Genetic dysfunction of serotonin 2A receptor hampers response to antidepressant drugs: A translational approach

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A R T I C L E   I N F O

Article history:
Received 25 September 2015
Received in revised form 23 December 2015
Accepted 24 December 2015
Available online 4 January 2016

Keywords:
Antidepressant
Serotonin transporter
Electrophysiology
Genetic
Hippocampus
MDD: major depressive disorder
MDE: major depressive episode
Polymorphism
SSRI
5-HT2A receptor
5-HT1A receptor
5-HT transporter

A B S T R A C T

Pharmacological studies have yielded valuable insights into the role of the serotonin 2A (5-HT2A) receptor in major depressive disorder (MDD) and antidepressant drugs (ADs) response. However, it is still unknown whether genetic variants in the HTR2A gene affect the therapeutic outcome of ADs and the mechanism underlying the regulation of such response remains poorly described. In this context, a translational human-mouse study offers a unique opportunity to address the possibility that variations in the HTR2A gene may represent a relevant marker to predict the efficacy of ADs.

In a first part of this study, we investigated in depressed patients the effect of three HTR2A single nucleotide polymorphisms (SNPs), selected for their potential functional consequences on 5-HT2A receptor (rs6313, rs6314 and rs7333412), on response and remission rates after 3 months of antidepressant treatments. We also explored the consequences of the constitutive genetic inactivation of the 5-HT2A receptor (i.e. in 5-HT2A−/− mice) on the activity of acute and prolonged administration of SSRIs.

Our clinical data indicate that GG patients for the rs7333412 SNP were less prone to respond to ADs than AA/AG patients. In the preclinical study, we demonstrated that the 5-HT2A receptor exerts an inhibitory influence on the neuronal activity of the serotonergic system after acute administration of SSRIs. However, while the chronic administration of the SSRIs escitalopram or fluoxetine elicited a progressive increase in the firing rate of 5-HT neurons in 5-HT2A−/− mice, it failed to do so in 5-HT2A+/− mutants. These electrophysiological impairments were associated with a decreased ability of the chronic administration of fluoxetine to stimulate hippocampal plasticity and to produce antidepressant-like activities.

Genetic loss of the 5-HT2A receptor compromised the activity of chronic treatment with SSRIs, making this receptor a putative marker to predict ADs response.

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1. Introduction

Genetic factors have been shown to influence antidepressant drugs (ADs) response in major depressive disorder (MDD) (Klengel and Binder, 2013). In particular, variations in the gene encoding for the serotonin 2A (5-HT2A) receptor subtype have been associated...
with clinical outcome after antidepressant treatment. A recent meta-analysis (Lin et al., 2014) pointed out an association between the C allele of rs6313 and ADs response but also between the A allele of rs7997012 and the percentage of responders, as shown in the STAR*D study (McMahon et al., 2006). Naturalistic studies, in which all classes of ADs were administered using “real-world” treatment options revealed significant genotypic associations with remission for different SNPs of HTR2A (Fabbri et al., 2014; Horstmann et al., 2010; Noro et al., 2010). Interestingly, an association with rs7333412, known to be in linkage disequilibrium with rs7997012 has been recently proposed (Fabbri et al., 2014) and such findings have also been observed specifically with SSRIs (Choi et al., 2005; Kato et al., 2009; Wilkie et al., 2009; Kishi et al., 2010; Viikki et al., 2011). Despite these reports, negative results have been yielded (Lucae et al., 2010; Niitsu et al., 2013; Serretti et al., 2013) thereby requiring additional investigations to provide definitive conclusion. In addition to this lack of clear-cut evidence, association studies are of limited value owing to the lack of information about the consequences of SNP on 5-HT2A receptors expression and/or function. To circumvent this issue, studies aimed at exploring 5-HT2A receptor expression using positron emission tomography or post-mortem mRNA analysis were conducted in ADs-treated depressed patients. Again, the conclusions remain somewhat equivocal since some investigators reported decreased expression (Dean et al., 2014; Muguruza et al., 2014) while others found enhanced expression (Massou et al., 1997; Zanardi et al., 2001) or no difference compared to controls (Messa et al., 2003). Consequently, it is at this point uncertain whether a lower or a higher neurotransmission at the 5-HT2A receptor has an impact on clinical response to ADs.

Preclinical studies are of great relevance to further elucidate the role of the 5-HT2A receptor in the regulation of the serotonergic system and ADs response. In vivo recordings in the dorsal raphe nucleus (DRN) show that systemic administration of the preferential 5-HT2A receptor agonist DOI attenuates the firing rate of 5-HT neurons in rodents (Martin-Ruiz et al., 2001; Boothman and Sharp, 2005; Quesseveur et al., 2013) and reduces basal extracellular 5-HT levels at nerve terminals (Martin-Ruiz et al., 2001). Conversely, 5-HT2A receptor antagonists elicit antidepressant-like activities (Pandey et al., 2010; Zaniewska et al., 2010) and potentiate the antidepressant-like responses of SSRIs in rodents ( Marek et al., 2005). It is thus possible that inactivation of the 5-HT2A receptor might positively impact SSRIs response. However, these pharmacological studies do not necessarily predict the influence of genetic loss of the 5-HT2A receptor on ADs response.

The present study was conducted to examine the effect of three HTR2A SNPs selected for their potential functional consequences on 5-HT2A receptor (rs6313, rs6314 and rs7333412) (Ozaki et al., 1997; Polesskaya et al., 2006; Laje et al., 2010) on ADs response during a 3-month treatment period. In parallel, we also determined the role of the 5-HT2A receptor in the modulation of the serotonergic system and ascertain whether its genetic inactivation in 5-HT2A mice modifies the functional activity of acute and prolonged SSRIs administration at the electrophysiological, neurochemical, immunohistochemical and behavioral levels.

2. Material and methods

For more details on material and methods, see Supplemental Information.

3. Clinical study

3.1. Patients and antidepressant treatments

624 in- or out-patients, aged 18–65 years, with a diagnosis of current major depressive episodes (MDE) in a context of MDD based on the Mini International Neuropsychiatric Interview (MINI) were included (Corruble et al., 2015). These patients required the initiation of a new antidepressant treatment. A minimum depression score of 18 on the 17-item Hamilton Depression Rating Scale (HDRS) was required to ensure that patients qualify for MDE. A monotherapy of antidepressant was chosen by the clinician, using “real world” treatment options. The antidepressant medication belonged to one of the four AD classes: SSRIs (n = 243 patients), serotonin norepinephrine reuptake inhibitors (SNRIs, n = 239), tricyclic antidepressants (TCAs, n = 55) and other antidepressant treatments (n = 87; mirtazapine n = 29, agomelatine n = 14, mianserin n = 5, iproniazide n = 5, moclobemide n = 1, tianeptine n = 1, electroconvulsiotherapy n = 30 or repetitive Transcranial Magnetic Stimulation n = 2). All patients signed a written informed consent for study participation and for genetic analyses.

3.2. Assessment of depression

Socio-demographic characteristics (age, sex) and MDD history (number of previous MDE, the lifetime duration of MDD since the beginning of the first MDE, history of prior antidepressant treatment) were gathered at inclusion. The HDRS was rated at baseline, one month, and three months after initiation of current antidepressant treatment. Responders were defined by a decrease in the HDRS score of at least 50% from baseline to follow-up. Remitters were defined as patients with a HDRS score inferior or equal to 7. Clinical assessments were performed blind to genotyping results. Each interview and diagnostic assignment was reviewed by a senior psychiatrist. Difference in HDRS scores, response and remission rates were both analyzed after 1 and 3 months of AD treatment.

