Neuropsychopharmacology (2015) 40, 2764–2773

www.neuropsychopharmacology.org

Mice Lacking the Serotonin *Htr*$_{2B}$ Receptor Gene Present an Antipsychotic-Sensitive Schizophrenic-Like Phenotype

Pothitos M Pitychoutis[1,2], Arnauld Belmer[1], Imane Moutkine[1], Joëlle Adrien[3] and Luc Maroteaux*[,1]

[1]INSERM UMR S-839, Université Pierre et Marie Curie-Paris6, Institut du Fer à Moulin, Paris, France; [2]Department of Biology and Center for Tissue Regeneration and Engineering, University of Dayton, Dayton, OH, USA; [3]INSERM UMR S-975, Universite Pierre et Marie Curie-Paris6, Paris, France

Impulsivity and hyperactivity share common ground with numerous mental disorders, including schizophrenia. Recently, a population-specific serotonin 2B (5-HT$_{2B}$) receptor stop codon (ie, *HTR2B* Q20*) was reported to segregate with severely impulsive individuals, whereas 5-HT$_{2B}$ mutant (*Htr*$_{2B}^{-/-}$) mice also showed high impulsivity. Interestingly, in the same cohort, early-onset schizophrenia was more prevalent in *HTR2B* Q*20 carriers. However, the putative role of 5-HT$_{2B}$ receptor in the neurobiology of schizophrenia has never been investigated. We assessed the effects of the genetic and the pharmacological ablation of 5-HT$_{2B}$ receptors in mice subjected to a comprehensive series of behavioral test screenings for schizophrenic-like symptoms and investigated relevant dopaminergic and glutamatergic neurochemical alterations in the cortex and the striatum. Domains related to the positive, negative, and cognitive symptom clusters of schizophrenia were affected in *Htr*$_{2B}^{-/-}$ mice, as shown by deficits in sensorimotor gating, in selective attention, in social interactions, and in learning and memory processes. In addition, *Htr*$_{2B}^{-/-}$ mice presented with enhanced locomotor response to the psychostimulants dizocilpine and amphetamine, and with robust alterations in sleep architecture. Moreover, ablation of 5-HT$_{2B}$ receptors induced a region-selective decrease of dopamine and glutamate concentrations in the dorsal striatum. Importantly, selected schizophrenic-like phenotypes and endophenotypes were rescued by chronic haloperidol treatment. We report herein that 5-HT$_{2B}$ receptor deficiency confers a wide spectrum of antipsychotic-sensitive schizophrenic-like behavioral and psychopharmacological phenotypes in mice and provide first evidence for a role of 5-HT$_{2B}$ receptors in the neurobiology of psychotic disorders.
Neuropsychopharmacology (2015) **40,** 2764–2773; doi:10.1038/npp.2015.126; published online 27 May 2015

INTRODUCTION

Schizophrenia is a devastating, complex, and costly neuropsychiatric disorder that affects ~1% of the world's population (Olesen *et al*, 2012). Schizophrenia is typically characterized by three symptom clusters: positive (eg, hallucinations, delusions, disordered thoughts), negative (eg, flattened affects, social withdrawal), and cognitive (eg, attention and working memory deficits) (Simpson *et al*, 2010). This disease has traditionally been associated with a deregulated dopaminergic system, mainly because of the fact that for many years typical antipsychotics (ie, strong dopamine (DA)-D$_2$ receptor antagonists such as haloperidol) have served as the mainstream pharmacotherapy for psychotic patients (Keshavan *et al*, 2008). However, it has long been known that the serotonergic system is also implicated in the pathogenesis of schizophrenia (Meltzer and Massey, 2011). Indeed, atypical antipsychotics, of which clozapine is the prototype, consist of agents that are more potent antagonists of serotonin 2A (5-HT$_{2A}$) receptors over D$_2$ receptors (Gonzalez-Maeso *et al*, 2008). Many atypical antipsychotics show similar affinity for 5-HT$_{2B}$ and 5-HT$_{2A}$ receptors (eg, clozapine, amisulpride, asenapine, aripiprazole, or cariprazine) (Abbas *et al*, 2009; Kiss *et al*, 2010; Shahid *et al*, 2009; Shapiro *et al*, 2003), although the contribution of the 5-HT$_{2B}$ receptor to the action of antipsychotic compounds has never been reported. Notably, the elucidation of the intricate mechanisms underlying the neurobiology of schizophrenia is imperative in order to develop novel drugs with improved therapeutic efficacy (Kvajo *et al*, 2012). In this direction, models of genetic deletion in mice have been indispensable in characterizing the contribution of specific genes to disease pathophysiology (Arguello and Gogos, 2006).

Impulsivity, very broadly defined as action without foresight, novelty seeking, and hyperlocomotion share common ground with numerous mental disorders, including chronic substance abuse, attention deficit hyperactivity disorder, and schizophrenia (Fineberg *et al*, 2010; Humby and Wilkinson, 2011). In a recent study conducted in a Finish cohort, we reported that a population-specific 5-HT$_{2B}$ receptor stop codon (*HTR2B* Q20*) segregates with severe impulsivity, psychosis, and early-onset schizophrenia (Bevilacqua *et al*, 2010). Moreover, mice in which the *Htr*$_{2B}$ gene has been genetically inactivated (ie, *Htr*$_{2B}^{-/-}$) exhibit a hyperlocomotor phenotype as well as impulsivity, as assessed in the delay discounting task (Bevilacqua *et al*, 2010; Doly *et al*, 2008).

*Correspondence: Dr L Maroteaux, INSERM UMR S-839, Université Pierre et Marie Curie, Institut du Fer à Moulin, 17 rue du Fer à Moulin, Paris 75005, France, Tel: +33 1 45876123, Fax: +33 1 45876132, E-mail: luc.maroteaux@upmc.fr
Received 26 January 2015; revised 8 April 2015; accepted 25 April 2015; accepted article preview online 4 May 2015

However, the extent to which 5-HT_{2B} receptor is implicated in the neurobiology of schizophrenia has never been investigated.

In this study, we assessed the effects of the genetic ablation of Htr_{2B} in mice subjected to a comprehensive series of behavioral tests screening for positive, negative, and cognitive schizophrenic-like symptoms. Following the demonstration that loss of function of the 5-HT_{2B} receptor in $Htr_{2B}^{-/-}$ mice confers a wide spectrum of schizophrenic-like behavioral phenotypes, we show that selected phenotypes are induced by acute pharmacological blockade of 5-HT_{2B} receptors with RS127445 (selective 5-HT_{2B} receptor antagonist) and rescued by chronic antipsychotic treatment with the typical antipsychotic drug haloperidol. The dorsal striatum (dSTR) and its connections with the prefrontal cortex (PFC) have been implicated in the pathogenesis of both the positive and the cognitive symptoms of schizophrenia (Howes *et al*, 2009; Simpson *et al*, 2010). Indeed, neurochemical alterations in the dopaminergic and glutamatergic corticostriatal circuits underlie a substantial portion of the dysfunction seen in schizophrenic patients (Gordon, 2010; Howes *et al*, 2009; Keshavan *et al*, 2008; Simpson *et al*, 2010). Thus, we further investigated relevant dopaminergic and glutamatergic neurochemical alterations in the PFC and the dSTR of $Htr_{2B}^{-/-}$ mice. Altogether, our findings present the first evidence for a role of 5-HT_{2B} receptors in the neurobiology of schizophrenia.

MATERIALS AND METHODS

Animals

Male $Htr_{2B}^{-/-}$ mice used in these experiments were in a 129S2/SvPas (129S2) background, and wild-type ($Htr_{2B}^{+/+}$) 129S2 mice (8–12 weeks old) used as controls were bred in our animal facility under standard 12-h light/dark schedule, and housed in groups of 3–5 of the same genetic background and sex after weaning (Diaz *et al*, 2012). Animal experiments were conducted in accordance with standard ethical guidelines (National Institutes of Health's 'Guide for the Care and Use of Laboratory animals', and European Directive 2010/63/UE) and were approved by the Ethics Committee for Animal Experiments (No. 1170.01).

