CHAPTER 5

Early handling modulates outcome of neonatal glucocorticoid exposure

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ABSTRACT

Synthetic glucocorticoids are frequently used to prevent respiratory disorders in prematurely born infants. Besides the short-term benefit on lung function, numerous human and animal follow-up studies have reported adverse neurodevelopmental side effects. In contrast to these reports we recently showed a relatively mild outcome after neonatal dexamethasone treatment using a rat model. The aim of the current study was to investigate whether neonatal handling, which was an inevitable component of our experimental design, might serve as an intervention strategy modulating the adverse effects of dexamethasone treatment.

Rat pups were injected with dexamethasone or saline on postnatal days 1, 2 and 3, and additionally daily handled or left undisturbed until postnatal day 21. Maternal care was observed during the first week of life and was enhanced in response to handling. Eye opening was accelerated and body weight reduced in dexamethasone treated animals. In adulthood, we report that although dexamethasone treatment and handling yielded comparable effects on stress-induced CORT response and startle reactivity, acquisition of fear was only affected by handling. Dexamethasone treatment reduced the sensitivity for beneficial effects of handling on pre-pulse inhibition. Non-handled animals appeared more susceptible to the impact of dexamethasone treatment compared to handled animals, as was demonstrated for spatial learning in the water maze. Moreover, dexamethasone treatment only impaired spatial orientation in the T-maze in non-handled animals.

These findings emphasize that the outcome of neonatal glucocorticoid exposure is not deterministic and strongly interacts with other components of the postnatal environment.

INTRODUCTION

Synthetic glucocorticoids such as dexamethasone (DEX) are frequently used to enhance lung function in prematurely born infants. Although some studies indeed showed beneficial effects of glucocorticoid treatment (1) leading to a decreased incidence and severity of bronchopulmonary dysplasia, others however failed to do so or showed only modest effects (2). Moreover, follow-up studies of prematurely born infants treated with glucocorticoids have shown pervasive adverse neurodevelopmental effects (3, 4). Randomized placebo-controlled trials reported that glucocorticoid treatment led to an increased incidence of neurodevelopmental impairment (3), and resulted in poor motor skills as well as lower IQ scores compared to untreated controls (5). Therefore there has been growing concern whether the short-term benefits of glucocorticoid treatment outweigh the adverse side effects leading to neurodevelopmental impairment (6).

In line with evidence from human studies, rodent studies demonstrated that perinatal glucocorticoid treatment resulted in long-lasting alterations in cognitive performance and hippocampal function (7-10), stress responsiveness (11, 12), social behaviour (13) and to a significantly shortened lifespan (10, 14). Previous rodent studies in our laboratory did show developmental alterations after neonatal DEX treatment in terms of brain development, body weight and eye opening. However, the long term consequences for behavioural phenotype were relatively mild and in some cases even beneficial (chapter 4 of this thesis).

Interestingly, our experimental design consisted, besides manipulations necessary for drug treatment, of a substantial amount of neonatal handling during the full postnatal period. This was the result of daily weighing and marking for discrimination between individual pups receiving different treatments according to a within-litter design. Other studies investigating the long-term effects of neonatal DEX-treatment have used either a between-litter design (14) or a within-litter design with use of another type of marking (15) reducing the amount of daily handling.

It is well known that daily handling during the postnatal period attenuates stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis (16-19), reduces emotionality (18), and enhances cognitive performance (20). Moreover, we have previously reported the long-lasting impact of neonatal handling - inherent to the dexamethasone-treatment design - throughout the lifespan, suggesting an interaction with the effects of DEX treatment (chapter 4 of this thesis). Therefore, it is hypothesized that the effects of handling might have potentially compensated for certain DEX-induced alterations. To investigate whether handling of the neonate can indeed reverse neonatal DEX-induced alterations in adult phenotype we have studied the effects of glucocorticoid treatment in a handling vs a non handling context.

MATERIAL AND METHODS

Animals

Adult female and male Long Evans rats from our breeding population (originally obtained from Janvier, France) were used as breeders. Two females were mated with one male for 10 days in type IV polycarbonate cages (59x38x20cm) containing sawdust bedding and tissues. Food (RM3, Special Diet Services, Witham, Essex, UK) and water (8 ml 25% HCl /10 L tap water) were provided *ad libitum*. Animals were maintained on a 11-h light : 13-h dark cycle with lights on at 08.30h, in a temperature (21± 2°C) and humidity (55 ± 5%) controlled room. After breeding, pregnant females were individually housed. Females were checked daily for presence of pups. If pups were present, the day of birth for that particular litter was defined as postnatal day 0 (pnd 0). On pnd 1, litters were culled to 8 pups (4 male and 4 female) and randomly assigned to the Handling (H) or Non Handling (NH) group. Cages were cleaned once on pnd 10 and after weaning once weekly. After weaning (pnd 22) animals were group housed with same sex littermates. 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).