3.3. SNP genotyping

A sample of 5 mL of whole blood was collected at baseline. DNA from lymphocytes was extracted from 1 mL of blood sample using a Puregene Kit (Gentra systems, Minneapolis, USA) and cryopreserved at −20 °C. Three HTR2A SNPs (rs6313, rs6314 and rs7333412) were genotyped. SNPs genotyping was performed by a TaqMan SNP genotyping assay (assay ID: C_3042197_1 for rs6313, C_11696920_20 for rs6314 and C_29235757_10 for rs7333412, Applied Biosystems) according to the manufacturer’s instructions. Allelic discrimination was performed with the ABI prism 7900HT Sequence Detection System (Applied biosystems, Courtaboeuf, France). Genotyping was performed blind to clinical data. DNA sample was obtained for 569 patients, including 521 (92%) Caucasian patients, 36 (6%) Afro-Caribbean patients and, 9 (2%) Asian patients. Ethnicity was not available for 3 patients. For rs6313, genotyping results were available for 562 patients. The minor allele frequency (MAF) for T allele was 0.45. For rs6314, genotyping results were available for 562 patients. The MAF for T allele was 0.09. For rs7333412, genotyping results were available for 539 patients. The MAF for G allele was 0.28. Underdetermined genotyping results were due to technical issues (lack of DNA in initial sample or lack of amplification). No significant deviation from the Hardy–Weinberg (HW) equilibrium was detected (χ2 = 1.6, p = 0.20 for rs6313; χ2 = 0.75, p = 0.38 for rs6314 χ2 = 3.78, p = 0.052 for rs7333412). For rs6313, patients were classified into TT group (patients with TT genotype) and CC/CT group (patients with CC and CT genotypes). CC/CT patients may have an impaired HTR2A expression, based on a previous study (Polesskaya et al., 2006). For rs6314, patients were classified into CC group and TT/CT group. TT/CT patients may have a lower intracellular signaling from 5-HT2A*R based on a previous study (Ozaki et al., 1997). For rs7333412, patients were classified into GG group and AA/AG group. AA/AG patients are
known to have the lower SHTT binding potential (Laje et al., 2010).

4. Preclinical study

4.1. Animals

Adult male wild type and mutant mice for the 5-HT2A or the 5-HT1A receptor (5-HT2A/5-HT2A; 5-HT1A/5-HT1A) were respectively bred on a S129/Sv-C5Bl6J and on C5Bl6J genetic background. All mice were 10–14 weeks old (25–35 g) at the beginning of the experiments. They were maintained on a 12L:12D schedule and housed five per cage. All testing was conducted in compliance with the NIH laboratory animal care guidelines and with protocols approved by the Institutional Animal Care and Use Committee (Counil directive # 87- 848, October 19, 1987, Ministere de l’Agriculture et de la Foret, Service Veterinaire de la Sant et de la Protection Animale, permissions # 92-196 to Alain Gardier).

4.2. Drugs and treatments

For acute studies, escitalopram-hydrochloride (4 mg/kg, free base; Biotrend; Switzerland), MDL100907 (2 mg/kg, free base; Tocris; UK), 8-OHDPAT (50–250 μg/kg, free base) or WAY100635 (0.5 mg/kg, free base), purchased from Sigma–Aldrich (France), were dissolved in saline and administered subcutaneously (s.c.). For chronic studies, escitalopram-hydrochloride (10 mg/kg, free base; s.c.) or fluoxetine-hydrochloride (18 mg/kg, free base; s.c.; Biotrend) were dissolved in saline and administered in mice for 2, 21 and 28 days in the electrophysiological experiments and for 28 days for the immunohistochemistry and behavioral experiments. Subcutaneously implanted osmotic minipumps (models: 1007D and 1002, Alzet, Direct Corporation, USA) were preloaded with escitalopram or fluoxetine and all experiments were performed with osmotic mini-pumps in place.

4.3. In vivo extracellular recordings of DRN 5-HT neurons

Mice were anesthetized with chloral hydrate (400 m/kg; i.p.) and mounted in a stereotaxic frame. The presumed DRN 5-HT neurons were identified according to the criteria previously described (Aghajanian and Vandermaelen, 1982) i.e., a slow (0.5–2.5 Hz) and regular firing rate and long-duration (2–5 ms) bior triphasic extracellular waveform. Each neuron was recorded during 2 min in real time using Spike2 software (Cambridge Electronic Design, UK).

4.4. In vivo intracerebral microdialysis

Mice were anesthetized with chloral hydrate (400 m/kg; i.p.) and implanted with the microdialysis probes into the ventral hippocampus (vHPC) (coordinates in mm from bregma: anterior = –2.5, lateral = +2.5, ventral = –2.5). The next day, the probes were continuously perfused with artificial cerebrospinal fluid. Four fractions of dialysate samples were collected to determine basal values (mean ± SEM) of extracellular 5-HT levels ([5-HT]sex). The systemic administration of saline, escitalopram or the combination of WAY100635/escitalopram was then performed and the effects of these drugs were evaluated from the collection of eight additional samples.

4.5. 8-OHDPAT-induced hypothermia

Body temperature was assessed intracutally, using a lubricated probe (Bio-BRET-3) inserted approximately 2 cm and monitored with a thermometer (Bioseb, France). Three baseline body temperature measurements were taken as a control measure. Ten minutes after the third baseline measurement, animals received 8-OH-DPAT (100 μg/kg s.c.), and body temperature was measured every 10 min after the injection during 1 h (Bill et al., 1991).

4.6. Immunohistochemistry

The brains were removed and cryoprotected in 30% sucrose PBS with 0.1% Na3 and stored at 4 °C. Serial sections (35 μm) were cut through the entire hippocampus (plate 41–61; Paxinos and Franklin and, 2001) on a cryostat and stored in PBS with 0.1% NaN3. Details of the protocol used to determine 5-bromo-2-deoxyuridine (BrdU) labeling for survival study and Doublecortin (DCX) for maturation of newborn neurons are presented in Supplemental Methods.

4.7. Behavior

The same animals were subjected to the novelty suppressed feeding (NSF) paradigm and tail suspension test (TST) with a 2 days period between the assessments of each behavior. The NSF paradigm is a conflict test that elicits competing motivations: the drive to eat and the fear of venturing into the center of a brightly lit arena (Surget et al., 2008). Next, we assessed immobility during exposure to inescapable stress using the TST, a test classically used to evaluate the efficacy of antidepressant drugs (Steru et al., 1985).

5. Data analysis

5.1. Clinical data

Genotype frequencies and deviation from the Hardy–Weinberg equilibrium were assessed using the Chi2 test. Two sample t-tests were used to compare quantitative variables according to genotype groups. The differences in socio-demographic and MDD history characteristics were tested between these groups (Supplemental Table 1). Variables showing significant differences (p < 0.05) or with a tendency towards difference (p < 0.1) between groups were selected as covariates for regression models. Thus, linear and logistic regression models used to assess the impact of genotypes on HDRS scores or the percentage of responders and remitters to ADs were performed with sex and class of antidepressant medication as covariates. Repeated measures ANOVA were performed to analyze the influence of genotypes on HDRS scores over time. The number of patients needed to detect a clinically relevant difference of 4 points on the HDRS score between genotype groups was calculated, with a two-sided type I error of 0.05 and a power of 80%. On the basis of these calculations, we estimated that 98 patients were needed (49 in each group). Difference in HDRS scores, response and remission rates were both analyzed after 1 and 3 months of AD treatment.

5.2. Preclinical data

Data were expressed as mean ± SEM except for the latency to feed in the NSF for which data were expressed as median with interquartile range (IQR) because they do not follow a normal distribution. Depending on the data analyzed, statistical comparisons between groups were performed using Student’s (un)paired test, one or two-way ANOVA or Kruskal–Wallis test (for the NSF). ANOVA were followed by appropriate post-hoc test and all p-values were corrected for multiple comparisons using Bonferroni or Dunn’s correction. A p-value of less than 0.05 was considered statistically significant.
6. Results

6.1. Impact of HTR2A genetic single nucleotide polymorphisms (SNPs) on antidepressant response in depressed patients

Among the 624 patients included in this cohort study, 152 dropped out prematurely before one month, and 293 before 3 months. The main reasons for drop-outs were antidepressant monotherapy change, use of unauthorized drugs, and lost to follow up. As genotyping results were initially available for 569 patients, the analyses were performed on 427 patients after one month and 308 patients after 3 months of treatment.

No significant difference between genotypes groups was observed for socio-demographic characteristics, MDD history (number of previous MDE, the lifetime duration of MDD since the beginning of the first MDE, history of prior antidepressant treatment) and severity of depression as measured by the HDRS score at baseline (Supplemental Table 1). Of note, for rs7333412, since patients of the GC group tended to be more often women ($p < 0.054$) and received more often TCA antidepressants and less often SNRI antidepressants ($p = 0.03$; Table 1), sex and antidepressant treatment were used as covariates in multivariate models for rs7333412.

No significant difference was shown in antidepressant response according to rs7333412 polymorphisms (Supplemental Table 2). The analysis of antidepressant response according to rs7333412 genotype groups is shown in Table 1. In bivariate analyses, after one month of antidepressant treatment, GC patients were less prone to respond to antidepressant medication than AA/AG patients: GC patients had higher HDRS score ($p = 0.02$), lower percentage of HDRS improvement from baseline ($p = 0.01$) and lower percentage of responders ($p = 0.009$). After 3 months of antidepressant treatment, GC patients had significantly higher HDRS scores ($p = 0.049$). GC patients also tended to have a lower percentage of HDRS improvement from baseline ($p = 0.09$). Accordingly, repeated measures ANOVA showed an influence of rs7333412 on the HDRS score over time ($p = 0.016$). After one month of treatment, linear and logistic regression models showed that GC patients had significantly higher HDRS scores ($p = 0.03$), lower percentage of HDRS improvement from baseline ($p = 0.007$) and lower response rates ($p = 0.026$) than AA/AG patients, regardless of sex and antidepressant medication class.

