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Sex differences in response to chronic mild stress and congenital serotonin deficiency

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Summary Women exhibit a nearly twofold increased risk of developing depression and anxiety disorders when compared to men, a fact that has been hypothesized to result in part from increased stress susceptibility. Here, we used the tryptophan hydroxylase-2 R439H knock-in mouse (Tph2KI) and the chronic unpredictable mild stress (CMS) model to examine sex differences in response to congenital 5-HT deficiency and chronic stress. Our results demonstrate that female mice, but not 5-HT-deficient animals, exhibit significantly increased susceptibility to CMS-induced despair-like behavior in the forced swim test. In addition, female 5-HT-deficient mice exhibit anhedonia-like behavior in the sucrose preference test, whereas male 5-HT-deficient animals do not, suggesting that females exhibit increased sensitivity to at least some of the effects of congenital 5-HT deficiency. Although CMS did not reduce cell proliferation in the hippocampus, low levels of brain 5-HT were associated with increased hippocampal cell proliferation, an effect that was predominantly observed in females. Overall, these results highlight the importance of interactions between psychiatric disease risk factors such as sex, chronic stress and congenital 5-HT deficiency in the development of aberrant emotional behavior.

1. Introduction

The diathesis—stress hypothesis of psychiatric disease proposes that mental illnesses, such as depression and anxiety disorders, stem from interactions between biological vulnerability factors and exposure to environmental stressors. However, the factors that influence stress susceptibility remain largely unknown. The brain serotonin (5-HT) system has been widely implicated in the etiology of depression and anxiety disorders, largely due to the fact that most antidepressants increase extracellular 5-HT. Support for the 5-HT deficiency theory of depression has also come from clinical studies identifying 5-HT-deficiency-like biomarker alterations in depression patients (Mann et al., 1996; Asberg, 1997) and genetic studies identifying mutations in tryptophan hydroxylase 2 (Tph2), the rate limiting enzyme in brain 5-HT

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synthesis, in suicide victims and patients with affective disorders, such as major depression (Zill et al., 2004a,b; Zhang et al., 2005; Cichon et al., 2008). However, the factors that regulate susceptibility to the consequences of 5-HT deficiency have not been widely explored, and the effects of congenital 5-HT deficiency on stress vulnerability have not been elucidated.

Women exhibit a nearly two-fold higher incidence of depression and anxiety disorders when compared to men (Breslau et al., 1997; Young and Korszun, 2010), and they have been reported to have increased sensitivity to some of the effects of stress (Weiss et al., 1999) and tryptophan depletion, an experimental model of 5-HT deficiency (Booij et al., 2002). To investigate the impact of sex on susceptibility to chronic stress and congenital 5-HT deficiency, we used the tryptophan hydroxylase-2 R439H knock-in (Tph2KI) mouse line, which harbors a loss-of-function mutation in the 5-HT synthesis enzyme, *tryptophan hydroxylase-2* (*Tph2*). Tph2KI mice have been shown to exhibit alterations in several depression- and anxiety-like behaviors and 60—80% reductions in 5-HT in the frontal cortex, hippocampus, striatum and amygdala (Beaulieu et al., 2008; Jacobsen et al., 2012a,b; Sachs et al., 2013a,b).

In the current work, we employed the chronic unpredictable mild stress (CMS) model, which has been previously shown to induce depression- and anxiety-like behavior in rodents (Willner et al., 1987; Surget et al., 2009; Griebel et al., 2002). Here, we examined the responses of Tph2KI mice to CMS to evaluate the combinatorial effects of sex, stress and 5-HT deficiency on depression- and anxiety-like behavior and on hippocampal neurogenesis. Chronic stress has been reported to inhibit adult hippocampal neurogenesis (Gould et al., 1997, 1998; Malberg and Duman, 2003), but the importance of hippocampal neurogenesis for depression- and anxiety-like behavior remains controversial (Petrik et al., 2012). The current study provides preclinical support for the diathesis—stress model of psychiatric disease and sheds new light on the complex interactions that regulate emotional behavior and the factors that influence susceptibility to pathological conditions, such as chronic stress and 5-HT deficiency.

2. Materials and methods

2.1. Animals

The generation of Tph2KI mice, which are on a mixed background (c57BL6/J — 129S6/SvEvTac), has been described previously (Beaulieu et al., 2008). Age-matched (10—12 weeks old at the start of the experiments) WT and Tph2KI littermates were used for all experiments. Animals used for this study were derived from heterozygous—heterozygous breeding pairs to prevent any potential confounding effects of maternal/paternal behavior. Mice were housed 4—5 per cage except for sucrose preference tests (SPTs), during which they were singly housed. CMS mice (but not controls) were also periodically housed in isolation overnight as part of the CMS paradigm (see below). Control mice were maintained on a 12 h light—dark cycle in a temperature-controlled facility and had *ad libitum* access to food and water. Experiments were conducted during the light phase. All experiments were performed in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals and were covered by a protocol that had been approved by the Duke University Institutional Animal Care and Use Committee.

2.2. Chronic mild stress

The CMS paradigm involved randomly exposing mice to two of the following stressors each day (except for behavioral testing days, on which mice were not exposed to stressors) for seven weeks (one in the morning between ~9—11 AM, and one in the afternoon between 3—6 PM): single housing (overnight), damp bedding (8 h), cage tilting (2 h), cage shaking (~1 Hz, 1 h), swim stress (10 min), lights on overnight, lights off during the day (3 h) and restraint stress (1 h). Other than for the single housing stressor and for sucrose preference tests, CMS mice were group housed 4—5/cage. The mice were not subjected to any food or water deprivation longer than 1 h. A timeline of stress exposure and behavioral testing can be found in Fig. 1.

2.3. Behavioral tests

For the elevated plus maze (EPM), mice were video-recorded and the location and total distance traveled of each mouse over a 5 min period were analyzed using EthoVision, version 7.0 (Noldus, The Netherlands), as described previously (Kumar et al., 2013). The forced swim test (FST) was performed by placing a mouse in a 3 L beaker of water (23 °C) for 6 min and videotaping its behavior. Immobility was scored using EthoVision over the full 6 min. The novel open field (NOF) test was performed by videotaping animals in activity chambers (45 cm × 45 cm, ~125 lx) and analyzing the total distance traveled and the time spent and the distance traveled in the center of the arena over a 20 min period using EthoVision. The EPM, FST and NOF were conducted between 1 PM and 6 PM. For the SPT, mice were individually housed and given access to two sipper bottles, one with standard drinking water and the other with standard drinking water plus 1% sucrose. The volume of both solutions consumed over an 18 h period (5 PM—11 AM) was recorded. All bottles were checked for leaks, and any leaking bottles were not included in the final analysis.

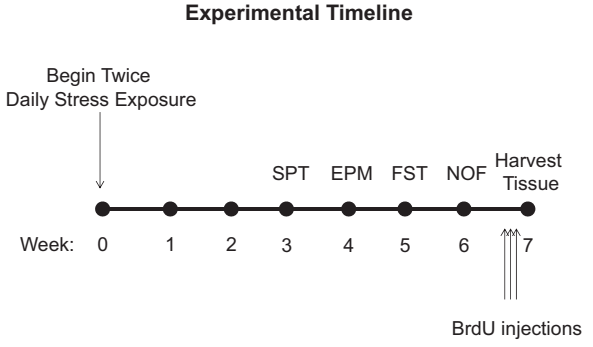

Figure 1 A timeline of experiments. SPT = sucrose preference test, EPM = elevated plus maze, FST = forced swim test, NOF = novel open field test.