Drug Treatment

Male pups were randomly assigned to either the saline (SAL) or the dexamethasone (DEX) group using a within litter design. Pups in the DEX group were subcutaneously (SC) injected with dexamethasone-21-phosphate (Sigma Aldrich, Zwijndrecht, The Netherlands) on pnd 1 (0,5 µg/g body weight), pnd 2 (0,3 µg/g) and pnd 3 (0,1 µg/g). Pups in the SAL groups were injected with equivalent volumes of sterile and pyrogen free saline (SAL). In order to prevent bias for the dam to show enhanced attention for injected or non-injected offspring, all female littermates were injected with SAL on pnd 1, 2 and 3. Pups were marked for identification with a toe clip on pnd 1. Drug treatment always took place between 9:00 and 11:00.

Handling

From pnd 1-21 H pups underwent daily 15 min separations from the dam. At the onset of each separation, pups were removed altogether from the home cage, and placed in a type IIL polycarbonate cage (36,5x20,5x14cm) that was brought to an adjacent room, where it was placed on a heating pad. Following 15 min of separation, all pups were returned to the housing room and reunited with the dam in the home cage. Handling always took place between 12:00 and 13:00. NH pups were left undisturbed from pnd 1-21 except for a cage change on pnd 10.

Maternal Care

The maternal behaviour of each dam was observed and scored for five 60 minute periods per day during the first 7 days postpartum using a procedure as described by Champagne *et al* (2003) (21). Observations were performed at three periods

during the light phase (13:00, 15.30, 18:00) and two periods during the dark phase (07:00 and 20:00) of the light cycle. Within each observation period the behaviour of each mother was scored every 3 minutes (20 observations per period, 100 observations per day, 700 observations for the first 7 days postpartum). The following maternal behaviours were scored: a) Licking and grooming (LG) of the pups, b) Arched back nursing/blanket nursing/passive nursing, c) Mother away from nest/no maternal contact. The data were analyzed as the percentage of observations in which dams displayed one of the behaviours described above.

Postnatal development

Postnatal development (body weight and eye opening) was monitored in H pups during H procedure. For eye opening a scale from 0-2 was used indicating: 0 = closed, 1 = partial opening, 2 = fully open (adapted from Flagel, Vazquez, 2001).

Adult Phenotype

Animals were tested according to the schedule depicted in figure 1.

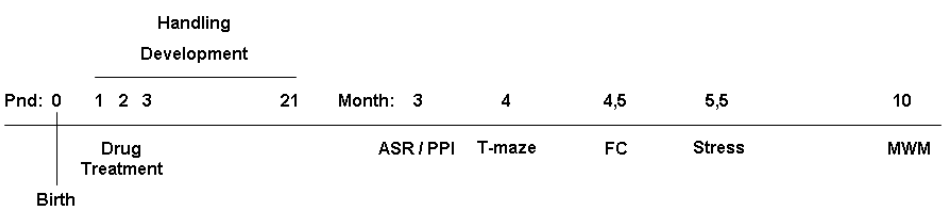

Fig 1. Timeline experiments. Pnd: postnatal day; ASR: acoustic startle reactivity; PPI: prepulse inhibition; FC: fear conditioning; Stress: endocrine response to restraint stress; MWM: morris water maze.

Acoustic Startle Reactivity and Prepulse Inhibition

Prepulse inhibition was measured at 3 months of age. Animals were brought to the testing room and allowed to habituate for 45 min. After habituation they were placed in a startle recording apparatus (SR-LAB, San Diego Instruments, CA, USA) containing a transparent Plexiglas tube (diameter 8.7 cm, length 20.5 cm) mounted on a Plexiglas base. Sounds were presented by a speaker and movement of the animals was detected by a piezoelectric accelerometer mounted below the Plexiglas tube and recorded by a computer. Testing started with a 5 min habituation session with background white noise of 70 dB[A]. Animals were first presented with six pulse alone trials (117 dB[A]) followed by 39 trials comprising different trial types according to a pseudo-randomized schedule with an inter-trial interval of 10-20 sec. Trial types: background white noise alone, prepulse alone using intensities of 2, 4, 8, 16 dB[A] above background noise (i.e. 72, 74, 78 and 86 dB[A]), pulse alone (117 dB[A]) or a combination of one of the four prepulses plus pulse. Finally, animals were again exposed to five pulse alone trials. The

duration of the prepulses was 20 ms, duration of the pulses was 40 ms. Prepulse to pulse interval was 100 ms. Startle activity was measured during 100 ms after onset of the pulse. The percentage of PPI at the different prepulse intensities was calculated as [100-(100 x startle amplitude at prepulse trial)/ (startle amplitude at startle pulse-alone trial)]. Speakers were calibrated every day, and boxes were cleaned with a 10% ethanol solution after every session to eliminate odour cues. Experiments were performed between 09:00 and 13:00 to minimize circadian influence.