### Table 1

Antidepressant response according to the rs6313, rs6314 and rs7333412. HDRS: Hamilton Depression Rating Scale, M1: one month, M3: 3 months, SSRI: selective serotonin reuptake inhibitors, SNRI: serotonin norepinephrin reuptake inhibitors, TCA: tricyclic antidepressants, m: mean, sd: standard deviation, $p$<sup>(*)</sup>: $p$ in logistic regression models with sex and class of antidepressant treatment as covariates, $p < 0.05$.

<table>
<thead>
<tr>
<th>rs7333412 (n = 538)</th>
<th>AA/AG (n = 486)</th>
<th>GG (n = 52)</th>
<th>$p$</th>
<th>$p$&lt;sup&gt;(*)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antidepressant treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCA</td>
<td>37 (7.6%)</td>
<td>8 (15.4%)</td>
<td>$p = 0.03^*$</td>
<td></td>
</tr>
<tr>
<td>SNRI</td>
<td>196 (40.3%)</td>
<td>10 (19.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSRI</td>
<td>185 (38%)</td>
<td>24 (46.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>69 (14.1%)</td>
<td>10 (19.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>After one month of antidepressant treatment</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>n = 378</td>
<td>n = 49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDRS score (m(sd))</td>
<td>14.2 (±6.9)</td>
<td>16.6 (±6.8)</td>
<td>$p = 0.02^*$</td>
<td>$p = 0.03^*$</td>
</tr>
<tr>
<td>Percentage of HDRS improvement from baseline (m(sd))</td>
<td>41.5 (±27.2)</td>
<td>29.3 (±20.2)</td>
<td>$p = 0.01^*$</td>
<td>$p = 0.007^*$</td>
</tr>
<tr>
<td>Responders (%)</td>
<td>158 (41.8%)</td>
<td>11 (22.4%)</td>
<td>$p = 0.009^*$</td>
<td>$p = 0.026^*$</td>
</tr>
<tr>
<td>Remitters (%)</td>
<td>74 (19.6%)</td>
<td>6 (12.2%)</td>
<td>$p = 0.21$</td>
<td>$p = 0.30$</td>
</tr>
<tr>
<td><strong>After three months of antidepressant treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 276</td>
<td>n = 32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDRS score (m(sd))</td>
<td>12.4 (±7.4)</td>
<td>15.1 (±7.7)</td>
<td>$p = 0.049^*$</td>
<td>$p = 0.11$</td>
</tr>
<tr>
<td>Percentage of HDRS improvement from baseline (m(sd))</td>
<td>48.7 (±20.9)</td>
<td>40.1 (±25.8)</td>
<td>$p = 0.09$</td>
<td>$p = 0.24$</td>
</tr>
<tr>
<td>Responders (%)</td>
<td>148 (53.6%)</td>
<td>13 (40.6%)</td>
<td>$p = 0.16$</td>
<td>$p = 0.26$</td>
</tr>
<tr>
<td>Remitters (%)</td>
<td>75 (27.2%)</td>
<td>6 (18.7%)</td>
<td>$p = 0.30$</td>
<td>$p = 0.35$</td>
</tr>
</tbody>
</table>

6.2. Role of the 5-HT<sub>2A</sub> receptor in the modulation of the serotonergic system and in response to the acute administration of SSRIs

A complete statistical summary is included in Supplemental Table 3.

To determine to what extent the 5-HT<sub>2A</sub> receptor participates in SSRI-induced inhibition of the serotonergic system, DRN 5-HT neuronal activity was examined in mice displaying a constitutive genetic or a pharmacological inactivation of the 5-HT<sub>1A</sub> receptor. As expected, in wild-type 5-HT<sub>1A</sub><sup>+</sup> mice, escitalopram significantly decreased the firing rate of DRN 5-HT neurons by ~80% relative to baseline (Fig. 1AB; $p < 0.001$). In 5-HT<sub>1A</sub><sup>−</sup> mice, escitalopram administration also elicited a decrease in the firing rate of DRN 5-HT neurons by ~65% relative to baseline ($p = 0.003$) and this inhibitory response was reversed by the 5-HT<sub>2A</sub> receptor antagonist MDL100907. Indeed, the mean firing rate after the administration of MDL100907 was no longer different from baseline ($p = 0.18$) (Fig. 1CD). We performed additional electrophysiological recordings in 5-HT<sub>1A</sub><sup>−</sup> mice pre-treated with the 5-HT<sub>1A</sub> receptor antagonist WAY100635. In these conditions, escitalopram still inhibited the firing rate of DRN 5-HT neurons by ~40% relative to baseline ($p = 0.004$) whereas the addition of MDL100907 reversed this effect (Fig. 1EF). Importantly, in 5-HT<sub>1A</sub> or wild-type mice pre-treated with WAY100635, the 5-HT<sub>1A</sub> receptor agonist 8-OHDPAT failed to inhibit DRN 5-HT neuronal activity (Fig. 1C−E) demonstrating that 5-HT<sub>1A</sub> autoreceptors were indeed inactivated under our experimental conditions. As previously described in rat (Boothman et al., 2003; Sotty et al., 2009), we also verified that MDL100907 did not modify the spontaneous firing rate of DRN 5-HT neurons in 5-HT<sub>1A</sub><sup>−</sup> mice compared to baseline ($p = 0.054$) and we extended this observation to the 5-HT<sub>1A</sub><sup>−</sup> mice (p = 0.39) and 5-HT<sub>1A</sub><sup>+</sup> mice pre-treated with WAY100635 (p = 0.71; Supplemental Fig. 1A−C).

In microdialysis experiments, acute treatment with escitalopram increased hippocampal [5-HT]<sub>ext</sub> by ~325% in 5-HT<sub>1A</sub><sup>−</sup> (p < 0.001) and by ~280% in 5-HT<sub>1A</sub><sup>+</sup> mice (p = 0.026) compared to their corresponding vehicle-treated control groups (Fig. 2A−C). No differences between both genotypes were noticed (p = 0.81; Fig. 2C). However, WAY100635 significantly potentiated the increase in [5-HT]<sub>ext</sub> induced by escitalopram in 5-HT<sub>1A</sub><sup>−</sup> mice (p = 0.04), but not in 5-HT<sub>1A</sub><sup>+</sup> mice (p = 0.07), compared to the
respective groups of mice injected with the SSRI alone (Fig. 2C). As expected WAY100635 alone did not affect extracellular 5-HT levels in the hippocampus of 5-HT_2A^+/+ and 5-HT_2A^-/- mice compared to baseline (p = 0.51 and p = 0.67; respectively, Supplemental Fig. 2A–2D).

To test for the sensitivity of the 5-HT_1A autoreceptor, we compared the ability of the 5-HT_1A receptor agonist 8-OHDPAT to inhibit the in vivo firing rate of DRN 5-HT neurons in 5-HT_2A^+/+ and 5-HT_2A^-/- mice. As previously reported, a marked and dose-dependent inhibition of DRN 5-HT neuronal activity was observed in response to the administration of cumulative doses of 8-OHDPAT in both 5-HT_2A^+/+ and 5-HT_2A^-/- mice with ED_{50} of 111 ± 14

Fig. 1. Role of the 5-HT_2A receptor in acute SSRI-induced inhibition of DRN 5-HT neuronal activity. In these experiments, escitalopram (ESC: 4 mg/kg) was administered subcutaneously (s.c.). Data are means ± SEM of firing frequency (Hz) of 5-HT neurons recorded in response to ESC in 5-HT_1A^+/+ mice (A), in 5-HT_1A^-/- mice (C) or in 5-HT_1A^+/+ mice pre-treated with the 5-HT_1A receptor antagonist WAY100635 (0.5 mg/kg; s.c.) (E). (B, D and F) Typical recordings of 5-HT neurons in which the arrows indicate the time when drugs were injected. In mice displaying an inactivation of the 5-HT_1A receptor, a single injection of the 5-HT_1A receptor agonist 8-OHDPAT (250 mg/kg; s.c.) and 5-HT_2A receptor antagonist (MDL100907: 2 mg/kg; s.c.) was performed before and after ESC administration; respectively. (n = 5–10 mice per group). **p < 0.01 and ***p < 0.001: significantly different from baseline. #p < 0.05: significantly different from ESC.
and 53 ± 5 μg/kg; respectively (Fig. 2D,E). Comparisons of the AUC values revealed a more pronounced inhibitory effect of the agonist in 5-HT$_2A$ mice compared to their wild-type littermates (p = 0.014) (Fig. 2E,F). To confirm these results, we used the 8-OHDPAT-induced hypothermia test as a relevant paradigm to evaluate the sensitivity of the 5-HT$_{1A}$ autoreceptor (Richardson-
6.3. Effects of the sustained administration of SSRIs in mice lacking the 5-HT2A Receptor