Drug Treatments

The selective 5-HT_{2B} receptor antagonist RS127445 (RS; 0.5 mg/kg; Tocris, France) was administered acutely (ie, 1 h before testing) and intraperitoneally (i.p.), as in previous studies from our group (Diaz *et al*, 2012; Doly *et al*, 2008). The glutamatergic psychostimulant and *N*-methyl-D-aspartic acid (NMDA) receptor antagonist dizocilpine (MK-801; 0.5 mg/kg; i.p.; Sigma-Aldrich, France) and the dopaminergic psychostimulant amphetamine (10 mg/kg; i.p.; Sigma-Aldrich, France) were dissolved in saline and administered at a rate 10 ml/kg. Different cohorts of $Htr_{2B}^{+/+}$ and $Htr_{2B}^{-/-}$ mice were chronically treated (4 weeks) with haloperidol (Sigma-Aldrich) in drinking water (2.0 mg/kg/day) or vehicle, as described before (Terry *et al*, 2007). Following chronic antipsychotic treatment, locomotor activity and prepulse inhibition (PPI) were tested, as described below. Separate mouse cohorts were used for the assessment of sleep architecture and for social interaction behavior and neurochemical estimations.

Locomotor Response to Novelty and to Psychostimulants

Hyperactivity in response to novelty and locomotor hypersensitivity to acute administration of psychostimulants are considered useful correlates of positive schizophrenic-like symptoms in mice (Arguello and Gogos, 2006). Locomotor activity was measured in a circular corridor with four infrared beams placed at every 90° (Imetronic, Passac, France), as previously described (Blundell *et al*, 2010; Doly *et al*, 2008). Mice were injected with saline and individually placed in the activity box for 2 h during four consecutive days in order to habituate to the apparatus. On day 5, mice were placed in the activity box for 1 h and then treated with dizocilpine or amphetamine. Locomotor activity was then screened for 1–6 h.

PPI of Acoustic Startle

PPI has been considered an operational measure of sensorimotor gating and preattentive processing, and its deficit has been consistently reported in schizophrenic patients and in relevant animal models (Meyer *et al*, 2005). Sensorimotor gating was assessed as previously described (Yadav *et al*, 2011). PPI was indexed by the percentage inhibition of the startle response at each level of prepulse intensity by using the following formula: % PPI = ((mean reactivity on pulse-alone trials − mean reactivity on prepulse-pulse trials)/mean reactivity on pulse-alone trials) × 100%. % Average PPI values were calculated from all PPI values across all the four prepulse intensities.

Sociability and Preference for Social Novelty

Decreased interactions with conspecifics are often used to model social withdrawal, a negative symptom of schizophrenia (Arguello and Gogos, 2006). The three-chamber social test was performed as previously described (Peca *et al*, 2011). Target subjects (social I and social II) were adult $Htr_{2B}^{+/+}$ males. For the sociability test, the test animal was introduced into the middle chamber and was left to habituate for 5 min, after what an unfamiliar mouse (social I) was introduced into a wire cage in one of the side -chambers and an empty wire cage (EC) on the other side chamber. The dividers were then opened and the test animal was allowed to freely explore all three chambers over a 10-min session. Preference for social novelty was assayed during a second 10-min session in which a novel stranger mouse (social II) was inserted into the previously empty wire cage. The time spent in close interaction with the two cages was scored manually.

Novel Object Recognition (NOR)

Cognitive deficits, including working memory impairment, are typically observed in animal models of schizophrenia (Arguello and Gogos, 2006; Meyer *et al*, 2005). The NOR task represents the most useful method for studying cognitive impairment in schizophrenia (Lyon *et al*, 2012). The NOR

test was performed as previously described (Carlini *et al*, 2008) with retention tests performed at 1 and 24 h intervals in order to assess short- and long-term memory, respectively. Each trial lasted for 5 min. The time that the animals spent exploring the novel and the familiar objects were measured; results are expressed as % time of exploration of the novel object.

Contextual and Cued Fear Conditioning

Cognitive deficits in associative fear memory have been reported in mice displaying schizophrenic-like phenotypes (Arguello and Gogos, 2006; Gleason *et al*, 2012). Fear conditioning was assessed in computer-controlled operant chambers (Imetronic), as previously described (Blundell *et al*, 2010; Cai *et al*, 2006; Powell *et al*, 2004). All results were expressed as % time freezing.

Latent Inhibition (LI)

Latent inhibition (LI) refers to the retardation in learning about the significance of a stimulus as a result of its prior repeated preexposures without consequence (Lubow and Moore, 1959). LI was assessed in a conditioned freezing paradigm, as previously described (Meyer *et al*, 2005; Willi *et al*, 2010). The test procedures consisted of four phases: preexposure, conditioning, context test, and tone test. Animals were randomly allocated to either the preexposed (PE) or non-preexposed (NPE) condition. The establishment of LI (ie, difference in freezing between NPE and PE groups) reflects the ability to ignore stimuli that historically predict nonsignificant consequences. Thus, LI deficiency is a translational model that assesses attentional deficits in animal models of schizophrenia (Arguello and Gogos, 2006; Meyer *et al*, 2005).

Sleep/Wakefulness Patterns

A battery of evidence shows that schizophrenia is associated with alterations in sleep architecture (Keshavan *et al*, 2008; Monti and Monti, 2004). Sleep architecture and assessment and spectral analysis of the electroencephalogram (EEG) recording were performed as previously described (Boutrel *et al*, 1999). Polygraphic recordings were scored visually every 15 s epoch as wakefulness, nonrapid eye movement (NREM) sleep, or rapid eye movement (REM) sleep, following classical criteria (Lena *et al*, 2004; Tobler *et al*, 1997), using Somnologica. For analysis of the spontaneous sleep–wakefulness patterns, the amounts of vigilance states for each animal were calculated for every hour throughout 48 h and summed over 24 h. REM sleep latency was calculated as the time elapsing from sleep onset after the animal had been awakened to the first episode of REM sleep (Popa *et al*, 2005).

Neurochemical Assessments

DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and glutamate tissue concentrations in the PFC and the dSTR of $Htr_{2B}^{+/+}$ and $Htr_{2B}^{-/-}$ mice were assessed *ex vivo* by means of high-performance liquid chromatography (HPLC) as previously described (Banas *et al*, 2011; Pitychoutis *et al*, 2011). A real-time qPCR-based approach was implemented to examine the mRNA expression of relevant key receptor genes pertaining to the dopaminergic and the glutamatergic neurochemical systems. qPCR was performed as previously described (Diaz *et al*, 2012).

Statistical Analysis

Differences between experimental groups were analyzed by unpaired *t*-tests and one-way or two-way analysis of variance (ANOVA) with genotype and treatment as main factors, depending on the experimental design. Bonferroni's *post hoc* test was applied in order to elucidate specific differences between groups. Repeated measures ANOVAs were implemented in order to analyze locomotor activity and sleep architecture data. Statistical analyses of the results are summarized in Supplementary Tables S1 and S2. In graphs/tables, the values represent means ± SEM and $p < 0.05$ was considered statistically significant.

RESULTS

Deficits in the PPI of the Startle Reflex

Genetic ablation of the 5-HT_{2B} receptor induced a global deficit in the PPI of the startle reflex (Figure 1a) and a reduction in startle amplitude (Supplementary Figure S1a). Moreover, acute treatment of $Htr_{2B}^{+/+}$ mice with the 5-HT_{2B} receptor antagonist RS127445 phenocopied the effects of genetic ablation of 5-HT_{2B} receptor in the PPI (Figure 1b and Supplementary Figure S1b).

