Programming of hippocampal structure and function by early-life stress: Opportunities for nutritional intervention
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Preface

Early-life stress shapes the brain for life
EARLY-LIFE STRESS SHAPES THE BRAIN FOR LIFE

DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

Early-life is a critical developmental period that plays an important role in determining adult health. This concept has been formulated as the developmental origins of health and disease (DOHaD) hypothesis [1-3], and implies that the quality of the early life environment will have a lasting impact on our health and vulnerability to develop diseases later in life. While this hypothesis was originally formulated as a framework to explain the differential vulnerability to cardiovascular [4,5] and metabolic [6,7] diseases, it is also of major importance for mental health [8,9]. Indeed, emerging clinical and preclinical evidence firmly establishes the association between adverse early-life experiences and predisposition to develop psychopathology (e.g. depression, schizophrenia) and impaired cognitive functioning in adulthood.

The concept that brain structure and function can be shaped ‘for life’ by experiences during the perinatal period is termed ‘programming’. The implications of this concept are major and imply that, once we understand the exact processes involved in this programming, we can potentially intervene and prevent the development of deleterious effects. Until the 80's, programming of the offspring's brain has often been viewed as the result of the unidirectional influence of parental care, described by e.g. psychological conflict, parental attachment and ‘toxic’ parenting theories. Nowadays these concepts are increasingly being replaced by (neuro-)biological theories focusing on a more complex interplay between the genetic makeup of the offspring and his/her environment (including parental care) during critical developmental periods [10]. However, while the theories have evolved, the exact underlying mechanisms responsible for the life-long effects of early experiences remain largely elusive.

Sadly, early-life stress (ES) is no exception in the contemporary world and many children experience one or more forms of ES, ranging from e.g. physical/sexual abuse, to neglect, exposure to war, poverty, illness or institutionalization (e.g. in the US over 3.7 million cases are referred to child protective services every year [11]). Prevention of ES is in most cases not feasible and currently no intervention strategies are available to protect a vulnerable population against the lasting ES-induced consequences for mental health. Today's children are tomorrow's future, so there is a high need for effective interventions, both from a societal as well as an economic point of view. In order to develop these, we need a better understanding of the processes involved in programming of the brain by ES.
OUTSTANDING QUESTIONS
The main goal of this thesis is to provide insight into some of the biological processes at play when experience, hormones and nutrition act together during early sensitive postnatal period(s) in life and confer enduring effects on later brain structure and cognitive and emotional function. The following outstanding questions will be addressed:
1. **Which key elements of the early-life environment are critical in programming ES effects?**
2. **Are the ES effects progressive or irreversible alterations that impact the brain from the start, and can we define critical time-windows for possible intervention?**
3. **Is ES differentially affecting males and females, and if so, what could be the possible underlying biological substrates?**

Answering these fundamental questions can have high translational value. In fact, in the past decades, insights from preclinical ES research have had important implications for human health. For instance, knowledge obtained from rodent studies addressing the role of early maternal sensory stimulation in regulating the offspring's stress system [12-15] has led to changes in clinical practice, facilitating and promoting contact between parent and infant in the stressful environment of the newborn IC unit. Indeed massage, kangaroo care (skin-to-skin contact between mother and infant) and other forms of sensory stimulation are now often applied in these clinical settings and have beneficial effects on pain responsiveness [16], reactivity to stress [17] and short-term weight gain [18].

