Hormones and Behavior 66 (2014) 713–723

Contents lists available at ScienceDirect

Hormones and Behavior

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

Regular article

Sex differences in the behavioural and hypothalamic–pituitary–adrenal response to contextual fear conditioning in rats

Núria Daviu [a,b], Raül Andero [a,b,1], Antonio Armario [a,b,*,2], Roser Nadal [a,c,**,2]

[a] Institut de Neurociències and Red de Transtornos Adictivos (RTA), Universitat Autònoma de Barcelona, Cerdanyola del Vallès 08193, Spain
[b] Animal Physiology (School of Biosciences), Universitat Autònoma de Barcelona, Cerdanyola del Vallès 08193, Spain
[c] Psychobiology (School of Psychology), Universitat Autònoma de Barcelona, Cerdanyola del Vallès 08193, Spain

ARTICLE INFO

Article history:
Received 18 April 2014
Revised 2 August 2014
Accepted 30 September 2014
Available online 13 October 2014

Keywords:
Sex differences
ACTH
Corticosterone
HPA axis
Anxiety
Freezing
Fear generalization

ABSTRACT

In recent years, special attention is being paid to sex differences in susceptibility to disease. In this regard, there is evidence that male rats present higher levels of both cued and contextual fear conditioning than females. However, little is known about the concomitant hypothalamic–pituitary–adrenal (HPA) axis response to those situations which are critical in emotional memories. Here, we studied the behavioural and HPA responses of male and female Wistar rats to context fear conditioning using electric footshock as the aversive stimulus. Fear-conditioned rats showed a much greater ACTH and corticosterone response than those merely exposed to the fear conditioning chamber without receiving shocks. Moreover, males presented higher levels of freezing whereas HPA axis response was greater in females. Accordingly, during the fear extinction tests, female rats consistently showed less freezing and higher extinction rate, but greater HPA activation than males. Exposure to an open-field resulted in lower activity/exploration in fear-conditioned males, but not in females, suggesting greater conditioned cognitive generalization in males than females. It can be concluded that important sex differences in fear conditioning are observed in both freezing and HPA activation, but the two sets of variables are affected in the opposite direction: enhanced behavioural impact in males, but enhanced HPA responsiveness in females. Thus, the role of sex differences on fear-related stimuli may depend on the variables chosen to evaluate it, the greater responsiveness of the HPA axis in females perhaps being an important factor to be further explored.

Introduction

Exposure to electric footshock (FS) is the most extensively used experimental paradigm for the study of emotional memory and fear conditioning. One or a few FS exposures in a single session result in the development of long-lasting fear conditioning to the FS context (i.e. Fendt and Fanselow, 1999; Le Doux, 2003; Maren, 2008). More recently, FS exposure has gained interest as a putative animal model for post-traumatic stress disorder (PTSD) because it may induce long-lasting behavioural changes reminiscent of those typically observed in PTSD patients: avoidance of places and other cues associated to the traumatic event, generalization of fear/anxiety to context/cues having certain similarities with the original ones and sensitization to other putative stressful situations (Armario et al., 2008; Siegmund and Wotjak, 2007).

One typical consequence of exposure of rats to brief sessions of FS, widely described by several labs in the 90s, is a long-lasting (days to weeks) reduction of activity/exploration (herein hypoactivity) in novel environments (Bruijnzeel et al., 2001a,b; Van den Berg et al., 1998; Van Dijken et al., 1992a,b,c). Although such hypoactivity could be at first sight interpreted in terms of FS-induced unconditioned increases in anxiety, the experimental evidence supporting this is scarce (Bruijnzeel et al., 2001a; Pijlman et al., 2003; Pijlman and Van Ree, 2002). In fact, mice previously exposed to FS showed hypoactivity in the elevated plus-maze (EPM), a classical test of anxiety, in accordance with earlier reports in rats. However, time spent in the open arms of the EPM was increased rather than reduced (Radulovic et al., 1998), arguing against enhanced anxiety. Importantly, in both mice and rats FS-induced hypoactivity in novel environments appears to be dependent on the development of fear to the original context because generalization did not develop when animals were shocked immediately after being placed into the chamber (Daviu et al., 2010, 2012; Radulovic et al., 1998), a procedure known to prevent contextual fear