5-HT2A+/+ and 5-HT2A−/− mice were then treated for 0 (basal), 2, 21 and 28 days with escitalopram. The mean spontaneous basal firing rate of DRN 5-HT neurons was significantly higher in 5-HT2A+/+ mice than in 5-HT2A−/− littermates (p < 0.001). Two days’ treatment with escitalopram resulted in a −40% and −65% decrease in DRN 5-HT neuronal firing rate relative to baseline in 5-HT2A+/+ and 5-HT2A−/− mice (p < 0.001), respectively (Fig. 3A). No significant differences were noticed between both genotypes (p = 0.24). In contrast, although sustained treatment with escitalopram after 21 days produced a complete recovery of DRN 5-HT neuronal firing rate to basal level in 5-HT2A+/+ as shown by the lack of significant difference with baseline (p = 0.85), it failed to do so in 5-HT2A−/− mice. Indeed after 21 days (p < 0.001) but also 28 days; p < 0.001) of treatment in mutants, the firing rates of DRN 5-HT neurons remained significantly lower than baseline (Fig. 3A) but also than the corresponding group of wild-types (p < 0.0033 and p = 0.0035; respectively).

These important results were extended to another SSRI, fluoxetine. Indeed, two days’ treatment with fluoxetine resulted in a −75% and −70% decrease in DRN 5-HT neuronal firing rate in 5-HT2A+/+ (p < 0.001) and 5-HT2A−/− mice (p < 0.001) relative to baseline (Fig. 3B), but no significant differences were observed between both genotypes. In contrast, after 28 days of fluoxetine treatment, a complete recovery of DRN 5-HT neuronal firing rate to basal level was observed in 5-HT2A+/+ (p = 0.45) but not in 5-HT2A−/− mice (p < 0.001) (Fig. 3B). Again, after 21 days of treatment with fluoxetine, significant lower firing rates of DRN 5-HT neurons were noticed in 5-HT2A−/− mice compared to wild-type littermates (p = 0.0035).

At the postsynaptic level, a part of chronic SSRI action relies on their ability to stimulate brain plasticity in the adult hippocampus (Santarelli et al., 2003). The chronic administration of fluoxetine for 28 days induced a significant increase in cell survival in the hippocampal subgranular zone of 5-HT2A+/+ (p = 0.03), but not in 5-HT2A−/− mice (p = 0.99) (Fig. 4A). Interestingly, neuronal maturation was increased in 5-HT2A+/+ (p = 0.003) but not significantly in 5-HT2A−/− (p = 0.21; Fig. 4B,C).

Finally, we determined whether such electrophysiological and neurogenic impairments might be sufficient to influence behavioral activities of SSRIs in these mutants subjected the novelty suppressed feeding (NSF) and tail suspension test (TST) in which chronic antidepressant treatments are supposed to be dependent and independent of the hippocampus, respectively (David et al., 2009). In the NSF, chronic fluoxetine produced anxiolytic-antidepressant-like effects in 5-HT2A+/+ mice compared to controls as shown by its ability to decrease the latency to feed (median = 113.7 s, IQ [91; 176] vs median = 69.5 s, IQ [59.25; 88.75]; p = 0.009). On the contrary this treatment had no effect in 5-HT2A−/− mutant mice compared to controls (median = 132 s, IQ [110; 198] vs median = 143 s to feed, IQ [73; 239]; p = 0.64; Fig. 4D,E). Importantly, food intake after the test measured over 5 min in animals’ home cage was not different between groups (VEH-5-HT2A+/+: 8 ± 0.8; FLX-5-HT2A+/+: 7.6 ± 0.9; VEH-5-HT2A−/−: 7.3 ± 0.7; FLX-5-HT2A−/−: 7.1 ± 0.6 mg/g of body weight; Two-way ANOVA). In the TST, chronic fluoxetine significantly decreased the time of immobility in 5-HT2A+/+ mice subjected to the TST (p = 0.027) whereas it produced an opposite effect in 5-HT2A−/− mice compared to their respective control groups (p = 0.006; Fig. 4F).

Fig. 3. Partial recovery of DRN 5-HT neuronal activity after long-term treatment with SSRI in 5-HT2A+/+ mice. (A–B) Data are means ± SEM of firing rate (Hz) of 5-HT neurons recorded in the DRN of 5-HT2A+/+ and 5-HT2A−/− mice administered with escitalopram (ESC: 4 mg/kg; s.c.)/(A) or fluoxetine (FLX: 18 mg/kg; s.c.)/(B) for 0 (basal), 2, 21 and 28 days. (n = 3–5 mice per group). *p < 0.05; **p < 0.01 and ***p < 0.001: significantly different from the basal firing rate of the corresponding genotype. ##p<0.01 and ###p<0.001: significantly different from 5-HT2A−/− mice for the same duration of SSRI treatment.

7. Discussion

In this study, we addressed the role of the 5-HT2A receptor in ADs response by evaluating the influence of three HTR2A SNPs (rs6313, rs6314 and rs7333412) during a 3-month treatment period in depressed patients and then by comparing the chronic effects of SSRIs in 5-HT2A+/+ and 5-HT2A−/− mice. The most remarkable result obtained herein is the fact that GC patients for the rs7333412 were less prone to respond to antidepressant medication than AA/AG patients. In addition, genetic inactivation of 5-HT2A Receptor in mice compromised the activity of SSRIs making this receptor a putative marker to predict response and remission after antidepressant treatment.

Previous studies reported that one of the 5-HT2A receptor polymorphism (rs6311), the only variant located in the 5-HT2A...
receptor gene promoter influencing the promoter’s activity, could lead to different ADs response (Kato and Serretti, 2010). Interestingly, rs6313 is in complete linkage disequilibrium with rs6311 (Myers et al., 2007; Spurlock et al., 1998) and the C allele has been associated with a lower mRNA levels in post-mortem brains (Polesskaya and Sokolov, 2002; Polesskaya et al., 2006). As for rs6314, the T allele would be associated with a decreased 5-HT$_{2A}$ receptor-mediated intracellular signaling (Ozaki et al., 1997).

