Early life experience : neuroendocrine adaptations to maternal absence

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Effects of maternal deprivation on performance in the water maze and swim stress

Chapter 4

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4.1 Abstract

Rat pups subjected to a single 24 hours maternal deprivation show altered stress responsiveness and cognitive performance in the water maze at adulthood. Here we show in 6 months old male CD1 mice deprived of maternal care for 24 hours at postnatal day 8, an initial impairement in reversal learning: relocating the platform revealed perseverance in search for the former platform location. Spatial learning, long-term memory and swim-induced corticosterone responses were not affected. We conclude that reduced flexibility is a subtle long-lasting behavioural change induced by maternal deprivation.

4.2 Introduction

Traumatic early life events can program the susceptibility of the stress system for stressful experiences later in life and influence both behavioural and endocrine responses [18 - 20, 28, 33]. Therefore, they are considered risk factors for the development of mood disorders [2, 9]. To study the consequences of a disruption of postnatal development in rodents maternal deprivation paradigms are used. Maternal deprivation is the separation of mother and pups for a single period of 24 hours during the stress hypo-responsive period (SHRP). In rats, this paradigm resulted in long-lasting effects on cognition and endocrine responses to stressful stimuli [18 - 20, 28, 33].

For example, maternal deprivation of Brown Norway rats delayed acquisition of a spatial learning task until adulthood (at 3 and 12 months of age) and caused a higher degree of persistent behaviour. With increasing age, group differences in performance were gone at 32 months of age. However, the inter-individual differences in the senescent population increased. While control rats showed a normal Gausian distribution for learning, maternal deprivation resulted in an inverted U-shape distribution with more rats performing either very poor or very good [18].

Cognitive impairment as a consequence of aging is influenced by stress experienced throughout life. Though corticosteroids are essential for cognitive performance [3, 16], excessive corticosterone responses to stress have been associated with impaired cognitive performance in various learning tasks [3, 4, 21, 27]. Maternal deprivation results in both immediately increased basal ACTH and corticosterone [19, 28], but also in an age-dependent attenuated or hyper-responsive hypothalamic-pituitary-adrenal axis (HPA axis) [20, 33]. Thus, also a possibly affected endocrine responsiveness has to be taken into account when investigating the consequences of maternal deprivation on spatial learning.

Genetically manipulated mice are becoming more and more important subjects of research. To understand the effects of genetic manipulations and dissociate them from environmental contingencies, knowledge on normal development and the consequences of early life events are crucial. In mice the immediate short-term consequences of maternal deprivation generally show the same effects as observed for rats (for a comparison see [25]). In contrast, long-term effects of a single 24 hours maternal deprivation on cognition or endocrine responses to stress have to

our knowledge, as of yet, not been investigated in mice. Therefore, we studied the consequences of a single 24 hours maternal deprivation at postnatal day (pnd) 8 on both cognition and corticosterone responsiveness in CD1 mice at adulthood (6 months of age). Taking the overlap in immediate effects of maternal deprivation at pnd 8 in CD1 mice with those observed in rats into account [5, 25, 26, 30, 31] we hypothesised that the long-term consequences will also be comparable. We expected a delayed acquisition of spatial learning in the water maze and a hyper-responsive HPA axis to novelty exposure.

4.3 Materials and Methods

4.3.1 Animals

Male offspring of CD1 mice (obtained from Charles River, The Netherlands) was used. Four females were mated with one male in type 3 polycarbonate cages containing sawdust bedding and tissue to provide nest-building material; food (SRM-A, Hope Farms, The Netherlands) and water (containing 6% HCl) *ad libitum*; lights on from 7:00 to 19:00 hours in a temperature (21 ± 1°C) and humidity (55 ± 5%) controlled room. Pregnant females were individually transferred to new type 3 polycarbonate cages during the last week of gestation. Cages were controlled for litters daily between 9:00 and 9:30 hours. If litters were found, the day of birth was defined as postnatal day 0 (= pnd 0). On the day after parturition, pnd 1, litters were culled to 4 males and 4 females. One week before the endocrine response to swim stress or training in the water maze started, mice were housed individually in type 1 polycarbonate cages and weighed and handled daily.

Animal experiments were approved by the Local Committee for Animal Health, Ethics and Research of Leiden University and carried out in accordance with European Communities Council Directive 86/609/EEC.

