"Sapienza" University of Rome

Department of Psychology

Ph.D. program in Behavioral Neuroscience

Curriculum of Psychobiology and Psychopharmacology

A DISSERTATION IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BEHAVIORAL NEUROSCIENCE

Shared mechanisms underlying the impact of maternal psychophysical stress and obesity on offspring neurodevelopment

Chiara Musillo

Coordinator of the Ph.D. program

Prof. Cecilia Guariglia

Supervisor

Prof. Rossella Ventura

External Supervisors

Dr. Francesca Cirulli

Dr. Alessandra Berry

Table of contents

PREFACE

Vulnerability to mental illness might find its roots very early during development, already during fetal life. In fact, prenatal adversities can affect brain development by shaping neuronal circuits involved in stress responses, resulting in embedded biological traces that persist throughout life. In this perspective, maternal environment plays a pivotal role in driving fetal neurodevelopment, even more important than purely heritable genetic background.

Chapter 1 of this thesis introduces the concept that maternal obesity - a growing public health issue - can be considered as a stressor that, by contributing to establish a sub-optimal intrauterine environment, may derange fetal neurodevelopment. We reviewed in detail clinical and preclinical evidence showing an association between the prenatal exposure to an "obesogenic environment" and a higher risk for the occurrence of neurodevelopmental and psychiatric disorders.

An ever increasing body of evidence shows that similar mental health outcomes in the offspring have been observed as a result of either maternal obesity or maternal distress during pregnancy. Thus, in **Chapter 2** we propose a "funnel effect" model hypothesizing that prenatal stressors of different nature might trigger shared stress-responsive pathways affecting neuroendocrine system, immune-inflammatory processes and energy metabolism regulation, ultimately resulting in increased vulnerability to psychopathology.

Chapter 3 and Chapter 4 (original studies) investigate the shared biological mechanisms underlying the above-mentioned stressors and their effects during specific time windows across neurodevelopment in two C57Bl6/N mouse models of maternal psychophysical stress (PNS) and maternal obesity (mHFD). We focused on oxidative stress as a central player driving fetal brain programming by adverse prenatal conditions. Also for this reason in our mouse models, we administered as preventive strategy the antioxidant N-acetyl-cysteine (NAC) to protect fetal neurodevelopment from stress-derived derangements.

In particular, when we focused on the short-term effects, we found a widespread pro-inflammatory profile in fetal brains exposed either to PNS or mHFD - with females being more susceptible - to be associated to placental dysfunctions (**Chapter 3**). Moreover, investigation of the long-term effects of PNS and mHFD specifically during adolescence showed similar effects in the offspring, characterized by reduced brain anti-oxidant defenses and impairments in hippocampal *Bdnf* levels, overall leading to alterations in the emotional behavior and hypothalamic-pituitary-adrenal axis functionality, in a sex-dependent fashion. Maternal NAC administration, by restoring the redox balance, showed long-term protective effects on brain development (**Chapter 4**).

Together, our findings contribute to support our original "funnel effect" model to explain the converging effects of different stressors on offspring brain development. Above all, a pivotal role of redox signalling was highlighted as the orchestrator of a synchronized response to early adversities by the neuroendocrine and the immune system, among others. In addition, we unveil clear sex-specific differences that drive the programming effects of prenatal stressors on neurodevelopment.

CHAPTER 1

Maternal Obesity as a Risk Factor for Brain Development and Mental Health in the Offspring

Francesca Cirulli[1]*, Chiara Musillo[1,2] and Alessandra Berry[1]

[1]Center for Behavioral Sciences and Mental Health, Istituto Superiore di Sanita`, Rome, Italy. [2]PhD Program in Behavioral Neuroscience, Department of Psychology, Sapienza University of Rome, Rome, Italy.

Neuroscience (2020) 447:122-135. doi: 10.1016/j.neuroscience.2020.01.023

Abstract

Maternal obesity plays a key role in the health trajectory of the offspring. Although research on this topic has largely focused on the potential of this condition to increase the risk for child obesity, it is becoming more and more evident that it can also significantly impact cognitive function and mental health. The mechanisms underlying these effects are starting to be elucidated and point to the placenta as a critical organ that may mediate changes in the response to stress, immune function and oxidative stress. Long-term effects of maternal obesity may rely upon epigenetic changes in selected genes that are involved in metabolic and trophic regulations of the brain. More recent evidence also indicates the gut microbiota as a potential mediator of these effects. Overall, understanding cause-effect relationships can allow the development of preventive measures that could rely upon dietary changes in the mother and the offspring. Addressing diets appears more feasible than developing new pharmacological targets and has the potential to affect the multiple interconnected physiological pathways engaged by these complex regulations, allowing prevention of both metabolic and mental disorders.

Keywords

Maternal obesity, Pregnancy, Fetal programming, Mood disorders, Placenta, Oxidative stress

Introduction

Overweight and obesity are dramatically rising in low- and middle-income areas, particularly in urban settings (https://www.who.int/end-childhood-obesity/publications/ echo-report/en/). The prevalence of obesity is increasing across all populations and age groups: although genetic factors may play a role in modulating vulnerability to weight gain and fat accumulation, they cannot explain the exponential increase in obesity we are currently witnessing (Congdon, 2019). Indeed, globalization and urbanization have gradually led to the so-called nutrition transition i.e. a reduction of physical activity associated with the increase in consumption of low-cost and easily accessible ultra-processed, energy-dense, nutrient-poor foods (https://www.who.int/end-childhood-obesity/publications/ echo-report/en/). Such a spread of unhealthy lifestyles results in energy unbalance that favors fat storage, eventually strengthening the ground for the settlement of obesogenic environment (Townshend and Lake, 2017).

Maternal obesity affects 30% of pregnant women and excess weight gain occurs in 40% of gestations. The ‘‘Commission on Ending Childhood Obesity” (established by WHO) in its 2016 final report, has tackled the early life environment (including preconception and pregnancy) as a critical time for long-lasting and trans-generational effects of the metabolic derangement underlying obesity – and the associated comorbidities – as well as a window of opportunity to prevent them (https://www.who.int/end-childhoodobesity/ publications/echo-report/en/). Although a great deal of research has focused on the mechanisms that can lead to offspring obesity, there is also evidence for an effect of maternal obesity on cognition and mental health of the offspring (Rodriguez, 2010; Buss et al., 2012; Hinkle et al., 2012, 2013; Casas et al., 2013; Rivera et al., 2015). Indeed, exposure to maternal obesity or to an unhealthy maternal diet and metabolic diseases (diabetes and hypertension), can all increase the risk of for later-life cognitive disabilities and psychiatric disorders such as attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (ASDs), anxiety, depression, schizophrenia and eating disorders in the offspring (Buss et al., 2012, Hinkle et al., 2012, Hinkle et al., 2013, Yatsunenko et al., 2012, Rivera et al., 2015, Dinan and Cryan, 2017).

Obesity in pregnancy results in neuroendocrine, metabolic and immune/inflammatory changes which can affect fetal exposure to hormones and nutrients, disrupting the development of those neural pathways critical for the regulation of behavior and cognition. Inflammation during pregnancy may alter functional connectivity and may be associated with altered behavioral regulation and reduced working memory performance in early childhood. Changes in microbiota composition as a result of maternal obesity may play a role in these effects. Indeed, the gut microbiota develops and stabilizes in early life phases (Yatsunenko et al., 2012), which are crucial for the programming of tissues and organs, including the brain by e.g. modulation of the immune system (El Aidy et al., 2016, Dinan and Cryan, 2017) hormones, neurotransmitters and metabolites acting on brain physiology.

Postnatal nutrition (also targeting microbiota) may represent an important strategy to counteract metabolic-associated cognitive impairment. In fact, the beneficial effects of diets, nutrients and foods (e.g. Mediterranean diets, omega-3) on mental health and cognitive performance are starting to be recognized (Bruce et al., 2002,

Dimmitt et al., 2010, Davari et al., 2013, Bellisario et al., 2014, Mrizak et al., 2014, Dinan and Cryan, 2016, Kelly et al., 2016, Milior et al., 2016, Patterson et al., 2016, Sherwin et al., 2016).

This review will focus on the contribution played by maternal obesity in shaping the metabolic and behavioral phenotype of the offspring by affecting the developmental programming of the fetus. Main emphasis will be given to changes in oxidative stress (OS) pathways and epigenetic modifications as possible mechanisms mediating the effect of maternal obesity and early exposure to high caloric diets (e.g. westernized diet rich in fats and sugars, high fat diet – HFD). The most recent evidence coming from epidemiological and preclinical studies will be reviewed taking also into account the role played by maternal obesity in early programming of the offspring gut microbiota. Finally, the potentiality of strategies aimed at preventing/counteracting the negative effects of prenatal obesity will be considered, including antioxidants and probiotics/prebiotic administration during pregnancy and to the offspring.

Developmental Origin of Health and Disease (DOHaD) Evolutionary and epidemiological perspectives

Mammalian development unfolds as a gradual process, which is continuously adjusted to the needs and challenges posed by the environment (in this review we will specifically focus on the intrauterine development) within the constraints of the genetic asset of the individual. From an evolutionary perspective, the elevated plasticity characterizing the developmental program is perfectly suited at generating a plethora of best adapted phenotypes that allow the perpetuation of the species with regard to the eco-ethological niches they will colonize (Bateson, 2001, Bateson et al., 2004). While such plasticity helps the individual to adjust to changes in the environment, it can also provide a substrate for increased vulnerability later in life, thus resulting in a double edge sward. The overall outcome of the developmental program will depend heavily upon the interplay among the genetic background of the organism, the intrauterine milieu and the stability of the extra-uterine (external) environmental conditions with respect to those that primed fetal developmental trajectories (Bateson et al., 2004).

Indeed, early life experiences have the potential to become embedded biological traces in the animal's physiology, resulting either in increased vulnerability or in a greater resilience for the onset of disease states. The seminal studies of Barker and colleagues have contributed to provide evidence for a strong association between environmental challenges during pregnancy, altered fetal growth and health outcomes later in life (Barker et al., 1993, Seckl, 1998) giving rise to the concept of the DOHaD. In fact, the observation that those regions in England characterized by the highest infant mortality (due to reduced birth weight) showed the highest rates of adult mortality from cardiovascular disease (CVD), led to the hypothesis that those babies who survived were at greater risk of CVD later in life (Barker and Osmond, 1986, Barker et al., 1989, Barker et al., 1993). Most intriguingly, the relationship between reduced birth weight and the onset of diseases at adult age (e.g. hypertension or type 2 diabetes - T2D) was unrelated to lifestyle risk factors (Osmond et al., 1993, Cirulli

et al., 1994, Leon et al., 1996, Harris and Seckl, 2011). In addition, studies on twins provided scarce evidence for a main role of genes in these associations (Baird et al., 2001, Bateson et al., 2004) indicating that vulnerability to adult diseases might rely upon fetal life experiences as a result of the redirection of the developmental trajectories.

Although for historical reasons Barker's theory was focused on the effects of prenatal undernutrition (see for instance the studies on the "Dutch famine" e.g. (Roseboom, 2019) and references therein), nowadays it is rather maternal obesity and its sequelae for metabolic and mental health that poses serious public health concerns (Jehn and Brewis, 2009, Congdon, 2019). Indeed, recent studies clearly suggest that maternal obesity, both before becoming pregnant and throughout gestation, is associated with negative short- and long-term health outcomes. Among all, a general developmental delay in children and poorer physical and mental abilities during aging are most often observed (see for instance the studies carried out on the Helsinki Birth Cohort (Berry et al., 2018a, Berry et al., 2018b, Eriksson et al., 2014, LifeCycle Project-Maternal et al., 2019, Mina et al., 2017, Westberg et al., 2016) with OS playing a key role as a mediator of both short and long-term effects (Berry et al., 2018a, Berry et al., 2018b, Edlow, 2017). Higher maternal body mass index (BMI) has been also associated with shorter telomeres length as assessed in the leukocytes of elderly women (Guzzardi et al., 2016). Moreover, umbilical cord gene expression profiling has identified patterns consistent with neurodegeneration/premature brain aging in fetuses of obese women (Edlow et al., 2016b) suggesting long-term sequelae of early metabolic stress. To this regard, Alzheimer's disease (AD), a neurodegenerative pathology affecting old age, has been recently defined as 'Type-3-Diabetes'. In fact, recent evidence point to common molecular and cellular mechanisms linking type 1 diabetes, T2D and insulin resistance to cognitive deficits in old subjects, suggesting that an early obesogenic environment might set the stage for a shared vulnerability to both metabolic and mental health issues (Kandimalla et al., 2017).

The obesogenic womb and its sequelae

While for the mother the negative effects of obesity are readily observed as they may affect the ability to become pregnant and may also lead to obstetric complications, the effects on the offspring may not be immediately evident at birth although they have the potential to have both short- and long-term consequences (Alfaradhi and Ozanne, 2011, Tenenbaum-Gavish and Hod, 2013, Iozzo et al., 2014, Moussa et al., 2016, Contu and Hawkes, 2017).

Most importantly, in addition to the effects on metabolic programming, long-term longitudinal and associative studies provide evidence for a direct association between prenatal metabolic stress and an increased risk to develop neuropsychiatric, mood disorders and cognitive disabilities (see Howell and Powell, 2017 and references therein). It should be mentioned that being obese during childhood may, by itself, trigger the onset of behavioral and emotional issues leading in turn to stigmatization and poor socialization, all conditions that may reduce educational attainment and trigger the onset or precipitation of psychiatric disorders (Pizzi and

Vroman, 2013, Miller et al., 2015). However, there is now evidence that, independently from offspring obesity, maternal obesity is associated with neurobehavioral problems in the offspring, although proving cause-effect mechanisms is rather hard in humans, given the confounds deriving from the many intervening (genetic and environmental) variables involved in these effects. Nonetheless, there are now quite a number of epidemiological studies indicating that compared to children born from mothers having normal weight, children of obese mothers have a greater chance to show behavioral problems or being diagnosed with a neurodevelopmental disorder, such as ADHD (Godfrey et al., 2017). Regarding cognitive deficits, studies on the UK Millennium cohort have provided evidence for a negative relationship between maternal BMI and children's general cognitive ability at 7 years (Basatemur et al., 2013). A large synthesis and meta-analyses of the literature has more recently evaluated the association between pre-pregnancy overweight or obesity (in relation to normal weight) and subsequent childhood neurodevelopmental outcomes (Sanchez et al., 2018). This meta-analysis supports previous preclinical and observational studies indicating that children born from mothers obese prior to and during pregnancy are at increased risk for neurodevelopmental disorders.

Notwithstanding the above-mentioned evidence, it is rather difficult to establish whether maternal obesity affects neurobehavioral development in the offspring directly. In addition, maternal intelligence quotient, socio-economic status, breastfeeding vs. formula, maternal mental health, maternal diet and other postnatal lifestyle influences may concur to effects of maternal obesity (Soubry et al., 2013, Contu and Hawkes, 2017, Godfrey et al., 2017). These questions can be more easily asked using animal models which allow to distinguish the confounding variables present in human studies, which are mostly observational in nature. In animal models, maternal obesity is mainly modelled by feeding dams before and/or during gestation with HFD. Overall results from these studies enlarge and strengthen clinical and epidemiological evidence, indicating that offspring of obese mothers are characterized by social impairments, anxiety and depressive-like symptoms, in addition to cognitive disability and hyperactivity (Sullivan et al., 2015). One main important question that has being asked through preclinical models is whether pre-pregnancy obesity and maternal obesity exert the same effects on the offspring. This is an important point that can redirect prevention policies: it is much easier to address weight gain problems during pregnancy than acting upon an obesity condition that might be acquired much before gestation. A recent preclinical study appears to support epidemiological evidence indicating that prenatal and pregnancy windows have independent programming effects on the offspring. Preconception exposure affects body composition and adiposity while gestation exposure affects metabolism and tissue immune cell phenotypes (Chang et al., 2019). Overall, the current evidence suggests that pre-pregnancy obesity, rather than weight gain during gestation, may be most harmful to the fetus.

The field is very complex as also shown by inconclusive evidence provided by a very recent meta-analysis addressing results coming from animal models (Menting et al., 2019). Indeed, while clear effects of maternal obesity were observed on locomotor activity and anxiety, both increased by the metabolic maternal challenge, no significant effect was revealed by the meta-analysis on learning and memory performance in the offspring (Menting et al., 2019). Although animal models may have some limitations, they are fundamental for

understanding that the effects of an obesogenic environment experienced during fetal life depend upon multiple and not-exclusive pathways, which may lead to long-term pathological outcomes.

Mechanisms underlying fetal programming by maternal obesity

In complex organisms there is a finely-tuned crosstalk between physiological and behavioral responses, ultimately leading to the avoidance or to the adaptation to challenges. While acute responses to short-term stressors are pivotal to restore homeostasis, the management of a chronic stress condition imposes a non-negligible burden to the organisms ("allostatic load") that might affect growth, metabolism, reproduction, inflammatory/immune and neuroendocrine function (McEwen, 1998, Seckl, 2004, de Kloet et al., 2005, Maccari and Morley-Fletcher, 2007).

Pregnancy is a highly demanding task involving physiological adaptations leading to a shift in the homeostatic balance (Mannaerts et al., 2018). Such changes, which would result in a pathological state in non-pregnant women, are (usually) well tolerated in healthy (pregnant) subjects. Although obesity per se cannot be considered a pathological condition, it represents a major risk factor for the onset and/or precipitation of many metabolic and cardiovascular non-communicable diseases in addition to mood disorders (Chaput et al., 2012, Armani et al., 2017, Wurtman and Wurtman, 2018). Thus, being pregnant and obese at the same time may turn out to be an extremely stressful condition leading to a maternal allostatic load that will be affecting the fetus. This will engage multiple, not mutually exclusive, mechanisms (during sensitive developmental phases), affecting tissue organization and organs' physiology in the offspring (Harris and Seckl, 2011, Iozzo et al., 2014). Among these mechanisms, hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and the associated excessive glucocorticoids (GCs) secretion, OS and inflammation, as well as gut microbiota dysbiosis (just to mention a few) may all contribute to mediate the effects of maternal obesity, ultimately leading to vulnerability to neurodevelopmental and psychiatric morbidity in the offspring which could be embedded, through epigenetic changes, in selected genes.

Maternal metabolic stress shaping brain development

There is abundant preclinical and clinical evidence to indicate that adverse socio-economic conditions, in addition to physical, emotional or sexual abuse, are well-established risk factors for mood disorders (McEwen, 2000, Roth and Sullivan, 2005, Raikkonen and Pesonen, 2009, Ehlert, 2013, McLaughlin et al., 2015, Roseboom, 2019). As the studies of Barker and colleagues (Barker et al., 1993, Seckl, 1998), previously detailed in this review, have listed body weight at birth within the set of risk factors that can increase susceptibility for adult diseases, maternal obesity needs to be listed as another yet adverse condition capable to affect offspring's development. Although, counterintuitively, obesity may involve a form of

malnourishment, as it entails "poor nutrition". Indeed, one has to consider that, even when food is relatively prevalent, it may lack vitamins, minerals or other fundamental nutrients.

In order to understand the mechanisms underlying the detrimental effects of maternal obesity on brain developmental trajectories, we need to consider that this condition is likely to rely upon the same mechanisms regulating responses to stress (McEwen, 2000, Cirulli, 2017). Acute activation of this pathway represents an adaptive stress response, allowing the organism to face an acute threat. Under circumstances of chronic or overwhelming adversities, prolonged responses to stress may become maladaptive for the individual and toxic for the organism leading to pathological states (or "allostatic load") (McEwen et al., 2015). The pathways deranged during early development and involved in the elaboration of stressful and metabolic signals might be further modified in their function when a "second hit" occurs during critical periods, such as adolescence or adulthood (Bock et al., 2014, Boersma et al., 2014, Cirulli et al., 2003, Daskalakis et al., 2013, Ehlert, 2013, Entringer et al., 2015, Provencal and Binder, 2015). Thus, the effects of metabolic or psychological stressors experienced at later stages will be more disruptive in those individuals who have been exposed to stressors early during development.

Comorbidity between metabolic and behavioral disorders suggests shared developmental pathways and common mediators. Our group, together with many others, have clearly identified neurotrophins, such as BDNF, and GCs as main effectors of brain plasticity and metabolic regulations in response to stressful events (Cirulli et al., 2003, Cirulli and Alleva, 2009, Levine, 1957, McEwen, 2000, Meaney et al., 1989).

There is a plethora of data from clinical and preclinical studies suggesting that the type and quantity of micronutrients, high levels of leptin and insulin as well as elevations in inflammatory mediators, such as interleukins and tumor necrosis factor, can play a fundamental role in affecting developmental trajectories of the fetus by crossing the placental barrier (Godfrey et al., 2017). Central to the maternal-fetal regulation is indeed the placenta (Fig. 1). This temporary organ, the main mother-fetus interface, is able to convey stress signals to the fetus by modulating the entry of hormones, glucose, amino acids, vitamins and ions necessary for fetal growth and development (Fowden et al., 2009, Bale et al., 2010, Cirulli, 2017). Since elevated levels of GCs can be detrimental for the fetus, a specific enzyme, the 11ß-hydroxysteroid dehydrogenase type 2 (11β-HSD-2), acts as a shield, guaranteeing their rapid inactivation and allowing an optimal amount of these hormones to be transferred to the fetus for organs maturation. It is rather interesting that psychological stressors and metabolic/nutritional challenges can both affect the expression or the activity of the 11β-HSD-2 enzyme, with high levels of GCs reaching the fetus, resulting in increased susceptibility for psychiatric disorders and metabolic complications linked to an increased risk of insulin resistance and T2D, in addition to CVD and metabolic disorders associated with obesity (Eriksson et al., 2014, Mansur et al., 2015, Mina et al., 2015, Rivera et al., 2015, Stout et al., 2015).

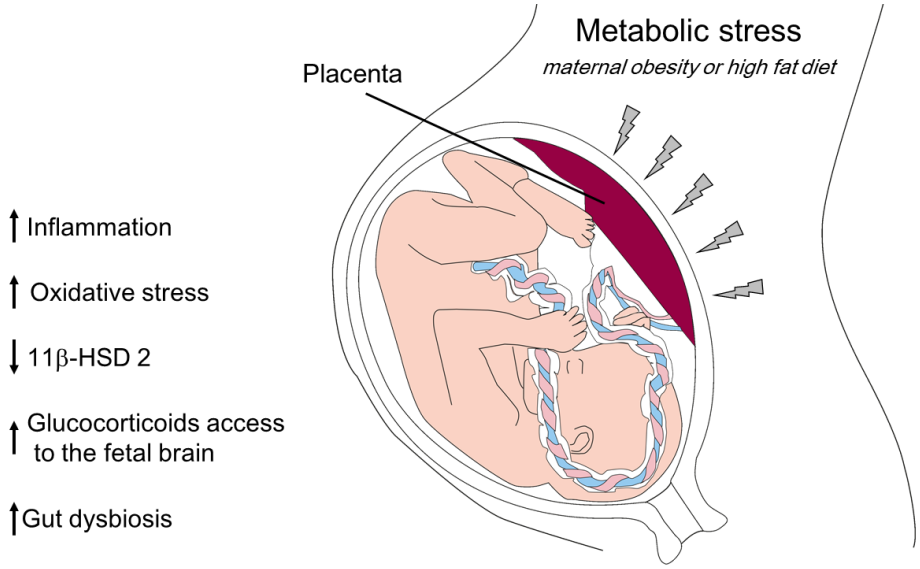


Fig. 1. Potential mechanisms underlying the effects of maternal obesity on fetal development. The placenta is the critical mother-fetus interface transducing metabolic stress into changes in the developing organism underlying the short- and long-term effects of maternal obesity (see text for a detailed description).

In recent studies we have clearly shown that HFD feeding during pregnancy (in mouse models), independently from maternal obesity, is a stressful challenge that is able to influence negatively the fetus, which is already exposed to excess GCs as a result of maternal pregnancy status (Bellisario et al., 2014, Eriksson et al., 2014, Bellisario et al., 2015). HFD-feeding in female mice before conception and during pregnancy, in fact, increases levels of maternal stress hormones and is associated with both reduced 11β-HSD-2 enzymatic activity and expression of the 11β-HSD-1 gene in the placenta (Bellisario et al., 2015). Exposure to a diet with high fat content has also been shown to disrupt maternal behavior at parturition with deleterious consequences on the offspring (Bellisario et al., 2015). These dysregulations, observed in HFD-fed dams, might be caused by changes in neural activity in brain regions responsible for olfactory processing and social recognition, ultimately leading to inappropriate maternal behavior (Bellisario et al., 2015). These effects are less apparent in animal models of reduced OS, especially in female subjects, suggesting that diet-induced metabolic dysfunction, neuroendocrine response and sex/gender all play a role in these regulations (see later in this review) (Bellisario et al., 2014).

Worth noticing, these data also confirm clinical evidence showing that the presence of high levels of GCs during gestation can affect the expression of GCs-sensitive genes in the central nervous system, as well as in the periphery, with important effects on HPA axis function and regulation (Mina et al., 2015, Mina et al., 2017). These effects are reminiscent of a solid literature in animal models indicating that prenatal maternal stress affects HPA axis activity and stress regulation through changes in GCs receptors in limbic regions of the brain (Welberg and Seckl, 2001, McGowan et al., 2008, Brunton and Russell, 2010). We can thus

hypothesize that, by affecting the amount of GCs reaching the fetus, HFD during pregnancy could lead to similar short- and long-term effects already described for prenatal stress.

A further intriguing aspect is related to the modulating effect of the HPA axis on immune function and inflammation. The group of Sasaki (Sasaki et al., 2014) investigated the expression of several inflammatory genes linked to GCs signaling (Sorrells et al., 2009) as a result of perinatal exposure to HFD (Bilbo and Tsang, 2010). Results suggest a dysregulation of pro- and anti-inflammatory genes linked to HFD exposure in utero with NF-kB and IL-6 transcripts being increased in the hippocampus in HFD-exposed offspring. Moreover, increased expression of the cytokine IL-6 in the hypothalamus (De Souza et al., 2005) and cortex (White et al., 2009) is an established consequence of prolonged HFD exposure in utero.

Chronic systemic inflammation characterizes both maternal obesity and pregnancy. Obese pregnant women have higher levels of circulating pro-inflammatory cytokines while maternal BMI is directly correlated with (maternal) pro-inflammatory cytokines concentrations and the activation of pro-inflammatory placental pathways. Changes in fetal cytokines expression, fetal neuronal damage and changes in gene expression in the neonatal brain have been previously related to placental and intrauterine inflammation (Edlow, 2017). Inflammation during pregnancy might alter the connectivity of brain networks and be associated with altered neurobehavioral regulations and reduced memory performance in early childhood. Indeed, it has been recently reported that maternal IL-6 during pregnancy correlates with newborn brain connectivity and can predict future working memory in offspring (Rudolph et al., 2018). Thus, changes in inflammatory mediators could be important biomarkers to be used to estimate long-term effects of maternal obesity on brain function.

Role of the microbiota

Long-lasting effects on fetus and newborn resulting from exposure to maternal obesity during pregnancy could also be explained by an alteration in the composition of the gut microbiota during critical developmental windows (Cryan, 2016). Each individual owns a unique gut microbiota profile composed by different strains of bacteria. Changes in this balance may lead to a condition of dysbiosis (Rinninella et al., 2019). Recent studies associate dysbiosis with different gastrointestinal and metabolic diseases such as diabetes and obesity but also with neurodegenerative and mental disorders, including AD and Parkinson's disease (PD), ASDs, schizophrenia and depression (Heiss and Olofsson, 2019). This suggests that the balance of the gut microbial composition may play a key role in the regulation of different developmental processes (De Palma et al., 2015, Dinan and Cryan, 2016, El Aidy et al., 2016, Foster et al., 2016, Kelly et al., 2016, Luczynski et al., 2016).

The early life phases are crucial for the development of the microbiota and to define its ultimate role in the programming of tissues and organs (Dimmitt et al., 2010). A number of studies show that the gut microbiota influences brain function (Dinan and Cryan, 2016, El Aidy et al., 2016, Foster et al., 2016). Notwithstanding the fact that the infant gut microbiota has been worldwide considered to be determined after birth, recent evidences have shown how the whole intrauterine environment (placenta, amniotic fluid, meconium and

umbilical cord blood) is actually colonized by specific bacterial species (Collado et al., 2016). These data robustly suggest the transmission of microorganisms from the mother to the fetus ahead of birth and this plays a pivotal role in the determination of the offspring microbiota (Satokari et al., 2009, Aagaard et al., 2014, Koleva et al., 2015, Collado et al., 2016). Transfer of microbes and microbiome metabolites between mother and infant can also occur during delivery and lactation and can be affected by maternal health and metabolism, in addition to mode of delivery and use of antibiotics (Soderborg et al., 2016). Hence, dysbiosis and obesity during pregnancy can dramatically affect microbiota composition of the mother as well as microbial transmission to the fetus (Basu et al., 2011), maternal diet having one of the largest known impacts on these regulations.

The maternal gut microbiota differs in maternal obesity, particularly in the latter half of pregnancy, with overweight women being characterized by an increase in the Firmicutes phylum (Staphylococcus) as well as in some Proteobacteria (Soderborg et al., 2016). Elevated levels of microbe-derived plasma endotoxin appear to be one potential mechanism by which maternal microbes in obesity could affect the developing fetus; this might increase translocation of bacteria-derived products across the intestinal mucosa, contributing to systemic and placental inflammation and insulin resistance (Basu et al., 2011).

Maternal obesity or excess gestational weight gain have been linked to inflammation in the placenta. Although the specific mechanisms through which the bacterial transmission is carried out are not currently well known, a new concept which is developing is that the placenta microbiota may be the intermediary of the microbial passage from the mother to the baby and may affect fetal development (Pelzer et al., 2017). An important subject for future studies is the role of placenta microbiota and its interaction with other maternally-derived variables, including life style, diet, BMI and pregnancy complications, which could all alter placental microbiota (Pelzer et al., 2017).

Role of oxidative stress

OS characterizes biological systems in aerobic conditions; it results from an unbalance between pro-oxidant and anti-oxidant molecules, with oxidants overriding the defense system of the organism. During normal healthy pregnancy, OS and its mediators – reactive oxygen species (ROS) – are increased within certain boundaries as the result of a physiological mild inflammatory state, to stimulate cell proliferation and proper fetal development (Dennery, 2007, Mannaerts et al., 2018). However, if OS overrides safety levels, complications might arise both for the mother and the offspring (Hracsko et al., 2008, Edlow, 2017, Mannaerts et al., 2018). OS is increased in many different pathological conditions including obesity, T2D and metabolic syndrome and can be triggered by chronic consumption of HFD. There is clear evidence for disrupted oxidative signaling in psychiatric disorders. Alterations in multiple biomarkers of OS have been observed in ADHD, bipolar disorder, ASDs, depression, and schizophrenia (Hovatta et al., 2010). Markers of oxidative damage to neurons have also been observed in post-mortem samples in several psychiatric diseases. It is of interest that

mitochondrial disorders with a clear genetic origin are also associated with an elevated incidence of psychiatric disorders, especially mood disorders and psychosis. Increased ROS generation might lead to random damage to proteins, lipids and DNA and excessive OS during pregnancy might result in pathological conditions of the placenta, the embryo, and the fetus, also leading to epigenetic changes and altered gene expression in the fetus (Del Rio et al., 2005, Mannaerts et al., 2018). The placenta is a main source of OS and this organ, in obese women, is characterized by greater levels of OS markers when compared to that of lean women (Saben et al., 2014). Edlow and colleagues found an upregulation of genes related to OS response as a result a global gene expression profiling carried out in the amniotic fluid from obese women (Edlow et al., 2014). Intriguingly, among these, Apolipoprotein D, a protein that is highly expressed in the brain and is upregulated in psychiatric conditions and neurological disorders (Sutcliffe and Thomas, 2002, Muffat and Walker, 2010), was found to be increased by nine-fold in fetuses of obese compared with lean women (Edlow et al., 2014, Edlow, 2017).

