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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.

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Key words: hippocampus, prefrontal cortex, contextual memory, depression, 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 programming 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 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 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 Task (rIGT; Van den Bos et al., 2014); and

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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.

(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, 2013; Oomen et al., 2011, 2014](#)).

The peripubertal period is considered a critical time window in which programming 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 μ L 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 $55 \pm 15\%$, 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 $\times$ 10-cm wide) and two enclosed arms (50-cm long $\times$ 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 5 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

risk-taking behavior). Absolute data for frequency of visits and time spent in the arms was cumulated over arm categories (open and closed). From this a ratio was constructed dividing the frequency or duration in the open arm divided by its respective total value. For latency, the smallest number (not zero) for the open arm entries was used for statistical analyses.

rIGT

Experiments were performed as described elsewhere (De Visser et al., 2011). In short, rats entered the rIGT at ~12 weeks of age. The rIGT apparatus consisted of a start box, choice area and four arms. Before testing, rats were habituated to the apparatus in a 10-min free exploration trial. Two days later they were mildly food deprived (90–95% of free feeding body weight) and tested for a period of 10 days (two 5-day periods, 9 am–3 pm, on weekdays; food freely available on weekend days).

Of the four arms in the maze, two arms were baited and two arms were empty. The two empty arms were included to measure non-reward related exploration (De Visser et al., 2011). The two baited arms consisted of a long-term disadvantageous arm (“bad arm”) and a long-term advantageous arm (“good arm”). In the disadvantageous arm, rats received occasional big rewards (three sugar pellets in one out of 10 trials) among frequent punishments (three quinine-treated sugar pellets in nine out of 10 trials). In the advantageous arm, rats frequently received small rewards (one sugar pellet in eight out of 10 trials) and infrequent punishments (one quinine-treated sugar pellet in two out of 10 trials). The positions of the baited and empty arms, as well as the advantageous and disadvantageous arm were counterbalanced across rats. To help rats differentiate arms, distinct visual cues (10 × 10 cm; cross or circle in black or white) were placed to the side of the wall at the entrance of each arm. The chosen arm was only closed when the rat had entered a choice arm with its full body, including the tail.

Each trial had a maximum duration of 6 min (inter-trial interval: 30 s). Rats received a total of 120 trials across 10 days. Rewards were 45 mg sugar pellets (F0042, Bio-serv Inc., Frenchtown, NJ, USA); punishments were quinine-treated sugar pellets that were unpalatable but not uneatable. Rats were habituated to the sugar pellets in the week prior to the first rIGT session in their home cage daily, followed by a single session of providing six sugar pellets in a novel empty Macrolon type-III cage, which all rats did eat. During rIGT testing most rats consumed the quinine-treated sugar pellets once, but left them uneaten after tasting them briefly. Typically, rats that consistently eat quinine-treated sugar pellets are removed from statistical analyses, but these rats were not encountered in the current study.

The main parameter of interest was the number of choices for the advantageous option as a fraction of the total number of trials. This was expressed in blocks of 20 trials to study task progression. Scores in the last session (trial 101–120) were taken as a measure of final IGT performance of rats. The total number of sucrose pellets collected during the task (trial 1–120) was used as a measure of overall task performance to reflect the

final “budget”. Furthermore, the number of switches between different arms was calculated as a measure of exploratory behavior. This was expressed per block of 20 trials to study the change in exploratory behavior across the task. When rats encountered a sugar reward, their subsequent choice was scored as a win-stay when they revisited the same arm. When rats encountered a quinine punishment, their subsequent choice was scored as a lose-shift when they switched to another arm. Win-stay and lose-shift were calculated as a fraction of the total number of encounters with sugar (win) and quinine (loss) respectively.

Novel ORT and OLT

At 13 weeks of age, rats’ memory was tested in a novel ORT for non-spatial – presumably hippocampus-independent (Barker and Warburton, 2011) – recognition memory (Bevins and Besheer, 2006), and at 15 weeks old in an OLT to assess spatial memory (Ennaceur, 2010). Both tests took place in an infrared light setting. The arenas were made of solid black plastic with dimensions of 73 cm by 73 cm with 50-cm high walls. Objects used in the ORT (three copies each) were constructed out of Duplo™ (approx. 62.4 mm × 93.6 mm × 137.9 mm). For the OLT two identical objects were created comprised of a glass laboratory staining dish (10.7 cm × 8.7 cm × 7 cm), with a red light bulb diagonally placed into the dish. No object familiarization sessions were performed.

The ORT testing protocol consisted of two habituation sessions, a training phase, and two testing trials (1 h and 24 h after training). In all sessions rats were placed facing the left corner of the box closest to the experimenter. After habituation on day 1, animals were presented on day 2 with two identical objects in a 5-min trial in which they were allowed to freely explore. After a 1-h retention interval rats were placed back into the arena for five minutes in which they now encountered both a familiar object and a novel object. The set-up was counterbalanced. On day 3, rats were placed into the arena for another 5-min trial in which the object used in the first (training) phase was replaced by a new object.

For the OLT, we used the same scheme (two habituation sessions, one training phase, a 1-h and 24-h testing trial), now using two identical objects. Rats were always placed with their nose against the wall, in between from where the objects would be located during the training phase. One hour after training, one of the objects was relocated in the diagonal corner relative to the other object. On the 24-h trial the object that hadn’t moved yet, was laterally moved, so that both objects were now opposite to where they were in the training phase. We counterbalanced which of the two objects (the left or right object) was relocated first in the 1-h trial as well as the wall upon which it was organized.

All sessions were recorded using an infrared-sensitive camera. Videos were later analyzed manually using Observer XT 9 (Noldus, Wageningen, The Netherlands). Exploration of an object was defined similar to other studies (Akkerman et al., 2012) as time spent attending to the object within a distance of 2 cm, focusing on three

variables: (1) amount of visits to the object (frequency), (2) time spent exploring the object (duration), and (3) latency to approach the object (latency). The discrimination ratio was determined by dividing the time (frequency, latency) spent with the novel object or in the novel location by total exploration for the given variable. We report here only on the results of the total 5 minutes' analyses of the 1-h test, as results after 24 h were very similar.

Structural analysis

Two weeks following the novel ORT, rats were anaesthetized with pentobarbital sodium salt (0.5 ml i.p.) and perfused transcardially with saline (0.9 % NaCl) followed by 4% paraformaldehyde (PFA) in phosphate buffer (PB; 0.1 M; pH 7.4). Brains were post-fixed in the skull and stored in the refrigerator (4 °C) for 1 day in 4% before they were removed and preserved in the refrigerator (4 °C) in a 25 ml solution of PB with 0.01% Sodium Azide.

Four days prior to slicing, brains were washed and placed in a 15% sucrose solution in 0.1 M PB for 1 day, followed by 3 days in a 30% sucrose solution. For 32 animals, brains were frozen and hippocampal sections (40 µm) were cut using a sliding microtome, washed and stored in PB with 0.01% Sodium Azide. Sections were collected and stored at 4 °C in 15 ml vials in a one-in-ten series, such that each vial contained approximately 10 hippocampal sections that were systematically sampled throughout the entire hippocampus.

DG granular cell layer volume and cell count

Sections were mounted on Superfrost™ glass slides (75-mm × 25-mm) and Nissl-stained with 0.25% Cresyl Violet-acetate in distilled water and 1% acetic acid, then dehydrated and coverslipped. For all sections (with a maximum of 10 sections per animal), the total number of granular cells in the DG as well as the – manually traced – volume of the DG was estimated using a systematic random sampling method performed with the StereoInvestigator set-up (Microbrightfield, Magdeburg, Germany). Also, we expressed cell density by dividing the optical fractionator estimated DG granular cell count by the total volume of the DG (in mm). Sections were counted unilaterally, as any hemispheric bias would be unlikely. The optical fractionator settings (with a 100 × objective) were: grid size 150 × 150, counting frame 15 × 15, resulting in on average 396 ± SD 50 markers over 10 sections per animal (see also [Oomen et al., 2011](#)).

Adult neurogenesis and proliferation

Immunohistochemistry was performed for doublecortin (DCX), a marker for immature, migratory neurons, and for Ki-67, a marker for proliferation, using methods described before ([Oomen et al., 2010, 2011](#)). Briefly, for DCX, an endogenous peroxidase activity block was followed by incubation in 2% milk powder (Elk, Campina) in TBS. Next, sections were incubated in goat anti-DCX

(sc-8066, Santa Cruz Biotechnology, Dallas, Texas, USA, diluted 1:800 in TBS-0.1% Triton, 0.25% gelatin incubation mix) for 1 h at room temperature and then overnight at 4 °C. The secondary antibody was biotinylated donkey anti-goat (1:500, Jackson Immunoresearch, West Grove, PA, USA), followed by a 2-h incubation in Avidin–Biotin Complex Peroxidase (1:800) (Vectastain ABC Elite, Vector Laboratories, Burlingame, CA, USA). Further amplification was with biotinylated tyramide (1:500) with 0.01% hydrogen peroxide, followed by 1.5 h of incubation in ABC Elite (1:800). Chromogen development involved a standard DAB-HRP reaction. Finally, stained sections were counterstained with hematoxylin, dehydrated and coverslipped using Entellan.

