

RESEARCH PAPER

Response of *Htr3a* knockout mice to antidepressant treatment and chronic stress

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BACKGROUND AND PURPOSE

It has recently been suggested that 5-HT₃ receptor blockade enhances the efficacy of selective 5-HT (serotonin) reuptake inhibitor (SSRI) antidepressants and may reverse stress-induced deficits in rodents.

EXPERIMENTAL APPROACH

To further explore this hypothesis, we used mice lacking the 5-HT₃ receptor (*Htr3a* KO) and their wild-type (WT) controls to assess their response in behavioural paradigms relevant to anxiety and depression. Mice were studied under basal, antidepressant treatments and chronic social defeat stress (CSDS) conditions.

KEY RESULTS

In basal conditions, *Htr3a* KO mice displayed anxiolytic- and antidepressant-like behaviours in the elevated plus maze, the social interaction and the forced swim tests (FST), but behaved as WT mice in response to acute citalopram in the FST. However, the effects of fluoxetine were blunted in *Htr3a* KO mice in these same tests. In an *in vitro* electrophysiological paradigm, a low-dose citalopram treatment triggered 5-HT_{1A} receptor desensitization only in the dorsal raphe nucleus of *Htr3a* KO, although a high dose desensitized 5-HT_{1A} autoreceptor function equally in *Htr3a* KO and WT mice, suggesting that citalopram may become effective at lower doses when 5-HT₃ receptors are inactivated. In addition, *Htr3a* deletion blocked CSDS-induced modification in the cortical expression of two genes involved in oxidative stress, *CaMKIIa* and *SOD1*.

CONCLUSIONS AND IMPLICATIONS

Taken together, these data show that *Htr3a* deletion promotes SSRI efficacy and prevents the occurrence of stress-induced deleterious effects, suggesting that the 5-HT₃ receptor may represent an interesting target for the treatment of stress-related disorders.

Abbreviations

5-CT, 5-carboxamidotryptamine; 5-HIAA, 5-hydroxyindolacetic acid; SERT (5-HTT), 5-HT transporter; CaMKIIa, calmodulin-dependent protein kinase II α ; CSDS, chronic social defeat stress; DRN, dorsal raphe nucleus; EPM, elevated plus maze; FST, forced swim test; GIRK, G-protein-gated inwardly rectifying K⁺; KO, knockout; NTS, nucleus tractus solitarius; OF, open field test; SI, social interaction test; SSRI, selective 5-HT (serotonin) reuptake inhibitor; WT, wild-type

Introduction

Selective **5-HT** (serotonin) reuptake inhibitors (SSRIs) are currently the compounds most prescribed to treat major depression. Although they display a good clinical efficacy, about 50% of patients do not respond correctly to this first-line therapy (Hamon and Blier, 2013) and they induce dose-dependent negative side-effects in several patients that can lead to treatment interruption (Zajacka *et al.*, 2010). Among the new generation of drugs in development, **vortioxetine** is a recently (2014) released multimodal antidepressant, which, besides being a **5-HT transporter (SERT; 5-HTT)** inhibitor, a **5-HT_{1A}** receptor agonist and a **5-HT_{1B}** receptor partial agonist is also a **5-HT₇** and **5-HT₃** receptor antagonist (Mork *et al.*, 2012), the latter property being crucial in its mechanism of action (Sanchez *et al.*, 2015). Multimodal actions and in particular those involving 5-HT₃ receptor blockade may facilitate the antidepressant efficacy of 5-HT reuptake blockers at lower doses.

The 5-HT₃ receptor, the only ligand-gated cation channel among the 5-HT receptor family, is located on brain structures relevant to depression and antidepressant responses (Laporte *et al.*, 1992). It has long been argued that 5-HT₃ receptor antagonists possess antidepressant and anxiolytic properties (Martin *et al.*, 1992; Mahesh *et al.*, 2013) and recent data showed that they could potentiate the effects of an SSRI in preclinical behavioural tests (Ramamoorthy *et al.*, 2008; Bétry *et al.*, 2015). Interestingly, chronic vortioxetine has been shown to induce a faster recovery of serotonergic neurons' firing than **fluoxetine** treatment (Bétry *et al.*, 2013). These effects of vortioxetine were attributed to its capacity to induce faster 5-HT_{1A} receptor desensitization, which is usually correlated to an antidepressant's delay of action (Le Poul *et al.*, 1995). However, the fact that in Bétry's study, 5-HT_{1A} receptor desensitization was indirectly measured and the effect of vortioxetine was blocked by 5-HT₃ receptor agonist ligands, which by themselves could induce a rapid desensitization of 5-HT₃ receptors (Thompson and Lummis, 2006) limited the conclusions of these pharmacological studies. It was therefore crucial to determine if the effect of SSRIs is effectively potentiated in the absence of 5-HT₃ receptors. The generation of mutant mice lacking the 5-HT₃ receptor subunit 3A, essential for the receptor channel function, was instrumental in confirming its involvement in anxiety-related behaviours (Kelley *et al.*, 2003; Bhatnagar *et al.*, 2004). However, although Bhatnagar and Vining (2004) suggested a blunted responsiveness of these mutant mice to acute stress, the effects of chronic stress and antidepressant treatments on 5-HT₃ receptor knockout (**Htr3a** KO) mice in assays related to depression had not yet been investigated.

Therefore, to thoroughly characterize the role of 5-HT₃ receptors in depression-related behaviours and antidepressant treatments, **Htr3a** KO and wild-type (WT) mice were exposed to acute and chronic SSRI antidepressant treatments and studied in the chronic social defeat stress (CSDS) paradigm, a validated model of depression (see Chaouloff, 2013). We assessed the effects of 5-HT₃ receptor genetic invalidation in behavioural tests related to anxiety and depression and the effects of acute **citalopram** and fluoxetine in these mutant mice. *In vitro* electrophysiological studies were used to assess the citalopram-induced 5-HT_{1A} autoreceptor desensitization in

mice lacking 5-HT₃ receptors. Finally, as oxidative stress is an early event putatively involved in the pathogenesis of depression, in these mutant mice we assessed the effect of the CSDS paradigm on two oxidative stress markers, SOD1 and the **calmodulin**-dependent protein kinase II α (CaMKII α), recently found to be regulated by 5-HT₃ receptors (Bhatt *et al.*, 2014; Hutchinson *et al.*, 2015).

Methods

Animals

Experiments were performed using male homozygous **Htr3a** KO and WT littermates born from heterozygous mutants on a C57BL/6 J genetic background (>10 generations) and genotyped as described by Zeitz *et al.* (2002). After they had been weaned and sexed, males were housed separately in groups of four to six animals per cage and maintained under standard laboratory conditions (22 \pm 1°C, 60% relative humidity, 12 h light/dark cycle, food and water available *ad libitum*). Male mice of each genotype were used at 2 months of age when their body weight equally reached about 25 g. Mice were always used for a single experiment, were all naïve of previous test or treatment and randomly distributed among the different groups (see Experimental protocol in supplementary information). The number of animals (6–15) per experiment was based on a power analysis (Charan and Kantharia, 2013) corrected by the mouse breeding availability. For most of the experiments, no animals were excluded from the results. However, in the splash test, two mice were removed due to the poor quality of the video recording. Some animals were also excluded from CSDS RT-PCR studies due to sample processing issues. All studies, performed by a blind experimenter, followed the editorial on experimental design and analysis in pharmacology (Curtis *et al.*, 2015). All procedures concerning animal care and treatment were carried out in accordance with the protocols approved by the ethical committee # C2EA -05 Charles Darwin for the use of experimental animals and were licensed by the Directorate General for Research and Innovation (French Ministère de l'Enseignement Supérieur et de la Recherche), under protocol authorization # 00966.02. Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath and Lilley, 2015).

