

Stimulation of 5-HT_{2C} Receptors Improves Cognitive Deficits Induced by Human Tryptophan Hydroxylase 2 Loss of Function Mutation

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Polymorphisms in the gene encoding the serotonin synthesis enzyme Tph2 have been identified in mental illnesses, including bipolar disorder, major depression, autism, schizophrenia, and ADHD. Deficits in cognitive flexibility and perseverative behaviors are shared common symptoms in these disorders. However, little is known about the impact of Tph2 gene variants on cognition. Mice expressing a human *TPH2* variant (Tph2-KI) were used to investigate cognitive consequences of *TPH2* loss of function and pharmacological treatments. We applied a recently developed behavioral assay, the automated H-maze, to study cognitive functions in Tph2-KI mice. This assay involves the consecutive discovery of three different rules: a delayed alternation task, a non-alternation task, and a delayed reversal task. Possible contribution of locomotion, reward, and sensory perception were also investigated. The expression of loss-of-function mutant Tph2 in mice was associated with impairments in reversal learning and cognitive flexibility, accompanied by perseverative behaviors similar to those observed in human clinical studies. Pharmacological restoration of 5-HT synthesis with 5-hydroxytryptophan or treatment with the 5-HT_{2C} receptor agonist CP809.101 reduced cognitive deficits in Tph2-KI mice and abolished perseveration. In contrast, treatment with the psychostimulant methylphenidate exacerbated cognitive deficits in mutant mice. Results from this study suggest a contribution of *TPH2* in the regulation of cognition. Furthermore, identification of a role for a 5-HT₂ receptor agonist as a cognition-enhancing agent in mutant mice suggests a potential avenue to explore for the personalized treatment of cognitive symptoms in humans with reduced 5-HT synthesis and *TPH2* polymorphisms.

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INTRODUCTION

Tryptophan hydroxylase 2 (Tph2) is the rate-limiting enzyme for brain serotonin (5-HT) synthesis (Walther *et al*, 2003; Zhang *et al*, 2004). This member of the amino-acid hydroxylase family catalyzes the formation of the direct 5-HT precursor, 5-hydroxy-tryptophan (5-HTP) from tryptophan (Walther *et al*, 2003). Multiple noncoding or loss-of-function polymorphisms in the *TPH2* gene have been identified as potential genetic risk factors for several psychiatric illnesses, including bipolar disorder, major depression, attention deficit hyperactivity disorder (ADHD),

and schizophrenia (Popova and Kulikov, 2010; Russo *et al*, 2009; Waider *et al*, 2011).

Cognitive dysfunctions, including impaired reversal learning, deficits in cognitive flexibility, and perseverative behaviors are shared symptoms in these illnesses (Iosifescu, 2012). Interestingly, studies have shown that acute 5-HT depletion, in humans and animals, may contribute to the development of these cognitive symptoms (Clarke *et al*, 2004; Izquierdo *et al*, 2012). Noncoding *TPH2* single nucleotide polymorphisms (SNP) have also been identified in humans exhibiting such cognitive deficits (Strobel *et al*, 2007). However, the consequences on cognition of a chronic alteration in Tph2 enzymatic activity resulting from loss of function mutations have remained unexplored.

The R439H Tph2-KI mice carry a rare coding single point mutation (SNP) identified in a cohort of humans with major depression as well as in one individual with low 5-HT synthesis (Zhang *et al*, 2005). This mutation results in a reduction of brain 5-HT synthesis by ~40% in

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R/H heterozygous (HET) and ~80% in H/H homozygous (HO) animals (Beaulieu *et al*, 2008). Tph2-KI mice replicate alterations in mood disorder and serotonin-related biomarkers observed in human pathologies (Jacobsen *et al*, 2012b; Lavoie *et al*, 2013), which provide the opportunity to use R439H Tph2-KI mice as a model with construct, face, and predictive validity to investigate consequences of 5-HT depletions (Dzirasa *et al*, 2013; Jacobsen *et al*, 2012a).

Here we investigate the consequences of chronic 5-HT deficiency and pharmacological treatments on cognitive functions in R439H Tph2-KI mice with the automated H-maze (Del'Guidice *et al*, 2009; Girard *et al*, 2012). This behavioral test is a recently developed cognitive assay that involves a delayed reaction paradigm (Belhaoues *et al*, 2005), consisting in the consecutive discovery of three different rules: a delayed alternation task, a non-alternation task, and a delayed reversal task requiring a stronger mobilization of cognitive resources (Del'Guidice *et al*, 2009). The inclusion of this task was used as an analog to observations made in humans for whom the successive challenges of inhibiting a rule and finding a new one become more and more difficult with repetitions (Baddeley, 2012). These tasks, respectively, evaluate rule learning, cognitive flexibility/reversal learning and adaptability to increasingly complex problem-solving conditions. Our results showed that long-lasting 5-HT synthesis reduction in Tph2-KI mice is associated with altered cognitive flexibility, deficits in reversal learning and the development of perseverative behaviors. Acute rescue of 5-HT synthesis in these mutant mice using the 5-HT precursor 5-HTP or the stimulation of 5HT_{2C} receptors, with the agonist CP809.101, improved cognitive functions and abolished perseverative behaviors.

MATERIAL AND METHODS

Animals

R439H Tph2-KI mice have been described previously (Beaulieu *et al*, 2008). Respective wild-type R/R (WT) littermates were used as controls for mutant mice and all mice were 3–4 months old. Experiments were carried out using cohorts composed of 50% male and 50% female mice. No behavioral differences were observed between sexes in any of the tests. Different groups of animals were used for each behavioral test. All mice were housed 3–4 per cage and maintained on a 12-h light/dark cycle (lights on at 0700 hours) in a humidity-controlled room at 23 °C. Mice were kept with food and water available *ad libitum* throughout the experiments, except for habituation and training periods. All procedures were conducted in accordance with guidelines from the Canadian Council on Animal Care and approved by Laval University Animal Care Committee.