4.3.2 Maternal deprivation and weaning

Mothers nursing litters selected for maternal deprivation were removed from their cage and placed in clean type 3 polycarbonate cages at 9:00 hours on pnd 8. The home cage containing the pups was placed in an adjacent room on a heating pad (30–33°C) to control for pup body temperature. Pups were not fed during the deprivation period. After 24 hours (at 9:00 hours on pnd 9), the mothers were reunited with their pups and left undisturbed. Control litters were left undisturbed. At weaning (pnd 28) the pups were placed in all male and all female groups with littermates from the same nest (n=4 per cage) and left undisturbed until testing.

4.3.3 Water maze schedule and procedure

At 6 months of age mice (n=10 per treatment) were tested in the water maze for their spatial learning abilities.

Handling of the animals: Particular attention was paid to handle the mice gently and quietly. Mice were picked up at the base of their tail and placed in the water maze. When search latencies

exceeded 60 seconds, a metal grid (5 x 20 cm) was used to guide the animals to the platform of the water maze and later to remove them from the platform. Upon presentation of the grid, animals climbed onto it and could easily be transported to their home cage. Any unwanted punishment for finding the platform or chasing the mouse through the pool was thus avoided.

Water maze procedure: A pool (white, diameter 140 cm) was filled with warm water (26 ± 1°C) and made opaque by the addition of chalk. A platform (8 cm in diameter) was situated 1 cm below the water surface, invisible for the animal (spatial condition). One free swim trial with no platform present was run before training. The mouse was placed in the middle of the pool and allowed to swim for 120 seconds. For training trials the pool was divided into 4 quadrants with the platform in the middle of one of the quadrants. For each trial, the mouse was placed in the water, with the head facing the wall, at one of four possible starting positions at an intersection between two quadrants. A maximum of 60 seconds was allowed, during which the mouse had to find the platform and climb onto it and remain there for 10 seconds. If the animal did not find the platform, it was guided there with a grid and allowed to stay for 10 seconds. Animals were tested sequentially with an inter-trial interval of approximately 5 minutes. After each trial, mice were placed individually in type 1 polycarbonate cages containing paper sheets under a red-light heating lamp to dry for approximately 3 minutes. Thereafter, mice were either placed in the water for another trial or back in their home cage.

Training schedule: Three days before spatial training in the water maze started, the pool was filled with approximately 2 cm of warm water (26 ± 1°C) and a large flat object to climb on. This was the mice's first contact with water and each mouse was allowed to move around for 120 seconds (water adaptation trial). Water maze training on day 1 started with a 120 seconds free swim in the absence of the platform (before training). This allowed estimation of the ability of the mice to swim and to determine the pre-training swim pattern, *i.e.* their exploratory strategy, indicative for any preferences for a certain part of the pool. Directly thereafter, spatial training with a submerged platform started. Mice performed 26 trials distributed over eight test days. Within a training day, inter-trial intervals were 5 minutes. After the second trial on day 4 (trial 12) the platform was moved to the opposite quadrant to test reversal learning abilities. Between the last trial on day 5 (trial 17) and the first trial on day 20 (trial 18) a two weeks break was introduced to test long-term memory.

For all training trials we assessed the time needed (seconds), speed (cm/s) and distance swum (cm) to find and climb on the platform. Thereafter, we calculated the mean performance of each mouse per day. Total traveled distance indicated the level of general activity. Behaviour was recorded on videotape and analysed by EthoVision 1.95 (Noldus Information & Technology BV, Wageningen, The Netherlands).

4.3.4 Swim stress, blood sampling and corticosterone measurement

At 6 months of age a separate group of male mice (n=10 per group) was tested for their stress responsiveness to swim stress. One day before the actual swim stress a basal blood sample was

