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Research report

The effects of sex and neonatal maternal separation on fear-potentiated and light-enhanced startle

Reinoud de Jongh [a], Mark A. Geyer [c], Berend Olivier [a,b], Lucianne Groenink [a,*]

[a] *Department of Psychopharmacology, Utrecht Institute for Pharmaceutical Sciences and Rudolf Magnus Institute of Neuroscience, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands*
[b] *Yale University School of Medicine, Department of Psychiatry, 34 Park Street, New Haven, CT 06508, USA*
[c] *University of California at San Diego, Department of Psychiatry 0804, 9500 Gilman Drive, La Jolla, CA 92093-0804, USA*

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Abstract

This study was based on the higher prevalence of anxiety disorders in women than in men, and on the finding that early adverse experiences are a major risk factor for the development of anxiety disorders later in life. The object of this study was to investigate in rats, the sensitivities of the light-enhanced startle (LES) and fear-potentiated startle (FPS) paradigms to sex differences and to determine the effects of maternal separation (MS) on the baseline startle magnitude and potentiated startle response in these paradigms.

Pups in the MS group were separated daily from their mother for 180 min/day from postnatal day 2 (PND2) to PND14. Control litters remained undisturbed. The adult male and female progeny were tested in the FPS and LES. As predicted, females showed a significantly greater potentiation of startle than males in the FPS, and a strong trend towards greater startle potentiation in the LES. Contrary to predictions, MS had no effect on startle potentiation in the FPS and severely disrupted LES in female, but not male rats.

The observed sex differences add to the validity of the FPS and LES as animal paradigms of fear and anxiety. The findings indicate that these paradigms can be used to study the biological basis of sex differences in fear and anxiety. In contrast, the effects of MS on startle potentiation argue against the idea that MS provides a robust model for the predicted influences of early adverse effects on these startle potentiation measures of fear and anxiety.

Keywords: Light-enhanced startle; Fear-potentiated startle; Sex difference; Maternal separation

1. Introduction

Anxiety disorders are the most frequent psychiatric disorders with a lifetime rate of prevalence exceeding 10% of the population [22]. Women have a higher prevalence of anxiety disorders than men, the ratio of women to men being 2 to 1 for generalized anxiety disorder and specific phobia [22], suggesting that the mechanisms underlying anxiety are sex-dependent. Considering these sex differences in anxiety, one might also expect female rats to be more anxious than male rats. Indeed, female rats show greater defensiveness in situations involving potential threat [2] and are more anxious in the social interaction and Vogel conflict tests [19]. Female rats were less anxious than male rats, however, in an open field [1,30] and on the elevated plus-maze [19,32,40].

Early adverse experiences, such as childhood abuse and parental loss, are a major risk factor for the development of anxiety disorders [16]. In rodents, neonatal maternal separation has been used as an animal model of the effects of early adverse experience, and has been shown to result in increased anxiety-like behaviour in adult rats in behavioural paradigms such as the elevated-plus maze and the social interaction test [18,21,27,39].

* Corresponding author. Tel.: +31 30 2534549; fax: +31 30 2537387.
E-mail address: l.groenink@pharm.uu.nl (L. Groenink).

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In the present study, the sensitivity of the light-enhanced and fear-potentiated startle paradigms to both sex differences and early adverse experience was investigated. The fear-potentiated startle (FPS) paradigm was first described by Brown et al. [3] and has since then greatly increased our understanding of the neural and pharmacological mechanisms that influence conditioned fear (for review see [10,25]). In the FPS, the acoustic startle response is increased by presenting the startle-eliciting noise in the presence of a cue previously paired with foot shock.

More recently, it was shown that the startle response could also be increased by bright light, an unconditioned anxiogenic stimulus in rats [35]. In this procedure, the light-enhanced startle (LES) paradigm, rats show a potentiated startle response in a brightly illuminated environment, compared to a dark environment. Interestingly, Grillon et al. [13] showed that in humans, startle is increased when tested in the dark and that this increase appears to be the result of fear or anxiety and not an attentional process. It is suggested that the increase of startle in rats tested in bright light and in humans tested in the dark has an evolutionary basis, that is, rats are nocturnal and are more vulnerable in the light, whereas humans are diurnal and more vulnerable in the dark [13,35].

