Hormones and Behavior 103 (2018) 7–18

Contents lists available at ScienceDirect

Hormones and Behavior

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

Early life stress in rats sex-dependently affects remote endocrine rather than behavioral consequences of adult exposure to contextual fear conditioning

Sílvia Fuentes[a,c], Núria Daviu[a,b,1], Humberto Gagliano[a,b], Xavier Belda[a,b], Antonio Armario[a,b,d,*,2], Roser Nadal[a,c,d,*,2]

[a] Institut de Neurociències, Universitat Autònoma de Barcelona, Spain
[b] Psychobiology Unit, School of Psychology, Universitat Autònoma de Barcelona, Spain
[c] Animal Physiology Unit, School of Biosciences, Universitat Autònoma de Barcelona, Spain
[d] CIBERSAM, Instituto de Salud Carlos III, Spain

ARTICLE INFO

Keywords:
Fear memory
Fear generalization
Sex differences
ACTH
Corticosterone
PTSD models

ABSTRACT

Exposure to electric foot-shocks can induce in rodents contextual fear conditioning, generalization of fear to other contexts and sensitization of the hypothalamic-pituitary-adrenal (HPA) axis to further stressors. All these aspects are relevant for the study of post-traumatic stress disorder. In the present work we evaluated in rats the sex differences and the role of early life stress (ELS) in fear memories, generalization and sensitization. During the first postnatal days subjects were exposed to restriction of nesting material along with exposure to a "substitute" mother. In the adulthood they were exposed to (i) a contextual fear conditioning to evaluate long-term memory and extinction and (ii) to a novel environment to study cognitive fear generalization and HPA axis heterotypic sensitization. ELS did not alter acquisition, expression or extinction of context fear conditioned behavior (freezing) in either sex, but reduced activity in novel environments only in males. Fear conditioning associated hypoactivity in novel environments (cognitive generalization) was greater in males than females but was not specifically affected by ELS. Although overall females showed greater basal and stress-induced levels of ACTH and corticosterone, an interaction between ELS, shock exposure and sex was found regarding HPA hormones. In males, ELS did not affect ACTH response in any situation, whereas in females, ELS reduced both shock-induced sensitization of ACTH and its conditioned response to the shock context. Also, shock-induced sensitization of corticosterone was only observed in males and ELS specifically reduced corticosterone response to stressors in males but not females. In conclusion, ELS seems to have only a minor impact on shock-induced behavioral conditioning, while affecting the unconditioned and conditioned responses of HPA hormones in a sex-dependent manner.

1. Introduction

The development of fear to contexts associated with danger is a biological adaptive process. However, excessive or permanent contextual fear memories may lead to psychopathology (Maren et al., 2013), particularly to fear and anxiety disorders that have an estimated lifetime prevalence close to 29% of the population (Kessler et al., 2005). In rodents, fear memories can be studied by means of the Pavlovian contextual fear conditioning paradigm where an unconditioned stimulus or US (usually a shock) that elicits fear (unconditioned response), is paired with a particular context (conditioned stimulus, CS) (Fanselow and Poulos, 2005; Maren et al., 2013; Ledoux, 2014). When animals are later exposed to the context without shock, freezing is measured as an index of fear. Generally, the test is done 24–48 h after the acquisition of conditioning aiming to evaluating "recent" fear memories. However, recent and remote contextual fear memories may engage different brain structures and biochemical mechanisms (Frankland et al., 2006; Restivo et al., 2009; Xu et al., 2012; Tayler et al., 2013; Graff et al., 2014; Einarsson et al., 2015).