Drug Administration

All drugs were administered intraperitoneally (i.p.) in a volume of 10 ml/kg saline. Methylphenidate (MPH; Tocris Cookson, Ballwin, MI) was injected at 30 mg/kg as described (Beaulieu *et al*, 2006). 5-carboxamidotryptamine (5-CT; Sigma-Aldrich, Oakville, Canada) and CP809.101 (Tocris Cookson, Ballwin, MI) were administered at 0.1 and 1 mg/kg,

respectively as described (Beaulieu *et al*, 2006; Siuciak *et al*, 2007). 5-hydroxy-L-tryptophan (5-HTP; Sigma-Aldrich, Oakville, Canada) was administered at a 20 mg/kg dose (Jacobsen *et al*, 2012b). Drugs were administered 30 min before the different tests.

Automated H-Maze

The automated H-maze was performed as described previously in (Del'Guidice *et al*, 2009), see Supplementary Information for Material And Methods. Briefly, mice were assessed in three consecutive delayed response tasks (Belhaoues *et al*, 2005)—Alternance (ALT), Nonalternance (N-ALT), and Reversal (REV). In the first task (ALT), mice had to learn to alternately move between both chambers of the H-maze to induce the automatic delivery of a hydric reward (turn left, then turn right, in alternation) (Supplementary Figure S1A). In the second task, mice had to accomplish a response inhibition of the first task in order to learn the second rule (N-ALT) that consisted in always turning to the same side to enter each chamber (Supplementary Figure S1A). In the third task (REV), mice had to execute the opposite strategy of the one learned in the N-ALT task and reward could only be obtained by learning the new strategy (Supplementary Figure S1A). For each task, the overall completion or 'rate of survival' as well as the number of trials taken to complete the task were measured. Occurrence of specific behavioral strategies was also quantified. Shifting deficits, and indication of a deficit in reversal learning, were defined as maintenance of the ALT strategy at the beginning of the N-ALT task (Supplementary Figure S1B). Perseveration errors were defined as episodes of more than six repetitive failed attempts indicating that the mice display a deficit in the test by repetitively applying an invalid strategy (Del'Guidice *et al*, 2009).

Cross Maze Test

The cross-maze procedure was based on previous behavioral investigations (Marquis *et al*, 2006, 2008), see Supplementary Information for material and methods.

Olfactory Perception in a Novelty-Related Test

The olfactory perception test was performed as described (Breton-Provencher *et al*, 2009), see Supplementary Information for material and methods.

Sucrose Preference and Quinine Aversion Tests

Sucrose preference and quinine avoidance tests were performed as described (Ninomiya *et al*, 1995), see Supplementary Information for material and methods.

Locomotion in a Novel Environment

Locomotion was assessed under illuminated conditions in an automated Omnitech Digiscan apparatus (AccuScan Instruments, Columbus, OH) as described (Beaulieu *et al*, 2004). Mice were injected 30 min before the test and then placed into the activity monitor for 30 min. Locomotor activity was expressed as the total distance covered.

Motor Coordination in the Rotarod Test

Motor abilities of mice were assessed on a rotarod (Med-Associates, St Albans, VT) using the 2 to 20 RPM acceleration program as described (Xi *et al*, 2005).

Statistical Analysis

To compare performances between mice from the three genotypes in the H-maze, data were processed in analysis of variance (ANOVA) followed, when necessary, by Newman-Keuls *post hoc* comparisons, using the Prism statistic software 2007 (GraphPad Software, La Jolla, CA). To compare the effects of drugs on cognition in the H-maze, olfactory perception, preference/avoidance tests, and motor tests, we proceeded with two-way ANOVAs followed by Bonferroni *post hoc* tests to compare replicate means by genotype. Nonparametric Mann-Whitney *U* test has been used to compare percentage of cumulative successes. Two-tailed *t*-tests were also used to compare the performances between vehicle WT and vehicle HO mice used as control for drug treatments. The *p*-levels ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$) in the figures are denoted with asterisks (*, **, and ***, respectively). Only significant statistical analyses are presented.

RESULTS

Tph2-KI Mice Show Reversal Learning Deficits and Perseverative Behaviors in the H-Maze

Performances of Tph2-KI mice were evaluated in a traditional, non-automated cross-maze paradigm involving the sequential learning of two rules (Marquis *et al*, 2006, 2008). All WT mice learned the tasks in this test, whereas only 82% of HO mice completed both tasks. No significant difference was found between groups in the number of trial taken to complete each task (two-way ANOVA: $F(1,58) = 0.83$, NS; Bonferroni *post hoc* test: NS) (Supplementary Figure S2A). However, HO mice showed a trend toward perseverative strategies (Supplementary Figure S2B). Nevertheless, this trend remained nonsignificant (two-way ANOVA: $F(1,58) = 0.83$, NS; Bonferroni *post hoc* test: NS), possibly due to elevated variability between experimental groups. This suggested that application of a more stringent behavioral test might uncover cognitive differences between WT and Tph2-KI mice.

Mice were then tested using the automated H-maze (Supplementary Figure S1). Individual performances of each mouse were analyzed, for each consecutive task (Figure 1). The performances of representative untreated WT and HET mice that completed the three tasks were similar (Figure 1a and b, Supplementary Figure S1A). In contrast, the few HO R439H Tph2-KI mice that completed the tasks did it within more trials than mice from other genotypes (Figure 1c). Analysis of HO mice strategies showed two types of perseverative behaviors that were first manifested at the transition between the ALT and N-ALT task. Immediately after learning the first task, HO mice continued with the same alternation strategy for several trials (Figure 1c and d, Supplementary Figure S1B), although this pattern was only reinforced half of the time (ANOVA: $F(2,27) = 4.84$, $p \leq 0.05$). The second behavior was

observed in both the N-ALT and REV tasks only in HO mice and consisted in perseveration errors characterized by repetitive, consecutive, and nonrewarded trials (Figure 1c).

