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Increased stress reactivity is associated with cognitive deficits and decreased hippocampal brain-derived neurotrophic factor in a mouse model of affective disorders

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ABSTRACT

Cognitive deficits are a common feature of major depression (MD), with largely unknown biological underpinnings. In addition to the affective and cognitive symptoms of MD, a dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis is commonly observed in these patients. Increased plasma glucocorticoid levels are known to render the hippocampus susceptible to neuronal damage. This structure is important for learning and memory, creating a potential link between HPA axis dysregulation and cognitive deficits in depression. In order to further elucidate how altered stress responsiveness may contribute to the etiology of MD, three mouse lines with high (HR), intermediate (IR), or low (LR) stress reactivity were generated by selective breeding.

The aim of the present study was to investigate whether increased stress reactivity is associated with deficits in hippocampus-dependent memory tests. To this end, we subjected mice from the HR, IR, and LR breeding lines to tests of recognition memory, spatial memory, and depression-like behavior. In addition, measurements of brain-derived neurotrophic factor (BDNF) in the hippocampus and plasma of these animals were conducted.

Our results demonstrate that HR mice exhibit hippocampus-dependent memory deficits along with decreased hippocampal, but not plasma, BDNF levels. Thus, the stress reactivity mouse lines are a promising animal model of the cognitive deficits in MD with the unique feature of a genetic predisposition for an altered HPA axis reactivity, which provides the opportunity to explore the progression of the symptoms of MD, predisposing genetic factors as well as new treatment strategies.

1. Introduction

Patients suffering from MD often complain of cognitive impairment, mainly memory impairment and executive functioning deficits (Austin et al., 2001; Porter et al., 2003; Reppermund et al., 2007). It has been reported that divided attention and verbal working memory deficits were present in the majority of subjects, both on admission and at discharge (Reppermund et al., 2007). Furthermore, low episodic memory performance in healthy subjects has been associated with an increased risk of developing depression, and can thus be seen as a premorbid marker of MD (Airaksinen et al., 2007).

There are also physiological correlates of MD, such as a dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis. MD is generally associated with a hyperresponsive HPA axis (Holsboer, 2000; Ising et al., 2005). However, there are also depressed patients exhibiting a hypofunction of the hypothalamic corticotrophin-releasing hormone (CRH) system along with other symptoms characteristic of this subtype of MD (Angst et al., 2002; Antonijevic, 2006; Gold and Chrousos, 2002; Gold et al., 1995). In the context of cognitive deficits in MD, it is relevant to distinguish between the patients with a hypoactive and hyper-active HPA axis as cognitive dysfunction, in particular memory deficits, have been associated with hypercortisolemia in depressed patients (Belanoff et al., 2001; Bremner et al., 2004). The relationship between memory dysfunction and hypercortisolemia is presumably brought about by the negative influence of glucocorticoids on the hippocampus, a structure that is important for learning and memory (Eichenbaum, 2000; Sapolsky et al., 1990; Scoville and Milner, 1957). Repeated stress and chronically increased glucocorticoid levels have been reported to be associated with a reduction in hippocampal volume, dendritic atrophy in the CA3 region of the hippocampus and reduced birth of new granule cells in the dentate gyrus of the hippocampus (Lyons et al., 2007; McKittrick et al., 2000; Sapolsky et al., 1990; Sousa et al., 1999; Watanabe et al., 1992). Using *in vivo* imaging, hippocampal volume has been found to be decreased in patients suffering from MD (Bremner et al.,

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2000; Frodl et al., 2006; Holsboer and Ising 2010; MacQueen et al., 2003; Sheline et al., 1996) and in patients suffering from Cushing's syndrome, where hypercortisolemia is a cardinal symptom (Starkman et al., 1992).

Glucocorticoids have an acute effect on memory processing. Stress and glucocorticoids are believed to enhance memory consolidation, whereas increased glucocorticoid levels impair memory retrieval (Buchanan and Lovallo, 2001; de Quervain et al., 1998; Flood et al., 1978; Holsboer and Ising, 2010; Kovacs et al., 1977; Kuhlmann and Wolf, 2006; Oitzl et al., 1998; Roozendaal, 2000). The effects of stress on memory function are also dependent on the type of memory being studied (Luethi et al., 2008). Furthermore, studies using a standardized psychosocial stressor have confirmed a correlation between cortisol reactivity and memory impairment in healthy adults (Kirschbaum et al., 1996).

Moreover, studies on rodents have shown that glucocorticoid excess decreases the level of brain-derived neurotrophic factor (BDNF) in the hippocampus (Jacobsen and Mork, 2006; Murakami et al., 2005; Schaaf et al., 1998, 1997), which has been strongly implicated in the regulation of hippocampal long-term potentiation (LTP) (Chen et al., 1999; Korte et al., 1996; Patterson et al., 1996). Reduced hippocampal BDNF levels have furthermore been shown to impair memory performance in a number of animal studies (Gorski et al., 2003; Monteggia et al., 2004; Schaaf et al., 2001). Interestingly, BDNF levels are also reduced in the plasma of depressed patients and tend to increase in response to antidepressant treatment (Huang et al., 2008; Lee et al., 2007; Monteleone et al., 2008; Nibuya et al., 1996; Santarelli et al., 2003). It is believed that said increase in BDNF may play a key role in the effectiveness of the antidepressant treatment (Wang et al., 2008). However, the changes in brain BDNF concentration demonstrated in animal studies and the plasma BDNF concentrations of patients may merely reflect the treatment-induced decrease in plasma corticosterone/cortisol level (Greden et al., 1983; Holsboer et al., 1982; Ising et al., 2007). Furthermore, low plasma BDNF levels have been associated with memory impairment in MD (Grassi-Oliveira et al., 2008). However, it is important to note that plasma BDNF levels are not necessarily a reflection of brain BDNF levels.