For Ki-67 immunohistochemistry, sections were first mounted on Superfrost slides. Following an endogenous peroxidase block, the nuclear Ki-67 antigen was retrieved in 0.01 M citrate buffer pH 6 using a microwave oven set to 800 W until boiling, then lowered to 400 W and 280 W, each for five minutes. After cooling down, blockade of aspecific binding sites was as above, and the incubation of the sections with first antibody was in rabbit anti-Ki67p 1:5000 (Novocastra, Newcastle upon Tyne, United Kingdom). The next day, incubation was done with biotinylated goat-anti rabbit (1:200, Vector Laboratories) in incubation mix. Further signal amplification and chromogen development were as described above.

The number of DCX-immunopositive cells was quantified unilaterally in 9 ± 1 sections using the optical fractionator method on a StereoInvestigator setup (Microbrightfield, Germany/USA) coupled to a Zeiss microscope with a 40× objective, using a 140 × 80 grid size and 50 × 50 counting frame, as described before ([Oomen et al., 2010](#)). In [Fig. 4](#), results are presented for the bilateral hippocampus. Quantification of Ki-67 immunopositive cell numbers was done manually in both sides of the hippocampus in 10 ± 1 sections at 400× magnification using a light microscope and multiplied by ten to obtain the total number of Ki67-immunopositive cells for the entire hippocampus.

Statistical analysis

Unless stated otherwise all data (structural and cognitive) were analyzed using a two-way independent ANOVA, followed by post hoc analysis with a Bonferroni test ($p < .05$ considered to be significant). The rearing condition (MD or NMD) and intervention (VEH or MIF) were used as independent variables. If necessary for post hoc analysis, one-way ANOVAs were conducted grouping the condition and intervention together in a group variable. Group performance based on discrimination ratios in the OLT and ORT, as tested with one-sample *t*-test against test statistic 0.5, is a common method for analyses of discrimination ratios in memory tests. However, not taking standard deviation into account in chance performance results in a less stringent approach to analyze these data, thereby overestimating the significance levels of each group's performance, thus increasing the probability for type I error ([Akkerman et al., 2012](#)). Therefore, for those values

that were significant on a one-sample *t*-test against .5, a fictional chance level variable ($\mu = .5$, $\sigma =$ population variance) was constructed using SPSS with the compute variable function to check these results from the one-sample *T*-test more stringently on an independent samples *T*-tests. All statistical analyses were conducted using SPSS version 20.0 (IBM, United States).

Literature review

To review the effects of ELS on female rodents we gathered published papers through a thorough PubMed search. We used the following search query: “(female OR sex-spec* OR gender) AND (matern*) AND (separ* OR depri*) AND (postnatal OR neonatal OR prenatal) AND (stress) AND ((memor* OR learn* OR spatial OR fear memory) OR (anxiety OR fear) OR (goal-direct* OR decision*) OR (emotionality)) AND (behavior) AND (rat OR mice).” This search query resulted in 158 hits. After carefully screening the abstracts, 40 papers were found to match the following criteria: (1) the experiment was conducted at least in female mice or rats; (2) the manipulation was presumed to reflect ELS (before PND 21); papers describing stress through injection with a neurochemical or psychoactive substance were excluded; (3) behavioral studies pertained to one of five domains: anxiety-related behavior, stressful-learning tasks, non-stressful (hippocampus) learning, depressive-like behavior and social behavior. Additionally, 24 additional journal articles were found through references in the extracted papers and added to the data base.

For each behavioral endpoint of a paper we listed the type of task, age at which the rodents were tested, ELS paradigm, and age when ELS was applied. Subsequently, significant ($p < .05$) results were denoted based on the interpretation given by the authors, with “↑” constituting an enhancement, “↓” an impairment, and “–” no significant effect among the groups; when available, effects of males were also extracted. Results from 212 experimental series in 64 publications are listed in [Tables 1–5](#), according to the behavioral domains: anxiety-related behavior, stressful-learning tasks, non-stressful (hippocampus) learning, depressive-like behavior and social behavior. These categories are arbitrary and depend on the interpretation of the tasks. For instance, memory formation in a Morris Water Maze is relatively non-stressful (hippocampus-dependent) when the water temperature is 30 °C but becomes stressful (emotional) when the temperature is considerably lower, e.g. 22 °C ([Akirav et al., 2004](#)). As another example, behavior in a forced swim test is often considered to reflect depressive-like behavior, but can also be interpreted as a highly adaptive behavior to an inescapable stressor. In general, we followed the interpretation of the authors when assigning experimental series to a behavioral domain.

Results from the same paper are listed as separate entries (experimental series) when tested in different strains, at different ages or subjected to different early-life manipulations. Notably, in some papers neonatal handling (MS; 15-m/day) was used as a control condition (e.g. [Frankola et al., 2010](#)) while in other experiments neonatal handling constituted a brief

maternal separation next to a control condition (e.g. [Eklund and Arborelius, 2006](#)). In the latter case, the separation condition was added as a separate entry.

RESULTS

Early-life intervention

Bodyweight for all 64 animals in the first batch (rats of the second batch were food-deprived) was analyzed from week 5 (weight $\mu = 97.23$ g $\pm$ $\sigma = 8.8$ g) through week 13 (weight $\mu = 217.52$ g $\pm$ $\sigma = 11.1$ g) as dependent variables, and the groups (NMD-VEH, MD-VEH, NMD-MIF, MD-MIF) as between-groups factor in a repeated measures ANOVA. A significant interaction effect was found for time (in weeks) $\times$ rearing condition effects ($F[8,53] = 2.550$, $p = .020$) as well as a main effect for time in weeks independently ($F[8,53] = 1272.6$, $p < .001$). Examination of the effects of the groups showed a significant difference between the two rearing conditions (NMD and MD; $F[1,60] = 27.16$, $p < .001$).

These results suggest that, regardless of intervention, body weight of MD rats stayed significantly lower from the beginning of measurement in week 5 until the end of the measurements, supporting effective early-life intervention by MD. Investigation of the absolute and percentage weekly mean increase in weight with a two-way ANOVA did not yield any significant main or interaction effects. This suggests an equal growth rate for each group.

EPM

Two-way ANOVAs of the parameters observed within the EPM yielded no significant differences between the groups. For instance, examination of the time spent in the open arms revealed no significant condition $\times$ intervention interaction ($F[1,60] = 1.227$, $p = .272$), nor main effects of condition ($p = .742$) or intervention ($p = .182$). Similarly, no significant differences were found for frequency of visits (open, closed and total arm entries), time spent in closed arm, latency to first visit an open arm and number of head dips (data not shown).

These results suggest that neither MD nor intervention of the putative effects thereof had an effect on anxiety-like behavior as measured by EPM performance. Addition of the cycle stage as a covariate did not show any significant effect of cycle stage in the performance in the EPM, e.g. for time spent in the open arm ($p = .159$).

rIGT

Female rats in all groups ($n = 8$ – 12 per group) were able to learn the task (chance level for the advantageous option is 0.25; see [Fig. 1](#)). Examination of the fraction of visits to the advantageous arm during the final r-IGT session revealed no significant condition $\times$ intervention interaction ($F[3,38] = .758$, $p = .389$), nor main effects of condition ($F[3,38] = .991$, $p = .326$) or intervention ($F[3,38] = 1.175$, $p = .285$).

Similarly no significant condition $\times$ intervention interaction ($F[3,38] = 1.320$, $p = .258$), nor main effects

Fig. 1. Female rats were able to learn the rodent Iowa Gambling Task (chance level for the advantageous option is 0.25; horizontal striped line), as is evident from the fraction of visits to the advantageous arm during the final 60 trials. No significant condition $\times$ intervention interaction nor main effects of condition (maternal deprivation = MD; no maternal deprivation = NMD) or intervention (mifepristone = MIF; vehicle = VEH) were observed during the final session (see main text for statistical details). Data show mean $\pm$ SEM, based on $n = 8$ –12 animals per group.

of condition ($F[3,38] = .56$, $p = .815$) or intervention ($F[3,38] = 2.349$, $p = .134$) was observed for the total number of sugar pellets obtained during the 120 trials; for the fraction of visits to the disadvantageous arm (condition $\times$ intervention interaction ($F[3,38] = 1.247$, $p = .271$), condition ($F[3,38] = .101$, $p = .752$), intervention ($F[3,38] = .333$, $p = .567$)); and for the visits to the empty arms (condition $\times$ intervention interaction ($F[3,38] = .10$, $p = .919$), condition ($F[3,38] = 3.231$, $p = .080$), intervention ($F[3,38] = 2.266$, $p = .141$)).