Impact of tph2 Genotype on Performances for each of the Three Different Tasks

Mice from all genotypes were able to reach the criterion of four consecutive successful trials in the ALT task (Figure 2a and b). However, there were statistically significant differences between the three groups (Figure 2a: ANOVA: $F(2,27) = 5.51$, $p \leq 0.01$). Compared with the performances of WT mice, HET, and HO mice required more trials to learn the first rule (Newman-Keuls test: $p \leq 0.05$ and $p \leq 0.01$, respectively).

A significant difference was found between the three genotypes in the N-ALT task (ANOVA: $F(2,219) = 13.47$, $p \leq 0.001$) (Figure 2c). HO mice required approximately three times more trials than mice from the WT and HET groups to learn the second task (Newman-Keuls test: $p \leq 0.001$). Furthermore, only 60% of HET and HO mice completed the N-ALT task (Figure 2d).

In the REV task (Figure 2e and f), no significant difference was found between the WT and the remaining mice of the HET group (Student's *t*-test, NS). No statistical analysis was ran on HO mice, as only two of them completed the REV task.

Olfactory Perception is not Affected in Tph2-KI Mice

As the H-maze relies on odor-assisted learning (Del'Guidice *et al*, 2009), an olfactory perception test (Breton-Provencher *et al*, 2009) was carried out to establish whether reduced performances in mutant mice could be attributed to altered olfactory sensitivity. Mice from all genotypes showed a similar increase in odor detection between 10^{-6} and 10^{-5} $\mu\text{l/l}$ for both odors (Figure 3a and b). Therefore, no difference in odor detection was observed between the three groups across two different odors. Furthermore, no significant difference in exploration was found between genotypes during the habituation sessions.

Normal Sucrose Preference and Quinine Avoidance in Tph2-KI Mice

Sucrose preference and a quinine avoidance test were also carried out to establish if differences in the performances observed in the automated H-maze could be attributed to deficits in reward or avoidance (Figure 3c and d). No difference was observed between the three genotypes in the two tests (two-way ANOVA: $F(2,54) = 1.11$ and 0.3 , NS, respectively). Mice from all genotypes showed a significant preference for sucrose (two-way ANOVA: $CF(2,54) = 377.3$, $p \leq 0.001$, Figure 3c) and an aversion to quinine (two-way ANOVA: $CF(254) = 167$, $p \leq 0.001$, Figure 3d) as compared with water.

Deterioration of Cognitive Performances Following Methylphenidate Treatment

As the neurotransmitter dopamine (DA) modulates the 5-HT system (Alex and Pehek, 2007), we first attempted

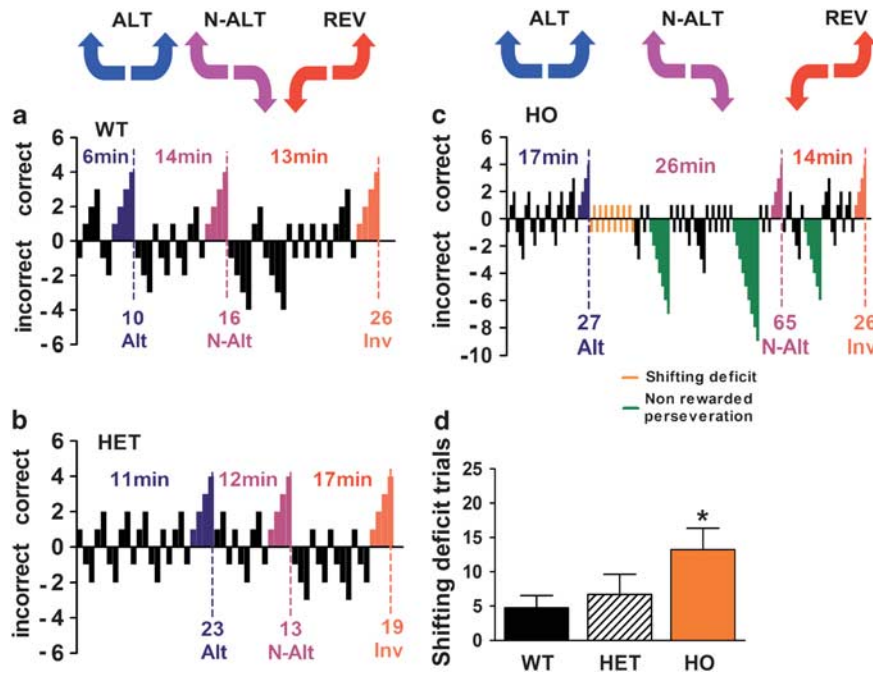


Figure 1 Tph2-KI mice show perseveration and strong impairments in reversal learning in the H-maze. (a–c) Representative examples of the performance of WT (a), HET (b) and HO (c) mice that have completed the three tasks. Each positive (top) and negative (bottom) bar corresponds to a rewarded or a failed trial, respectively. Dotted lines indicate that the mouse has reached the criterion of four consecutive successful trials for the task as defined (30). Expected correct behaviors are indicated above each task (ALT: blue; N-ALT: purple; REV: red, see Supplementary Figure S1A for details). Numbers on top of each task indicate the time required for the mouse to complete the task. Numbers on the bottom part indicate the number of trials required to complete each task. In c, the shifting deficits (orange bars) and perseveration errors (green bars) are indicated. Shifting deficits were defined as maintenance of the ALT strategy at the beginning of the N-ALT task (see Supplementary Figure S1B for details). Perseveration errors were defined as episodes of more than six repetitive failed attempts as defined (Del'Guidice *et al*, 2009). (d) Quantification of shifting deficit from ALT to N-ALT task for each Tph2-KI mouse genotype. It should be noted here that the term perseveration errors is an operational definition that can only be applied to describe a strategy used by a mouse in the test. The occurrence or nonoccurrence of such errors should not be construed as a measurement of all perseverative or repetitive behaviors (Del'guidice *et al*, 2013). Data are means \pm SEM of number of trials corresponding to a correct ALT strategy at the beginning of the N-ALT task. $n = 10$ mice per genotype. (Detailed gender repartition of mice used for the different automated H-Maze experiments are listed in Supplementary Table S2), $*p \leq 0.05$, one-way ANOVA with Newman–Keuls *post hoc* test.

to improve cognitive functions of Tph2-KI mice using the dopaminergic psychostimulant MPH. This drug is used for the treatment of attention deficit hyperactivity disorder (ADHD) and is known to increase some cognitive abilities in humans (Arnsten and Dudley, 2005). Furthermore, recent data from our group have shown that acute treatment with methylphenidate restore the cognitive performance of dopamine transporter knockout (DAT-KO) mice in the automated H-maze (Del'guidice *et al*, 2013).