Enhanced Locomotor Response to Psychostimulants

Administration of NMDA receptor antagonists (eg, dizocilpine) and DA releasers (eg, amphetamine) exacerbate existing symptoms in schizophrenic patients and cause psychotic-like symptoms in humans and in rodents (Arguello and Gogos, 2006). As shown herein and before, $Htr_{2B}^{-/-}$ mice exhibit enhanced locomotor response to novelty (Figure 1c) (Doly *et al*, 2008). The locomotor response of $Htr_{2B}^{-/-}$ mice to both dizocilpine (Figure 1d) and amphetamine (Figure 1e) was significantly enhanced as compared with $Htr_{2B}^{+/+}$ mice. Several other behaviors appear to be completely unaltered in $Htr_{2B}^{-/-}$ mice, including motor coordination on the Rotarod and nociception assessed in the hot-plate test (Supplementary Figure S1c and d).

Defect in Social Interaction

According to our data, both $Htr_{2B}^{-/-}$ mice and RS-treated $Htr_{2B}^{+/+}$ mice displayed dysfunctional social interaction behavior (Figure 1f), as measured by observing the time mice spent in close interaction with the social partner *vs* the inanimate stimulus (ie, EC). During the second session, preference for social novelty was evaluated by inserting a novel social stimulus (social II) in the previously empty cage and measuring the time spent interacting with social I and social II stimuli (Figure 1g). All three groups showed preference for social novelty as revealed by the greater % time interacting with social II *vs* social I (Supplementary Figure S1e). Social memory was assessed by comparing the

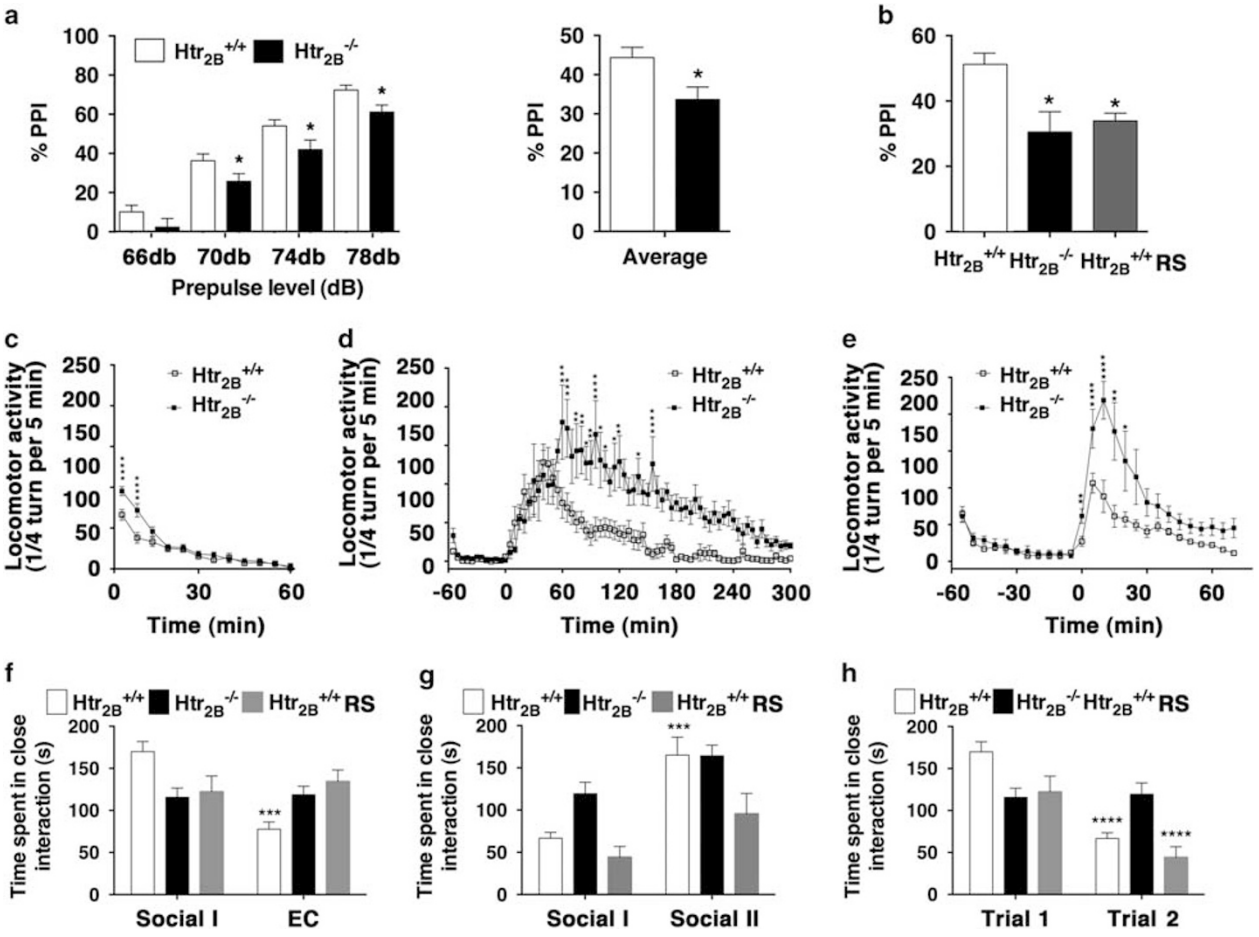

Figure 1 Prepulse inhibition (PPI) is impaired in $Htr_{2B}^{-/-}$ mice across prepulse intensities over 66 db and in average of all intensities ($N=27$ per group) (a). Acute injection of RS127445 (0.5 mg/kg), a selective 5-HT_{2B} receptor antagonist (RS), also impaired PPI in $Htr_{2B}^{+/+}$ mice ($N=8$ per group) (b). Genetic ablation of the 5-HT_{2B} receptor enhanced novelty-induced locomotion during the first 10 min of testing ($N=8$ per group) (c). Genetic blockade of the 5-HT_{2B} receptor enhanced locomotor responses to the glutamatergic psychostimulant and noncompetitive NMDA receptor antagonist dizocilpine (MK-801; 0.5 mg/kg; i.p.) ($N=8$ per group) (d) and to the dopaminergic psychostimulant amphetamine (10 mg/kg; i.p.) ($N=8$ per group) (e). Genetic and pharmacological ablation of the 5-HT_{2B} receptor impaired social interactions, as evidenced by the lack of preference for the social partner (social I *vs* the empty wire cage (EC)) ($N=7$–9 per group) (f). $Htr_{2B}^{+/+}$ mice spent significantly more time interacting with the novel social stimulus (social II *vs* social I), but this was not the case for $Htr_{2B}^{-/-}$ mice or $Htr_{2B}^{+/+}$-RS-treated mice ($N=7$–9 per group) (g). Genetic ablation of 5-HT_{2B} receptor impaired social memory, as $Htr_{2B}^{-/-}$ mice spent similar time interacting with social I partner during both trials, whereas both $Htr_{2B}^{+/+}$ and $Htr_{2B}^{+/+}$-RS-treated mice spent significantly less time interacting with social I partner in the second trial ($N=7$–9 per group) (h). $^{*}P<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ significantly different from vehicle-treated $Htr_{2B}^{+/+}$ mice as determined by unpaired *t*-test, one-way ANOVA, or two-way ANOVA; full statistical analysis is presented in Supplementary Table S1.

time mice spent in close interaction with social I stimulus during the two different trials. This analysis showed that genetic ablation of 5-HT_{2B} receptor impaired social memory, as $Htr_{2B}^{-/-}$ mice spent similar time interacting with social I partner during both trials (Figure 1h). Social memory in both $Htr_{2B}^{+/+}$ and $Htr_{2B}^{+/+}$-RS-treated mice appeared to be intact. In addition, social interaction with a juvenile conspecific was also impaired in $Htr_{2B}^{-/-}$ mice (Supplementary Figure S1f). Of note, the differences observed in social interaction could not be attributed to altered olfactory function because of the genetic ablation of 5-HT_{2B} receptor in mice (Supplementary Figure S1g and h).

