Programming the brain : towards intervention strategies

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SUMMARY

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It is well documented that early-life experiences are involved in shaping later-life phenotypes. Both human and animal studies have reported the impact of (adverse) early experiences on the development of the stress system, and its consequences for the development of stress-related disorders. The aim of the research described in this thesis was to explore the acute and long-lasting consequences of two distinct types of postnatal experience, varying substantially in nature and severity. In one study, the outcome of very subtle differences in maternal environment was examined. In another series of experiments, exposure to synthetic glucocorticoids during the early postnatal period was investigated. Furthermore, two potential intervention strategies to prevent the frequently reported adverse effects of glucocorticoid-induced disruption of normal development and brain maturation were evaluated.

In **chapter 1** an overview was given of important concepts for the study of developmental programming. Additionally, several animal models used in experiments described in this thesis were introduced.

In **chapter 2** we tested the hypothesis that maternal care is equally distributed across littermates and results in the development of a uniform stress phenotype with the litter. To investigate the distribution of maternal care across individual pups within the litter, we used an adapted version of a frequently used model of naturally-occurring variations in maternal care (Champagne et al. 2003). This allowed the study of within-litter differences in single pup-directed maternal licking and grooming. We reported that maternal care is not homogeneously distributed across littermates. Thus, besides differences in maternal care between litters, also variation within the litter exists. Next, the endocrine response to an acute novelty stressor was investigated at adolescence and adulthood in pups receiving low versus high levels of maternal care within the litter. We reported that these very subtle differences in early-life experience have long-lasting impact on the offspring's later-life endocrine stress phenotype. Interestingly, we observed that rat mothers show a preference for male over female pups, resulting in a bias in the distribution of males and females over the low and high maternal care group. Therefore, future studies are needed to investigate the gender-specific impact of within-litter differences in maternal care on later-life stress phenotype.

In **chapter 3** we investigated the acute consequences of neonatal glucocorticoid treatment on cell proliferation and glial activity in the developing brain. To investigate the impact of neonatal exposure to synthetic glucocorticoids, Long Evans rat pups were injected with dexamethasone on postnatal day 1, 2 and 3, according to a protocol based on the treatment regimen for premature infants used in clinical settings. We reported that neonatal glucocorticoid exposure acutely, but transiently reduced hippocampal cell proliferation. Additionally, the number of glial cells in the corpus callosum and hippocampus was reduced in dexamethasone-treated animals one week post-treatment. These short-term alterations in the developing brain might contribute to the frequently reported detrimental impact of neonatal dexamethasone treatment on adult phenotype. Next, we tested the possibility to prevent these alterations by pharmacological

intervention using central GR antagonist administration prior to dexamethasone treatment. We reported that although central mifepristone administration did not prevent the reduction in hippocampal cell proliferation, the dexamethasone-induced reduction in number of glial cells was fully normalized by central mifepristone pre-treatment, which was suggested as a potential intervention strategy to protect the developing brain against certain detrimental effects of glucocorticoid exposure.

Then, we investigated in **chapter 4** the consequences of this treatment for postnatal development, as well as the young adult, middle aged and senescent endocrine and behavioural phenotype, in order to test the hypothesis that neonatal glucocorticoid exposure results in long-lasting alterations in endocrine and behavioural reactivity as well as in a shortening of the lifespan. We reported that neonatal dexamethasone treatment leads to developmental alterations, such as growth retardation and accelerated eye opening. The frequently reported adverse effects on adult endocrine and behavioural phenotype were however not observed. Neonatal dexamethasone treatment did not result in spatial learning impairments, nor did it result in altered stress-induced HPA axis activation and acquisition of fear. Additionally, we did not report a shorter lifespan in dexamethasone- compared to saline-treated animals. It was suggested that - contrary to our expectations - postnatal handling of the neonate, as a result of our within-litter treatment design, modulates - and potentially overrides - the outcome of neonatal dexamethasone exposure. Indeed, additional studies showing reduced pre-pulse inhibition, motor performance and spatial learning, as well as prolonged stress-induced HPA axis activation in animals that experienced a totally undisturbed postnatal environment compared to handled animals (both saline and dexamethasone treated), strengthen this hypothesis, which was finally tested in **chapter 5**.

In **chapter 5** we investigated whether neonatal handling could serve as a second - behavioural - intervention strategy to rescue the dexamethasone-induced phenotype. We reported that neonatal handling enhanced maternal care upon reunion, and that the effects of handling indeed interacted with those of neonatal dexamethasone treatment in shaping the adult endocrine and behavioural phenotype. Although dexamethasone treatment and handling yielded comparable effects on the restraint stress-induced CORT response and acoustic startle reactivity, acquisition of fear was only affected by handling without an effect of dexamethasone exposure. Interestingly, it could be concluded that dexamethasone treatment reduced the sensitivity for beneficial effects of handling, as was observed for pre-pulse inhibition. Additionally, non-handled animals appeared to be more susceptible to the impact of neonatal dexamethasone treatment compared to handled animals, as was demonstrated for spatial learning in the water maze. Moreover, the impairing effects of dexamethasone treatment in spatial orientation in the T-maze were only observed in non-handled animals. Overall it appears that dexamethasone-treatment mostly affects non-handled animals, specifically for tasks with a cognitive component.

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

In **chapter 6**, the experimental findings described in this thesis were summarized and evaluated, and the implications for the field of developmental programming were discussed. We concluded that dexamethasone-induced effects interact strongly with other environmental factors during development and are therefore susceptible to intervention strategies, contrary to our expectations that the impact of neonatal dexamethasone exposure would overrule any other environmental effect. These observations might be valuable for the clinic, creating awareness for the important contribution of environmental influences, such as the degree of contact to the caregiver, in mediating glucocorticoid-induced effects.

Additionally, the finding that very subtle within-litter differences in maternal care have long-lasting impact on later-life stress phenotypes, highlights the important contribution of all components of the postnatal environment, no matter how subtle, in shaping the adult phenotype.