Measurement of 5-HT turnover

Tissue levels of 5-HT and 5-hydroxyindolacetic acid (5-HIAA) were determined using HPLC with electrochemical detection (HPLC-ED) in prefrontal cortex, hippocampus and brainstem levels of both naïve **Htr3a** KO and WT mice as previously described (Salomon *et al.*, 2006; Mongeau *et al.*, 2010). Those regions have been chosen because of both their strong serotonergic innervation and of their involvement in the pathogenesis of depression.

Quantitative autoradiography of 5-HT_{1A} receptor stimulation-induced [³⁵S]-GTP- γ -S binding

Autoradiographic measurement of 5-HT_{1A} receptor-stimulated [³⁵S]-GTP- γ -S binding in the dorsal raphe nucleus (DRN) was performed in naïve **Htr3a** KO and WT mice as

previously described by Froger *et al.* (2004). [³⁵S]-GTP- γ -S binding is expressed as percentage over the baseline: [(stimulated-basal)/basal] \times 100.

Quantification of RNA levels by quantitative RT-PCR

Animals were killed by cervical dislocation, and the prefrontal cortex, hippocampus and blocks containing the raphe or the nucleus tractus solitarius (NTS) were quickly dissected, frozen in liquid nitrogen and kept at -80°C for molecular analysis. Total mRNA was extracted using TRI Reagent Solution (Life Technologies, Saint Aubin, France) following the manufacturer's instructions. Reverse transcription was performed with a High Capacity cDNA Reverse Transcription kit (Applied Biosystem, Courtaboeuf, France) with the following cycling protocol: 25°C for 10 min, 37°C for 2 h and 85°C for 5 s. cDNA samples were stored at -20°C . Amplification reactions were performed with KAPA SYBR FAST qPCR Master Mix (Clinisciences, Nanterre, France) following manufacturer's instructions using the 7300 Real Time PCR System (Applied Biosystem, Courtaboeuf, France). The following cycling protocol was applied: 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. The following primers were used: 5-HT_{1A} receptor, ACCCCAACGAGTGCACCAT CAG (sense), GCAGGCGGGGACATAGGAG (antisense); 5HT3A subunit, GTGGACTCCTGAGGACTTCGACAA (sense), AGATGTCAAGGCTACAGGCGGTCA (antisense); CaMKIIa (Ca²⁺/calmodulin-dependent protein kinase IIa), GAGACCAAAGCACGGAGAG (sense), GGGTTGGCTC TTGTATGGA (antisense); SOD1 CACTTCGAGCAGAAGG CAAG (sense), CCCCATACTGATGGACGTGG (antisense). β -actin (sense: CCACCATGTACCCAGGCATT, antisense: CGGACTCATCGTACTCCTGC) was used as housekeeping gene for normalizing gene expression results. The $2^{\Delta\Delta\text{CT}}$ (Delta Delta Comparative Threshold) method was used to quantify the fold change in mRNA expression of *Htr3a* KO naïve mice with WT naïve mice and socially defeated WT, Control and defeated KO mice groups with the control WT mice group.

Electrophysiological studies

Extracellular recordings of DRN serotonergic neurons firing were made as previously detailed elsewhere (Lanfume *et al.*, 1999). Briefly, animals were killed by cervical dislocation, and brains from either naïve or SSRI-treated mice were rapidly removed, and a block of brainstem containing the DRN was cut into 400 μm thick coronal sections using a vibratome and immersed in an ice-cold artificial CSF (aCSF). Extracellular recordings of characterized 5-HT_{1A} autoreceptor-expressing serotonergic neurons in the DRN were obtained using glass microelectrodes filled with 2 M NaCl (10–15 M Ω) (Lanfume *et al.*, 1999). Baseline activity was recorded for 5–10 min prior to the application of drugs via a three-way tap system. The duration of each application of the 5-HT_{1A} receptor agonist **ipsapirone** (0–0.3 μM) was 3 min. The effect of ipsapirone was evaluated by comparing the mean discharge frequency for 2 min prior to its addition to the superfusing aCSF with that recorded at the peak of drug action. One to three cells were recorded per animal.

Behavioural studies

The behavioural tests were adapted from existing protocols (see Boule *et al.*, 2014 and Mongeau *et al.*, 2010). Classical tests for measuring depression-relevant behaviours (forced swim test – FST), locomotor activity (open field – OF) and anxiety-like behaviours (elevated plus maze – EPM; social interaction test – SI) were performed in different groups of mice.

Elevated plus maze. The apparatus consisted of an elevated plus maze (EPM) composed by two open arms and two closed arms (40 \times 5 cm), elevated 50 cm from an infrared floor. Animals were placed at the centre of the plus maze facing a closed arm and left free to explore for 10 min. The number of entries, the time spent in the open arms and the total distance covered in the entire maze were measured using a video tracking system (Viewpoint, Lyon, France).

Social interaction test (SIT). Mice were habituated for 20 min to the open field (50 \times 50 cm) for two consecutive days. The third day, two mice of the same genotype, age and weight but unfamiliar to each other were paired and placed in the open field for 10 min. Total time spent in active social interactions (defined as sniffing, fighting, chasing, grooming or crawling under and over each other) was scored from a recorded video using XNote StopWatch software.

Forced swim test (FST). Mice were placed for 6 min into Plexiglas cylinders (25 \times 15 cm) filled with water ($25 \pm 0.5^{\circ}\text{C}$) to a depth of 15 cm and placed in front of an infrared plate. The immobility (defined as the absence of movement and/or small movements necessary to keep the head above the water) was scored using a video tracking system (Viewpoint, Lyon, France) during the last 4 min of the test. For antidepressant response testing, citalopram (2.5, 5.0 mg $\cdot\text{kg}^{-1}$), fluoxetine (20.0 mg $\cdot\text{kg}^{-1}$) or saline were injected i.p. 30 min before the test.

Chronic social defeat stress (CSDS). Chronic social defeat stress protocol was adapted from Krishnan *et al.* (2007), with slight modifications.

The stress procedure was conducted on naïve *Htr3a* KO and WT mice from day 1 to day 10. Mice were weighed before the beginning of the first defeat session (day 1) and on day 11. Open field and social avoidance tests were performed at day 11 on socially defeated and control animals to measure their anxio-depressive phenotype. The next day (day 12), the state of their fur and grooming behaviour were evaluated as an index of a depression-like state (Smolinsky *et al.*, 2009). Finally, mice were killed by cervical dislocation and their brain dissected on day 13 between 09:00 and 12:00 h. Adrenal glands were dissected out and weighed to estimate the effect of stress.

Male CD-1 retired breeder mice were screened for aggressive behavioural response based on the following criterion: latency to initial aggression less than 60 s in at least two consecutive 180 s screening sessions (Golden *et al.*, 2011). At least 24 h before the first defeat, CD-1 mice were singly housed in a standard cage on one side of a transparent

perforated Plexiglas divider, which allowed sensory but not physical contact. One experimental mouse was exposed to a CD-1 aggressor mouse for 10 min, during which the intruder WT or *Htr3a* KO mouse was attacked and displayed subordinate posturing. To avoid physical injury, mice were briefly separated in cases of over-aggressive behaviour from the CD-1 mouse. In cases of mild injury, which occurred in less than 25% of the mice (wounds or bites not exceeding 0.2 cm of length), the mice were kept in the study and treated with topical antiseptic (chlorhexidine 0.25%). After the 10 min defeat session, the experimental mouse was placed for the rest of the day on the opposite side of the divider. This procedure was repeated for 10 consecutive days (days 1–10), using a different aggressor CD-1 mouse each day, between 10:00 and 11:00 h. Control mice were housed in pairs in a similar cage separated by a Plexiglas divider and never exposed to CD-1 mice. Immediately after the last defeat session, stressed and control mice were singly housed in a new cage.

Open field test (OF). Twenty-four hours after the last defeat session, mouse anxiety levels and locomotor activity were tested in the open field. The apparatus consisted of four open arenas (50 × 50 cm) separated by Plexiglas walls and equipped with an infrared floor for automatic animals exploratory behaviour detection. A virtual zone (20 × 20 cm) was delimited in the centre of the open field. Mice were placed in the open field boxes, and left free to explore for 45 min under low light conditions (5 lux). The number of entries, the time spent at the centre and the total distance covered in the entire open field were measured using a video tracking system (Viewpoint, Lyon, France).