Analysis of win-stay behavior also revealed no differences between groups (win-stay, trial block: condition $\times$ intervention interaction ($F[3,38] = .894$, $p = .350$), condition ($F[3,38] = .000$, $p = .986$), intervention ($F[3,38] = 1.325$, $p = .257$)); nor did we see significant effects for lose-shift behavior (condition $\times$ intervention interaction ($F[3,38] = .229$, $p = .635$), condition ($F[3,38] = 1.248$, $p = .271$), intervention ($F[3,38] = .229$, $p = .635$)).

To study exploratory behavior we analyzed the number of switches between different arms. Also for this parameter, ELS did not affect behavior: condition $\times$ intervention interaction ($F[3,38] = .035$, $p = .853$), main effect of condition ($F[3,38] = .145$, $p = .706$), main effect of intervention ($F[3,38] = 1.304$, $p = .261$). The results suggest that, in this particular reward-based decision making test, no aspect in behavioral performance of female rats is affected by stress early in life.

ORT

Animals explored the arena for 86 ± 16 s ($n = 16$ per group) during training and, as expected, this decreased significantly over subsequent trials (RM-ANOVA; $F[2,120] = 7.692$, $p = .001$). The decrease was observed in all groups alike, as indicated by a non-significant group $\times$ time interaction ($F[6,120] = 1.159$, $p = .333$). RM-ANOVA pointed out that there was no significant

group effect ($F[3,60] = 1.245$, $p = .301$) suggesting similar exploration times for the groups during the ORT.

Memory performance per group was assessed through an independent samples t -test against a fictional chance level group (based on population SD for 1-h duration discrimination indices (DI) $\sigma = .135$; $n = 16$, $\mu = .49$, $\sigma = .11$; and frequency DI $\sigma = .117$; $n = 16$, $\mu = .44$, $\sigma = .12$). This showed that NMD-VEH ($t[30] = 2.629$, $p = .013$; $t[30] = 3.224$, $p = .003$) and MD-MIF animals ($t[30] = 2.738$, $p = .010$; $t[30] = 3.833$, $p = .001$) performed significantly better than chance in the ORT based on the DI of duration, even when the population variance was taken into account (see Fig. 2a).

When comparing all groups, analysis of the DI revealed no main effect of rearing condition ($F[1,60] = .523$, $p = .473$ at 1 h), intervention ($F[1,60] = .192$, $p = .663$), nor a rearing condition $\times$ intervention interaction ($F[1,60] = 1.388$, $p = .243$) regarding duration as well as frequency (data not shown). Addition of the cycle stage as a covariate had no significant effect on the performance in the ORT, e.g. for DI duration in the 1-h test ($p = .404$). No significant effects were found for the number of approaches toward the new object in the 1-h test, for the old object visits, time spent with either of the objects, or latency to the new objects (data not shown).

Thus, while the NMD-VEH and MD-MIF group performed above chance on the ORT task in the 1-h test, the between-group differences in performance in terms of DI were minimal.

Object location task

On average, animals explored the arena for 117 ± 18 s ($n = 63$) during the OLT training phase, which decreased significantly during the test phases to 100 ± 19 for the 1-h test, respectively (RM-ANOVA; main effect of time; $F[2,58] = 26.103$, $p < .001$). Bonferroni-corrected pairwise comparisons revealed significantly different exploration durations ($p < .001$) between the training phase ($n = 63$, $\mu = 117.1$, $\sigma = 17.9$) and the test phases separately (1-h test: $n = 63$, $\mu = 100.3$, $\sigma = 19.4$) but not among the test phases themselves. Independently, the groups did not differ on exploration time ($F[3,59] = 1.377$).

Independent sample T -tests were used to assess memory performance per group. Total sample variances were .085 and .086 for 1-h frequency and duration DI respectively, as these values were used to create fictional chance level groups (1-h frequency DI: $n = 16$, $\mu = .47$, $\sigma = .11$; 1-h duration DI: $n = 16$, $\mu = .50$, $\sigma = .08$). Independent T -tests showed significant preference for the novel object location during the 1-h test based on discrimination by duration for NMD-VEH ($t[30] = 2.252$, $p = .032$), MD-VEH ($t[30] = 2.501$, $p = .018$), and MD-MIF ($t[30] = 2.405$, $p = .023$) but not for the 1-h duration DI of the NMD-MIF group ($t[30] = 1.163$, $p = .254$, see also Fig. 2b). Investigation of frequency DI only yielded trends of MD-VEH ($t[30] = 1.914$, $p = .065$) and MD-MIF ($t[30] = 1.941$, $p = .062$).

Fig. 2. (a) Maternal deprivation and/or mifepristone treatment did not alter behavior of adult female rats in the Object Recognition Memory task. Data show the mean + SEM ($n = 16$ rats per group) for the discrimination index during the 5-min period of the 1-h test. $p < 0.05$ for memory performance per group, assessed through an independent samples t -test against a fictional chance level group. Horizontal striped line indicates chance level performance. See main text for statistical details. (b) Two-way ANOVAs of the 1-h DI's duration did not reveal any significant main effect for condition, intervention, nor a significant condition $\times$ intervention interaction effect in the object-in-location task.

Two-way ANOVAs of the 1-h DI duration did not reveal any significant main effect for condition ($p = .457$), intervention ($p = .573$), nor a significant condition $\times$ intervention interaction effect ($p = .434$) (see Fig. 2b). This was also confirmed when a two-way ANOVA of the 1-h DI for frequency yielded no significant main effects for condition ($p = .420$), intervention ($p = .899$), nor an interaction effect ($p = .626$). However, a significant condition $\times$ intervention interaction effect was observed for latency to approach the object in a novel location on the 1-h test ($F[1,60] = 4.725$, $p = .034$) indicating that MIF alters initial behavior differentially in each of the rearing conditions, lowering latency in control rats, while increasing latency in MD rats. As no post hoc can be done with a two-way ANOVA with dichotomous group variables, a one-way ANOVA was conducted with groups as factor, but this did not reveal a significant difference between the groups ($F[3,60] = 2.269$, $p = .090$). Addition of the cycle stage as a covariate did

not show any significant effect in the performance, e.g. there was no significant main effect of cycle stage on DI duration on the 1-h test ($p = .349$).

In summary, while all groups (except NMD-MIF) showed preference for the novel location in the 1-h test, no differences *between* groups were found on spatial memory performance.

DG volume and plasticity

Six animals were excluded from analysis for various reasons, like missing sections or discolored samples. To assess DG volume, a total of 493.253 ± 51.379 granular cells were estimated on average ($n = 27$) over an area of approximately 1.38 mm^3 . No main effects (nine sections) were found for condition, intervention or condition $\times$ intervention interaction for cell count (condition: $F[1,23] = .996$, $p = .329$; intervention: $F[1,23] = .557$, $p = .463$; condition $\times$ intervention: $F[1,23] = .554$, $p = .464$) or DG volume (condition: $F[1,23] = .764$, $p = .391$; intervention: $F[1,23] = .002$, $p = .967$; condition $\times$ intervention: $F[1,23] = .056$, $p = .814$). Similarly, cell density was comparable for all groups (condition: $F[1,23] = .007$, $p = .936$; intervention: $F[1,23] = .894$, $p = .354$; condition $\times$ intervention: $F[1,23] = 1.312$, $p = .264$) (see Fig. 3).

Neurogenesis quantification of DCX + cell numbers showed no significant differences between the groups (condition: $F[3,28] = .010$, $p = .920$; intervention: $F[3,28] = .053$, $p = .820$; condition $\times$ intervention: $F[3,28] = 1.037$, $p = .317$; see Fig. 4). Quantification of adult cell proliferation, as based on Ki-67 + cell numbers, also revealed no significant differences (condition: $F[3,27] = .016$, $p = .899$; intervention: $F[3,27] = .071$, $p = .792$; condition $\times$ intervention: $F[3,27] = 1.713$, $p = .202$).

Review of literature

Since 24-h MD on PND3 did not appear to affect any of the behavioral parameters tested by us in female rats, we wondered to what extent this is a general finding and thus how this general lack of behavioral effects in females relates to the literature. To this end, we performed an extensive review of existing literature on the behavioral consequences of ELS in female rodents. We focused on five behavioral domains: anxiety-related behavior (EPM; light–dark preference; open field behavior); depressive-like behavior (forced swim test; sucrose preference; tail suspension); stressful/emotional learning and memory (active or inhibitory avoidance; cued and/or contextual fear conditioning); non-stressful learning and memory (object location task; Morris watermaze at 30°C); and social behavior (social approach, interaction, recognition; social play). In total, 212 experimental results were extracted from 64 studies. The results are summarized in Tables 1–5.

When combining the results in all domains – and disregarding differences in early-life adversity, species, strain or age of testing – almost two thirds of the experimental series failed to show significant effects of early-life adversity in females (64%), which was higher

Fig. 3. (a) Example of Nissl-stained histological section, illustrating the area delineated to determine volume of the dentate gyrus. (b) Higher magnification used to establish cell count. (c) The four experimental groups did not differ in the cell count per volume unit.