A growing body of evidence suggest that OS may play a role in the etiology of mood disorders (Hovatta et al., 2010). A number of studies indicates that exposure to chronic stress may perturb the overall body energy balance acting not only on the neuroendocrine system but also on mitochondrial remodeling, affecting OS balance in the body and in the brain (Picard and McEwen, 2014, Picard and McEwen, 2018, Picard et al., 2018). Recent work suggests that isoprostanes (biomarkers of lipid peroxidation) are selectively upregulated in adolescents who have experienced early childhood adversities, suggesting that dysregulation of stress-sensitive systems during early life stages can have persisting, and potentially deleterious, impact on brain structure–function development acting through OS-linked mechanisms (Horn et al., 2019). The mammalian brain is very sensitive to OS insults being characterized by high metabolic rate, poor antioxidant defenses and reduced capacity for cellular regeneration (Floyd and Carney, 1992). Thus, a prenatal insult such as an obesogenic womb, characterized by elevated levels of inflammation and OS, has the potential to affect dramatically the neurodevelopmental programming of the fetus, setting the stage for later life vulnerability to psychiatric disorders.

Preclinical data enlarge and corroborate this body of evidence and the p66Shc−/− mouse model – that our group has thoroughly characterized – provides a striking example of how prenatal HFD feeding might impinge upon OS pathways to affect fetal programming and healthspan (Berry et al., 2007, Berry et al., 2008, Berry et al., 2010, Berry et al., 2012, Berry and Cirulli, 2013). p66Shc is a mammalian gene that regulates apoptosis by increasing intracellular OS and affects lipid metabolism by promoting fat storage (Berniakovich et al., 2008, Trinei et al., 2009). p66Shc is a protein acting specifically in the mitochondrion as a redox enzyme that generates H2O2 whose function is in a cause-effect relationship with that of insulin/IGF1(Berniakovich et al., 2008). Genetically modified mice lacking the p66Shc gene (p66Shc−/−, knock out mice – KO) are resistant to OS insults and to HFD-induced obesity, resulting overall in a healthier and long-lived phenotype, showing greater brain and behavioral plasticity associated to increased central levels of the neurotrophin BDNF (Berry et al., 2007, Berry et al., 2008, Berry et al., 2010, Berry et al., 2012, Berry and Cirulli, 2013). Prenatal exposure to HFD in these KO mice leads to a gender-specific resilience to both stressful and metabolic challenges

(Bellisario et al., 2014). Administration of a HFD during peri-conceptional time and throughout pregnancy resulted, in fact, in reduced body weight at birth and into a greater catch-up, particularly in males, while female offspring showed increased BMI as well as higher leptin levels. By contrast, p66Shc−/− subjects were overall protected (Bellisario et al., 2014), KO females being characterized by improved ability to cope with both metabolic and neuroendocrine stressors and showing enhanced glucose tolerance and insulin resistance. Moreover, while prenatal HFD led to a hyperactive HPA axis in wild type offspring, this was not observed in KO mice (particularly in females) overall suggesting that sex-hormones might play an important part in directing the effects of a metabolically stressful maternal challenge during fetal development (Bellisario et al., 2014). Data on gender differences have been further extended by Edlow and colleagues (Edlow et al., 2016a) who found that maternal obesity results specifically associated with sex-specific differences in fetal size and fetal brain gene expression signatures, males being the most vulnerable sex. In these studies, ROS metabolism was found to be affected by HFD (Edlow et al., 2016a) further strengthening the important role played by OS mediators in maternal obesity.

Translating these preclinical findings to humans, investigating a group of women enrolled from the Helsinki Birth Cohort, we found a long-term increase in p66Shc mRNA in peripheral blood mononuclear cells (PBMC) of old frail subjects born from obese mothers (Berry et al., 2018a, Berry et al., 2018b). Moreover, when these women were stratified according to their BMI, PBMC p66Shc expression levels were reduced in subjects with a BMI ≥30.4 kg/m2 following physical exercise. This piece of data is particularly interesting for a number of reasons. First of all, it confirms a role for oxidative stress in human subjects in the signaling pathway linking maternal obesity to the health outcome in the offspring. Secondly it suggests that maternal obesity may play a role in the long-term programming of the mitochondrial function and metabolism (also through changes in the p66Shc expression) affecting the aging process. Moreover, it indicates that metabolic-challenging stimuli, such as physical exercise, may affect the expression of p66Shc, particularly in obese subjects, pointing to this molecule as a potential new target for therapeutic intervention studies (Berry et al., 2018a, Berry et al., 2018b, Berry and Cirulli, 2013).

Long-lasting effects of maternal obesity - epigenetic signatures

Epigenetic modifications have been proposed as a key causal mechanism linking maternal adiposity and offspring health outcome. Long-term effects of early nutritional experiences are potentially mediated by post-translational modifications of DNA, post-translational modification of histones and non-coding RNAs. One of the most interesting consequences of these mechanisms is that they can account for transgenerational transmission of traits (Szyf et al., 2005, Szyf, 2012, Daskalakis et al., 2013, Bock et al., 2014, Turecki et al., 2014, Provencal and Binder, 2015).

To date, very little work has been performed to determine epigenetic changes in the brains of human offspring born to obese mothers. DNA methylation changes have been reported in cord blood and microRNA levels in

amniotic fluid in human studies of maternal obesity, indicating a potential role for these epigenetic mechanisms in the long-term effects of maternal obesity on the offspring (Godfrey et al., 2017). Cohort studies analyzing BMI extremes in these data sets found associations between maternal BMI and offspring DNA methylation at birth and at 3 years of age. However, more work is needed to study these regulations.

In candidate gene approach studies, one of the most important findings concerns the observation that aryl-hydrocarbon receptor repressor (AHRR) DNA methylation is 2.1% higher in offspring of obese vs. normal weight mothers; as robust links have been found between maternal smoking and offspring AHRR methylation (Reynolds et al., 2015), these observations suggest that methylation of these gene may be involved in the link between multiple adverse conditions, including maternal obesity, on offspring outcomes.

Among the classical epigenetic mechanisms, DNA methylation is an attractive target for investigation because levels of folic acid, a co-factor in the production of the methyl donor methionine, are decreased in the amniotic fluid of obese pregnant women (Mohd-Shukri et al., 2015, Contu and Hawkes, 2017). In clinical studies, maternal depressive symptoms during pregnancy have been found to correlate with increased DNA methylation of the GR (NR3C1) in male infants and to result in decreased BDNF exon IV DNA methylation in both sexes at 2 months of age (Braithwaite et al., 2015). BDNF plays also a critical role in the integration and optimization of behavioral and metabolic responses by acting in the brain and periphery, it increases insulin sensitivity and parasympathetic tone (Cirulli and Alleva, 2009). Low levels of circulating BDNF characterize individuals with obesity and T2D, which implies a main role for this neurotrophin in obesity and metabolism (Mou et al., 2015). While methylation of the NRC31 has been related to prenatal early adversity in numerous studies (van der Knaap et al., 2015), these results indicate that genes involved both in stress responsivity and in feeding behavior are epigenetically regulated, supporting the notion of joint programming of these stress-activated pathways and allowing for more detailed studies of the mechanisms underlying comorbidity of mental and metabolic disorders (Rivera et al., 2015).

Preclinical studies performed in animal models (Vucetic et al., 2010) have indicated decreased DNA methylation in the promoter regions of the mu-opioid receptor genes as well as globally in brain regions associated with reward such as the ventral tegmental area (VTA), prefrontal cortex (PFC) and nucleus accumbens (NAc) of offspring born to obese mothers (Carlin et al., 2013). Supplementation of maternal HFD with methyl donors during gestation and lactation was able to restore some of these effects (Carlin et al., 2013). Prenatal methyl supplementation has also been shown to reverse deficits in motivated behavior in offspring exposed to a HFD indicating that early life is a sensitive time during which dietary methyl donor supplementation can alter PFC-dependent cognitive behaviors (McKee et al., 2017). Differential expression of 37 microRNAs, rather than changes in DNA methylation (Edlow, 2017), have been reported in the brains of embryonic mice born to mothers fed a HFD vs. control diet.

Preventive strategies

As mentioned above, maternal obesity affects multiple interconnected physiological pathways engaged by complex regulations. Thus, addressing diets and dietary and supplementation (particularly antioxidants) might provide a "broad-spectrum" of promising and feasible strategy to prevent/counteract the disruptive effects of the obesogenic womb, especially considering the difficulty in the development of new target-specific pharmacological interventions. In this paragraph we will specifically focus on some of the most promising dietary intervention that include also oral supplementation with the antioxidant N-Acetyl-Cysteine (NAC).

NAC is the rate-limiting substrate in the biosynthesis of glutathione (GSH). It is a ROS scavenger and its clinical efficacy as well as safety have been recently documented in many pathological conditions (see Mokhtari et al., 2017 and references therein for a complete review). NAC is currently one of the most promising targets for neuropsychiatric disorders. Its most interesting feature is that its efficacy appears cross-diagnostic (acting on neurotransmitter systems such as glutamate, antioxidants as well as inflammatory pathways, see Berk et al., 2013), while being relatively safe. Determining precisely how antioxidants – in particular NAC -work is crucial both to understand the basic biology of mental disorders as well as to devise adjunctive therapies that can act on these pathways. Indeed, the apparent universality of NAC action is intriguing and implies that it may target downstream pathways underlying co-morbidity between stress and metabolic responses. Although there is only preliminary data of the efficacy of NAC in many of psychiatric disorders, the field is expanding with many additional trials that could be of interest also in the field of maternal obesity pathways (Berk et al., 2013).

So far, some preclinical studies have focused on the effects of this molecule in the context of maternal obesity. NAC administration during pregnancy in mice might counteract apoptosis and ROS-related genotoxicity by increasing glutathione levels and decreasing mitochondrial membrane depolarization (Amin et al., 2008). Indeed, mice lacking the rate-limiting enzyme for GSH synthesis show a range of behavioral disorders and treatment with NAC reverses some of these deficits, restoring GSH levels (Berk et al., 2013). NAC may also contribute to maintain oxidative balance through the action of the cysteine/cystine cycle (Elshorbagy et al., 2012). We have recently observed that the administration of NAC in animal models throughout pregnancy is able to buffer the effects of HFD on both the mother and the offspring. In our studies, offspring of mice supplemented with NAC during fetal life showed improved glucose tolerance, in addition to a reduced activation of the HPA axis when exposed to stress (Berry et al., 2018a, Berry et al., 2018b). Worth noticing, results obtained through in vivo NAC supplementation parallel the protective effects observed in the p66Shc−/− mice (a mouse model of reduced oxidative stress; see above) strengthening the link among metabolic stress experienced during fetal life, fetal programming and OS/mitochondrial pathways (Berry et al., 2018a, Berry et al., 2018b, Berry et al., 2007, Berry and Cirulli, 2013, Giorgio et al., 2012, Hovatta et al., 2010).

In addition to NAC, plant-based-antioxidant-rich diets could protect against free radical production and oxidative damage, potentially preventing obesity and comorbidities. As an example, luteolin supplementation of obese, HFD-fed adult mice has been found to be associated with better cardiometabolic features and reduced inflammatory and oxidative stress markers (Gentile et al., 2018). Research on these diets could extend to the prenatal period in the future.

Poor prenatal diets have been often associated with poorer maternal and offspring mental health outcomes. Thus, the quality of maternal diet represents a potentially important and modifiable target for reducing the risk of mental disorders both in mothers and in the offspring (Lindsay et al., 2017). To this regard, longitudinal studies in clinical populations are warranted in order to provide further insights for the development and design of future and more effective intervention trials for improved maternal and child health outcomes (Lindsay et al., 2017). So far, observational data in humans has pointed to polyunsaturated fatty acids (PUFAs), including omega-3 and omega-6 fatty acids, as possible candidate therapeutics in maternal obesity. In particular, in human pregnancy studies data suggest that omega-3 PUFA may play a critical role not only on fetal neurodevelopment, but also for supporting positive maternal mood, decreasing stress, anxiety and depression in the pre- and postnatal periods (Lindsay et al., 2017). Maternal omega-3 fatty acid deficiency has been associated with increased risk of offspring ASDs and ADHD. Human pilot studies relying on the supplementation of obese pregnant women with omega-3 fatty acids demonstrated a reduction in maternal and placental inflammation (see Lindsay et al., 2017 for a review).

In preclinical studies it has been shown that prenatal exposure to dietary omega-3 fatty acids may provide key relationships between metabolic signals and BDNF methylation, creating an epigenetic memory and a reservoir of neuroplasticity that can protect the brain against later insults (Tyagi et al., 2015). In animal models, early life methyl donor supplementation has been used as a strategy to alter one carbon metabolism and DNA methylation in a sex-dependent manner (McKee et al., 2017). In particular, supplementation of methyl donors in HFD fed dams has been able to attenuate weight gain and decrease fat preference (males) as well as to change gene expression and global hypomethylation in the brain of the offspring (McKee et al., 2017). A methyl-balanced diet can also prevent the effects of prenatal stress on binge eating behavior (Schroeder et al., 2017). In addition, early micronutrient supplementation can protect from early life stress-induced cognitive impairments, thus reinforcing the notion of common pathways that are engaged by early nutrition and early life stress (Naninck et al., 2017).

We have previously argued that the negative effects of maternal obesity on mental disorders in the offspring may be mediated, at least in part, by gut dysbiosis. Changes in the microbiome and its metabolites offer the opportunity for non-invasive risk-screening and risk-reduction by tailored dietary formulations and diets, since earliest stages of life. Changing the composition of the gut microbiota through the use of probiotics and prebiotics could become a new strategy for reducing the risk of metabolic disorders in both the mother and offspring. In order to do this, it is necessary to identify clinically relevant markers and build early predictive models, which might help tailoring appropriate dietary interventions. Dietary interventions aimed at modifying

gut microbiota development and composition in the first phases of life may promote cognitive and emotional development. The use of pre- and probiotics has been shown to rapidly modify the microbial community and reduce (at least temporarily) adiposity and chronic inflammation in animal models of obesity and in limited human studies. As far as clinical studies are concerned, there have been only a handful of randomized clinical trials (RCTs) that examined the effects of probiotics administered during pregnancy with the aim of improving insulin sensitivity and reducing gestational diabetes mellitus diagnosis, with limited success. In one RCT either Bifidobacterium lactis alone or B. lactis plus Lactobacillus rhamnoses GG probiotic was administered to pregnant women 14 days before a scheduled Caesarean delivery (Baldassarre et al., 2018). Modulation of the gut microbiota by prebiotics, probiotics or by fecal microbiota transplantation has been used in the management of certain neurological disorders, including ASDs and depression, as well as their associated gastrointestinal symptoms, and increasing numbers of clinical trials suggest the beneficial effects of such treatments. Fermentable fiber influences gut microbiota composition and the production of short chain fatty acids; these, in turn, have been demonstrated to affect mood in animal models, as well as influence neurotransmitters such as serotonin although additional studies are needed to determine the role of different microbes in the physiology of the host and pathogenesis, as well as how the gut microbiota can be modulated for beneficial effects. Most of the interventions used mainly concern the prenatal phases, but they could be extended postnatally. It is rather difficult to discern the effects of prenatal maternal obesity to maternal obesity during lactation. Based upon an animal model, as an example, we were able to show that mice fed a HFD during pregnancy (and not lactation) still showed inappropriate maternal behavior after parturition (Bellisario et al., 2015). There is evidence from epidemiological studies that overweight and obese women are less likely to breastfeed than normal weight women (Amir and Donath, 2007).

It is especially important to stress here is that all the data above mentioned have to be taken with due caution given the relative paucity and high levels of heterogeneity of the studies present in the literature. In particular, it is imperative that further controlled trials be performed in order to determine the type of antioxidants vs pre- and probiotics and the most appropriate dose.

Based upon the above-mentioned considerations, and data gathered through RCTs, we can draw some hypothetical scenarios on the proper timing of an ideal intervention and their possible consequences. We expect that the negative effects of being exposed to maternal obesity might manifest themselves at different time points throughout life, also in relation to a second hit (being this a stressful event or another metabolic challenge), starting from the early developmental phases to adolescence, up until aging. We hypothesize different potential windows of intervention: the earlier we intervene the greater and long-lasting the benefit. Ideally, the timing of the intervention should be set during brain development (before critical periods are closed) and hopefully before the end of adolescence/puberty. Adolescence is a critical time for brain and behavioral development ultimately leading to the transition from childhood to adulthood. This peculiar time is characterized by a massive neuronal remodeling powerfully shaped by hormones, thus representing a very vulnerable time in life as well as a window of opportunity. With late interventions (middle-age or old age) we

might still be able to act on residual neuronal plasticity, ameliorating the outcome of neurodegenerative disorders such as AD or PD (see Fig. 2).

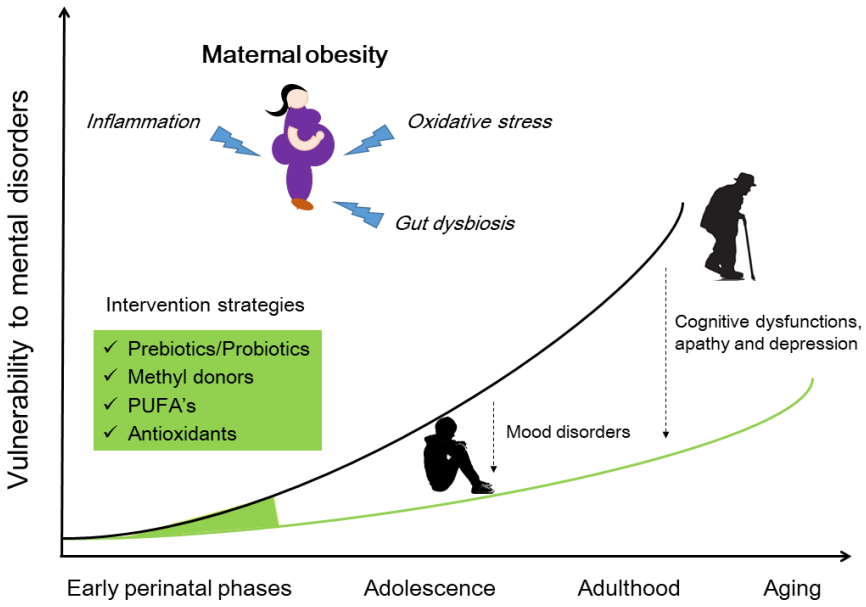


Fig. 2. Hypothetical scenarios on the proper timing of the interventions and their possible consequences. The ability of the organism to cope with the sequelae of the obesogenic womb (oxidative stress, gut dysbiosis and inflammation) decreases throughout life as a result of the physiological decrease in neuronal and behavioral plasticity. Different windows of intervention may be foreseen. Earlier timing (perinatal phases until adolescence) may lead to greater and longer-lasting benefits, later interventions (adulthood until aging) may be able to act only on residual neuronal plasticity ameliorating the outcome of age-related neurodegenerative disorders. The earlier the intervention (green area within the curves) the greater the potential to reduce disease risk later in life (dashed arrows). Figure modified from Godfrey KM, Gluckman PD, Hanson MA. Developmental origins of metabolic disease: life course and intergenerational perspectives. Trends in endocrinology and metabolism: TEM. 2010;21(4):199–205.

Although initial research linking prenatal development with major non-communicable disorders in later life focused on the effects of fetal undernutrition, nowadays it is rather maternal obesity and its sequelae for metabolic and mental health that poses serious public health concerns throughout the world (Jehn and Brewis, 2009, Congdon, 2019). Indeed, an ever-increasing body of epidemiological data – that are corroborated by preclinical evidence – suggests that exposure to an obesogenic womb might strongly impact offspring healthspan throughout life, setting the stage for increased vulnerability to a number of pathological conditions including obesity, CVD, stroke, T2D and asthma (just to mention few), in addition to neuropsychiatric disorders. As recently pointed out by Godfrey and colleagues (Godfrey et al., 2017), there is a need of thorough large-scale studies tackling, for example, the differential impact played by maternal diet and maternal obesity as well as maternal obesity during pregnancy vs obesity during the lactation period. There may be a mix of psychological, behavioral and cultural factors underlying such effects.

These studies should be performed including populations differing in terms of culture as well as ethnicity and collecting detailed data on the cognitive and behavioral phenotype of both parents and offspring; in addition, a comprehensive assessment of diet and of measures of adiposity should be performed (Godfrey et al., 2017).

From an evolutionary perspective, foraging behavior in harsh environments, a condition opposed to maternal obesity and characterized by limited and intermittent food availability, has most likely contributed to shape our brain to deal more effectively with spatial navigation, decision-making, social relationships and even creativity (Berry and Cirulli, 2013, Mattson, 2019). Globalization and urbanization, by favoring sedentary lifestyle and the consumption of high-caloric foods, are gradually leading to a change in the energetic niche experienced both prenatally and early postnatally by humans with consequences that are hard to predict. The ultimate outcome of such a metabolic shift is now starting to show an impact on pre- as well as post-natal developmental trajectories with potential disruptive effects on cognitive processes and susceptibility to mental disorders. Epigenetic changes and dysbiosis linked to such processes have the potential to propagate these modifications to future generations.

While public health countermeasures that can rapidly break the vicious cycle of maternal and offspring obesity are mandatory, we are definitively in need of basic knowledge and appropriate longitudinal, controlled studies that can address the basic mechanisms underlying such regulation, allowing for appropriate intervention but, more importantly, indicating appropriate biomarkers and effective preventive measures.

Acknowledgments

We kindly acknowledge Luigia Cancemi and Stella Falsini for literature search and EndNote bibliography.

Funding

This work was supported by the European Union's Horizon 2020 Research And Innovation Programme under Grant agreement No 633589 (Aging with Elegans), ERANET-NEURON JTC 2018 (Mental Disorders) Project "EMBED" and JPI HDHL-INTIMIC-085-GUTMOM project within the ERA-NET Cofund HDHL-INTIMIC (INtesTInal MICrobiomics) programme.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J (2014) The placenta harbors a unique microbiome. Sci Transl Med 6:237ra265.

Alfaradhi MZ, Ozanne SE (2011) Developmental programming in response to maternal overnutrition. Front Genet 2:27.

Amir LH, Donath S (2007) A systematic review of maternal obesity and breastfeeding intention, initiation and duration. BMC Pregnancy Childbirth 7:9.

Armani A, Berry A, Cirulli F, Caprio M (2017) Molecular mechanisms underlying metabolic syndrome: the expanding role of the adipocyte. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 31:4240-4255.

Baird J, Osmond C, MacGregor A, Snieder H, Hales CN, Phillips DI (2001) Testing the fetal origins hypothesis in twins: the Birmingham twin study. Diabetologia 44:33-39.

Bale TL, Baram TZ, Brown AS, Goldstein JM, Insel TR, McCarthy MM, Nemeroff CB, Reyes TM, et al. (2010) Early life programming and neurodevelopmental disorders. Biol Psychiatry 68:314-319.

Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS (1993) Fetal nutrition and cardiovascular disease in adult life. Lancet 341:938-941.

Barker DJ, Osmond C (1986) Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet 1:1077-1081.

Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ (1989) Weight in infancy and death from ischaemic heart disease. Lancet 2:577-580.

Basatemur E, Gardiner J, Williams C, Melhuish E, Barnes J, Sutcliffe A (2013) Maternal prepregnancy BMI and child cognition: a longitudinal cohort study. Pediatrics 131:56-63.

Basu S, Haghiac M, Surace P, Challier JC, Guerre-Millo M, Singh K, Waters T, Minium J, et al. (2011) Pregravid obesity associates with increased maternal endotoxemia and metabolic inflammation. Obesity (Silver Spring) 19:476-482.

Bateson P (2001) Fetal experience and good adult design. International journal of epidemiology 30:928-934.

Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J (2014) The placenta harbors a unique microbiome. Sci Transl Med 6:237ra265.

Alfaradhi MZ, Ozanne SE (2011) Developmental programming in response to maternal overnutrition. Front Genet 2:27.

Amir LH, Donath S (2007) A systematic review of maternal obesity and breastfeeding intention, initiation and duration. BMC Pregnancy Childbirth 7:9.

Armani A, Berry A, Cirulli F, Caprio M (2017) Molecular mechanisms underlying metabolic syndrome: the expanding role of the adipocyte. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 31:4240-4255.

Baird J, Osmond C, MacGregor A, Snieder H, Hales CN, Phillips DI (2001) Testing the fetal origins hypothesis in twins: the Birmingham twin study. Diabetologia 44:33-39.

Bale TL, Baram TZ, Brown AS, Goldstein JM, Insel TR, McCarthy MM, Nemeroff CB, Reyes TM, et al. (2010) Early life programming and neurodevelopmental disorders. Biol Psychiatry 68:314-319.

Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS (1993) Fetal nutrition and cardiovascular disease in adult life. Lancet 341:938-941.

Barker DJ, Osmond C (1986) Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet 1:1077-1081.

Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ (1989) Weight in infancy and death from ischaemic heart disease. Lancet 2:577-580.

Basatemur E, Gardiner J, Williams C, Melhuish E, Barnes J, Sutcliffe A (2013) Maternal prepregnancy BMI and child cognition: a longitudinal cohort study. Pediatrics 131:56-63.

Basu S, Haghiac M, Surace P, Challier JC, Guerre-Millo M, Singh K, Waters T, Minium J, et al. (2011) Pregravid obesity associates with increased maternal endotoxemia and metabolic inflammation. Obesity (Silver Spring) 19:476-482.

Bateson P (2001) Fetal experience and good adult design. International journal of epidemiology 30:928-934.

Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B, Foley RA, Gluckman P, Godfrey K, et al. (2004) Developmental plasticity and human health. Nature 430:419-421.

Bellisario V, Berry A, Capoccia S, Raggi C, Panetta P, Branchi I, Piccaro G, Giorgio M, et al. (2014) Gender-dependent resiliency to stressful and metabolic challenges following prenatal exposure to high-fat diet in the p66(Shc-/-) mouse. Front Behav Neurosci 8:285.

Bellisario V, Panetta P, Balsevich G, Baumann V, Noble J, Raggi C, Berry A, Seckl J, et al. (2015) High-fat diet during pregnancy acts as a stressor increasing maternal glucocorticoids' signaling to the fetus and disrupting maternal behavior in a mouse model. Psychoneuroendocrinology 61:10-10.

Berk M, Malhi GS, Gray LJ, Dean OM (2013) The promise of N-acetylcysteine in neuropsychiatry. Trends Pharmacol Sci 34:167-177.

Berniakovich I, Trinei M, Stendardo M, Migliaccio E, Minucci S, Bernardi P, Pelicci PG, Giorgio M (2008) p66Shc-generated oxidative signal promotes fat accumulation. J Biol Chem 283:34283-34293.

Berry A, Amrein I, Notzli S, Lazic SE, Bellisario V, Giorgio M, Pelicci PG, Alleva E, et al. (2012) Sustained hippocampal neurogenesis in females is amplified in P66(Shc-/-) mice: An animal model of healthy aging. Hippocampus 22:2249-2259.

Berry A, Bellisario V, Panetta P, Raggi C, Magnifico MC, Arese M, Cirulli F (2018) Administration of the Antioxidant N-Acetyl-Cysteine in Pregnant Mice Has Long-Term Positive Effects on Metabolic and Behavioral Endpoints of Male and Female Offspring Prenatally Exposed to a High-Fat Diet. Front Behav Neurosci 12.

Berry A, Bucci M, Raggi C, Eriksson JG, Guzzardi MA, Nuutila P, Huovinen V, Iozzo P, et al. (2018) Dynamic changes in p66Shc mRNA expression in peripheral blood mononuclear cells following resistance training intervention in old frail women born to obese mothers: a pilot study. Aging Clin Exp Res 30:871-876.

Berry A, Capone F, Giorgio M, Pelicci PG, de Kloet ER, Alleva E, Minghetti L, Cirulli F (2007) Deletion of the life span determinant p66Shc prevents age-dependent increases in emotionality and pain sensitivity in mice. Exp Gerontol 42:37-45.

Berry A, Carnevale D, Giorgio M, Pelicci PG, de Kloet ER, Alleva E, Minghetti L, Cirulli F (2010) Greater resistance to inflammation at adulthood could contribute to extended life span of p66(Shc-/-) mice. Exp Gerontol 45:343-350.

Berry A, Cirulli F (2013) The p66(Shc) gene paves the way for healthspan: evolutionary and mechanistic perspectives. Neurosci Biobehav Rev 37:790-802.

Berry A, Greco A, Giorgio M, Pelicci PG, de Kloet R, Alleva E, Minghetti L, Cirulli F (2008) Deletion of the lifespan determinant p66(Shc) improves performance in a spatial memory task, decreases levels of oxidative stress markers in the hippocampus and increases levels of the neurotrophin BDNF in adult mice. Exp Gerontol 43:200-208.

Bilbo SD, Tsang V (2010) Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 24:2104-2115.

Bock J, Rether K, Groger N, Xie L, Braun K (2014) Perinatal programming of emotional brain circuits: an integrative view from systems to molecules. Frontiers in neuroscience 8:11.

Boersma GJ, Lee RS, Cordner ZA, Ewald ER, Purcell RH, Moghadam AA, Tamashiro KL (2014) Prenatal stress decreases Bdnf expression and increases methylation of Bdnf exon IV in rats. Epigenetics 9:437-447.

Braithwaite EC, Kundakovic M, Ramchandani PG, Murphy SE, Champagne FA (2015) Maternal prenatal depressive symptoms predict infant NR3C1 1F and BDNF IV DNA methylation. Epigenetics 10:408-417.

Bruce J, Davis EP, Gunnar MR (2002) Individual differences in children's cortisol response to the beginning of a new school year. Psychoneuroendocrinology 27:635-650.

Brunton PJ, Russell JA (2010) Prenatal social stress in the rat programmes neuroendocrine and behavioural responses to stress in the adult offspring: sex-specific effects. J Neuroendocrinol 22:258-271.

Buss C, Entringer S, Davis EP, Hobel CJ, Swanson JM, Wadhwa PD, Sandman CA (2012) Impaired executive function mediates the association between maternal pre-pregnancy body mass index and child ADHD symptoms. PloS one 7:e37758.

Carlin J, George R, Reyes TM (2013) Methyl donor supplementation blocks the adverse effects of maternal high fat diet on offspring physiology. PloS one 8:e63549.

Casas M, Chatzi L, Carsin AE, Amiano P, Guxens M, Kogevinas M, Koutra K, Lertxundi N, et al. (2013) Maternal pre-pregnancy overweight and obesity, and child neuropsychological development: two Southern European birth cohort studies. International journal of epidemiology 42:506-517.

Chang E, Hafner H, Varghese M, Griffin C, Clemente J, Islam M, Carlson Z, Zhu A, et al. (2019) Programming effects of maternal and gestational obesity on offspring metabolism and metabolic inflammation. Scientific reports 9:16027.

Chaput JP, Doucet E, Tremblay A (2012) Obesity: a disease or a biological adaptation? An update. Obes Rev 13:681-691.

Cirulli F (2017) Interactions between early life stress and metabolic stress in programming of mental and metabolic health. Curr Opin Behav Sci 14:65-71.

Cirulli F, Alleva E (2009) The NGF saga: from animal models of psychosocial stress to stress-related psychopathology. Front Neuroendocrinol 30:379-395.

Cirulli F, Berry A, Alleva E (2003) Early disruption of the mother-infant relationship: effects on brain plasticity and implications for psychopathology. Neurosci Biobehav R 27:73-82.

Cirulli F, Berry A, Alleva E. (2003a) Early disruption of the mother-infant relationship: effects on brain plasticity and implications for psychopathology. Neurosci Biobehav Rev 27:73-82.

Cirulli F, Santucci D, Laviola G, Alleva E, Levine S (1994) Behavioral and hormonal responses to stress in the newborn mouse: effects of maternal deprivation and chlordiazepoxide. Developmental psychobiology 27:301-316.

Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S (2016) Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Scientific reports 6:23129.

