Antidepressant treatment differentially affects the phenotype of high and low stress reactive mice

Alexandre Surget [a, b, c, *], Petra S. Van Nieuwenhuijzen [a, b, 1], Jan-Michael Heinzmann [d, 1], Alana Knapman [d], Silja McIlwrick [d], Willy-Paul Westphal [a, b], Chadi Touma [d, e, 2], Catherine Belzung [a, b, 2]

[a] U930 "Imaging and Brain", Inserm, Tours, France
[b] UFR Sciences et Techniques, Université François Rabelais, Tours, France
[c] Institute of Neuroscience and Psychology, University of Glasgow, Glasgow, UK
[d] Research Group of Psychoneuroendocrinology, Max Planck Institute of Psychiatry, Munich, Germany
[e] Department of Behavioural Biology, University of Osnabrück, Germany

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ABSTRACT

Modelling key endophenotypes can be a powerful approach to gain insight into mechanisms underlying the aetiology and pathophysiology of neuropsychiatric disorders. Based on evidence of stress hormone system dysregulations in depression, the Stress Reactivity (SR) mouse model has been generated by a selective breeding approach for extremes in HPA axis reactivity, resulting in high (HR), intermediate (IR) and low (LR) reactive mice. The characterisation of their phenotypic alterations has highlighted many similarities of HR and LR mice with the melancholic and atypical depression, respectively. We therefore aimed to examine whether the antidepressant fluoxetine (10 mg/kg/day i.p., 4—5 weeks) can ameliorate the phenotypic characteristics of HR and LR mice in neuroendocrine functions (HPA axis basal activity, stress reactivity, negative feedback), emotional reactivity/coping-strategy (open field, forced swim tests), spatial learning/memory (Morris water-maze) and hippocampal neurogenesis. Line differences in HPA axis reactivity were maintained under fluoxetine treatment. However, we observed fluoxetine effects on glucocorticoid-induced negative feedback, stress-coping behaviours, cognitive functions and neurogenesis. Specifically, our results revealed line-dependent consequences of fluoxetine treatment: (1) an amelioration of the 'melancholic-like' features of HR mice (reversing the negative feedback resistance, the hyperactive coping style and the memory deficits; increasing hippocampal neurogenesis); (2) an exacerbation of the phenotypic deviations of LR mice (increasing their pronounced negative feedback and passive coping style). Thus, these findings support the predictive validity of antidepressant treatment in the HR mouse line and emphasize the translational value of the SR mouse model for the development of therapeutic strategies based on endophenotype-driven classifications.

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1. Introduction

Animal models are essential to explore neural circuits and dynamic neurobiological processes that are not directly accessible from human subjects. However, the lack of knowledge on major depressive disorders (MDDs) limits the capacity to objectively assess the quality of the models and question their validity (van der Staay et al., 2009). Moreover, considering the multifactorial origins of depression along with its symptomatic variability and the subjective nature of some symptoms (Ostergaard et al., 2011), trying to recapitulate the full depressive syndrome in animals is certainly unrealistic. In order to overcome these limitations, an alternative approach focusing on endophenotypes has increasingly gained support (Hasler et al., 2004). Endophenotypes are assumed to represent more basic phenomena that are related either to a single component of the clinical symptomatology or a biological trait associated with MDDs. As more elementary phenotypes, they are supposed to involve fewer genes, to underlie more straightforward pathophysiological processes and to improve the translation from

* Corresponding author. U930 "Imaging and Brain" INSERM and Université F. Rabelais, Bât. L (S300) Parc de Grandmont, 37200 Tours, France.
E-mail addresses: alexandre.surget@univ-tours.fr, alex.surget@gmail.com (A. Surget).
[1] These authors contributed equally.
[2] These authors contributed equally.

preclinical to clinical research.

Selective breeding is a valuable approach to isolate specific endophenotypes. It has recently been used to select CD-1 mice for extremes in hypothalamic-pituitary-adrenocortical (HPA) axis reactivity, thereby establishing the so-called 'stress reactivity' (SR) mouse model (Touma et al., 2008). Indeed, dysfunction of the stress hormone system is one of the most consistent physiological alterations reported in MDDs (de Kloet et al., 2005; Nemeroff et al., 1984; Stetler and Miller, 2011). In particular, HPA axis abnormalities are commonly observed in depressed patients and can be characterized by altered circadian activity, aberrant glucocorticoid (GC) release in response to stressors, and/or an impaired negative feedback (Holsboer, 2000; Holsboer and Ising, 2010). It is noteworthy that the directionality of HPA axis dysregulations depends on the MDD subtypes: hyperactivity and disturbed negative feedback are frequently found in melancholic depression, while hypoactivity and exaggerated negative feedback have been linked to atypical depression (Antonijevic, 2006; Gold and Chrousos, 2002). In the SR mouse model, three breeding lines with different levels of corticosterone (CORT) release in response to a psychological stressor (15 min restraint) have been established: the high (HR), intermediate (IR) and low (LR) reactivity mice. In addition to their neuroendocrine disturbances, HR and LR mice display a number of other phenotypic similarities with MDDs (Heinzmann et al., 2014; Knapman et al., 2010b; Touma et al., 2008; Touma et al., 2009). For instance, increased HPA axis activity and decreased glucocorticoid-induced negative feedback in the HR line are associated with lower body weight, disturbed sleep architecture, cognitive deficits, increased emotional reactivity and hyperactive stress-coping behaviour. Such impairments are reminiscent of many characteristics of the melancholic subtype of depression. In contrast, the LR mice recapitulate some features associated with atypical depression: exacerbated HPA axis negative feedback, higher body weight, reduced emotional reactivity and passive coping style. Altogether, these previous works enabled the identification of many phenotypic consequences of the genetic predisposition for extremes in HPA axis reactivity. However, the question whether the different neuroendocrine and behavioural phenotypes of HR and LR mice can be reversed by antidepressant treatments had not been addressed yet.