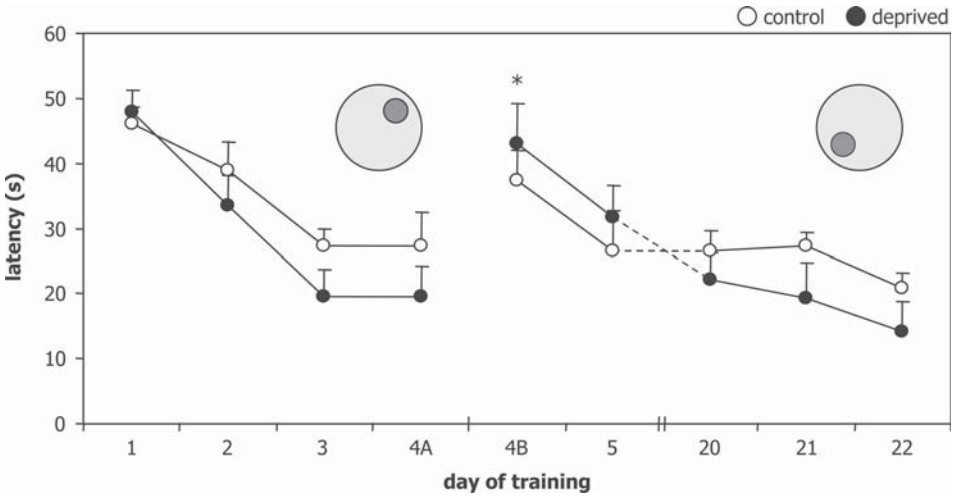

Figure 4.1
Performance of maternally deprived and control CD1 mice in the water maze, presented as the latency (in seconds) to reach the platform. Between 'day 4A' and 'day 4B' the platform position was reversed to the opposite quadrant from the position for 'day 4A'. The change in platform position is illustrated by the inset indicating a water maze with the respective platform positions. The dashed line (---) represents the two weeks delay in between 'day 5' and 'day 20'. Data represent mean ± S.E.M. of daily trials, * $P<0.05$ significant from 'day 4A' within the same group.

collected via tail incision [6, 7]: a small incision at the base of the tail with a razor blade allowed collection of a 50-100 µl blood sample within 90 seconds after opening of the animal's cage. At the day of testing, the mouse was released in the middle of the pool used for water maze testing. After one minute, the mouse was removed from the water using a grid and placed in its home cage under a red-light heating lamp for another three minutes. At 15, 30, 60, 90 and 180 minutes after the mouse was placed in the water maze a blood sample was collected.

Blood plasma was collected individually in potassium-EDTA coated 10 ml tubes (1.6 mg EDTA/ml blood; Sarstedt, Germany). All samples were kept on ice and later centrifuged at 13000 rpm for 15 minutes at 4°C. Blood plasma was transferred to Eppendorf tubes for corticosterone determination and stored at –20°C until further analysis. Corticosterone was measured using a commercially available radio immunoassay (RIA) kit containing ^{125}Iodine labelled corticosterone (MP Biomedicals INC., CA, USA). Vials were counted for 2 minutes in a gamma-scintillation counter (Packard Minaxi Gamma counter, Series 5000).

4.3.5 Statistical analysis

Performance in the water maze and corticosterone responses to stress were analysed using a general linear model. TREATMENT (control, deprived) was the between subjects factor. The within subjects factor was DAY for water maze performance and TIME (minutes) for the response to swim stress. Comparisons at different time points were tested *post hoc* with independent samples t-tests (between groups), or Wilcoxon paired samples tests (between days). Statistical significance was accepted at $P<0.05$. The performance of the water maze is presented in average performance per day. All data are presented as mean ± S.E.M.