Social avoidance testing. Social avoidance was assessed 3 h after the open field test, as previously described (Berton *et al.*, 2006; Krishnan *et al.*, 2007). The test consisted of two consecutive 150 s sessions, under low light conditions (5 lux). In the first session, experimental mice were allowed to explore the open field containing an empty circular wire cage (18 × 9 cm) located at one end of the field. In the second session, conditions were identical except that the circular cage contained a CD-1 aggressive mouse (defined as 'target'). A virtual interaction zone (area projecting 8 cm around wire cage) was delimited, and the time spent in this zone was scored during both sessions using a video tracking system (Viewpoint, Lyon, France). Social interaction behaviour was estimated with the time spent in the interaction zone with the target present and with the following interaction ratio: (time spent in the interaction zone in the presence of target / time spent in the interaction zone in the absence of target) × 100.

Coat state evaluation. The total score of the coat state resulted from the sum of scores recorded at four different parts of the mouse body: head, neck, dorsal and ventral coat. For each area, a score of 0 was given for a well-groomed coat and 1 for an unkempt coat. Potential wounds induced by social defeat and treated as described above were not considered for coat state evaluation.

Splash test. The splash test consists in spraying a 10% sucrose solution on the back of mice in their home cage. The sucrose solution induces grooming behaviour. The total time spent in grooming behaviour and the latency for the first grooming event to occur were scored from a recorded video using XNote StopWatch software over a 5 min period.

Drugs

For electrophysiological experiments, *Htr3a* KO and WT mice were treated either with saline (10 mL·kg⁻¹) or citalopram 5 or 20 mg·kg⁻¹·day⁻¹ i.p. for 14 days.

For antidepressant response testing, citalopram (2.5, 5.0 mg·kg⁻¹), fluoxetine (20.0 mg·kg⁻¹) or saline (10 mL·kg⁻¹) were injected i.p. 30 min before the test.

Citalopram was purchased from Biotrend (Cologne, Germany) and fluoxetine from Santa Cruz (Dallas, USA).

Statistical analysis

All data (displayed as mean ± SEM) were analysed using Prism 5 Software (GraphPad, San Diego, USA). For the comparison of two groups, Student's unpaired *t*-test was used, with Welch's correction if needed. All remaining data were compared using a two-way ANOVA, followed by Bonferroni *post hoc* test in case of significant factor interaction. The level of significance was set at $P < 0.05$. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis *et al.*, 2015).

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015a,b,c).

Results

Effect of 5-HT₃ receptor deletion on the serotonergic activity

5-HT turnover was evaluated in the prefrontal cortex, the hippocampus and the brainstem to investigate the serotonergic activity in naïve *Htr3a* KO and WT mice. A two-way ANOVA revealed no brain area × genotype interaction [$F(2,32) = 0.79$, $P = 0.46$], but there was a significant overall effect of genotype [$F(1,32) = 4.95$, $P = 0.03$], which was associated with an overall diminution of the 5-HIAA/5-HT ratio (−9%, prefrontal cortex; −16%, hippocampus; −20%, brainstem; Figure 1A).

Because 5-HT_{1A} receptors are known to regulate 5-HT turnover (Hamon *et al.*, 1991), their coupling capacity to G-protein was assessed in the DRN of naïve *Htr3a* KO and WT mice by measuring [³⁵S]-GTP-γ-S binding after stimulation with the 5-HT_{1A} receptor agonist 5-carboxamidotryptamine (5-CT). A two-way ANOVA revealed a significant effect of genotype [$F(1,40) = 13.37$, $P = 0.0007$], linked to an increased [³⁵S]-GTP-γ-S binding of about 30% for each 5-CT concentration (Figure 1B). No 5-

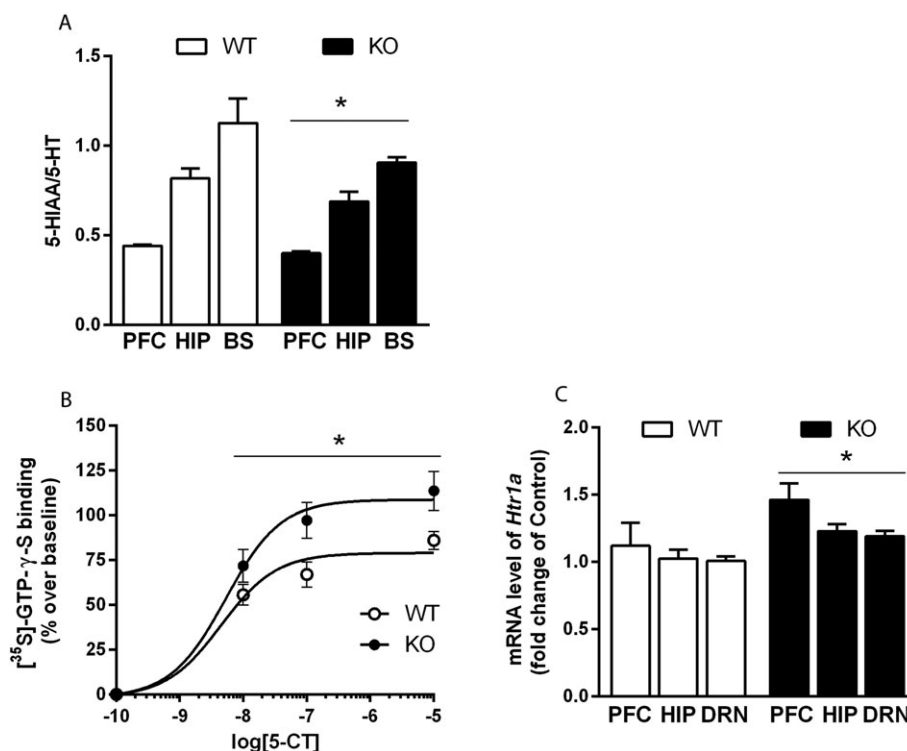


Figure 1

Effect of 5-HT₃ receptor deletion on 5-HT_{1A} receptor function and 5-HT turnover (A) 5-HT turnover, determined by the 5-HIAA/5-HT ratio, was evaluated in the prefrontal cortex, the hippocampus and the brainstem of naïve *Htr3a* KO and WT mice. 5-HT turnover was significantly decreased in *Htr3a* KO compared with WT mice. Each bar was the mean ± SEM of $n = 7$ WT and $n = 6$ KO mice. $*P < 0.05$, effect of genotype; two-way ANOVA. BS, brain stem. (B) 5-HT_{1A} receptor coupling to G protein was assessed in the DRN of naïve *Htr3a* KO and WT mice using quantitative autoradiography for measuring 5-HT_{1A} receptor-stimulated [³⁵S]-GTP-γ-S binding. Binding of [³⁵S]-GTP-γ-S was increased in the DRN of *Htr3a* mutants compared with WT mice. Each bar was the mean ± SEM of $n = 6$ mice per group. $*P < 0.05$, effect of genotype, two-way ANOVA. (C) 5-HT_{1A} receptor gene (*Htr1A*) mRNA expression was determined by quantitative qRT-PCR in the raphe, the prefrontal cortex and the hippocampus of naïve *Htr3a* KO and WT mice. *Htr1A* expression was higher in *Htr3a* KO compared with WT mice. Each bar was the mean ± SEM of $n = 10$ WT and $n = 9$ KO mice. $*P < 0.05$, effect of genotype; two-way ANOVA. β-actin was used as housekeeping gene. PFC, prefrontal cortex; HIP, hippocampus.

CT concentration × genotype interaction was found [$F(3,40) = 1.85$, $P = 0.15$].