T-maze

Spatial orientation in the T-maze was investigated at 4 months of age. The T-maze consisted of three unequally sized arms made of Plexiglas. The length of the start arm was 75 cm, whereas the other arms were both 32 cm in length. The width and height of the arms were 12 and 20 cm respectively. During training, rats were placed in the start arm facing the outer wall and were allowed to explore the start arm and one of the two other arms for 10 minutes. Half of the animals were allowed to visit the left and the other half the right arm to reduce the influence of a preference of the rats for one side. After a delay of 2.5 hours, rats were placed back in the T-maze and were allowed to explore all three arms for 5 minutes. The duration and frequency of visiting the familiar or new arm during re-exposure were measured using EthoVision XT (Noldus Information Technology, Wageningen, The Netherlands). T-maze experiments were performed between 09:00 and 13:30 to minimize circadian influence. The experimental apparatus was cleaned with a 10% ethanol solution between all sessions to eliminate odour cues. To reduce the effect of stress due to testing order within the cage, one animal per litter was tested per day.

Contextual Fear Conditioning

Contextual fear memory was assessed at 4,5 months of age. The shock box (40x40x50 cm) consisted of black Plexiglas walls and a stainless steel rod floor, connected to a shock generator. On day 1 the animal was transferred from the housing room to the adjacent test room where it was placed in the shock box. After a 2 min delay it was exposed to a foot shock of 0.6 mA (duration 2 sec). After the shock the animal remained in the shock box for 2 minutes, after which it was removed from the box and transferred back to the home cage.

24 hours later (day 2), the animal was re-exposed to the shock box for 4 min, however without receiving a foot shock. An overhead video camera recorded behaviour of the animals throughout all sessions. Behaviour was analyzed by an observer unaware of treatment conditions using The Observer 9.0 XT (Noldus Information Technology, Wageningen, The Netherlands). The following behaviours were scored (1) freezing (lack of all body movement except those necessary for breathing), (2) scanning (lack of movements, except lateral head movements and movements necessary for breathing), (3) rearing, (4) walking, and (5) sitting. Behaviour was analyzed during 3 distinct time periods: (1) 2 min before

shock, (2) 2 min after shock, (3) 4 min re-exposure. All testing took place between 10:00 and 13:00 to minimize circadian influence. The shock box was cleaned with a 10% ethanol solution between all sessions to eliminate odour cues. To reduce the effect of stress due to testing order within the cage, one animal per litter was tested per day.

Endocrine response to restraint stress

At 5,5 months of age, endocrine response to an acute restraint stressor was tested. One day prior to restraint stress a basal blood sample was taken from the tail vein. The next day animals were exposed to 10 min restraint stress by placing them in a custom made restrainer restricting body movement. Blood samples were taken at 2, 5, 10, 30, 60, and 120 min.

Hormone Analysis

Blood samples were collected in EDTA coated tubes (Microvette CB 300 K2E, Sarstedt, Germany). Samples were kept on ice and centrifuged for 15 min at 13000 rpm at 4°C. Plasma was transferred to Eppendorf tubes and stored at -20°C until further analysis. Plasma and corticosterone (CORT) concentration was measured using a commercially available radio immuno assay (RIA) kit containing ^{125}Iodine labelled CORT (MP Biomedicals Inc., USA). All samples were processed in the same assay to exclude inter-assay variability.

Water maze performance

At 10 months of age spatial learning in a water maze was tested. Animals were put, without prior training in a pool (150 cm diameter) filled with 30 cm of water (21°C) made opaque by adding 3 spoons of latex paint. A platform (10 cm diameter) was hidden 1 cm below the surface of the water and was positioned in the NE quadrant. Animals were given 3 daily trials (inter-trial interval 15 min) to find the platform on day 1, 2 and 3. On day 4, two more training trials were given, followed by a probe trial without platform present. On day 5, animals were given 3 reversal trials with the platform located in the position opposite (SW quadrant) from where it was during training. Trials always started in one of the 3 quadrants where the platform was not located in a pseudo-randomized fashion, following the same order of start position for every animal. If an animal did not reach the platform within 2 minutes, it was gently guided there by the experimenter. Animals were allowed to stay on the platform for 15 sec after finding it or being guided there. Swim patterns were tracked by an overhead camera and later analysed using EthoVision XT (Noldus Information Technology, Wageningen, The Netherlands). Latency and distance to platform as well as latency, frequency and duration in target and other quadrants, or in close proximity to the platform (or the location where the platform in previous trials) were analyzed during training and reversal trials, as well as swim patterns during probe trials. After every swim trial, animals were dried with a towel and kept in a clean cage lined with clean dry tissue paper

that was placed on a heating pad. All trials took place between 10:00 and 13:00 to minimize circadian influence.