It is however important to realize that it is not one single element of the early-life environment, but rather the synergistic action of various components (e.g. maternal sensory stimuli, stress hormones, neuropeptides and nutrition) that programs the brain during early-life, at least in part via epigenetic mechanisms. Epigenetic modulation of DNA and chromatin reflects a way for environmental factors to lastingly, or transiently, modify the degree of gene transcription [15]. While the essential role of maternal care in programming the brain is well established, the role of early nutrition has been largely ignored. However, early-life is a period of high nutritional demand, during which diet not only provides the building blocks required for growth and (brain) development, but also the essential elements for many biochemical processes, such as the production of neurotransmitters and epigenetic modifications. Therefore, in this thesis, the role of nutritional elements, their interactions with the stress system and their influence on the epigenetic machinery are addressed. Understanding the role and influence of early nutrition can alter the way we think about programming of the brain by ES and may create a platform for novel nutritional interventions, which are typically non-invasive and easily applicable.
For optimal intervention, it is important to identify the critical time windows during which the brain is most sensitive to early-life experiences. Therefore we need to understand if programming is mediated by acute and/or lasting effects on the stress-neuroendocrine and cognitive systems, which are still developing at that time. Such insights have important ramifications, not only for the design of specific interventions but also for policymaking. As an example, in the case of institutionalized children, researchers have tried to identify an optimal time for placement in foster families, resulting in evidence that the beneficial effects on behavior and cognitive function are much larger with earlier placements [19]. Accordingly, for many neurobiological mechanisms during development, optimal periods for intervention exist as well.

Another issue that needs clarification is the sex-specific sensitivity to early-life experiences. There is an obvious difference in the sex-ratio of many (ES-associated) psychopathologies; e.g. depression occurs twice as often in girls as in boys [20,21], while autism and attention deficit/hyperactivity disorders are 3-7 times more prevalent in boys than in girls [22]. However, so far surprisingly little is known as to how early-life experiences can alter postnatal or adolescent brain development and plasticity in a sexually dimorphic manner. Is there a difference between males and females in the vulnerability to ES experiences? What determines this, are sex hormones involved, and could this also relate to the sex differences in later susceptibility to neurodevelopmental diseases and psychopathology? Answering these questions will help to identify populations that are at risk and will provide more insight into how we can optimize differential intervention strategies for males versus females.

THE SCOPE OF THIS THESIS
To enable the investigation of processes involved in the programming of brain structure and function by ES, we have established and validated a mouse model of chronic ES (based on [23]). In this model, chronic ES is induced by fragmentation of maternal care via limiting the amount of nesting and bedding material in the cage during the first postnatal week. This model recapitulates important aspects of human neglect/abuse situations and, in contrast to related mother-pup separation paradigms, here the mother is present but stressed and unable to provide the appropriate care [24].

As causality between ES and long-term outcomes is difficult to prove in clinical research, animal models provide a clear advantage as they do allow us to directly address the above-mentioned questions. In addition, working with mice enables us to control for genetic background and life history and allows behavioral testing with direct access to brain material (within the same individual), for e.g. the assessment of hormone/nutrient content, neuronal
plasticity measures, gene expression and epigenetic modifications within specific brain regions of interest under well-controlled conditions.

The key brain region that we will focus on in this thesis, is the hippocampus, an extremely plastic brain structure important for cognition [25] and regulation of the stress response [26,27]. There is clinical evidence that ES exposure is not only associated with an impaired cognitive function in adulthood [28-30] but that this impairment also correlates with reductions in hippocampal volume [31-33]. In addition, reduced hippocampal volumes have been found in adults born with a low birth weight and are very common in ES-associated disorders, including major depression [34]. A possible explanation for the heightened sensitivity of particularly the hippocampus to early-life environments, is its extended development and postnatal maturation; the hippocampus continues to develop until 2 years of age in humans and up to 2 weeks after birth in rodents [35,36].

The dentate gyrus (DG), the hippocampal subregion that develops last, is of our particular interest, not only because its development largely coincides with the period of ES [36,37], but also because the DG exhibits a unique form of hippocampal plasticity, termed adult neurogenesis (AN). AN comprises the generation of new neurons from neuronal precursor cells in the adult brain, a process limited to very few brain regions, that occurs in most mammals (including human and rodents) and is implicated in cognitive functioning [38]. Although other forms of hippocampal plasticity might also contribute to ES-induced cognitive impairments, we focus here on AN as it is strongly modulated by life experiences and environmental influences. For example, physical activity [39], treatment with antidepressant drugs [40,41] and hippocampus-dependent learning [42] can increase AN, while ageing [43] and exposure to stress are potent inhibitors of AN [44,45]. It has been suggested that early-life experiences can also have large, even more persistent, effects on hippocampal plasticity [46] and that these play a role in mediating ES-induced later cognitive impairments. In that respect, the remarkable responsiveness of AN to adult life experiences might offer possibilities to restore cognitive function via modulation of AN via environmental stimuli later in life.