Fig. 4. Impairment of long-term treatment with SSRI-induced stimulation of hippocampal plasticity and antidepressant-like activities in 5-HT$_{2A}^{-/-}$ mice. (A) Cell survival. Data are expressed as mean ± SEM of the number of BrdU-positive cells in 5-HT$_{2A}^{-/-}$ and 5-HT$_{2A}^{+/+}$ mice treated with fluoxetine (FLX: 18 mg/kg; s.c.) for 28 days. (B,C) Neuronal maturation. Data are expressed as mean ± SEM of the number of DCX-positive cells in 5-HT$_{2A}^{-/-}$ and 5-HT$_{2A}^{+/+}$ mice treated with fluoxetine (FLX: 18 mg/kg; s.c.) for 28 days (n = 3–5 mice per group). (D,E) Novelty suppressed feeding test. Anxiolytic/antidepressant-like activity is expressed as median with interquartile range of the latency time to feed in 5-HT$_{2A}^{-/-}$ and 5-HT$_{2A}^{+/+}$ mice treated with saline or fluoxetine. (F) Tail suspension test. Antidepressant-like activity is expressed as mean ± SEM of immobility time in 5-HT$_{2A}^{-/-}$ and 5-HT$_{2A}^{+/+}$ mice treated with saline or fluoxetine. White bars represent data obtained for control animals and grey bars those obtained for animals treated with chronic fluoxetine (FLX: 18 mg/kg; s.c. for 28 days). *p < 0.05; **p < 0.01: significantly different from the corresponding control groups treated with the vehicle. ns p > 0.05: not significant from the corresponding control groups treated with the vehicle. (n = 3–5 per group for immunohistochemical studies and n = 8–15 mice per group for the behavioral studies).
However, no influence of both SNPs was detected on ADs response. Although our results with rs6313 are consistent with a recent meta-analysis (Niitsu et al., 2013), they stand in contrast with another meta-analysis showing that MDD patients with the 5-HT2A receptor rs6313 CT polymorphism display a lower ADs response (Lin et al., 2014). The negative result obtained with rs6314 may be due to the very low frequency of the TT genotype (3 out of 563 genotyped patients). Indeed the minor allele frequency for T allele is inferior to 10%, which corresponds to an uncommon variant. In this context, influence on ADs response may be difficult to demonstrate in an association study. In contrast to the results reported with rs6313 and rs6314, we showed an association between rs7333412 and ADs response in patients with MDD: GG patients displayed a significant lower percentage of responders than AA/AG patients. This is in line with recent reports resulting an association between rs7333412 and remission after citalopram treatment in the STAR*D cohort (Fabbrri et al., 2014). Unfortunately, functional consequences of this SNP on 5-HT2A receptor-mediated transmission is currently unknown. This SNP is located in an untranslated region of HTR2A, comprised in a larger region of HTR2A gene spreading from 3' UTR to intrinsic region and previously associated with variation in SSRI response (McMahon et al., 2008; Fabbrri et al., 2014). There is no precise data describing the possibly link between the SNPs present in this untranslated region and 5-HT2A receptor expression levels. A first hint towards understanding is that rs7333412 has been associated with 5-HTT binding potential (BP) variation (Laje et al., 2010). Although no precise mechanism was described in the latter study, the authors hypothesized that a trans-regulatory effect might be involved implying that low levels of 5-HT2A receptor would result in a higher 5-HTT expression. It is noteworthy that GG patients display a higher 5-HTT BP (i.e., with higher density and/or affinity of 5-HTT) compared to AA patients, AG patients having an intermediate 5-HTT BP. Thus, patients with the apparent higher 5-HTT binding potential are less prone to respond to ADs. Although speculative, these results suggest that GG patients for the rs7333412 would have lower basal 5-HT transmission and consequently would not benefit as much from the enhancing SSRIs properties. This hypothesis is in line with our previous investigations demonstrating that a lower neurotransmission at the 5-HT2A receptor may favor the susceptibility to MDD and the severity of MDD (Petit et al., 2014). Alternatively, in these GG patients the percentage of brain 5-HTT occupancy to reach therapeutically extracellular 5-HT concentrations could require higher doses of treatments and thereby explain a lower rate of responders. Despite these considerations, it is important to note that these hypotheses contrast with clinical evidence showing that depressed patients with the highest levels of 5-HTT display a better response to SSRIs. The 5-HTT gene gives rise to a bi-allelic polymorphism designated long (L) and short (S) and there is now strong evidence that the L allele is associated with approximately 2-fold higher levels of transcriptional activity and rate of 5-HT uptake compared with the S variant (Greenberg et al., 1999; Lesch et al., 1996). Results of pharmacogenetic studies have demonstrated that patients with L/L genotype display a better response to SSRIs than S/L individuals either in term of latency to recover or proportions of responders (Reynolds et al., 2014).

To further explore the link between the 5-HT2A receptor and ADs response, we conducted a full comprehensive analysis of the role of this receptor on SSRIs response in wild-type 5-HT2A mice and 5-HT2A−/− mutant mice. It is now well accepted that the over-activation of the inhibitory 5-HT1A autoreceptor, particularly the first day of SSR treatment, inhibits the firing rate of 5-HT neurons in the DRN and this would delay the onset of therapeutic effects (Gardier et al., 1996). However, other G protein-coupled receptors contribute to the modulation of the serotonergic system (Maejima et al., 2013; Quessevere et al., 2013). Accordingly, a single administration of the SSRI escitalopram decreased DRN 5-HT neuronal activity in animals displaying a genetic or a pharmacological inactivation of the 5-HT1A receptor. This observation together with the fact that such inhibitory response was reversed by MDL100907 demonstrated the negative influence of the 5-HT2A receptor on the serotonergic system. However, there are data showing that the acute administration of MDL100907 reduced 5-HT synthesis in Human (Hasegawa et al., 2012) but this parameter does not necessarily predict changes in 5-HT neuronal activity. In support of the latter assertion, the intrinsic electrophysiological properties of 5-HT neurons have been recently examined in mice lacking brain 5-HT, i.e. tryptophan hydroxylase-2 null mice (TpH2−/−) and the authors demonstrated that the firing rate of DRN 5-HT neurons remained essentially unchanged across TpH2 genotypes (Montalbano et al., 2015). It should be noticed that growing evidence suggests the existence of specialized sub-populations of 5-HT neurons based on of their anatomical location within the DRN, their projection areas (Kiyasova and Gaspar, 2011) but also on their gene expression (Okaty et al., 2015) and electrophysiological properties (Fernandez et al., 2015). Consequently, we cannot rule out the possibility that 5-HT2A receptor-mediated inhibition of neuronal activity in the DRN is a common feature of the whole population of serotonergic cells but restricted to those displaying a low and regular firing rate. Moreover, the mechanism by which 5-HT2A receptors influence 5-HT neuronal activity is unclear. It is well known that the DRN receives serotonergic input from recurrent collaterals (Lechin et al., 2006) and evidence suggests that the rise in endogenous 5-HT after SSRI promote the activation of local 5-HT2A receptor expressed on GABAergic interneurons (Boothman and Sharp, 2005). This would contribute to dampen the neuronal activity of the serotonergic system. Nevertheless, other mechanisms could contribute to the electrophysiological effects of escitalopram through the recruitment of the 5-HT2A receptors. We recently demonstrated that the ability of the preferential 5-HT2A receptor agonist DOI to decrease the firing rate of 5-HT neurons was attenuated in mice displaying a lesion of the noradrenergic system (Quessevere et al., 2013) suggesting that the activation of the 5-HT2A receptor in the locus coeruleus dampens the activity of the DRN as previously proposed (Blier and Szabo, 2005). Moreover, the medial prefrontal cortex (mPFC) is endowed with a high density of 5-HT2A receptors expressed on excitatory pyramidal neurons projecting on DRN 5-HT neurons. However, it has been repeatedly showed that the local application of DOI in mPFC increased the firing rate of 5-HT neurons through a direct pathway (Martin-Ruiz et al., 2001; Bortolozzi et al., 2003) or via the ventral tegmental area projecting to the DRN (Vazquez-Borsetti et al., 2011). Indeed, electrophysiological studies showed that the systemic or intracritical injection of stimulate VTA DA firing rate (Bortolozzi et al., 2005), whereas several studies emphasized the excitatory impact of DA on 5-HT neurons (Haj-Dahmane, 2001; Aman et al., 2007). We then explored the functional consequence of 5-HT2A receptor genetic inactivation on hippocampal [5-HT]ext. Despite the recruitment of the 5-HT2A receptor in the inhibitory effect of acute SSRI on 5-HT neurons, the ability of escitalopram to increase hippocampal [5-HT]ext was similar between 5-HT2A−/− and 5-HT2A+/+ mice. However, the blockade of the 5-HT1A autoreceptor by WAY106035 potentiated SSR1-induced increase in hippocampal [5-HT]ext specifically in 5-HT2A−/− mice. These results along with the observations that 8-OHDPAT-induced hypothermia or inhibition of DRN 5-HT neuronal were more pronounced in mutants than in wild-types, provided clear-cut evidence that the constitutive lack of 5-HT2A receptor is associated with a hypersensitization/upregulation of the 5-HT1A autoreceptor. They also suggest that the combined inactivation of both receptors is required to produce optimal
effects on hippocampal 5-HT availability.