Data analysis

Statistical analysis was performed using SPSS 17.0. Data are presented as mean +/- SEM. Data were analysed using two-way repeated measures ANOVA with time (age, trial, day or min) as within and drug treatment and handling as between subject factors, one-way ANOVA to compare H to NH animals (in case of maternal LG data), or two-way ANOVA to measure a main effect of drug treatment (SAL, DEX) and handling (NH, H), or an interaction between drug treatment and handling. If an interaction was observed, post-hoc pair-wise comparisons were performed. For the T-maze experiments, differences within each group were tested using a paired t-test. Level of significance was set at $p < .05$. Where needed corrections for multiple testing and violation of sphericity were conducted. Graphs were made using GraphPad Prism®.

RESULTS

Maternal care

H pups received significantly higher levels of maternal LG compared to NH pups directly following exposure to the handling procedure ($F(1,39) = 12.72$, $p = .001$, fig 2A). However, overall level of LG did not differ significantly between NH and H litters (fig 2B).

Body weight and postnatal development

DEX treated animals weighted significantly less throughout the postnatal period ($F(1,20) = 90.25$, $p < .001$, fig 3A). For obvious reasons this was monitored only in H animals. During the post-weaning period (fig 3B), NH animals tended to

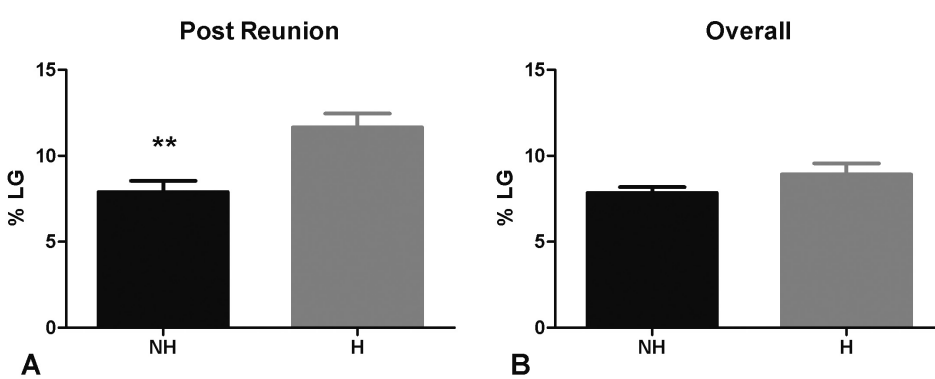

Fig 2. Maternal licking and grooming (LG) directly following the handling procedure (A) and overall (B). Displayed are mean +/- SEM. ** $p < .01$

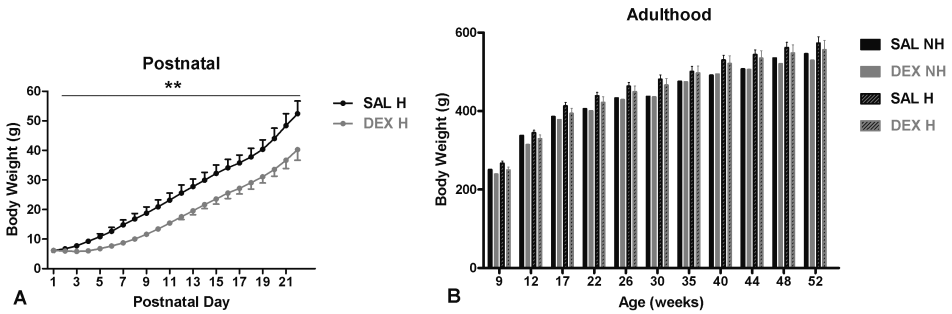

Fig 3. DEX treatment significantly reduced body weight throughout the postnatal period (A). In adulthood, no effect of DEX treatment was observed. H however tended (ns) to result in higher body weight (B). Data represent mean ± SEM. ** p < .01

have a lower body weight than H animals, although this effect was not statistically significant (F(1,32) = 4.12, p = .051).

For eye opening we observed a significant main effect of drug treatment (F(1,19) = 40.66, p < .001, fig 4) indicating that DEX treated animals show an acceleration in eye opening. For obvious reasons this was monitored only in H animals. We also observed a significant time x drug treatment interaction (F(20,380) = 17.29, p < .001). Post-hoc analysis per time point revealed that DEX treated animals showed enhanced levels of eye opening compared to SAL treated animals on pnd 8 (p = .019), 9, 10, 11, 12 (p < .01).