Fig. 4. Proliferation and neurogenesis in the hippocampal dentate gyrus. (a) Ki-67 immunocytochemistry reveals Ki-67 immunopositive cells in black (arrow), reflecting proliferation, located at the border zone of the granular cell layer (GCL, in blue) and the hilus (h). (b) Numerous DCX-immunopositive cells (in brown) are seen in the granular cell layer, reflecting young migratory neurons. DCX + cell bodies are located at the subgranular zone (arrows) and their protrusions pass through the GCL (blue) up into the molecular layer (m). Bar in (b) represents 50 μ m (same magnification for (a)). (c) For each animal, we counted the number of Ki67 + cells in every 10th section and from this the total number of Ki67 + cells per animal was inferred. All bars represent the mean + SEM per group ($n = 6-8$) animals per group. (d) For each animal, the number of DCX + cells was counted in every 10th section, sampled in an unbiased stereological manner, based on a total of 9 ± 1 sections per animal. Results are expressed for both hemispheres of the hippocampus (see text for details). All bars in (c) and (d) represent the mean + SEM per group ($n = 7-9$) animals per group. VEH; vehicle, MIF; mifepristone treatment, MD; maternally deprived animals, NMD; non-deprived, control animals.

Table 1. Literature overview of the early-life adversity effects on anxiety-related behavior in female rodents

Anxiety-related Behavior							
Test	Age (test)	Early-life Stress Type	Rodent	Strain	♀	♂	Ref. #
Elevated Plus Maze (EPM)	PND26	MS; 3-h/day (PND2–14)	Rat	LE-H	–	–	Frankola et al. (2010)
Elevated Plus Maze (EPM)	PND26	MS; 3-h/day (PND2–14)	Rat	LE-H	↓	↓	Grissom et al. (2012)
Elevated Plus Maze (EPM)	PND28	MS; 3-h/day (PND2–14)	Rat	WKY	–	–	Sterley et al. (2011)
Elevated Plus Maze (EPM)	PND28	MS; 3-h/day (PND2–14)	Rat	SHR	–	–	Sterley et al. (2011)
Elevated Plus Maze (EPM)	PND30	MD; 24-h (PND9)	Rat	WIS	–	↑	Llorente et al. (2007)
Elevated Plus Maze (EPM)	PND30–40	MS; 3-h/day (PND3–21)	Rat	LE	–	↓	Muhammad and Kolb (2011)
Elevated Plus Maze (EPM)	PND35–45	MS; 15-m (PND1–10)	Rat	SD	↑	↑	Koehnle and Rinaman (2010)
Elevated Plus Maze (EPM)	PND35–45	MS; 3-h/day (PND1–10)	Rat	SD	–	–	Koehnle and Rinaman (2010)
Elevated Plus Maze (EPM)	PND42	MD; 24-h (PND9)	Rat	WIS	–	–	Marco et al. (2013)
Elevated Plus Maze (EPM)	PDN44	MD; 24-h (PND9)	Rat	WIS	–	–	Llorente et al. (2011)
Elevated Plus Maze (EPM)	PDN44	MD; 24-h + CUS (PND9 + 28–43)	Rat	WIS	–	–	Llorente et al. (2011)
Elevated Plus Maze (EPM)	1 month	MS; 15-m/day (PND2–21)	Rat	SD	↑	↑	McIntosh et al. (1999)
Elevated Plus Maze (EPM)	1 month	MS; 3-h/day (PND2–21)	Rat	SD	↑	–	McIntosh et al. (1999)
Elevated Plus Maze (EPM)	1.5 months	MS; 3-h/day (PND2–14)	Rat	WIS	↓	↓	Salzberg et al. (2007)
Elevated Plus Maze (EPM)	2 months	EW (PND16)	Rat	WIS	–	↓	Ito et al. (2006)
Elevated Plus Maze (EPM)	2 months	MS; 3-h/day (PND3–14)	Rat	WIS	↓	–	Park et al. (2005)
Elevated Plus Maze (EPM)	2 months	MS; 2 × 3-h/day (PND1–21)	Rat	WIS	↑	–	León Rodríguez and Dueñas (2013)
Elevated Plus Maze (EPM)	2 months	MS; 4.5-h/day (PND1–21)	Rat	WIS	–	–	Renard et al. (2005)
Elevated Plus Maze (EPM) + stress	2 months	MS; 4.5-h/day (PND1–21)	Rat	WIS	↑	–	Renard et al. (2005)
Elevated Plus Maze (EPM)	2.5 months	MS; 3-h/day (PND2–14)	Rat	SD	–	–	Xiong et al. (2014)
Elevated Plus Maze (EPM) + stress	2.5 months	MS; 3-h/day (PND2–14)	Rat	WIS	–	–	James et al. (2014)
Elevated Plus Maze (EPM)	2.5 months	MD; 24-h (PND3)	Rat	WIS	–	–	Oomen et al. (2011)
Elevated Plus Maze (EPM)	2.5–3 months	MS; 3-h/day (PND1–14)	Mouse	B6	–	↓	Romeo et al. (2003)
Elevated Plus Maze (EPM)	3 months	MS; 3-h/day (PND1–14)	Mouse	B6	↓	↓	Veenema et al. (2007a)
Elevated Plus Maze (EPM)	3.5 months	LN (PND2–9)	Rat	SD	–	↓	Prusator and Greenwood-Van Meerveld (2015)
Elevated Plus Maze (EPM)	3–4 months	MS; 3-h/day (PND3–15)	Rat	LE-H	–	–	Slotten et al. (2006)
Elevated Plus Maze (EPM)	3–4 months	MD; 24-h (PND9)	Rat		–	–	Barna et al. (2003)
Elevated Plus Maze (EPM) + stress	3–4 months	MD; 24-h (PND9)	Rat		–	↓	Barna et al. (2003)
Elevated Plus Maze (EPM)	4 months	MS; 3-h/day (PND2–14)	Rat	LE-H	↓	↓	Kalinichev et al. (2002)
Elevated Plus Maze (EPM)	4 months	MS; 15-m/day (PND2–14)	Rat	LE-H	↓	↓	Kalinichev et al. (2002)
Elevated Plus Maze (EPM)	4–4.5 months	MS; 6-h/day (PND2–6)	Rat	LE-H	–	–	Zimmerberg and Kajunski (2004)

(continued on next page)

Table 1 (continued)