The expression of 5-HT_{1A} receptor mRNA (*Htr1A*) was also quantified by qRT-PCR in several brain regions of naïve *Htr3a* KO and WT mice. Although there was no brain structure × genotype interaction [$F(2,53) = 0.39$, $P = 0.68$], a two-way ANOVA revealed a significant effect of genotype [$F(2,53) = 13.88$, $P = 0.003$], associated with an increased *Htr1A* expression in each brain structure (+23.4%, prefrontal cortex; +18.5%, hippocampus; +16.7%, raphe; Figure 1C).

Effect of 5-HT₃ receptor deletion on anxiety-related behaviours

The phenotype of naïve *Htr3a* KO versus WT mice was assessed in tests relevant to anxiety-related behaviours. In the EPM (Figure 2A), *Htr3a* KO mice displayed less anxiety-like behaviours, defined as a two times higher exploration and time spent in the open arms. Locomotor activity in the entire maze was similar in the two groups (Figure S1A).

In the SI test, *Htr3a* KO mice showed an increased time spent in active social interactions compared with control mice (Figure 2B), suggesting again an anxiolytic-like phenotype. This effect was not related to an increase in

locomotion because activity of the two groups did not differ in the open field test (Figure S1B). In addition, in various group of mice subjected to the CSDS procedure for 10 days and submitted to the latter test, a two-way ANOVA revealed a significant effect of stress (number of entries in the centre, $F(1,47) = 37.87$, $P < 0.0001$; time spent in the centre, $F(1,47) = 19.80$, $P < 0.0001$ and locomotor activity, $F(1,47) = 80.00$, $P < 0.0001$), but no effect of genotype and no significant interaction (Figures 2C and S1B).

Effect of 5-HT₃ receptor deletion on acute SSRI-induced antidepressant-like response and in depression-related behavioural tests

Behaviours of naïve *Htr3a* KO and WT mice were evaluated in the FST: *Htr3a* KO mice spent less time immobile than WT mice (Figure 3A, left).

Htr3a KO and WT mice responses to acute antidepressant treatments were investigated in this test using two different SSRI: citalopram, a highly selective compound (Hyttel, 1982) in order to block 5-HTT only, and fluoxetine, which possesses moderate 5-HT₃ receptor antagonist properties (Fan, 1994). Indeed, different classes of antidepressants such as fluoxetine act as functional antagonists at the

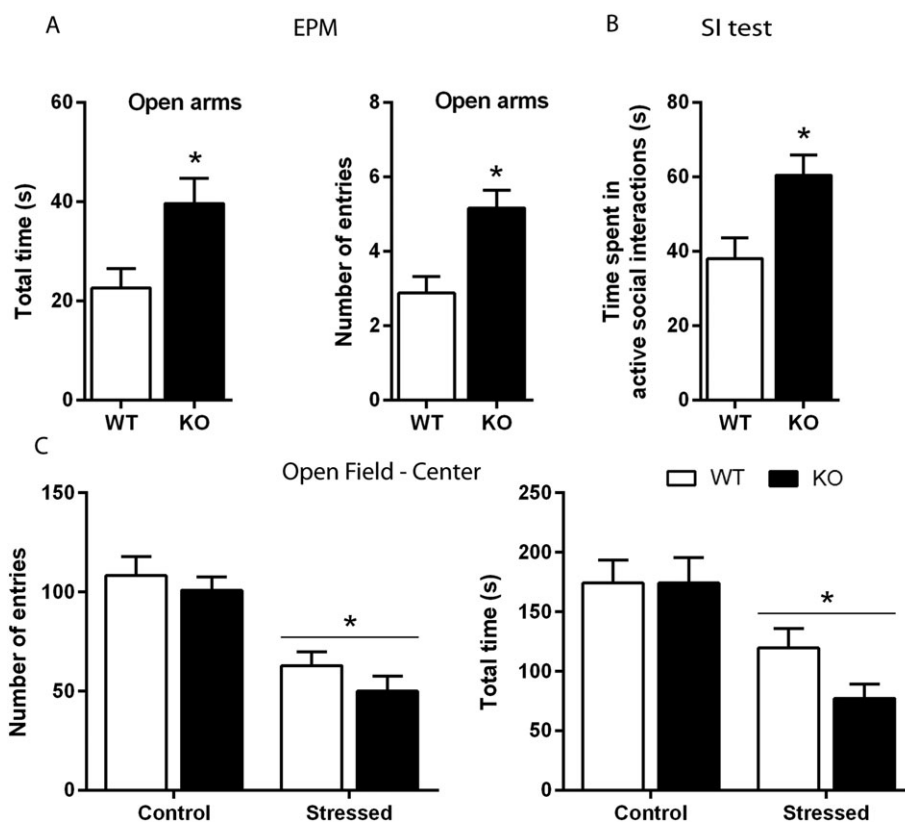


Figure 2

Effect of 5-HT₃ receptor genetic invalidation on anxiety-related behaviours in naïve and socially defeated mice. (A) Naïve *Htr3a* KO and WT mice were placed for 10 min in an EPM. The exploration of the open arms (measured as the time spent and the number of entries) was higher in *Htr3a* KO compared with WT animals. Each bar was the mean \pm SEM of $n = 8$ WT and $n = 6$ KO mice. $*P < 0.05$, 'WT' versus 'KO', Student's unpaired *t*-test. (B) The total time spent in active social interactions during a 10 min period was significantly increased in *Htr3a* KO compared with paired WT mice. Each bar was the mean \pm SEM of $n = 7$ WT and $n = 9$ KO mice. $*P < 0.05$ 'WT' versus 'KO', Student's unpaired *t*-test. (C) After 10 days of CSDS, there was a similar decrease in the exploration of the centre of an open field in both *Htr3a* KO and WT mice, as shown by the diminution in the number of entries and time spent in this zone. Each bar was the mean \pm SEM. of $n = 11$ Control WT, 12 Stressed WT, 11 Control KO and 15 Stressed KO mice. $*P < 0.05$, effect of stress, two-way ANOVA.

human 5-HT_{3A} receptor stably expressed in HEK 293 cells and at endogenous 5-HT₃ receptors of rat hippocampal neurons and of mouse N1E-115 neuroblastoma cells (Eisensamer *et al.*, 2003). A two-way ANOVA revealed a significant effect of treatment [FST, $F(2,49) = 6.25$, $P = 0.03$] in mice acutely treated with citalopram (2.5 and 5.0 mg·kg⁻¹ i.p.). There was no significant effect of genotype or treatment \times genotype interaction suggesting a similar response to acute citalopram in *Htr3a* KO and WT (Figure 3A, middle). In mice acutely treated with fluoxetine (20 mg·kg⁻¹, i.p.), there was a significant treatment \times genotype interaction [$F(1,28) = 10.04$, $P < 0.004$] in the FST (Figure 3A, right), as fluoxetine induced a decrease in the immobility time in WT mice, but not *Htr3a* KO mice ($P < 0.05$ and $P > 0.05$, respectively, two-way ANOVA followed by Bonferroni *post hoc* test).

In groups of mice subjected to the CSDS procedure for 10 days, coat state scoring showed a significant effect of stress in both *Htr3a* KO and WT mice [$F(1,47) = 71.65$, $P < 0.0001$, effect of stress, two-way ANOVA], but no effect of genotype [$F(1,47) = 0.71$, $P = 0.40$] or stress \times genotype interaction [$F(1,47) = 0.007$, $P = 0.93$] (Figure 3B).

In the splash test (Figure 3C), a two-way ANOVA indicated a significant overall effect of stress on time spent grooming, which was increased in both stressed *Htr3a* KO and WT mice [$F(1,45) = 5.64$, $P = 0.02$]. There was no effect of genotype [$F(1,45) = 2.57$, $P = 0.12$] or stress \times genotype interaction [$F(1,45) = 1.06$, $P = 0.31$]. However, a significant stress \times genotype interaction [$F(1,44) = 5.69$, $P = 0.02$] in the latency to groom was found. Bonferroni *post hoc* test revealed that the latency to groom was lower in control *Htr3a* KO compared with WT mice. CSDS shortened this latency in stressed WT mice but, interestingly, not in mutant animals.