Figure 4a and b show representative examples of vehicle-treated WT and HO mice. Most MPH-treated WT mice failed to complete the three tasks (Figure 4c, Figure 5a) (Mann–Whitney test: $U = 8$, $p \leq 0.01$). This treatment also reduced the success rate of Tph2-KI HO animals in the N-ALT and REV tasks (Figure 5b). One contributing factor to this further deterioration of performances may come from a dramatic increase in the occurrence of perseveration errors in Tph2-KI mice treated with MPH (Figure 4d, Figure 5c) (ANOVA: $F(4,39) = 14.40$, $p \leq 0.001$; Newman–Keuls test: $p \leq 0.001$). Interestingly, while showing reduced performances, WT mice treated with MPH did not display the perseverative behaviors observed in vehicle-treated HO-mutant mice (Figure 4b and c), suggesting that

enhanced DA tones and reduction of 5-HT synthesis have different effects on cognitive abilities, at least in the H-maze.

Acute 5-HT Restoration Therapy Improves Cognitive Performances in Tph2-KI Mice

We then explored the effect of an acute restoration of 5-HT synthesis on cognitive performances in Tph2-KI mice. To achieve this, mice were treated with a dose of the 5-HT precursor 5-HTP that has been shown to restore normal 5-HT levels in R439H Tph2-KI mice (Siesser *et al*, 2013). Administration of 5-HTP did neither affect the performances nor success rates of WT mice in the automated H-Maze (Figure 4e, Figure 5a). In contrast, this treatment completely abolished the perseverative behaviors (Figure 4f, Figure 5c) (Newman–Keuls test: $p \leq 0.001$) and improved overall completion of the three tasks in HO mice (Figure 5b) (Mann–Whitney test: $U = 16$, $p \leq 0.05$). As visual observation of 5-HTP-treated mice suggested an effect on locomotion, the effect of treatment on spontaneous and forced locomotion was evaluated using a locomotor activity monitor and an accelerating rotarod test (Supplementary Figure S3A–B). These tests

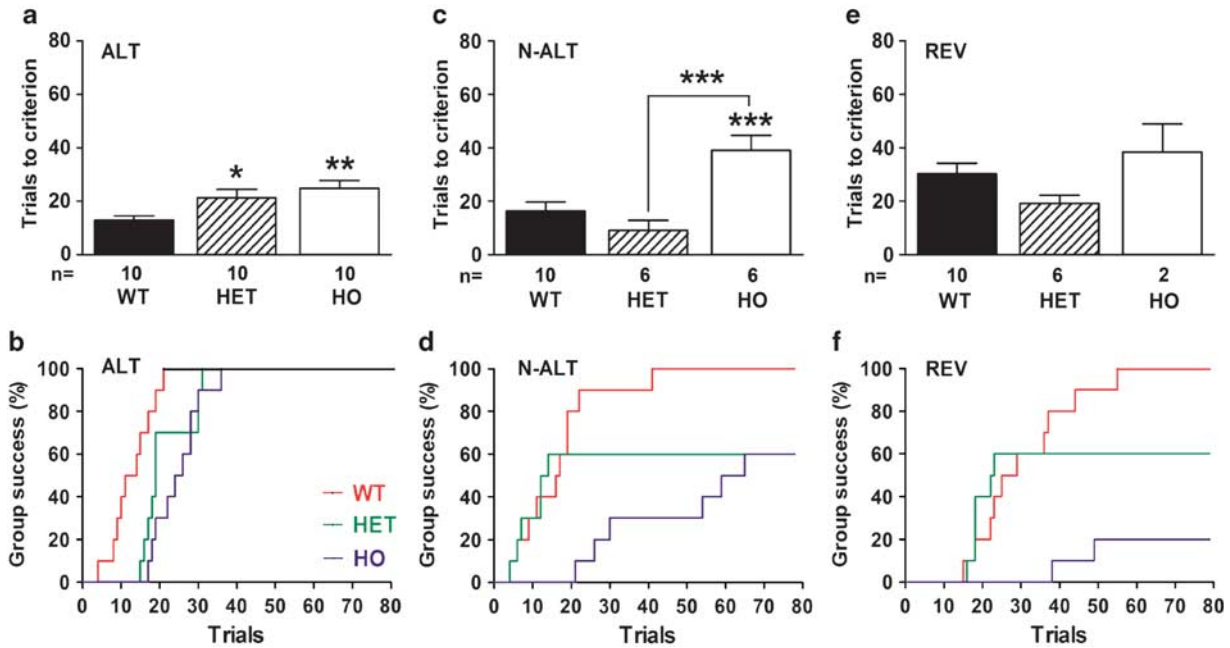


Figure 2 Performances per genotype of Tph2-KI mice in each task of the automated H-Maze. (a, c, and e) Average number of trials (\pm SEM) needed to reach the criterion of four consecutive successful trials for mice of the WT, HET, and HO genotype in (a) the ALT task, (c) the N-ALT task and (e) the REV task. Average number of trials was calculated only from the performances of mice that completed each task. (b, d, and f) Survival curves representing the number of trials required to reach maximum group success for genotype in each task (b) the ALT, (d) the N-ALT and (f) the REV tasks. Initial $n = 10$ mice per genotype, number of remaining animals used to calculate average number of trials are indicated below each condition. * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$, one-way ANOVA test with Newman-Keuls *post hoc* test.

