Programming of the Brain by Metabolic and Nutritional Factors After Early-Life Stress: Modulations by Early Nutritional Interventions

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ENGLISH SUMMARY

Early life is a critical and sensitive period during which the developing brain can be 'programmed' for life. Several studies have shown that exposure to early-life stress (ES), such as childhood abuse or neglect, leads to a higher vulnerability to develop brain disorders in adulthood. These include for example cognitive impairments, anxiety and depression. Interestingly, these disorders often occur in a sex-specific manner and, notably, are often comorbid with metabolic diseases, e.g. obesity and diabetes. It is furthermore remarkable that also nutrition-related changes occurring during the early life period, like the Dutch Hunger Winter of 1944, are associated with similar metabolic disorders later in life. This indicates a close interplay between stress and nutrition in programming the adult body and brain. So far, however, it is unclear if and how peripheral metabolic and nutritional factors are involved in the ES-induced programming of the brain. Also, if this is the case, there are currently no effective nutritional interventions available that may possibly modify or prevent these diseases.

Hence, the **main goals** of this thesis were to; i) gain insight into the short- and/or long-term sex-specific effects of ES on the brain and metabolism. We focused on the metabolic leptin and ghrelin systems, and also investigated if ES-exposed mice were metabolically more vulnerable when challenged with an early western-style diet. We further assessed if ii) ES affects nutritional availability by studying the essential fatty acids centrally and peripherally, and iii) whether nutritional supplementation with a low omega (ω)-6/ω-3 diet during the early life period, might prevent the ES-induced cognitive impairments and later metabolic alterations. We further investigated some of the possible (neuro)biological mechanisms that could underlie the beneficial effects of diet on cognition; we included the central and peripheral fatty acid compositions, hippocampal neurogenesis, microglial phagocytosis, neuronal plasticity, apoptosis and maternal care.

In **chapter 1**, we provide a comprehensive overview of the available clinical and preclinical evidence that both ES and early-life malnutrition lead to similar lasting changes in the adult neuroendocrine systems and emotional functions. We propose the concept that stress and nutritional factors are closely interrelated and affect one another, and result in lasting outcomes on the brain. In addition, we suggest a role for metabolic hormones and epigenetic mechanisms in regulating the stress system and brain functions.

In **chapter 2**, we use a well-established chronic ES mouse model, in which nesting and bedding material is limited from postnatal day (P)2 to 9, which leads to lasting cognitive impairments. Concomitantly, ES lastingly affected the adipose tissue and leptin system in both male and female mice. This demonstrated; i) short-term increases in brown adipose tissue mass and browning of white adipose tissue, ii) long-term reductions in white adipose tissue mass, circulating leptin levels and leptin gene expression in the white adipose tissue of both males and females, whereas iii) the body fat distribution, e.g. the ratio of mesenteric white adipose tissue versus the other white adipose tissues, was higher only in females after ES exposure. In addition, iv) the reductions in white adipose tissue were correlated with an impaired cognitive performance after ES in males only, and v) ES was found to increase the leptin receptor gene expression in the choroid plexus, while it was

unaltered in the hippocampus in adulthood. This suggests a possible adaptation at the central level to the reduction in peripheral leptin.

Interestingly, we also showed that an early exposure to a western-style diet resulted in a higher body fat accumulation in ES-exposed mice when compared to controls in adulthood. Taken together, chronic ES lastingly affects the adipose tissue and leptin system, which are possibly involved in the ES-induced cognitive impairments. Moreover, our data indicate that ES increases the vulnerability to develop metabolic alterations when exposed to a moderate obesogenic environment.

Next to the role of the metabolic hormone leptin, we addressed sex-specific effects of ES on the ghrelin system in **chapter 3**. In this study, we focused on the hypothalamic neuropeptide Y (NPY) and agouti-related protein (AgRP) feeding circuitry in young postnatal male and female mice. We found that ES results in; i) alterations in the two forms of ghrelin, which were more pronounced in ES-exposed females than ES males, ii) increases in NPY and AgRP expression in the hypothalamic region in a similar pattern in both sexes, iii) and the hypothalamic receptor for ghrelin was unaltered. Our study is the first to show differential effects of chronic ES on the different forms of ghrelin, which might program the hypothalamic circuits leading to lasting sex-specific metabolic disorders.

In **chapter 4**, we studied the short- and long-term effects of ES on the central and peripheral fatty acid (FA) profile, and on the hepatic triglycerides and lipid metabolism. We show that ES; i) changes the FA composition in central (hippocampus) and peripheral (erythrocytes, liver) tissues at P9 and P180, ii) without affecting the hepatic triglycerides or lipid metabolism (i.e. the expression of genes involved in FA uptake and oxidation, de novo lipogenesis and very low-density lipoprotein secretion).

We further applied early nutritional intervention using a low ω-6/ω-3 diet for a short period from P2 to P42 in control and ES-exposed male mice. By increasing the relative ω-3 availability with a low ω-6/ω-3 diet, we show that; iii) the diet has no effects on the ES-induced reductions in fat depositions and leptin levels throughout life at P9, P42, P180 and P245. Interestingly, while the metabolic alterations were unaffected by the low ω-6/ω-3 diet, iv) it prevented the ES-induced cognitive impairments in adulthood. Additionally, we investigated potential mechanisms underlying the beneficial effects of this diet on cognition, and demonstrated that; v) the diet also prevented ES-induced reductions in adult hippocampal 'neurogenesis' (a unique feature of the hippocampus that refers to the capacity to generate new neurons during adulthood) and increases in microglial phagocytic function in adulthood.

vi) Other mechanisms we investigated were related to maternal care in the dams as well as to the central and peripheral FA profiles, hippocampal proliferation, neuronal plasticity or apoptosis at P9, P42, P180 and/or P245, which were generally neither affected by ES nor by the low ω-6/ω-3 diet. Thus, we demonstrated that the ES-induced cognitive impairments can be prevented by an early nutritional intervention using a low ω-6/ω-3 diet, and that the beneficial effects of the diet are, at least partly, mediated by modifications of hippocampal neurogenesis and microglia. This

study creates promising novel opportunities for non-invasive, nutritional interventions that lend themselves to application in clinical settings, particularly in vulnerable populations that are exposed to stress during early life.

In **chapter 5**, we discuss our findings from a broader perspective and involve other potential mechanisms, including the role of the blood-brain barrier and additional key metabolic hormones/ nutrients, its interactions as well as the clinical relevance of our research findings.