In addition, in the social avoidance test performed 24 h after the CSDS procedure, a two-way ANOVA on the time spent in the interaction zone with the target present did not show any stress \times genotype interaction nor a significant stress effect [$F(1,45) = 0.52$, $P = 0.47$ and $F(1,45) = 1.52$, $P = 0.23$, respectively]. Nevertheless, there was a significant effect of genotype [$F(1,45) = 4.86$, $P = 0.03$], as both non-stressed and stressed *Htr3a* KO mice spent more time interacting with the target than paired controls, suggesting again an anxiolytic-like behaviour (Table S1).

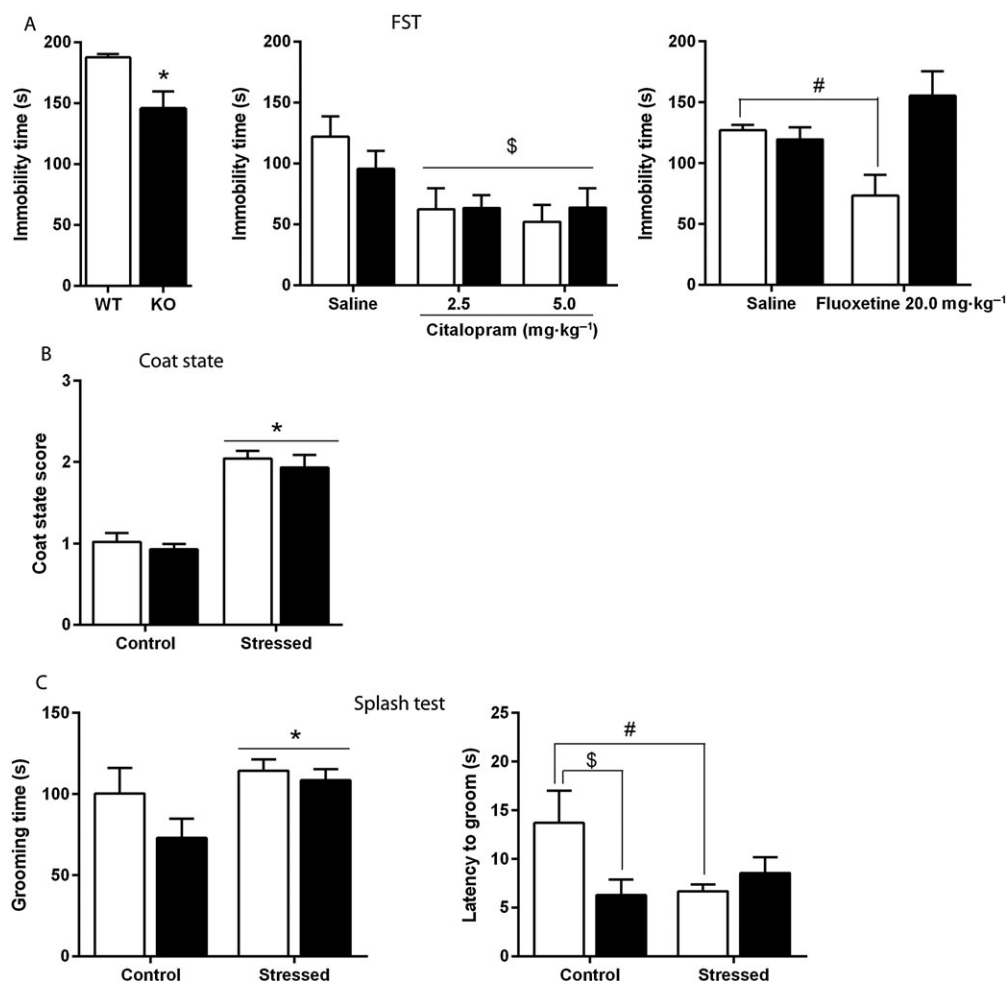


Figure 3

Effect of 5-HT₃ receptor deletion on depression-related behaviours in naïve, SSRI-treated and socially defeated mice. (A) At basal level, *Htr3a* KO mice were less immobile than WT mice in the FST (A, left). Acute administrations of citalopram (2.5, 5.0 mg·kg⁻¹ i.p.) induced a decreased immobility in both genotypes, and no differences were found between mutants and WT mice (A, middle). When injected with fluoxetine (20.0 mg·kg⁻¹ i.p.), WT mice displayed less immobility than saline-treated paired controls, but this effect was absent in *Htr3a* KO mice (A, right). Each bar is the mean ± SEM of *n* = 12 naïve WT, 14 naïve KO (A, left); 7 saline-treated WT, 9 citalopram 2.5-treated WT, 9 citalopram 5.0-treated WT, 13 saline-treated KO, 8 citalopram 2.5-treated KO, 9 citalopram 5.0-treated KO (A, middle); 8 saline-treated WT, 9 fluoxetine-treated WT, 8 saline-treated KO and 7 fluoxetine-treated KO mice (A, right). **P* < 0.05, 'WT' versus 'KO', Student's unpaired *t*-test; \$*P* < 0.05, effect of treatment, two-way ANOVA; #*P* < 0.05, 'WT fluoxetine 20 mg·kg⁻¹' versus 'WT saline', two-way ANOVA followed by Bonferroni *post hoc* test. (B) After 10 days of CSDS, the coat state score was equally increased in stressed *Htr3a* KO and WT mice. Each bar was the mean ± SEM of *n* = 11 Control WT, 12 Stressed WT, 11 Control KO and 15 Stressed KO mice. **P* < 0.05, effect of stress, two-way ANOVA. (C) After 10 days of CSDS, the time spent in grooming in a 5 min period following sucrose solution spraying (splash test) was increased in stressed animals (C, left). As for the latency to groom (C, right), a two-way ANOVA analysis revealed a significant stress × genotype interaction (*P* < 0.05). Grooming was lower in *Htr3a* KO compared with WT non-stressed mice. CSDS induced a significant decrease in the latency to groom in WT mice, but no change in mutant animals. Each bar was the mean ± SEM of *n* = 11 Control WT, 11 Stressed WT, 11 Control KO and 14 Stressed KO mice. **P* < 0.05 effect of stress, two-way ANOVA; \$*P* < 0.05, 'Control WT' versus 'Control KO'; #*P* < 0.05, 'Control WT' versus 'Stressed WT', two-way ANOVA followed by Bonferroni *post hoc* test.

Effect of 5-HT₃ receptor deletion on 5-HT neuronal activity and on chronic antidepressant-induced responses

Basal serotonergic neuronal firing activity was recorded in brainstem slices containing the DRN. This activity did not differ between naïve *Htr3a* KO and WT mice (Figure 4A). Ipsapirone, a 5-HT_{1A} receptor agonist, that induce about 50% firing inhibition of DRN 5-HT neurons at a dose 60 nM (EC₅₀; Lanfumey *et al.*, 1999), produced a similar

effect in brainstem slices of *Htr3a* KO and WT mice (Figure 4B).