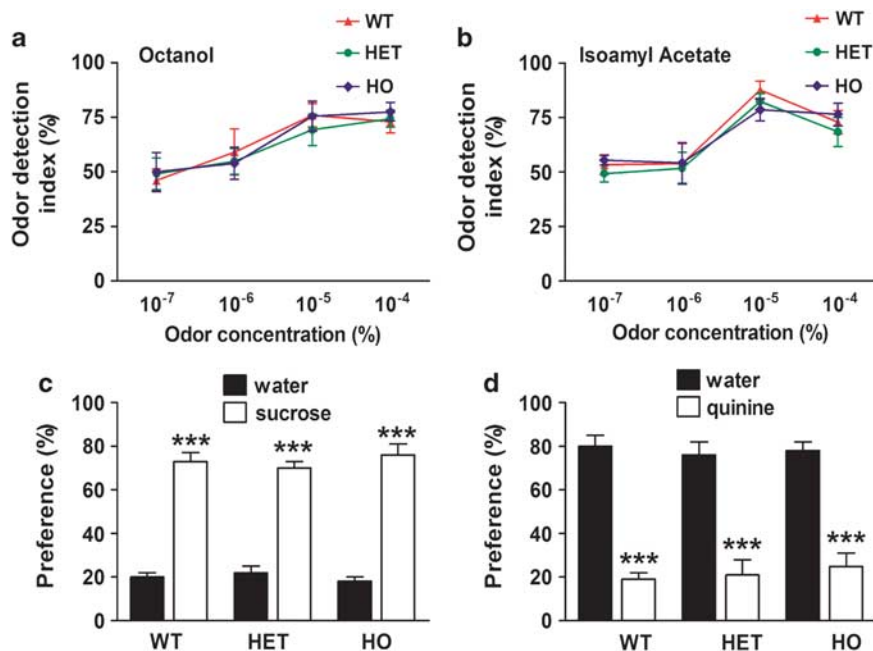


Figure 3 5-HT deficiency does not affect olfactory perception, sucrose preference, or quinine avoidance in Tph2-KI mice. (a and b) Odor detection threshold of WT, HET, and HO Tph2-KI mice for the two odors isoamyl acetate and octanol. Normalized values are expressed as the mean ratio between time spent investigating the odor and total sniffing time (ie, odor plus mineral oil). Data are presented as mean \pm SEM ($n = 8$ mice per group). (c and d) Preference/avoidance test in WT, HET, and HO Tph2-KI mice for two different tastes (C-sucrose and D-quinine). Normalized values are expressed as the mean ratio between the quantity of sucrose or quinine drunk and total volume of liquid absorbed (ie, water plus sucrose or water plus quinine). Data are presented as mean \pm SEM ($n = 17$ mice per group). Two-way ANOVA with Bonferroni *post hoc* tests. *** $p \leq 0.001$.