Adult phenotype

Acoustic Startle Reactivity

Acoustic startle reactivity (ASR) to the first five pulses revealed a main effect of handling (F(1,37) = 9.047, p = .005) and a drug treatment x handling interaction (F(1,37) = 13,497, p < .001; fig 5A, black bars). Post-hoc pair-wise comparisons revealed higher ASR in SAL NH animals compared to SAL H (p = .011) and DEX NH (p = .023), indicating that both H and DEX treatment reduced initial startle reactivity. No main effect of handling, drug treatment or an interaction effect was

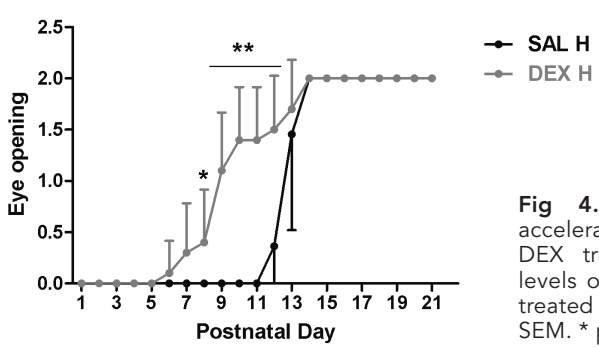

Fig 4. DEX treatment resulted in accelerated eye opening, on pnd 8-12 DEX treated animals show enhanced levels of eye opening compared to SAL treated animals. Data represent mean ± SEM. * p < .05, ** p < .01

observed for the last five pulses where all groups showed similar startle reactivity (fig 5A, white bars).

Habituation of ASR was calculated based on the difference in ASR to the first and the last five pulses. Main effects for handling ($F(1,33) = 8.478$, $p = .006$) and drug treatment ($F(1,33) = 10.901$, $p = .002$) were observed, indicating that both H and DEX treatment reduced habituation of the acoustic startle response (fig 5B), an effect that appears to be mostly driven by the high initial ASR in SAL NH animals.

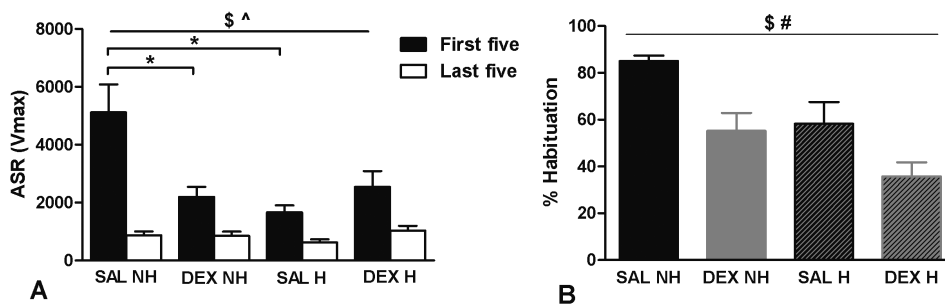

Fig 5. Acoustic startle reactivity (ASR) to the first five (black bars) and last five (white bars) pulses (A) and habituation of ASR (B). Data represent mean ± SEM. $ main effect of handling $p < .01$, # main effect of drug treatment $p < .01$, ^ drug treatment x handling interaction $p < .01$. Results of post-hoc pair-wise comparisons: * $p < 0.05$.

Prepulse Inhibition (PPI)

Analysis of PPI data showed a significant drug treatment x handling interaction ($F(1,35) = 4.81$, $p = .035$, fig 6) for PP2 (prepulse 2 dB[A] above background). Post-hoc pair-wise comparisons revealed significantly lower PPI in SAL NH animals as compared to SAL H animals ($p = .021$) indicating that H enhanced PPI in SAL treated but did not affect DEX treated animals. We did not observe main effects of drug treatment or handling on PPI. Effects were comparable for PP4 (4 dB[A] above background), however post-hoc testing showed a trend towards significance. No effects of DEX treatment or H were observed for PP8 and PP16.

Spatial orientation in the T-maze

Since exploratory behaviour was reduced after the first minute of re-exposure to the T-maze, we focused on behaviour during the first minute. All animals, except DEX NH, showed the expected preference for the new versus the familiar arm of the T-maze, indicating that this DEX-induced effect on hippocampal performance can be reversed by handling. Paired T-tests showed that all groups except for DEX NH spent significantly more time in the new compared to the familiar arm (SAL NH: $p = .028$, SAL H: $p = .002$, DEX H: $p < .001$, fig 7).

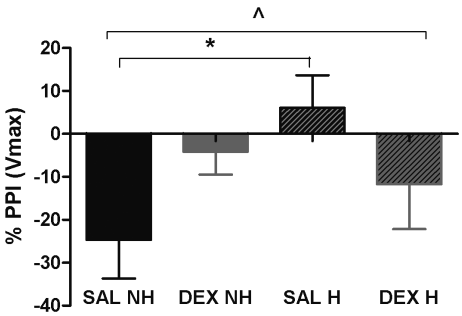

Fig 6. Prepulse inhibition for PP2 (2 dB[A] above background). Handling significantly enhanced PPI in SAL treated animals without affecting DEX treated animals. Data represent mean ± SEM. ^ drug treatment x handling interaction $p < .05$. Results of post-hoc pair-wise comparisons: * $p < .05$.