Congdon P (2019) Obesity and Urban Environments. Int J Environ Res Public Health 16.

Contu L, Hawkes CA (2017) A Review of the Impact of Maternal Obesity on the Cognitive Function and Mental Health of the Offspring. Int J Mol Sci 18.

Cryan JF (2016) Stress and the Microbiota-Gut-Brain Axis: An Evolving Concept in Psychiatry. Can J Psychiatry 61:201-203.

Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER (2013) The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. Psychoneuroendocrinology 38:1858-1873.

Davari S, Talaei SA, Alaei H, Salami M (2013) Probiotics treatment improves diabetes-induced impairment of synaptic activity and cognitive function: behavioral and electrophysiological proofs for microbiome-gut-brain axis. Neuroscience 240:287-296.

de Kloet ER, Joels M, Holsboer F (2005) Stress and the brain: from adaptation to disease. Nat Rev Neurosci 6:463-475.

De Palma G, Blennerhassett P, Lu J, Deng Y, Park AJ, Green W, Denou E, Silva MA, et al. (2015) Microbiota and host determinants of behavioural phenotype in maternally separated mice. Nature communications 6:7735.

De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, Saad MJ, Velloso LA (2005) Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology 146:4192-4199.

Del Rio D, Stewart AJ, Pellegrini N (2005) A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 15:316-328.

Dennery PA (2007) Effects of oxidative stress on embryonic development. Birth Defects Res C Embryo Today 81:155-162.

Dimmitt RA, Staley EM, Chuang G, Tanner SM, Soltau TD, Lorenz RG (2010) Role of postnatal acquisition of the intestinal microbiome in the early development of immune function. Journal of pediatric gastroenterology and nutrition 51:262-273.

Dinan TG, Cryan JF (2016) Mood by microbe: towards clinical translation. Genome Med 8:36.

Dinan TG, Cryan JF (2017) Microbes, Immunity, and Behavior: Psychoneuroimmunology Meets the Microbiome. Neuropsychopharmacology 42:178-192.

Edlow AG (2017) Maternal obesity and neurodevelopmental and psychiatric disorders in offspring. Prenatal diagnosis 37:95-110.

Edlow AG, Guedj F, Pennings JL, Sverdlov D, Neri C, Bianchi DW (2016) Males are from Mars, and females are from Venus: sex-specific fetal brain gene expression signatures in a mouse model of maternal diet-induced obesity. Am J Obstet Gynecol 214:623 e621-623 e610.

Edlow AG, Hui L, Wick HC, Fried I, Bianchi DW (2016b) Assessing the fetal effects of maternal obesity via transcriptomic analysis of cord blood: a prospective case-control study. BJOG 123:180-189.

Edlow AG, Vora NL, Hui L, Wick HC, Cowan JM, Bianchi DW (2014) Maternal obesity affects fetal neurodevelopmental and metabolic gene expression: a pilot study. PloS one 9:e88661.

Ehlert U (2013) Enduring psychobiological effects of childhood adversity. Psychoneuroendocrinology 38:1850-1857.

El Aidy S, Stilling R, Dinan TG, Cryan JF (2016) Microbiome to Brain: Unravelling the Multidirectional Axes of Communication. Adv Exp Med Biol 874:301-336.

Elshorbagy AK, Smith AD, Kozich V, Refsum H (2012) Cysteine and obesity. Obesity (Silver Spring) 20:473-481.

Entringer S, Buss C, Wadhwa PD (2015) Prenatal stress, development, health and disease risk: A psychobiological perspective-2015 Curt Richter Award Paper. Psychoneuroendocrinology 62:366-375.

Eriksson JG, Sandboge S, Salonen MK, Kajantie E, Osmond C (2014) Long-term consequences of maternal overweight in pregnancy on offspring later health: findings from the Helsinki Birth Cohort Study. Ann Med 46:434-438.

Floyd RA, Carney JM (1992) Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress. Ann Neurol 32 Suppl:S22-27.

Foster JA, Lyte M, Meyer E, Cryan JF (2016) Gut Microbiota and Brain Function: An Evolving Field in Neuroscience. Int J Neuropsychopharmacol 19.

Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M, Burton GJ (2009) Placental efficiency and adaptation: endocrine regulation. The Journal of physiology 587:3459-3472.

Gentile D, Fornai M, Pellegrini C, Colucci R, Benvenuti L, Duranti E, Masi S, Carpi S, et al. (2018) Luteolin Prevents Cardiometabolic Alterations and Vascular Dysfunction in Mice With HFD-Induced Obesity. Front Pharmacol 9:1094.

Giorgio M, Berry A, Berniakovich I, Poletaeva I, Trinei M, Stendardo M, Hagopian K, Ramsey JJ, et al. (2012) The p66Shc knocked out mice are short lived under natural condition. Aging Cell 11:162-168.

Godfrey KM, Reynolds RM, Prescott SL, Nyirenda M, Jaddoe VW, Eriksson JG, Broekman BF (2017) Influence of maternal obesity on the long-term health of offspring. The lancet Diabetes & endocrinology 5:53-64.

Guzzardi MA, Iozzo P, Salonen MK, Kajantie E, Eriksson JG (2016) Maternal adiposity and infancy growth predict later telomere length: a longitudinal cohort study. International journal of obesity 40:1063-1069.

Harris A, Seckl J (2011) Glucocorticoids, prenatal stress and the programming of disease. Hormones and Behavior 59:279-289.

Heiss CN, Olofsson LE (2019) The role of the gut microbiota in development, function and disorders of the central nervous system and the enteric nervous system. J Neuroendocrinol 31:e12684.

Hinkle SN, Schieve LA, Stein AD, Swan DW, Ramakrishnan U, Sharma AJ (2012) Associations between maternal prepregnancy body mass index and child neurodevelopment at 2 years of age. International journal of obesity 36:1312-1319.

Hinkle SN, Sharma AJ, Kim SY, Schieve LA (2013) Maternal prepregnancy weight status and associations with children's development and disabilities at kindergarten. International journal of obesity 37:1344-1351.

Horn SR, Leve LD, Levitt P, Fisher PA (2019) Childhood adversity, mental health, and oxidative stress: A pilot study. PloS one 14:e0215085.

Hovatta I, Juhila J, Donner J (2010) Oxidative stress in anxiety and comorbid disorders. Neuroscience research 68:261-275.

Howell KR, Powell TL (2017) Effects of maternal obesity on placental function and fetal development. Reproduction 153:R97-R108.

Hracsko Z, Orvos H, Novak Z, Pal A, Varga IS (2008) Evaluation of oxidative stress markers in neonates with intra-uterine growth retardation. Redox Rep 13:11-16.

Iozzo P, Holmes M, Schmidt MV, Cirulli F, Guzzardi MA, Berry A, Balsevich G, Andreassi MG, et al. (2014) Developmental ORIgins of Healthy and Unhealthy AgeiNg: the role of maternal obesity--introduction to DORIAN. Obes Facts 7:130-151.

Jehn M, Brewis A (2009) Paradoxical malnutrition in mother-child pairs: untangling the phenomenon of over- and under-nutrition in underdeveloped economies. Econ Hum Biol 7:28-35.

Kandimalla R, Thirumala V, Reddy PH (2017) Is Alzheimer's disease a Type 3 Diabetes? A critical appraisal. Biochim Biophys Acta Mol Basis Dis 1863:1078-1089.

Kelly JR, Clarke G, Cryan JF, Dinan TG (2016) Brain-gut-microbiota axis: challenges for translation in psychiatry. Ann Epidemiol 26:366-372.

Koleva PT, Kim JS, Scott JA, Kozyrskyj AL (2015) Microbial programming of health and disease starts during fetal life. Birth Defects Res C Embryo Today 105:265-277.

Leon DA, Koupilova I, Lithell HO, Berglund L, Mohsen R, Vagero D, Lithell UB, McKeigue PM (1996) Failure to realise growth potential in utero and adult obesity in relation to blood pressure in 50 year old Swedish men. Bmj 312:401-406.

Levine S (1957) Infantile experience and resistance to physiological stress. Science 126:405.

LifeCycle Project-Maternal O, Childhood Outcomes Study G, Voerman E, Santos S, Inskip H, Amiano P, Barros H, Charles MA, et al. (2019) Association of Gestational Weight Gain With Adverse Maternal and Infant Outcomes. JAMA 321:1702-1715.

Lindsay KL, Buss C, Wadhwa PD, Entringer S (2017) The Interplay between Maternal Nutrition and Stress during Pregnancy: Issues and Considerations. Ann Nutr Metab 70:191-200.

Luczynski P, McVey Neufeld KA, Oriach CS, Clarke G, Dinan TG, Cryan JF (2016) Growing up in a Bubble: Using Germ-Free Animals to Assess the Influence of the Gut Microbiota on Brain and Behavior. Int J Neuropsychopharmacol.

Maccari S, Morley-Fletcher S (2007) Effects of prenatal restraint stress on the hypothalamus-pituitary-adrenal axis and related behavioural and neurobiological alterations. Psychoneuroendocrinology 32 Suppl 1:S10-15.

Mannaerts D, Faes E, Cos P, Briede JJ, Gyselaers W, Cornette J, Gorbanev Y, Bogaerts A, et al. (2018) Oxidative stress in healthy pregnancy and preeclampsia is linked to chronic inflammation, iron status and vascular function. PloS one 13:e0202919.

Mansur RB, Brietzke E, McIntyre RS (2015) Is there a "metabolic-mood syndrome"? A review of the relationship between obesity and mood disorders. Neurosci Biobehav Rev 52:89-104.

Mattson MP (2019) An Evolutionary Perspective on Why Food Overconsumption Impairs Cognition. Trends Cogn Sci 23:200-212.

McEwen BS (1998) Protective and damaging effects of stress mediators. N Engl J Med 338:171-179.

McEwen BS (2000) Effects of adverse experiences for brain structure and function. Biol Psychiatry 48:721-731.

McEwen BS, Gray JD, Nasca C (2015) 60 YEARS OF NEUROENDOCRINOLOGY: Redefining neuroendocrinology: stress, sex and cognitive and emotional regulation. The Journal of endocrinology 226:T67-83.

McGowan PO, Meaney MJ, Szyf M (2008) Diet and the epigenetic (re)programming of phenotypic differences in behavior. Brain research 1237:12-24.

McKee SE, Grissom NM, Herdt CT, Reyes TM (2017) Methyl donor supplementation alters cognitive performance and motivation in female offspring from high-fat diet-fed dams. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 31:2352-2363.

McLaughlin KA, Sheridan MA, Tibu F, Fox NA, Zeanah CH, Nelson CA, 3rd (2015) Causal effects of the early caregiving environment on development of stress response systems in children. Proceedings of the National Academy of Sciences of the United States of America 112:5637-5642.

Meaney MJ, Aitken DH, Viau V, Sharma S, Sarrieau A (1989) Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal type II glucocorticoid receptor binding in the rat. Neuroendocrinology 50:597-604.

Menting MD, van de Beek C, Mintjens S, Wever KE, Korosi A, Ozanne SE, Limpens J, Roseboom TJ, et al. (2019) The link between maternal obesity and offspring neurobehavior: A systematic review of animal experiments. Neurosci Biobehav Rev 98:107-121.

Milior G, Lecours C, Samson L, Bisht K, Poggini S, Pagani F, Deflorio C, Lauro C, et al. (2016) Fractalkine receptor deficiency impairs microglial and neuronal responsiveness to chronic stress. Brain, behavior, and immunity 55:114-125.

Miller AL, Lee HJ, Lumeng JC (2015) Obesity-associated biomarkers and executive function in children. Pediatr Res 77:143-147.

Mina TH, Lahti M, Drake AJ, Denison FC, Raikkonen K, Norman JE, Reynolds RM (2017) Prenatal exposure to maternal very severe obesity is associated with impaired neurodevelopment and executive functioning in children. Pediatr Res 82:47-54.

Mina TH, Raikkonen K, Riley SC, Norman JE, Reynolds RM (2015) Maternal distress associates with placental genes regulating fetal glucocorticoid exposure and IGF2: Role of obesity and sex. Psychoneuroendocrinology 59:112-122.

Mohd-Shukri NA, Duncan A, Denison FC, Forbes S, Walker BR, Norman JE, Reynolds RM (2015) Health Behaviours during Pregnancy in Women with Very Severe Obesity. Nutrients 7:8431-8443.

Mou Z, Hyde TM, Lipska BK, Martinowich K, Wei P, Ong CJ, Hunter LA, Palaguachi GI, et al. (2015) Human Obesity Associated with an Intronic SNP in the Brain-Derived Neurotrophic Factor Locus. Cell reports 13:1073-1080.

Moussa HN, Alrais MA, Leon MG, Abbas EL, Sibai BM (2016) Obesity epidemic: impact from preconception to postpartum. Future Sci OA 2:FSO137.

Mrizak I, Grissa O, Henault B, Fekih M, Bouslema A, Boumaiza I, Zaouali M, Tabka Z, et al. (2014) Placental infiltration of inflammatory markers in gestational diabetic women. General physiology and biophysics 33:169-176.

Muffat J, Walker DW (2010) Apolipoprotein D: an overview of its role in aging and age-related diseases. Cell Cycle 9:269-273.

Naninck EF, Oosterink JE, Yam KY, de Vries LP, Schierbeek H, van Goudoever JB, Verkaik-Schakel RN, Plantinga JA, et al. (2017) Early micronutrient supplementation protects against early stress-induced cognitive impairments. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 31:505-518.

Osmond C, Barker DJ, Winter PD, Fall CH, Simmonds SJ (1993) Early growth and death from cardiovascular disease in women. Bmj 307:1519-1524.

Patterson E, Ryan PM, Cryan JF, Dinan TG, Ross RP, Fitzgerald GF, Stanton C (2016) Gut microbiota, obesity and diabetes. Postgrad Med J 92:286-300.

Pelzer E, Gomez-Arango LF, Barrett HL, Nitert MD (2017) Review: Maternal health and the placental microbiome. Placenta 54:30-37.

Picard M, McEwen BS (2014) Mitochondria impact brain function and cognition. Proceedings of the National Academy of Sciences of the United States of America 111:7-8.

Picard M, McEwen BS (2018) Psychological Stress and Mitochondria: A Conceptual Framework. Psychosom Med 80:126-140.

Picard M, McEwen BS, Epel ES, Sandi C (2018) An energetic view of stress: Focus on mitochondria. Front Neuroendocrinol 49:72-85.

Pizzi MA, Vroman K (2013) Childhood obesity: effects on children's participation, mental health, and psychosocial development. Occup Ther Health Care 27:99-112.

Provencal N, Binder EB (2015) The effects of early life stress on the epigenome: From the womb to adulthood and even before. Experimental neurology 268:10-20.

Raikkonen K, Pesonen AK (2009) Early life origins of psychological development and mental health. Scand J Psychol 50:583-591.

Reynolds LM, Wan M, Ding J, Taylor JR, Lohman K, Su D, Bennett BD, Porter DK, et al. (2015) DNA Methylation of the Aryl Hydrocarbon Receptor Repressor Associations With Cigarette Smoking and Subclinical Atherosclerosis. Circ Cardiovasc Genet 8:707-716.

Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, Mele MC (2019) What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms 7.

Rivera HM, Christiansen KJ, Sullivan EL (2015) The role of maternal obesity in the risk of neuropsychiatric disorders. Frontiers in neuroscience 9:194.

Rodriguez A (2010) Maternal pre-pregnancy obesity and risk for inattention and negative emotionality in children. Journal of child psychology and psychiatry, and allied disciplines 51:134-143.

Roseboom TJ (2019) Epidemiological evidence for the developmental origins of health and disease: effects of prenatal undernutrition in humans. The Journal of endocrinology 242:T135-T144.

Roth TL, Sullivan RM (2005) Memory of early maltreatment: neonatal behavioral and neural correlates of maternal maltreatment within the context of classical conditioning. Biol Psychiatry 57:823-831.

Rudolph MD, Graham AM, Feczko E, Miranda-Dominguez O, Rasmussen JM, Nardos R, Entringer S, Wadhwa PD, et al. (2018) Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring. Nature neuroscience 21:765-772.

Saben J, Lindsey F, Zhong Y, Thakali K, Badger TM, Andres A, Gomez-Acevedo H, Shankar K (2014) Maternal obesity is associated with a lipotoxic placental environment. Placenta 35:171-177.

Sanchez CE, Barry C, Sabhlok A, Russell K, Majors A, Kollins SH, Fuemmeler BF (2018) Maternal pre-pregnancy obesity and child neurodevelopmental outcomes: a meta-analysis. Obes Rev 19:464-484.

Sasaki A, de Vega W, Sivanathan S, St-Cyr S, McGowan PO (2014) Maternal high-fat diet alters anxiety behavior and glucocorticoid signaling in adolescent offspring. Neuroscience 272:92-101.

Satokari R, Gronroos T, Laitinen K, Salminen S, Isolauri E (2009) Bifidobacterium and Lactobacillus DNA in the human placenta. Lett Appl Microbiol 48:8-12.

Schroeder M, Jakovcevski M, Polacheck T, Lebow M, Drori Y, Engel M, Ben-Dor S, Chen A (2017) A Methyl-Balanced Diet Prevents CRF-Induced Prenatal Stress-Triggered Predisposition to Binge Eating-like Phenotype. Cell Metab 25:1269-1281 e1266.

Seckl JR (1998) Physiologic programming of the fetus. Clin Perinatol 25:939-962, vii.

Seckl JR (2004) Prenatal glucocorticoids and long-term programming. Eur J Endocrinol 151 Suppl 3:U49-62.

Sherwin E, Rea K, Dinan TG, Cryan JF (2016) A gut (microbiome) feeling about the brain. Curr Opin Gastroenterol 32:96-102.

Soderborg TK, Borengasser SJ, Barbour LA, Friedman JE (2016) Microbial transmission from mothers with obesity or diabetes to infants: an innovative opportunity to interrupt a vicious cycle. Diabetologia 59:895-906.

Sorrells SF, Caso JR, Munhoz CD, Sapolsky RM (2009) The stressed CNS: when glucocorticoids aggravate inflammation. Neuron 64:33-39.

Soubry A, Schildkraut JM, Murtha A, Wang F, Huang Z, Bernal A, Kurtzberg J, Jirtle RL, et al. (2013) Paternal obesity is associated with IGF2 hypomethylation in newborns: results from a Newborn Epigenetics Study (NEST) cohort. BMC Med 11:29.

Stout SA, Espel EV, Sandman CA, Glynn LM, Davis EP (2015) Fetal programming of children's obesity risk. Psychoneuroendocrinology 53:29-39.

Sullivan EL, Riper KM, Lockard R, Valleau JC (2015) Maternal high-fat diet programming of the neuroendocrine system and behavior. Horm Behav 76:153-161.

Sutcliffe JG, Thomas EA (2002) The neurobiology of apolipoproteins in psychiatric disorders. Mol Neurobiol 26:369-388.

Szyf M (2012) The early-life social environment and DNA methylation. Clin Genet 81:341-349.

Szyf M, Weaver IC, Champagne FA, Diorio J, Meaney MJ (2005) Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. Front Neuroendocrinol 26:139-162.

Tenenbaum-Gavish K, Hod M (2013) Impact of maternal obesity on fetal health. Fetal Diagn Ther 34:1-7.

Townshend T, Lake A (2017) Obesogenic environments: current evidence of the built and food environments. Perspect Public Health 137:38-44.

Trinei M, Berniakovich I, Beltrami E, Migliaccio E, Fassina A, Pelicci P, Giorgio M (2009) P66Shc signals to age. Aging (Albany NY) 1:503-510.

Turecki G, Ota VK, Belangero SI, Jackowski A, Kaufman J (2014) Early life adversity, genomic plasticity, and psychopathology. The lancet Psychiatry 1:461-466.

Tyagi E, Zhuang Y, Agrawal R, Ying Z, Gomez-Pinilla F (2015) Interactive actions of Bdnf methylation and cell metabolism for building neural resilience under the influence of diet. Neurobiology of disease 73:307-318.

van der Knaap LJ, Oldehinkel AJ, Verhulst FC, van Oort FV, Riese H (2015) Glucocorticoid receptor gene methylation and HPA-axis regulation in adolescents. The TRAILS study. Psychoneuroendocrinology 58:46-50.

Vucetic Z, Kimmel J, Totoki K, Hollenbeck E, Reyes TM (2010) Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. Endocrinology 151:4756-4764.

Welberg LA, Seckl JR (2001) Prenatal stress, glucocorticoids and the programming of the brain. J Neuroendocrinol 13:113-128.

Westberg AP, Salonen MK, von Bonsdorff M, Kajantie E, Eriksson JG (2016) Maternal body mass index in pregnancy and offspring physical and psychosocial functioning in older age: findings from the Helsinki Birth Cohort Study (HBCS). Ann Med 48:268-274.

White CL, Purpera MN, Morrison CD (2009) Maternal obesity is necessary for programming effect of high-fat diet on offspring. American journal of physiology Regulatory, integrative and comparative physiology 296:R1464-1472.

Wurtman J, Wurtman R (2018) The Trajectory from Mood to Obesity. Curr Obes Rep 7:1-5.

Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, et al. (2012) Human gut microbiome viewed across age and geography. Nature 486:222-227.

CHAPTER 2

Prenatal psychological or metabolic stress increases the risk for psychiatric disorders: the "funnel effect" model

Chiara Musillo [a,b], Alessandra Berry [a]*, Francesca Cirulli [a]

[a] Center for Behavioral Sciences and Mental Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. [b] PhD Program in Behavioral Neuroscience, Department of Psychology, Sapienza University of Rome, Rome, Italy

Neuroscience and Biobehavioral Reviews (2022) 136:104624. doi: 10.1016/j.neubiorev.2022.104624

Abstract

Adverse stressful experiences in utero can redirect fetal brain development, ultimately leading to increased risk for psychiatric disorders. Obesity during pregnancy can have similar effects as maternal stress, affecting mental health in the offspring. In order to explain how similar outcomes may originate from different prenatal conditions, we propose a "funnel effect" model whereby maternal psychological or metabolic stress triggers the same evolutionarily conserved response pathways, increasing vulnerability for psychopathology. In this context, the placenta, which is the main mother-fetus interface, appears to facilitate such convergence, re-directing "stress" signals to the fetus. Characterizing converging pathways activated by different adverse environmental conditions is fundamental to assess the emergence of risk signatures of major psychiatric disorders, which might enable preventive measures in risk populations, and open up new diagnostics, and potentially therapeutic approaches for disease prevention and health promotion already during pregnancy.

Keywords

Pregnancy, Maternal obesity, Placenta, Glucocorticoids, Inflammation, Metabolism, Oxidative stress, Mental health, Animal model, Clinical studies, Major depression.

1. Introduction

Adverse events experienced during early life can affect brain development setting the stage for increased vulnerability to mental disorders. The developmental process is characterized by multiple time windows in which the organism is extremely plastic and sensitive to environmental stimuli. The ability of the organism to shape itself to best fit with the external environment results in an evolutionary advantage, leading to functional adaptations. However, such developmental plasticity provides, at the same time, the ground for increased vulnerability to later life diseases, resulting overall in a double edge sward. Thus, the overall success or detrimental result of the developmental program will depend upon the genetic background of the organism and on the stability of environmental conditions with respect to those that contributed to prime its developmental trajectories and will produce endurable biological modifications in the individual (Bateson et al., 2004; Cirulli and Berry, 2013).

As far as the mammalian brain is concerned, adverse events can impair the maturation of structures involved in the emotional and endocrine responses to stress, eventually increasing the risk for the onset of psychiatric disorders later in life (Agorastos et al., 2019; Calkins and Devaskar, 2011; Cirulli et al., 2003; Cirulli and Alleva, 2009). Research performed over many decades has now provided evidence that early exposure to conditions such as physical or sexual abuse, maltreatment, neglect or separation experienced during childhood or adolescence, results in a variety of negative health outcomes (Cirulli et al., 2009; Heim and Nemeroff, 1999; Hughes et al., 2017; LeMoult et al., 2020; McLaughlin et al., 2019; Pechtel and Pizzagalli, 2011). In particular, it has been observed that social and emotional development, as well as responses to stress, are often dysregulated in children exposed to early adversities contributing to increase vulnerability to psychiatric disorders and anti-social behaviors later in life (Bick and Nelson, 2015). As an example, the Adverse Childhood Experiences project (ACEs - https:// www.acesaware.org/) has been the first large scale study showing the impact of early postnatal life stressors (including physical, emotional, sexual abuse, neglect and many others) on physical and mental health problems in over 17,000 adults (Felitti et al., 1998). More in detail, this study provided evidence for a strong relationship between child exposure to stressful conditions and the long-term effects on health risk factors (smoking, severe obesity, physical inactivity, depressed mood and suicide attempt), incidence of diseases (heart attack, cancer, stroke, chronic bronchitis or emphysema, diabetes) and mortality (Felitti et al., 1998). Notably, Felitti and co-workers reported that the number of adverse events children were exposed to was directly related to the occurrence of adult diseases, suggesting that experiencing multiple traumatic/stressful events might greatly contribute to set the vulnerability for later life diseases (Felitti et al., 1998; Guinosso et al., 2016).

While studies have been most often focusing on the postnatal effects of early adverse experiences on mental health outcomes, it is now clear that exposure to postnatal stress is often a pursuit of pre-existing maternal adverse conditions affecting sensitive developmental windows already during fetal life. Beyond the well-characterized effects of psychological stress, other environmental challenges, such as unbalanced nutritional habits, are emerging as risk factors contributing to thwart the intrauterine environment, and consequently the

developing fetus. To this regard, David Barker's work (see later in this manuscript) gave rise to an entirely new field of study known initially as the "Fetal Origins of Adult Disease - FOAD" and later renamed as the "Developmental Origins of Health and Disease - DOHaD" to include the whole developmental process (from pre-natal to post-natal phases) and also to take into account the effects of stressors of different nature (Entringer et al., 2010; Krontira et al., 2020; Provenzi et al., 2021; Gluckman et al., 2015). As a result of these studies, unbalanced maternal nutritional habits, similarly to early life stressors, came to be recognized over the years as risk factors contributing to threat the intrauterine environment, setting the stage for the onset of psychiatric pathologies in the offspring (al-Haddad et al., 2019; Banderali et al., 2015; Cirulli et al., 2020; Leijser et al., 2018).

In this manuscript we will review studies that indicate that there is now convincing evidence that very different prenatal conditions, such as prenatal maternal stress and maternal obesity can both independently represent risk factors for offspring development and mental health outcomes (Bellisario et al., 2015; Berry et al., 2021, 2018a, 2015; Luoni et al., 2016a, 2014; Panetta et al., 2017).

In order to explain how different environmental conditions can give rise to similar effects on mental health, we will argue that maternal obesity acts as a stressor, thus engaging the same evolutionary conserved response pathways as classical psychological stressors (Bellisario et al., 2015). Glucocorticoids (GCs), in particular, which play a major role in the response to stress, also mobilize energy reserves, exemplifying the main role played by metabolic mediators in the stress response, providing an explanation for the observed co-morbidity between metabolic and psychiatric disorders (Milaneschi et al., 2019). An ever increasing body of evidence reports that systems involved in homeostatic adjustments such as the neuroendocrine, the immuno-inflammatory and the energy metabolism (insulin and leptin) are greatly altered both in psychiatric and metabolic disorders (Milaneschi et al., 2019). Such conditions, and the related alterations, are even more dramatic in the context of pregnancy, contributing to the establishment of a sub-optimal intrauterine environment.

We propose a "funnel effect" model whereby maternal psychological and metabolic stress trigger the same response pathways engaging common biological mediators, increasing vulnerability for psychopathology (Cirulli and Berry, 2013). In this context, the placenta, which is the main mother-fetus interface, appears to facilitate such convergence, re-directing stress signals to the fetus.

This review will provide evidence for comparable mental health outcomes following prenatal exposure to either maternal stressful life events or maternal obesity and discuss the possible biological mechanisms underlying these effects taking into account both clinical and preclinical evidence. Moreover, the proposed "funnel effect" model and the underlying driving forces will be discussed also considering evolutionary adaptations modulating physiological coordinated responses towards adversities.

2. Prenatal adverse events shape brain development

While in the short term, stress-activated responses are essential for the maintenance of homeostasis and survival, over longer time periods, they can become detrimental, resulting in a burden (allostatic load) that affects growth, metabolism, inflammatory/immune and neuroendocrine responses (Cirulli and Berry, 2013; De Kloet et al., 2005; McEwen, 1998). The allostatic load experienced during gestation involves not only the mother, but also the growing fetus, with negative consequences for fetal programming (see Box 1). However, the specific effects of adverse conditions on the growing organism will greatly depend upon the timing of exposure to stress, since different organs show specific sensitivity at different times across pregnancy (Harris and Seckl, 2011). A striking example of the time specificity of the effects of prenatal insults is provided by the studies on the Dutch famine cohort showing that the same kind of stressor is associated to pervasive derangements of metabolic programming or brain development, depending upon the pregnancy trimester of exposure to undernutrition, leading to a higher prevalence of metabolic or psychiatric disorders later in life (Hoek et al., 1998; Roseboom et al., 2006, 2011).

As far as the brain is concerned, stress during pregnancy might greatly affect the development of those structures that will play a role in later life responsiveness to stress and emotional adaptation, setting the stage for vulnerability to psychiatric disorders (Fig. 1). In the following paragraphs, we will specifically focus on the programming effects of maternal stress and maternal obesity on fetal brain development.

Box 1

The mother-fetus dyad: from allostasis to allostatic load

Pregnancy is a time of great plasticity in women's life, characterized by a number of physiological changes that take place from the conception, partially lasting after childbirth. Because of the multitude of complex structural and functional adaptations, pregnancy may be described as a condition of "allostasis" (allostasis = maintaining stability through changes) where both the mother and the fetus cooperate for mutual stability and wellbeing (Khambadkone et al., 2020; Russell and Brunton, 2019). If this fine modulation is perturbed by environmental challenges, pregnancy could lose its dynamic balance resulting into a state of "allostatic load" (McEwen, 1998) that turns the plasticity of the mother-fetus dyad into a risk factor for vulnerability to further insults. As far as environmental challenges are concerned, these can impact both psychophysical and metabolic aspects of the maternal physiology activating signaling pathways that in turn affect fetal growth (Harris and Seckl, 2011). As for example, GCs are essential hormones involved in many physiological functions ranging from metabolic balance to stress responsiveness and mood regulations. During mid- and late pregnancy a physiological rise of maternal GCs levels occurs ensuring the proper maturation of developing fetal organs by promoting cell differentiation (Duthie and Reynolds, 2013; Solano and Arck, 2020). Given the toxic effect deriving from an excessive or prolonged exposure to GCs, such a rise needs to be tightly regulated to prevent negative outcomes in the fetus. Psychological stress, changes in mood or full-blown

psychiatric disorders have been associated with disruptions of the neuroendocrine system function and increased GCs secretion. Thus, during pregnancy, psychiatric conditions, and more in general psychological distress, may shift the balance of mother-fetus dyad into a condition of "allostatic load", representing a great risk factor for fetal neurodevelopment. Likewise, among the several metabolic adaptations occurring during gestation, glucose homeostasis plays a key role for a healthy pregnancy. Indeed, the increase in the fetal demand for glucose supply that accompanies the progression of pregnancy is crucial for the correct development of the growing organism. Such a demand is entirely satisfied by the mother through an increased endogenous glucose production accompanied by a physiological decrease in insulin sensitivity (Armistead et al., 2020). Thus, metabolic stressors such as overnutrition or obesity might greatly challenge the metabolic balance providing also in this case an "allostatic load" in the mother-fetus dyad (Jeyabalan, 2013; Tenenbaum-Gavish and Hod, 2013).

