Progress in Neuro-Psychopharmacology & Biological Psychiatry 35 (2011) 1461–1471

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Vulnerability to chronic subordination stress-induced depression-like disorders in adult 129SvEv male mice

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ARTICLE INFO

Article history:
Received 15 July 2010
Received in revised form 4 November 2010
Accepted 5 November 2010
Available online 17 November 2010

Keywords:
Anxiety
Anhedonia
Knockout mice
Major depression
Strain comparison
Stress

ABSTRACT

Exposure to stressful life events is intimately linked with vulnerability to neuropsychiatric disorders such as major depression. Pre-clinical animal models offer an effective tool to disentangle the underlying molecular mechanisms. In particular, the 129SvEv strain is often used to develop transgenic mouse models but poorly characterized as far as behavior and neuroendocrine functions are concerned. Here we present a comprehensive characterization of 129SvEv male mice's vulnerability to social stress-induced depression-like disorders and physiological comorbidities. We employed a well characterized mouse model of chronic social stress based on social defeat and subordination. Subordinate 129SvEv mice showed body weight gain, hyperphagia, increased adipose fat pads weight and basal plasma corticosterone. Home cage phenotyping revealed a suppression of spontaneous locomotor activity and transient hyperthermia. Subordinate 129SvEv mice also showed marked fearfulness, anhedonic-like response toward a novel but palatable food, increased anxiety in the elevated plus maze and social avoidance of an unfamiliar male mouse. A direct measured effect of the stressfulness of the living environment, i.e. the amount of daily aggression received, predicted the degree of corticosterone level and locomotor activity but not of the other parameters. This is the first study validating a chronic subordination stress paradigm in 129SvEv male mice. Results demonstrated remarkable stress vulnerability and establish the validity to use this mouse strain as a model for depression-like disorders.

1. Introduction

Major depression is a severe, life-threatening, and widespread psychiatric disorder. The link between exposure to stressful life events and increased risk for major depression or depression-like disorders has been confirmed in several studies (McEwen 2007; de Kloet et al 2005; Caspi et al., 2003; Kendler et al., 1999; Bartolomucci and Leopardi, 2009; Schmidt et al., 2008). Maladaptive mechanisms by which stressor exposure could increase risk for depression have been proposed, including deficient neuromodulatory homeostasis, alterations of neuronal structure and function, hyperactive hypothalamus-pituitary-adrenocortical (HPA) axis and hyperactive immune system (Krishnan and Nestler 2008; McEwen, 1999, 2007; Lupien et al., 2009; Sapolsky et al., 2000; Dantzer et al., 2008). It has been shown that certain individuals are better able to cope with stressful life events by engaging protective psychological strategies (Feder et al., 2009; Kaufman et al., 2004). Identifying individual susceptibility to stress, or inability to engage successful coping strategies, have the potential to uncover the molecular mechanisms involved in stress vulnerability and could suggest novel therapeutic approaches to neuropsychiatric disorders (Caspi et al., 2010). Genetic basis of individual vulnerability has been addressed by comparing genetically homogeneous inbred strains of mice (Fuller 1960; Crawley et al., 1997; Parmigiani et al., 1999). This approach has now been paralleled by direct genetic manipulation, e.g. gene knockout, inserting point mutations, etc., which are usually performed by means of homologous recombination in embryonic stem cells often obtained from various substrains of the murine 129 strain lineage (Thomas and Capecchi, 1987; Gerlai 2001; Capecchi 2005). The 129 strain behavioral and neuroendocrine phenotype, however, is poorly characterized partly because of experimental evidence of abnormal phenotypic features (Wahlsten 1982; Koike et al. 2006; Wolfer et al. 1997). Recent studies conducted on different 129 substrains reported high trait anxiety (Rodgers et al., 2002; Dulawa

Abbreviations: HPA, Hypothalamus-pituitary-adrenocortical; EPM, Elevated plus maze; NPF, Novel/palatable food test; ACTH, Adrenocorticotropic hormone; HA, high-aggression-received; LA, low-aggression-received).

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doi:10.1016/j.pnpbp.2010.11.016

et al., 2004; Salomons et al., 2010; Chourbaji et al., 2010), impaired fear extinction (Hefner et al., 2008; Camp et al., 2009), and altered prepulse inhibition (Ralph et al., 2001). Of main interest, Chourbaji et al. (2010) recently demonstrated that 129SvEv mice (both males and females) show a remarkable behavioral despair in the learned helplessness model of depression and a passive coping style in the forced swim and tail suspension tests for antidepressant drug screening. Overall, recent studies established a potential depression-vulnerable phenotype for the 129SvEv strain. Aim of the present study was to investigate behavioral and physiological vulnerability of adult 129SvEv male mice to a validated naturalistic model of chronic subordination stress-induced depression-like disorders (Bartolomucci et al., 2001, 2004, 2009, 2010). Results of the present study demonstrated that 129SvEv male are highly vulnerable to social stress, showing physiological and behavioral alterations such as body weight gain, increased adiposity and plasma corticosterone, psychomotor disturbances, increased fearfulness, anxiety and social avoidance.