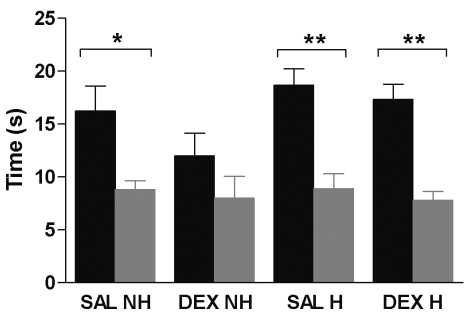

Fig 7. Spatial orientation in the T-Maze. All groups except DEX NH spent significantly more time in the new compared to the familiar arm during re-exposure to T-maze. Data represent mean ± SEM. * $p < .05$, ** $p < .01$.

Contextual Fear Conditioning

No significant differences in behaviour were observed between groups prior to shock administration**.** Directly after shock exposure, a main effect of handling was observed for freezing ($F(1,36) = 14{,}731$, $p < .001$, fig 8A) indicating that H animals showed less freezing compared to NH animals. During re-exposure (24h later), a main effect of handling was observed for freezing ($F(1,36) = 8.892$, $p = .005$, fig 8B) indicating that again, H animals showed less freezing compared to NH animals. Three animals did not receive a shock and were therefore excluded from the analysis.

Endocrine stress responsiveness

Repeated measures ANOVA revealed a main effect of time ($F(2.39, 69.20) = 184.73$, $p < .001$) and a time x drug treatment x handling interaction ($F(2.39,69.20) = 4.56$, $p = .010$). Post-hoc analysis per time point indicated at t = 120 min a significant drug treatment x handling interaction ($F(3,42) = 3.55$, $p = .023$). At t = 120 SAL NH animals showed significantly higher CORT levels compared to DEX NH ($p = .005$) and SAL H ($p = .009$). The difference compared to DEX H was not statistically significant ($p = .071$). This indicates that both handling and DEX treatment resulted in enhanced negative feedback of the HPA axis in response to acute restraint stress (fig 9).

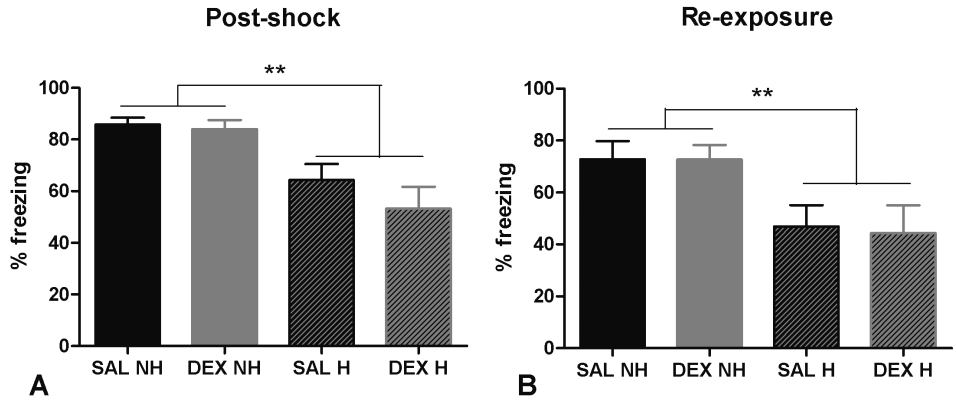

Fig 8. Freezing behaviour directly following shock exposure (A) and during re-exposure to shock context (B). Data represent mean ± SEM. Handled animals display significantly lower levels of freezing both directly after shock exposure and during re-exposure to the shock context. **: main effect of handling, $p < .01$

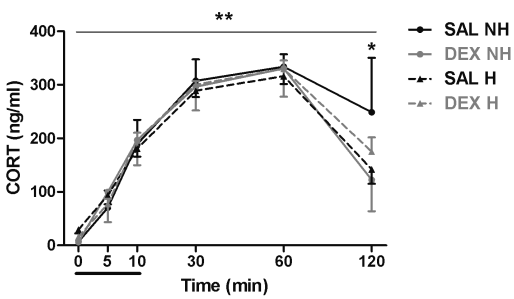

Fig 9. Corticosterone (CORT) levels before, during and following exposure to 10 min restraint stress (indicated with horizontal black bar). Both handling and DEX treatment result in enhanced negative feedback of the HPA axis at t = 120 min. Data represent mean ± SEM. ** time x drug treatment x handling interaction $p = .01$, * drug treatment x handling interaction at t = 120, $p < .05$