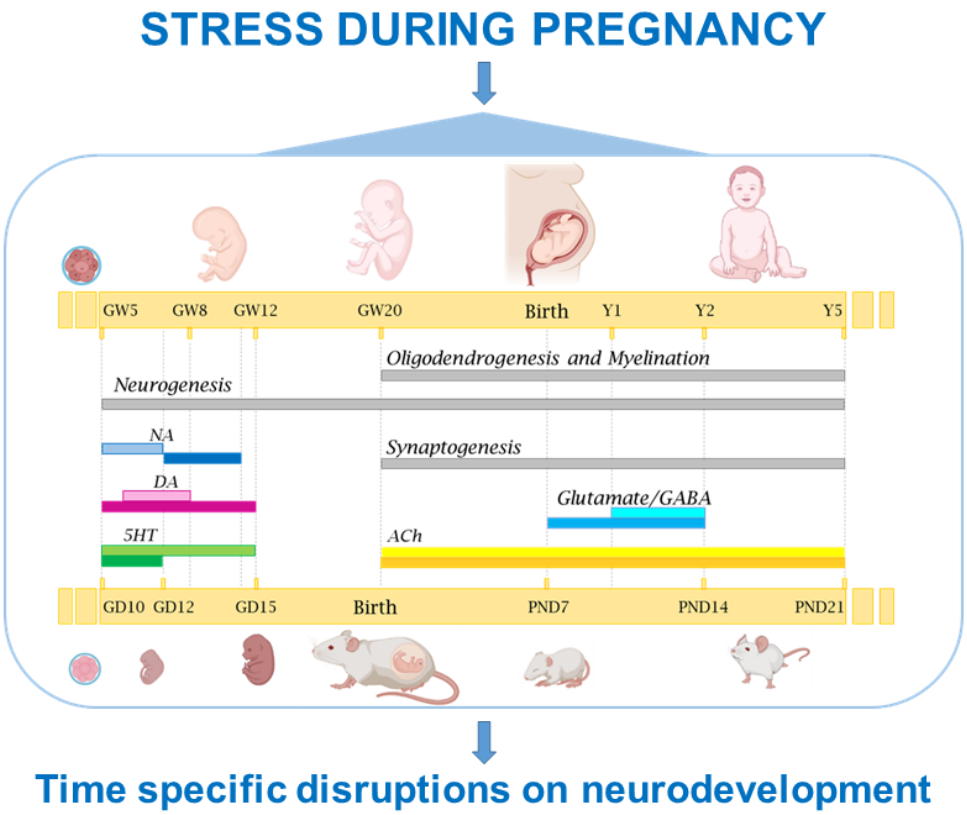


Fig. 1. Time-dependent impact of prenatal stress. Stress during pregnancy might greatly affect brain structures playing a role in stress responsiveness and emotional adaptation, setting the stage for later life vulnerability to psychiatric disorders. Since different brain structures show different sensitivity at different times across development, the magnitude and the impact of early life stress will greatly depend upon the timing of exposure. The cartoon shows a comparison between brain developmental stages in humans and mice. Abbreviations. 5HT: 5-hydroxytryptamine or serotonin; Ach: acetylcholine; DA: dopamine; GABA: gamma-aminobutyric acid; GD: gestational day; GW: gestational week; NA: noradrenalin; PND: post-natal day; Y: year.

2.1. Programming effects of maternal stress on fetal brain development

2.1.1. Human findings

World Health Organization (WHO) reports that worldwide about 10% of pregnant women experience a mental disorder, particularly anxiety and depression, reaching 15.6% in developing countries (htt ps://www.who.int/teams/mental-health-and-substance-use/materna l-mental-health). Clinical studies provide clear associations between maternal mental health issues during pregnancy and an increased risk for neurodevelopmental disorders in the offspring. Besides overt psychiatric conditions, maternal self-perceived feelings of stress, deriving from physical or sexual abuse, maltreatment, low socio-economic status, partner's loss or natural disaster (just to mention few), are sufficient to dramatically affect brain development, increasing the risk for pathologies, such as attention deficit hyperactivity disorder (ADHD), or emotional problems in the offspring (Brannigan et al., 2019; O'Connor et al., 2003; Send et al., 2017; Sosnowski et al., 2018; Van Den Bergh and Marcoen, 2004).

To this regard, retrospective studies initially conceived to investigate the consequences of natural disasters on individual stress perception were also the first to point out the association between stressful events experienced by the mother and neurodevelopmental disturbances in children. As an example, following the accident at the nuclear power plant in Chernobyl in 1986, many children - prenatally exposed to the disaster - were found to be characterized by a higher prevalence of developmental speech-language deficits and emotional disorders (Igumnov and Drozdovitch, 2000; Kolominsky et al., 1999). Such outcomes were associated with parental psychological stress deriving from the evacuation of the contaminated areas and relocation, independently from the effects of the radiations (Igumnov and Drozdovitch, 2000; Kolominsky et al., 1999).

Likewise, the Project Ice Storm (https://www.mcgill.ca/projetverg las/icestorm) provided evidence for the long-term effects of in utero stress exposure on fetal programming (Laplante et al., 2004). In particular, adolescents born from women who were exposed to severe social isolation during pregnancy (due to an ice storm in Quebec) showed increased externalizing behaviors when tested in a psychosocial stress-provoking paradigm, accompanied by elevated cortisol reactivity (Laplante et al., 2008; Yong Ping et al., 2020). Moreover, it has been observed that children born from depressed or anxious mothers showed suboptimal neurobehavioral function and hyperactive cortisol responses (Molenaar et al., 2019; Oberlander et al., 2008; Osborne et al., 2018). Furthermore, the New England Family Study revealed that children (at 4 months and 1 year of age) of those pregnant women experiencing socioeconomic hardship, were characterized by higher risk of neurologic abnormalities with regard to responses to specific stimuli and ratings of sensory and autonomic nervous system function (Gilman et al., 2017).

Epigenetic modifications of genes specifically involved in stress responses, that play also a role in psychiatric conditions, have been observed as a result of prenatal stress (Cao-Lei et al., 2016; Provençal and Binder, 2015; Provenzi et al., 2021; Sosnowski et al., 2018). A striking example is provided by data from the mother-child dyads in the Avon Longitudinal Study of Parents and Children (ALSPAC - htt p://www.bristol.ac.uk/alspac/)

showing that children born from methylated mapping to genes associated with brain development and psychiatric disorders (Viuff et al., 2018). Furthermore, there is evidence for an association between different prenatal stressors and the methylation of genes directly involved in stress response regulation and hypothalamic-pituitary-adrenal (HPA) axis function such as Nr3c1 (that encodes for glucocorticoid receptors - GRs) and its repressor FKBP51 (Fkbp5) (Serpeloni et al., 2019; Sosnowski et al., 2018). To this regard, using a thorough converging evolutionary cross-species genome-wide approach, we have shown that several regions in Morc1, a gene associated with major depressive disorder, were differentially methylated in CD34 + and CD3 + T immune cells in response to early life stress (Nieratschker et al., 2014). Using a similar cross-species approach - involving humans, non-human primates and rodents - we have also provided evidence for DNA methylation of Ank3, a gene associated with psychiatric disorders, as a result of early-life stress (Luoni et al., 2016b). Interestingly, this piece of data was further strengthened by the observation that those people carrying a polymorphism associated to Ank3 are more vulnerable to early life stress (Luoni et al., 2016b).

2.1.2. Preclinical evidence

Investigating the impact of maternal stress on fetal programming in humans is challenging due to difficulty related to the recruitment of stressed children and the retrospective self-reported traumatic history as well as the environmental confounding such as genetic background, maternal smoke/alcohol consumption or nutritional habits, often resulting in discordant results. Moreover, in humans, studies to investigate the influence of early-life stress on psychiatric disorders (with regard to specific epigenetic modifications as well as changes in gene expression) are limited by the lack of access to living brain tissues.

It is well-known that there is a variability in the incidence and the prevalence of psychiatric disorders between men and women however, the identification of specific gender-dependent risk factors is often hampered by the reduced statistical power and the poor compliance to the follow up in clinical studies. Also in this case, animal models provide a great advantage in filling this gap.

In the last decades, a plethora of studies involving animal models has remarkably contributed to expand our knowledge on the effects of early life stress on offspring health outcomes. In fact, animal models offer more controlled experimental conditions and allow the manipulation of very critical times during development (Cirulli et al., 2009, 2003; Maccari et al., 2014). As for prenatal stress, a wide number of paradigms - applied during pregnancy - have been developed ranging from restraint stress to chronic unpredictable mild stress or social stress (just to mention a few) involving the use of the most common laboratory rodents (mice and rats). Interestingly, regardless of the species and the paradigm used, negative long-lasting health outcomes dealing with increased anxiety- and depressive-like behaviors as well as impaired cognitive performance have been reported in the offspring. Moreover, altered physiological balance of brain inflammatory markers and oxidative stress (OS) mediators have been also reported in association with the afore-mentioned behavioral phenotype in a sex-dependent fashion (Darnaud´ery and Maccari, 2008; Vall´ee et al., 1999, 1997; Weinstock, 2017). Worth to notice, many of these features have been associated with permanent impairments of the HPA axis functionality. More in detail, prolonged corticosterone responses to stress were observed in adult rodents,

complemented by reduced levels of both GRs and mineralocorticoid receptors (MRs) in the hippocampus, overall affecting coping strategies towards stress throughout life (Brunton and Russell, 2010; Cirulli and Berry, 2013; Henry et al., 1994; Koehl et al., 1999).

A number of studies has found alterations in brain levels of Insulin-like Growth Factor (IGF), a trophic factor that plays a pivotal role in the development of the central nervous system, modulating neurogenesis, axonal growth and synaptogenesis. More in detail, a reduction of IGF upon prenatal stress was found to be associated with long-term impaired cognitive abilities and increased levels of plasma corticosterone in rats (Basta-Kaim et al., 2014; Guan et al., 2021).

Our research group has greatly contributed to identify specific derangements in brain development resulting from prenatal restraint stress in male and female rat offspring from the early postnatal phases throughout adolescence and adulthood. Overall, we found that prenatal stress decreased the expression levels of GRs to the same extent in adult male and female rats both the in prefrontal cortex and the hippocampus (Luoni et al., 2016a). In addition, we have shown that prenatally stressed rats were characterized by a reduction of affiliative behaviors and increased social anxiety during adolescence, a time in life when sociability plays a pivotal role in brain maturation and in the development of social skills (Berry et al., 2015). Such behavioral phenotype was associated with decreased levels of Brain-Derived Neurotrophic Factor (BDNF), a neurotrophin involved in neuronal plasticity as well as in the modulation of emotional and cognitive functions (Berry et al., 2015). In particular, changes in the expression levels of Bdnf turned out to be very sensitive to prenatal stress, being tightly modulated in different brain areas, according to sex and age (Luoni et al., 2014).

More recently, we have provided evidence that prenatal stress may increase the vulnerability to metabolic stressors. In particular, when challenged at adulthood with a metabolic insult (high-fat diet - HFD), male offspring of stressed mothers were characterized by decreased glucose tolerance and insulin sensitivity as well as by changes in eating behavior (Panetta et al., 2017). The metabolic challenge also resulted in increased anxiety and increased levels of pro-inflammatory cytokines such as Il-1β, Tnf-α and Il-6 in the dorsal and ventral hippocampus and in the prefrontal cortex (Berry et al., 2021). This piece of data is quite intriguing since it indicates that stressors as diverse as psychophysical and metabolic challenges converge on common signaling pathways involved in the regulation of mood and metabolism in a sex-dependent fashion.

2.2. Programming effects of maternal obesity on fetal brain

2.2.1. Human findings

Metabolic stress experienced during pregnancy also deserves to be listed as an adverse condition able to permanently alter fetal programming (Lippert and Brüning, 2021). To this regard, the pioneering studies of Barker and colleagues paved the way for this field of research (Barker and Osmond, 1986). Briefly, Barker observed that the regions in England showing the highest rates of infant mortality due to low birth weight were

also characterized by the highest rates of mortality from cardiovascular diseases. These observations led to the hypothesis that those babies who survived, despite their reduced body weight, developed a greater vulnerability to metabolic disorders later in life (Barker, 2007; Barker and Osmond, 1986). Interestingly, the relationship between reduced birth weight and the development of diseases was found to be dependent from maternal undernutrition during pregnancy, unveiling the powerful role of metabolism as a potential stress. Findings from a cohort study of infants born during the Dutch famine (1943–1945) strengthened and enlarged this body of evidence showing that prenatal metabolic stress not only shapes metabolism but also mental health in the offspring, depending upon the specific time windows of exposure to undernutrition (Roseboom et al., 2011). In fact, while any time of gestation can be susceptible to metabolic programming, perturbation of early developmental phases results in an increased vulnerability to psychiatric disorders such as schizophrenia and depression, increased responsiveness to stress and low cognitive performances (Hoek et al., 1998; Roseboom et al., 2006, 2011).

Very interestingly, a recent study on the Scottish Aberdeen Maternity and Neonatal Databank (https://www.abdn.ac.uk/iahs/research/obsgy nae/amnd/) has shown that both low as well as high maternal body mass index (BMI) are associated with offspring mental disorders (Lahti- Pulkkinen et al., 2021) suggesting that both undernutrition and overnutrition during pregnancy can alter fetal developmental trajectories.

We are currently experiencing times characterized by a progressive reduction of physical activity and increased consumption of low-cost ultra-processed high-caloric food, strengthening the foundation for the settlement of an obesogenic environment (Townshend and Lake, 2017). In this context, overnutrition and obesity during pregnancy are emerging as pressing public health issues since they have been associated with adverse health outcome in the offspring (Congdon, 2019; Kearney, 2010; Khambadkone et al., 2020; Ng and Popkin, 2012; Rosenheck, 2008). Accordingly, large-scale population studies provide evidence for an association between high maternal BMI before and during pregnancy and an increased risk for the development of neuropsychiatric and mood disorders (Cirulli et al., 2020; Edlow, 2017; Kawai et al., 2004; Schaefer et al., 2000). To this regard, recent systematic reviews and meta-analysis have showed that children born from overweight or obese mothers are characterized by higher risk for neurodevelopmental disorders including ADHD and autism spectrum disorders (ASDs), cognitive delay and behavioral problems (Kong et al., 2020; Sanchez et al., 2018). In addition, in the Western Australian Pregnancy Cohort Study (https://rainestudy.org.au/research-pa per/the-western-australian-pregnancy-cohort-raine-study-generation- 2/) it was found that children and adolescents born from overweight and obese mothers were at higher risk to develop persistent depressive symptoms (Robinson et al., 2013). An ever increasing body of evidence is indicating that children of obese women are characterized by an increased risk of schizophrenia, ASDs and cognitive delay (Aye et al., 2014; Brown et al., 2004; Buka et al., 2001; Goines et al., 2011; Van Der Burg et al., 2016).

Epigenetic mechanisms may underly developmental programming. Several clinical studies indicate that genome-wide DNA methylation is significantly affected in offspring born to overweight and obese mothers

(Morales et al., 2014). In fact, it is well-known that nutrition can directly affect one-carbon metabolism, which provides the methyl groups in the reactions involved in DNA methylation (Clare et al., 2019; Yajnik and Deshmukh, 2012). Thus, unbalanced diets leading to any excess or deficiency in the number of the methyl group donors can disrupt one-carbon metabolism resulting in embedded changes in the epigenome methylation. Substantial evidence supports the involvement of one-carbon cycle dysregulation in psychiatric disorders including depression and ASDs, possibly affecting the expression of key genes involved in the regulation of emotional and cognitive behavior (Sugden, 2006). An important co-factor in the production of the methyl donor methionine is the folic acid. Interestingly, Contu and Hawkes have provided evidence for obese pregnant women being characterized by lower levels of folic acid in the amniotic fluid (Contu and Hawkes, 2017). Moreover, changes in DNA methylation have been reported also in cord blood of children born to obese mothers, supporting the role of maternal obesity in altering methylation patterns (Contu and Hawkes, 2017; Nardelli et al., 2014).

2.2.2. Preclinical evidence

As far as gestational metabolic stressors are concerned, animal models of maternal obesity have been developed and widely studied. Different paradigms are commonly employed, ranging from the exposure to purely HFD to high-fat/high-sugar diets (the so called "Western diet" or "cafeteria diet") at different times during gestation or even starting largely from preconception. In our experience, maternal overweight, without overt obesity, is already sufficient to redirect fetal developmental trajectories leading to metabolic and behavioral phenotype alterations avoiding unwanted perinatal complications characterising maternal obesity. To this regard, we have clearly demonstrated that administration of a HFD before and during pregnancy, for a total of 13 weeks (from pre-conception until delivery), in C57Bl/6 mice substantially alters the intrauterine milieu with severe consequences for the developing fetus (Bellisario et al., 2015). Indeed, when the phenotype of the offspring was thoroughly characterized, we observed long-lasting and sex-dependent effects. In particular, prenatal HFD resulted in reduced body weight at birth and in a greater catch-up, particularly in male subjects at adult age, in association to increased anxiety-related behaviors in the elevated plus maze test; moreover, female offspring was characterized by impaired feedback response of the HPA axis activation following an acute restraint stress challenge (Bellisario et al., 2014). We have further shown that in this same model, prenatal exposure to HFD is able to reduce antioxidant defenses in the hypothalamus of male offspring, an effect that can be prevented by prenatal administration of the antioxidant N-acetyl-cysteine (NAC) (Berry et al., 2018a), suggesting that changes in OS underly these long-term effects and that the redox balance should be tightly regulated for a proper brain development.

Winther and co-workers observed that prenatal HFD led to anxiogenic behaviors in the offspring, increasing the time spent in the closed arms in the elevated plus maze (Winther et al., 2018). Moreover, Niu and co-workers found increased corticosterone and corticotrophin-releasing hormone levels both under basal conditions as well as after an acute challenge (restraint stress) upon prenatal HFD (Niu et al., 2019).

Interestingly, the effects of such metabolic stress on neuroendocrine fetal programming are rather long-lasting since Balsevich and colleagues observed an upregulation of both Nr3c1 and Fkbp5 in the paraventricular nucleus of the hypothalamus also in mice at middle-age (Balsevich et al., 2016).

There is also some evidence showing that intrauterine exposure to a HFD leads to poor cognitive performance in adulthood, affecting object recognition memory and spatial memory compared to offspring of chowfed dams (Cordner et al., 2019). However, a recent meta-analysis has pointed out the high heterogeneity in studies investigating the effects of maternal obesity on cognitive abilities in the offspring, often leading to inconsistent results, highlighting the need for further studies (Menting et al., 2019).

In rodents the exposure to a HFD during fetal life leads to alterations of the IGF pathway in the hypothalamus, a central regulator of feeding and mood, leading to central insulin resistance with more pronounced effects in male offspring, suggesting a common substrate that might link emotionality and metabolic balance (Dearden et al., 2020; Gomes et al., 2018; Schellong et al., 2019).

Also, immune modulation is impaired in animal models of maternal obesity. Indeed, it has been shown that HFD consumption during pregnancy results in an exacerbated inflammatory profile in the offspring (Bordeleau et al., 2020). More in detail, neonatal and adolescent offspring of obese dams showed an increased expression of microglial activation markers together with altered microglial morphology in the hippocampus, under basal conditions as well as in response to a lipopolysaccharide challenge (Bilbo and Tsang, 2010; Bordeleau et al., 2020). All these feature were accompanied by impaired acquisition and retention in a spatial navigation task as well as increased emotional reactivity in males (Bilbo and Tsang, 2010). These data suggest that inflammatory pathways are tightly regulated also in association with metabolic challenges and might affect fetal brain programming.

3. The “funnel effect” model: shared mechanisms underlying vulnerability to psychiatric disorders

Mental health problems and obesity are widespread conditions with major individual and public health concerns. Depression and anxiety during pregnancy affect about 10–15% of women, while maternal obesity affects 30% of pregnant women. As already mentioned, the mother and the fetus are a dyadic and integrated system, characterized by a state of “allostasis”, being particularly vulnerable to environmental challenges. Thus, alterations in the maternal physiology associated with psychological or metabolic stress result in an allostatic load with pervasive consequences on fetal brain programming. So far, we have provided clinical and preclinical evidence for similar mental health outcomes in the offspring as a result of both maternal obesity and maternal psychological stress, with regard to increased risk for neurodevelopmental and psychiatric disorders, as well as altered ability to cope with stress later in life (Kong et al., 2020; Molenaar et al., 2019; Oberlander et al., 2008; Robinson et al., 2013; Sanchez et al., 2018). Despite this evidence, there is a great need to understand the precise mechanisms of action through which different prenatal stressors can similarly

affect brain developmental trajectories. To this regard, it is important to underline that the behavioral outcomes observed in the offspring were found to be often associated with a dysregulated HPA axis, in addition to altered inflammatory responses and metabolic derangements, suggesting a main role for these system in brain development during fetal life.

Indeed, both psychiatric conditions, such as depression, and metabolic dysfunction, such as those characterizing obesity, show common features dealing with alterations in HPA axis function, immunoinflammatory activation and energy metabolism regulation including changes in leptin and insulin (Milaneschi et al., 2019). Such alterations are even more disruptive when occurring during pregnancy, a time characterized by multiple physiological adjustments aimed at supporting the growing fetus, greatly affecting the intrauterine environment. Thus, we hypothesize that whatever the maternal stress (psychological, metabolic) the effects will converge on the activation of shared signaling pathways ultimately affecting brain development through a "funnel effect" model where the placenta plays a key role (Fig. 2). More in detail, we suggest that the same homeostatic systems, that are found to be profoundly dysregulated in obese mothers, as well as in pregnant women suffering by severe stress or psychiatric disorders, might be responsible to prime those brain circuits involved in the regulation of emotion and stress responsiveness setting the stage for an increased vulnerability for mental health.

In this context, the placenta might play a pivotal role as a sensor of maternal stressors conveying them to the fetus. The placenta is a unique temporary organ in that it develops by the direct interaction between maternal and fetal tissues, representing a physical link between maternal physiology and the growing fetus. Placental formation starts early right after conception (6–7 days in humans) and progresses through continuous adaptations in order to support fetal growth and wellbeing. It provides oxygen and nutrients, but also regulates the transfer of neuroendocrine and immune mediators as well as metabolic factors in order to protect the fetus from excessive exposure to stress hormones and growth-regulating factors as well as pathogens (Behura et al., 2019; Khambadkone et al., 2020; Turco and Moffett, 2019). Both psychophysical and metabolic stress during pregnancy can disrupt the protective placental function with important consequences on proper fetal brain development (Khambadkone et al., 2020).

As far as neuroendocrine mechanisms are concerned, in healthy pregnancies, the placental enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD-2) transforms the active cortisol (corticosterone in rodents) into the inactive form (cortisone and 11β-dehydrocorticosterone), avoiding an excessive amount of maternal GCs reaching the fetus (Shearer et al., 2019). We have provided evidence from animal studies that both maternal psychophysical (restraint stress during the last week of gestation in rats) and metabolic stress (HFD consumption before and during pregnancy in mice) reduce placental 11β-HSD-2 enzymatic activity as well as placental weight thus allowing more GCs to reach the fetus overall disrupting placental function (Bellisario et al., 2015; Panetta et al., 2017). Moreover, among the long-term effects driven by overexposure to GCs, we have previously mentioned an altered ability to cope with further stressors during life that can be, at least in

part, explained by HPA axis developmental alteration reducing the number of both MRs and GRs (Maccari et al., 1995; Van Waes et al., 2006).

Inflammatory processes also represent an appealing mechanism linking placental dysfunctions due to prenatal psychological or metabolic stress and neurodevelopmental risk in the offspring. In fact, the intrauterine inflamed state is able to affect the physiological immune tolerance towards the fetus, activating placental pro-inflammatory pathways leading to increased inflammatory profile also in fetal brain (Aye et al., 2014; Bilbo and Tsang, 2010; Bolton and Bilbo, 2014). This effect could be in part explained considering that placental macrophages, deriving from the yolk-sac, migrate to the fetus through the blood stream and colonize the developing brain giving origin to microglial cells (Edlow et al., 2019; Takahashi et al., 1991). Thus, fetal exposure to inflammation could prime microglia towards a pro-inflammatory profile with severe implications in the onset of mental disorders across the lifespan (Bilimoria and Stevens, 2015; Edlow et al., 2019; Takahashi et al., 1991).

Energy metabolism mediators represent another pathway potentially driving the effects of different prenatal stressors on brain development. The coordinated action of insulin/IGF-1 and adipokines regulates mitochondrial function, protein synthesis, and the flux of glucose, amino acids and lipids across the placental barrier. A number of studies have highlighted changes in the expression of different members of the insulin/ IGF family (e.g.: IGF-1, IGF-2 and IGF binding proteins) in the placenta of male and female fetuses, under condition of both maternal stress and maternal obesity. Thus, it is possible to hypothesize that placental changes in this signaling pathway might be predictive of a specific sex-dependent vulnerability (Ferraro et al., 2012; Mina et al., 2015; Mueller and Bale, 2008; Rosario et al., 2016). As for adipokines, they are directly secreted by the placenta and play a key role in the modulation of placental-fetal immune tolerance and inflammatory processes (Dos Santos et al., 2015; Nogues et al., 2019). During gestation, a dysregulated production of leptin has been observed in obese mothers as well as a result of maternal stress. In particular, leptin acts as a pro-inflammatory factor on immune cells leading to a release of cytokines, supporting the hypothesis that high levels of leptin, that are observed in stressed and obese mothers, could represent a primer for placental inflammation (Challier et al., 2008; Tessier et al., 2013).

In this review, we have been mainly focusing on derangements of neuroendocrine, immune and metabolic systems upon maternal different stressors and their sequelae on fetal brain development, since these systems are strongly and tightly regulated and characterized by a mutual crosstalk. This feature supports the fundamental nature and the evolutionary adaptation underlying physiological coordinated responses to adversities as early challenges impinge upon energy balance regulatory systems, which, in many cases, overlap with stress-response systems (Cirulli, 2017; Cirulli et al., 2020; Lindsay et al., 2019; Milaneschi et al., 2019). In addition, different systems can be activated in response to adverse conditions: genes involved in stress reactivity as well as in the immune response are both modulated by different prenatal challenges, supporting the hypothesis of a synergistic action on fetal programming and their long-term effects.

It is worth to mention that other mechanisms might act as mediators of maternal stress, either of psychophysical or metabolic nature, one above all is OS. Indeed, a growing body of evidence suggest that OS might underlie the endurable effects of early life adversities on the offspring neurodevelopment by modulating neurogenesis, neuronal migration and pruning (Berry et al., 2018b; Bittle et al., 2019; Horn et al., 2019; Karanikas et al., 2021; Londono Tobon et al., 2016; Malti et al., 2014). Neurodevelopmental disorders have been related to increased levels of systemic OS. Moreover, experiencing stressful events (psychophysical stress) or overnutrition (metabolic stress) during pregnancy has been associated with increased OS both in the mother and in the offspring although the specific mechanisms underlying this increase in the newborn still need to be elucidated. To this regard, it is possible to hypothesize that the above-discussed disruptions in the signaling pathways related to GCs, inflammatory and metabolic mediators might all contribute to provide a pro-oxidant environment in the developing fetus (Buss, 2021). In our hands, buffering OS in animal models of maternal HFD-feeding is able to prevent long-term metabolic and behavioral derangements, suggesting a pivotal role for OS in fetal programming (Guan et al., 2021).

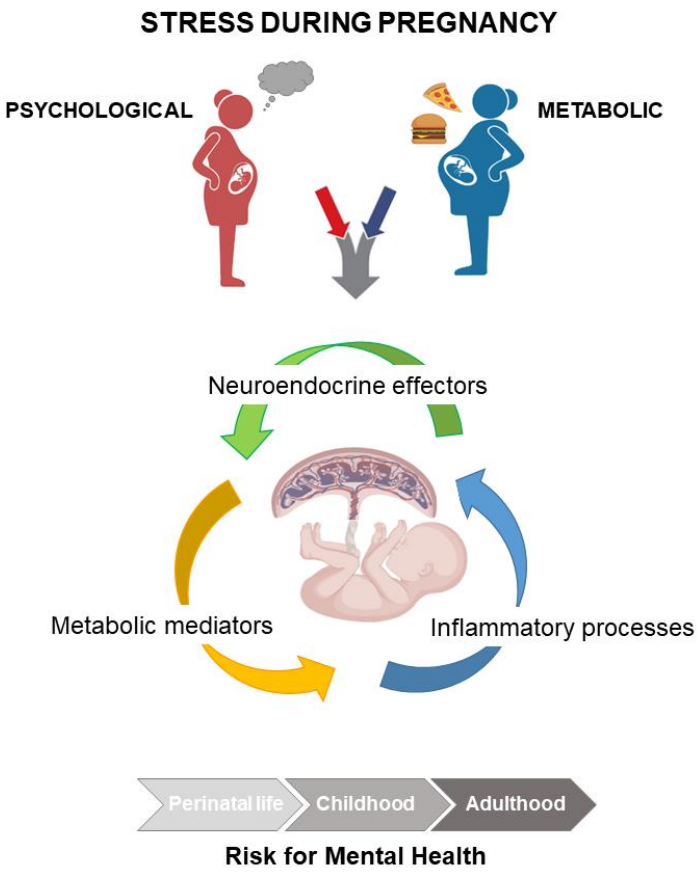


Fig. 2. The "funnel effect" model. Maternal psychophysical or metabolic stress may trigger shared signaling pathways dealing with neuroendocrine, inflammatory and metabolic mediators, disrupting fetal brain development. A "funnel effect" model might explain how such different maternal stressors may converge to similar effects on later life mental

health outcomes. To this regard, the placenta, which is the main mother-fetus interface, might play a pivotal role acting as a sensor of intrauterine adverse conditions and directing stress signals from the mother to the fetus.

4. Conclusive remarks and future perspectives

In this review, we have integrated clinical and preclinical evidence obtained over the past few decades indicating that both psychophysical and metabolic stress experienced during intrauterine life can threat fetal brain development with long-lasting effects on mental health. We have proposed a "funnel effect" model to explain how these different prenatal stressors impinge upon common pathways and converge through the placenta to increase risk for mental disorders in the offspring.

In general, maternal stress includes a multitude of other factors such as smoking, air pollution, metals exposure, viral agents as well as nutritional and metabolic status, that may all contribute to challenge the intrauterine environment and consequently the developing organism (al-Haddad et al., 2019; Banderali et al., 2015; Cirulli et al., 2020; Leijser et al., 2018). Worth noticing, these environmental challenges often co-occur targeting preferentially (but not exclusively) people with a low socioeconomic status, making overall poverty a multi-faceted risk factor for later life health outcomes, including psychiatric disorders (Martins et al., 2021; Palacios-Barrios and Hanson, 2019). As an example, the increase in consumption of easily accessible low cost food, characterized by high calorie content and poor nutrient availability, greatly contributes to the spread of obesity, increasing the incidence of the related metabolic pathologies particularly among people with low socio-economic status (Townshend and Lake, 2017). Thus, living in a poor environment has the potential to greatly synergize with the main mechanisms (neuroendocrine, immune/inflammatory and metabolic effectors) that we have hypothesized to mediate the effects of maternal stress contributing to negatively affect fetal development and vulnerability to mental disorders later in life.

Health promotion and disease prevention descend upon our ability to strengthen the foundations of health and to mitigate the adverse impact of stressors in the prenatal periods. Overall, identifying common converging pathways in fetal development can be crucial to unveil early risk biomarkers of mental disorders and open up novel early diagnostic and treatment avenues.

Acknowledgments

We are thankful to Sabrina Alviti and Paola Rossolino for management of external funding. This work was supported by ERANETNEURON JTC 2018 (Mental Disorders) Project ''EMBED" to F.C. HDHL-INTIMIC-085-GUTMOM project (co-PI F.C.) within the ERA-NET Co-fund HDHL-INTIMIC (INtesTInal MICrobiomics) programme; HDHLINTIMIC: Knowledge Platform on Food, Diet, Intestinal Microbiomics and Human Health (partner ISS); "Avvio alla Ricerca - Tipo 1" 2021 "Sapienza" University of Rome funding to C.M. and funding from the Italian Ministry of Health (Ricerca Corrente 2021 to F.C.).