2. Methods

2.1. Animals

129SvEv were derived from breeders supplied by Takeda Cambridge (U.K.). Swiss CD-1 mice were derived from an outbred stock originally obtained from Charles River Italia (Lecco, Italy). Mice were born and reared at the University of Parma in a 12-hr light–dark cycle (lights on at 7:00) and maintained at 22±2 °C. After weaning at postnatal day 25 (28 for CD-1) mice were housed in same-sex groups of siblings (3–6 per cage) in plexiglas cages (38×20×18 cm) with wood shaving bedding changed weekly. All efforts were made to minimize the number of animals used and their suffering. All animal experimentation was conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/EEC) and approved by the ethical committees of the University of Parma and the Italian Institute of Health.

2.2. Chronic subordination stress paradigm

The procedure used in the present experiment is a modified version of our standard Chronic Subordination Stress Paradigm (Bartolomucci et al., 2001; 2004). The modified experimental procedure has recently been introduced to investigate stress vulnerability of inbred and/or transgenic mouse strains (Bartolomucci et al., 2010) which are often characterized by a lower aggressive phenotype when compared to males of the outbred CD1 strain (Parmigiani et al., 1999; Bartolomucci et al., 2010). The modified procedure requires that the experimental mouse is housed in a home cage to establish territory ownership (resident) and is subsequently subordinated in his home cage by an intruder CD1 male (Bartolomucci et al., 2010). Resident mice experience thus loss of territory ownership and lowering of social status which has been demonstrated to induce the deepest effects of chronic stress exposure in mice (Bartolomucci et al., 2005; Bartolomucci 2005, 2007 for review).

Three-month old 129SvEv males (n=31) were individually housed in Plexiglas cages (38×20×18 cm) for a 7 days baseline period. The three week stress procedure started after baseline, when each resident mouse received in its home cage a same-age CD-1 male mice as intruder (n=31). After ten minutes of interaction, the two animals were separated by means of a wire mesh partition, which allowed continuous sensory contact but no physical interaction. Between 10:00 and 12:00 the partition was removed daily for a maximum of 10 min. Each 129SvEv-CD1 dyad was stable across the entire stress procedure. The social status was determined as follows: the chasing and biting animal was defined as 'Dominant' (129SvEv, N=8 and CD1, N=23; see Results section), while the mouse displaying upright posture flight behavior and squeaking vocalization was the 'Subordinate' (129SvEv, N=23 and CD1, N=8, see Results section). To give a rough estimate of mice aggressive behavior, the number of attack bouts performed by each animal was quantified by direct observations by a trained observer (Bartolomucci et al., 2001, 2009, in press). The numbers of attack bouts performed by each animal were quantified during the first four days than again at day 10 and 20 by direct observation. For ethical reasons, and to prevent injuries, the mice were separated by the partition if fighting escalated, i.e. when the dominant persistently bit the opponent on the back or tail. If the fight was interrupted, the number of attack bouts was computed according to the following formula: # of attacks received = (# of attacks received before mice were separated * 600)/time until mice were separated).

Age-matched 129SvEv mice housed in groups of 3 littermates were included as the non-stressed control group (n=30). This choice was based on previous experiments showing that grouped sibling mice maintain normal social behavior and social hierarchy while showing no metabolic, immune, endocrine, or behavioral evidences of stress activation or anxiety (see Bartolomucci et al., 2001, 2003, 2004, for details). Control mice were re-housed in group of 3 (from pre-existing groups of 4–6 animals per cage as described above) the same day in which the chronic stress procedure started and were manipulated exactly as the animals under stress as far as environmental factors are considered. Stressed and control males were littermates to reduce possible confounds of inter-litter variability.

A schematic overview of the experimental procedure is depicted in Fig. 1A (see below for full procedural details). Novel/palatable food (NPF) test was performed on days 12–15, the elevated plus maze (EPM) test was performed on day 17 and the social interaction test was performed on day 20. The order of testing was based on intrinsic stressfulness of each test: NPF<EPM<social avoidance. Mice were sacrificed by decapitation (preceded by brief CO_2 inhalation) on day 22 at 9:00 to obtain nadir value of corticosterone and ACTH (organ harvesting is scheduled accordingly).

2.3. Home cage phenotyping

Locomotor activity was monitored continuously while core body temperature was determined on a daily base. Body temperature was recorded always at 10:00 by using temperature-sensing subcutaneous transponders (Bio Medic Data Systems, Seaford, DE, USA). Sensors were implanted at least 15 days before the beginning of the experiment according to manufacturer instructions and previous experiments (Bartolomucci et al., 2009). Locomotor activity in the home cage was monitored by means of an automated system that uses small passive infrared sensors positioned on the top of each cage (TechnoSmart, Rome, Italy) as previously described (Bartolomucci et al., 2009).

2.4. Novel/palatable food test (NPF)

Mice were presented in the home cage with a novel but highly palatable food, i.e. half a peanut, on a petri dish once a day for 4 consecutive days (days 12–15) starting at 9:00. On day 4, when the latency to ingest the palatable food was expected to be decreased because of habituation to novelty and hedonic response to the palatable food, the peanuts were presented in a normal housing cage in which bedding was changed (following the rationale previously established for the novelty induced suppression of feeding test for anxiety (Merali et al., 2003)). Latency to eat the peanut was recorded with a cut-off time of 600 s. If mice did not eat the peanut after 600 s the peanut was left in the cage. NPF test was scheduled daily at 9:00 to avoid a direct effect of aggression received and far away from circadian maximum of food intake which occurs before lights off in mice.