Please cite this article in press as: Loi M et al. Effects of early-life stress on cognitive function and hippocampal structure in female rodents. Neuroscience (2015), http://dx.doi.org/10.1016/j.neuroscience.2015.08.024

Neuroscience xxx (2015) xxx–xxx

EFFECTS OF EARLY-LIFE STRESS ON COGNITIVE FUNCTION AND HIPPOCAMPAL STRUCTURE IN FEMALE RODENTS

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Abstract—We tested the effect of early-life stress (ELS) – 24 h maternal deprivation (MD) at postnatal day (PND) 3 – on cognitive performance and hippocampal structure in 12–17-week-old female rats. Behavioral performance was examined in: the Elevated Plus Maze, as an index for general anxiety; the rodent Iowa gambling test, probing reward-based decision making; and the object recognition and object-in-location task, to assess non-stressful contextual memory performance. We further determined hippocampal dentate gyrus (DG) volume and cell density as well as adult proliferation and neurogenesis rates. Half of the rats was treated with the glucocorticoid receptor antagonist mifepristone during a critical pre-pubertal developmental window (PNDs 26–28), in an attempt to ameliorate the potentially adverse behavioral consequences of ELS. Neither MD nor treatment with the glucocorticoid antagonist affected behavioral performance of the females in any of the tasks. Also, DG structure, proliferation and neurogenesis were not different between the groups. Lack of structural differences and a behavioral phenotype in non-stressful hippocampus dependent learning tasks fits with the lack of phenotype generally reported after ELS in female but less so in male rodents. As evident from an extensive literature review, female and male animals appear to respond more similarly to early-life adversity when tested in anxiety-related tasks. This agrees with recent findings in humans suggesting that females may be relatively resilient to the structural/ hippocampal effects of childhood maltreatment, but not to the anxiety and mood-related psychopathology for which childhood maltreatment is considered a risk factor.

***This article is part of a Special Issue entitled: Early Adversity*. © 2015 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: early- life stress, hippocampus, amygdala, prefrontal cortex, contextual memory, anxiety, depression, social behavior, neurogenesis, sex difference.

INTRODUCTION

Stress experienced early in life is a well-documented risk factor for the precipitation of psychiatric illnesses, including mood and anxiety disorders (Kessler et al., 2010). Interestingly, these disorders appear more prevalent in females than in males (Steel et al., 2014; Reynolds et al., 2015). Early-life stress (ELS) is thought to contribute to this risk through programing of the brain and the hypothalamus–pituitary–adrenal (HPA) axis (Meaney et al., 2007).

How ELS programs the brain and HPA axis is often studied in animal models, as these allow precise control over the genetic and early-life environment as well as a detailed investigation of the underlying mechanisms. Many models have been developed, including a 24-h maternal deprivation (MD) at postnatal day (PND) 3, a model of maternal neglect (Pryce et al., 2005; Marco et al., 2015). Adult male rats that were subjected to a 24-h MD at PND 3 demonstrate reduced neurogenesis, impaired spatial memory but enhanced memory formation under stressful learning conditions (Oomen et al., 2010). The behavioral phenotype in female rats appeared to be more subtle and confined to amygdala-dependent learning paradigms (Oomen et al., 2011). Literature on cognitive performance in adult females exposed early in life to this or other types of ELS is generally less extensive than literature on males. Given the prevalence of many mood and anxiety disturbances in human female subjects, a more in-depth study of early-life adversity in female rodents is nonetheless highly relevant.

We therefore set out to study the effects of 24-h MD at PND 3 in three cognitive domains and on hippocampal structural measures. More specifically, we tested: (1) general anxiety behavior in an Elevated Plus Maze (EPM, Rodgers and Dalvi, 1997); (2) reward-based decision making in a rodent version of the Iowa Gambling

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Abbreviations: DCX, doublecortin; DG, dentate gyrus; DI, discrimination indices; ELS, early-life stress; EPM, Elevated Plus Maze; HPA, hypothalamus–pituitary–adrenal; MD, maternal deprivation; MIF, mifepristone; NMD, non-maternally deprived; OLT, object-in-location task; ORT, object recognition task; PB, phosphate buffer; PND, postnatal day; rIGT, rodent Iowa Gambling Task; VEH, vehicle.

http://dx.doi.org/10.1016/j.neuroscience.2015.08.024
0306-4522/

Task (rIGT; Van den Bos et al., 2014); and (3) non-stressful contextual learning in an object recognition task (ORT, Bevins and Besheer, 2006) and an object-in-location task (OLT, adapted from Ennaceur et al., 2005). Hippocampal structural measures included dentate gyrus (DG) volume, proliferation and neurogenesis that can be altered by (early life) stress and are involved in aspects of cognition (Lucassen et al., 2010; Oomen et al., 2011, 2014).