Htr3a KO and WT animals were treated with citalopram (5 or 20 mg·kg⁻¹·day⁻¹, i.p.) or saline for 14 days, and the activity of 5-HT neurons in response to ipsapirone 60 nM was assessed. As illustrated in Figure 4C, a two-way ANOVA showed a significant interaction between genotype and citalopram treatment [*F*(2,30) = 4.24, *P* = 0.02]. Bonferroni *post hoc* tests showed that while a 14 day treatment with 20 mg·kg⁻¹·day⁻¹ citalopram significantly decreased the

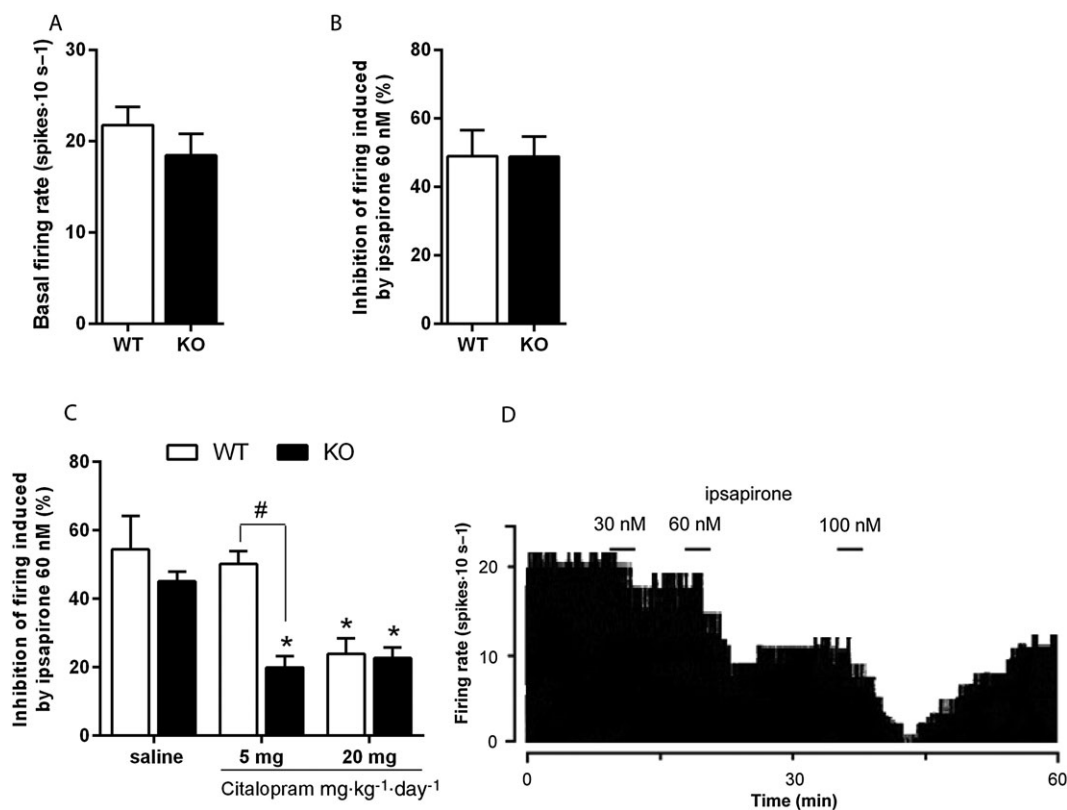


Figure 4

Effect of 5-HT₃ receptor deletion on 5-HT_{1A} receptor function under chronic citalopram. In basal conditions, no difference in either the basal firing rate of DRN serotonergic neurons (A) or the efficacy of ipsapirone 60 nM (B) to inhibit their activity was observed between naïve *Htr3a* KO and WT mice. Each bar was the mean \pm SEM of $n = 13$ WT and 10 KO cells (five WT and four KO mice). (C) Effects of chronic citalopram treatment. In *Htr3a* KO mice, a 14 day treatment with citalopram with either a 5 or 20 mg·kg⁻¹·day⁻¹ regimen decreased 5-HT firing inhibition induced by ipsapirone 60 nM, while in WT mice, only the 20 mg·kg⁻¹·day⁻¹ regimen decreased ipsapirone-induced inhibition of firing. Each bar was the mean \pm SEM of $n = 6$ saline-treated WT, 7 citalopram 5-treated WT, 5 citalopram 20-treated WT, 4 saline-treated KO, 6 citalopram 5-treated KO and 9 citalopram 20-treated KO cells (respectively 5, 6, 4, 4, 5 and 6 mice). * $P < 0.05$, 'Citalopram' versus paired 'Saline' and # $P < 0.05$, 'KO mice treated with citalopram 5 mg·kg⁻¹·day⁻¹' versus paired 'WT mice', two-way ANOVA followed by Bonferroni *post hoc* test. (D) Integrated firing rate histogram (in spikes·10 s⁻¹) showing the effect of increasing concentrations of ipsapirone on the electrical activity of a DRN 5-HT neurons.

effect of ipsapirone 60 nM in both *Htr3a* KO and WT mice, 5 mg·kg⁻¹·day⁻¹ citalopram for 14 days decreased the inhibitory effect of ipsapirone in *Htr3a* KO mice only, suggesting that at this low dose, citalopram more effectively desensitizes 5-HT_{1A} receptor in the mutant mice.

Effect of 5-HT₃ receptor deletion on weight alterations and CSDS-induced cellular stress

In mice subjected to the CSDS procedure, adrenal weight was significantly greater [$F(1,37) = 46.27$, $P < 0.0001$, two-way ANOVA, effect of stress] than in non-stressed mice for both genotypes (Figure S2A).

Difference in body weight between D1 and D11 (Figure S2B) showed a significant stress \times genotype interaction [$F(1,44) = 4.69$, $P = 0.04$]. Bonferroni *post hoc* test showed that stressed WT mice displayed a gain of body weight compared with non-stressed WT mice, an effect absent in mutant mice.

The effect of CSDS on the 5-HT_{3A} subunit gene (*Htr3a*) mRNA expression in WT mice was evaluated using qRT-PCR in various brain regions including the NTS, a region strongly

enriched in 5-HT_{3A}R (Laporte *et al.*, 1992). A two-way ANOVA revealed a significant stress \times brain area interaction [$F(2,48) = 10.25$, $P = 0.02$] in *Htr3a* gene expression. *Post hoc* test showed that *Htr3a* was significantly up-regulated in the NTS of stressed mice (Table 1).

Table 1

5-HT₃ receptor subunit 3A gene expression after CSDS in WT mice

	Control WT	Stressed WT
Hippocampus	1.00 \pm 0.03	1.03 \pm 0.03
Prefrontal Cortex	1.00 \pm 0.04	1.14 \pm 0.04
NTS	1.01 \pm 0.07	1.53 \pm 0.15 ^a

A two-way ANOVA revealed a significant stress \times brain area interaction in *Htr3a* expression, and a *post hoc* test showed that *Htr3a* was significantly up-regulated in the NTS. Each value was the mean \pm SEM of $n = 7$ Control and 10 Stressed WT mice.

^a $P < 0.05$ 'Control WT' versus 'Stressed WT', two-way ANOVA followed by Bonferroni *post hoc* test.

The mRNA expression of *CaMKIIa* and *SOD1*, genes linked to oxidative stress and regulated by 5-HT₃ receptors (Bhatt *et al.*, 2014; Hutchinson *et al.*, 2015), was also quantified using qRT-PCR in CSDS-subjected *Htr3a* KO and WT mice prefrontal cortex, a region commonly studied in relation with depression-related behaviours (Ressler and Mayberg, 2007). A two-way ANOVA showed a significant stress × genotype interaction [$F(1,41) = 5.32$, $P = 0.03$] in *CaMKIIa* gene expression. While the Bonferroni *post hoc* test revealed that *CaMKIIa* mRNA level was higher in stressed compared with control WT mice, there was no difference in *Htr3a* KO mice (Figure 5A). Analysis of the *SOD1* gene expression also revealed a significant stress × genotype interaction [$F(1,33) = 6.69$, $P = 0.01$]. *Post hoc* analyses showed that *SOD1* mRNA level was lower in control KO than in control WT mice and that stress induced a down-regulation of this gene only in WT animals (Figure 5B).

Discussion

The pivotal role of 5-HT_{1A} receptors in both depression-related behaviours and antidepressant responses have well been established several years ago (see in Celada *et al.*, 2013); however, that of 5-HT₃ receptors remained controversial. The present data clearly show its involvement in anxiety- and depression-related behaviours. Furthermore, they suggest that SSRI treatments may be effective at lower doses when 5-HT₃ receptors are inactivated. 5-HT₃ receptors are thus of considerable interest in the development of new therapeutic strategies with lower levels of 5-HT reuptake inhibition to increase treatment tolerability while preserving maximal effectiveness (Jakubovski *et al.*, 2016).