In light of these results, we questioned how a functional change influenced the long-term mechanism of action of SSRI. As previously demonstrated in mice, we reported that subchronic (2 days) administration of escitalopram or fluoxetine decreased the firing rate of DRN 5-HT neurons while their prolonged administration was associated with a progressive recovery to normal activity (Guard et al., 2012). Interestingly, 5-HT neurons did not regain their baseline firing activity in 5-HT2A− mice after administration of both SSRIs for 28 days. These results are congruent with the hypersensitization/up-regulation of the 5-HT1A autoreceptor detected in 5-HT2A− mice. Indeed, since the complete recovery of 5-HT firing has been correlated with a 5-HT1A desensitization (Blier, 2003), one would expect a delayed recovery in mice displaying, on the contrary, 5-HT1A hypersensitization/up-regulation. At the post-synaptic level, previous data correlated the antidepressant-like activity of prolonged SSRI administration with their ability to stimulate different cellular plasticity related-processes in the hippocampus (David et al., 2009). In this part of our study we decided to focus our attention on fluoxetine because the modalities of its use after chronic administration have been well characterized in our laboratory (David et al., 2009; Rainer et al., 2012; Le Dantec et al., 2014; Petit et al., 2014). Here, we reported an attenuation of chronic fluoxetine to induce cell survival and neuronal maturation in 5-HT2A− mice although the participation of this receptor on hippocampal plasticity remains poorly investigated. In agreement with the findings in mutant mice, pharmacological studies reported that the acute administration of the 5-HT2A receptor antagonist ketanserin decreased cell proliferation (Banas et al., 2004; Iha et al., 2008). However, the sustained administration of ketanserin produced opposite effects (Jha et al., 2008) and the fact that atypical antipsychotics, which also display 5-HT2A receptor antagonistic activity, potentiate SSRI-induced hippocampal cell proliferation in animal models of depression (Xu et al., 2006; Wang et al., 2013) contrast with our present data. It is therefore possible that the hypersensitization/upregulation of the 5-HT1A autoreceptor detected in 5-HT2A− mice would play a major role in attenuating such SSRI response. In keeping with this hypothesis, impaired cell survival and neuronal maturation observed in 5-HT2A− mice treated with fluoxetine might result from an attenuated ability of this SSRI to increase of 5-HT extracellular levels and therefore hippocampal neurogenesis. This is further strengthened by the observation that the 5-HT1A receptor agonist 8-OHDPAT increases cell proliferation or survival in the hippocampus through a post-rather than a pre-synaptic effect (Santarelli et al., 2003; Banas et al., 2004; Huang and Herbert, 2005, 2006; Soumier et al., 2010) whereas 5-HT1A antagonist decreases these processes (Radley and Jacobs, 2002).

Such beneficial effects of post-synaptic 5-HT1A receptor on hippocampal neurogenesis has been recently confirmed in a study demonstrating that deletion of this receptor in dentate gyrus granule cells attenuates SSRI-induced increase in RNA expression levels of neurotrophic factors such as VEGF and BDNF (Samuels et al., 2015). Interestingly, although the neurotrophic activity of SSRI on cell proliferation, differentiation and survival in the hippocampus was attributed to the local synthesis and release of BDNF (Sairanen et al., 2005; Tallaz et al., 2010; Quesseveur et al., 2013), it has been shown that ketanserin blocked stress-induced down-regulation of BDNF mRNA in the rat hippocampus (Vaidya et al., 1997). Discrepancies between these findings and our results are unclear but in agreement with an attenuation of the ability of fluoxetine to stimulate cell survival and neuronal maturation in 5-HT2A− mice, we reported that its chronic administration also failed to elicit anxiodepressive like activities in these mutants subjected to the NSF. This should be analyzed in light of recent data showing that manipulation contributing to block adult hippocampal neurogenesis limit the efficacy of ADs (Santarelli et al., 2003; Surget et al., 2008; David et al., 2009). In the TST, although chronic fluoxetine exerted antidepressant-like effects in wild-type mice, it elicited opposite activity in mutant mice. The latter results cannot be interpreted as a pro-depressive-like effect since the TST has not been developed to unveil pathological states. Moreover, the observation that the chronic administration of another SSRI, escitalopram, failed to induce antidepressant-like effects in 5-HT2A− mice subjected to the same paradigm (Supplemental Fig. 3) strengthens our interpretation. Several hypotheses can be advanced in an attempt to explain that the disruption of the 5-HT2A receptor gene impaired behavioral responses. As mentioned earlier, it is possible that the constitutive loss of 5-HT2A receptors leads to developmental and compensatory effects that are not observed after treatments with 5-HT2A receptor antagonists in wild-type mice. The hypersensitization/upregulation of the 5-HT1A receptor reported in 5-HT2A− mice clearly illustrates such a possibility and several examples in the literature demonstrate that a higher density and/or functional activity of the 5-HT1A receptor mitigate the antidepressant-activity of SSRI in clinical (Lemonde et al., 2004) and preclinical (Richardson-Jones et al., 2010) studies. Alternatively, it is possible that the lack of 5-HT2A receptor itself is a cause of chronic SSRI non-response. Although the inactivation of 5-HT2A receptor in the DRN prevents the counter-productive acute inhibitory action of SSRI on the serotonergic activity, the impact of its inactivation at the nerve terminals in the hippocampus, the amygdala and/or the frontal cortex remains poorly examined and should draw our attention for future investigations.

This study has some limitations. The drop-out rate in the clinical study is relatively high (25.9% before M1, 21.8% between M1 and M3). However, it is similar to other naturalistic studies, as the STAR*D study (Warden et al., AJP, 2007). As we studied only 3 SNPs, we did not use multiple-testing correction in the statistical analysis of the clinical data. Even with Bonferroni corrections (p = 0.05/3 = 0.016), the results of the percentage of HDRS improvement from baseline to M1 remained statistically significant in bivariate and regression analyses. We considered that this clinical signal was strong enough to justify the preclinical investigations performed in this work. But, we cannot rule out that some of the tests performed in the clinical dataset show significant p values by chance. In the preclinical investigations, although the therapeutic activity of ADs can be reliably unveiled in naive animals (Lucy, 1997) our electrophysiological, histological and behavioral studies could have been carried out in an animal model of depression to reinforce the parallel with the clinical data and to ascertain that the altered ADs response reported herein persists under pathological conditions. This is particularly important in light of the present results since the expression and functional activity of both 5-HT1A and 5-HT2A receptors can be modified after corticosterone exposure (Fernandes et al., 1997; Rainer et al., 2012) or unpredictable chronic mild stress (Rasul et al., 2013). These models would be probably more relevant to draw future lines of pharmacogenetic researches.

8. Conclusion

This translational study provides compelling evidence for a role of the 5-HT2A receptor on serotonergic ADs response. Our results indicate that the rs7334125 SNP in the 5-HT2A receptor gene may influence the response to ADs. Together with preclinical data, they shed some light on the fact that the genetic inactivation of 5-HT2A receptor may compromised the antidepressant effects of SSRIs and this is particularly important in order to determine the appropriate antidepressant strategy according to patients’ genotype.
Competing interest

None declared for G.Q, ACP, HTN, L.D; R.C, I.S, JPG, CV, L.B. AM.G and DJ.D have received grant funding from Servier, Lundbeck, Takeda and P. Fabre laboratories. EC has received consulting fees from Astra-Zeneca, Eisi, Servier, Lundbeck, Otsuka, Sanofi. BP.G has received grant funding from Lundbeck, Theranexus and Neuroresearch.

Acknowledgments

We would like to thank Ms Solange Domergue from the animal care facility of the “Institut Fédératif de Recherche-IFR141” of the Paris Sud University for her assistance in the 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> mice breeding.

Appendix ASupplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.neuropharm.2015.12.022.

References


1. **Clinical study**

**Study design**

The data of the present article are based on a prospective, multicentric, real-world cohort study in psychiatry settings. This study, conducted in accordance with the International Helsinki Declaration, was registered by the French National Agency for Medicine and Health Products Safety and the “Commission Nationale Informatique et Libertés”, was approved by the Ethics Committee of Paris-Boulogne.

**Exclusion criteria**

Patients with MDE with psychotic symptoms, bipolar disorders, psychotic disorders, eating disorders, current substance abuse or dependence (DSM-IV-TR), pregnancy, breast feeding, organic brain syndromes or severe unstable medical conditions were not included. Patients receiving antipsychotics or mood stabilizers during the month preceding inclusion and/or for 4 months or more during the last year preceding inclusion were not included. Antipsychotics, mood stabilizers, stimulants were not permitted during the study.

2. **Preclinical study**

**In vivo extracellular recordings of DRN 5-HT neurons**

The extracellular recordings of the 5-HT neurons in the DRN were performed using single-barreled glass micropipettes (Stoelting, Dublin, Ireland) pulled on a pipette puller (Narishige, Tokyo, Japan) and preloaded with a 2 M NaCl solution. Their impedance typically ranged between 2.5 and 5 MΩ. The single-barreled glass micropipettes were positioned 0.2–0.5 mm posterior to the interaural line on the midline and lowered using a hydraulic micropositioner (Kopf Instruments) into the
DRN, usually attained at a depth between 2.5 and 3.5 mm from the brain surface. To increase the signal-to-noise ratio, we used a current amplifier (BAK Electronics, Mount Airy, MD, USA) connected to the active filter Humbug (Quest scientific, DIPSI, Châtillon, France). For all dose–response curves, only one neuron per mouse was recorded. For chronic experiments, several 5-HT neurons per mouse were recorded to calculate the mean ± SEM of basal firing rate in the different treatment conditions.