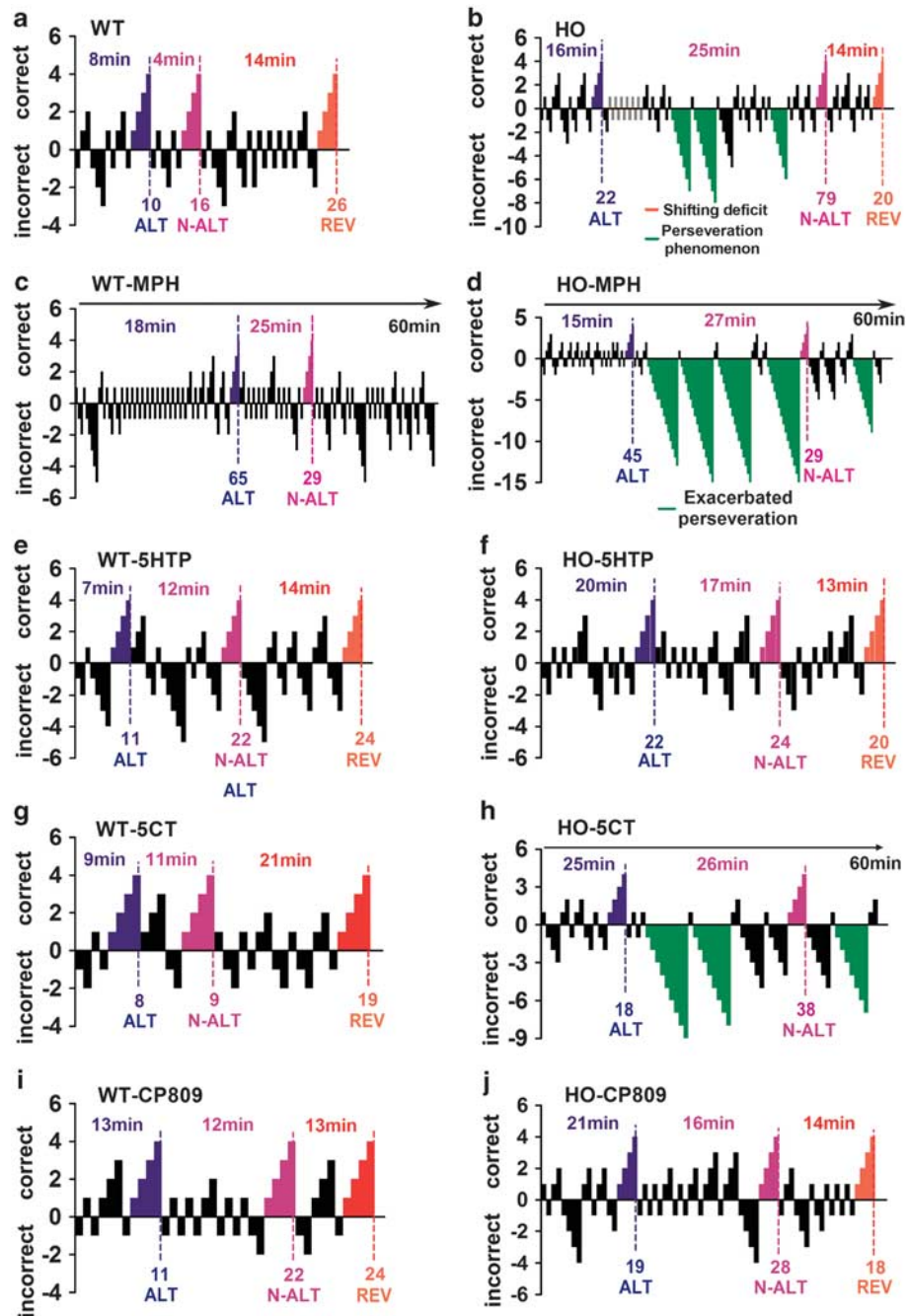


Figure 4 Representative example of the effects of monoaminergic drugs on cognitive performances of Tph2-KI mice. (a and b) Vehicle-treated WT (a) and HO (b) mice. (c and d) MPH-treated WT (c) and HO (d) mice. (e and f) 5-HTP-treated WT (e) and HO (f) mice. (g and h) 5-CT-treated WT (g) and HO (h) mice. (i and j) CP809.101-treated WT (i) and HO (j) mice. Each positive (top) and negative (bottom) bar corresponds to a rewarded or a failed trial, respectively. Dotted lines indicate that the mouse has reached the criterion of four consecutive successful trials for the task as defined (Del'Guidice *et al*, 2009). Expected correct behaviors are indicated above each task (ALT: blue; N-ALT: purple; REV: red, see Supplementary Figure S1A for details). Numbers on top of each task indicate the time required for the mouse to complete the task. Numbers on the bottom part indicate the number of trials required to complete each task. Shifting deficits (orange bars) and perseveration errors (green bars) are indicated. Shifting deficits were defined as maintenance of the ALT strategy at the beginning of the N-ALT task (see Supplementary Figure S1B for details). Perseveration errors were defined as episodes of more than six repetitive failed attempts as described (Del'Guidice *et al*, 2009). See method section for a description of drug administration conditions and doses. $n = 8$ mice per conditions, detailed gender repartition and effectiveness of mice used for the different automated H-Maze experiments are listed in Supplementary Table S2. Relative affinity of 5-CT and CP809.101 for different 5-HT receptors is listed in Supplementary Table S3.

confirmed that 5-HTP reduced spontaneous locomotor activity only in HO mice (two-way ANOVA: $F(1,28) = 4.75$, $p \leq 0.05$; Bonferroni *post hoc* test: $p \leq 0.01$) without affecting motor functions as evaluated with the accelerating rotarod (Supplementary Figure S3B).

5-HT2C Agonist Improves Cognitive Performances in Tph2-KI Mice

Different agonists were then used to identify the 5-HT receptor types involved in the regulation of cognitive performances in Tph2-KI mice. Co-stimulation of the