Spatial learning in the Morris Water Maze

Repeated measures ANOVA of spatial learning revealed a main effect of time ($F(2.09,77.35) = 127.62$, $p < .001$) and a main effect of drug treatment ($F(1,37) = 5.41$, $p = .026$), indicating that SAL treated animals show overall a longer latency to find the platform compared to DEX treated animals during spatial learning on days 1 to 4. There was a trend towards a drug treatment x handling interaction ($F(1,37) = 4.07$, $p = .051$). Post-hoc analysis showed that overall SAL NH animals need more time to find the platform compared to DEX NH ($p = .026$), an effect that is mostly driven by performance on day 1 (fig 10A). When day 1 is analysed into more detail (per trial, see fig 10B) we observed a main effect of time ($F(2,74) = 26.71$, $p < .001$), and drug treatment ($F(1,37) = 8.74$, $p = .005$) and a drug treatment x handling interaction ($F(1,37) = 6.80$, $p = .013$). Post-hoc analysis shows that SAL NH animals need significantly more time to find the platform compared to DEX NH animals ($p = .003$) whereas the other groups do not differ significantly. This indicates that DEX treatment affects mostly NH animals. However, although

DEX NH animals are significantly faster compared to SAL NH, the slope of the learning curves is highly comparable.

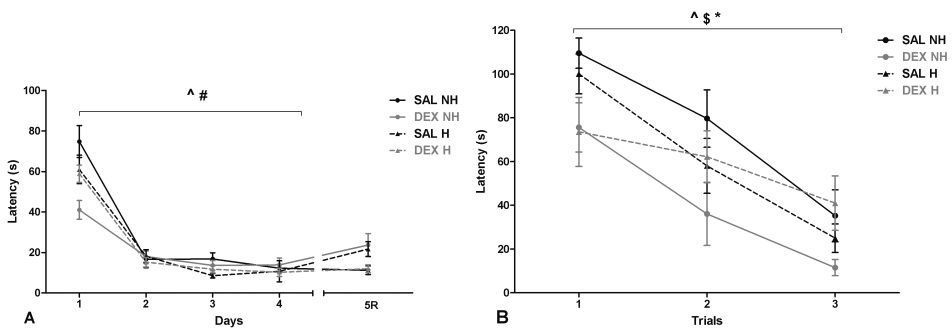

Fig 10. Spatial learning in the water maze. Overall, DEX treated animals had a shorter latency to find the platform compared to SAL treated animals. This effect was mostly driven by a difference between SAL NH and DEX NH on training day 1 (A). When day 1 was analysed per trial we observed that DEX treatment affected NH animals, without affecting H animals. DEX NH animals are faster on all trials, including trial 1 (B). ^ main effect of time $p < .01$, # main effect of drug treatment $p < .05$, $ main effect of drug treatment $p < .01$ * drug treatment x handling interaction $p < .05$.

DISCUSSION

The aim of this study was to investigate the impact of neonatal glucocorticoid treatment in an H vs NH context, in order to answer the question if H compensates for the effects of neonatal DEX treatment. We observed DEX-induced developmental alterations, in terms of reduced body weight and accelerated eye opening, which were comparable to previous findings from our laboratory. For adult and middle aged behavioural and endocrine phenotype we observed that the outcome was determined by various interactions between neonatal DEX treatment and H.

Effects of dexamethasone treatment and handling work in same direction

For some characteristics of the animal's phenotype the effects of DEX treatment and H point in the same direction. SAL NH animals show an extremely high initial ASR which is substantially lower in both DEX-treated and H animals. Interestingly, startle reactivity in SAL NH animals is reduced to levels comparable to the other groups towards the end of the startle protocol, resulting in a high degree of habituation in these animals. All groups show startle habituation, but since initial startle was lower in DEX-treated and H animals, the degree of habituation is also lower in these groups. Overall, both DEX and H appear to reduce acoustic startle reactivity. A similar phenomenon has been shown in previous studies from our group (Claessens et al, unpublished), however different control groups were used. Others have also reported that H animals show reduced ASR compared to NH individuals (22). The effects of neonatal glucocorticoid treatment on ASR have not

been frequently studied, but Ferguson and colleagues showed no effect of DEX treatment on postnatal day 7 on adult ASR (23).

The impact of DEX treatment and H on CORT responsiveness follows a comparable pattern: both DEX treatment and H enhance negative feedback of the HPA axis, leaving SAL NH animals with a significantly prolonged CORT response. A suppressed CORT response following acute restraint - and other types of - stress in adult rats that were neonatally exposed to DEX has been reported before (12, 24), as well as the impact of H leading to enhanced stress recovery (19, 25). Although functional outcome is similar, the effects of DEX treatment and H are likely to be mediated via different mechanisms. H animals show increased expression of GR compared to NH animals (26), whereas such an effect has never been shown in response to postnatal DEX treatment. However, prenatal DEX treatment has been reported to result in an increase in hippocampal GR density (27).

Overall it can be concluded that SAL NH animals display a 'reactive' phenotype. As expected, the experience of H reduces this reactivity. Interestingly, neonatal exposure to glucocorticoids has a comparable effect.