The aim of the present study was to further the understanding of the relationship between HPA axis hyperresponsiveness or hyporesponsiveness and memory function. We approached this question by using a recently established animal model generated by selectively inbreeding mice for extremes in stress reactivity (Touma et al., 2008). A founder population of CD-1 mice was subjected to a standardized stressor (15 min restraint stress) and their HPA axis reactivity in response to this stressor was determined by subtracting the corticosterone level of the initial blood sample, taken before the stressor, from the corticosterone level of a blood sample taken immediately after the restraint stress. Males and females with very high stress reactivity (HR) were then mated, as were males and females with extremely low stress reactivity (LR). Their offspring were tested for their stress reactivity in the same manner and so forth for each generation to come (for details see (Touma et al., 2008)). An intermediate (IR) reactivity line was additionally established to serve as a control group with the same inbreeding status as the other two lines. The IR mice present a corticosterone response similar to the mean of the founder population of CD-1 mice (Touma et al., 2008).

These mouse lines have previously been characterized with respect to several endophenotypes relevant to MD. In summary, the HR mice display several characteristics found in patients suffering from melancholic depression, such as a hyperreactive HPA axis, a flattened circadian glucocorticoid rhythm, a hyper-active coping style, increased rapid eye movement sleep, and decreased body weight. On the other hand the LR mice display similarities to the atypical subtype of depression, such as a hyporeactive HPA axis, a more passive coping style, increased aggressive behavior and increased body weight (Touma et al., 2008, 2009). In order to investigate the cognitive abilities of this stress reactivity mouse model, we implemented a novel object recognition test to assess recognition memory and a Y-maze free choice exploration paradigm to assess spatial learning (Dellu et al., 2000). Furthermore, coping behavior was assessed using the forced swim test (FST). When the behavioral testing was completed the brains were collected and the amount of BDNF in the hippocampus was quantified.

The great advantage of this mouse model over others investigating the role of the HPA axis in depression is that it allows for the investigation of two pathological states i.e. an extremely high HPA axis reactivity and an extremely low HPA axis reactivity. In addition, it is possible to apply a top down approach and go from a known endophenotype to investigate the genotype. Another unique advantage is that knowing that the animals from a specific line will develop a certain phenotype enables the possibility to study the progression of the symptoms of the disease.

2. Methods

2.1. Subjects

Animals derived from the eighth generation of the stress reactivity (SR) mouse model (for details see (Touma et al., 2008)) were used in these experiments. Twelve male and 12 female mice from the HR, IR, and LR breeding lines, were selected according to their corticosterone increase in the stress reactivity test (SRT) (described below). In order to control for litter effects the mice were selected from a minimum of six different litters per line. During the behavioral testing period, animals were single housed in transparent polycarbonate cages (standard macrolon cages type II, $26 \times 20 \times 14$ cm) with food and water available *ad libitum*. A 12:12 h light–dark cycle with lights on at 8 a.m. was maintained throughout breeding and testing. The housing rooms and experimental rooms were kept at a constant temperature (22 ± 1 °C) and humidity ($55 \pm 10\%$). Animals were between 3 and 5 months of age during the period of behavioral testing. All experiments were performed during the trough of the circadian rhythm of glucocorticoid secretion (between 9 and 12 a.m.) and the order of testing was counterbalanced across the different breeding lines.

The presented work complies with current regulations covering animal experimentation in Germany and the EU (European Communities Council Directive 86/609/EEC). All experiments were announced to the appropriate local authority and were approved by the 'Animal Welfare Officer' of the Max Planck Institute of Psychiatry.

2.2. Stress reactivity test and plasma corticosterone measurements

At the age of approximately 8 weeks, the mice were subjected to a stress reactivity test (SRT) in order to assess the reactivity of their hypothalamic–pituitary–adrenal (HPA) axis. The SRT is described in detail elsewhere (Touma et al., 2008). Briefly, an initial blood sample for corticosterone measurement was collected from a small incision in the ventral tail vessel. The mouse was then placed in a restraint tube for 15 min. Following the restraint stress, a second blood sample, the reaction sample, was obtained from a second incision in the tail vessel. The corticosterone increase in response to this stressor was calculated by subtracting the corticosterone concentration in the initial sample from the corticosterone concentration in the reaction sample.

Plasma corticosterone concentrations were determined using a commercial radioimmunoassay (RIA) kit (MP Biomedicals, Solon, Ohio, USA), with slight modifications to the manufacturer's