3 nM	>1 μM
5-HT1B	5.13 nM	>1 μM
5-HT1D	0.9 nM	136 nM
5-HT3	>1 μM	195 nM
5-HT5	15.84 nM	>1 μM
5-HT7	0.45 nM	661 nM

Ki for CP809,101 are reported from Siuciak et al., 2007
Ki for 5-CT were obtained from the PDSP database (http://pdsp.med.unc.edu/kidb.php)