Memory Deficits in the NOR and the Fear Conditioning

5-HT_{2B} receptor deficiency induced a significant impairment of both short-tem (1 h; Figure 2a) and long-term NOR memory (24 h; Figure 2b). Similarly, $Htr_{2B}^{-/-}$ mice exhibited impaired fear learning and memory, as evidenced by the reduced time they spent freezing in response to the context (Figure 2c) and the cue in a fear conditioning paradigm (Figure 2d). These cognitive deficits appear to be independent of object exploration behavior or sensitivity to foot shocks. Indeed, $Htr_{2B}^{-/-}$ and $Htr_{2B}^{+/+}$ mice spent similar time exploring the objects during both NOR trials (Supplementary Figure S1i) and showed comparable freezing behavior in response to foot shock during training in the fear conditioning paradigm (Supplementary Figure S1j).

Attention Deficits in the LI Paradigm

In the test of the conditioned response to the tone, the presence of LI in $Htr_{2B}^{+/+}$ mice was demonstrated by a clear reduction in the amount of freezing to the tone in PE mice relative to the NPE mice. However, LI was not established in $Htr_{2B}^{-/-}$ mice (Figure 2e).

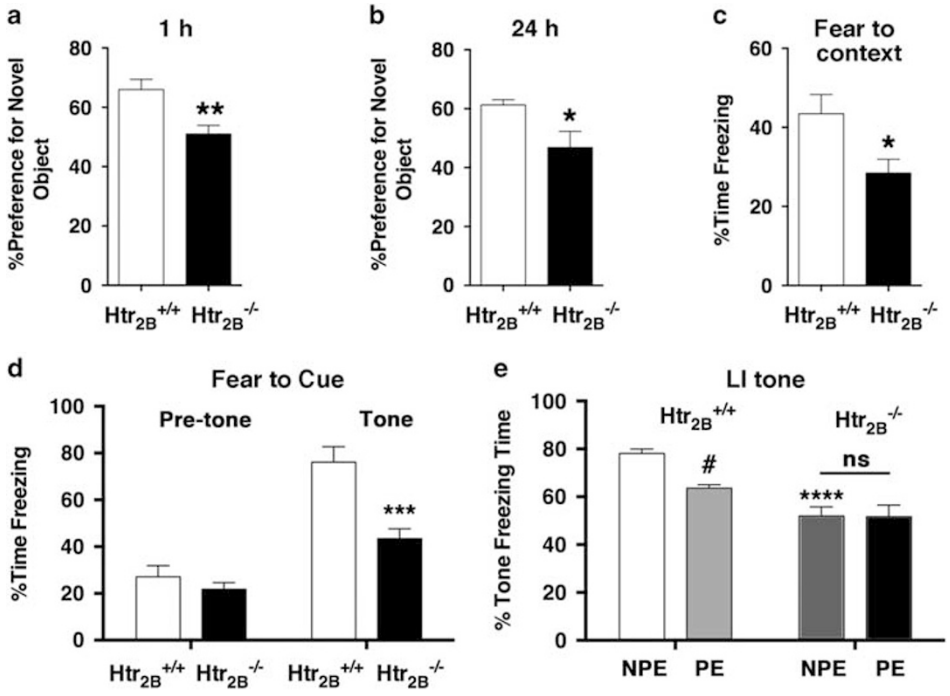

Figure 2 $Htr_{2B}^{-/-}$ mice present with impaired short-term (a) and long-term (b) memory for a novel object, as assessed in the NOR test ($N = 8$ per group). Contextual (c) and cued (d) fear memory is impaired in $Htr_{2B}^{-/-}$ mice ($N = 6$–7 per group). In the latent inhibition (LI) paradigm, (e) LI was established in $Htr_{2B}^{+/+}$ but not in $Htr_{2B}^{-/-}$ mice as indicated by the absence of difference in freezing between NPE and PE groups ($N = 7$ per group). *$P < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ significantly different from $Htr_{2B}^{+/+}$ mice following unpaired *t*-test or two-way ANOVA; $^{\#}p < 0.05$ significantly different from NPE in two-way ANOVA; full statistical analysis is presented in Supplementary Table S1.

Alterations in Sleep Architecture

$Htr_{2B}^{-/-}$ mice exhibited increased wakefulness duration at the expense of NREM sleep (Figure 3a), as well as decreased latency to REM sleep (Figure 3b). Under baseline conditions, $Htr_{2B}^{+/+}$ and $Htr_{2B}^{-/-}$ mice exhibited the typical polyphasic structure of vigilance states found in rodents and a diurnal rhythm of sleep and wakefulness, with larger amounts of sleep during the light period classically observed in nocturnal species (Figure 3c–e). However, $Htr_{2B}^{-/-}$ mice exhibited significantly increased amounts of wakefulness, at the expense of NREM sleep, throughout the dark period and at the onset of light period (Figure 3c and d), whereas REM sleep was not affected (Figure 3e).

Chronic Haloperidol Treatment Rescued Selected Schizophrenic-Like Phenotypes in $Htr_{2B}^{-/-}$ Mice

Haloperidol was selected herein on the basis of its minor binding capacity to 5-HT_2 receptors including murine 5-HT_{2B} receptors unlike many other antipsychotics (Meltzer, 2013) (Supplementary Figure S1k). Chronic oral intake of haloperidol (2 mg/kg/day) for 4 weeks restored the balance between wakefulness and NREM sleep (Figure 3c–e) in $Htr_{2B}^{-/-}$ mice. Moreover, chronic haloperidol treatment normalized the PPI deficits (Figure 3f) and the psychomotor agitation (Figure 3g) conferred by 5-HT_{2B} receptor deletion. However, haloperidol treatment did not ameliorate the social deficit observed in $Htr_{2B}^{-/-}$ mice in the three-chamber social interaction test (Figure 3h and i).

Neurochemical Alterations in $Htr_{2B}^{-/-}$ Mice

In our experimental setup, DA and glutamate tissue levels were found decreased in the dSTR of $Htr_{2B}^{-/-}$ mice, whereas no major neurochemical alterations were observed in the PFC (Figure 4a and b). Moreover, our results showed that the genetic ablation of the 5-HT_{2B} receptor resulted in a differential regulation of mRNA expression of DA and glutamate receptors in the PFC and the dSTR. In particular in the dSTR, D_2R mRNA expression was decreased (Figure 4c), whereas GluR2 was upregulated in the PFC (Supplementary Figure S2f). It is noteworthy that chronic haloperidol treatment normalized the observed neurochemical alterations in DA and glutamate concentrations (Figure 4a and b) and D_2R receptor expression in the dSTR (Figure 4c).