Effect of the 5-HT_{3A} receptor mutation on anxiety- and depression- related behaviours

The present series of behavioural explorations conducted in *Htr3a* KO mice generally confirmed that genetic inactivation

of 5-HT₃ receptors induces an anxiolytic-like phenotype (Kelley *et al.*, 2003; Bhatnagar *et al.*, 2004), consistent with the anxiolytic properties of 5-HT₃ receptor antagonists (Mahesh *et al.*, 2013). Nevertheless, the anxiogenic effect generated by social defeat was not altered by the *Htr3a* mutation. *Htr3a* KO mice displayed less immobility behaviour than WT mice in the FST, consistently with the effect of 5-HT₃ receptor antagonists in these tests used to screen antidepressant-like activity (Ramamoorthy *et al.*, 2008; Bétry *et al.*, 2015). Therefore, the bulk of data show that 5-HT₃ receptor inactivation can lead to both anxiolytic- and antidepressant-like behaviours. As expected under acute treatment conditions, the highly selective SSRI citalopram reduced immobility in the FST, and this effect was not altered by the genotype, contrasting with previous studies that had shown that 5-HT₃ receptor antagonists potentiate behavioural effects induced by various SSRIs in the mice FST (Redrobe and Bourin, 1997; Ramamoorthy *et al.*, 2008). Constitutive KO cannot be considered always similar to acute receptor blockade, as is the case with 5-HT₃ receptor antagonists such as **ondansetron** (Saynor and Dixon, 1989). Several 5-HT system compensations, like those documented in the present study at the 5-HT_{1A} receptor level, could intervene during the development of mutant mice (Rudmann and Durham, 1999).

In the FST, fluoxetine induced a significant antidepressant effect only in WT but not in *Htr3a* KO mice. Interestingly, in addition to its 5-HTT blocking effect, fluoxetine, in contrast to citalopram, possesses 5-HT₃ receptor antagonist properties within the micromolar affinity range (Fan, 1994). This suggests that in these conditions of treatment, the antidepressant-like activity of fluoxetine in the FST is at least partly mediated by 5-HT₃ receptors. This result finds an echo in the work of Smit-Rigter *et al.* (2012), in which the effect of prenatal exposure to fluoxetine was blunted in *Htr3a* KO mice in the novelty-suppressed feeding test, a paradigm frequently used to screen the anxiolytic properties of antidepressant treatments.

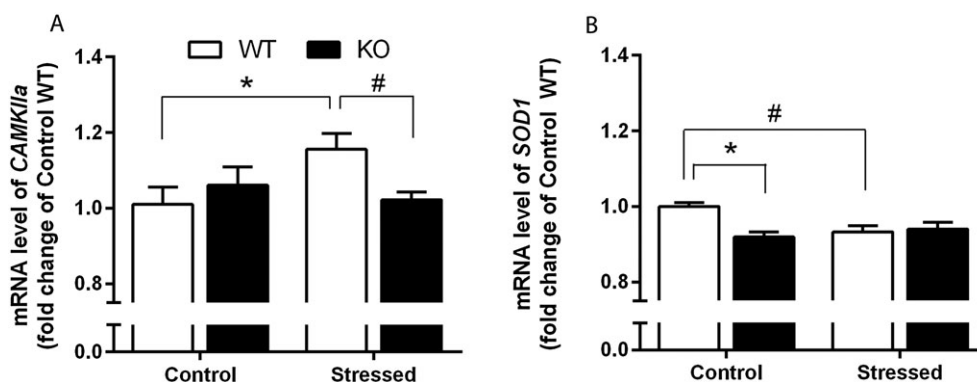


Figure 5

Effect of CSDS on oxidative stress-related gene expression. (A) CSDS increased *CaMKIIa* mRNA level in WT mice but not in *Htr3a* KO mice. Each bar was the mean ± SEM of $n = 9$ Control WT, 14 Stressed WT, 10 Control KO and 14 Stressed KO mice. * $P < 0.05$, 'Control WT' versus 'Stressed WT'; # $P < 0.05$, 'Stressed WT' versus 'Stressed KO', two-way ANOVA followed by Bonferroni *post hoc* test. β -actin was used as housekeeping gene. (B) *SOD1* mRNA level was significantly lower in control KO compared with paired WT mice. CSDS decreased *SOD1* gene expression in WT animals but not in mutant mice. Each bar was the mean ± SEM. $n = 6$ Control WT, 13 Stressed WT, 8 Control KO and 11 Stressed KO mice. * $P < 0.05$, 'Control WT' versus 'Control KO'; # $P < 0.05$, 'Control WT' versus 'Stressed WT', two-way ANOVA followed by Bonferroni *post hoc* test. β -actin was used as housekeeping gene.

We have also assessed a depression-like behaviour using the stress-induced fur coat state alteration (Smolinsky *et al.*, 2009). The *Htr3a* mutation did not affect the stress-induced behavioural alteration in this paradigm. In the splash test, CSDS induced an overall stimulating effect in grooming in both *Htr3a* KO and WT animals. These data are consistent with those describing an increase of self-grooming behaviour in socially defeated rodents (Smolinsky *et al.*, 2009; Denmark *et al.*, 2010), whereas chronic mild stress is usually followed by a decrease in grooming (Bouille *et al.*, 2014) and/or an increased latency for the first grooming event (Siopi *et al.*, 2016). Interestingly, the later parameter was reduced in WT mice, but not in *Htr3a* KO stressed mice, suggesting that 5-HT₃ receptor genetic inactivation reduced the sensitivity to social defeat stress.

Effect of the 5-HT_{3A} receptor mutation on chronic antidepressant treatment responses

The delay for SSRI-induced clinical efficacy is partly linked to the time course of somatodendritic 5-HT_{1A} receptor desensitization (Le Poul *et al.*, 1995), and 5-HT₃ receptor blockade has recently been proposed to accelerate this phenomenon (Bétry *et al.*, 2013). We thus conducted *in vitro* single cell recording to directly measure 5-HT_{1A} receptor responses in *Htr3a* KO mice.

First, we showed that 5-HT₃ receptor invalidation affected neither basal 5-HT neuronal firing nor its response to 5-HT_{1A} receptor stimulation, which is in accord with previous studies where 5-HT₃ receptor antagonists did not modify DRN 5-HT neuron firing (Adrien *et al.*, 1992). However, this conclusion contrasts with previous results indicating that 5-HT₃ receptor stimulation impaired 5-HT_{1A} receptor function in mice (Kondaurova *et al.*, 2012) and with the data obtained here at the mRNA expression and G-protein coupling levels. Indeed, our receptor expression studies demonstrated a 5-HT_{1A} receptor up-regulation in *Htr3a* KO mice, associated with both a higher degree of coupling between 5-HT_{1A} receptors and G proteins within the DRN and a decrease in 5-HT turnover. The 5-HT_{1A} receptor up-regulation observed in *Htr3a* KO mice was not translated at the neural activity level under the ipsapirone challenge. The electrophysiological response of raphe 5-HT cells is not exclusively related to 5-HT_{1A} autoreceptor activity, as it is also driven by the G-protein-gated inwardly rectifying K⁺ (GIRK) channels coupled to these receptors (Haj-Dahmane *et al.*, 1991), and thus, a regulation at GIRK itself may have compensated for 5-HT_{1A} receptor up-regulation. Further experiments are necessary to address this issue.

Although not modified by the mutation at basal level, 5-HT_{1A} receptor sensitivity was shown to be differently affected by chronic citalopram treatment. While 5 mg·kg⁻¹ × 14 days citalopram treatment failed to produce any change in 5-HT_{1A} receptor sensitivity in WT mice, it did produce strong 5-HT_{1A} receptor desensitization in *Htr3a* KO mice. These results are in line with those of Bétry *et al.* (2013) who have hypothesized that the fast action of vortioxetine, an SSRI with strong 5-HT₃ receptor antagonist properties, is mediated by its ability to block 5-HT₃ receptors. Moreover, a recent *in vivo* electrophysiological study in rats revealed that a 5-HT₃ receptor antagonist (ondansetron) is

able to counteract the 5-HT_{1A} receptor-mediated inhibitory effect of the SSRI **paroxetine** on DRN 5-HT neurons basal firing (Bétry *et al.*, 2015). The latter and present data both argue for an important regulatory action of 5-HT₃ receptors on the 5-HT_{1A} receptor-desensitization process induced by SSRI.