After Pavlovian contextual fear conditioning, animals not only fear the shock-paired context, but other contexts that have partial resemblance with the original one. This phenomenon is called fear

* Corresponding authors at: Institut de Neurociències, Universitat Autònoma de Barcelona, 08193, Cerdanyola del Vallès, Spain.
E-mail addresses: antonio.armario@uab.es (A. Armario), roser.nadal@uab.es (R. Nadal).
[1] Present address: Department of Physiology and Pharmacology, Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1, Canada.
[2] Both authors contributed equally.

https://doi.org/10.1016/j.yhbeh.2018.05.017
Received 31 January 2018; Received in revised form 9 April 2018; Accepted 22 May 2018

generalization and interestingly remote fear memories are more prone to generalization (Houston et al., 1999; Balogh and Wehner, 2003; Biedenkapp and Rudy, 2007; Wiltgen and Silva, 2007; Wiltgen et al., 2010; Poulos et al., 2016). Importantly, context fear conditioning causes hypoactivity in novel environments that have no resemblance at all with the context. This hypoactivity is dependent on the development of contextual fear learning rather than the mere exposure to shocks (Radulovic et al., 1998; Daviu et al., 2010). We have termed this phenomenon cognitive generalization as it does not appear to involve perceptual processes (Daviu et al., 2014). The study of fear generalization in animal models is particularly useful for translational research because patients with post-traumatic stress disorder (PTSD) show overgeneralization of fear and an inability to inhibit fear in front of safe cues (Jovanovic et al., 2012; Briscione et al., 2014). In addition, in animals, exposure to shocks can induce sensitization of the hypothalamic-pituitary-adrenal (HPA) axis (Johnson et al., 2002; Belda et al., 2016), the prototypical stress system in all vertebrates, suggesting that prior experience with shocks can induce both behavioral and endocrine sensitization.

Considering that behavioral consequences of exposure to shock can model PTSD, it is of note that different PTSD symptoms have been reported in men versus women (Carmassi et al., 2014; Carragher et al., 2016), and preliminary data suggest that increased generalization may be an important correlate of PTSD symptoms in females but less so in males (Radell et al., 2017). In animal models, sex differences in fear conditioning have been previously described (see Dalla and Shors, 2009 for a review), but the direction of the differences are not always concordant with those observed in humans. Males have stronger contextual recent fear memory, as measured by freezing (e.g. Barker and Galea, 2010; Daviu et al., 2014), but the conditioned HPA axis response is stronger in females (Daviu et al., 2014). Moreover, cognitive generalization of fear was higher in males than females (Daviu et al., 2014). All these data together suggest that fear is a multidimensional construct and thus, not all the indexes of fear may always develop in the same direction.

Early life experiences have a long-lasting impact on central nervous system functioning, behavior and cognition (Heim and Nemeroff, 2001; Shea et al., 2005; Morgan and Fisher, 2007). More specifically, fear memories are profoundly modulated by early life experiences and childhood trauma has been reported to increase vulnerability to PTSD in adulthood (Bremner et al., 1993; Breslau et al., 1999; Cougle et al., 2010). In animal models, early life postnatal stressful experiences (ELS) have also a long-term effect in emotional behavior. One of the most extensively studied models is maternal separation/deprivation that has been found to potentiate in adulthood the formation of recent contextual and/or auditory fear memories (Oomen et al., 2010; Diehl et al., 2014; Sampath et al., 2014; Toda et al., 2014), but opposite results have been found in other studies (Guijarro et al., 2007; Wang et al., 2011). Moreover, maternal separation increases at adulthood fear generalization to other contexts and cues—e.g. a tone not paired with shocks in a paradigm of auditory fear conditioning—(Sampath et al., 2014). In contrast, consistent decreases in fear memory have been reported after early exposure to “protective” treatments such as postnatal handling (Meerlo et al., 1999; Claessens et al., 2012) or environmental enrichment (Barbelivien et al., 2006). Most of these studies have been performed in male rats and only in few cases both sexes were included (Wang et al., 2011; Diehl et al., 2014). On the other hand, one of the aspects that have deserved scarce attention is the impact of ELS on remote fear memories and generalization. In PTSD patients, the probability to interact again with the same contextual cues than the ones paired to the trauma is lower than the probability to be exposed to only partially similar cues. Therefore, the study in animal models of how ELS modulates fear generalization to different contexts and cues deserves more attention.