In this thesis we address if altered hippocampal plasticity is one of the neurobiological substrates of ES-induced cognitive impairments and we study if neurogenic capacity in adulthood is programmed by early-life experiences.
**THESIS OUTLINE**

The main objective of this thesis is to gain more insight in the biological processes at play during the programming of the brain by early-life experiences. By understanding how sensory stimuli, hormones and nutrition confer enduring effects on brain structure and function, and by testing the efficacy of interventions that are designed on this acquired knowledge, we hope to generate new ideas for intervention strategies that can prevent or restore lasting effects of ES on brain function and structure. To achieve the highest translational value, this research concentrates on clarifying unresolved issues regarding the programming of hippocampal structure and function by chronic ES. They concern: the complexity of the early-life environment, critical time-windows and sex-specific vulnerability.

**Chapter 1** provides an introduction into the perinatal programming effects of stress, nutrition and epigenetics on adult hippocampal structure and functions, including cognition and emotion. Next to an extensive overview of the literature, we hypothesize that stress during gestation, or early lactation, affects nutrient availability in the dam and her offspring. We propose that this may persistently modulate gene expression via epigenetic programming and thereby alters hippocampal properties.

In **Chapter 2**, we validate the chronic early life stress model in mice, a paradigm inducing chronic ES by fragmentation of maternal care, which is induced by an impoverished cage environment. We characterize the direct and lasting effects of this ES paradigm on postnatal and adult hippocampal neurogenesis and cognitive functions in male and female mice. ES induced alterations in neurogenesis appear (at least partly) involved in the later development of cognitive deficits. In addition, we describe a sex-specific effect of ES, with males being more vulnerable than females.

Next, we set out to determine if essential micronutrients are critically involved in programming hippocampal structure and function by ES. The focus is on methyl-donors (methionine, homocysteine, vitamins B₆, B₁₂, B₉ and their metabolites), as these are required for neuronal development and functioning of the epigenetic machinery, which has been implicated in lasting effects of ES. As described in **Chapter 3**, we first developed a novel methodology for the detection and exact measurement of these nutrients in milk, plasma and brain of neonatal mice. With this method, simultaneous and highly precise measurement of these micronutrients is now possible in these three different matrices, allowing us to study their concentration in maternal milk, their absorption by the offspring and their uptake in the brain under healthy or pathological conditions.
In chapter 4, we use our newly developed methodology to test how exposure to early-life stress affects the availability, absorption and uptake of these essential micronutrients. We show, for the first time, that ES affects levels of methionine in the plasma and brain of the offspring. Next, we show that supplementing the maternal diet during ES with methyl donors can restore nutrient levels in the offspring and can prevent ES-induced alterations. This short and early nutritional supplementation, with a specific group of micronutrients, is thus already able to ameliorate ES-induced cognitive impairments in adulthood, and thereby presents a novel ‘window of opportunity’ to modulate programming effects on the pup brain. To understand what mediates the effects of this successful dietary intervention, we further investigated maternal behavior, offspring HPA-axis activity, hippocampal neurogenesis, gene expression and DNA methylation.

In chapter 5, we investigate the potential of other environmental factors to stimulate brain plasticity and thereby protect against the lasting consequences of ES. While we focused on males in the previous chapter, we here specifically study female mice. As females seem more resilient to ES-effects than males under basal conditions (chapter 2), we were interested whether the neurogenic capacity of the adult female brain was differentially affected by ES in response to stimuli known to modulate AN and cognition. We tested the effects of: i) exercise in adulthood and ii) dietary supplementation with essential micronutrients in early life.

In chapter 6 we review and discuss the sex-specific vulnerability to develop psychopathologies and address whether such sex specific susceptibility could be explained by differences in sex hormones during specific developmental time windows.

The main findings of this thesis and their implications are summarized and discussed in chapter 7.
REFERENCES


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