Author's contribution

CM drafted the initial version of the manuscript; the final draft was critically revised by FC and AB. All authors approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

Agorastos, A., Pervanidou, P., Chrousos, G.P., Baker, D.G., 2019. Developmental trajectories of early life stress and trauma: A narrative review on neurobiological aspects beyond stress system dysregulation. Front. Psychiatry. https://doi.org/10.3389/fpsyt.2019.00118

al-Haddad, B.J.S., Oler, E., Armistead, B., Elsayed, N.A., Weinberger, D.R., Bernier, R., Burd, I., Kapur, R., Jacobsson, B., Wang, C., Mysorekar, I., Rajagopal, L., Adams Waldorf, K.M., 2019. The fetal origins of mental illness. Am. J. Obstet. Gynecol. https://doi.org/10.1016/j.ajog.2019.06.013

Agorastos, A., Pervanidou, P., Chrousos, G.P., Baker, D.G., 2019. Developmental trajectories of early life stress and trauma: A narrative review on neurobiological aspects beyond stress system dysregulation. Front. Psychiatry. https://doi.org/10.3389/fpsyt.2019.00118

al-Haddad, B.J.S., Oler, E., Armistead, B., Elsayed, N.A., Weinberger, D.R., Bernier, R., Burd, I., Kapur, R., Jacobsson, B., Wang, C., Mysorekar, I., Rajagopal, L., Adams Waldorf, K.M., 2019. The fetal origins of mental illness. Am. J. Obstet. Gynecol. https://doi.org/10.1016/j.ajog.2019.06.013

Armistead, B., Johnson, E., Vanderkamp, R., Kula-Eversole, E., Kadam, L., Drewlo, S., Kohan-Ghadr, H.R., 2020. Placental Regulation of Energy Homeostasis during Human Pregnancy. Endocrinol. (United States). https://doi.org/10.1210/endocr/bqaa076

Aye, I.L.M.H., Lager, S., Ramirez, V.I., Gaccioli, F., Dudley, D.J., Jansson, T., Powell, T.L., 2014. Increasing maternal body mass index is associated with systemic inflammation in the mother and the activation of distinct placental inflammatory pathways. Biol. Reprod. 90. https://doi.org/10.1095/biolreprod.113.116186

Balsevich, G., Baumann, V., Uribe, A., Chen, A., Schmidt, M. V., 2016. Prenatal Exposure to Maternal Obesity Alters Anxiety and Stress Coping Behaviors in Aged Mice. Neuroendocrinology 103, 354–368. https://doi.org/10.1159/000439087

Banderali, G., Martelli, A., Landi, M., Moretti, F., Betti, F., Radaelli, G., Lassandro, C., Verduci, E., 2015. Short and long term health effects of parental tobacco smoking during pregnancy and lactation: A descriptive review. J. Transl. Med. https://doi.org/10.1186/s12967-015-0690-y

Barker, D.J.P., 2007. The origins of the developmental origins theory, in: Journal of Internal Medicine. J Intern Med, pp. 412–417. https://doi.org/10.1111/j.1365-2796.2007.01809.x

Barker, D.J.P., Osmond, C., 1986. Infant mortality, childhood nutrition, and ischaemic heart disease in England and wales. Lancet 327, 1077–1081. https://doi.org/10.1016/S0140-6736(86)91340-1

Bateson, P., Barker, D., Clutton-Brock, T., Deb, D., D'Udine, B., Foley, R.A., Gluckman, P., Godfrey, K., Kirkwood, T., Lahr, M.M., McNamara, J., Metcalfe, N.B., Monaghan, P., Spencer, H.G., Sultan, S.E., 2004. Developmental plasticity and human health. Nature 430, 419–421. https://doi.org/10.1038/nature02725

Behura, S.K., Dhakal, P., Kelleher, A.M., Balboula, A., Patterson, A., Spencer, T.E., 2019. The brain-placental axis: Therapeutic and pharmacological relevancy to pregnancy. Pharmacol. Res. https://doi.org/10.1016/j.phrs.2019.104468

Bellisario, V., Berry, A., Capoccia, S., Raggi, C., Panetta, P., Branchi, I., Piccaro, G., Giorgio, M., Pelicci, P.G., Cirulli, F., 2014. Gender-dependent resiliency to stressful and metabolic challenges following prenatal exposure to high-fat diet in the p66Shc-/- mouse. Front. Behav. Neurosci. 8. https://doi.org/10.3389/fnbeh.2014.00285

Bellisario, V., Panetta, P., Balsevich, G., Baumann, V., Noble, J., Raggi, C., Nathan, O., Berry, A., Seckl, J., Schmidt, M., Holmes, M., Cirulli, F., 2015. Maternal high-fat diet acts as a stressor increasing maternal glucocorticoids' signaling to the fetus and disrupting maternal behavior and brain activation in C57BL/6J mice. Psychoneuroendocrinology 60, 138–150. https://doi.org/10.1016/j.psyneuen.2015.06.012

Berry, A., Bellisario, V., Panetta, P., Raggi, C., Magnifico, M.C., Arese, M., Cirulli, F., 2018a. Administration of the antioxidant n-acetyl-cysteine in pregnant mice has long-term positive effects on metabolic and behavioral endpoints of male and female offspring prenatally exposed to a high-fat diet. Front. Behav. Neurosci. 12. https://doi.org/10.3389/fnbeh.2018.00048

Berry, A., Bucci, M., Raggi, C., Eriksson, J.G., Guzzardi, M.A., Nuutila, P., Huovinen, V., Iozzo, P., Cirulli, F., 2018b. Dynamic changes in p66Shc mRNA expression in peripheral blood mononuclear cells following resistance training intervention in old frail women born to obese mothers: a pilot study. Aging Clin. Exp. Res. 30, 871–876. https://doi.org/10.1007/s40520-017-0834-4

Berry, A., Mazzelli, M., Musillo, C., Riva, M.A., Cattaneo, A., Cirulli, F., 2021. High-fat diet during adulthood interacts with prenatal stress, affecting both brain inflammatory and neuroendocrine markers in male rats. Eur. J. Neurosci. https://doi.org/10.1111/ejn.15181

Berry, A., Panetta, P., Luoni, A., Bellisario, V., Capoccia, S., Andrea Riva, M., Cirulli, F., 2015. Decreased Bdnf expression and reduced social behavior in periadolescent rats following prenatal stress. Dev. Psychobiol. 57, 365–373. https://doi.org/10.1002/dev.21297

Bick, J., Nelson, C.A., 2015. Early Adverse Experiences and the Developing Brain. Neuropsychopharmacol. 2016 411 41, 177–196. https://doi.org/10.1038/npp.2015.252

Bilbo, S.D., Tsang, V., 2010. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. FASEB J. 24, 2104–2115. https://doi.org/10.1096/fj.09-144014

Bilimoria, P.M., Stevens, B., 2015. Microglia function during brain development: New insights from animal models. Brain Res. https://doi.org/10.1016/j.brainres.2014.11.032

Bittle, J., Menezes, E.C., McCormick, M.L., Spitz, D.R., Dailey, M., Stevens, H.E., 2019. The Role of Redox Dysregulation in the Effects of Prenatal Stress on Embryonic Interneuron Migration. Cereb. Cortex 29, 5116–5130. https://doi.org/10.1093/cercor/bhz052

Bolton, J.L., Bilbo, S.D., 2014. Developmental programming of brain and behavior by perinatal diet: Focus on inflammatory mechanisms. Dialogues Clin. Neurosci. 16, 307–320. https://doi.org/10.31887/dcns.2014.16.3/jbolton

Bordeleau, M., Lacabanne, C., Fernández De Cossío, L., Vernoux, N., Savage, J.C., González-Ibáñez, F., Tremblay, M.È., 2020. Microglial and peripheral immune priming is partially sexually dimorphic in adolescent mouse offspring exposed to maternal high-fat diet. J. Neuroinflammation 17. https://doi.org/10.1186/s12974-020-01914-1

Brannigan, R., Cannon, M., Tanskanen, A., Huttunen, M.O., Leacy, F.P., Clarke, M.C., 2019. The association between subjective maternal stress during pregnancy and offspring clinically diagnosed psychiatric disorders. Acta Psychiatr. Scand. 139, 304–310. https://doi.org/10.1111/acps.12996

Brown, A.S., Hooton, J., Schaefer, C.A., Zhang, H., Petkova, E., Babulas, V., Perrin, M., Gorman, J.M., Susser, E.S., 2004. Elevated Maternal Interleukin-8 Levels and Risk of Schizophrenia in Adult Offspring. Am. J. Psychiatry 161, 889–895. https://doi.org/10.1176/appi.ajp.161.5.889

Brunton, P.J., Russell, J.A., 2010. Prenatal social stress in the rat programmes neuroendocrine and behavioural responses to stress in the adult offspring: Sex-specific effects. J. Neuroendocrinol. 22, 258–271. https://doi.org/10.1111/j.1365-2826.2010.01969.x

Buka, S.L., Tsuang, M.T., Torrey, E.F., Klebanoff, M.A., Wagner, R.L., Yolken, R.H., 2001. Maternal cytokine levels during pregnancy and adult psychosis. Brain. Behav. Immun. 15, 411–420. https://doi.org/10.1006/brbi.2001.0644

Buss, C., 2021. Maternal oxidative stress during pregnancy and offspring neurodevelopment. Brain. Behav. Immun. 93, 6–7. https://doi.org/10.1016/J.BBI.2021.01.007

Calkins, K., Devaskar, S.U., 2011. Fetal origins of adult disease. Curr. Probl. Pediatr. Adolesc. Health Care 41, 158–176. https://doi.org/10.1016/j.cppeds.2011.01.001

Cao-Lei, L., Laplante, D.P., King, S., 2016. Prenatal Maternal Stress and Epigenetics: Review of the Human Research. Curr. Mol. Biol. Reports 2, 16–25. https://doi.org/10.1007/s40610-016-0030-x

Challier, J.C., Basu, S., Bintein, T., Minium, J., Hotmire, K., Catalano, P.M., Hauguel-de Mouzon, S., 2008. Obesity in Pregnancy Stimulates Macrophage Accumulation and Inflammation in the Placenta. Placenta 29, 274–281. https://doi.org/10.1016/j.placenta.2007.12.010

Cirulli, F., 2017. Interactions between early life stress and metabolic stress in programming of mental and metabolic health. Curr. Opin. Behav. Sci. https://doi.org/10.1016/j.cobeha.2016.12.009

Cirulli, F., Alleva, E., 2009. The NGF saga: From animal models of psychosocial stress to stress-related psychopathology. Front. Neuroendocrinol. 30, 379–395. https://doi.org/10.1016/J.YFRNE.2009.05.002

Cirulli, F., Berry, A., 2013. Early developmental trajectories of brain development: New directions in the search for early determinants of health and longevity, in: Laviola, G., Macrì, S. (Eds.), Adaptive and Maladaptive Aspects of Developmental Stress. Springer New York, pp. 1–281. https://doi.org/10.1007/978-1-4614-5605-6

Cirulli, F., Berry, A., Alleva, E., 2003. Early disruption of the mother-infant relationship: Effects on brain plasticity and implications for psychopathology, in: Neuroscience and Biobehavioral Reviews. Elsevier Ltd, pp. 73–82. https://doi.org/10.1016/S0149-7634(03)00010-1

Cirulli, F., Francia, N., Berry, A., Aloe, L., Alleva, E., Suomi, S.J., 2009. Early life stress as a risk factor for mental health: Role of neurotrophins from rodents to non-human primates. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2008.09.001

Cirulli, F., Musillo, C., Berry, A., 2020. Maternal Obesity as a Risk Factor for Brain Development and Mental Health in the Offspring. Neuroscience. https://doi.org/10.1016/j.neuroscience.2020.01.023

Clare, C.E., Brassington, A.H., Kwong, W.Y., Sinclair, K.D., 2019. One-Carbon Metabolism: Linking Nutritional Biochemistry to Epigenetic Programming of Long-Term Development. Annu. Rev. Anim. Biosci. https://doi.org/10.1146/annurev-animal-020518-115206

Congdon, P., 2019. Obesity and urban environments. Int. J. Environ. Res. Public Health. https://doi.org/10.3390/ijerph16030464

Contu, L., Hawkes, C.A., 2017. A review of the impact of maternal obesity on the cognitive function and mental health of the offspring. Int. J. Mol. Sci. https://doi.org/10.3390/ijms18051093

Cordner, Z.A., Khambadkone, S.G., Boersma, G.J., Song, L., Summers, T.N., Moran, T.H., Tamashiro, K.L.K., 2019. Maternal high-fat diet results in cognitive impairment and hippocampal gene expression changes in rat offspring. Exp. Neurol. 318, 92–100. https://doi.org/10.1016/j.expneurol.2019.04.018

Cuffe, J.S.M., Walton, S.L., Moritz, K.M., 2015. The Developmental Origins of Renal Dysfunction, in: The Epigenome and Developmental Origins of Health and Disease. Elsevier Inc., pp. 291–314. https://doi.org/10.1016/B978-0-12-801383-0.00015-3

Darnaudéry, M., Maccari, S., 2008. Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. Brain Res. Rev. https://doi.org/10.1016/j.brainresrev.2007.11.004

De Kloet, E.R., Joëls, M., Holsboer, F., 2005. Stress and the brain: From adaptation to disease. Nat. Rev. Neurosci. https://doi.org/10.1038/nrn1683

Dearden, L., Buller, S., Furigo, I.C., Fernandez-Twinn, D.S., Ozanne, S.E., 2020. Maternal obesity causes fetal hypothalamic insulin resistance and disrupts development of hypothalamic feeding pathways. Mol. Metab. 42. https://doi.org/10.1016/j.molmet.2020.101079

Dos Santos, E., Duval, F., Vialard, F., Dieudonné, M.N., 2015. The roles of leptin and adiponectin at the fetal-maternal interface in humans. Horm. Mol. Biol. Clin. Investig. 24, 47–63. https://doi.org/10.1515/hmbci-2015-0031

Duthie, L., Reynolds, R.M., 2013. E-Mail At the Cutting Edge Changes in the Maternal Hypothalamic-Pituitary-Adrenal Axis in Pregnancy and Postpartum: Influences on Maternal and Fetal Outcomes. Neuroendocrinology 98, 106–115. https://doi.org/10.1159/000354702

Edlow, A.G., 2017. Maternal obesity and neurodevelopmental and psychiatric disorders in offspring. Prenat. Diagn. 37, 95–110. https://doi.org/10.1002/pd.4932

Edlow, A.G., Glass, R.M., Smith, C.J., Tran, P.K., James, K., Bilbo, S., 2019. Placental Macrophages: A Window Into Fetal Microglial Function in Maternal Obesity. Int. J. Dev. Neurosci. 77, 60–68. https://doi.org/10.1016/j.ijdevneu.2018.11.004

Entringer, S., Buss, C., Wadhwa, P.D., 2010. Prenatal stress and developmental programming of human health and disease risk: Concepts and integration of empirical findings. Curr. Opin. Endocrinol. Diabetes Obes. https://doi.org/10.1097/MED.0b013e3283405921

Felitti, V.J., Anda, R.F., Nordenberg, D., Williamson, D.F., Spitz, A.M., Edwards, V., Koss, M.P., Marks, J.S., 1998. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: The adverse childhood experiences (ACE) study. Am. J. Prev. Med. 14, 245–258. https://doi.org/10.1016/S0749-3797(98)00017-8

Ferraro, Z.M., Qiu, Q., Gruslin, A., Adamo, K.B., 2012. Characterization of the insulin-like growth factor axis in term pregnancies complicated by maternal obesity. Hum. Reprod. 27, 2467–2475. https://doi.org/10.1093/humrep/des189

Gilman, S.E., Hornig, M., Ghassabian, A., Hahn, J., Cherkerzian, S., Albert, P.S., Buka, S.L., Goldstein, J.M., 2017. Socioeconomic disadvantage, gestational immune activity, and neurodevelopment in early childhood. Proc. Natl. Acad. Sci. U. S. A. 114, 6728–6733. https://doi.org/10.1073/pnas.1617698114

Goines, P.E., Croen, L.A., Braunschweig, D., Yoshida, C.K., Grether, J., Hansen, R., Kharrazi, M., Ashwood, P., Van De Water, J., 2011. Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: A case-control study. Mol. Autism 2. https://doi.org/10.1186/2040-2392-2-13

Gomes, R.M., Bueno, F.G., Schamber, C.R., de Mello, J.C.P., de Oliveira, J.C., Francisco, F.A., Moreira, V.M., Junior, M.D.F., Pedrino, G.R., de Freitas Mathias, P.C., Miranda, R.A., de Moraes, S.M.F., Natali, M.R.M., 2018. Maternal diet-induced obesity during suckling period programs offspring obese phenotype and hypothalamic leptin/insulin resistance. J. Nutr. Biochem. 61, 24–32. https://doi.org/10.1016/j.jnutbio.2018.07.006

Guinosso, S.A., Johnson, S.B., Riley, A.W., 2016. Multiple adverse experiences and child cognitive development. https://doi.org/10.1038/pr.2015.195

Harris, A., Seckl, J., 2011. Glucocorticoids, prenatal stress and the programming of disease. Horm. Behav. https://doi.org/10.1016/j.yhbeh.2010.06.007

Heim, C., Nemeroff, C.B., 1999. The Impact of Early Adverse Experiences on Brain Systems Involved in the Pathophysiology of Anxiety and Affective Disorders.

Henry, C., Kabbaj, M., Simon, H., Le Moal, M., Maccari, S., 1994. Prenatal Stress Increases the Hypothalamo-Pituitary-Adrenal Axis Response in Young and Adult Rats. J. Neuroendocrinol. 6, 341–345. https://doi.org/10.1111/j.1365-2826.1994.tb00591.x

Hoek, H.W., Brown, A.S., Susser, E., 1998. The Dutch Famine and schizophrenia spectrum disorders, in: Social Psychiatry and Psychiatric Epidemiology. Springer, pp. 373–379. https://doi.org/10.1007/s001270050068

Horn, S.R., Leve, L.D., Levitt, P., Fisher, P.A., 2019. Childhood adversity, mental health, and oxidative stress: A pilot study. PLoS One 14. https://doi.org/10.1371/journal.pone.0215085

Hughes, K., Bellis, M.A., Hardcastle, K.A., Sethi, D., Butchart, A., Mikton, C., Jones, L., Dunne, M.P., 2017. The effect of multiple adverse childhood experiences on health: a systematic review and meta-analysis. Lancet Public Heal. 2, e356–e366. https://doi.org/10.1016/S2468-2667(17)30118-4

Igumnov, S., Drozdovitch, V., 2000. The intellectual development, mental and behavioural disorders in children from Belarus exposed in utero following the Chernobyl accident.

Jeyabalan, A., 2013. Epidemiology of preeclampsia: Impact of obesity. Nutr. Rev. 71. https://doi.org/10.1111/nure.12055

Karanikas, E., Daskalakis, N.P., Agorastos, A., 2021. Oxidative dysregulation in early life stress and posttraumatic stress disorder: A comprehensive review. Brain Sci. https://doi.org/10.3390/brainsci11060723

Kawai, M., Minabe, Y., Takagai, S., Ogai, M., Matsumoto, H., Mori, N., Takei, N., 2004. Poor maternal care and high maternal body mass index in pregnancy as a risk factor for schizophrenia in offspring. Acta Psychiatr. Scand. 110, 257–263. https://doi.org/10.1111/j.1600-0447.2004.00380.x

Kearney, J., 2010. Food consumption trends and drivers. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2010.0149

Khambadkone, S.G., Cordner, Z.A., Tamashiro, K.L.K., 2020. Maternal stressors and the developmental origins of neuropsychiatric risk. Front. Neuroendocrinol. https://doi.org/10.1016/j.yfrne.2020.100834

Koehl, M., Darnaudé Ry, M., Dulluc, J., Reeth, O. Van, Le Moal, M., Maccari, S., 1999. Prenatal Stress Alters Circadian Activity of Hypothalamo-Pituitary-Adrenal Axis and Hippocampal Corticosteroid Receptors in Adult Rats of Both Gender, J Neurobiol. https://doi.org/10.1002/(SICI)1097-4695(19990905)40:3

Kolominsky, Y., Igumnov, S., Drozdovitch, V., 1999. The psychological development of children from Belarus exposed in the prenatal period to radiation from the Chernobyl atomic power plant. J. Child Psychol. Psychiatry Allied Discip. 40, 299–305. https://doi.org/10.1017/S0021963098003369

Kong, L., Chen, X., Gissler, M., Lavebratt, C., 2020. Relationship of prenatal maternal obesity and diabetes to offspring neurodevelopmental and psychiatric disorders: a narrative review. Int. J. Obes. https://doi.org/10.1038/s41366-020-0609-4

Krontira, A.C., Cruceanu, C., Binder, E.B., 2020. Glucocorticoids as Mediators of Adverse Outcomes of Prenatal Stress. Trends Neurosci. https://doi.org/10.1016/j.tins.2020.03.008

Lahti-Pulkkinen, M., Räikkönen, K., Bhattacharya, S., Reynolds, R.M., 2021. Maternal body mass index in pregnancy and mental disorders in adult offspring: a record linkage study in Aberdeen, Scotland. Sci. Rep. 11, 15132. https://doi.org/10.1038/S41598-021-94511-Y

Laplante, D.P., Barr, R.G., Brunet, A., Du Fort, G.G., Meaney, M.L., Saucier, J.F., Zelazo, P.R., King, S., 2004. Stress during pregnancy affects general intellectual and language functioning in human toddlers. Pediatr. Res. 56, 400–410. https://doi.org/10.1203/01.PDR.0000136281.34035.44

Laplante, D.P., Brunet, A., Schmitz, N., Ciampi, A., King, S., 2008. Project Ice Storm: Prenatal Maternal Stress Affects Cognitive and Linguistic Functioning in 5Â½-Year-Old Children. J. Am. Acad. Child Adolesc. Psychiatry 47, 1063–1072. https://doi.org/10.1097/CHI.0b013e31817eec80

Leijser, L.M., Siddiqi, A., Miller, S.P., 2018. Imaging Evidence of the Effect of Socio-Economic Status on Brain Structure and Development. Semin. Pediatr. Neurol. 27, 26–34. https://doi.org/10.1016/j.spen.2018.03.004

LeMoult, J., Humphreys, K.L., Tracy, A., Hoffmeister, J.A., Ip, E., Gotlib, I.H., 2020. Meta-analysis: Exposure to Early Life Stress and Risk for Depression in Childhood and Adolescence. J. Am. Acad. Child Adolesc. Psychiatry. https://doi.org/10.1016/j.jaac.2019.10.011

Lindsay, K.L., Buss, C., Wadhwa, P.D., Entringer, S., 2019. The Interplay Between Nutrition and Stress in Pregnancy: Implications for Fetal Programming of Brain Development. Biol. Psychiatry. https://doi.org/10.1016/j.biopsych.2018.06.021

Lippert, R.N., Brüning, J.C., 2021. Maternal Metabolic Programming of the Developing Central Nervous System: Unified Pathways to Metabolic and Psychiatric Disorders. Biol. Psychiatry. https://doi.org/10.1016/j.biopsych.2021.06.002

Londono Tobon, A., Diaz Stransky, A., Ross, D.A., Stevens, H.E., 2016. Effects of Maternal Prenatal Stress: Mechanisms, Implications, and Novel Therapeutic Interventions. Biol. Psychiatry. https://doi.org/10.1016/j.biopsych.2016.09.011

Luoni, A., Berry, A., Calabrese, F., Capoccia, S., Bellisario, V., Gass, P., Cirulli, F., Riva, M.A., 2014. Delayed BDNF alterations in the prefrontal cortex of rats exposed to prenatal stress: Preventive effect of lurasidone treatment during adolescence. Eur. Neuropsychopharmacol. 24, 986–995. https://doi.org/10.1016/j.euroneuro.2013.12.010

Luoni, A., Berry, A., Raggi, C., Bellisario, V., Cirulli, F., Riva, M.A., 2016a. Sex-Specific Effects of Prenatal Stress on Bdnf Expression in Response to an Acute Challenge in Rats: a Role for Gadd45β. Mol. Neurobiol. 53, 7037–7047. https://doi.org/10.1007/s12035-015-9569-4

Luoni, A., Massart, R., Nieratschker, V., Nemoda, Z., Blasi, G., Gilles, M., Witt, S.H., Suderman, M.J., Suomi, S.J., Porcelli, A., Rizzo, G., Fazio, L., Torretta, S., Rampino, A., Berry, A., Gass, P., Cirulli, F., Rietschel, M., Bertolino, A., Deuschle, M., Szyf, M., Riva, M.A., 2016b. Ankyrin-3 as a molecular marker of early-life stress and vulnerability to psychiatric disorders. Transl. Psychiatry 6. https://doi.org/10.1038/tp.2016.211

Maccari, S., Krugers, H.J., Morley-Fletcher, S., Szyf, M., Brunton, P.J., 2014. The consequences of early-life adversity: Neurobiological, behavioural and epigenetic adaptations. J. Neuroendocrinol. https://doi.org/10.1111/jne.12175

Maccari, S., Piazza, P.V., Kabbaj, M., Barbazanges, A., Simon, H., Le Moal, M., 1995. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. J. Neurosci. 15, 110–116. https://doi.org/10.1523/jneurosci.15-01-00110.1995

Malti, N., Merzouk, H., Merzouk, S.A., Loukidi, B., Karaouzene, N., Malti, A., Narce, M., 2014. Oxidative stress and maternal obesity: Feto-placental unit interaction. Placenta 35, 411–416. https://doi.org/10.1016/j.placenta.2014.03.010

Martins, J., Czamara, D., Sauer, S., Rex-Haffner, M., Dittrich, K., Dörr, P., de Punder, K., Overfeld, J., Knop, A., Dammering, F., Entringer, S., Winter, S.M., Buss, C., Heim, C., Binder, E.B., 2021. Childhood adversity correlates with stable changes in DNA methylation trajectories in children and converges with epigenetic signatures of prenatal stress. Neurobiol. Stress 15. https://doi.org/10.1016/j.ynstr.2021.100336

McEwen, B.S., 1998. Stress, Adaptation, and Disease: Allostasis and Allostatic Load. Ann. N. Y. Acad. Sci. 840, 33–44. https://doi.org/10.1111/j.1749-6632.1998.tb09546.x

McLaughlin, K.A., Weissman, D., Bitrán, D., 2019. Childhood Adversity and Neural Development: A Systematic Review. Annu. Rev. Dev. Psychol. 1, 277–312. https://doi.org/10.1146/annurev-devpsych-121318-084950

Menting, M.D., van de Beek, C., Mintjens, S., Wever, K.E., Korosi, A., Ozanne, S.E., Limpens, J., Roseboom, T.J., Hooijmans, C., Painter, R.C., 2019. The link between maternal obesity and offspring neurobehavior: A systematic review of animal experiments. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2018.12.023

Milaneschi, Y., Simmons, W.K., van Rossum, E.F.C., Penninx, B.W., 2019. Depression and obesity: evidence of shared biological mechanisms. Mol. Psychiatry. https://doi.org/10.1038/s41380-018-0017-5

Mina, T.H., Räikkönen, K., Riley, S.C., Norman, J.E., Reynolds, R.M., 2015. Maternal distress associates with placental genes regulating fetal glucocorticoid exposure and IGF2: Role of obesity and sex. Psychoneuroendocrinology 59, 112–122. https://doi.org/10.1016/j.psyneuen.2015.05.004

Molenaar, N.M., Tiemeier, H., van Rossum, E.F.C., Hillegers, M.H.J., Bockting, C.L.H., Hoogendijk, W.J.G., van den Akker, E.L., Lambregtse-van den Berg, M.P., El Marroun, H., 2019. Prenatal maternal psychopathology and stress and offspring HPA axis function at 6 years. Psychoneuroendocrinology 99, 120–127. https://doi.org/10.1016/j.psyneuen.2018.09.003

Morales, E., Groom, A., Lawlor, D.A., Relton, C.L., 2014. DNA methylation signatures in cord blood associated with maternal gestational weight gain: Results from the ALSPAC cohort. BMC Res. Notes 7, 1–10. https://doi.org/10.1186/1756-0500-7-278

Mueller, B.R., Bale, T.L., 2008. Sex-specific programming of offspring emotionality after stress early in pregnancy. J. Neurosci. 28, 9055–9065. https://doi.org/10.1523/JNEUROSCI.1424-08.2008

Nardelli, C., Iaffaldano, L., Ferrigno, M., Labruna, G., Maruotti, G.M., Quaglia, F., Capobianco, V., Di Noto, R., Del Vecchio, L., Martinelli, P., Pastore, L., Sacchetti, L., 2014. Characterization and predicted role of the microRNA expression profile in amnion from obese pregnant women. Int. J. Obes. 38, 466–469. https://doi.org/10.1038/ijo.2013.121

Ng, S.W., Popkin, B.M., 2012. Time use and physical activity: A shift away from movement across the globe. Obes. Rev. 13, 659–680. https://doi.org/10.1111/j.1467-789X.2011.00982.x

Nieratschker, V., Massart, R., Gilles, M., Luoni, A., Suderman, M.J., Krumm, B., Meier, S., Witt, S.H., Nöthen, M.M., Suomi, S.J., Peus, V., Scharnholz, B., Dukal, H., Hohmeyer, C., Wolf, I.A.-C., Cirulli, F., Gass, P., Sütterlin, M.W., Filsinger, B., Laucht, M., Riva, M.A., Rietschel, M., Deuschle, M., Szyf, M., 2014. MORC1 exhibits cross-species differential methylation in association with early life stress as well as genome-wide association with MDD. Transl. Psychiatry 4, e429–e429. https://doi.org/10.1038/tp.2014.75

Niu, X.T., Wu, X.Y., Ying, A.N., Shao, B., Li, X.F., Zhang, W.L., Lin, C.C., Lin, Y.S., 2019. Maternal high fat diet programs hypothalamic-pituitary-adrenal function in adult rat offspring. Psychoneuroendocrinology 102, 128–138. https://doi.org/10.1016/j.psyneuen.2018.12.003

Nogues, P., Dos Santos, E., Jammes, H., Berveiller, P., Arnould, L., Vialard, F., Dieudonné, M.N., 2019. Maternal obesity influences expression and DNA methylation of the adiponectin and leptin systems in human third-trimester placenta. Clin. Epigenetics 11. https://doi.org/10.1186/s13148-019-0612-6

O'Connor, T.G., Heron, J., Golding, J., Glover, V., 2003. Maternal antenatal anxiety and behavioural/emotional problems in children: A test of a programming hypothesis. J. Child Psychol. Psychiatry Allied Discip. 44, 1025–1036. https://doi.org/10.1111/1469-7610.00187

Oberlander, T.F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., Devlin, A.M., 2008. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. Epigenetics 3, 97–106. https://doi.org/10.4161/epi.3.2.6034

Osborne, S., Biaggi, A., Chua, T.E., Du Preez, A., Hazelgrove, K., Nikkheslat, N., Previti, G., Zunszain, P.A., Conroy, S., Pariante, C.M., 2018. Antenatal depression programs cortisol stress reactivity in offspring through increased maternal inflammation and cortisol in pregnancy: The Psychiatry Research and Motherhood – Depression (PRAM-D) Study. Psychoneuroendocrinology 98, 211–221. https://doi.org/10.1016/j.psyneuen.2018.06.017

Palacios-Barrios, E.E., Hanson, J.L., 2019. Poverty and self-regulation: Connecting psychosocial processes, neurobiology, and the risk for psychopathology. Compr. Psychiatry 90, 52–64. https://doi.org/10.1016/j.comppsych.2018.12.012

Panetta, P., Berry, A., Bellisario, V., Capoccia, S., Raggi, C., Luoni, A., Longo, L., Riva, M.A., Cirulli, F., 2017. Long-term sex-dependent vulnerability to metabolic challenges in prenatally stressed rats. Front. Behav. Neurosci. 11. https://doi.org/10.3389/fnbeh.2017.00113