**In vivo intracerebral microdialysis**

Concentric dialysis probes were made of cuprophan fibers and constructed as described previously (Bert et al., 2004). All probes (×0.30 mm outer diameter) presented an active length of 1.5 mm within the vHPC with aCSF (composition in mM: NaCl 147, KCl 3.5, CaCl₂ 1.0, MgCl₂ 1.2, NaH₂PO₄ 1.0, NaHCO₃ 25.0, pH 7.4) at a flow rate of 1.5 µl/min, using a CMA/100 pump (Carnegie Medicine, Stockholm, Sweden). Dialysate samples were collected every 15 min in small Eppendorf tubes for the measurement of their 5-HT contents using HPLC (XL-ODS column; 4.6 × 7.0 mm, particle size 3 µm; Beckman) coupled to amperometric detection (1049A; Hewlett-Packard, Les Ulis, France). The limit of sensitivity for 5-HT was ~0.5 fmol per sample (signal-to-noise ratio=2).

**Immunohistochemistry**

*5-bromo-2-deoxyuridine (BrdU) labeling for survival study*

Mice were administered BrdU (150mg/kg, i.p. b.i.d. for 3 days) 4 weeks before sacrifice. We then proceeded as described by Xia and collaborators (Xia et al., 2012). For DAB staining, sections were mounted on slides and boiled in citric acid (pH 6.0) for 5 min, rinsed with PBS, and treated with 0.01% trypsin in Tris/CaCl₂ for 10 min. Brain sections were incubated for 30 min with 2N HCl and blocked with 5% NGS. Sections were then incubated overnight at room temperature with anti-mouse BrdU (1:100) (Beckman-Dickson, France). After washing with PBS, sections were incubated for 1 hr with secondary antibody (1:200 biotinylated goat anti-mouse) followed by amplification with an avidin-biotin complex. The staining was visualized with DAB. For the quantification of BrdU labeling, a stereological procedure was used using an Olympus BX51 microscope (Germany).
Maturation of newborn neurons

For doublecortin (DCX) staining, the procedure consisted of the following steps: 1 hr incubation in 0.1M TBS with 0.5% Triton X-100 and 10% normal donkey serum (NDS), followed by goat anti-doublecortin primary antibody (1:100) in TBS/Tx/NDS for 24 hrs at 4°C. The secondary antibody was biotinylated donkey anti-goat (1:500) in TBS/NDS for 1 hr at room temperature, followed by a 1hr amplification step using an avidin-biotin complex (Vector, USA). The immunohistochemistry protocol was adapted from David and collaborators (David et al., 2009). For the quantification of DCX labeling, a stereological procedure was used using an Olympus BX51 microscope (Germany).

Behavior

The novelty suppressed paradigm (NSF)

Animals were food-deprived for 24h prior to the test. Testing was performed in a 50x50x20-cm box covered with bedding and illuminated by a 70-watt lamp. The NSF test was carried out during a 10 min period as described by Santarelli and collaborators (Santerelli et al., 2003). At the time of testing, a single pellet of food (regular chow) was placed on a white paper platform positioned in the centre of the box. Mice were tested individually by placing them in the corner of the maze, and a stopwatch was immediately started. The latency to eat (defined as the mouse sitting on its haunches and biting the pellet with the use of forepaws) was timed. Immediately afterwards, the animal was transferred to its home cage and the amount of food consumed during the subsequent 5 min was measured, serving as a control for change in appetite as a possible confounding factor (home cage food consumption), because antidepressants are known to affect appetite.

The Tail suspension test (TST)

Mice were suspended by their tails with tape. In such a position animals cannot escape or hold on to nearby surfaces. During the test, typically 6 minutes in duration, the resulting escape oriented behaviors are quantified using the Bioseb TST software (Bioseb, Vitrolles, France). A specific strain gauge linked to a computer quantifies properly the time of mobility and immobility. The latter parameter was scored and used in the present study as a measure of despair.

## SUPPLEMENTAL TABLE 1.

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<td>P</td>
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<td><strong>Number of previous MDE</strong></td>
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<td></td>
<td>37 (37%)</td>
</tr>
<tr>
<td>SSRI</td>
<td>183 (40.1%)</td>
<td>34 (32.4%)</td>
<td></td>
<td>38 (38%)</td>
</tr>
<tr>
<td>Others</td>
<td>70 (15.2%)</td>
<td>13 (12.4%)</td>
<td></td>
<td>14 (14%)</td>
</tr>
</tbody>
</table>

#### After one month of antidepressant treatment

<table>
<thead>
<tr>
<th></th>
<th>n=364</th>
<th>n=81</th>
<th>n=85</th>
<th>n=359</th>
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<tbody>
<tr>
<td>Percentage of HDRS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>improvement from baseline (m(sd))</td>
<td>40.4 (±27.9)</td>
<td>38.2 (±27.5)</td>
<td>40.3 (±30.4)</td>
<td>39.9 (±27.2)</td>
</tr>
<tr>
<td>Responders (%)</td>
<td>143 (39.2%)</td>
<td>31 (38.3%)</td>
<td>36 (42.3%)</td>
<td>138 (38.4%)</td>
</tr>
<tr>
<td>Remitters (%)</td>
<td>69 (18.9%)</td>
<td>14 (17.3%)</td>
<td>20 (23.5%)</td>
<td>63 (17.5%)</td>
</tr>
</tbody>
</table>

#### After three months of antidepressant treatment

<table>
<thead>
<tr>
<th></th>
<th>n=269</th>
<th>n=53</th>
<th>n=56</th>
<th>n=266</th>
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</thead>
<tbody>
<tr>
<td>Percentage of HDRS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>improvement from baseline (m(sd))</td>
<td>47.8 (±30.2)</td>
<td>48.7 (±27.8)</td>
<td>49.7 (±29.9)</td>
<td>47.5 (±29.9)</td>
</tr>
<tr>
<td>Responders (%)</td>
<td>141 (52.4%)</td>
<td>28 (52.8%)</td>
<td>31 (55.3%)</td>
<td>138 (51.2%)</td>
</tr>
<tr>
<td>Remitters (%)</td>
<td>73 (27.1%)</td>
<td>14 (26.4%)</td>
<td>18 (32.1%)</td>
<td>69 (25.9%)</td>
</tr>
</tbody>
</table>
**SUPPLEMENTAL TABLE 3.**

<table>
<thead>
<tr>
<th>Test</th>
<th>parameter</th>
<th>Statistical test</th>
<th>F value</th>
<th>Comparison</th>
<th>P value</th>
<th>Fig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ephy (5-HT_{1A}^{+/−})</td>
<td>DR Firing rate</td>
<td>paired t-test</td>
<td>_</td>
<td>baseline vs ESC</td>
<td>_</td>
<td>1A</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ephy (5-HT_{1A}^{−/−})</td>
<td>DR Firing rate</td>
<td>1-way ANOVA followed by Bonferroni post hoc test.</td>
<td>F_{3,26}=4.1 p=0.01</td>
<td>vehicle vs ESC, vehicle vs MDL, ESC vs MDL, vehicle vs DPAT</td>
<td>p&lt;0.001, p=0.1851, p=0.047, p=0.6949</td>
<td>1C</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ephy (WAY100635 in 5-HT_{1A}^{−/−})</td>
<td>DR Firing rate</td>
<td>1-way ANOVA followed by Bonferroni post hoc test.</td>
<td>F_{3,23}=3.8 p=0.02</td>
<td>vehicle vs ESC, vehicle vs MDL, ESC vs MDL, vehicle vs DPAT</td>
<td>p&lt;0.001, p=0.2821, p=0.039, p=0.6765</td>
<td>1E</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Microalalyis (5-HT_{2A}^{+/−})</td>
<td>[5-HT]_{ext}</td>
<td>2-way ANOVA followed by Bonferroni post hoc test.</td>
<td>Genotype, 5-HT_{2A}^{+/−} vs 5-HT_{2A}^{−/−}</td>
<td>saline, ESC, ESC+WAY</td>
<td>p=0.3456, p=0.8125, p=0.0449</td>
<td>2C</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treatment, In 5-HT_{2A}^{+/−}</td>
<td>saline vs ESC, ESC vs WAY+ESC, saline vs WAY+ESC</td>
<td>p&lt;0.001, p=0.0706, p&lt;0.001</td>
<td>2C</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treatment, In 5-HT_{2A}^{−/−}</td>
<td>saline vs ESC, ESC vs WAY+ESC, saline vs WAY+ESC</td>
<td>p=0.0260, p=0.0401, p&lt;0.001</td>
<td>2C</td>
</tr>
<tr>
<td></td>
<td>Firing rate</td>
<td>Unpaired t-test</td>
<td>Interaction</td>
<td>Genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------</td>
<td>----------------</td>
<td>--------------</td>
<td>-------------------------------</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Ephy 5-HT<sub>2A</sub> +/- and 5-HT<sub>2A</sub> -/- | F<sub>2,30</sub>=4.2  
  p=0.02 | _           | 5-HT<sub>2A</sub> +/- vs 5-HT<sub>2A</sub> -/- | t<sub>1,7</sub>=0.0145 |
| Hypothermia       | F<sub>2,30</sub>=4.2  
  p=0.02 | _           | 5-HT<sub>2A</sub> +/- vs 5-HT<sub>2A</sub> -/- | t<sub>1,9</sub>=0.0168 |
| Firing rate with ESC 5-HT<sub>2A</sub> +/- and 5-HT<sub>2A</sub> -/- | F<sub>3,353</sub>=9.5  
  p<0.001 | _           | _             | _                                      |