Anxiety-related Behavior							
Test	Age (test)	Early-life Stress Type	Rodent	Strain	♀	♂	Ref. #
Elevated Plus Maze (EPM)	4–5 months	MS; 3-h/day (PND3–12)	Rat	WIS	↓	↓	Wigger and Neumann (1999)
Elevated Plus Maze (EPM)	5 months	MD; 24-h (PND4)	Rat	WIS	–	–	Lehmann et al. (1999)
Elevated Plus Maze (EPM)	5 months	MD; 24-h (PND9)	Rat	WIS	–	–	Lehmann et al. (1999)
Elevated Plus Maze (EPM)	5 months	MD; 24-h (PND18)	Rat	WIS	–	–	Lehmann et al. (1999)
Elevated Plus Maze (EPM)	5 months	LN (PND2–9)	Mouse	B6J	–	–	Naninck et al. (2015)
Elevated Plus Maze (EPM)	Adulthood	MD; 24-h (PND11)	Rat	WIS	–	–	Barbosa Neto et al. (2012)
Elevated Plus Maze (EPM)	Adulthood	MD; 24-h (PND9)	Rat	WIS	–	↑	Llorente-Berzal et al. (2011)
Elevated T-Maze (ETM)	3–4 months	MS; 3-h/day (PND3–15)	Rat	LE-H	–	↑	Slotten et al. (2006)
Open Field (OF)	PND26	MS; 3-h/day (PND2–14)	Rat	LE-H	–	–	Grissom et al. (2012)
Open Field (OF)	PND26–28	MS; 3-h/day (PND2–14)	Rat	LE-H	–	–	Frankola et al. (2010)
Open Field (OF)	PND28	MS; 6-h/day (PND2–6 + 9–13)	Rat	SD	↑	↑	Spivey et al. (2009)
Open Field (OF)	PND28	MS; 6-h/day (PND2–6 + 9–13)	Rat	HO	↓	↓	Spivey et al. (2008a)
Open Field (OF)	PND30	ED; 5-h/day (PND2–14)	Rat	SD	–	–	Rees et al. (2006)
Open Field (OF)	PND30	MS; 5-h/day (PND2–14)	Rat	SD	–	–	Rees et al. (2006)
Open Field (OF) – familiar	PND30	MS; 6-h/day (PND2–6 + 9–13)	Rat	HO	↑	↑	Spivey et al. (2008a)
Open Field (OF)	PND30–40	MS; 3-h/day (PND3–21)	Rat	LE	–	–	Muhammad and Kolb (2011)
Open Field (OF)	PND35	MS; 2-h/day (PND1–14)	Mouse	B6J	–	↑	Kundakovic et al. (2013)
Open Field (OF)	PND35	MS; 2-h/day (PND1–14)	Mouse	B/c	↑	–	Kundakovic et al. (2013)
Open Field (OF)	PND37	MD; 24-h (PND9)	Rat	WIS	–	–	Marco et al. (2013)
Open Field (OF)	1.5 months	MS; 6-h/day (PND2–6 + 9–13)	Rat	SD	↑	↑	Spivey et al. (2008b)
Open Field (OF)	2 months	Res.; 3 × 45-m/day (G14–21)	Rat	SD	↓	–	Bowman et al. (2004)
Open Field (OF)	2.5 months	PS (G1–7/8–14/15–21)	Mouse	B6:129	–	–	Mueller and Bale (2008)
Open Field (OF)	2.5 months	MS; 3-h/day (PND2–14)	Rat	SD	–	–	Xiong et al. (2014)
Open Field (OF) – stress	2.5 months	MS; 3-h/day (PND2–14)	Rat	WIS	↓	↓	James et al. (2014)
Open Field (OF)	2.5–3 months	MS; 3-h/day (PND1–14)	Mouse	B6	↑	↓	Romeo et al. (2003)
Open Field (OF)	2.5–3 months	MS; 5-h/day (PND2–14)	Rat	SD	–	–	Rees et al. (2006)
Open Field (OF)	2.5–3 months	ED; 5-h/day (PND2–14)	Rat	SD	–	–	Rees et al. (2006)
Open Field (OF)	3 months	Res.; 3 × 30-m/day (G15–22)	Rat	WIS	↓	–	Lehmann et al. (2000)
Open Field (OF)	3 months	Res.; 3 × 30-m/day + MS; 6-h/day (G15–22 + PND12,14,16,18)	Rat	WIS	↓	–	Lehmann et al. (2000)
Open Field (OF)	3 months	MS; 15-m/day (PND1–14)	Mouse	CD1	–	–	Zanettini et al. (2010)
Open Field (OF)	3 months	MS; 3-h/day (PND1–10)	Rat	WIS	–	↓	Diehl et al. (2007)
Open Field (OF)	3 months	MS; 3-h/day (PND1–14)	Mouse	B6J	↓	–	Tsuda and Ogawa (2012)
Open Field (OF)	3 months	MS; 3-h/day (PND1–14)	Mouse	B6	↓	–	Veenema et al. (2007a)
Open Field (OF)	3 months	MS; 3-h/day (PND2–15)	Mouse	B/cJ	–	–	Wang et al. (2011)
Open Field (OF)	3 months	MS; 6-h/day (PND12,14,16,18)	Rat	WIS	–	–	Lehmann et al. (2000)
Open Field (OF)	4 months	MS; 12-h/day (PND9,11)	Rat	WIS	↑	↓	Takase et al. (2012)
Open Field (OF)	3 months	MD; 24-h (PND4)	Rat	WIS	–	–	Lehmann et al. (1999)
Open Field (OF)	3 months	MD; 24-h (PND9)	Rat	WIS	–	–	Lehmann et al. (1999)
Open Field (OF)	3 months	MD; 24-h (PND18)	Rat	WIS	–	–	Lehmann et al. (1999)
Open Field (OF)	3.5 months	LN (PND2–9)	Rat	SD	–	↓	Prusator and Greenwood-Van Meerveld (2015)
Exploration/Holeboard	2 months	MS; 4-h/day (PND1–15)	Rat	WIS	–	–	Marmendal et al. (2004)
Holeboard	Adulthood	MD; 24-h (PND9)	Rat	WIS	–	–	Llorente-Berzal et al. (2011)
Holeboard	PND30	MD; 24-h (PND9)	Rat	WIS	–	–	Llorente et al. (2007)
Spontaneous Motor Activity	8–9 months	MS; 2 × 3-h/day (PND1–13)	Rat	WIS	–	–	Eklund and Arborelius (2006)
Spontaneous Motor Activity	8–9 months	MS; 15-m (PND1–13)	Rat	WIS	↓	–	Eklund and Arborelius (2006)
Defensive Withdrawal Test (DWT)	4 months	MS; 15-m (PND1–13)	Rat	WIS	–	–	Eklund and Arborelius (2006)

Table 1 (continued)

Anxiety-related Behavior							
Test	Age (test)	Early-life Stress Type	Rodent	Strain	♀	♂	Ref. #
Defensive Withdrawal Test (DWT)	4 months	MS; 2 × 3-h/day (PND1–13)	Rat	WIS	↑	–	Eklund and Arborelius (2006)
Light/Dark Test	PND29	MS; 6-h/day (PND2–6 + 9–13)	Rat	HO	↓	↓	Spivey et al. (2008a)
Light/Dark Test	3 months	MS; 3-h/day (PND1–14)	Mouse	B6J	–	–	Tsuda and Ogawa (2012)
Light/Dark Test	3 months	MS; 3-h/day (PND2–15)	Mouse	B/cJ	–	–	Wang et al. (2011)
Light/Dark Test	4 months	MS; 12-h/day (PND9,11)	Rat	WIS	–	–	Takase et al. (2012)
Novelty Test	2.5 months	EW (PND16)	Rat	WIS	–	↑	Ito et al. (2006)
Novel Object Exploration	3 months	MS; 3-h/day (PND1–14)	Mouse	B6	↓	↓	Veenema et al. (2007a)
Novelty-induced Suppression of Drinking	2.5 months	MS; 15-m/day (PND1–13)	Rat	LH	↑	↑	Macri et al. (2004)
Novelty-induced Suppression of Drinking	2.5 months	MS; 4-h/day (PND1–13)	Rat	LH	–	–	Macri et al. (2004)
Novelty-suppressed Feeding Test	PND24	MS; 3-h/day (PND2–14)	Rat	LE-H	↓	↓	Grissom et al. (2012)
Novelty-suppressed Feeding Test	2 months	LN (PND2–9)	Rat	WIS	↓	–	Machado et al. (2013)
Swim Stress Exposure	2.5 months	MS; 3-h/day (PND2–14)	Rat	SD	–	–	Stiller et al. (2011)
Swim Stress Exposure	2.5 months	EW; PND16 (PND16)	Rat	SD	↓	↓	Stiller et al. (2011)
Open Space Swimming Test (OSST)	4.5 months	MS; 3-h/day (PND1–14)	Rat	LE	↓	–	Mourlon et al. (2010)
Fleeing and Freezing	PND20	MS; 4-h/day (PND1–15)	Rat	WIS	–	–	Marmendal et al. (2004)
Fleeing and Freezing	2 months	MS; 4-h/day (PND1–15)	Rat	WIS	–	–	Marmendal et al. (2004)
Defensive Behaviors	PND26–32	MS; 3-h/day (PND2–13)	Rat	LE-H	↓	↓	Litvin et al. (2010)
Defensive Behaviors	PND26–32	MS; 6-h/day (PND11–13)	Rat	LE-H	↓	↓	Litvin et al. (2010)
Acoustic Startle Response	4 months	MS; 15-m/day (PND2–14)	Rat	LE-H	–	–	Kalinichev et al. (2002)
Acoustic Startle Response	4 months	MS; 3-h/day (PND2–14)	Rat	LE-H	–	↓	Kalinichev et al. (2002)
Acoustic Startle Response	5 months	MS; 6-h/day (PND12,14,16,18)	Rat	WIS	–	↑	Lehmann et al. (2000)
Acoustic Startle Response	5 months	Res.; 3 × 30-m/day (G15–22)	Rat	WIS	–	–	Lehmann et al. (2000)
Acoustic Startle Response	5 months	Res.; 3 × 30-m/day + MS; 6-h/day (G15–22 + PND12,14,16,18)	Rat	WIS	–	↑	Lehmann et al. (2000)

Abbreviations: AR = Artificial Rearing; B6 = C57BL/6; B/c = Balb/c; CUS = Chronic Unpredictable Stress; G = Gestational day; ED = Early Deprivation; EW = Early Weaning; LE = Long-Evans; LE-H = Long-Evans Hooded; HO = Holtzman; LG = Licking/Grooming; LN = Limited Nesting/bedding material; MD = Maternal Deprivation; MS = Maternal Separation; MSS + FS = Maternal Sound Stress + Forced Swim; PND = Postnatal Day; PS = Prenatal Stress; Res. = Restraint; SD = Sprague-Dawley; SHR = Spontaneously Hypertensive Rat; WIS = Wistar; WIS-H = Wistar-Han; WKY = Wistar-Kyoto.

–: no significant effect; ↑: significant increase; ↓: significant decrease.

than in males (55%). Also, a higher percentage of studies reported significantly *impaired* behavioral performance after early-life adversity in males (32%) than in females (25%). Interestingly, of all experimental series in which both males and females were tested ($n = 190$), one third (32%) was found to be affected by adverse early-life conditions in a sex-dependent manner; this was even 59% when experimental series ($n = 86$) were excluded showing no significant effect in both sexes. When series showing sex-dependent differences were analyzed ($n = 61$), the majority (64%) showed significant effects in males but not in females. Only in about one third of cases (30%) did females but not males display significant effects, whereas significant effects in opposite direction were seldom encountered (7%).