Both paradigms are sensitive to a range of anxiolytic agents (for FPS [4,17,20,28], for LES [8,35,37]). These paradigms differ markedly, however, in both their eliciting stimuli and the time course of the response. The specific threat stimulus and rapid onset and offset of the response [5,6,9], have led to the suggestion that FPS models fear [7]. In contrast, the potential threat (for a nocturnal animal in daylight, there is the potential risk of being attacked by a predator) and the slow onset and offset of the response [9,38] suggest that LES could model anxiety [7]. Moreover, the potentiations of the startle response in FPS and LES appear to be mediated by different brain regions. Infusions of the a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist 2,3-dihydroxy-6-nitro-7-sulphamoylbenzo(F)-quinoxaline (NBQX) into the central nucleus of the amygdala (CeA) blocked FPS but not LES, whereas infusions into the bed nucleus of the stria terminalis (BNST) blocked LES but not FPS [36].

The first aim of the present study was to determine the effects of maternal separation (MS) on the baseline startle magnitude and potentiated startle response in these paradigms, and determine the extent to which these effects are gender-dependent. Considering that early adverse experiences are a major risk factor for the development of anxiety disorders, MS rats were expected to have a higher baseline startle response and/or a higher potentiation of the startle response than control rats. The second aim was to investigate the sensitivities of the LES and FPS paradigms to sex differences. Considering the fact that women have a higher prevalence of anxiety disorders than men, female rats were expected to show a higher potentiation of the startle response than male rats in both paradigms.

2. Materials and methods

2.1. Animal care and maternal separation

Animals were housed in a temperature ($21 \pm 2\,^{\circ}C$), humidity ($55 \pm 5\%$), and light controlled environment (lights on from 6 a.m. to 6 p.m.). Food and water were freely available. Timed pregnant Wistar rats (Harlan, Horst, The Netherlands) arrived at Utrecht University on day 13 of gestation. On the day after delivery (postnatal day 1 or PND1), all pups were pooled, sexed, culled to litters of five males and females, and placed in clean cages. Litters were assigned randomly to either the maternal separation (MS) or control groups. Pups in the MS group were separated daily from their mother for 180 min between 0900 and 1200 h on PND2 to PND14. During separation, the pups of each litter were kept together in clean plastic cages (12 cm × 18 cm) filled with bedding and placed on a 37 °C heating blanket. Control litters remained undisturbed, except for cage changing once a week. On PND21, pups were weaned and housed in groups of four. Male and female rats were housed in separate colony rooms. Starting on PND46, rats were handled daily for one week. Testing occurred from PND53 to PND67. The study was approved by the ethical committee of the Faculties of Pharmaceutical Sciences, Chemistry and Biology (DEC/FSB), Utrecht University, The Netherlands.

2.2. Apparatus

Four startle devices were used simultaneously (SR-Lab, San Diego Instruments, San Diego, CA, USA). The startle devices consisted of a Plexiglas cylinder (8.8 cm in diameter and 20.3 cm in length) with a stainless steel grid floor placed on a Plexiglas base. Each startle device was placed in a ventilated sound-attenuated cubicle. Cage movements were measured with a piezoelectric film attached to the Plexiglas base of the startle device. A calibration system (San Diego Instruments) was used to ensure comparable startle magnitudes across the four devices throughout the experiment. Startle stimuli, consisting of 50 ms white-noise bursts, were presented through a piezoelectric tweeter situated 15.2 cm from the top of the cylinder. Background noise was 55 dB. Sound intensities were measured using a microphone, which was placed on top of the Plexiglas cylinder and fitted to a Bruel and Kjaer sound level meter (Type 2226). Startle magnitudes were sampled each millisecond during a period of 65 ms beginning at the onset of the startle stimulus. Each startle device was equipped with a white fluorescent bulb (9 W) on the back wall of the sound-attenuated cubicle and a stimulus light in the ceiling situated 15.2 cm from the top of the cylinder. The fluorescent bulb produced an illumination level of approximately 900 lx and the stimulus light an illumination level of approximately 180 lx, both measured from inside the Plexiglas cylinder using a Gossen luxmeter (MAVOLUX 5032C). There was no background illumination in any of the experiments.

2.3. Procedure

2.3.1. Light-enhanced startle

Rats of one group ($n = 74$; 36 males: 18 controls and 18 MS/38 females: 19 controls and 19 MS) were placed in the startle chamber and, after a 5 min acclimation period, presented with 30 startle stimuli, 10 each at 90, 95 and 105 dB, with an inter-stimulus interval (ISI) of 30 s. Within every block of three stimuli, the three