**Treatment in 5-HT<sub>2A</sub> +/-**

- ESC J0 vs ESC J2  
  p=0.0031
- ESC J0 vs ESC J21  
  p=0.8554
- ESC J0 vs ESC J28  
  p=0.0022

**Treatment in 5-HT<sub>2A</sub> -/-**

- ESC J0 vs ESC J2  
  p<0.001
- ESC J0 vs ESC J21  
  p<0.001
- ESC J0 vs ESC J28  
  p<0.001

**Interaction**

F<sub>3,353</sub>=9.5  
  p<0.001

In 5-HT<sub>2A</sub> +/-

- ESC J0 vs ESC J2  
  p=0.0031
- ESC J0 vs ESC J21  
  p=0.8554
- ESC J0 vs ESC J28  
  p=0.0022

In 5-HT<sub>2A</sub> -/-

- ESC J0 vs ESC J2  
  p<0.001
- ESC J0 vs ESC J21  
  p<0.001
- ESC J0 vs ESC J28  
  p<0.001
<table>
<thead>
<tr>
<th>Ephy</th>
<th>Firing rate with FLX</th>
<th>2-way ANOVA followed by Bonferroni post hoc test.</th>
<th>Genotype</th>
<th>5-HT\textsubscript{2A}\textsuperscript{+/−} vs 5-HT\textsubscript{2A}\textsuperscript{−/−}</th>
<th>3B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Genotype</td>
<td>F\textsubscript{3,310}=0.03, p=0.8577</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Genotype</td>
<td>FLX J0 (baseline)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Genotype</td>
<td>FLX J2</td>
<td>p=0.1512</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Genotype</td>
<td>FLX J21</td>
<td>p=0.3687</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Genotype</td>
<td>FLX J28</td>
<td>p=0.0035</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neurogenesis</th>
<th>Cell survival BrdU-positive cells</th>
<th>2-way ANOVA followed by Bonferroni post hoc test.</th>
<th>Genotype</th>
<th>Saline vs FLX:</th>
<th>4A</th>
</tr>
</thead>
<tbody>
<tr>
<td>(5-HT\textsubscript{2A}\textsuperscript{+/−} and 5-HT\textsubscript{2A}\textsuperscript{−/−})</td>
<td></td>
<td></td>
<td>Genotype</td>
<td>In 5-HT\textsubscript{2A}\textsuperscript{+/−} p=0.032</td>
<td></td>
</tr>
<tr>
<td>(5-HT\textsubscript{2A}\textsuperscript{+/−} and 5-HT\textsubscript{2A}\textsuperscript{−/−})</td>
<td></td>
<td></td>
<td>Genotype</td>
<td>In 5-HT\textsubscript{2A}\textsuperscript{−/−} p=0.995</td>
<td></td>
</tr>
<tr>
<td>(5-HT\textsubscript{2A}\textsuperscript{+/−} and 5-HT\textsubscript{2A}\textsuperscript{−/−})</td>
<td></td>
<td></td>
<td>Genotype</td>
<td>F\textsubscript{1,14}=11.9, p=0.004</td>
<td></td>
</tr>
<tr>
<td>(5-HT\textsubscript{2A}\textsuperscript{+/−} and 5-HT\textsubscript{2A}\textsuperscript{−/−})</td>
<td></td>
<td></td>
<td>Genotype</td>
<td>Treatment: F\textsubscript{1,14}=6.4, p=0.024</td>
<td></td>
</tr>
<tr>
<td>(5-HT\textsubscript{2A}\textsuperscript{+/−} and 5-HT\textsubscript{2A}\textsuperscript{−/−})</td>
<td></td>
<td></td>
<td>Genotype</td>
<td>Interaction: F\textsubscript{1,14}=4.95</td>
<td></td>
</tr>
</tbody>
</table>

<p>| Neurogenesis (5-HT2A&lt;sup&gt;+/−&lt;/sup&gt; and 5-HT2A&lt;sup&gt;−/−&lt;/sup&gt;) | Cell proliferation DCX-positive cells | 2-way ANOVA followed by Bonferroni post hoc test. | Genotype | $F_{1,14}=8.3$ | p=0.012 | Treatment | $F_{1,14}=22.1$ | p&lt;0.001 | Interaction | $F_{1,14}=3.8$ | p=0.07 | Saline vs FLX: | In 5-HT2A&lt;sup&gt;+/+&lt;/sup&gt; | p=0.003 | In 5-HT2A&lt;sup&gt;−/−&lt;/sup&gt; | p=0.201 | 4B |
| Behavior (5-HT2A&lt;sup&gt;+/−&lt;/sup&gt; and 5-HT2A&lt;sup&gt;−/−&lt;/sup&gt;) | NSF Latency to feed with FLX | Kruskal-Wallis test, followed by Dunn’s post hoc test | KW=9.92 | p=0.019 | Saline vs FLX: | In 5-HT2A&lt;sup&gt;+/+&lt;/sup&gt; | p=0.009 | In 5-HT2A&lt;sup&gt;−/−&lt;/sup&gt; | p=0.64 | 4D |
| Behavior (5-HT2A&lt;sup&gt;+/−&lt;/sup&gt; and 5-HT2A&lt;sup&gt;−/−&lt;/sup&gt;) | TST Time of immobility with FLX | 2-way ANOVA followed by Bonferroni post hoc test. | Genotype | $F_{1,49}=31.2$ | p&lt;0.001 | Treatment | $F_{1,49}=0.04$ | p=0.84 | Interaction | $F_{1,49}=17.2$ | p&lt;0.001 | Saline vs FLX: | In 5-HT2A&lt;sup&gt;+/+&lt;/sup&gt; | p=0.027 | In 5-HT2A&lt;sup&gt;−/−&lt;/sup&gt; | p=0.006 | 4F |
| Ephy 5-HT1A&lt;sup&gt;+/+&lt;/sup&gt; | DR Firing rate | paired t-test | _ | baseline vs ESC | $t_{4}=0.54$ | 4A |
| Ephy 5-HT1A&lt;sup&gt;−/−&lt;/sup&gt; | | | | | | 4B |
| Ephy 5-HT1A&lt;sup&gt;+/+&lt;/sup&gt; | | | | | | 4C |</p>
<table>
<thead>
<tr>
<th>Microialysis (5-HT\textsubscript{2A}+)</th>
<th>([5\text{-HT}]_{\text{ext}})</th>
<th>paired t-test</th>
<th>Saline vs WAY:</th>
</tr>
</thead>
</table>
| [5-HT\textsubscript{2A}]\textsuperscript{+/+} | In 5-HT\textsubscript{2A}+/
| t\textsubscript{1,12}=0.51 | In 5-HT\textsubscript{2A}+/ |
| [5-HT\textsubscript{2B}]\textsuperscript{+/+} | In 5-HT\textsubscript{2A}+/ |
| [5-HT\textsubscript{2B}]\textsuperscript{+/+} | In 5-HT\textsubscript{2A}+/ |

<table>
<thead>
<tr>
<th>Behavior (5-HT\textsubscript{2A}+/ and 5-HT\textsubscript{2A}−/−)</th>
<th>TST</th>
<th>2-way ANOVA followed by Bonferroni post hoc test.</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of immobility with ESC</td>
<td>(F_{1,30}=25.2)</td>
<td>p&lt;0.001</td>
<td>Saline vs ESC:</td>
</tr>
<tr>
<td></td>
<td>(F_{1,30}=6.04)</td>
<td>p=0.048</td>
<td>(p=0.033)</td>
</tr>
<tr>
<td></td>
<td>(F_{1,30}=15.5)</td>
<td>p&lt;0.001</td>
<td>(p=0.86)</td>
</tr>
</tbody>
</table>

\(S\text{2A}\) \(S\text{2B}\) \(S\text{3}\)