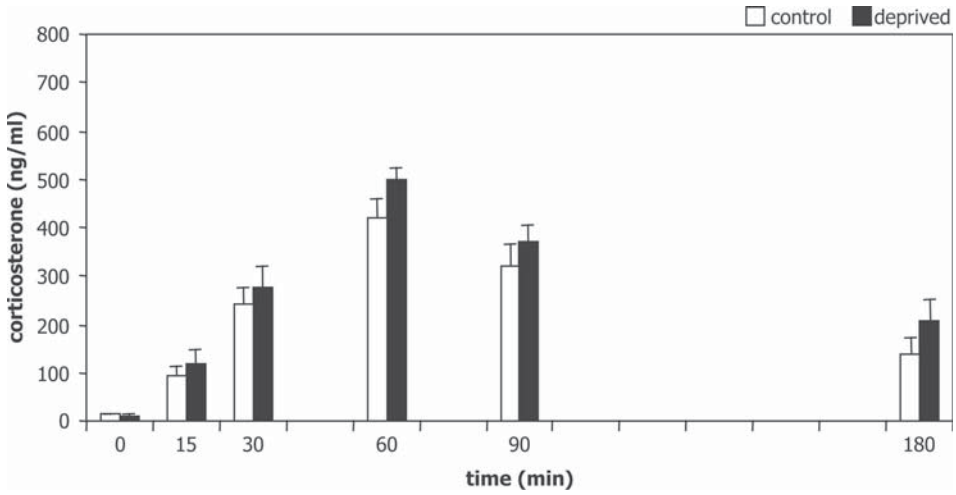

Figure 4.2
Basal and swim stress-induced plasma corticosterone levels (ng/ml) in control and deprived CD1 mice. Data represent mean ± S.E.M.

4.4 Results

4.4.1 Water maze performance (***Figure 4.1***)

In the free swim trial before training, mice of either treatment group showed a randomly distributed exploration pattern of the pool (data not shown). Distances swum were comparable as well (distance (cm): control 1333.76 ± 47.50; deprived 1250.77 ± 67.58). Latency to locate the platform indicated that all mice learned the task (days 1 to 4A; DAY: $F(3,51)17.04$, $P<0.001$), reflected by the decreasing time swum. This performance was not influenced by maternal deprivation. When on the second half of day 4 the platform was moved to the opposite quadrant (*i.e.* day 4B), it took deprived mice significantly longer ($P<0.05$) than controls to find this new position. With further training all mice learned the new position (days 4B to 22; DAY: $F(4,68)11.25$, $P<0.001$). A two weeks break of the training schedule (between days 5 and 20) did not affect the performance.

With increasing numbers of trials mice swum faster (days 1 to 4A; DAY: $F(3,54)6.15$, $P<0.001$). At day 4 mice had reached their maximum speed, which was neither affected by the reversal trial nor by the two weeks break ($P>0.05$; mean velocity (cm/s) at pnd 4B: control 23.18 ± 0.78, deprived 23.04 ± 1.23; at pnd 22: control 23.69 ± 1.31, deprived 21.80 ± 1.43). Though the first four test days the swim speed increased, the decreasing distance traveled indicated that mice were more goal-directed (days 1 to 4A; DAY: $F(3,51)9.84$, $P<0.001$). This was even more prominent during the reversal training (days 4B to 22; DAY: $F(4,68)9.96$, $P<0.001$). When the platform position was switched during the reversal trial deprived mice swum longer distances ($P<0.05$) than control animals ($P=0.44$).

4.4.2 Swim stress (***Figure 4.2***)

The basal corticosterone concentrations were comparable between deprived and control mice. All mice responded similarly to swim stress: with an increase in corticosterone secretion

(TIME: F(5,90)65.25, $P<0.001$). After 3 hours corticosterone had returned to basal levels with no differences between the groups.

4.5 Discussion

CD1 mice readily learned this spatial navigation task, indicated by the decreased latency and distance swam to locate the submerged platform. Maternal deprivation did not influence the acquisition of this task.

Maternally deprived and control mice showed similar learning curves for latency and distance swum. Thus, unlike maternally deprived rats [18], maternally deprived mice did not show a delayed acquisition of the water maze. Interesting is the swim pattern of deprived mice when the platform was relocated opposite to the trained position. In this reversal trial, mice returned persistently to the trained platform position before searching for an escape option elsewhere. They took longer latencies and swam longer distances to find the new platform position, which is in accordance with rat data at 12 months of age [18]. Also in these 6 months old mice we observed a less flexible behavioural pattern. It is the first response to the altered environmental condition, as the learning curve for this new position and the long-term memory established by continuing training after a two weeks break were not affected. We might consider this persistent behaviour adaptive, as long as environmental conditions remain stable.

Corticosteroids facilitate as well as impair cognitive performance [3, 11, 16]. While a context-dependent increase of corticosterone is mainly related to facilitation of learning and memory, long-lasting elevations of corticosterone and periods of stress have rather been associated with impaired cognitive performance [4, 21, 27]. Here, corticosterone responses to swimming were comparably high in maternally deprived and control mice. It is most likely that this task-related increased corticosterone potentiated the consolidation of information and resulted in comparable learning curves and long-term memory in both groups.