DISCUSSION

Herein we investigated the effects of the genetic ablation of 5-HT_{2B} receptor across a battery of translational behavioral paradigms relevant for assessing face validity of animal models of schizophrenia (Arguello and Gogos, 2006; Meyer *et al*, 2005; Willi *et al*, 2010). We report herein that loss of function of 5-HT_{2B} receptor confers a wide spectrum of schizophrenic-like behavioral and psychopharmacological phenotypes in mice. Importantly, domains related to the positive, negative, and cognitive symptom clusters of schizophrenia appear to be affected upon 5-HT_{2B} receptor gene ablation (Table 1). $Htr_{2B}^{-/-}$ mice display a global deficit in sensorimotor gating (ie, impaired PPI performance) as

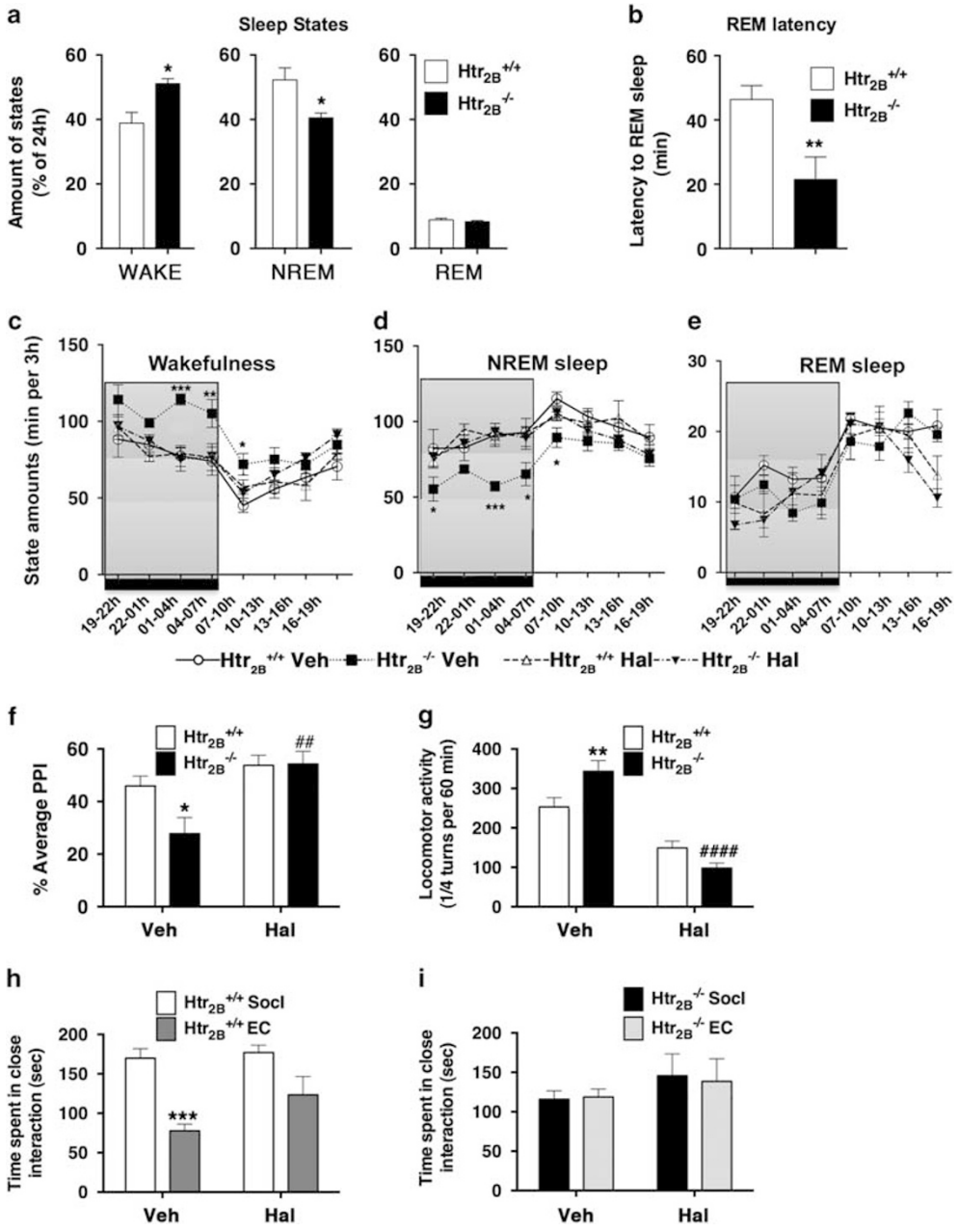

Figure 3 In $Htr_{2B}^{-/-}$ mice across 24 h, the total amount of wakefulness is increased while that of NREM sleep is decreased (a), as is the latency to REM sleep, calculated as the time elapsing from sleep onset after the animal had been awakened to the first episode of REM sleep (b) ($N = 4$–5 per group). $Htr_{2B}^{-/-}$ mice exhibit significantly increased amounts of wakefulness at the expense of NREM sleep throughout the dark period and at the onset of the light period as assessed by the amount of vigilance states, expressed as min per 3 h (c, d) but REM sleep is not affected (e). Chronic oral haloperidol treatment (Hal, 2 mg/kg/day during 4 weeks) normalizes sleep–wakefulness states across the light/dark cycle ($N = 4$–5 per group) (c–e). The effects of chronic oral haloperidol treatment were also tested in the prepulse inhibition (f) and locomotor activity (g) ($N = 8$–10 per group); social interaction was assessed in the three-chamber sociability test by comparing the time $Htr_{2B}^{+/+}$ (h) and $Htr_{2B}^{-/-}$ mice (i) spent interacting with a novel mouse (Soc I) *vs* an empty wire cage (EC; $N = 7$–9 per group). $^{*}P < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ significantly different from the Veh-treated $Htr_{2B}^{+/+}$ mice; $^{\#\#}p < 0.01$; $^{\#\#\#\#}p < 0.0001$ significantly different from the Veh-treated $Htr_{2B}^{-/-}$ mice; full statistical analysis is presented in Supplementary Table S1.

well as psychomotor agitation (ie, novelty-induced hyperlocomotion) and psychostimulant hypersensitivity (ie, enhanced locomotor response to psychostimulants), all phenotypes that have been related to the positive symptoms of schizophrenia (Gunduz-Bruce, 2009; Meyer *et al*, 2005; Pratt *et al*, 2012). Moreover, 5-HT_{2B} receptor gene ablation impaired social interaction behavior with conspecifics, a trait used commonly to model negative symptoms of schizophrenia in rodents (Arguello and Gogos, 2006; Pratt *et al*, 2012). Most importantly, $Htr_{2B}^{-/-}$ mice present with selective attention deficits (ie, lack of LI establishment) and learning and memory impairments (ie, poor NOR and fear conditioning performance and social memory deficit) that closely resemble the cognitive deficits observed in