Pechtel, P., Pizzagalli, D.A., 2011. Effects of Early Life Stress on Cognitive and Affective Function: An Integrated Review of Human Literature. https://doi.org/10.1007/s00213-010-2009-2

Provençal, N., Binder, E.B., 2015. The effects of early life stress on the epigenome: From the womb to adulthood and even before. Exp. Neurol. https://doi.org/10.1016/j.expneurol.2014.09.001

Provenzi, L., Guida, E., Montirosso, R., 2021. Applying behavioral epigenetic principles to preterm birth and early stress exposure, in: Provenzi, L., Montirosso, R. (Eds.), Developmental Human Behavioral Epigenetics. pp. 91–104. https://doi.org/10.1016/c2018-0-04683-6

Robinson, M., Zubrick, S.R., Pennell, C.E., Van Lieshout, R.J., Jacoby, P., Beilin, L.J., Mori, T.A., Stanley, F.J., Newnham, J.P., Oddy, W.H., 2013. Pre-pregnancy maternal overweight and obesity increase the risk for affective disorders in offspring. J. Dev. Orig. Health Dis. 4, 42–48. https://doi.org/10.1017/S2040174412000578

Rosario, F.J., Powell, T.L., Jansson, T., 2016. Activation of placental insulin and mTOR signaling in a mouse model of maternal obesity associated with fetal overgrowth. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 310, R87–R93. https://doi.org/10.1152/ajpregu.00356.2015

Roseboom, T., de Rooij, S., Painter, R., 2006. The Dutch famine and its long-term consequences for adult health. Early Hum. Dev. https://doi.org/10.1016/j.earlhumdev.2006.07.001

Roseboom, T.J., Painter, R.C., Van Abeelen, A.F.M., Veenendaal, M.V.E., De Rooij, S.R., 2011. Hungry in the womb: What are the consequences? Lessons from the Dutch famine. Maturitas. https://doi.org/10.1016/j.maturitas.2011.06.017

Rosenheck, R., 2008. Fast food consumption and increased caloric intake: A systematic review of a trajectory towards weight gain and obesity risk. Obes. Rev. https://doi.org/10.1111/j.1467-789X.2008.00477.x

Russell, J.A., Brunton, P.J., 2019. Giving a good start to a new life via maternal brain allostatic adaptations in pregnancy. Front. Neuroendocrinol. https://doi.org/10.1016/j.yfrne.2019.02.003

Sanchez, C.E., Barry, C., Sabhlok, A., Russell, K., Majors, A., Kollins, S.H., Fuemmeler, B.F., 2018. Maternal pre-pregnancy obesity and child neurodevelopmental outcomes: a meta-analysis. Obes. Rev. https://doi.org/10.1111/obr.12643

Schaefer, C.A., Brown, A.S., Wyatt, R.J., Kline, J., Begg, M.D., Bresnahan, M.A., Susser, E.S., 2000. Maternal prepregnant body mass and risk of schizophrenia in adult offspring. Schizophr. Bull. 26, 275–286. https://doi.org/10.1093/oxfordjournals.schbul.a033452

Schellong, K., Melchior, K., Ziska, T., Ott, R., Henrich, W., Rancourt, R.C., Plagemann, A., 2019. Hypothalamic insulin receptor expression and DNA promoter methylation are sex-specifically altered in adult offspring of high-fat diet (HFD)-overfed mother rats. J. Nutr. Biochem. 67, 28–35. https://doi.org/10.1016/j.jnutbio.2019.01.014

Send, T.S., Gilles, M., Codd, V., Wolf, I., Bardtke, S., Streit, F., Strohmaier, J., Frank, J., Schendel, D., Sütterlin, M.W., Denniff, M., Laucht, M., Samani, N.J., Deuschle, M., Rietschel, M., Witt, S.H., 2017. Telomere length in newborns is related to maternal stress during pregnancy. Neuropsychopharmacology 42, 2407–2413. https://doi.org/10.1038/npp.2017.73

Serpeloni, F., Radtke, K.M., Hecker, T., Sill, J., Vukojevic, V., De Assis, S.G., Schauer, M., Elbert, T., Nätt, D., 2019. Does prenatal stress shape postnatal resilience? An epigenome-wide study on violence and mental health in humans. Front. Genet. 10. https://doi.org/10.3389/fgene.2019.00269

Shearer, F.J.G., Wyrwoll, C.S., Holmes, M.C., 2019. The Role of 11β-Hydroxy Steroid Dehydrogenase Type 2 in Glucocorticoid Programming of Affective and Cognitive Behaviours. Neuroendocrinology 109, 257–265. https://doi.org/10.1159/000499660

Solano, M.E., Arck, P.C., 2020. Steroids, Pregnancy and Fetal Development. Front. Immunol. https://doi.org/10.3389/fimmu.2019.03017

Sosnowski, D.W., Booth, C., York, T.P., Amstadter, A.B., Kliewer, W., 2018. Maternal prenatal stress and infant DNA methylation: A systematic review. Dev. Psychobiol. 60, 127–139. https://doi.org/10.1002/dev.21604

Sugden, C., 2006. One-carbon metabolism in psychiatric illness. Nutr. Res. Rev. 19, 117–136. https://doi.org/10.1079/nrr2006119

Takahashi, K., Naito, M., Katabuchi, H., Higashi, K., 1991. Development, differentiation, and maturation of macrophages in the chorionic villi of mouse placenta with special reference to the origin of Hofbauer cells. J. Leukoc. Biol. 50, 57–68. https://doi.org/10.1002/jlb.50.1.57

Tenenbaum-Gavish, K., Hod, M., 2013. Impact of maternal obesity on fetal health. Fetal Diagn. Ther. https://doi.org/10.1159/000350170

Tessier, D.R., Ferraro, Z.M., Gruslin, A., 2013. Role of leptin in pregnancy: Consequences of maternal obesity. Placenta 34, 205–211. https://doi.org/10.1016/j.placenta.2012.11.035

Townshend, T., Lake, A., 2017. Obesogenic environments: Current evidence of the built and food environments. Perspect. Public Health. https://doi.org/10.1177/1757913916679860

Turco, M.Y., Moffett, A., 2019. Development of the human placenta. Dev. https://doi.org/10.1242/dev.163428

Vallée, M., Maccari, S., Dellu, F., Simon, H., Le Moal, M., Mayo, W., 1999. Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: A longitudinal study in the rat. Eur. J. Neurosci. 11, 2906–2916. https://doi.org/10.1046/j.1460-9568.1999.00705.x

Vallée, M., Mayo, W., Dellu, F., Moal, M. Le, Simon, H., Maccari, S., 1997. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: Correlation with stress-induced corticosterone secretion. J. Neurosci. 17, 2626–2636. https://doi.org/10.1523/jneurosci.17-07-02626.1997

Van Den Bergh, B.R.H., Marcoen, A., 2004. High antenatal maternal anxiety is related to ADHD symptoms, externalizing problems, and anxiety in 8- and 9-year-olds. Child Dev. https://doi.org/10.1111/j.1467-8624.2004.00727.x

Van Der Burg, J.W., Sen, S., Chomitz, V.R., Seidell, J.C., Leviton, A., Dammann, O., 2016. The role of systemic inflammation linking maternal BMI to neurodevelopment in children. Pediatr. Res. https://doi.org/10.1038/pr.2015.179

Van Waes, V., Enache, M., Dutriez, I., Lesage, J., Morley-Fletcher, S., Vinner, E., Lhermitte, M., Vieau, D., Maccari, S., Darnaudéry, M., 2006. Hypo-response of the hypothalamic-pituitary-adrenocortical axis after an ethanol challenge in prenatally stressed adolescent male rats. Eur. J. Neurosci. 24, 1193–1200. https://doi.org/10.1111/j.1460-9568.2006.04973.x

Viuff, A.C., Sharp, G.C., Rai, D., Henriksen, T.B., Pedersen, L.H., Kyng, K.J., Staunstrup, N.H., Cortes, A., Neumann, A., Felix, J.F., Tiemeier, H., Jaddoe, V.W.V., Relton, C.L., 2018. Maternal depression during pregnancy and cord blood DNA methylation: findings from the Avon Longitudinal Study of Parents and Children. Transl. Psychiatry 8. https://doi.org/10.1038/s41398-018-0286-4

Weinstock, M., 2017. Prenatal stressors in rodents: Effects on behavior. Neurobiol. Stress. https://doi.org/10.1016/j.ynstr.2016.08.004

Winther, G., Elfving, B., Müller, H.K., Lund, S., Wegener, G., 2018. Maternal High-fat Diet Programs Offspring Emotional Behavior in Adulthood. Neuroscience 388, 87–101. https://doi.org/10.1016/j.neuroscience.2018.07.014

Yajnik, C.S., Deshmukh, U.S., 2012. Fetal programming: Maternal nutrition and role of one-carbon metabolism. Rev. Endocr. Metab. Disord. https://doi.org/10.1007/s11154-012-9214-8

Yong Ping, E., Laplante, D.P., Elgbeili, G., Jones, S.L., Brunet, A., King, S., 2020. Disaster-related prenatal maternal stress predicts HPA reactivity and psychopathology in adolescent offspring: Project Ice Storm. Psychoneuroendocrinology 117, 104697. https://doi.org/10.1016/j.psyneuen.2020.104697

CHAPTER 3

Prenatal psychophysical and metabolic stress disrupt oxidative stress balance and placental function leading to a sex-dimorphic increase in fetal brain inflammatory profile

Chiara Musillo[1,2], Maria Antonietta Ajmone-Cat[3], Roberta De Simone[3], Federica Fratini[4], Egidio Iorio[5], Roberta Tassinari[6], Francesca Maranghi[6], Eleonora M. Pieroni[1], Maria Elena Pisanu[5], Mattea Chirico[5], Rachel N. Lippert[7,8,9], Thorsten Henning[9,10], Daniela Weber[9,10], Alessandro Giuliani[11], Alessandra Berry[1], Francesca Cirulli[1]

[1]Center for Behavioral Sciences and Mental Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. [2]Ph.D. Program in Behavioral Neuroscience, Department of Psychology, Sapienza University of Rome, 00185 Rome, Italy. [3]National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. [4]FAST Core facility - Proteomics Mass Spec Unit, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. [5]FAST Core facility - NMR Unit, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. [6]Center for Gender-Specific Medicine, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. [7]Neurocure Cluster of Excellence, Charité - Universitätsmedizin Berlin, Berlin Germany. [8]German Center for Diabetes Research (DZD), Neuherberg, Germany. [9]German Institute for Human Nutrition Potsdam-Rehbruecke, Nuthetal Germany, 14558. [10]Food4Future (F4F), c/o Leibniz Institute of Vegetable and Ornamental Crops (IGZ), 14979 Grossbeeren, Germany. [11]Department of Health and Environment (DAMSA) Mechanisms, Biomarkers and Models Section, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy.

In preparation

Abstract

Maternal obesity during pregnancy represents a stressful condition capable to affect fetal brain development to an extent that is very similar to psychophysical maternal stress, setting the stage for increased vulnerability to neurodevelopmental and psychiatric disorders. By means of two C57Bl6/N mouse models, we compared the effects of maternal psychophysical stress (PNS) and maternal obesity (mHFD) in E17.5 embryos (late gestation), investigating the role of the placenta in conveying maternal stressful stimuli to the developing fetal brain. We hypothesized that derangements in the oxidative stress balance during pregnancy might underlie the effects of both PNS and mHFD on neurodevelopment. Thus, we investigated the protective effects of the antioxidant N-acetyl-cysteine (NAC) administered to pregnant dams. We used a multi-disciplinary approach combining histological and lipidomic analysis to characterize structural and functional changes of the placenta accompanied by a targeted transcriptomic analysis to define oxidative stress and inflammatory related changes in the brains of male and female fetuses. Overall, we found that both PNS and mHFD disrupt maternal redox balance resulting in a sex-dimorphic and fetal brain phenotype characterized by a pro-oxidant and pro-inflammatory milieu, unravelling a greater vulnerability of female offspring. These effects are associated with specific changes in placental morphology as well as with a reduced efficiency in placental lipid metabolism. Prenatal administration of NAC is able to partially preserve placental functionality and to prevent brain developmental derangements.

Keywords

Maternal obesity, maternal stress, placenta, fetal programming, brain development, inflammation, sex, oxidative stress, mouse model

Introduction

Psychological stressors experienced during pregnancy may increase the risk of neuropsychiatric problems in the offspring, suggesting that mental illness might find its roots very early during development, already during *in utero* fetal life (al-Haddad et al., 2019; Brannigan et al., 2019; Calkins & Devaskar, 2011; E. P. Davis et al., 2020; Gilman et al., 2017; Khambadkone et al., 2020; Srinivasan et al., 2020; Tuovinen et al., 2021; Van den Bergh et al., 2020). As the mammalian brain is highly plastic and sensitive to external stimuli, adverse prenatal conditions may become embedded biological traces, shaping neuronal circuits involved in the response to stress, ultimately affecting mental health in the offspring (Barker, 1995; Barker & Osmond, 1986; Cirulli & Berry, 2013). In addition to psychological stressors, other early life adversities may entail a greater vulnerability to mental illness (Musillo et al., 2022). Thus, it is possible to hypothesize that adverse experiences of different nature may impinge upon common pathways involved in the response to stress. A number of preclinical and clinical studies have indicated that obesity during pregnancy might be considered as a stressor holding similar risks for negative mental health outcomes in the offspring as prenatal - psychological - stress (al-Haddad et al., 2019; Brannigan et al., 2019; Calkins & Devaskar, 2011; Cirulli et al., 2020; J. Davis & Mire, 2021; Gilman et al., 2017; Khambadkone et al., 2020; Sanchez et al., 2018). In particular, maternal obesity has been associated with a higher risk for neurodevelopmental disorders such as attention deficit hyperactivity and autism spectrum disorders, cognitive delay but also mood disorders and depression (Cirulli et al., 2020; J. Davis & Mire, 2021; Sanchez et al., 2018).

The mechanisms explaining how different maternal stressors might possibly impact the developing fetal brain, accounting for similar sequelae, still need to be studied in detail. In this scenario, the placenta is emerging as a main target of different stressful conditions, conveying environmental adversities to the fetus. Indeed, disruptions in placental function has been associated with abnormal fetal development (Behura et al., 2019; Bronson & Bale, 2016). Interestingly, Kratimenos and Penn have recently coined the term "neuroplacentology", referring to a novel and expanding field of research focusing on the sequelae of placental dysfunctions specifically on brain development (Kratimenos & Penn, 2019; Vacher et al., 2021). To this regard, the weakening of the placental barrier as a result of stressful conditions has been proposed as a suitable mechanism explaining how this organ might be the key orchestrator in mediating signals of stress to the fetus (Bronson & Bale, 2016; Khambadkone et al., 2020). In agreement with this hypothesis, we have provided evidence that, in rodents, metabolic stress (high-fat diet consumption) reduces the placental 11β-HSD-2 (11β-Hydroxysteroid dehydrogenase 2) enzymatic activity to a very similar extent than psychophysical stress (restraint stress), resulting in excessive amounts of glucocorticoids (GCs) reaching the fetus, leading to long-term negative outcomes in the offspring (Bellisario et al., 2015; Panetta et al., 2017).

Beyond resulting in an excessive amount of maternal GCs, prenatal stress and obesity may lead to increased levels of systemic inflammation and oxidative stress (OS) in the womb, activating placental pro-inflammatory pathways (Bilbo & Tsang, 2010; Bolton & Bilbo, 2014; Cirulli et al., 2022). Within the fetal brain, a main target of inflammation and OS is represented by microglia which plays a key role during development as

regulator of neurogenesis, plasticity and neuronal apoptosis (Hammond et al., 2021). It is worth noticing that newborn microglial cells find their origin in the placental macrophages that, deriving from the yolk-sac, migrate through the maternal blood stream and colonize the developing brain and could be affected by a dysregulated intrauterine environment. Thus, a widespread *in utero* inflammatory environment could prime microglial cells towards an aberrant activation, eventually reprogramming brain development (Edlow et al., 2019; Musillo et al., 2022; Takahashi et al., 1991).

During inflammatory processes, immune cells produce a great amount of reactive oxygen and nitrogen species (ROS and NOS), overall increasing levels of OS and nitrosative stress (NS). On the other hand, the establishment of a pro-oxidant environment enhances the pro-inflammatory phenotype, leading to a condition of mutual reinforcement, often characterizing the pathophysiology of many health issues including obesity and psychological stress (Biswas, 2016). Oxidative balance is also crucial for a proper brain development, regulating neurogenesis, neuronal migration, and pruning; moreover increased systemic OS has been associated with a higher risk for neurodevelopmental disorders (Bittle et al., 2019; Londono Tobon et al., 2016). In line with this evidence, a role of OS during critical phases of brain development has been proposed as a possible mechanism mediating the effects of different maternal stressors on offspring brain development and mental health (C. Buss, 2021).

N-acetyl-cysteine (NAC) is emerging as a promising drug as it is a powerful antioxidant with also anti-inflammatory properties (Chiew et al., 2018; Wilkes et al., 2005). Interestingly, there is a growing body of clinical evidence supporting beneficial effects of NAC in psychiatric patients, making this drug a valid candidate for the treatment of a wide range of neuropsychiatric disorders (Berk et al., 2013). As we have previously shown, NAC has the ability to boost the antioxidant defenses in the offspring of a mouse model of maternal obesity by increasing levels of glutathione (Berry et al., 2018). Thus, this compound could modulate the increase in the production of ROS, a possible etiopathological mechanism characterizing both psychophysical stress and maternal obesity.

Our main working hypothesis was that maternal stressors as different as psychophysical stress (prenatal stress - PNS) and obesity (maternal high-fat diet - mHFD) might share common inflammatory/OS related pathways, disrupting fetal brain development, by primarily affecting placental function and morphology. To test such hypothesis, we used two different mouse models mimicking, respectively, maternal psychophysical stress and maternal obesity. In both models we evaluated: i) structural and functional placental alterations; ii) disruptions of the inflammatory/OS related pathways in fetal brains; iii) sex-specific differences in fetal programming; iv) the efficacy of the prenatal administration of NAC to preserve placental functionality and brain development against stressors occurring early during life.

Materials and methods

This study was reported in conformity with ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) (du Sert et al., 2020).

Animal handling

All experimental procedures were approved by the ethical body of the Istituto Superiore di Sanità for animal welfare and conducted in conformity with the European Directive 2010/63/EU and the Italian legislation on animal experimentation, D.Lgs. 26/2014. They were authorized by the Italian Ministry of Health.

Two-month-old C57BL/6N mice, 140 females and 70 males, were purchased from a commercial breeder (Charles River, Italy) and housed two/cage in transparent Plexiglas cages provided by Tecniplast, in an air-conditioned room (temperature 21 ± 1°C, relative humidity 60 ± 10%), under a reversed 12/12 h light/dark cycle with lights off from 07:00 a.m. to 07:00 p.m. Fresh tap-water and standard chow (energy 3.3 kcal/g, fat 17%, carbohydrate 60% and protein 23% provided by Mucedola, Italy) were continuously available. After three weeks of habituation, female breeders were allocated into the experimental groups (see below for further details).

Prenatal stressors

Female breeders were allocated into two models of maternal stress as described below.

Psychophysical stress (maternal restraint - PNS). Pregnant females were assigned to the control (CTRL) or the PNS groups and counterbalanced to avoid bias due to body weight. Females assigned to the PNS group were individually restrained in a transparent Plexiglas cylinder (11.5 × 3 cm) and contextually exposed to bright light as an additional stressor (6.500 lux) for 30 minutes, three times daily, from gestational day (G) 12.5 until G17.5. Stress sessions were conducted during the dark phase (07:00 a.m. to 07:00 p.m.) at different hours during the day in order to prevent habituation to the repeated procedure (Maccari et al., 1995). Females assigned to the CTRL group were left undisturbed during the entire pregnancy period. The final number of dams was: CTRL-Vehicle = 9; CTRL-NAC = 7; PNS-Vehicle = 9; PNS-NAC = 7.

Metabolic stress (maternal HFD - mHFD). Female breeders were assigned to the control diet (CD, n = 35) or the high-fat diet (HFD, n = 35) groups and counterbalanced to avoid bias due to body weight. Diets were administered *ad libitum* for 10 weeks before mating and throughout gestation (for a total of 13 weeks), until sacrifice at G17.5. HFD (D12331, energy 5.56 kcal/g, fat 58%, carbohydrate 25.5% and protein 16.4) and CD (D12328, energy 4.07 kcal/g, fat 10.5%, carbohydrate 73.1% and protein 16.4%) were provided by Research Diets Inc., New Brunswick, NJ, USA. The final number of dams was: CD-Vehicle = 9; CD-NAC = 12; HFD-Vehicle = 6; HFD-NAC = 8.

N-acetyl-cysteine (NAC) administration

Five weeks before mating, female breeders were divided in two groups receiving either the antioxidant NAC treatment (n = 35 for the PNS model and 35 for the mHFD model) or tap water as vehicle (n = 35 for the PNS model and 35 for the mHFD model), until sacrifice at G17.5 (for a total of 8 weeks). Treatments were allocated through a minimization approach to avoid bias due to body weight (Altman & Bland, 2005). In order to minimize stress due to excessive handling procedure, NAC was daily administered in drinking water to yield an average dose of 1 g/kg body weight (Berry et al., 2018). Solution consumption was recorded weekly throughout the entire experiment. NAC was purchased as powder by Sigma Aldrich.

Mating, pregnancy and tissues collection

After 5 weeks on NAC administration (and 10 weeks on diets), all females were bred (see Bellisario et al., 2015 for further details on the breeding procedure). Body weight gain was monitored once a week before mating and during pregnancy at G7, at G14 and at G17.5. Females that did not get pregnant underwent a second 48 h mating with a different male breeder.

At G17.5, the day before the expected delivery date, dams were sacrificed by cervical dislocation and trunk blood was collected into heparinized tubes to obtain plasma for measuring NAC levels and markers of OS. Immediately, a caesarean (c)-section was performed opening the uterine horns, then each amniotic sac was exposed by removing the maternal uterine wall and placentas, fetal brains and tails were dissected-out. From each pregnant dam, placentas of one male and one female were fixed for histopathological evaluations. Additional placentas of one male and one female as well as all the fetal brains and the tails were frozen in 2-Methylbutane then stored at -80 °C for proteomic/lipidomic/metabolomic analysis on the placentas, gene expression analysis on the fetal brains and genetic sex determination on the tails, respectively.

The investigators could not be blinded to the prenatal conditions but all the tissues collected (blood, placentas, tails and brains) were coded and analyzed in blind.

Peripheral circulating levels of NAC and markers of OS in pregnant dams

NAC measurements were performed after (Henning et al., 2022) with modifications. Briefly 10 µL murine plasma were spiked with 40 µL internal standard (d3-NAC,300 nM in 90% acetonitrile) and vortexed for 30 s. To support protein precipitation, samples were stored at -20 °C for 10 min in a precooled rack and then centrifuged at 30.000×g and 4 °C for 10 min. Next, 40 µL of supernatant were transferred into a vial with a 100 µL glass insert and 5 µL were injected into the LC system. Chromatography was performed using an ACQUITY UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 µm) equipped with a VanGuard BEH Amid pre-column (2.1 mm × 5 mm, 1.7 µm). Mobile phase A consisted of acetonitrile/MilliQ water (9:1, v/v)

containing 5 mM and 0.3% formic acid (v/v). Mobile phase B consisted of MilliQ water containing 25 mM ammonium formate (pH 6). Gradient elution started at 100% A and was held for 2 min. Thereafter, the proportion of solvent B was increased to 40% within 0.1 min. 40% were kept constant for 2 minutes until column was re-equilibrated with initial conditions until minute 6. The flow rate was constantly set at 0.25 mL/min. Samples were kept in the autosampler at 6 °C and column temperature was set at 35°C. For detection a Waters Xevo TQ-XS was used in ESI+ mode. Monitored mass transitions were 164.1 > 122.0 and 164.1 > 146.1 for NAC and 167.1 > 123.0 and 167.1 > 149.0 for d3-NAC. Plasma malondialdehyde was determined after derivatization with thiobarbituric acid following separation by RP-HPLC followed and subsequent fluorescence detection as described by (Wong et al., 1987) with modifications (Weber et al., 2014). The analyses of protein carbonyls and 3-nitrotyrosine in plasma by two in-house ELISA methods have been described elsewhere (H. Buss et al., 1997; Weber et al., 2014). Protein concentration was measured by the Bradford method prior to the respective ELISAs.

Sex determination by PCR Reactions

Genomic DNA was extracted from embryo mice tails (about 1 cm) using DNeasy Blood & Tissue kit (Qiagen), according to the manufacturer instructions, and the genetic sex of mice was determined by Sly/Xlr PCR with the SX_F, 5′-GATGATTTGAGTGGAAATGTGAGGTA-3′ and SX_R, 5′-CTTATGTTTATAGGCATGCACCATGTA-3′ primer pairs, according to (McFarlane et al., 2013). 15 µL of PCR products were electrophoresed together with a DNA ladder (GelPilot MidRange ladder, Qiagen) and GelRed nucleic acid stain (Biotium) on 2% agarose gels, and visualized with the Bio-Rad ChemiDoc XRS system.

Histomorphometric analysis of mouse placenta

Placentas were fixed in paraformaldehyde solution 4% in PBS, stored in 80% ethyl alcohol, sliced transversally through their center with a scalpel and embedded in paraffin by tissue processor (Shandon Excelsior ES, Thermo Scientific). Placentas were cut into 5 µm sections using the Microm HM 325 (Thermo Scientific) and stained with hematoxylin-eosin. Slides were examined under a light microscope (Nikon Microphot FX, Amsterdam, Netherlands) for histological changes. For quantitative histomorphometrical analyses, placentas were examined by an image analysis system (Nis-Elements Br) applied to the optical microscope (Nikon Microphot FX). Thickness of layers (decidua basalis, junctional zone and labyrinth) was measured using a 10 x lens. For each layer, the mean thickness and the ratio with the whole placenta thickness were calculated using three serial sections/sample (Tait et al., 2015).

Lipidomic and Metabolomic analysis of mouse placenta by 1H-NMR spectroscopy

Extraction of aqueous and organic metabolites. Frozen placentas were pulverized and subjected to methyl-tert-butyl ether/methanol extraction of lipids, metabolites and proteins (Coman et al., 2016). The polar phase containing water soluble cellular metabolites was evaporated under nitrogen gas and lyophilized whilst the organic fraction (lipid phase) was evaporated under nitrogen gas flow. Both phases of cell extracts were stored at -20°C.

Metabolomics analyses by Nuclear Magnetic Resonance (NMR) spectroscopy. The aqueous fraction from cells were reconstituted in 700 µL D2O using TSP (0.1mM) as NMR internal standard whereas lipid fraction from cells was resuspended in a CD3OD/CDCl3 solution (2:1 v/v) with 0.05%of tetramethylsilane (TMS) as internal reference. High-resolution 1H-NMR analyses were performed at 25 °C at 700 MHz (14.09 T Bruker AVANCE NEO spectrometer; Karlsruhe, Germany, Europe) on aqueous and organic cell extracts using acquisition pulses, water pre-saturation, data processing, and peak area deconvolution as previously described (Saulle et al., 2021). Relative quantification of lipid signals in organic fractions was referred to the signal at 1.6 ppm (as a measure of total acyl chain and referred to 100). The integrals of characteristic lipid signals were compared to this value. *Reagents*: methanol (CD3OD), chloroform (CDCl3); deuterium oxide (D2O) (Cambridge Isotope Laboratories, Inc.); 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt (TSP) (Merck & Co, Montreal, Canada).

Gene expression on fetal brains

One male and one female fetal brain (G17.5) from each pregnant dam - for a total of 6 males and 6 females per experimental group - were removed from the skull on ice and homogenized in Trizol reagent (Thermo Fisher Scientific) using the TissueLyser LT (Qiagen) for 5 min at 50 Hz. Total RNA purification was performed according to the Trizol reagent user guide, and the quality and concentration of RNA was measured at Nanodrop. All samples were stored at -20°C until gene expression analyses.

cDNA was reverse transcribed from 1 µg of RNA using the High-Capacity cDNA Reverse Transcription Kit and the Gene Amp PCR System 9700 (Applied Biosystems, Thermo Fisher Scientific). Real-time PCR was performed on the reverse transcription products using the AB 7500 Real Time PCR System, with TaqMan master mix and the following TaqMan™Gene Expression Assays (Applied Biosystems, Thermo Fisher Scientific): hypoxanthine guanine phosphoribosyl transferase (HPRT, Mm00446968_m1), interleukin-1β (IL-1β, Mm00434228_m1), interleukin-6 (IL-6, Mm00446190_m1), tumor necrosis factor alpha (TNF-α, Mm00443258_m1), transforming growth factor beta 1 (TGF-β1, Mm01178820_m1), insulin-like growth factor 1 (IGF-1, Mm00439560_m1), inducible nitric oxide synthase (iNOS, Mm00440502_m1), arginase 1 (Arg-1, Mm00475988_m1), uncoupling protein 2 (Ucp2, Mm00627599_m1), cluster of differentiation 68 (CD68, Mm03047343_m1), triggering receptor expressed on myeloid cells 2 (TREM 2, Mm04209424_g1), transmembrane protein 119 (TMEM 119, Mm00525305_m1). Annealing temperature was 60 °C for all the

primer pairs listed. All samples were run in duplicate, and each PCR well contained 20 µl as a final volume of reaction, including 2 µl of complementary DNA corresponding to approximately 60 ng total RNA, 750 nM of each primer, and 10 µl PCR master mix. Thermal cycling conditions were as follows: 1 cycle at 95 °C for 10 min, 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The relative expression level of each mRNA (normalized to HPRT used as the housekeeping gene, and relative to the appropriate male mice vehicle group) was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA). All the data were graphically presented as mean ± SEM. Maternal parameters were analyzed by means of a two-way ANOVA with stressful condition (PNS/CTRL or HFD/CD) and treatment (NAC/Vehicle) as between-subject factors and repeated measure (body weight) as within subject factors. As regards the thickness of placental layers, male and female mice were analyzed separately by means of a two-way ANOVA with stressful condition (PNS/CTRL or HFD/CD) and treatment (NAC/Vehicle) as between-subject factors. Post hoc comparisons among groups were performed using the Tukey's test. Gene expression data were analyzed by means of a three-way ANOVA with prenatal condition (PNS/CTRL or HFD/CD), prenatal treatment (NAC/Vehicle) and sex (male or female) as between-subject factors. ANOVA analysis was followed by post-hoc Šídák's multiple comparisons test on significant effects. Grubb's test, using 5% significance level critical values, was used to detect outliers (Grubbs, 1950). A level of probability set a $p<0.05$ was used as statistically significant. To analyze lipidome content, we preliminarily conducted a principal component analysis (PCA) on 17 lipid signals. The PCA is a factorial method that allows correlation analysis of a set of n standardized variables by extracting $k < n$ orthogonal factors as linear combinations of the original variables. These factors were then named after the domain that recapitulates the variables with the highest factor loadings (at least >0.5 in absolute value). Once the factors were defined, the loadings of the 17 variables were multiplied by the standardized values and then added to identify the coordinates in a new multidimensional space. These new values (reflecting the score of each individual for each orthogonal factor) were then analyzed through an unpaired t-test.

Results

Dams' body weight

Body weight of dams was recorded once a week from the beginning of the experiment (1st week) and throughout pregnancy until sacrifice at G17.5.

Psychophysical stress. NAC administration, that started five weeks before mating, overall reduced dams' body weight (main effect of treatment: $F(1,28)=36.299$, $p<0.0001$). In particular, the interaction between treatment and time showed that this effect started from the 5th week of administration and continues throughout the entire pregnancy ($F(1,8)=23.01$, $p<0.0001$; post hoc comparisons: $p<0.01$ NAC vs Vehicle). When the restraint stress was applied, it was able to reduce dams' body weight (main effect of stress: $F(1,28)=7.512$, $p=0.0106$). More in detail, an interaction between stress and time revealed that PNS reduced dams' body weight during pregnancy specifically at G14 and G17.5 ($F(1,8)=4.156$, $p=0.0001$; post hoc comparisons: $p<0.01$ PNS vs CTRL), see Figure 1A.