Given all the above, the general aim in the present study was to characterize how a model of ELS in rats is affecting at adulthood in a sex-dependent manner: (i) contextual fear acquisition and remote memory and extinction. To asses that, freezing, as a behavioral measure, and HPA axis functioning (measured by adrenocorticotrophic hormone (ACTH) and corticosterone plasma levels), as an endocrine measure, were used. (ii) generalization of fear to a novel environment, using hypoactivity as a behavioral measure, and (iii) HPA axis response to the novel environment that acted as a heterotypic stressor. As a model of ELS we used, as previously (Fuentes et al., 2014), a combination of restriction of nesting material (Molet et al., 2014) and exposure to a substitute mother (Roth et al., 2009). This ELS procedure has been shown to induce, at adulthood, a mixture of detrimental and adaptive effects in several behaviors and in the functioning of the HPA axis. In our hands, this model induces sex-dependent cognitive and emotional changes in adulthood and increases maternal care provided by the biological mother, which may “buffer” some of the “negative” effects of stress (Fuentes et al., 2014).

2. Material and methods

2.1. Subjects

Long-Evans (RjOrl:LE) outbred rats were housed in Makrolon transparent polycarbonate wire-topped cages with solid bottom (26.5 × 42.5 × 18.5 cm, Ref. 1291 Eurostandard Type III H) containing sawdust bedding (Lignocel 3/4, Harlan) in a climate-controlled environment at 20–21 °C on a 12-hour light–dark cycle (lights on at 8:00 am). Behavioral studies were carried out during the light cycle. Food (SAFE-diet A04, Panlab S.L.U., Barcelona, Spain) and filtered tap-water were available ad libitum. All animal protocols were in accordance with the European Communities Council Directive 2010-63-EU and the Spanish legislation (BOE53-2013) and approved by the Ethics Committee for Human and Animal Research of the Universitat Autònoma de Barcelona and by the Generalitat de Catalunya. No specific environmental enrichment program was used in the animal facility. A maximal effort was done to minimize the number and suffering of animals. A detailed timeline of the study is provided in Fig. 1.

2.2. Early life treatment

The pups of the present study represent a particular cohort of offspring coming from the same dams used in Fuentes et al. (2014), being the offspring a different cohort of rats. As mentioned (Fuentes et al., 2014), 30 mated pregnant dams arrived from Janvier (France) at GD 15. Each dam (approximately 8 weeks old and primiparous) was paired with a different male (1 male/1 female). In this specific experiment 28 different dams were used. All of the dams were at the same vivarium, special care was taken to restrict access to the room and the day of delivery was termed postnatal day (PND) 0. The next day, the pups were weighed, counted, sexed and those litters higher than 12 were culled to 12 (maintaining, if possible, a sex ratio between 0.4 and 0.6) and weighed. Cross-fostering was never performed. At PND 1, the dams were assigned to control (CTR) or ELS conditions. Final litter size and sex ratio was not different between groups [litter size CTR: 9.3 ± 0.5; litter size ELS: 9.6 ± 0.5; sex ratio (males to females) CTR: 0.59 ± 0.04; sex ratio ELS: 0.52 ± 0.03]. The bedding was not changed until PND 8 when the pups were again weighed. As mentioned (Fuentes et al., 2014), prior to treatment (PND 1) body weight was not affected by group and at PND 8 ELS decreased weight in, both, males and females. The ELS consisted of a combination of 2 different treatments (i) restriction of nesting material and (ii) exposure for 1 h/day to a “substitute” mother (see Fuentes et al., 2014 for more details). The ELS treatment lasted 7 days (PND 1-7), and after that all dams were returned to control conditions. Control dams remained undisturbed with the litter during all the lactation period, until weaning at PND 21. Maternal behavior was measured between PND 1 and 7, and again at PND 13 and PND 18. As it can be seen in Fuentes et al. (2014), ELS