* Correspondence to: A. Amario, Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain. Fax: +34 935812390.
** Correspondence to: R. Nadal, Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain. Fax: +34 935812390.
E-mail addresses: Antonio.armario@uab.es (A. Armario), roser.nadal@uab.es (R. Nadal).
[1] Present address: Department of Psychiatry and Behavioural Sciences, Center for Behavioural Neuroscience, Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329, USA.
[2] Both authors contributed equally.

http://dx.doi.org/10.1016/j.yhbeh.2014.09.015
0018-506X/

conditioning. It thus appears that hypoactivity is not explained by unconditioned (sensitization) and requires the development of contextual fear conditioning. This process has been termed conditioned generalization (Radulovic et al., 1998). Conditioned generalization has also been observed in mice after cued (tone) fear conditioning in that such enhanced response was not observed in the groups that received unpaired tones and FS during the training session (Laxmi et al., 2003).

Shock-induced hypoactivity in novel environments could reflect a weak degree of fear generalization, not intense enough to be detected with classical tests of anxiety. Activation of the hypothalamic–pituitary–adrenal (HPA) axis, a consistent biological marker of stress intensity (see Armario et al., 2012 for review), does not support that previous exposure to shock greatly enhances fear/anxiety in a novel environment. Thus, while exposure to the FS context results in high levels of freezing and greater HPA activation in prior shocked rats than controls, FS-induced hypoactivity in different novel environments is not associated with greater HPA response in male rats (Daviu et al., 2010). We interpreted those results as the development of a long-lasting cautious behaviour associated to any unknown environment, which was not enough fear-provoking to induce activation of the HPA axis. We then propose the term of "conditioned cognitive generalization" to describe such a phenomenon to emphasize that this generalization is not based on configurational similarities with the original fear conditioning chamber.

The characterization of sex differences in responsiveness to stress and stress-related pathologies has gained considerable interest in the last years. There is general consensus that women are more vulnerable to develop anxiety disorders including PTSD and depression (Toufexis et al., 2006) but the neurobiological bases for this vulnerability remains elusive. Thus, the enhanced vulnerability is consonant with the higher sensitivity of females to emotional events and emotional memory (i.e. Canli et al., 2002), but fear conditioning appears to be sex-independent or greater in men than in women (i.e. Fredrikson et al., 1976; Milad et al., 2010; Schell et al., 1991). Similarly in animals, both contextual and cue fear conditioning, as evaluated by freezing, are higher in male than in female rats and mice (see Dalla and Shors, 2009 for a review). These sex differences are more consistent with contextual fear conditioning than with cued fear conditioning (Anagnostaras et al., 1998). In addition, male rats also show longer retention of fear memory (Gresack et al., 2009) and present reduced extinction of contextual fear conditioning (Brunzell et al., 2002; Chang et al., 2009) than females.

The fact that fear conditioning is greater in males than females, measuring freezing, does not preclude that other consequences of exposure to fear conditioning or other fear-sensitive parameters follow the same pattern. In this regard, whether or not conditioned cognitive generalization differs in male and female is not known. Moreover, although there is an extensive literature on sex differences in the HPA axis functioning in rodents (Rhodes and Rubin, 1999), there is no study about sex differences in HPA response to fear conditioning. This may be of special relevance because it is widely known that the role of the HPA axis is crucial in emotional memories. Although most studies on stress focus on plasma levels of corticosterone rather than ACTH, the latter hormone is a more direct reflection of stress-induced brain activation and has some advantages as a stress biomarker (Armario et al., 2012). Sex-differences in the endocrine response to fear conditioning are important for two main reasons. First, sex differences in ACTH response to stress in rats appear to be dependent on the particular type of stressor studied (Babb et al., 2013b; Spinedi et al., 1994; Watanobe, 2002). Second, characterization of ACTH response to fear-conditioning can contribute to determine whether or not females are actually less sensitive than males to fear conditioning. Thus, the aims of the present work were to compare male and female rats regarding: (i) the acquisition and the extinction of contextual fear conditioning as assessed by freezing and HPA axis activation, (ii) the degree of HPA sensitization and hypoactivity induced in a novel environment markedly different from the fear conditioning chamber as a consequence of previous exposure to shock.