Metabolic stress. Overall, HFD feeding increased dams' body weight already before mating as well as during pregnancy (main effect of diet: $F(1,31)=23.579$, $p<0.0001$). Interestingly, an interaction among time, diet and treatment was found showing that the administration of NAC was effective in containing HFD-induced body weight gain, starting from the 10th experimental week and through gestation ($F(13,403)=2.014$, $p=0.0186$, post hoc comparisons: $p<0.01$ HFD-NAC vs HFD-Vehicle), see Figure 1B.

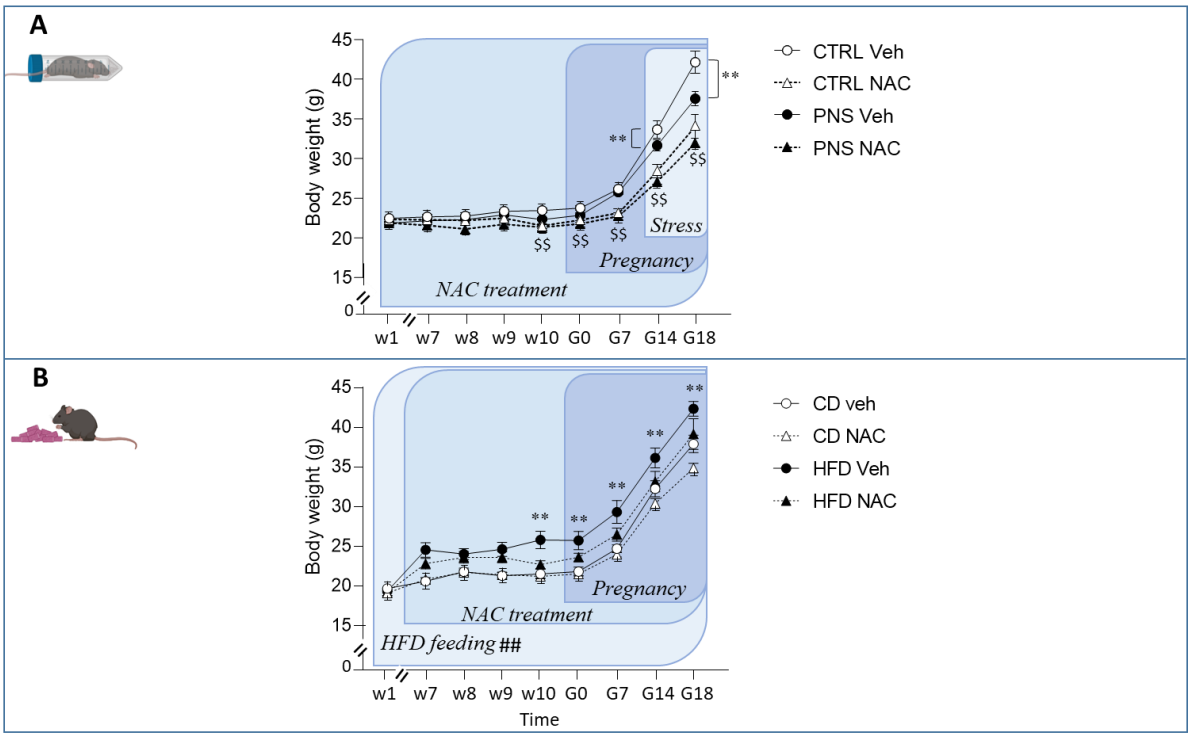


Figure 1 Dams' body weight. A) PNS as well as NAC administration reduced dams' body weight. $$ p<0.01 Tukey's test NAC vs Veh; ** p<0.01 Tukey's test PNS vs CTRL. **B)** NAC administration contained body weight gain in HFD-fed dams. ## p<0.01 main effect of diet; ** p<0.01 Tukey's test HFD-NAC vs HFD-Veh. Data are mean ± SEM. Number of subjects: 6-12 within each experimental group.

Peripheral circulating levels of NAC and markers of OS in pregnant dams

At G17.5 all dams were sacrificed and plasma was collected to measure NAC levels and markers of OS.

Circulating levels of NAC

Psychophysical stress. No main effect of stress was found in maternal circulating levels of NAC ($F(1,8)=2.255$, $p=1.1716$), see Table1.

Metabolic stress. No main effect of diet was found in maternal circulating levels of NAC ($F(1,8)=0.086$, $p=0.7767$), see Table 1.

Table 1 Circulating levels of NAC (nM) in pregnant females administered with the compound.

	NAC (nM)
CTRL-NAC	1.853 ± 0.672
PNS-NAC	4.768 ± 2.213
CD-NAC	2.761 ± 0.774
HFD-NAC	3.087 ± 0.693

Data are mean ± SEM. Number of subjects: 4-6 within each experimental group

Proteins and markers of OS in peripheral circulation of pregnant dams

Psychophysical stress. NAC treatment as well as PNS had no significant effect on total circulating protein levels ($F(1,28)=0.7007$, $p=0.4096$; see Figure 2A). Protein carbonyls were also not altered between treatment groups ($F(1,28)=2.241$, $p=0.1456$; see Figure 2A). However, markers of OS, 3-nitrotyrosine and malondialdehyde showed varying effects dependent on NAC treatment or stress exposure. Specifically, 3-Nitrotyrosine showed a significant interaction between stress and treatment, and a tendency toward a reduction in the PNS-NAC group that did not reach statistical significance ($F(1,28)=4.553$, $p=0.0418$, See Figure 2A). Malondialdehyde showed a significant suppression in the presence of NAC treatment in both control and PNS groups (See Figure 2A ($F(1,28)=32.35$, $p<0.0001$, post hoc comparisons: $p<0.01$ CTRL-NAC vs CTRL-Vehicle; $p<0.001$ PNS-NAC vs PNS-Vehicle).

Metabolic stress. Exposure to HFD had a significant effect in decreasing total circulating protein levels that was not further exacerbated by NAC treatment (main effect of diet: $F(1,26)=4.15$, $p=0.0478$; see Figure 2B). Malondialdehyde and protein carbonyls were also not altered between treatment groups ($F(1,26)=0.2211$, $p=0.6422$ and $F(1,26)=1.133$, $p=0.2970$, respectively). However, 3-Nitrotyrosine showed a significant interaction between diet exposure and NAC treatment revealing a tendency toward a reduction within the HFD-NAC group that did not reach statistical significance ($F(1,26)=4.330$, $p=0.474$); see Figure 2B).

In general, psychophysical and metabolic stress exerted similar effects, affecting 3-Nitrotyrosine levels and shifting the homeostatic balance towards a pro-oxidant maternal environment.

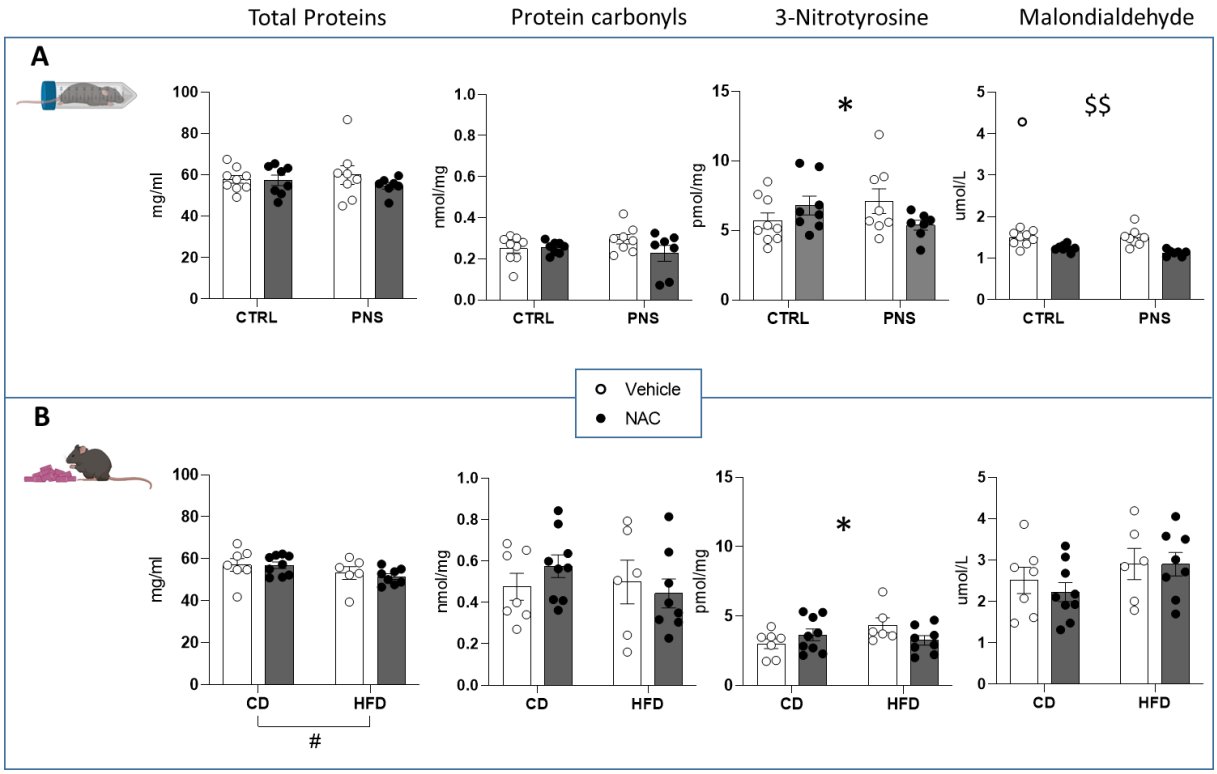


Figure 2 Total proteins and markers of OS in dams' peripheral circulation. 3-Nitrotyrosine levels were found to be affected by both PNS and HFD. **A)** $$ main effect of treatment $p<0.01$; * Interaction between stress and treatment. **B)** # main effect of diet $p<0.05$; * Interaction between stress and treatment. Data are mean ± SEM. Number of subjects: 6-9 within each experimental group.

Histomorphometric analysis on placenta

Psychophysical stress. When the placental layers were measured, we found sex dimorphic changes. In particular, PNS reduced the thickness of both the labyrinth and the decidua basalis only in male placentas (main effect of stress - labyrinth: $F(1,21)=7.095$, $p=0.0145$; decidua: $F(1,21)=5.688$, $p=0.0266$), see Figure

3A. The junctional zone resulted to be reduced by NAC treatment specifically in females, while no effect was observed in male placentas (main effect of NAC: F(1,21)=15.90, p=0.0007).

Metabolic stress. The exposure to mHFD increased the thickness of the junctional zone specifically in male placentas, while NAC treatment was able to prevent this effect (interaction between diet and treatment: F(1,19)=4.368, p=0.050; post hoc comparisons: p<0.05 HFD-Vehicle vs CD-Vehicle; p<0.01 HFD-NAC vs HFD-Vehicle), see Figure 3B. As for female placentas, we observed a reduction of the junctional zone as a result of the antioxidant treatment (Main effect of NAC: F(1,19)=14.09, p=0.0013).

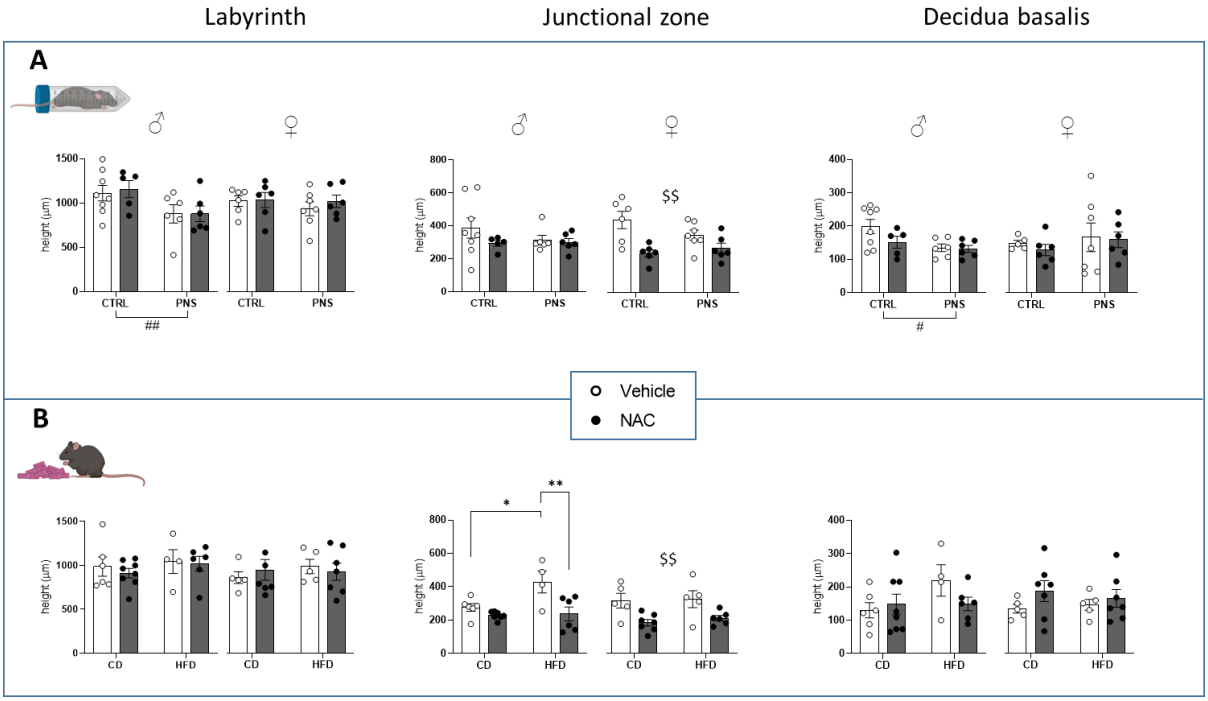


Figure 3 Thickness of the placental layers. A) PNS reduced labyrinth and decidua basalis specifically in males. Main effect of stress #p<0.05; ## p<0.01; $$ Main effect of NAC p<0.01. **B)** mHFD increased junctional zone in males only. Tukey's test *p<0.05 HFD-Vehicle vs CD-Vehicle, **p<0.01 HFD-NAC vs HFD-Veh; $$ Main effect of NAC p<0.01. Data are mean ± SEM. Number of subjects: 4-8 within each experimental group.

Placental lipidome profile

Psychophysical stress. When the z-score (relative to PC1) accounting for the effects on phospholipids and lipid metabolism was analyzed, we found that exposure to PNS reduced this parameter compared to CTRL (p=0.0184).

Metabolic stress. In placentas exposed to mHFD we found similar results to PNS showing a reduced z-score (relative to PC1) compared to CD (p=0.0262).

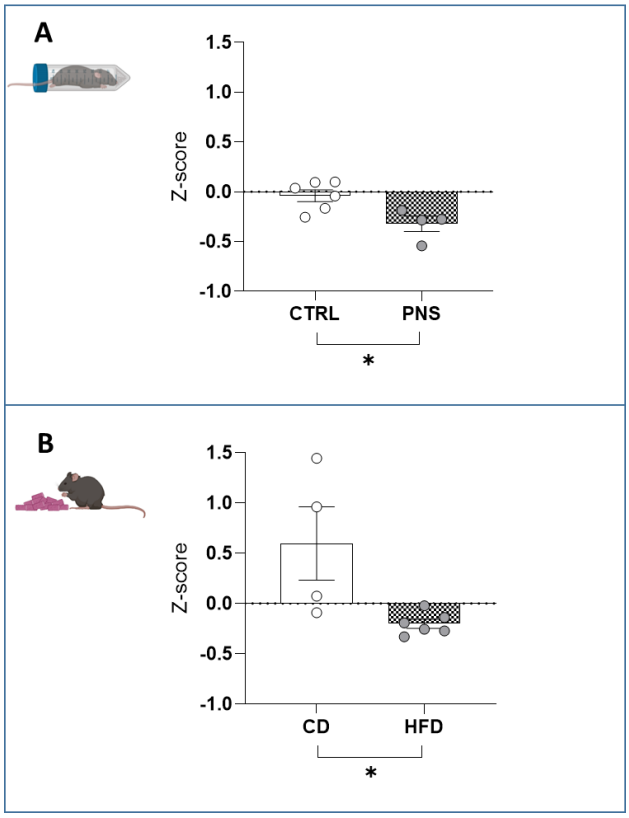


Figure 4 Placental lipidome content. Both PNS and mHFD reduced the z-score accounting for the effects on phospholipids and lipid metabolism.

Neuroinflammation and microglia function in fetal brains

We analyzed the transcript levels of genes involved in homeostatic and immune functions of microglia and markers of neuroinflammation: the inflammatory cytokines IL-1β, IL-6, TNF-α, TGF-β; the growth factor IGF-1; the inflammatory/oxidative stress-related proteins iNOS, ARG-1 and the mitochondrial uncoupling protein UCP2; the microglia/macrophage markers CD68, TREM 2, and TMEM 119.

Psychophysical stress. When the gene expression levels of a panel of microglia markers and neuroinflammatory mediators were measured, we found that PNS as well as the prenatal administration of NAC exerted sex-dependent effects on fetal brains. In particular, PNS had a dimorphic effect on *IL-1β* levels, increasing this cytokine specifically in females compared to male mice (post hoc comparisons: $p<0.05$ males PNS-Veh vs females PNS-Veh). In addition, prenatal administration of NAC seemed to rebalance this parameter, reducing *IL-1β* expression in females and increasing it in males (interaction between sex and treatment: $F(1,38)=7.06$, $p=0.0115$; post hoc comparisons did not reach statistical significance), see Figure 5A. As for *IL-6*, PNS increased the expression of this cytokine specifically in females (interaction between stress and sex: $F(1,39)=5.54$, $p=0.0237$; post hoc comparisons: $p<0.01$ PNS-Veh vs CTRL-Veh), while prenatal NAC treatment was able to prevent this effect (interaction between stress and treatment: $F(1,39)=8.34$, $p=0.0063$; post hoc comparisons: $p<0.05$ PNS-NAC vs PNS-Veh), see Figure 5A. A main effect of sex revealed increased

mRNA levels of *TNF-α* in male fetal brains compared to females ($F(1,38)=4.34$, $p=0.0439$), see Figure 5A. Expression levels of *TGF-β* and *IGF-1* were not significantly affected by PNS nor NAC treatment.

CD68 and *TREM2* mRNAs were not altered by PNS, NAC nor their combination in either sexes. In the case of *TMEM119*, a significant interaction between stress and treatment suggested that NAC enhanced its expression in CTRL mice, regardless of sex ($F(1,36)=6.25$, $p=0.0171$, although post hoc comparisons did not reach statistical significance), see Figure 5C.

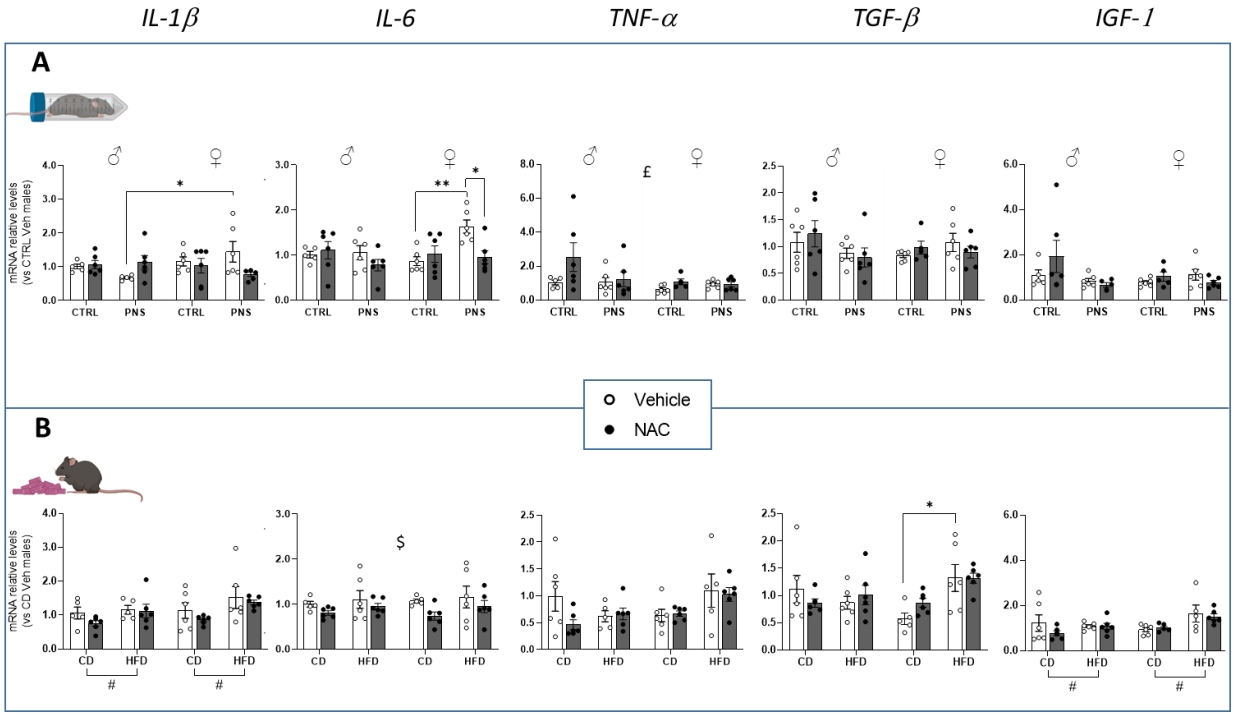
When the *iNOS/Arg-1* mRNA ratio was assessed, NAC treatment revealed a tendency toward a reduction within the PNS males (interaction between stress, sex and treatment: $F(1,36)=5.79$, $p=0.0214$), although post hoc analyses did not yield significance, see Figure 5E. Also for *UCP-2* mRNA expression, an interaction among stress, sex and treatment was found ($F(1,40)=5.14$, $p=0.0288$), suggesting enhanced *UCP2* expression in CTRL-NAC females compared to their control, see Figure 5E.

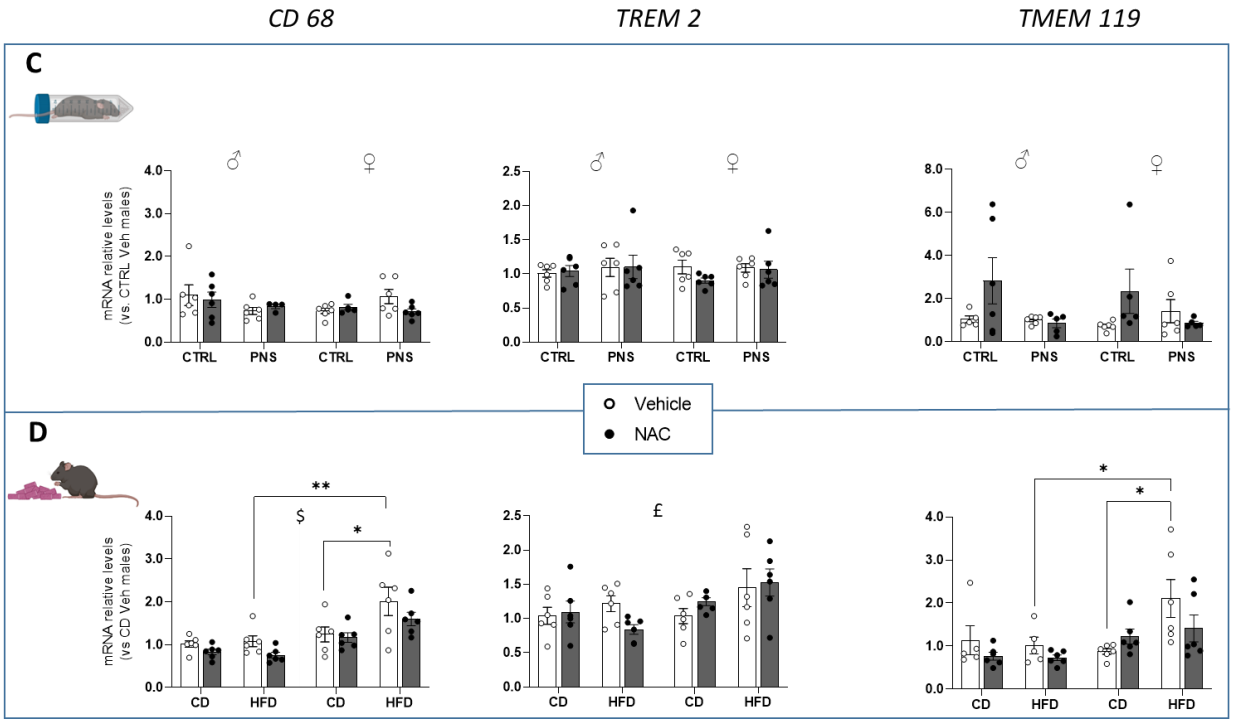
<u>*Metabolic stress*</u>. Overall, fetal brains exposed to mHFD showed altered expression of inflammatory and microglial related genes. More in detail, when cytokine levels were assessed, we found that mHFD increased *IL-1β* and *IGF-1* expression in both sexes (main effect of diet: $F(1,38)=7.03$, $p=0.0116$ and $F(1,39)=6.01$, $p=0.0188$, respectively for *IL-1β* and *IGF-1*), see Figure 5B. A significant interaction between sex and diet was found both for *TNF-α* and *TGF-β* mRNAs (*TNF-α*: $F(1,38)=4.45$, $p=0.0415$ and *TGF-β*: $F(1,39)=8.09$, $p=0.0070$). Post hoc comparisons showed increased levels of *TGF-β* in mHFD females when compared to their counterpart ($p<0.05$ HFD-Veh vs CD-Veh). As for *TNF-α*, a similar trend was observed although post hoc comparisons did not reach statistical significance, see Figure 5B. Prenatal exposure to NAC did not revert the HFD-induced changes of all the transcripts considered but *IL-6*. More in detail, NAC overall reduced its expression levels, regardless of diet and sex (main effect of treatment: $F(1,38)=5.43$, $p=0.0252$), see Figure 5B.

mHFD affected *CD68* and *TMEM119* expression in a sex-dependent manner. In particular, while no effect was observed in males, female fetal brains were characterized by increased mRNA levels when compared to both CD females as well as mHFD males (interaction between diet and sex - CD68: $F(1, 40)=7.15$, $p=0.0108$; post hoc comparisons: $p<0.05$ females HFD-Veh vs CD-Veh; $p<0.01$ males HFD-Veh vs females HFD-Veh; and TMEM119: $F(1,38)=5.13$, $p=0.0293$); post hoc comparisons: $p<0.05$ females HFD-Veh vs CD-Veh; males HFD-Veh vs females HFD-Veh). Maternal NAC administration was effective in overall reducing the expression of *CD68* (main effect of treatment: $F(1,40)=4.79$, $p=0.0345$), while it had no effects on *TMEM119* regulation (Figure 5D).

mHFD increased also the *iNOS/Arg-1* ratio in fetal brains (main effect of diet: $F(1,38)=10.3$, $p=0.0027$). As for *UCP2*, a significant interaction among diet, sex and treatment was found ($F(1,34)=6.37$, $p=0.0165$) showing that overall control males had higher levels of *UCP2* compared to control female subjects ($p<0.05$). In addition, it was shown that mHFD reduced *UCP2* in fetal male brains but not in females ($p<0.01$), while prenatal NAC was effective in decreasing this parameter in control male fetuses ($p<0.01$). See Figure 5F.

Overall, PNS and mHFD activated immuno-inflammatory pathways in fetal brains leading to increased expression levels of cytokines such as *IL-1β* and *IL-6*. A greater vulnerability of female fetuses was observed as a result of both the stressors. Furthermore, mHFD seemed to specifically target microglia markers and genes involved in the regulation of brain OS balance.





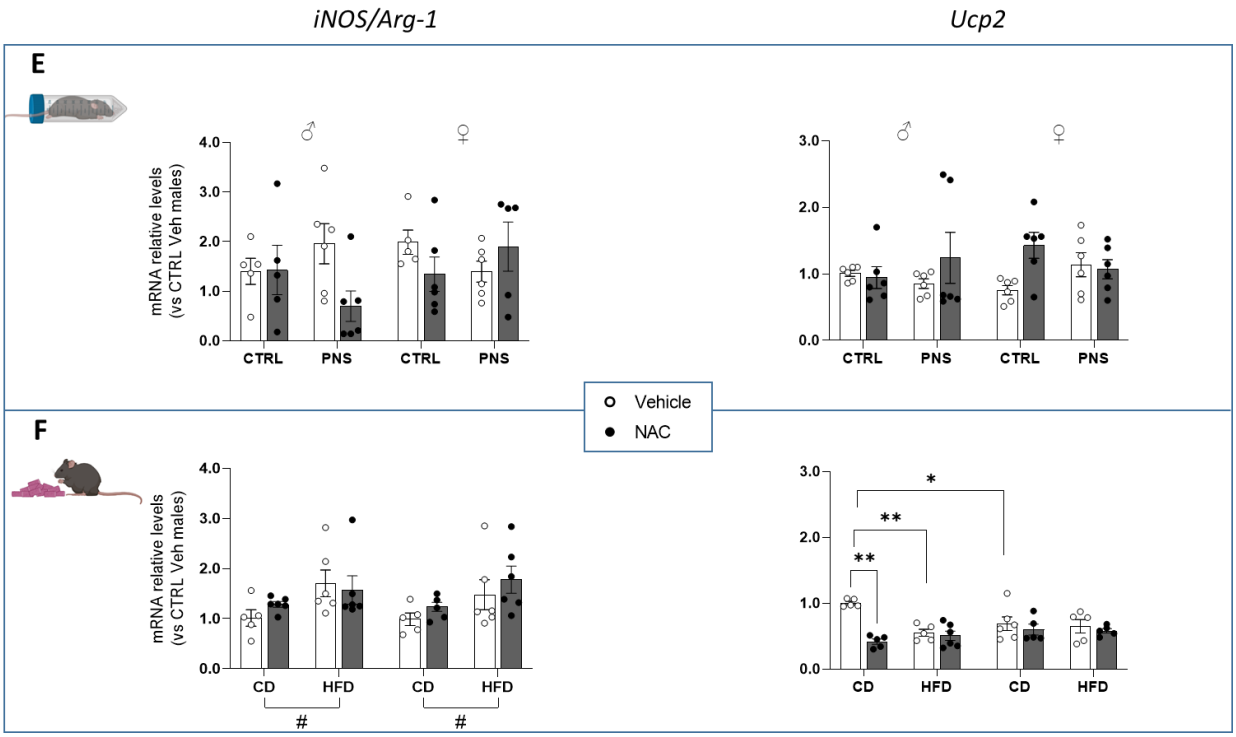


Figure 5 Neuroinflammation and microglia functions in male and female fetal brains at E18. A) Cytokines panel in fetal brains following a prenatal exposure to psychophysical stress. PNS increased levels of *IL-1β* and *IL-6* specifically in female fetal brains, while NAC prevented this effect. *IL-1β*: *p<0.05 Sidak's test males PNS vs females PNS; *IL-6*: *p<0.05 Sidak's test females PNS-NAC vs PNS-Veh; **p<0.01 Sidak's test females PNS-Veh vs female CTRL-Veh; *TNF-α*: £ main effect of sex p<0.05. **B)** Cytokines panel in mHFD fetal brains. mHFD increased levels of *IL-1β* and *IGF-1* in both sexes and increased *TGF-β* expression only in female fetuses. *IL-1β* and *IGF-1*: # main effect of diet p<0.05; $ main effect of treatment p<0.05; *p<0.05 Sidak's test female HFD-Veh vs CD-Veh. **C)** Markers of homeostatic and immune functions of microglia were not affected in PNS fetal brains. **D)** Markers of homeostatic and immune functions of microglia in mHFD fetal brains. mHFD had sex-dependent effects increasing levels of *CD68* and *TMEM119* specifically in female fetuses, while NAC buffered this change. *CD68*: *p<0.05 Sidak's test females HFD-Veh vs CD-Veh; **p<0.01 Sidak's test females HFD-Veh vs males HFD-Veh; **p<0.01 Sidak's test females HFD-NAC vs males HFD-NAC; *TMEM119*: *p<0.05 Sidak's test females HFD-Veh vs CD-Veh; females HFD-Veh vs males HFD-Veh. **E)** mRNA levels of *iNOS/Arg-1* and *UCP2* were not affected in fetal brains following PNS. **F)** The *iNOS/Arg*-1 ratio was found to be increased in mHFD fetal brains in both sexes; ## main effect of diet p<0.01. mHFD as well as NAC exposure decreased the expression of *UCP2* mRNA specifically in female fetal brains; **p<0.01 Sidak's test males HFD-Veh vs CD-Veh; CD-NAC vs CD-Veh. Data are mean ± SEM. Number of subjects: 5-6 within each experimental group.