Dexamethasone treatment interacts with handling

H enhanced PPI in SAL treated animals, but did not affect DEX treated animals. Although Pryce and colleagues did not report differences in PPI after neonatal H (22), the current PPI enhancing effect of H is in line with previous findings from our laboratory (chapter 4 of this thesis), although in that study the interaction with DEX treatment was not investigated. Zhang and colleagues have reported enhanced PPI in animals receiving high compared to low levels of maternal care during infancy (28). This is in line with our observation that maternal care is enhanced upon reunion following H. Neonatal glucocorticoid treatment has resulted in inconsistent findings regarding PPI phenotype. Ferguson and colleagues report no effect of DEX treatment (23) whereas Hauser and colleagues report an increase in PPI after prenatal DEX, which was not replicated (29). Our data indicate no overall effect of DEX treatment on PPI. However, DEX treatment appears to reduce the susceptibility to H effects.

A comparable type of interaction was observed for spatial learning in the water maze at middle age: DEX treatment altered performance of NH animals, whereas in H animals no effect of DEX treatment was observed. Several studies have shown the adverse effects of neonatal DEX treatment on water maze performance (8). We observed that DEX NH animals need significantly less time to find the platform compared to SAL NH animals, especially on the first day of training. Brabham and colleagues also showed that animals exposed prenatally to DEX (although raised by a non DEX-treated foster mother) perform better than other (SAL-treated) groups in the water maze (27). These findings were unexpected and the authors have suggested an important role for postnatal maternal care which might be different in mothers exposed to DEX or vehicle during pregnancy.

Similarly, there might have been differences in maternal care directed towards DEX vs SAL offspring in our current study. Since we observed maternal care

directed towards the entire litter containing both SAL and DEX treated offspring, we cannot be sure whether treatment-specific differences exist and/or contribute to the adult phenotype. Although H decreases the impact of DEX treatment, we did not find an overall effect of H on water maze performance. While H has been shown to improve spatial learning in the water maze, an effect that lasts up to old age (20, 30), our data are in line with those from several other studies which do not indicate improved circular maze performance in H compared to NH animals (25, 31, 32).

Neonatal glucocorticoid exposure reduced the sensitivity to the beneficial effects of H on PPI, whereas H reduced the sensitivity to the beneficial effects of DEX treatment on spatial learning.

Handling compensates for dexamethasone-induced effects

A different interaction between DEX treatment and H was observed for spatial orientation in the T-maze. DEX NH animals do not discriminate between the new and familiar arm upon re-exposure to the same extent as other groups do, in favour of the new arm. This DEX-induced effect can be reversed by H. The impact of neonatal H on T-maze performance has been reported before (33) and appears to result in a higher discrimination rate compared to NH animals, especially with increasing age (25). The impact of neonatal DEX treatment on T-maze learning has, to our knowledge, not been described.

Spatial orientation with a long-term memory component is affected by DEX treatment and can be fully restored by neonatal H.

Handling effect – no dexamethasone effect

Not all behaviours are affected by both DEX treatment and H. For the behavioural phenotype observed in the fear conditioning paradigm, we found that immediate reactivity, in terms of freezing, to the foot shock was higher in NH compared to H animals. NH animals continue to show more freezing during re-exposure to the shock context 24h later. Although H has been reported to enhance contextual fear conditioning (34), our findings cannot be interpreted as a difference in contextual fear learning or memory, due to differences in responsiveness directly following shock exposure, and are more likely to indicate a difference in coping style (35). We do not report a main effect of, or an interaction with DEX treatment, which is in line with findings from Kamphuis and colleagues (12).

CONCLUSIONS

We report that the outcome of neonatal DEX treatment was determined by interactions with the effects of H. Overall, SAL NH animals appear to be 'challenged' the most during behavioural testing in adulthood. They show: (1) extreme startle reactivity, (2) low PPI, (3) high freezing in response to a foot shock (although similar to DEX NH), (4) low negative feedback of the HPA axis in response to acute stress, and (5) impaired spatial learning in the water maze. As expected, neonatal

H reduced startle reactivity and enhanced glucocorticoid feedback of the HPA axis. Neonatal DEX treatment, although expected to have detrimental effects on phenotype, resulted in effects comparable to H for several of the parameters studied. DEX treatment led to reduced ASR, enhanced feedback of the HPA axis and improved performance in the water maze. Whereas H reduced the impact of DEX in the T-maze and the water maze, DEX treatment reduced the sensitivity for H effects as observed for PPI.

These findings clearly show that the outcome of neonatal DEX-treatment: 1) is not deterministic, 2) highly depends on other characteristics of the postnatal environment, and 3) is potentially mediated by alterations in mother-pup interaction. Moreover, they highlight the importance of interaction between individual components of the early postnatal environment.

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