Psychoneuroendocrinology (2007) 32, 417–429

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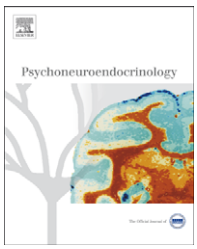

Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence

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Received 21 July 2006; received in revised form 9 February 2007; accepted 18 February 2007

KEYWORDS
Chronic stress;
Mouse;
HPA axis;
Anxiety;
DMP696;
Paroxetine

Summary
Chronic stress is widely regarded as a key risk factor for a variety of diseases. A large number of paradigms have been used to induce chronic stress in rodents. However, many of these paradigms do not consider the etiology of human stress-associated disorders, where the stressors involved are mostly of social nature and the effects of the stress exposure persist even if the stressor is discontinued. In addition, many chronic stress paradigms are problematic with regard to stress adaptation, continuity, duration and applicability. Here we describe and validate a novel chronic social stress paradigm in male mice during adolescence. We demonstrate persistent effects of chronic social stress after 1 week of rest, including altered adrenal sensitivity, decreased expression of corticosteroid receptors in the hippocampus and increased anxiety. In addition, pharmacological treatments with the antidepressant paroxetine (SSRI) or with the corticotropin-releasing hormone receptor 1 antagonist DMP696 were able to prevent aversive long-term consequences of chronic social stress. In conclusion, this novel chronic stress paradigm results in persistent alterations of hypothalamus–pituitary–adrenal axis function and behavior, which are reversible by pharmacological treatment. Moreover, this paradigm allows to investigate the interaction of genetic susceptibility and environmental risk factors.

1. Introduction

Chronic stress is generally considered a key risk factor for the development of a variety of human diseases (Anisman and Zacharko, 1992). Specifically anxiety and depressive disorders have frequently been associated with preceding

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doi:10.1016/j.psyneuen.2007.02.011

periods of chronic stress or stressful life events (Post, 1992; Amat et al., 2005). In addition to psychiatric disorders, chronic or severe stress has also been implicated in a variety of other diseases, such as the metabolic syndrome (including diabetes type II), cardiovascular disease and hypertension (Henry and Stephens, 1977; Pickering, 2001; Tennant, 2001).

To study the multiplicity of neurochemical pathways that are affected by chronic stress, a wide range of stimuli and paradigms have been used to induce a state of stress pathology. The more frequently used rodent paradigms for chronic stress often consist of the repeated exposure of the animal to physical stressors, such as electric shock, water immersion or restraint. However, large differences exist in the duration of the stressor, ranging from 2 to 16 h per day for 7–28 days (Ader and Grota, 1970; Ottenweller et al., 1989; Aguilera, 1994; Conrad et al., 1996; Anisman et al., 1998). Despite the intensity of the stressors, many of these paradigms do not fulfill the criteria of chronic stress, as the stressors are only applied for a relatively short time per day or for only a few consecutive days. In order to circumvent the additional problem of habituation and predictability, Willner and colleagues developed a paradigm of chronic mild stress (CMS), where different types of mild stressors are alternated (Willner, 2005). Mice or rats subjected to CMS have been shown to display a variety of behavioral changes mimicking clinical symptoms of depressed patients (Willner, 2005). However, these procedures do not take into account the etiology of human stress-associated disorders, as the majority of stressful stimuli in humans that have been shown to increase the risk of psychiatric disorders are of a social nature (Brown and Prudo, 1981). In an attempt to investigate the neurobiological mechanisms underlying disease susceptibility caused by social stress, it is crucial that the animal model meets the requirements of construct validity and, therefore, investigates the consequences of social stress in rodents (Geyer and Markou, 1995).

Social stress is probably one of the most pervasive stressors in humans and social animals (Bartolomucci et al., 2005; Fuchs, 2005). Animals exposed to inescapable social stress situations for a long time show extreme increases of their hypothalamic–pituitary–adrenal (HPA) system activity and a high mortality rate (Albeck et al., 1997). There are a number of experimental designs relying on social stress, such as the social defeat paradigm in rats and mice, the sensory contact model in mice or the visible burrow system in rats (Ader, 1969; Blanchard et al., 1995; Albeck et al., 1997; Koolhaas et al., 1997; Ahima et al., 1998; Stefanski, 2000, 2001; Aguilera et al., 2001; Veenema et al., 2003). A number of other species have also been extensively studied with regard to the behavioral and neuroendocrine consequences of chronic stress and social status, such as the guinea pig, the marmoset or the squirrel monkey (Sachser et al., 1998; Dettling et al., 2002; Levine and Mody, 2003; Parker et al., 2005). Few studies have also indicated that some of the observed neuroendocrine and behavioral effects evoked by these stressors persist even if the stress is discontinued, which is a crucial factor with regard to human pathology (Tsankova et al., 2006). A major drawback of these paradigms is that they are relatively work intensive and space consuming so that only a few animals per group can be tested in a specific experiment. This is of particular importance in view of individual differences in the susceptibility to chronic stress and stress-induced pathologies due to genetic or epigenetic variability.

An important characteristic of chronic stress paradigms is the age, at which the animals are exposed to the stressor. There are many indications that across the life span there are specific windows of vulnerability, where high levels of stress have an increased impact on further development (Heim and Nemeroff, 2001; Spencer et al., 2006; Avital et al., 2006). Many of the available chronic stress paradigms have been tested in young adult mice and rats (about 3 months of age). However, a high vulnerability has also been postulated in the adolescent period, due to the many still ongoing hormonal and neurodevelopmental processes (Tsoory et al., 2007). Especially social stressors are likely to have a high impact during this time as puberty seems crucial for the acquisition of social skills, which form the basis of social interactions and stability during adulthood (Sachser et al., 1998). Thus, the adolescent period is a highly adaptive period, where behavioral and neuroendocrine set points are fine-tuned.

In the current studies we therefore developed a novel mouse paradigm, which utilizes chronic social stress as a key pathogenic factor during adolescence. Based on early work by the groups of LeMoal and Henry in rats (Henry and Stephens, 1977; Klein et al., 1992; Arai and Widmaier, 1993), this paradigm creates an unstable social environment for a prolonged period of time. In this paradigm the animals are exposed to a continuous stressful situation, which they cannot escape from and which they are unable to adapt to. Another important advantage is the applicability of this paradigm to a large number of animals. Here, we characterize the paradigm with respect to effects on neuroendocrine and behavioral functions. We report persistent effects of this novel paradigm on stress-related physiological, neuroendocrine and behavioral parameters and their reversibility by a clinical efficacious antidepressant (the selective serotonin-reuptake inhibitor paroxetine) or CRH receptor type 1 (CRHR1)-antagonist treatment.

2. Methods

2.1. Animals

Experiments were carried out with male CD1 mice from Charles River Laboratories (Maastricht, the Netherlands), as this outbred strain would also allow for the possibility to identify genetic vulnerability markers. The animals were 26–28 days old on the day of arrival. All animals were housed in groups of four per cage ($45 \times 25 \times 20$ cm) under a 12L:12D cycle (lights on at 0600 h) and constant temperature ($23 \pm 2\,^{\circ}$C) conditions. Food and water were provided ad libitum. These experiments were carried out in the animal facilities of the Max Planck Institute of Psychiatry in Munich, Germany.

The experiments were carried out in accordance with the European Communities' Council Directive 86/609/EEC. All efforts were made to minimize animal suffering during the experiments. The protocols were approved by the committee for the Care and Use of Laboratory Animals of the Government of Upper Bavaria, Germany.