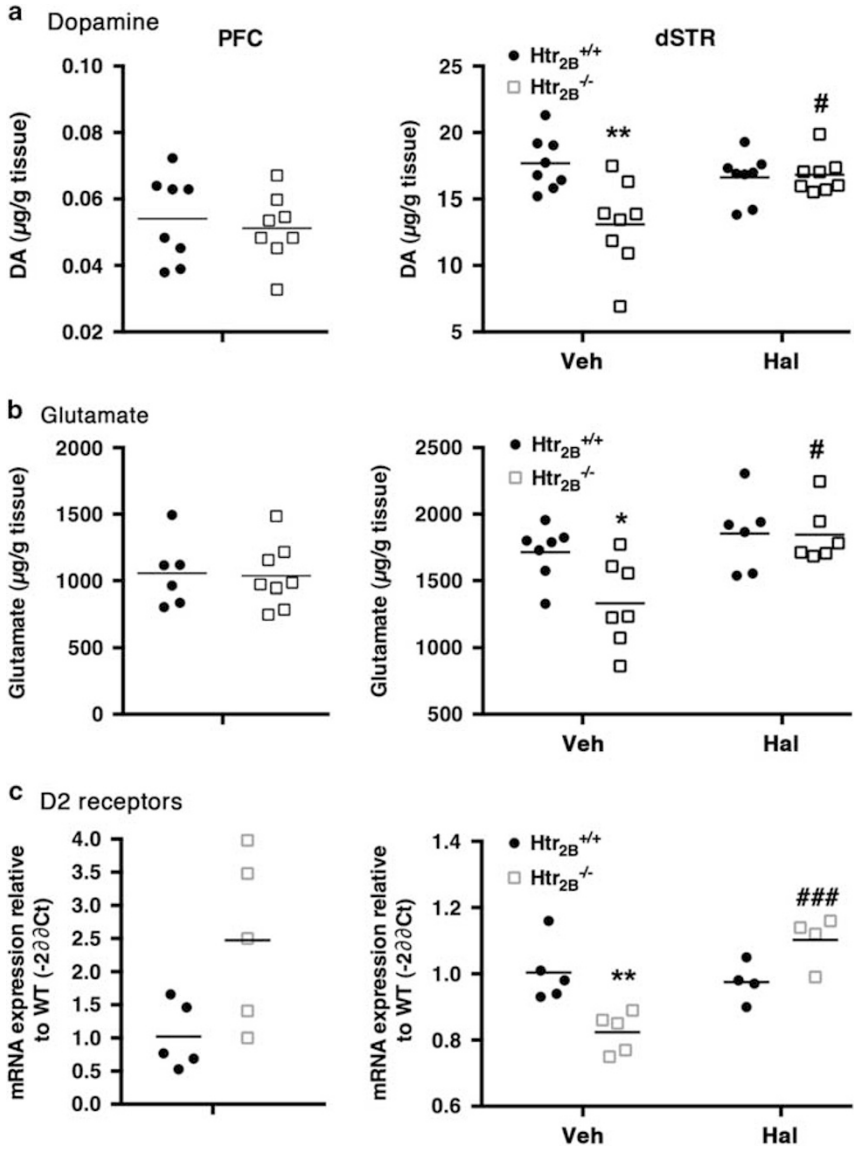

Figure 4 Region-distinctive neurochemical profile in the prefrontal cortex (PFC-left) and the dorsal striatum (dSTR-right) of $Htr_{2B}^{+/+}$ (black circle) and $Htr_{2B}^{-/-}$ (open square) mice. $Htr_{2B}^{-/-}$ mice present lower tissue concentrations of DA (a) and glutamate (b) in the dSTR ($N = 6$–8 per group). Genetic ablation of the 5-HT_{2B} receptor was associated with a downregulation of D_2R mRNA expression in dSTR (c) ($N = 4$–5 per group). Neurochemical alterations in the dSTR are effectively reversed upon chronic haloperidol treatment (2 mg/kg/day 4 weeks) (Hal) (a–c). $^{*}p < 0.05$; $^{**}p < 0.01$; significantly different from vehicle (Veh)-treated $Htr_{2B}^{+/+}$ mice in unpaired *t*-test or two-way ANOVA; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ significant effect from Veh-treated $Htr_{2B}^{-/-}$ mice in two-way ANOVA; full statistical analysis is presented in Supplementary Table S1.

schizophrenic patients (Keshavan *et al*, 2008). Remarkably, the alterations in sleep architecture observed in $Htr_{2B}^{-/-}$ mice (ie, reduced duration of NREM sleep and decreased latency to REM sleep) mimic the sleep abnormalities often seen in schizophrenic patients (Keshavan *et al*, 2008). The list of behavioral paradigms implemented herein, although not exhaustive, is consistent with schizophrenia-like behavioral abnormalities. Moreover, selected schizophrenic-like phenotypes (ie, PPI and social interaction deficits) were phenocopied in $Htr_{2B}^{+/+}$ mice by acute pharmacological ablation of 5-HT_{2B} receptors with the selective receptor antagonist RS127445.

We next assessed whether selected schizophrenic-like phenotypes could be rescued by chronic antipsychotic treatment. For this reason, $Htr_{2B}^{-/-}$ and $Htr_{2B}^{+/+}$ were chronically treated with the typical antipsychotic haloperidol. Of note, chronic antipsychotic treatment completely abolished the observed PPI deficits and alterations in sleep architecture. As expected, haloperidol treatment suppressed psychomotor agitation in $Htr_{2B}^{-/-}$ mice, but was ineffective against social withdrawal, a negative symptom of schizophrenia. Moreover, chronic haloperidol treatment also had an effect in $Htr_{2B}^{+/+}$ mice, normalizing the differences observed between the two genotypes during locomotor activity and social interaction testing.

How 5-HT_{2B} receptor gene deficiency may lead to the observed schizophrenic-like phenotype can only be speculated at present as the function of this receptor in the brain

Table 1 Schizophrenia-Like Behavioral Phenotypes in $Htr_{2B}^{-/-}$ Mice

Functional domain	Factor tested	$Htr_{2B}^{-/-}$	Reference
Behavior			
Locomotor activity	Response to novelty	↑	Figure 1c
	Response to dizocilpine	↑	Figure 1d
	Response to amphetamine	↑	Figure 1e
Sensorimotor gating	Prepulse inhibition	↓	Figure 1a and b
Attention	Latent Inhibition	×	Figure 2e
Cognitive function	Novel object recognition memory (short term)	↓	Figure 2a
	Novel object recognition memory (long term)	↓	Figure 2b
	Contextual fear memory	↓	Figure 2c
	Cued fear memory	↓	Figure 2d
	Social memory	↓	Figure 1h
Social interaction	Sociability	↓	Figure 1f
	Juvenile conspecific	↓	Supplementary Figure S1f
Sleep architecture	Wakefulness	↑	Figure 3a and c
	NREM sleep	↓	Figure 3a and d
	REM sleep	—	Figure 3a and e
	REM latency	↓	Figure 3b

remains largely uninvestigated. It is noteworthy that 5-HT_{2B} receptors have been shown to modulate aspects of both serotonergic and dopaminergic neurotransmission (Bevilacqua *et al*, 2010; Diaz and Maroteaux, 2011; Doly *et al*, 2008). In particular, *ex vivo* studies have indicated that 5-HT_{2B} receptors may serve as positive autoregulator of the serotonergic tone through modulating the 5-HT transporter (SERT) in raphe neurons (Launay *et al*, 2006), and *in vivo* studies in mice further confirmed that 5-HT_{2B} receptors contribute to the behavioral effects of the SERT-targeting 5-HT releasers, MDMA (ie, ecstasy) and dexfenfluramine (Banas *et al*, 2011; Doly *et al*, 2009; Doly *et al*, 2008). Recently, Auclair *et al* (2010) reported that 5-HT_{2B} receptors control the mesoaccumbal DA pathway activity, as an acute systemic administration of the 5-HT_{2B} subtype-selective antagonist RS127445 in rats induced a region-selective reduction of extracellular DA levels in the NAC in response to amphetamine challenge.

In light of the schizophrenic-like phenotype observed herein, the relevant neurochemical alterations found are not surprising. Indeed, the enhanced psychomotor response of $Htr_{2B}^{-/-}$ mice to dizocilpine and to amphetamine indicates that the glutamatergic and/or the dopaminergic systems must, at least to some extent, be altered in $Htr_{2B}^{-/-}$ mice. Schizophrenia has been largely associated with dopaminergic hypofunction in the PFC and hyperfunction in the basal ganglia; indeed numerous post-mortem studies report increased D_2R densities in the dSTR (Hirvonen *et al*, 2005; Seeman and Kapur, 2000) and decreased D_2R levels in the PFC of schizophrenic patients (Takahashi *et al*, 2006). Moreover, aberrations in glutamate-mediated neurotransmission through NMDA receptors in schizophrenia is supported by the reduced glutamate levels in the cerebrospinal fluid (CSF) and NMDA receptor expression in the PFC of schizophrenic patients (Gordon, 2010; Keshavan *et al*, 2008). In our experimental setup, DA and glutamate tissue concentrations and D_2R mRNA expression were found decreased in the dSTR of $Htr_{2B}^{-/-}$ mice. In accordance with our findings, DA tissue concentrations were also found decreased in the dSTR of *Nogo-A*$^{-/-}$ mice that also present with a schizophrenic-like phenotype (Willi *et al*, 2010). Intriguingly, the observed dopaminergic and glutamatergic alterations in the dSTR were corrected upon chronic antipsychotic treatment. The latter finding indicates that both D_2R-related and unrelated defects in $Htr_{2B}^{-/-}$ mice are reversed by haloperidol treatment and provide evidence that the effects of 5-HT_{2B} receptors extend beyond the serotonergic and the dopaminergic systems.