The sex-dependent differences were quite obvious in some domains, i.e. particularly for social behavior (57%), non-stressful learning and memory (41%) and depressive-like behavior (38%). Less marked differences between males and females were observed in the emotional realm, i.e. for anxiety related behavior (30%) or particularly stressful learning and memory (15%). This differentiation over domains was also evident from the prevalence of significant changes after early-life adverse conditions in females: depressive-like behavior (50%) > stressful learning (41%) > anxiety-related behavior (36%) > social behavior (33%) > non-stressful learning (29%); compared to males: depressive-like behavior (69%) > non-stressful learning (50%) > stressful learning (45%) > social behavior (43%) > anxiety-related behavior (40%).

Table 2. Literature overview of the early-life adversity effects on depressive-like behavior in female rodents

Depression Related Behavior							
Test	Age (test)		Rodent	Strain	♀	♂	Ref. #
Forced Swim Task (FST)	PND30–38	MS; 3-h/day (PND1–14)	Rat	SD	↓	↓	Zhang et al. (2013)
Forced Swim Task (FST)	PND31	MS; 3-h/day (PND2–14)	Rat	WKY	–	–	Sterley et al. (2011)
Forced Swim Task (FST)	PND31	MS; 3-h/day (PND2–14)	Rat	SHR	–	–	Sterley et al. (2011)
Forced Swim Task (FST)	PND34	MD; 24-h (PND9)	Rat	WIS	↓	↓	Llorente et al. (2007)
Forced Swim Task (FST)	2.5 months	PS (G1–7/8–14/15–21)	Mouse	B6:129	–	↓	Mueller and Bale (2008)
Forced Swim Task (FST)	2-months	MD; 24-h (PND9)	Rat	SD	↓	↓	Zamberletti et al. (2012)
Forced Swim Task (FST)	4.5 months	MS; 3-h/day (PND1–14)	Rat	LE	–	↓	Mourlon et al. (2010)
Forced Swim Task (FST)	4–5 months	MS; 3-h/day (PND3–12)	Rat	WIS	–	–	Wigger and Neumann (1999)
Forced Swim Task (FST)	5 months	LN (PND2–9)	Mouse	B6J	–	–	Naninck et al. (2015)
Sucrose Preference (SP)	PND30–38	MS; 3-h/day (PND1–14)	Rat	SD	↓	↓	Zhang et al. (2013)
Sucrose Preference (SP)	PND36	MS; 2-h/day (PND1–14)	Mouse	B6J	↓	↓	Kundakovic et al. (2013)
Sucrose Preference (SP)	PND36	MS; 2-h/day (PND1–14)	Mouse	B/c	↑	↓	Kundakovic et al. (2013)
Sucrose Preference (SP)	4.5 months	MS; 3-h/day (PND1–14)	Rat	LE	–	↑	Mourlon et al. (2010)
Learned Helplessness	PND36–38	MS; 4-h/day (PND2–9)	Rat	SD	↑	–	Freund et al. (2013)
Learned Helplessness	PND36–38	MS; 4-h/day (PND9–16)	Rat	SD	↑	↑	Freund et al. (2013)
Tail Suspension Test	2.5 months	PS (G1–7/8–14/15–21)	Mouse	B6:129	–	↓	Mueller and Bale (2008)

–: no significant effect; ↑: significant increase; ↓: significant decrease.

DISCUSSION

In this study we set out to examine the behavioral performance of 12–17-week-old female rats that had been subject to 24-h MD on PND3, focusing on three behavioral domains and DG structure. Given that early-life adversity is a common denominator in the vulnerability to mood- and anxiety disorders and that many of these disorders are more prevalent in females, we expected to see clear behavioral effects in this severe model of early-life adversity, and wondered whether this could possibly be ameliorated by a brief intervention with the powerful glucocorticoid receptor antagonist MIF. However, in females neither MD nor MIF treatment affected any of the studied behavioral indices, or the structural integrity or plasticity of the DG, an area relevant for performance in contextual memory tasks (Kheirbek et al., 2013).

This particular early-life intervention, 24-h MD at PND3, was earlier applied by us in male rats (Oomen et al., 2009, 2010). The paradigm is considered to be rather adverse and thought to be a model for severe maternal neglect (Pryce et al., 2005; Marco et al., 2015). Although we did not extensively monitor the neuroendocrine consequences of the model in the current study, we noticed a clear and significant attenuation in body weight gain, one of the characteristics earlier reported for this model in male rats (Oomen et al., 2009, 2010). We therefore have no reason to doubt the effectiveness of the early-life intervention.

The main outcome of the study is that 24-h MD at PND3 does not seem to change performance in any of the behavioral tasks to which the female rats were subjected. First, the behavior of female rats in the EPM did not differ among the groups. Second, with respect to the rIGT, female MD rats did not show any impairment compared to the control group, whereas an earlier study in male rats had demonstrated a correlation between the amount of maternal care and rIGT performance

(Van Hasselt et al., 2012). This underscores that behavioral performance of female rodents -and their vulnerability to early-life adversity- in the rIGT may differ from that of males, which agrees with the earlier reported sex differences in this task (Van den Bos et al., 2012). In males, the effects of corticosteroids seem to affect particularly the later stage of the task, when animals switch from an exploratory phase that involves affective and motivational circuits, to a cognitive control loop that depends (among other things) on prefrontal areas (De Visser et al., 2011). It is possible that compared to males, female rats had not yet fully reached the level at which they switch, and we cannot exclude that effects of MD might have been visible when rats would have been trained for a longer period of time. Third, while female (non-MD) rats were able to learn the ORT, we observed no consistent effects of MD or MIF. Possibly this is due to the fact that this task does not specifically depend on hippocampal function, except when the memory contains a temporal or object recency component (Barker and Warburton, 2011; Warburton and Brown, 2015). To circumvent this, we also tested animals in the OLT, which specifically measures hippocampal function (Barker and Warburton, 2011). Performance in similar sensitive hippocampus-dependent learning and memory tasks was earlier found to be affected by early-life environment in males but also in females (Frankola et al., 2010; Mourlon et al., 2010; Naninck et al., 2015). It should be noted that in our study DI values in most groups were only just above chance. We cannot fully exclude the possibility that the lack of effect in females is caused by a 'floor-effect'.

The current behavioral results were accompanied by a similar absence of differences between the groups in the measures for DG volume, proliferation (Ki-67) and neurogenesis (DCX). Exposure to stress (hormone) at adult or adolescent ages induces subtle sex-specific differences in neurogenesis (Brummelte and Galea, 2010), which depend in part on reproductive status, female cycle, age and genetic background

Table 3. Literature overview of the early-life adversity effects on stressful or emotional learning and memory tasks in female rodents