Methods

Animals

We used 60 days old male and female Wistar rats, from Harlan Laboratories breeders (Sant Feliu de Codines, Spain). They were housed in pairs in a 1000 cm^3 plastic cages with sawdust bedding (Lignocel, Panlab S.L.U., Barcelona, Spain) in standard conditions of temperature (21 ± 1 °C) and in a 12-h light/12-h dark schedule (lights on at 08.00 h). Males and females were housed in the same vivarium. Food (SAFE-diet A04, Panlab S.L.U., Barcelona, Spain) and water were available ad libitum. The experimental protocol was approved by the Committee of Ethics of the Universitat Autònoma de Barcelona and the Generalitat of Catalunya, followed the "Principles of laboratory animal care" and was carried out in accordance with the European Communities Council Directives (2010/63/EU) and the Spanish Legislation (RD 53/2013).

General procedure

The experimental procedures were always performed in the morning and handling started two days after being housed in pairs. Animals were handled for three consecutive days for approximately 2 min a day. On the last day of handling, animals were subjected to a tail nick procedure to habituate animals to blood sampling procedure. The tail nick consisted of gently wrapping the animals with a cloth, making a 2 mm incision at the end of the tail veins and then massaging the tail while collecting, within 2 min, 300 μl of blood into ice-cold EDTA capillary tubes (Stardest, Granollers Spain). The cage-mates were sampled simultaneously (two experimenters were sampling at the same time and a third was gently holding the two rats). This procedure is extensively used in our lab and by others because low resting levels of hormones are obtained (e.g. Andero et al., 2012; Belda et al., 2004; Vahl et al., 2005).

Two days later, animals were randomly assigned to the experimental groups: Control Male (n = 8), Shock Male (n = 7), Control Female (n = 8) and Shock Female (n = 8). Wistar rats present equivalent basal levels of plasmatic ACTH across the different stages of the estrous cycle (Atkinson and Waddell, 1997) and there is no consistent evidence for a different HPA response to stress during the estrous cycle when samples are taken in the morning (Babb et al., 2013a; Iwasaki-Sekino et al., 2009; Viau and Meaney, 1991). Moreover, monitoring the estrous cycle may have added some degree of stress which could interfere with the HPA axis measures of this study. For all these reasons, the estrous cycle was not monitored. The two animals of the same home-cage had the same treatment. On day 1 (fear acquisition), all animals were exposed to the fear conditioning chambers. Two identical fear conditioning chambers (Panlab S.L.U., Barcelona, Spain) were used, one for each cage-mate. Each chamber (25 cm × 25 cm × 25 cm) had a clear *Plexiglas* door and black aluminium sidewalls. The floor, composed of 19 stainless steel rods (3 mm in diameter), spaced 1 cm centre to centre, was wired to a shock generator and scrambler. A white house light (4 cm diameter) was placed in the right wall at 17.5 cm to the floor. The software (Freezing 1.3.0.1, Panlab S.L.U., Barcelona, Spain) controlled the house light and FS administration. The chambers were inside the sound attenuating box (67 cm × 53 cm × 55 cm, LE 111-118.8-116) provided with a fan that helps to mitigate strange sounds. The front door of this box allowed video camera recording and the image was transferred to a digital video recorder (JVC VR-716) and from there to a computer for manual analysis by stopwatch by a blind experimenter to the treatment. Freezing, a reliable measure of learned fear was assessed during the fear acquisition, context and extinction tests. Freezing involved the absence of all movements other than respiratory-related movements (Blanchard and Blanchard, 1969). The chambers were placed in a black painted room next to the vivarium which was illuminated by an indirect white 25 W bulb and the animals were transported to the test