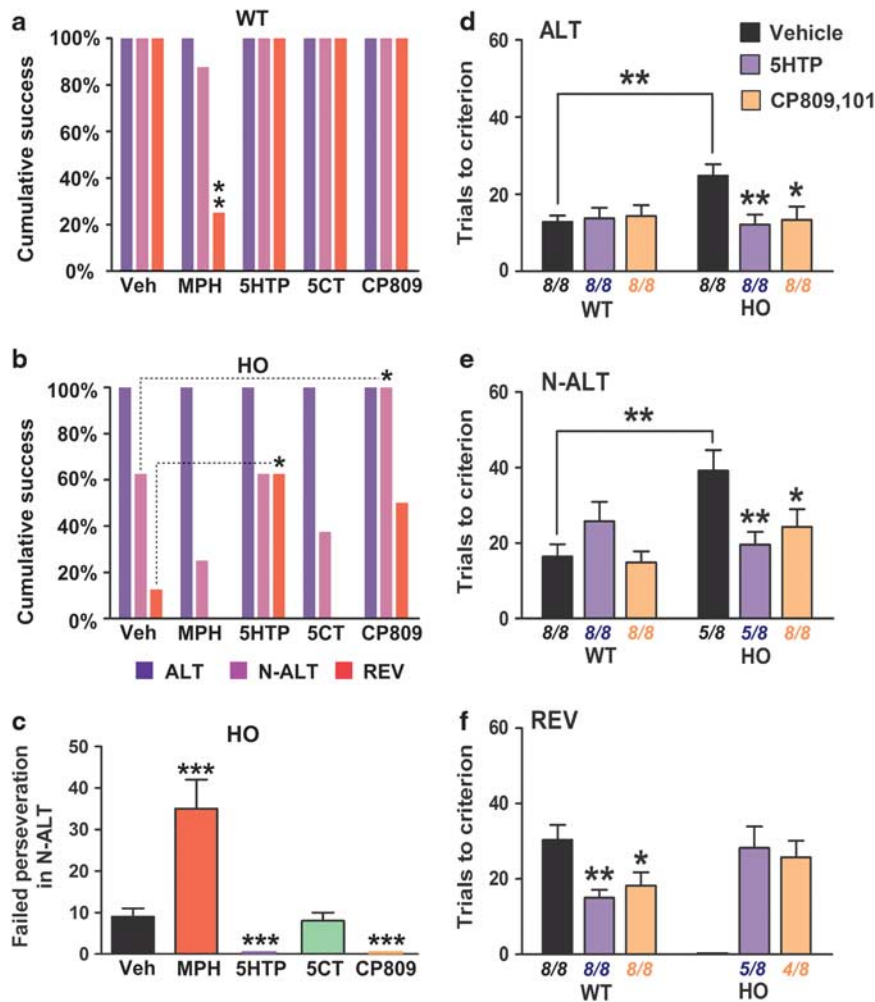


Figure 5 Effects of monoaminergic drug treatment on cumulative success rates, perseveration, and task performances in the automated H-maze. (a and b) Cumulative success rates (%) for WT (a) and HO (b) Tph2-KI mice in all three tasks of the automated H-maze following treatment with: vehicle, MPH, 5-HTP, 5-CT, or CP809.101. See method section for a description of drug administration conditions and doses. Data are presented as % of animals from each initial group that succeeded in completing each tasks. Initial $n = 8$ mice per group. (c) Average number (\pm SEM) of perseveration errors for HO Tph2-KI mice during the N-ALT task. Perseveration errors were defined as episodes of more than six repetitive failed attempts as defined (Del'Guidice *et al*, 2009). Calculation of perseveration errors included all animals independently of their ability to complete the N-ALT task. $n = 8$ animals per group. (d–f) Average number of trials (\pm SEM) required for reaching the criterion of 4 consecutive successful trials for mice of the WT and HO genotype after treatment with vehicle, 5-HTP or CP809.101. See method section for a description of drug administration conditions and doses. (d) the ALT task, (e) the N-ALT task, and (f) the REV task. Average number of trials was calculated only from the performances of mice that completed each task. Initial $n = 8$ mice per genotype, number of remaining animals used to calculate average numbers of trials are indicated below each condition. * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$, a and b, Mann–Whitney test; c, one-way ANOVA test with Newman–Keuls *post hoc* test, d–f, two-way ANOVA with Bonferroni *post hoc* tests; f, one-way ANOVA test with Newman–Keuls *post hoc* test.

5-HT₁, 5-HT₅, and 5-HT₇ receptors with 5-CT did not improve overall completion of the test by WT or HO mice (Figure 4g and h, Figure 5a and b). Furthermore, this drug had no effect on the perseverative behaviors of HO mice (Figure 5c) while reducing spontaneous locomotor activity in both WT and HO mice (Supplementary Figure S3C) (two-way ANOVA: $F(1,28) = 33.64$, $p \leq 0.001$). In contrast, the 5-HT_{2C} preferring agonist CP809.101 had no effect on WT mice performances (Figure 4i, Figure 5a) but reinstated the reversal learning and shifting abilities of HO mice (Figure 4j), increased overall completion of the three tasks (Figure 5b) (Mann–Whitney test: $U = 20$, $p \leq 0.05$) and completely abolished perseveration (Figure 5c) (Newman–Keuls test: $p \leq 0.001$). This compound also reduced locomotor activity in mice from both genotypes (Supplementary

Figure S3E) (two-way ANOVA: $F(1,28) = 19.16$, $p \leq 0.001$). None of these two serotonergic drugs impaired motor coordination or skills of WT and HO mice as measured by an accelerating rotarod test (Supplementary Figure S3D, F).

Impact of 5-HTP and CP809.101 Treatments on Tph2-KI Mice Performances in the Three Different Tasks

Beyond their effects on perseveration and overall completion of the different tasks, we then verified the impact of 5-HTP and CP809.101 treatments on learning performances. In the first task (ALT) (Figure 5d), a significant difference in the number of trials required to complete the task was found between the different treatments (two-way ANOVA: $F(2,42) = 3.88$, $p \leq 0.05$). Vehicle HO mice required

significantly more trials to complete the ALT task as compared with vehicle WT mice (Two-tailed *t*-test: $p \leq 0.01$). 5-HTP and CP809.101 significantly improved performances of HO mice (Bonferroni post hoc test: $p \leq 0.01$ and $p \leq 0.05$, respectively).

In the N-ALT task (Figure 5e), the interaction between genotypes and treatments was significant for the number of trials required to complete the task (two-way ANOVA: $F(2,39) = 5.85$, $p \leq 0.01$). Only 60% of vehicle HO mice completed the task within more trials than animals from the WT group (Two-tailed *t*-test: $p \leq 0.01$). Treatment with 5-HTP or CP809.101 reduced the number of trials required by Tph2-KI HO mice to complete the task as compared with vehicle-treated mice (Bonferroni *post hoc* test: $p \leq 0.01$ and $p \leq 0.05$, respectively).

In REV task (Figure 5f), a significant reduction was found between the number of trials required by WT mice to complete the task after 5-HTP and CP809.101 treatments as compared with vehicle (ANOVA: $F(2,23) = 6.04$, $p \leq 0.01$ followed by Newman-Keuls multiple comparison test: $p \leq 0.01$ and $p \leq 0.05$, respectively). In HO mice, while only one vehicle-treated mouse found the rule, 60% of the 5-HTP-treated HO mice and 50% of CP809.101-treated HO mice learned the third rule within a number of trials that was equivalent to those required by vehicle-treated WT mice, therefore confirming the efficacy of these two treatments on cognitive performances across the three tasks of the automated H-Maze.

DISCUSSION

The results presented here show that a single nucleotide loss of function polymorphism in *tph2* is sufficient to induce gene-dose-dependent cognitive deficits in mice. Furthermore, acute restoration of 5-HT levels through administration of 5-HTP or the 5-HT_{2C} receptor agonist CP809.101 can improve cognitive flexibility and reversal learning abilities independently from their effects on locomotion.

Mice with *tph2* loss of function mutation displayed shifting deficit accompanied by unrewarded perseveration strategies. Perseveration is reminiscent of broad cognitive symptoms observed in several neurological and psychiatric illnesses (Hauser, 1999; Joseph, 1999). Similar patterns of cognitive deficits have previously been associated with prefrontal cortex (PFC) dysfunctions in humans and mice with PFC-lesions (Del'Guidice *et al*, 2009; Verin *et al*, 1993). Recent characterization of brain circuit dysfunctions in Tph2-KI mice (Dzirasa *et al*, 2013) revealed increased synchrony between the amygdala and the PFC. Furthermore, amygdala lesions have been shown to facilitate reversal learning in rats (Izquierdo *et al*, 2012). Taken together with our observations, this suggests that cognitive deficits associated with *tph2* loss of function mutation in mice may arise, at least in part, from increased PFC amygdala connectivity resulting from 5-HT deficiency.

