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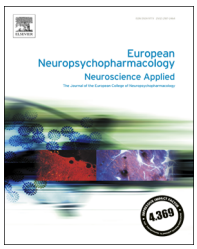

Mice lacking the serotonin 5-HT$_{2B}$ receptor as an animal model of resistance to selective serotonin reuptake inhibitors antidepressants

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Abstract
Depressive disorders are among the most prevalent neuropsychiatric dysfunctions worldwide, with high rates of resistance to antidepressant treatment. Genetic factors clearly contribute to the manifestation of depression as well as to the response to antidepressants. Transgenic mouse models appear as seminal tools to disentangle this complex disorder. Here, we analyzed new key aspects of the phenotype of knock-out mice for the gene encoding the serotonin 2B receptor ($Htr_{2B}^{-/-}$), including basal phenotype, ability to develop a depressive-like phenotype upon chronic isolation, and effect of chronic exposure to fluoxetine on chronically stressed $Htr_{2B}^{-/-}$ mice. We find, here, that $Htr_{2B}^{-/-}$ mice display an antidepressant-like phenotype, which includes reduced latency to feed in the novelty suppressed feeding test, basal increase in hippocampal BDNF levels, no change in TrkB and p75 protein levels, and an increased preference for sucrose consumption compared to wild type ($Htr_{2B}^{+/+}$) mice. Nevertheless, we show that these mice can develop depressive-like behaviors when socially isolated during four weeks. Selective serotonin reuptake inhibitors (SSRI) have been previously shown to be ineffective in non-stressed $Htr_{2B}^{-/-}$ mice. We evaluated, here, the effects of the SSRI fluoxetine in chronically stressed $Htr_{2B}^{-/-}$ mice and similarly no behavioral or plastic effect was induced by

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this antidepressant. All together, these results highlight the suitability to study resistance to SSRI antidepressants of this mouse model displaying panoply of conditions among which behavioral, neurotrophic and plastic causative factors can be analyzed.

1. Introduction

Major depressive disorder is one of the neuropsychiatric illnesses producing high economic burden, with depressed patients hampered to perform daily tasks. Given that an important proportion of depressed patients does not respond to available treatments, increasing efforts in research have been devoted to disentangling the ethiopatogeny around this illness and search for new pharmacological targets. However, only mild progress has been achieved in any of these two aspects (Kupfer et al., 2012). Depression is a complex health issue with genetic, social, physical and environmental factors contributing to its etiology. Therefore, animal models cannot completely recapitulate this heterogeneous illness. Nevertheless, available paradigms and tests can provide key and relevant neurobiological insights to bring light to the understanding of this pathology (Southwick and Charney, 2012).

We have recently shown that mice with genetic or pharmacological ablation of the serotonin receptor 2B subtype (Htr_{2B}) do not respond to selective serotonin reuptake inhibitor (SSRI) antidepressants (Diaz et al., 2012; Diaz and Maroteaux, 2011). Indeed, neither acute nor chronic classical SSRI effects have been observed in knockout mice for the 5-HT_{2B} receptor gene ($Htr_{2B}^{-/-}$). Interestingly, the basal response of $Htr_{2B}^{-/-}$ mice in the novelty suppressed feeding (NSF) test is similar to that of chronically SSRI-treated mice (Diaz et al., 2012). An antidepressant-like phenotype can be described as an animal expressing a repertoire of signs similar to those induced by the chronic administration of antidepressants. Therefore, the phenotype of the $Htr_{2B}^{-/-}$ mice in the NSF test could represent part of an antidepressant-like phenotype, but a better characterization is required.

Built on the observation that stressful events can precipitate a depressive state in humans, several animal models have been settled to study depressive disorder (for a review see Cryan and Mombereau 2004). Specifically, the unpredictable chronic mild stress (UCMS) originally developed in rats (Willner et al., 1992), and adapted to mice (Ducottet et al., 2004; Santarelli et al., 2003), is a paradigm in which animals are made to face unpredictable stressors during several weeks. As a result, animals develop anhedonic signs, and hence, a depressive-like state could be characterized. We have recently demonstrated that the UCMS protocol appears to have severe rather than mild effects in inbred 129S2/SvPas mice, the background strain of the embryonic stem cells used to generate $Htr_{2B}^{-/-}$ mice. In fact, 129S2/SvPas mice submitted to UCMS display marked features of stress and are irresponsive to antidepressants, whereas social isolation was revealed as a more confident and appropriate paradigm for 129 strains (Diaz and Maroteaux, 2015). In addition, as depression in humans is believed to be elicited by social stress rather than physical stress, the proposed social isolation model might better meet the etiological validity criteria (Blazer and Hybels, 2005).

The experiments presented herein were, therefore, conducted to address the following questions: (1) Do $Htr_{2B}^{-/-}$ mice present an antidepressant-like phenotype? (2) Are $Htr_{2B}^{-/-}$ mice able to develop a depressive-like state when submitted to experimental chronic isolation? (3) If $Htr_{2B}^{-/-}$ mice respond to the chronic isolation paradigm, are they able to respond to SSRI antidepressant treatment? To answer these questions, we analyzed key aspects of the $Htr_{2B}^{-/-}$ mice phenotype and an antidepressant-like phenotype was confirmed. We also demonstrated that $Htr_{2B}^{-/-}$ mice are able to develop a depressive-like phenotype when exposed to chronic isolation as wild type ($Htr_{2B}^{+/+}$) mice do. Finally, by classical assays to evaluate chronic antidepressant effects, we verified that chronically stressed $Htr_{2B}^{-/-}$ mice are still non-responsive to SSRIs therefore representing a useful model for the study of resistance to antidepressants.

2. Experimental procedures

2.1. Animals

For all the experiments, 7–9 week-old male mice were used. $Htr_{2B}^{+/+}$ and $Htr_{2B}^{-/-}$ mice were on a 129S2/SvPas background as the embryonic stem cells were used for homologous recombination. These animals were derived from heterozygote crosses and bred at the animal facilities of the Fer à Moulin Institute. Behavioral tests and animal care were conducted in accordance with standard ethical guidelines (National Institutes of Health's "Guide for the Care and Use of Laboratory animals", and European Directive 2010/63/UE), and have been approved by local ethical committee (N° 1170.01). All mice were maintained on a 12-light/ dark schedule (lights on at 8:00), temperature of (18–23 °C) with 40–60% humidity, and housed in groups of 3–5 of the same genetic background and sex after weaning until the beginning of the experimental protocol. Mice were randomly assigned to the different experimental groups. In all the studies, the observer was blind to the experimental conditions being measured.

2.2. In situ hybridization

Naïve mice (n=5–6 per genotype) were deeply anesthetized with xylazine (2 mg/kg i.p.) plus pentobarbital (50 mg/kg) and transcardially perfused with 5 ml of NaCl 0.9% and 50 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (1X PBS, pH 7.4) for 15 min. Brains were recovered and postfixed for 24 h at 4 °C in the same solution. Then, 50 μm thick coronal sections through the entire hippocampus and raphe were obtained on a vibratome. Sections were stored