Comparing the behavioural and endocrine data of maternally deprived rats and mice, we have to face basic methodological differences between the rat and mouse studies. Factors like species-dependent postnatal period of the SHRP, age of deprivation, age of testing and received maternal care after the deprivation period are likely to affect the outcome. First, independent of the species and postnatal day, the immediate effects of maternal deprivation are very similar. However, long-lasting effects seen in pituitary-adrenal responses to stress, emotionality and cognition that were observed weeks to months later were different. Rats deprived early (pnd 3) or late (pnd 11) during the SHRP showed either a hyper- or hypo-responsive ACTH response to stress at weaning (pnd 20) [29, 30]. In mice, an early (pnd 3) or late (pnd 8) deprivation resulted in a prolonged or unaffected corticosterone response at weaning (pnd 28), respectively [***Chapter 5***]. Avoidance learning in rats was reduced by maternal deprivation at pnd 4, whereas deprivation at pnd 9 (in the middle of the rat SHRP [13, 22, 34]) resulted in enhanced active avoidance and water maze learning [12]. Although the immediate effects of maternal deprivation are comparable

between rats and mice [25], nothing is known about the long-term consequences in mice. One might speculate that, since maternal deprivation at pnd 8 is at the end of the mouse SHRP [23], long-term effects might be less pronounced than expected for a deprivation earlier in the SHRP due to a more developed central nervous system at the time of deprivation.

Secondly, the age to evaluate the deprivation effects determines the observed outcome, at least in rats. For example, 12 months old rats maternally deprived at pnd 3 were hyper-responsive to novelty stress compared to non-deprived littermates, whereas both 3 months old and 30-32 months old rats were hypo-responsive [33]. If these age-dependent responses also hold true for mice we, in our 6 months old mice, might have been testing at the transition point from a hypo- to a hyper-responsive HPA axis, or *vice versa*, from a hyper- to a hyporesponsive HPA axis. Maternal deprivation induced impairment in acquisition of the water maze as observed in 3 and 12 months old rats was not observed anymore in 30-32 months old rats. In the senescent rat, we deal with an age-dependent cognitive decline together with a possible influence of the early life event [18]. However, in a larger subgroup of the maternally deprived 30-32 months old Brown Norway rat we detected a more severe impairment of cognitive function. The finding that mice, like rats, showed more behavioural persistence, thus a decreased flexibility to adapt to a changed environment might be of relevance for cognitive processes of learning and memory. We may argue that increased persistence compensates for a learning deficit. This is what we see in our 6 months old mice. Only examining the effects of maternal deprivation in different age groups will provide an answer to a generalisation of the effects of adverse early life event over species.

Finally, maternal care behaviour expressed by a different degree of licking and grooming of the pups, changes the behavioural and endocrine phenotype of the offspring in later life [8, 10, 17]. It has been shown in rats that early handling intensifies [14] and prenatal stress attenuates maternal care towards pups [1, 15, 32]. In the studies of Oitzl *et al.*, our main comparison regarding rat data, a split-litter deprivation design was used, leaving the hormonal state of the dam and maternal care behaviour undisturbed [18]. In the current mouse study we used a full-litter deprivation. A prolonged separation of the rat dam with her complete litter was shown to evoke compensatory maternal care upon reunion [15]. This deprivation-induced maternal care most likely did not occur in the split-litter deprivation, resulting in more detrimental and longer-lasting effects observed on pituitary-adrenal responsiveness and cognitive performance in the water maze. An alternative explanation lies in the age at deprivation in relation to maternal care. Licking and grooming of high- and low-care rat mothers differs in the first postnatal week, but is comparable four days before the end of the SHRP [14]. Mice were deprived at pnd 8, about four days before the end of the mouse SHRP [24] which makes it likely that maternal care again increased upon reunion.

Summarising, a single 24 hours of maternal deprivation at pnd 8 in mice indeed affected, like in rats, certain aspects of cognitive performance: the flexibility to adapt to a changed environment. However, main effects on learning and long-term memory were not observed. Corticosterone responses to swimming were strong and comparable in maternally deprived and

control mice and might have contributed to the fast acquisition of the spatial learning task. In rats, age-dependent non-linear dynamic changes in cognition and endocrinology were reported. Our study in mice showed that, like in rats, long-term effects most likely depend on the age when maternal deprivation is applied [29, 30, ***Chapter 5***] and on the age of examination in later life [18, 33].

4.6 Acknowledgements

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