Acute pharmacological ablation of 5-HT_{2B} receptors (ie, RS127445 treatment) phenocopied both positive (ie, PPI deficit) and negative (ie, impaired social interaction) symptoms observed in $Htr_{2B}^{-/-}$ mice. Moreover, in an earlier study, administration of another 5-HT_{2B} receptor-selective antagonist (ie, SB-215505) was shown to increase wakefulness at the expense of NREM and REM sleep in rats (Kantor *et al*, 2004), and in another study it was concluded that 5-HT exerts a 5-HT_{2B} receptor-mediated facilitation of NREM sleep (Popa *et al*, 2005), in accordance with our findings in $Htr_{2B}^{-/-}$ mice. Thus, based on these observations, we propose that the schizophrenic-like phenotype of $Htr_{2B}^{-/-}$ mice results from a combination of both the direct absence of 5-HT_{2B} receptor signaling and the neural adaptations triggered by the permanent lack of this receptor.

Antipsychotic drugs ameliorate hallucinations and delusions in patients with neuropsychiatric disorders, particularly schizophrenia and bipolar disorders. The two main classes are known as typical and atypical antipsychotics. Many atypical antipsychotics show similar affinity for 5-HT_{2B} and 5-HT_{2A} receptors (eg, clozapine, amisulpride, asenapine) (Abbas *et al*, 2009; Shahid *et al*, 2009) or even higher affinity

for 5-HT_{2B} receptors (eg, aripiprazole, cariprazine) (Kiss *et al*, 2010; Shapiro *et al*, 2003). Notably, it was recently shown that the efficacy of clozapine, but not haloperidol, is diminished in $Pet1^{-/-}$ mice that lack 5-HT neurons, and thus depends on an intact presynaptic serotonergic system (Yadav *et al*, 2011). Given that many marketed atypical antipsychotic drugs present high affinity for 5-HT_{2B} receptors (Shapiro *et al*, 2003) and that these receptors are expressed in 5-HT neurons (Diaz *et al*, 2012), our findings bear broader significance for the elucidation of the effects of these drugs in the treatment of psychotic disorders.

It should be borne in mind that the behavioral phenotype of $Htr_{2B}^{-/-}$ mice portrayed herein is not exclusive of a putative role of the 5-HT_{2B} receptor in the neurobiology of a broader range of neuropsychiatric diseases that share common domains of dysfunction with psychotic disorders. Interestingly, it was recently reported that two polymorphisms of the *HTR2B* gene were associated with intelligence quotation, intellectual disability, and language onset delay in a cohort of children and young adults suffering from autistic spectrum disorders (Hervas *et al*, 2014). In agreement, the present data strongly support a novel role for 5-HT_{2B} in the regulation of cognitive function and social behavior. Despite the fact that $Htr_{2B}^{-/-}$ mice display deficits in social interaction and in learning and memory processes that closely resemble autistic-like behavior, they also present with deficits in sensorimotor gating and locomotor hypersensitivity to psychostimulants that are core endophenotypes of schizophrenia (Arguello and Gogos, 2006; Powell *et al*, 2009).

Overall, this study revealed that 5-HT_{2B} receptor deficiency induces a wide spectrum of antipsychotic-sensitive schizophrenic-like phenotypes in mice. Further pharmacological validations with selective 5-HT_{2B} receptor antagonists (given acutely or chronically) that extend present findings to other behavioral tests, mouse strains and species, or other brain regions and neurochemical systems are clearly warranted to further dissect the role of the 5-HT_{2B} receptor in the neurobiology of psychotic spectrum disorders. This genetic mouse model holds additional value in further elucidating the schizophrenia-relevant neurodevelopmental, epigenetic, and physiological mechanisms that may be sensitive to 5-HT_{2B} receptor polymorphisms.

FUNDING AND DISCLOSURE

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Dr. Silvina L Diaz for help with mice, Dr. Véronique Fabre for support in sleep experiments, and Dr. Cédric Mombereau for advices in prepulse inhibition experiments. This work has been supported by funds from the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, the Université Pierre et Marie Curie, and by grants from the Fondation de France, the Fondation pour la Recherche Médicale 'Equipe FRM DEQ2014039529', the French Ministry of Research (Agence Nationale pour la Recherche ANR-12-BSV1-0015-01, and the Investissements d'Avenir program managed by the ANR under reference ANR-11-IDEX-0004-02. The team of LM is part of the École des Neurosciences de Paris Ile-de-France network and of the Bio-Psy Laboratory of Excellence. PMP was supported by a DIM NERF fellowship from the 'Région Ile-de-France' and by the University of Dayton.

REFERENCES

Abbas AI, Hedlund PB, Huang X-P, Tran TB, Meltzer HY, Roth BL (2009). Amisulpride is a potent 5-HT7 antagonist: relevance for antidepressant actions in vivo. *Psychopharmacology* **205**: 119–128.

Arguello PA, Gogos JA (2006). Modeling madness in mice: one piece at a time. *Neuron* **52**: 179–196.

Auclair AL, Cathala A, Sarrazin F, Depoortère R, Piazza PV, Newman-Tancredi A *et al* (2010). The central serotonin 2B receptor: a new pharmacological target to modulate the mesoaccumbens dopaminergic pathway activity. *J Neurochem* **114**: 1323–1332.

Banas S, Doly S, Boutourlinsky K, Diaz S, Belmer A, Callebert J *et al* (2011). Deconstructing antiobesity compound action: requirement of serotonin 5-HT2B receptors for dexfenfluramine anorectic effects. *Neuropsychopharmacology* **36**: 423–433.

Bevilacqua L, Doly S, Kaprio J, Yuan Q, Tikkanen R, Paunio T *et al* (2010). A population-specific HTR2B stop codon predisposes to severe impulsivity. *Nature* **468**: 1061–1066.

Blundell J, Blaiss CA, Etherton MR, Espinosa F, Tabuchi K, Walz C *et al* (2010). Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. *J Neurosci* **30**: 2115–2129.

Boutrel B, Franc B, Hen R, Hamon M, Adrien J (1999). Key role of 5-HT1B receptors in the regulation of paradoxical sleep as evidenced in 5-HT1B knock-out mice. *J Neurosci* **19**: 3204–3212.

Cai WH, Blundell J, Han J, Greene RW, Powell CM (2006). Postreactivation glucocorticoids impair recall of established fear memory. *J Neurosci* **26**: 9560–9566.

Carlini VP, Martini AC, Schioth HB, Ruiz RD, Fiol de Cuneo M, de Barioglio SR (2008). Decreased memory for novel object recognition in chronically food-restricted mice is reversed by acute ghrelin administration. *Neuroscience* **153**: 929–934.

Diaz SL, Doly S, Narboux-Nême N, Fernandez S, Mazot P, Banas S *et al* (2012). 5-HT2B receptors are required for serotonin-selective antidepressant actions. *Mol Psychiatry* **17**: 154–163.

Diaz SL, Maroteaux L (2011). Implication of 5-HT2B receptors in the serotonin syndrome. *Neuropharmacology* **61**: 495–502.

Doly S, Bertran-Gonzalez J, Callebert J, Bruneau A, Banas SM, Belmer A *et al* (2009). Role of serotonin via 5-HT2B receptors in the reinforcing effects of MDMA in mice. *PLoS One* **4**: e7952.