Stressful/Emotional Learning Tasks						
Test	Age (test)		Rodent	Strain	♀	♂ Ref. #
Active Avoidance (AA)	5 months	MS; 6-h/day (PND12,14,16,18)	Rat	WIS	↑	↑ Lehmann et al. (2000)
Active Avoidance (AA)	5 months	MD; 24-h (PND4)	Rat	WIS	–	↓ Lehmann et al. (1999)
Active Avoidance (AA)	5 months	MD; 24-h (PND9)	Rat	WIS	–	↑ Lehmann et al. (1999)
Active Avoidance (AA)	5 months	MD; 24-h (PND18)	Rat	WIS	–	– Lehmann et al. (1999)
Active Avoidance (AA)	5 months	Res.; 3 × 30-m/day (G15–22)	Rat	WIS	↓	↓ Lehmann et al. (2000)
Active Avoidance (AA)	5 months	Res.; 3 × 30-m/day + MS; 6-h/day (G15–22 + PND12,14,16,18)	Rat	WIS	↓	↓ Lehmann et al. (2000)
Inhibitory Avoidance (IA)		MS; 15-m/day (PND1–21)	Rat	SD	↓	↓ Kosten et al. (2007)
Auditory Fear Conditioning	PND37	MS; 3-h/day (PND1–14)	Rat	WIS	↓	↓ Chocyk et al. (2014)
Acoustic-Cued Fear Conditioning	PND42	MS; 1-h/day (PND2–9)	Rat	SD	–	– Kao et al. (2012)
Auditory Fear Memory Test	2 months	MS; 3-h/day (PND1–14)	Rat	WIS	–	↓ Chocyk et al. (2014)
Freezing: Tone	4 months	MD; 24-h (PND4)	Rat	WIS	–	– Lehmann et al. (1999)
Freezing: Tone	4 months	MD; 24-h (PND9)	Rat	WIS	–	– Lehmann et al. (1999)
Freezing: Tone	4 months	MD; 24-h (PND18)	Rat	WIS	–	– Lehmann et al. (1999)
Light-potentiated Fear Conditioning	P42	MS; 1-h/day (PND2–9)	Rat	SD	↓	– Kao et al. (2012)
Contextual Fear Conditioning	PND36	MS; 3-h/day (PND1–14)	Rat	WIS	↓	↓ Chocyk et al. (2014)
Contextual Fear Conditioning	2 months	MS; 3-h/day (PND1–10)	Rat	WIS	–	– Diehl et al. (2007)
Contextual Fear Conditioning	2 months	MS; 3-h/day (PND1–10)	Rat	WIS	↑	↑ Diehl et al. (2014)
Contextual Fear Conditioning	2.5 months	MS; 3-h/day (PND2–14)	Rat	SD	↓	– Xiong et al. (2014)
Contextual Fear Conditioning	3 months	MS; 6-h/day (PND1–21)	Rat	WIS	↓	– Sun et al. (2014)
Contextual Fear Conditioning		MS; 3-h/day (PND1–21)	Rat	SD	–	– Kosten et al. (2006)
Contextual Fear Conditioning		MS; 15-m/day (PND1–21)	Rat	SD	–	– Kosten et al. (2006)
Contextual Fear Memory Test	2 months	MS; 3-h/day (PND1–14)	Rat	WIS	↓	↓ Chocyk et al. (2014)
Context and Cued Fear Conditioning	3.5 months	MD; 24-h (PND3)	Rat	WIS	↓	– Oomen et al. (2011)
Freezing: Context	4 months	MD; 24-h (PND4)	Rat	WIS	–	– Lehmann et al. (1999)
Freezing: Context	4 months	MD; 24-h (PND9)	Rat	WIS	–	– Lehmann et al. (1999)
Freezing: Context	4 months	MD; 24-h (PND18)	Rat	WIS	–	– Lehmann et al. (1999)
Conditioned Freezing	4 months	MD; 24-h (PND9)	Rat	WIS	–	– Lehmann et al. (1999)
Conditioned Freezing	4 months	MD; 24-h (PND18)	Rat	WIS	–	– Lehmann et al. (1999)
Freezing: Conditioning	4 months	MD; 24-h (PND4)	Rat	WIS	–	– Lehmann et al. (1999)
Conditioned Emotional Response	4 months	MS; 6-h/day (PND12,14,16,18)	Rat	WIS	↓	↓ Lehmann et al. (2000)
Conditioned Emotional Response	4 months	Res.; 3 × 30-m/day (G15–22)	Rat	WIS	–	– Lehmann et al. (2000)
Conditioned Emotional Response	4 months	Res.; 3 × 30-m/day + MS; 6-h/day (G15–22 + PND12,14,16,18)	Rat	WIS	↓	↓ Lehmann et al. (2000)
Prepulse Inhibition (PPI)	Adulthood	MD; 24-h (PND9)	Rat	WIS	–	– Llorente-Berzal et al. (2011)
Prepulse Inhibition (PPI)	6 months	MS; 6-h/day (PND12,14,16,18)	Rat	WIS	–	– Lehmann et al. (2000)
Prepulse Inhibition (PPI)	6 months	Res.; 3 × 30-m/day (G15–22)	Rat	WIS	↑	↑ Lehmann et al. (2000)
Prepulse Inhibition (PPI)	6 months	Res.; 3 × 30-m/day + MS; 6-h/day (G15–22 + PND12,14,16,18)	Rat	WIS	–	– Lehmann et al. (2000)
Morris Water Maze (MWM) – 10 °C	12 months	Res.; 3 × 30-m/day (G15–19)	Rat	WIS-H	–	↓ Szuran et al. (2000)

–: no significant effect; ↑: significant increase; ↓: significant decrease.

(Kempermann et al., 2006; Veenema et al., 2007b; Pawluski et al., 2009; Barha et al., 2011). While the numbers we found here were largely in line with studies on rats of related ages (Oomen et al., 2010, 2011; Klomp et al., 2014), MD did not affect neurogenesis in female rats 17 weeks of age, indicating that the decreased neurogenesis found earlier in MD females at PND 21 (Oomen et al., 2009) does not last into later ages. The same applies to the earlier found decrease in DG cell number and density measured at 10 weeks after MD at PND3 (Oomen et al., 2011), which was not maintained in the animals we collected around 17 weeks of age.

One explanation could be the well-known age-related reduction in neurogenesis that – due to the progressive

slowing down of DG turnover at older ages (Heine et al., 2004) – may eventually normalize initial differences induced by ELS in DG cell number or density. The fact that MD also increases cell death in the infant rat hippocampus in a sex-dependent manner supports the concept of (transient) changes in turnover rate after MD, that may normalize at later ages (Zhang et al., 2002; Llorente et al., 2009). Alternatively, differences between the experimental design of the various studies may have contributed, as separate groups were used before for structural hippocampal analysis and for behavioral analysis (Oomen et al., 2009, 2011), whereas the present anatomical data were obtained from animals that had been used for behavioral analysis before. The latter

Table 4. Literature overview of the early-life adversity effects on non-stressful learning and memory tasks in female rodents

Non-stressful Hippocampus Learning						
Test	Age (test)		Rodent	Strain	♀	♂ Ref. #
Visible Platform Water Maze Task (VPWM)	PND27–29	MS; 3-h/day (PND2–14)	Rat	LE-H	–	– Grissom et al. (2012)
Morris Water Maze (MWM)* – reference mem.	PND27–30	MS; 3-h/day (PND2–14)	Rat	LE-H	–	↓ Frankola et al. (2010)
Morris Water Maze (MWM) – working mem.	PND31–33	MS; 3-h/day (PND2–14)	Rat	LE-H	↑	↓ Frankola et al. (2010)
Morris Water Maze (MWM)	3 months	MS; 6-h/day (PND1–21)	Rat	WIS	–	– Sun et al. (2014)
Morris Water Maze (MWM)	3 months	MD; 24-h (PND3)	Rat	WIS	–	– Oomen et al. (2011)
Morris Water Maze (MWM)	5 months	LN (PND2–9)	Mouse	B6J	–	↓ Naninck et al. (2015)
Morris Water Maze (MWM)	9 months	MD; 24-h (PND4)	Rat	WIS	–	– Lehmann et al. (1999)
Morris Water Maze (MWM)	9 months	MD; 24-h (PND9)	Rat	WIS	–	– Lehmann et al. (1999)
Morris Water Maze (MWM)	9 months	MD; 24-h (PND18)	Rat	WIS	–	– Lehmann et al. (1999)
Morris Water Maze (MWM) – 10-month mem.	10 months	AR (PND4–21)	Rat	SD	↑	– Lévy et al. (2003)
Morris Water Maze (MWM) – reversal test		AR (PND4–21)	Rat	SD	–	– Lévy et al. (2003)
Morris Water Maze (MWM) – small pool		AR (PND4–21)	Rat	SD	–	– Lévy et al. (2003)
Morris Water Maze (MWM) – large pool		AR (PND4–21)	Rat	SD	–	– Lévy et al. (2003)
Object Recognition Task (ORT)	PND28–33	MS; 3-h/day (PND2–14)	Rat	LE-H	–	↓ Frankola et al. (2010)
Object Recognition Task (ORT)	PND40	MD; 24-h (PND9)	Rat	WIS	↓	– Marco et al. (2013)
Object Recognition Task (ORT)	2 months	Res.; 3 × 45-m/day (G14–21)	Rat	SD	–	– Bowman et al. (2004)
Object Recognition Task (ORT)	♂ 2 ♀ 3.5 months	MS; 3-h/day (PND1–14)	Rat	LE	–	– Mourlon et al. (2010)
Object Recognition Task (ORT)	2 months	MD; 24-h (PND9)	Rat	SD	–	– Zamberletti et al. (2012)
Object Recognition Task (ORT)	2.5 months	MD; 24-h + CUS (PND9 + 28–43)	Rat	WIS	–	– Llorente et al. (2011)
Object Recognition Task (ORT)	3 months	MS; 3-h/day (PND2–15)	Mouse	B/cJ	↓	– Wang et al. (2011)
Object Recognition Task (ORT)	5 months	LN (PND2–9)	Mouse	B6J	↓	↓ Naninck et al. (2015)
Object Recognition Task (ORT)		MS; 12-h/day (PND9,11)	Rat	WIS	–	↓ Takase et al. (2012)
Object Recognition Task (ORT)		MS; 15-m/day (PND1–21)	Rat	SD	↑	↑ Kosten et al. (2007)
Novel Object Test	2.5 months	MD; 24-h (PND9)	Rat	WIS	–	↓ Llorente et al. (2011)
Object Location Task (OLT)	♂ 2 ♀ 3.5 months	MS; 3-h/day (PND1–14)	Rat	LE	↓	– Mourlon et al. (2010)
Object Location Task (OLT)	5 months	LN (PND2–9)	Mouse	B6J	↓	↓ Naninck et al. (2015)
Radial Arm Maze (RAM)	2 months	Res.; 3 × 45-m/day (G14–21)	Rat	SD	–	– Bowman et al. (2004)
Radial Arm Maze (RAM)	2.5 months	Low LG (PND1–21)	Rat	SD	↑	– Barha et al. (2007)
Radial Arm Maze (RAM)		AR (PND4–21)	Rat	SD	–	– Lévy et al. (2003)
T-Maze (TM) – water-filled	1.5 months	MSS + FS; 15-m/day (G10–18)	Rat	WIS/ST	–	↓ Nishio et al. (2001)
T-Maze (TM)	2 months	MS; 3-h/day (PND2–15)	Mouse	B/c	↓	↓ Mehta and Schmauss (2011)
T-Maze (TM)	2 months	MS; 3-h/day (PND2–15)	Mouse	B6J	–	– Mehta and Schmauss (2011)
T-Maze (TM) – reversal learning	3 months	MS; 3-h/day (PND2–15)	Mouse	B/cJ	–	↓ Wang et al. (2011)
T-Maze (TM) – spatial Learning	3 months	MS; 3-h/day (PND2–15)	Mouse	B/cJ	–	↓ Wang et al. (2011)
Y-Maze (YM)	PND27	MS; 3-h/day (PND2–14)	Rat	LE-H	↓	– Grissom et al. (2012)
Y-Maze (YM)	PND34–36	MS; 3-h/day (PND2–14)	Rat	LE-H	–	↓ Frankola et al. (2010)
Spontaneous Alternation Task (SAT)	PND35	MD; 24-h (PND9)	Rat	WIS	–	– Marco et al. (2013)
Spontaneous Alternation Task (SAT)	2 months	MD; 24-h (PND9)	Rat	WIS	–	↑ Llorente et al. (2011)
Spontaneous Alternation Task (SAT)	2 months	MD; 24-h + CUS (PND9 + 28–43)	Rat	WIS	↑	↑ Llorente et al. (2011)
Temporal Order Memory (TOM)	PND30–40	MS; 3-h/day (PND3–21)	Rat	LE	–	– Muhammad and Kolb (2011)
Circular Maze Learning		MS; 15-m/day (PND1–21)	Rat	SD	–	– Kosten et al. (2007)