Accordingly, this study aimed to assess the effects of antidepressant treatments in the SR mouse model. We investigated whether the selective serotonin reuptake inhibitor (SSRI) fluoxetine can ameliorate the HPA axis disturbances and other important phenotypic characteristics (emotional reactivity, spatial learning/memory) observed in HR and LR mice. Neuroendocrine functions were assessed via three distinct measures of HPA axis activity: basal activity, stress responsiveness and glucocorticoid-induced negative feedback as assessed in the combined dexamethasone/corticotropin-releasing hormone (Dex/CRH) test. The examination of emotional reactivity was based on stress-coping behaviour in the Forced Swim test (FST) as well as anxiety-related behaviours and exploratory drives in the Open Field test. Hippocampus-dependent spatial learning and memory were evaluated in the Morris watermaze (MWM). Finally, since hippocampal neurogenesis has been found to be reduced in depression (Lucassen et al., 2010) and critically involved in antidepressant response and regulation of the HPA axis (Surget et al., 2011), we also examined cell proliferation (Ki67+) and levels of immature neurons (doublecortin-positive, DCX+) in the dentate gyrus (DG) of the hippocampal formation.

2. Methods

2.1. Animals and housing conditions

The study used adult male mice from generations XV and XVII of the SR mouse model, which consists on three independent mouse lines derived from the CD-1 mouse strain and selectively bred for extreme HPA axis reactivity (see details about this approach in Touma et al., 2008). The mice were housed in groups of four animals in transparent polycarbonate cages (38 × 22 × 15 cm) with wood chips as bedding and wood shavings as nesting material. At the age of about eight weeks, HPA axis responsiveness to a stressor was assessed by means of the stress reactivity test (SRT) described below (see 2.3). The mice used in the experiments described below were 3–5 months of age and single housed (cage 23 × 16 × 14 cm) at least ten days before performing the experiments in order to avoid potential dominance hierarchy effects. Housing and experimental rooms were kept under standard laboratory conditions (12/12 h light/dark cycle, lights on 8:00 h; temperature: 22 ± 1 °C; relative humidity: 55 ± 10%). Commercial mouse diet (Altromin GmbH, Lage, Germany) and tap water were available ad libitum. All conducted experiments were in accordance with the current regulations covering animal experimentation (European Communities Council Directive 86/609/EEC) and approved by the appropriate local authorities.

2.2. Experimental design

A first cohort of mice was used to investigate the effects of chronic fluoxetine treatment on HPA activity and emotional reactivity (Fig. 1). Briefly, HR, IR and LR lines received a daily treatment with fluoxetine or vehicle (0.9% NaCl) for a total of 5 weeks. Fluoxetine was administered i.p. and at the concentration of 10 mg/kg/day based on previous experiments (Surget et al., 2008; Yalcin et al., 2008). After 25 days of treatment, baseline plasma CORT level was assessed from blood samples ('initial sample'). After 28 days of treatment, emotional reactivity was examined in a combined behavioural test comprising a 10-min open field test and a 6-min FST, which was immediately followed by a blood sampling to assess HPA axis reactivity to the swim stress ('reaction sample'). After 32 days of treatment, another blood sampling was carried out to obtain baseline plasma CORT values ('untreated sample') for the combined Dex/CRH test. Finally, after 35 days of treatment, the combined Dex/CRH test was performed.

A second cohort of mice was used to investigate the effects of chronic fluoxetine on spatial memory and hippocampal neurogenesis (Fig. 5a). HR, IR and LR mice received a daily treatment with fluoxetine (10 mg/kg/day, i.p.) or vehicle (0.9% NaCl) for a total of 33 days. After 26 days of treatment, all mice were subjected to the MWM test, consisting of a 4-day training stage followed by a probe trial. Four days later, the brain was collected to assess hippocampal cell proliferation/neurogenesis by immunohistochemistry.

2.3. Stress reactivity test (SRT)

The SRT is described in detail elsewhere (Touma et al., 2008). Briefly, the test consists of a 15-min restraint period and two tail blood samplings, one immediately before and one right after the restraint stressor. The animals' plasma CORT increase in response to the SRT served as criterion for selecting the animals over generations for the respective experimental groups of the three mouse lines.

2.4. Blood sampling

Blood sampling was performed as described previously (Touma et al., 2008). Blood samples obtained from the animals' ventral tail vessel were collected in EDTA-coated tubes (Microvette, Sarstedt, Nürnbrecht, Germany). Trunk blood was collected in EDTA-coated tubes (KABE Labortechnik GmbH, Nürnbrecht-Elsenroth,