Together with Bétry *et al.* (2013), we therefore suggest a synergistic effect of 5-HT₃ receptor blockade on SSRI-induced 5-HT_{1A} receptor desensitization occurring under low 5-HTT inhibition. Vortioxetine is able – in contrast to classical SSRIs – to increase 5-HT concentrations at low levels of 5-HTT occupancy (Pehrson *et al.*, 2013). In our conditions, 5-HT_{1A} receptor desensitization occurred in *Htr3a* KO mice, not WT mice, under the low-dose citalopram regimen (5 mg·kg⁻¹·day⁻¹). The amplitude of this desensitization was similar to that observed in WT mice with the 20 mg·kg⁻¹·day⁻¹ citalopram regimen (Martin *et al.*, 2015), commonly used in preclinical studies (Calcagno and Invernizzi, 2010). Actually, Ferrés-Coy *et al.* (2013) demonstrated that short-term partial reduction of 5-HTT expression by small interference RNA led to decreased in both 5-HT_{1A} autoreceptor expression and function. Interestingly, these effects appeared earlier and were of greater amplitude than those induced by fluoxetine at high dose. Therefore, we can hypothesize that 5-HT₃ receptor inactivation may possibly affect 5-HTT expression or internalization, leading to faster SSRI-induced effects on 5-HT neurotransmission when administered at low dose only.

Effect of the 5-HT_{3A} receptor mutation on CSDS-induced cellular stress and weight alterations

To further explore the role of 5-HT receptors in response to stress, we assessed the metabolic and molecular consequences of a CSDS in both *Htr3a* KO and WT mice.

The increased weight gain observed after CSDS in WT mice is consistent with most of the data reported in the literature (Goto *et al.*, 2014). Interestingly, this weight gain was blunted by the 5-HT₃ receptor KO, similar to what has been observed with 5-HT₃ receptor antagonists that reversed weight gain in response to chronic mild stress (Gupta *et al.*, 2014). Interestingly, the *Htr3a* KO had reduced weight gain in mice fed with a high-fat diet, suggesting a higher endogenous metabolism in *Htr3a* KO mice (Oh *et al.*, 2015).

In WT mice, CSDS up-regulated *Htr3a* in the NTS, a structure particularly enriched in 5-HT₃ receptors in the mouse brain (Laporte *et al.*, 1992) and involved in stress-related changes in feeding (Lam *et al.*, 2009).

In addition, CSDS triggered significant modulations of 5-HT₃ receptor-related markers involved in the cellular stress response, that is, the antioxidant enzyme SOD1, a marker of oxidative stress shown to be reversed by 5-HT₃ receptor antagonists (Bhatt *et al.*, 2014; Kurhe *et al.*, 2014) and the CaMKII α , recently found to be involved in 5-HT₃ receptor signalling (Hutchinson *et al.*, 2015). In the prefrontal cortex, a structure known to be involved in mood disorders (Ressler and Mayberg, 2007), CSDS down-regulated *SOD1* in WT mice, as expected from previous stress studies (Bhatt *et al.*, 2014; Kurhe *et al.*, 2014). The decrease in the levels of this antioxidant protective enzyme in stressed mice suggests that

stress could induce dysfunction in the antioxidant defence system. Interestingly, these metabolic CSDS effects were not observed in mutant *Htr3a* KO mice. These data are consistent with those showing that 5-HT₃ receptor antagonists can reverse chronic stress effects on both SOD1 expression and enzymatic activity in rodent brain (Bhatt *et al.*, 2014; Kurhe *et al.*, 2014), supporting the notion of a beneficial effect of the 5-HT₃ receptor inactivation in preventing stress-induced oxidative stress damages. Moreover, Uchihara *et al.* (2016) recently demonstrated that in mice, *SOD1* overexpression prevented depressive-like phenotype induced by chronic **corticosterone** administration. Although, in our study, *Htr3a* deletion did not prevent all CSDS-induced anxiety-depressive phenotypes, it can be suggested that its protective effect on *SOD1* expression may have contributed to the resistance of *Htr3a* KO mice to CSDS that we described earlier.

Regarding *CaMKIIa*, its expression was increased in socially defeated WT but not in *Htr3a* KO mice. Although the role of CaMKIIa in the responses to stress and to antidepressant drugs remains unclear, it is known to be activated by oxygen radicals, and this hyperactivation is suspected to play an important role in the development of several peripheral pathologies (Robison, 2014). Furthermore, excessive CaMKIIa activity contributes to oxygen radical formation (Anderson, 2015). The absence of stress-induced CaMKIIa mRNA up-regulation in *Htr3a* KO mice could prevent the oxygen radical formation induced by stress in these mice (Hutchinson *et al.*, 2015), corroborating the role of 5-HT₃ receptors in oxidative-related stress effects.

In summary, our data showed that the genetic inactivation of 5-HT₃ receptors promoted the efficacy of a low dose of SSRI to trigger 5-HT_{1A} receptor desensitization in the DRN. This is consistent with the anxiolytic- and antidepressant-like effects of deleting 5-HT₃ receptors. In the chronic social stress depressiogenic-like condition, we observed an up-regulation of the *Htr3a* subunit, while deletion of this subunit prevented the modification of oxidative stress markers. Although some caution should be taken when studying unconditional KO, as they might not reflect entirely an acute gene deletion, these results underline the role of 5-HT₃ receptors in stress-related disorders and the mechanism of action of antidepressant drugs.

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Author contributions

V.M., R.M. and L.L. conceived and designed the experiments. V.M., A.R., C.B. and J.P.T. performed the experiments. T.M. and B.F. provided technical support. C.S.C. analysed the data. V.M., R.M. and L.L. wrote the manuscript. All authors reviewed the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research recommended by funding agencies, publishers and other organisations engaged with supporting research.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

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Table S1 Social avoidance test. In the social avoidance test, there were no differences in terms of time interacting with the target between stressed and control animals. However, non-stressed and stressed Htr3a KO mice spent significantly more time than paired WT mice in the interaction zone when the target was present. Each value was the mean \pm S.E.M. of $n = n = 11$ Control WT, 12 Stressed WT, 11 Control KO and 15 Stressed KO mice. * $P < 0.05$, effect of genotype, 2-way ANOVA.

Figure S1 A: Locomotor activity in the elevated plus maze. Animals from both genotypes displayed a similar locomotion in the EPM. Each bar was the mean \pm S.E.M. of $n = 8$ WT and 6 KO mice. B: Locomotion of Htr3a KO and WT mice submitted to CSDS in the open field. Both stressed mutant and wild-type animals displayed a similar hypolocomotion in the open field. Each bar was the mean \pm S.E.M. of $n = 11$ Control WT, 12 Stressed WT, 11 Control KO and 15 Stressed KO mice. * $P < 0.05$, effect of stress, 2-way ANOVA.

Figure S2 Effect of CSDS on body and adrenal weight. (A) Effect of stress on adrenal gland weight. Stress similarly increased adrenal gland weight in both genotypes. There was no effect of genotype nor an interaction between the stress and genotype factors. Each bar was the mean \pm S.E.M. of $n = 11$ Control WT, 12 Stressed WT, 11 Control KO and 15 Stressed KO mice. * $P < 0.05$, effect of stress (two-way ANOVA). (B) Body weight gain was higher in stressed WT compared to non-stressed paired mice. Stress had no effect in Htr3a KO mice. Each bar was the mean \pm S.E.M. of $n = 11$ Control WT, 12 Stressed WT, 11 Control KO and 15 Stressed KO mice. * $P < 0.05$, 'Control WT' versus 'Stressed WT', # $P < 0.05$, 'Stressed WT' versus 'Stressed KO', 2-way ANOVA followed by Bonferroni *post hoc* test.