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Blunted HPA axis reactivity reveals glucocorticoid system dysbalance in a mouse model of high anxiety-related behavior

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Summary Depression and anxiety disorders are often characterized by altered hypothalamic—pituitary—adrenal (HPA) axis re-/activity. However, the presence of a molecular link between dysbalanced neuroendocrine regulation and psychopathologies is not yet fully established. Earlier, we reported that high (HAB), normal (NAB) and low (LAB) anxiety-related behavior mice express divergent anxiety-related and passive/active coping phenotypes. Here, we studied mechanisms that might contribute to the different HPA axis reactivity observed in HAB, NAB and LAB mice and their involvement in the regulation of anxiety-related behavior and passive/active coping style. We found that HAB mice respond with significantly reduced corticosterone (CORT) secretion to an acute stressful stimulus and a blunted response in the Dex/CRH test compared to NAB and LAB mice. At the molecular level, higher expression of the glucocorticoid receptor (GR/*Nr3c1*) and decreased corticotropin-releasing hormone receptor 1 (*CRHR1*) expression were observed in the pituitary of HAB mice. We further analyzed whether these stress mediators differed between the HAB, NAB and LAB lines in limbic system-associated brain regions and whether their interplay contributes to the phenotype. Interestingly, not only in the pituitary but also in almost all brain regions investigated, GR expression was significantly higher in HAB mice. In contrast, the amount of CORT in the brain structures analyzed was significantly lower in these animals. The expression of *CRHR1* varied in the prefrontal cortex only. Since glucocorticoids regulate both *GR* and *CRHR1*, we treated HAB and NAB mice chronically

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with CORT. After 6 weeks of administration, reduced anxiety- and depression-like behaviors were observed in HAB mice, whereas increased anxiety was found in NABs. In both groups, GR, but not *CRHR1*, were significantly reduced. Taken together, our study proposes HAB mice as an animal model of simultaneous features of increased anxiety-related and depression-like behaviors with blunted HPA axis reactivity suggesting a dysregulated GR/CORT system as one key mechanism behind their phenotype.

1. Introduction

The hypothalamic—pituitary—adrenal (HPA) axis plays a pivotal role in regulating the physiological responses induced by stressful events. Its activation prepares the body in life-threatening situations by mobilizing glucose in the muscles, activating the liver to replenish energy supplies and promoting anti-inflammatory effects to withdraw energy from processes not absolutely necessary at that very moment. Although the mechanisms and the functioning of the HPA axis are highly adaptive, its reaction has to be regulated within an appropriate time frame *via* negative feedback. Since the appearance of the first humans, environments and the nature of stressors have changed substantially. Everyday long-lasting unavoidable social stress of modern life has largely replaced acute stress insults of prehistoric people, thereby complicating a precise recognition of initiation and termination phases of stress responses. It has been hypothesized that this shift in stress conditions contributes to the current high prevalence of stress-related psychopathologies, *e.g.* depression and anxiety, which are associated with altered HPA axis activity.

Across a variety of psychopathologies, changes in the regulation of the HPA axis are not unidirectional and sometimes can be observed only for subtypes of diseases under specific conditions. Thus, in case of ''melancholic'' depression, the HPA system has been reported to be hyperactive, whereas ''atypical'' depression, in contrast, is associated with hypoactive HPA axis functioning (Hasler et al., 2004; Lamers et al., 2013). Frequently, a dysregulated neuroendocrine system cannot be observed under basal conditions, but can only be revealed following exposure to stressful stimuli (Handwerger, 2009). Thus, the need of ''personalized'' approaches for the treatment of individual psychiatric disorders is of high significance since each disease subtype might have a specific molecular signature and neuroendocrine profile. However, the question remains whether an impaired regulation of key stress mediators is limited to the neuroendocrine system only, or if their functioning is affected in other brain structures as well. Although much effort has been invested to study this question, the precise mechanisms are still largely unknown.

Animal models of psychiatric disorders are useful tools for elucidating associations between behavioral symptoms and biochemical/physiologic alterations to suggest possible treatment strategies for psychiatric diseases. Here, we used an animal model of extremes in trait anxiety that has been already repeatedly and successfully applied to reveal multiple risk factors of psychopathologies (Landgraf et al., 2007; Sartori et al., 2011a). The high (HAB), normal (NAB) and low (LAB) anxiety-related behavior mouse model was established by selective inbreeding of outbred CD-1 mice based on their behavior in the elevated plus-maze (EPM) (Krömer et al., 2005). Selective inbreeding induced a robust divergence of not only anxiety-related behavior, but also of a passive/active coping style indicative of depression-like behavior (Krömer et al., 2005; Sotnikov et al., 2011). Several molecular pathways were found to be commonly affected in both psychiatric patients and HAB mice (Hambsch et al., 2010; Erhardt et al., 2011).

Here, we studied the phenomenon of blunted HPA axis reactivity in HAB mice. Based on a series of experiments, we propose GR/CRHR1-mediated mechanisms to drive the blunted CORT response in this line. To further characterize the contribution of these factors, we investigated their expression in different brain regions and their possible involvement in the regulation of behavior in the HAB/NAB/LAB mouse model.

2. Materials and methods

2.1. Animals

All animal experiments on mice were approved by the Government of Upper Bavaria (AZ 55.2-1-54-2532-93-12 and AZ 55.2-1-54-2532-64-07) and conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and UK Animals (Scientific Procedures) Act of 1986. CD1 mice were purchased from Charles River (Sulzfeld, Germany) and kept separate from the testing room. Mice were selectively inbred from a CD-1 outbred population for high, ''normal'' (NAB) or low anxiety-related behavior for >50 generations, with percent time spent on the open arms as a key criterion (HAB < 15%, NAB 35—45%, LAB > 60%). Animals were kept under standard housing conditions and were provided with food and water *ad libitum* (for details see Supplementary Material). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to *in vivo* techniques, if available.

2.2. Behavioral phenotyping

Anxiety-related behavior was measured using the elevated plus-maze (EPM) and light—dark box (LDB) tests as described earlier (Krömer et al., 2005). Locomotor activity was evaluated additionally in the open field (OF) test. All experiments were video-tracked and analyzed using Any-maze (Version 4.60, Stoelting Co., Wood Dale, USA). Time spent floating in the forced swimming test (FST) was used as an indicator