In addition to the possible contribution of the PFC, Tph2-KI mice have been shown to display exaggerated emotional responses in several behavioral tests relevant to the assessment of 'depressive-like', 'anxiety-like', and aggressive behaviors (Beaulieu *et al*, 2008). In line with our observations, humans carrying *TPH2* non-coding SNPs (Strobel *et al*, 2007), patients with affective disorders and healthy

subject submitted to chronic stress and anxiety all tend to present deficits of memory, attention, and/or executive functions (Castaneda *et al*, 2008; Gass and Curiel, 2011; O'Toole and Pedersen, 2011). High levels of anxiety have also been associated with perseverative behaviors in a number of cognitive tasks in rodents (Gill *et al*, 2012; Jiao *et al*, 2011). Consequently, anxiety might also represent a mediating factor contributing to cognitive deficits in mice with *tph2* loss of function mutation.

Recent investigations have shown that an increase of 5-HT transmission may improve performances in different behavioral tests assessing cognitive flexibility in rodents (Bari *et al*, 2010; Charles *et al*, 2011). Furthermore, impaired reversal learning and perseveration have also been reported following acute tryptophan and 5-HT depletion in humans and other primates (Clarke *et al*, 2004; Evers *et al*, 2010). In line with this, the 5-HT precursor 5HTP or the 5HT_{2C} preferring receptor agonist CP809.101 improved cognitive flexibility and reversal learning in Tph2-KI mice (Supplementary Table S1). These two drugs also exerted a positive effect on WT mice performances, albeit only in the more complicated last task of the test. This indicates that cognitive deficits induced by a *tph2* loss of function variant are not fully explained by long-term adaptation to 5-HT depletion and can therefore be alleviated, at least in part, by an acute stimulation of 5-HT neurotransmission. Importantly, the effects of 5HTP and CP809.101 cannot be solely explained by a reduction of locomotor activity, as 5-CT had a comparable effect on locomotion without alleviating cognitive deficits in Tph2-KI mice.

Pharmacological and genetic studies have suggested that several 5-HT receptors may contribute to the regulation of PFC-mediated reversal learning and cognitive flexibility (Homberg, 2012; Jensen *et al*, 2010). The different effects of 5-CT and CP809.101 on cognitive deficits in Tph2-KI mice suggest that targeting 5HT_{2C} receptors may be a viable avenue to improve cognition in humans with *TPH2* mutations. CP809.101 is a recently developed 5HT_{2C} receptor agonist (Siuciak *et al*, 2007) (Supplementary Table S3). However, we cannot fully exclude potential effects of this drug on others 5-HT receptors as CP809.01 can also bind to the 5HT_{2A} receptors, albeit with approximately ninefold lesser affinity (Jensen *et al*, 2013). Furthermore, while CP809.101 was reported to improve cognitive functions in rodents without affecting emotional behavior (Siuciak *et al*, 2007), results obtained with several other 5HT_{2C} receptor agonists are variable (Dougherty and Oristaglio, 2013; Schmidt *et al*, 1995). Given the complex phenotype of Tph2-KI mice and the anxiolytic effects of several serotonergic drugs (Bandelow *et al*, 2012; Witkin, 2008), it could thus also be premature to conclude that the effect of CP809.101 on Tph2-KI mice in the H-maze is entirely independent from an effect on anxiety.

In contrast to CP809.101 and 5HTP, MPH worsened the cognitive deficits of Tph2-KI mice and exacerbated their perseverative behaviors (Supplementary Table S1). This result is in contrast with beneficial effect of the same drug at the same dose on the cognitive functions of DAT-KO mice in the H-maze (Del'guidice *et al*, 2013). Furthermore, we have also shown that CP809.101 does not rescue the cognitive deficits of DAT-KO mice in the same test (Del'guidice *et al*, 2013). We elected to test the effects of

MPH because *TPH2* polymorphisms have been implicated in reversal learning deficits observed in ADHD (Baehne et al, 2009). Furthermore, this drug is often the first line of pharmacological treatment for children with learning disabilities. If a drug can induce opposite cognitive effects at the same dose and in the same tests in mice carrying different mutations, it is also possible that similar divergent effects may also occur in humans. This underscores the need for studies of drug-gene interaction to develop better cognition enhancing agents and personalized treatments for cognitive symptoms in people with gene polymorphisms affecting monoamine-mediated neurotransmission (Baehne et al, 2009; Pasini et al, 2013; Serretti et al, 2011). Furthermore, the distinct cognitive profiles of DAT-KO and Tph2-KI mice observed in the H-maze denotes the needs to develop strategies to further study the specific impact of such polymorphisms on behavior by coupling the use of animals carrying specific human variants with new technical approaches like the H-maze.

Various *TPH2* polymorphisms are potential genetic risk factors for major depression, ADHD, bipolar disorder and schizophrenia (Baehne et al, 2009; Serretti et al, 2011). However, the specific impact of such polymorphisms on behavior needs to be further studied using animals carrying specific human variants in order to understand their contribution to psychiatric symptomatology and their potential usefulness as diagnostic tools (Millan et al, 2012). While limited to a single rare human *TPH2* gene variant, the results obtained using the automated H-Maze and R439H Tph2-KI mice indicate that *TPH2* loss of function polymorphisms might contribute to cognitive deficits. Furthermore, our observations on the relative efficacy of MPH and CP809.101 in Tph2-KI mice suggest that information about *TPH2* loss of function polymorphisms can potentially instruct treatment strategies. This may provide an opportunity to develop better cognition-enhancing agents and personalized treatments for cognitive symptoms in people with reduced 5-HT synthesis and *TPH2* polymorphisms.

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The authors declare no conflict of interest.

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