–: no significant effect; ↑: significant increase; ↓: significant decrease.

* MWM at low temperature are placed in Table 3.

Table 5. Literature overview of the early-life adversity effects on social behavior in female rodents

Social Behavior							
Test	Age (test)		Rodent	Strain	♀	♂	Ref. #
Social Recognition Task (SRT)	2 months	MS; 3-h/day (PND2–15)	Mouse	B/c	–	–	Mehta and Schmauss (2011)
Social Recognition Task (SRT)	2 months	MS; 3-h/day (PND2–15)	Mouse	B6J	–	–	Mehta and Schmauss (2011)
Social Recognition Task (SRT)		AR (PND4–21)	Rat	SD	↓		Lévy et al. (2003)
Social Interaction Test	PND25	MS; 4-h/day (PND2–20)	Rat	SD	↓	–	Holland et al. (2014)
Social Interaction Test	PND40	MS; 4-h/day (PND2–20)	Rat	SD	–	↓	Holland et al. (2014)
Social Interaction Test	2 months	MD; 24-h (PND9)	Rat	SD	↓	–	Zamberletti et al. (2012)
Social Interaction Test	3 months	MS; 3-h/day (PND1–14)	Mouse	B6J	↓		Tsuda and Ogawa (2012)
Social Interaction Test		MS; 12-h/day (PND9,11)	Rat	WIS	–	↓	Takase et al. (2012)
Social Interaction Test		MS; 12-h/day (PND9,11)	Rat	WIS	–	↓	Takase et al. (2012)
Dyadic Social Encounter	PND40	MS; 2-h/day (PND1–14)	Mouse	B6J	–	↑	Kundakovic et al. (2013)
Dyadic Social Encounter	PND40	MS; 2-h/day (PND1–14)	Mouse	B/c	–	↓	Kundakovic et al. (2013)
Social Approach Test	3.5 months	MS; 15-m/day (PND1–14)	Mouse	CD1	–	–	Zanettini et al. (2010)
Intruder Test	2.5 months	EW (PND16)	Rat	WIS	–	–	Ito et al. (2006)
Resident-Intruder Test	4 months	MS; 15-m/day (PND1–14)	Mouse	CD1	–	–	Zanettini et al. (2010)
Competitive Behavior	3 months	MS; 4-h/day (PND1–15)	Rat	WIS	–	–	Marmendal et al. (2004)
Play Fighting Behavior	PND30–40	MS; 3-h/day (PND3–21)	Rat	LE	–	↓	Muhammad and Kolb (2011)
Short-term Social Preference Test	3 months	MS; 3-h/day (PND1–14)	Mouse	B6J	↓		Tsuda and Ogawa (2012)
Social Learning of Food Preference		AR (PND4–21)	Rat	SD	↓		Lévy et al. (2003)

–: no significant effect; ↑: significant increase; ↓: significant decrease.

included, among other things, a shift in day-night cycle and various hippocampus-dependent paradigms that could, in theory, have interfered with the present anatomical and neurogenesis 'end-stage' data.

Changes in proliferation and DG structure may further affect downstream processing within the hippocampus and alterations in neurogenesis have indeed been linked to cognitive changes (Clelland et al., 2009; Creer et al., 2010; Aimone et al., 2011; Sahay et al., 2011a,b; Oomen et al., 2014). Neurogenesis may be relevant for some, but not all forms of hippocampal-dependent learning and memory (Saxe et al., 2006; Zhang et al., 2008; Clelland et al., 2009; Kitamura et al., 2009; Oomen et al., 2014). Alternatively, structure of the amygdala was not studied now, but may be altered by MD and stress in females (Vyas et al., 2006; Krugers et al., 2012), which could be consistent with clinical literature showing excessive amygdala activation to fearful situations in depressed individuals (Abercrombie et al., 1998; Leppänen, 2006).

Lack of any effect of MD on the behavioral paradigms that we applied came somewhat as a surprise to us. Therefore, we carried out an extensive review of existing literature in several behavioral domains. Despite the large variation in ELS models, species, age of testing and outcome parameters, the majority of studies reports only mild behavioral changes in females after early-life adversity, i.e. two third of the experimental series in female rodents did not show a significant change in behavior after early-life adversity. Possibly this number of positive effects is lower than the actual situation, because the influence of the hormonal cycle – which could have added to variation in the behavioral outcome – is not always taken into account. On the other hand, more likely than not the prevalence of significant effects, at least in animal studies, is over-estimated due to a publication bias toward positive findings (Korevaar et al., 2011). We did not attempt to

make a full survey of all ELS studies in male rodents, but even considering the results in male rodents in this sample of studies, significant effects were more often encountered in males than females. Interestingly, the *difference* in behavioral phenotype between males and females was not very outspoken in anxiety-related behavioral measures, but clearly noticeable in social behavior and in non-stressful learning and memory tasks, including the ORT and OLT in which we presently also did not observe any effect of MD in females. Thus, less than 30% of all experiments in females showed a significant change in this domain after ELS, compared to 50% in males. Possibly, this stronger male phenotype also holds true for reward-based decision-making tasks, but the restricted number of papers on this type of task (Van den Bos et al., 2012) prohibits any conclusion at this moment.

Given that early-life adversity is a well-established risk factor for the development of psychopathology in humans (Kessler et al., 2010), and these are more prevalent in women (Steel et al., 2014), it is remarkable that two thirds of the reported endpoints in females and more than half in males in our review of rodent studies did not reveal any effects of quite severe conditions of early-life adversity. Noteworthy, the prevalence of female patients in several – but certainly not all (Reynolds et al., 2015) – psychiatric domains may be less prominent than generally thought, due to e.g. sociocultural acceptance patterns (Targum et al., 2013). The putative discrepancy between rodent and human studies can be explained in several ways. First, the observations in humans are often based on large case-control or general population samples, whereas animal studies usually involve more homogeneous but still small group sizes and may therefore lack the power to reach significance. One approach to mend this is by performing meta-analyses on the animal data. Secondly, it is possible that the developmental trajectories and circuits involved in the various behavioral domains in

rodents are dissimilar to those in humans, so that early-life adversity in rodents does not adequately model conditions in humans (Lupien et al., 2009). This may certainly play a role in the rIGT, which strongly depends on the function of mPFC areas, which are not in all cases equivalent between rodents and humans (Uylings et al., 2003). Thirdly, parenting conditions for humans and rodents – where in most models care is exclusively provided by the mothers – are very dissimilar.

Our own data and the overview of existing rodent literature reveal that particularly non-stressful (hippocampus-dependent) learning tasks are less vulnerable to early-life adversity in female than male subjects, which is interesting in the light of several recent studies in humans. On the one hand, objective measurement of spatial memory in human adults with a history of childhood trauma demonstrated improved performance in males as opposed to impaired function in females (Syal et al., 2014). However, another study showed that childhood emotional abuse is associated with reduced hippocampus volume in males, but not in females (Samplin et al., 2013). This study also showed that emotional abuse was associated with higher levels of subclinical psychopathology in both males and females. This finding in humans corroborates the overall outcome of our review that females may be more resilient to the structural/hippocampal effects but not to the anxiety-related psychiatric symptoms associated with childhood maltreatment.

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