The peripubertal period is considered a critical time window in which programing of the brain and HPA axis can be primed or ameliorated, depending on the intervention (Tsoory and Richter-Levin, 2006). We tested the latter possibility, by treating ELS and control female rats with the glucocorticoid receptor antagonist mifepristone (MIF) twice daily during PND 26–28. This paradigm was earlier found to successfully ameliorate the behavioral phenotype associated with 24-h MD on PND 3 in male rats (M. Loi; unpublished observation). Moreover, temporary treatment with MIF in adulthood normalized structural plasticity measures in paradigms associated with prolonged elevation in corticosterone level (Mayer et al., 2006; Oomen et al., 2007; Hu et al., 2012). The outcome of the behavioral experiments was compared with existing literature on various ELS models, where we specifically focused on female rats or mice.

EXPERIMENTAL PROCEDURES

Animals

Prior to the start of the study all animal procedures were approved by the animal ethics committee at Utrecht University, the Netherlands. Adult male and female Wistar rats were purchased from Harlan (Zeist, The Netherlands) and habituated in pairs to the animal facilities for two weeks. For breeding, male rats were put together with female rats in a 1:2 ratio for a period of 10 days. Females were housed in pairs after mating until the last week of pregnancy when they were housed individually. Every morning before 9 am cages were checked for births, which upon birth were denoted as PND 0. Dams with litters were left undisturbed until PND 3.

On the morning of PND 3, litters were randomly assigned to either the MD condition or the control (non-maternally deprived; NMD) group. In the MD group, litters were separated from their mother after being culled; in the NMD group litters were merely culled. Litters contained on average 9 ± 1 pups, with 4–5 females. For MD, the mother was placed in a separate cage; the pups went back in the home cage and were placed on a heating pad (32 °C) in a separate room. MD litters were kept in this room for 24 h before being placed back with the dam, as described elsewhere (Oomen et al., 2011). No animals died during the MD procedure.

On PND 21 the litters were weaned and housed in same-sex groups of either two or three. In both conditions, animals were then treated from PND 26 through PND 28 with MIF (5 mg/100 g bodyweight) or its vehicle (VEH). Per rat, 4 mg MIF powder (Sigma–Aldrich Chemie B.V., Zwijndrecht, The Netherlands; or Corcept Pharmaceuticals) was dissolved in 15 μL 99% ethanol mixed with 1 mL coffee cream (Campina, Woerden, The Netherlands) at body-temperature, as described elsewhere (see e.g. Hu et al., 2012). Twice daily, the drugs were administered through oral gavage directly into the stomach (6 h in-between injections).

In total, 106 female rats were included in the present study. All were kept under standard housing conditions (dark/light phase 12:12, lights on at 8 am, humidity 55 ± 15%, temperature 20–22 °C) and received food and water *ad libitum* unless indicated otherwise (see below). They were weekly handled for 1 min and body weights were registered (once per week until week 13) from which the absolute and percentage weekly growth were determined. Two separate batches of animals were studied. The first batch consisted of 64 rats that underwent MD at PND3 and glucocorticoid receptor antagonist or VEH administration at PND 26–28. At week 10, the animals were tested in the EPM. One week later the animals were moved to another room in which they went into a reversed day-night cycle (lights off at 8 am). At week 13 they were tested in the novel ORT and at week 15 in the OLT. At week 17, all animals were sacrificed and the brains of 7–9 animals of each group were prepared for the morphology/structural analysis. The second batch consisted of 42 rats (n = 8–12 per group) that underwent MD at PND3 and glucocorticoid receptor antagonist or VEH administration at PND 26–28. At week 12, these animals entered the rIGT.

In the late afternoon (2 pm or later) of test days in the memory tasks (novel object recognition; object-in-location) as well as two days before and two days after the test day, vaginal smear samples were collected for later determination of the female cycle stage. Samples were stained for 20 min in a Giemsa 10% dilution and analyzed under a light microscope. Estrous cycle determination was done by cross referencing the samples to images representative of the cycle stages (pro-estrous, estrous, met-estrus and di-estrus). Cycle stage was introduced as a covariate in the statistical analyses of this spatial learning task. This was not possible in the rIGT which encompasses 2 weeks.

EPM

The EPM was made of gray plastic. The maze itself is elevated 60 cm from the ground and features two open arms (50-cm long × 10-cm wide) and two enclosed arms (50-cm long × 10-cm wide, walls 40-cm high) placed across from their respective counterparts. At the start, rats were placed in the middle, facing the open arms. Open arms were lit to 15 lux each, the middle part 10 lux, and the two closed arms at five lux.

Rats were allowed to freely explore for 5 min. The behavior of the rats was recorded by a video camera and manually analyzed with Observer XT 9 (Noldus, Wageningen, The Netherlands). We assessed: (1) frequency of visits to the open or closed arms, (2) duration in the arms, (3) latency to first enter an open arm, and (4) total number of head dips (referred to as

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