Doly S, Valjent E, Setola V, Callebert J, Herve D, Launay JM *et al* (2008). Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. *J Neurosci* **28**: 2933–2940.

Fineberg NA, Potenza MN, Chamberlain SR, Berlin HA, Menzies L, Bechara A *et al* (2010). Probing compulsive and impulsive behaviors, from animal models to endophenotypes: a narrative review. *Neuropsychopharmacology* **35**: 591–604.

Gleason KA, Birnbaum SG, Shukla A, Ghose S (2012). Susceptibility of the adolescent brain to cannabinoids: long-term hippocampal effects and relevance to schizophrenia. *Transl Psychiatry* **2**: e199.

Gonzalez-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, Lopez-Gimenez JF *et al* (2008). Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* **452**: 93–97.

Gordon JA (2010). Testing the glutamate hypothesis of schizophrenia. *Nat Neurosci* **13**: 2–4.

Gunduz-Bruce H (2009). The acute effects of NMDA antagonism: from the rodent to the human brain. *Brain Res Rev* **60**: 279–286.

Hervas A, Toma C, Romaris P, Ribases M, Salgado M, Bayes M *et al* (2014). The involvement of serotonin polymorphisms in autistic spectrum symptomatology. *Psychiatr Genet* **24**: 158–163.

Hirvonen J, van Erp TG, Huttunen J, Aalto S, Nagren K, Huttunen M *et al* (2005). Increased caudate dopamine D2 receptor availability as a genetic marker for schizophrenia. *Arch Gen Psychiatry* **62**: 371–378.

Howes OD, Montgomery AJ, Asselin MC, Murray RM, Valli I, Tabraham P *et al* (2009). Elevated striatal dopamine function linked to prodromal signs of schizophrenia. *Arch Gen Psychiatry* **66**: 13–20.

Humby T, Wilkinson LS (2011). Assaying dissociable elements of behavioural inhibition and impulsivity: translational utility of animal models. *Curr Opin Pharmacol* **11**: 534–539.

Kantor S, Jakus R, Balogh B, Benko A, Bagdy G (2004). Increased wakefulness, motor activity and decreased theta activity after blockade of the 5-HT2B receptor by the subtype-selective antagonist SB-215505. *Br J Pharmacol* **142**: 1332–1342.

Keshavan MS, Tandon R, Boutros NN, Nasrallah HA (2008). Schizophrenia, 'just the facts': what we know in 2008 Part 3: neurobiology. *Schizophr Res* **106**: 89–107.

Kiss B, Horváth A, Némethy Z, Schmidt E, Laszlovszky I, Bugovics G *et al* (2010). Cariprazine (RGH-188), a dopamine D (3) receptor-preferring, D(3)/D(2) dopamine receptor antagonist-partial agonist antipsychotic candidate: in vitro and neurochemical profile. *J Pharmacol Exp Ther* **333**: 328–340.

Kvajo M, McKellar H, Gogos JA (2012). Avoiding mouse traps in schizophrenia genetics: lessons and promises from current and emerging mouse models. *Neuroscience* **211**: 136–164.

Launay JM, Schneider B, Loric S, Da Prada M, Kellermann O (2006). Serotonin transport and serotonin transporter-mediated antidepressant recognition are controlled by 5-HT2B receptor signaling in serotonergic neuronal cells. *FASEB J* **20**: 1843–1854.

Lena C, Popa D, Grailhe R, Escourrou P, Changeux JP, Adrien J (2004). Beta2-containing nicotinic receptors contribute to the organization of sleep and regulate putative micro-arousals in mice. *J Neurosci* **24**: 5711–5718.

Lubow RE, Moore AU (1959). Latent inhibition: the effect of nonreinforced pre-exposure to the conditional stimulus. *J Comp Physiol Psychol* **52**: 415–419.

Lyon L, Saksida LM, Bussey TJ (2012). Spontaneous object recognition and its relevance to schizophrenia: a review of findings from pharmacological, genetic, lesion and developmental rodent models. *Psychopharmacology (Berl)* **220**: 647–672.

Meltzer HY (2013). Update on typical and atypical antipsychotic drugs. *Annu Rev Med* **64**: 393–406.

Meltzer HY, Massey BW (2011). The role of serotonin receptors in the action of atypical antipsychotic drugs. *Curr Opin Pharmacol* **11**: 59–67.

Meyer U, Feldon J, Schedlowski M, Yee BK (2005). Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. *Neurosci Biobehav Rev* **29**: 913–947.

Monti JM, Monti D (2004). Sleep in schizophrenia patients and the effects of antipsychotic drugs. *Sleep Med Rev* **8**: 133–148.

Olesen J, Gustavsson A, Svensson M, Wittchen HU, Jonsson B (2012). The economic cost of brain disorders in Europe. *Eur J Neurol* **19**: 155–162.

Peca J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN *et al* (2011). Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* **472**: 437–442.

Pitychoutis PM, Pallis EG, Mikail HG, Papadopoulou-Daifoti Z (2011). Individual differences in novelty-seeking predict differential responses to chronic antidepressant treatment through sex- and phenotype-dependent neurochemical signatures. *Behav Brain Res* **223**: 154–168.

Popa D, Lena C, Fabre V, Prenat C, Gingrich J, Escourrou P *et al* (2005). Contribution of 5-HT2 receptor subtypes to sleep-wakefulness and respiratory control, and functional adaptations in knock-out mice lacking 5-HT2A receptors. *J Neurosci* **25**: 11231–11238.

Powell CM, Schoch S, Monteggia L, Barrot M, Matos MF, Feldmann N *et al* (2004). The presynaptic active zone protein RIM1alpha is critical for normal learning and memory. *Neuron* **42**: 143–153.

Powell SB, Zhou X, Geyer MA (2009). Prepulse inhibition and genetic mouse models of schizophrenia. *Behav Brain Res* **204**: 282–294.

Pratt J, Winchester C, Dawson N, Morris B (2012). Advancing schizophrenia drug discovery: optimizing rodent models to bridge the translational gap. *Nat Rev Drug Discov* **11**: 560–579.

Seeman P, Kapur S (2000). Schizophrenia: more dopamine, more D2 receptors. *Proc Natl Acad Sci USA* **97**: 7673–7675.

Shahid M, Walker GB, Zorn SH, Wong EHF (2009). Asenapine: a novel psychopharmacologic agent with a unique human receptor signature. *J Psychopharm* **23**: 65–73.

Shapiro DA, Renock S, Arrington E, Chiodo LA, Liu LX, Sibley DR *et al* (2003). Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsychopharmacology* **28**: 1400–1411.

Simpson EH, Kellendonk C, Kandel E (2010). A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia. *Neuron* **65**: 585–596.

Takahashi H, Higuchi M, Suhara T (2006). The role of extrastriatal dopamine D2 receptors in schizophrenia. *Biol Psychiatry* **59**: 919–928.

Terry AV Jr, Gearhart DA, Warner SE, Zhang G, Bartlett MG, Middlemore ML *et al* (2007). Oral haloperidol or risperidone treatment in rats: temporal effects on nerve growth factor receptors, cholinergic neurons, and memory performance. *Neuroscience* **146**: 1316–1332.

Tobler I, Deboer T, Fischer M (1997). Sleep and sleep regulation in normal and prion protein-deficient mice. *J Neurosci* **17**: 1869–1879.

Willi R, Weinmann O, Winter C, Klein J, Sohr R, Schnell L *et al* (2010). Constitutive genetic deletion of the growth regulator Nogo-A induces schizophrenia-related endophenotypes. *J Neurosci* **30**: 556–567.

Yadav PN, Abbas AI, Farrell MS, Setola V, Sciaky N, Huang X-P *et al* (2011). The presynaptic component of the serotonergic system is required for clozapine's efficacy. *Neuropsychopharmacology* **36**: 638–651.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)