Discussion

Collectively, we provide important evidence supporting the hypothesis that prenatal stressors of psychophysical (PNS) and metabolic nature (mHFD), experienced by the mother, affect the brain development of the fetus to a similar extent, by triggering shared converging inflammatory/OS related pathways. In addition, we highlight clear sexually dimorphic phenotypes in response to PNS or mHFD, revealing an increased vulnerability of female offspring, at least during early developmental stages.

In this study, we used two paradigms of prenatal stress relying upon repeated sessions of restraint stress from G12.5 to G17.5 or feeding with a diet enriched in fats, prior to and during pregnancy, to mimic, respectively, a condition of maternal psychophysical stress or maternal obesity. When assessing body weight gain of the dams in these two conditions, and as expected, body weight was increased by the HFD. By contrast, PNS pregnant females showed a decreased body weight gain during pregnancy, specifically at G14 and G17.5, compared to CTRL females. This piece of data replicates the reduction of maternal body weight due to restraint stress that has been widely described (Kinsley & Svare, 1986; Lu et al., 2021; Weinstock, 2017). Interestingly, both maternal stress and obesity during gestation have been associated with neonatal complications, in particular with intrauterine growth restriction (IUGR), a condition defined as the failure of the fetus to reach its full genetic growth potential (Lesage et al., 2004; Lopez-Tello et al., 2019; Radulescu et al., 2013; Rondó et al., 2003; Tanner et al., 2022). Our group has previously provided evidence that support and confirm this association, showing that maternal stress or obesity similarly cause reduced fetal weight in late gestation, an effect that is consistent across different species (Bellisario et al., 2015; Panetta et al., 2017). As expected, NAC administration revealed a strong capacity to prevent the weight gain in HFD-fed dams. These results are in agreement with previous studies by Kim and colleagues showing that NAC can exert anti-obesity activity by blocking metallothionein-II protein and removing ROS, two elements that are essential during adipocyte differentiation, thus inhibiting lipid droplet formation (Kim et al., 2006).

In agreement with the fact that increased OS and NS result from a range of adverse stimuli, when maternal blood was analyzed, we found that 3-Nitrotyrosine levels were altered by both PNS and mHFD towards a pro-oxidant profile. Since maternal blood ultimately flows into the placenta, we can assume that also the intrauterine milieu and in particular the placenta suffers from PNS/mHFD-induced OS. We have previously shown that placentas of fetuses exposed to PNS or mHFD were similarly characterized by reduced weight in late pregnancy (Bellisario et al., 2015; Panetta et al., 2017). Here we tested the hypothesis that OS might have a direct impact on placental function and that variations in total tissue weight might reflect alterations in placental structure and physiology; thus we performed histomorphometric evaluations and lipidomic analysis. The labyrinth zone is the crucial site of nutrient exchange, from the maternal to the fetal side, and it is strongly implicated in the pathogenesis of IUGR, while the decidua basalis is a maternal derived tissue with specialized glycogen-storing cells (Woods et al., 2018). Here we found that PNS reduced the thickness of the labyrinth and the decidua in a sex-dependent fashion, specifically affecting male placentas. Although we did not observe differences in mHFD labyrinth, other studies reported a smaller labyrinth in mHFD male placentas (Napso et

al., 2022), suggesting sex-specific overlapping effects of PNS and mHFD on placental morphology and functionality. Moreover, the junctional zone, which is the main endocrine compartment regulating the maternal-fetal crosstalk, was found to be increased in mHFD males only. Similar findings suggest that this effect might be mediated by increased levels of placental IGF expression as a result of overexposure to maternal corticosterone (Cuffe et al., 2012). Interestingly, NAC treatment was able to counteract such increase possibly buffering the detrimental effects of mHFD on 11β-HSD-2 activity, thus preventing excessive amount of GCs to affect the fetus (Bellisario et al., 2015; Panetta et al., 2017; Shi et al., 2020).

Our preliminary data regarding the placental lipidome content revealed important similarities between PNS and mHFD in reducing the more important component of the PCA performed on these samples, the one that accounts for the effects on phospholipids and lipid metabolism. This piece of data is not surprising as lipids play a main role in the central nervous system being the constituent of brain cell membranes. As an example, polyunsaturated fatty acids (PUFA) participate in cell signaling, neurotransmission and neuroinflammation (Bazinet & Layé, 2014). Because of limited capacity of the fetus to synthesize lipids that are essential for the developing brain (e.g. PUFA), an altered transfer across the placenta might have a detrimental impact on neurodevelopment (Devarshi et al., 2019). Thus, our data indicate that two very different prenatal conditions converge in modifying lipid content in the fetal placenta, supporting the important role of this temporary organ in transmitting the effects of adverse conditions on fetal growth.

PNS and mHFD, by increasing OS and causing placental dysfunction, might be responsible for abnormal fetal brain development specifically affecting microglial functional maturation. In order to test this hypothesis, we assessed a panel of genes involved in homeostatic and immune functions of microglia and markers of neuroinflammation. Overall, in this study we provide evidence confirming our hypothesis, showing that both PNS and mHFD trigger shared inflammatory/OS related pathways, exerting sex-specific effects on brain immune function, with greater effects on female fetuses. More in detail, we found that PNS and mHFD increased levels of one of the most representative pro-inflammatory cytokines, *Il-1β* , in the fetal brain, resembling the condition known as maternal immune activation (Baines et al., 2020). PNS was particularly effective in increasing *Il-6* levels, a condition that has been associated with increased glutamatergic synapse density and disruption of hippocampal connectivity in adulthood (Mirabella et al., 2021). It is of interest that, compared to PNS, mHFD had more pervasive effects inducing a pro-inflammatory milieu by increasing mRNA expression of growth factors (i.e. *Tgf-β* and *Igf-1*) and markers of microglia activation (i.e. *Cd68* and *Tmem119*), specifically in female brains as previously described (Kang et al., 2014). Moreover, mHFD drove the fetal brain towards a pro-oxidant profile by increasing *iNOS/Arg-1* ratio as well as decreasing *Ucp2* expression. Maintaining cellular redox balance is fundamental for brain development, not only because fetal brain is particularly vulnerable to OS and antioxidant enzymes are low expressed, but also because it regulates microglia homeostasis through the NFkB-NRF2 mutual crosstalk (Akhtar et al., 2017; Rojo et al., 2014). In this context, NAC was only partially effective in preventing the effects here described. However, we have data

to show that NAC may have more long-term effects on these dysregulations (Musillo et al, in preparation - Chapter 3).

Conclusions and future perspectives

In conclusion, our study shows that the prenatal exposure to stressors as diverse as psychophysical (PNS) or metabolic stress (mHFD) impinge upon common stress responsive pathways, affecting the maternal-fetal dyad. In particular, placental dysfunctions associated to a widespread pro-inflammatory brain profile characterize fetuses exposed to PNS or mHFD, with female offspring being more affected. We point to redox mechanisms as central players to drive fetal brain programming by prenatal adverse conditions. Thus, these findings contribute to support the "funnel effect" model, previously proposed to explain the convergent effects of different stressors on offspring brain development (Musillo et al., 2022).

It has been recently proposed that extracellular vesicles derived from the placenta and the amniotic fluid might represent a fine mechanism mediating the maternal-fetal crosstalk. These elements are easily detectable in the maternal peripheral blood, carrying a peculiar cargo of lipids, proteins and RNAs that reflects the intrauterine environment. Thus, the study of these vesicles would be complementary to our findings, providing dynamic and non-invasive biomarkers to open up new insights into the mechanisms underlying fetal programming of adverse conditions (Buca et al., 2020; Kratimenos & Penn, 2019).

Acknowledgments

We are thankful to Marianna Samà and Letizia Giona for the support in tissues collection; Enrico Cardarelli, Andrea Giovannelli and Antonio Di Virgilio for animal care.

This work was supported by ERANET NEURON JTC 2018 (Mental Disorders) Project ''EMBED" to F.C. HDHL-INTIMIC-085-GUTMOM project (co-PI F.C.) within the ERA-NET Co-fund HDHL-INTIMIC (INtesTInal MICrobiomics) programme; "Avvio alla Ricerca - Tipo 1" 2021 "Sapienza" University of Rome funding to C.M. and funding from the Italian Ministry of Health (Ricerca Corrente 2021 to F.C.). ISS Project MOMINFLAM - Unique signatures underlying placental-fetal brain crosstalk in maternal obesity. Proj code ISS20-88bc10d8839b to F.C.

Conflict of interest

The authors declare no conflict of interest

References

Akhtar, F., Rouse, C. A., Catano, G., Montalvo, M., Ullevig, S. L., Asmis, R., Kharbanda, K., & Maffi, S. K. (2017). Acute maternal oxidant exposure causes susceptibility of the fetal brain to inflammation and oxidative stress. *Journal of Neuroinflammation*, *14*(1). https://doi.org/10.1186/s12974-017-0965-8

al-Haddad, B. J. S., Oler, E., Armistead, B., Elsayed, N. A., Weinberger, D. R., Bernier, R., Burd, I., Kapur, R., Jacobsson, B., Wang, C., Mysorekar, I., Rajagopal, L., & Adams Waldorf, K. M. (2019). The fetal origins of mental illness. In *American Journal of Obstetrics and Gynecology* (Vol. 221, Issue 6, pp. 549–562). Mosby Inc. https://doi.org/10.1016/j.ajog.2019.06.013

Altman, D. G., & Bland, J. M. (2005). Treatment allocation by minimisation. *BMJ*, *330*(7495), 843. https://doi.org/10.1136/bmj.330.7495.843

Baines, K. J., Hillier, D. M., Haddad, F. L., Rajakumar, N., Schmid, S., & Renaud, S. J. (2020). Maternal Immune Activation Alters Fetal Brain Development and Enhances Proliferation of Neural Precursor Cells in Rats. *Frontiers in Immunology*, *11*, 1145. https://doi.org/10.3389/fimmu.2020.01145

Barker, D. J. P. (1995). Intrauterine programming of adult disease. In *Molecular Medicine Today* (Vol. 1, Issue 9, pp. 418–423). Mol Med Today. https://doi.org/10.1016/S1357-4310(95)90793-9

Barker, D. J. P., & Osmond, C. (1986). Infant mortality, childhood nutrition, and ischaemic heart disease in England and wales. *The Lancet*, *327*(8489), 1077–1081. https://doi.org/10.1016/S0140-6736(86)91340-1

Bazinet, R. P., & Layé, S. (2014). Polyunsaturated fatty acids and their metabolites in brain function and disease. In *Nature Reviews Neuroscience* (Vol. 15, Issue 12, pp. 771–785). Nature Publishing Group. https://doi.org/10.1038/nrn3820

Behura, S. K., Dhakal, P., Kelleher, A. M., Balboula, A., Patterson, A., & Spencer, T. E. (2019). The brain-placental axis: Therapeutic and pharmacological relevancy to pregnancy. In *Pharmacological Research* (Vol. 149, p. 104468). Academic Press. https://doi.org/10.1016/j.phrs.2019.104468

Bellisario, V., Panetta, P., Balsevich, G., Baumann, V., Noble, J., Raggi, C., Nathan, O., Berry, A., Seckl, J., Schmidt, M., Holmes, M., & Cirulli, F. (2015). Maternal high-fat diet acts as a stressor increasing maternal glucocorticoids' signaling to the fetus and disrupting maternal behavior and brain activation in C57BL/6J mice. *Psychoneuroendocrinology*, *60*, 138–150. https://doi.org/10.1016/j.psyneuen.2015.06.012

Berk, M., Malhi, G. S., Gray, L. J., & Dean, O. M. (2013). The promise of N-acetylcysteine in neuropsychiatry. In *Trends in Pharmacological Sciences* (Vol. 34, Issue 3, pp. 167–177). Trends Pharmacol Sci. https://doi.org/10.1016/j.tips.2013.01.001

Berry, A., Bellisario, V., Panetta, P., Raggi, C., Magnifico, M. C., Arese, M., & Cirulli, F. (2018). Administration of the antioxidant n-acetyl-cysteine in pregnant mice has long-term positive effects on metabolic and behavioral endpoints of male and female offspring prenatally exposed to a high-fat diet. *Frontiers in Behavioral Neuroscience*, *12*. https://doi.org/10.3389/fnbeh.2018.00048

Bilbo, S. D., & Tsang, V. (2010). Enduring consequences of maternal obesity for brain inflammation and behavior of

offspring. *The FASEB Journal*, *24*(6), 2104–2115. https://doi.org/10.1096/fj.09-144014

Biswas, S. K. (2016). Does the Interdependence between Oxidative Stress and Inflammation Explain the Antioxidant Paradox? In *Oxidative Medicine and Cellular Longevity* (Vol. 2016). Hindawi Publishing Corporation. https://doi.org/10.1155/2016/5698931

Bittle, J., Menezes, E. C., McCormick, M. L., Spitz, D. R., Dailey, M., & Stevens, H. E. (2019). The Role of Redox Dysregulation in the Effects of Prenatal Stress on Embryonic Interneuron Migration. *Cerebral Cortex*, *29*(12), 5116–5130. https://doi.org/10.1093/cercor/bhz052

Bolton, J. L., & Bilbo, S. D. (2014). Developmental programming of brain and behavior by perinatal diet: Focus on inflammatory mechanisms. *Dialogues in Clinical Neuroscience*, *16*(3), 307–320. https://doi.org/10.31887/dcns.2014.16.3/jbolton

Brannigan, R., Cannon, M., Tanskanen, A., Huttunen, M. O., Leacy, F. P., & Clarke, M. C. (2019). The association between subjective maternal stress during pregnancy and offspring clinically diagnosed psychiatric disorders. *Acta Psychiatrica Scandinavica*, *139*(4), 304–310. https://doi.org/10.1111/acps.12996

Bronson, S. L., & Bale, T. L. (2016). The Placenta as a Mediator of Stress Effects on Neurodevelopmental Reprogramming. In *Neuropsychopharmacology* (Vol. 41, Issue 1, pp. 207–218). Nature Publishing Group. https://doi.org/10.1038/npp.2015.231

Buca, D., Bologna, G., D'amico, A., Cugini, S., Musca, F., Febbo, M., D'arcangelo, D., Buca, D., Simeone, P., Liberati, M., Vitacolonna, E., Miscia, S., D'antonio, F., & Lanuti, P. (2020). Extracellular vesicles in feto–maternal crosstalk and pregnancy disorders. In *International Journal of Molecular Sciences* (Vol. 21, Issue 6). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/ijms21062120

Buss, C. (2021). Maternal oxidative stress during pregnancy and offspring neurodevelopment. In *Brain, Behavior, and Immunity* (Vol. 93, pp. 6–7). Brain Behav Immun. https://doi.org/10.1016/j.bbi.2021.01.007

Buss, H., Chan, T. P., Sluis, K. B., Domigan, N. M., & Winterbourn, C. C. (1997). Protein carbonyl measurement by a sensitive ELISA method. *Free Radical Biology and Medicine*, *23*(3), 361–366. https://doi.org/10.1016/S0891-5849(97)00104-4

Calkins, K., & Devaskar, S. U. (2011). Fetal origins of adult disease. *Current Problems in Pediatric and Adolescent Health Care*, *41*(6), 158–176. https://doi.org/10.1016/j.cppeds.2011.01.001

Chiew, A. L., Gluud, C., Brok, J., & Buckley, N. A. (2018). Interventions for paracetamol (acetaminophen) overdose. In *Cochrane Database of Systematic Reviews* (Vol. 2018, Issue 2). John Wiley and Sons, Inc. and the Cochrane Library. https://doi.org/10.1002/14651858.CD003328.pub3

Cirulli, F., & Berry, A. (2013). Early developmental trajectories of brain development: New directions in the search for early determinants of health and longevity. In G. Laviola & S. Macrì (Eds.), *Adaptive and Maladaptive Aspects of Developmental Stress* (pp. 1–281). Springer New York. https://doi.org/10.1007/978-1-4614-5605-6

Cirulli, F., De Simone, R., Musillo, C., Ajmone-Cat, M. A., & Berry, A. (2022). Inflammatory Signatures of Maternal Obesity as Risk Factors for Neurodevelopmental Disorders: Role of Maternal Microbiota and Nutritional

Intervention Strategies. *Nutrients*, *14*(15). https://doi.org/10.3390/NU14153150

Cirulli, F., Musillo, C., & Berry, A. (2020). Maternal Obesity as a Risk Factor for Brain Development and Mental Health in the Offspring. In *Neuroscience* (Vol. 447, pp. 122–135). https://doi.org/10.1016/j.neuroscience.2020.01.023

Coman, C., Solari, F. A., Hentschel, A., Sickmann, A., Zahedi, R. P., & Ahrends, R. (2016). Simultaneous metabolite, protein, lipid extraction (SIMPLEX): A combinatorial multimolecular omics approach for systems biology. *Molecular and Cellular Proteomics*, *15*(4), 1453–1466. https://doi.org/10.1074/mcp.M115.053702

Cuffe, J. S. M., O'Sullivan, L., Simmons, D. G., Anderson, S. T., & Moritz, K. M. (2012). Maternal corticosterone exposure in the mouse has sex-specific effects on placental growth and mRNA expression. *Endocrinology*, *153*(11), 5500–5511. https://doi.org/10.1210/en.2012-1479

Davis, E. P., Hankin, B. L., Glynn, L. M., Head, K., Kim, D. J., & Sandman, C. A. (2020). Prenatal Maternal Stress, Child Cortical Thickness, and Adolescent Depressive Symptoms. *Child Development*, *91*(2), e432–e450. https://doi.org/10.1111/cdev.13252

Davis, J., & Mire, E. (2021). Maternal obesity and developmental programming of neuropsychiatric disorders: An inflammatory hypothesis. *Brain and Neuroscience Advances*, *5*, 239821282110034. https://doi.org/10.1177/23982128211003484

Devarshi, P. P., Grant, R. W., Ikonte, C. J., & Mitmesser, S. H. (2019). Maternal omega-3 nutrition, placental transfer and fetal brain development in gestational diabetes and preeclampsia. *Nutrients*, *11*(5). https://doi.org/10.3390/nu11051107

du Sert, N. P., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Hurst, V., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., … Würbel, H. (2020). Reporting animal research: Explanation and elaboration for the arrive guidelines 2.0. In *PLoS Biology* (Vol. 18, Issue 7, p. e3000411). Public Library of Science. https://doi.org/10.1371/journal.pbio.3000411

Edlow, A. G., Glass, R. M., Smith, C. J., Tran, P. K., James, K., & Bilbo, S. (2019). Placental Macrophages: A Window Into Fetal Microglial Function in Maternal Obesity. *International Journal of Developmental Neuroscience*, *77*, 60–68. https://doi.org/10.1016/j.ijdevneu.2018.11.004

Gilman, S. E., Hornig, M., Ghassabian, A., Hahn, J., Cherkerzian, S., Albert, P. S., Buka, S. L., & Goldstein, J. M. (2017). Socioeconomic disadvantage, gestational immune activity, and neurodevelopment in early childhood. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(26), 6728–6733. https://doi.org/10.1073/pnas.1617698114

Grubbs, F. E. (1950). Sample Criteria for Testing Outlying Observations. *The Annals of Mathematical Statistics*, *21*(1), 27–58. https://doi.org/10.1214/aoms/1177729885

Hammond, B. P., Manek, R., Kerr, B. J., Macauley, M. S., & Plemel, J. R. (2021). Regulation of microglia population dynamics throughout development, health, and disease. In *GLIA* (Vol. 69, Issue 12, pp. 2771–2797). John Wiley & Sons, Ltd. https://doi.org/10.1002/glia.24047

Henning, T., Kochlik, B., Kusch, P., Strauss, M., Jurić, V., Pignitter, M., Marusch, F., Grune, T., & Weber, D. (2022). Pre-Operative Assessment of Micronutrients, Amino Acids, Phospholipids and Oxidative Stress in Bariatric Surgery Candidates. *Antioxidants*, *11*(4), 774. https://doi.org/10.3390/antiox11040774

Kang, S. S., Kurti, A., Fair, D. A., & Fryer, J. D. (2014). Dietary intervention rescues maternal obesity induced behavior deficits and neuroinflammation in offspring. *Journal of Neuroinflammation*, *11*(1), 156–156. https://doi.org/10.1186/s12974-014-0156-9

Khambadkone, S. G., Cordner, Z. A., & Tamashiro, K. L. K. (2020). Maternal stressors and the developmental origins of neuropsychiatric risk. In *Frontiers in Neuroendocrinology* (Vol. 57). Academic Press Inc. https://doi.org/10.1016/j.yfrne.2020.100834

Kim, J. R., Ryu, H. H., Hyun, J. C., Joo, H. L., Sang, W. K., Woo, H. K., Baek, S. H., & Jung, H. K. (2006). Association of anti-obesity activity of N-acetylcysteine with metallothionein-II down-regulation. *Experimental and Molecular Medicine*, *38*(2), 162–172. https://doi.org/10.1038/emm.2006.20

Kinsley, C., & Svare, B. (1986). Prenatal stress effects: Are they mediated by reductions in maternal food and water intake and body weight gain? *Physiology and Behavior*, *37*(1), 191–193. https://doi.org/10.1016/0031-9384(86)90405-1

Kratimenos, P., & Penn, A. A. (2019). Placental programming of neuropsychiatric disease. In *Pediatric Research* (Vol. 86, Issue 2, pp. 157–164). https://doi.org/10.1038/s41390-019-0405-9

Lesage, J., Del-Favero, F., Leonhardt, M., Louvart, H., Maccari, S., Vieau, D., & Darnaudery, M. (2004). Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behaviour disturbances in the aged rat. *Journal of Endocrinology*, *181*(2), 291–296. https://doi.org/10.1677/joe.0.1810291

Londono Tobon, A., Diaz Stransky, A., Ross, D. A., & Stevens, H. E. (2016). Effects of Maternal Prenatal Stress: Mechanisms, Implications, and Novel Therapeutic Interventions. In *Biological Psychiatry* (Vol. 80, Issue 11, pp. e85–e87). Biol Psychiatry. https://doi.org/10.1016/j.biopsych.2016.09.011

Lopez-Tello, J., Arias-Alvarez, M., Gonzalez-Bulnes, A., & Sferuzzi-Perri, A. N. (2019). Models of Intrauterine growth restriction and fetal programming in rabbits. In *Molecular Reproduction and Development* (Vol. 86, Issue 12, pp. 1781–1809). Mol Reprod Dev. https://doi.org/10.1002/mrd.23271

Lu, J., Liu, G., Wang, Z., Cao, J., Chen, Y., & Dong, Y. (2021). Restraint stress induces uterine microenvironment disorder in mice during early pregnancy through the β2-AR/cAMP/PKA pathway. *Stress*, *24*(5), 514–528. https://doi.org/10.1080/10253890.2020.1855419

Maccari, S., Piazza, P. V., Kabbaj, M., Barbazanges, A., Simon, H., & Le Moal, M. (1995). Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. *Journal of Neuroscience*, *15*(1 I), 110–116. https://doi.org/10.1523/jneurosci.15-01-00110.1995

McFarlane, L., Truong, V., Palmer, J. S., & Wilhelm, D. (2013). Novel PCR assay for determining the genetic sex of mice. *Sexual Development*, *7*(4), 207–211. https://doi.org/10.1159/000348677

Mirabella, F., Desiato, G., Mancinelli, S., Fossati, G., Rasile, M., Morini, R., Markicevic, M., Grimm, C., Amegandjin,

C., Termanini, A., Peano, C., Kunderfranco, P., di Cristo, G., Zerbi, V., Menna, E., Lodato, S., Matteoli, M., & Pozzi, D. (2021). Prenatal interleukin 6 elevation increases glutamatergic synapse density and disrupts hippocampal connectivity in offspring. *Immunity*, *54*(11), 2611-2631.e8. https://doi.org/10.1016/j.immuni.2021.10.006

Musillo, C., Berry, A., & Cirulli, F. (2022). Prenatal psychological or metabolic stress increases the risk for psychiatric disorders: the "funnel effect" model. In *Neuroscience and Biobehavioral Reviews* (Vol. 136). Neurosci Biobehav Rev. https://doi.org/10.1016/j.neubiorev.2022.104624

Napso, T., Lean, S. C., Lu, M., Mort, E. J., Desforges, M., Moghimi, A., Bartels, B., El-Bacha, T., Fowden, A. L., Camm, E. J., & Sferruzzi-Perri, A. N. (2022). Diet-induced maternal obesity impacts feto-placental growth and induces sex-specific alterations in placental morphology, mitochondrial bioenergetics, dynamics, lipid metabolism and oxidative stress in mice. *Acta Physiologica*, *234*(4), e13795. https://doi.org/10.1111/apha.13795

Panetta, P., Berry, A., Bellisario, V., Capoccia, S., Raggi, C., Luoni, A., Longo, L., Riva, M. A., & Cirulli, F. (2017). Long-term sex-dependent vulnerability to metabolic challenges in prenatally stressed rats. *Frontiers in Behavioral Neuroscience*, *11*. https://doi.org/10.3389/fnbeh.2017.00113

Radulescu, L., Munteanu, O., Popa, F., & Cirstoiu, M. (2013). The implications and consequences of maternal obesity on fetal intrauterine growth restriction. *Journal of Medicine and Life*, *6*(3), 292–298. /pmc/articles/PMC3806033/

Rojo, A. I., McBean, G., Cindric, M., Egea, J., López, M. G., Rada, P., Zarkovic, N., & Cuadrado, A. (2014). Redox control of microglial function: Molecular mechanisms and functional significance. In *Antioxidants and Redox Signaling* (Vol. 21, Issue 12, pp. 1766–1801). Mary Ann Liebert, Inc. https://doi.org/10.1089/ars.2013.5745

Rondó, P. H. C., Ferreira, R. F., Nogueira, F., Ribeiro, M. C. N., Lobert, H., & Artes, R. (2003). Maternal psychological stress and distress as predictors of low birth weight, prematurity and intrauterine growth retardation. *European Journal of Clinical Nutrition*, *57*(2), 266–272. https://doi.org/10.1038/sj.ejcn.1601526

Sanchez, C. E., Barry, C., Sabhlok, A., Russell, K., Majors, A., Kollins, S. H., & Fuemmeler, B. F. (2018). Maternal pre-pregnancy obesity and child neurodevelopmental outcomes: a meta-analysis. In *Obesity Reviews* (Vol. 19, Issue 4, pp. 464–484). Blackwell Publishing Ltd. https://doi.org/10.1111/obr.12643

Saulle, E., Spinello, I., Quaranta, M. T., Pasquini, L., Pelosi, E., Iorio, E., Castelli, G., Chirico, M., Pisanu, M. E., Ottone, T., Voso, M. T., Testa, U., & Labbaye, C. (2021). Targeting Lactate Metabolism by Inhibiting MCT1 or MCT4 Impairs Leukemic Cell Proliferation, Induces Two Different Related Death-Pathways and Increases Chemotherapeutic Sensitivity of Acute Myeloid Leukemia Cells. *Frontiers in Oncology*, *10*, 1. https://doi.org/10.3389/fonc.2020.621458

Shi, X. T., Zhu, H. L., Xiong, Y. W., Liu, W. B., Zhou, G. X., Cao, X. L., Yi, S. J., Dai, L. M., Zhang, C., Gao, L., Xu, D. X., & Wang, H. (2020). Cadmium down-regulates 11β-HSD2 expression and elevates active glucocorticoid level via PERK/p-eIF2α pathway in placental trophoblasts. *Chemosphere*, *254*. https://doi.org/10.1016/j.chemosphere.2020.126785

Srinivasan, R., Pearson, R. M., Johnson, S., Lewis, G., & Lewis, G. (2020). Maternal perinatal depressive symptoms and offspring psychotic experiences at 18 years of age: a longitudinal study. *The Lancet Psychiatry*, *7*(5), 431–440. https://doi.org/10.1016/S2215-0366(20)30132-2

Tait, S., Tassinari, R., Maranghi, F., & Mantovani, A. (2015). Bisphenol A affects placental layers morphology and angiogenesis during early pregnancy phase in mice. *Journal of Applied Toxicology*, *35*(11), 1278–1291. https://doi.org/10.1002/jat.3176

Takahashi, K., Naito, M., Katabuchi, H., & Higashi, K. (1991). Development, differentiation, and maturation of macrophages in the chorionic villi of mouse placenta with special reference to the origin of Hofbauer cells. *Journal of Leukocyte Biology*, *50*(1), 57–68. https://doi.org/10.1002/jlb.50.1.57

Tanner, L. D., Brockand, C., & Chauhan, S. P. (2022). Severity of fetal growth restriction stratified according to maternal obesity. *Journal of Maternal-Fetal and Neonatal Medicine*, *35*(10), 1886–1890. https://doi.org/10.1080/14767058.2020.1773427

Tuovinen, S., Lahti-Pulkkinen, M., Girchenko, P., Heinonen, K., Lahti, J., Reynolds, R. M., Hämäläinen, E., Villa, P. M., Kajantie, E., Laivuor, H., & Raikkonen, K. (2021). Maternal antenatal stress and mental and behavioral disorders in their children. *Journal of Affective Disorders*, *278*, 57–65. https://doi.org/10.1016/j.jad.2020.09.063

Vacher, C. M., Lacaille, H., O'Reilly, J. J., Salzbank, J., Bakalar, D., Sebaoui, S., Liere, P., Clarkson-Paredes, C., Sasaki, T., Sathyanesan, A., Kratimenos, P., Ellegood, J., Lerch, J. P., Imamura, Y., Popratiloff, A., Hashimoto-Torii, K., Gallo, V., Schumacher, M., & Penn, A. A. (2021). Placental endocrine function shapes cerebellar development and social behavior. *Nature Neuroscience*, *24*(10), 1392–1401. https://doi.org/10.1038/s41593-021-00896-4

Van den Bergh, B. R. H., van den Heuvel, M. I., Lahti, M., Braeken, M., de Rooij, S. R., Entringer, S., Hoyer, D., Roseboom, T., Räikkönen, K., King, S., & Schwab, M. (2020). Prenatal developmental origins of behavior and mental health: The influence of maternal stress in pregnancy. In *Neuroscience and Biobehavioral Reviews* (Vol. 117, pp. 26–64). Elsevier Ltd. https://doi.org/10.1016/j.neubiorev.2017.07.003

Weber, D., Stuetz, W., Bernhard, W., Franz, A., Raith, M., Grune, T., & Breusing, N. (2014). Oxidative stress markers and micronutrients in maternal and cord blood in relation to neonatal outcome. *European Journal of Clinical Nutrition*, *68*(2), 215–222. https://doi.org/10.1038/ejcn.2013.263

Weinstock, M. (2017). Prenatal stressors in rodents: Effects on behavior. In *Neurobiology of Stress* (Vol. 6, pp. 3–13). Elsevier Inc. https://doi.org/10.1016/j.ynstr.2016.08.004

Wilkes, J. M., Clark, L. E., & Herrera, J. L. (2005). Acetaminophen overdose in pregnancy. In *Southern Medical Journal* (Vol. 98, Issue 11, pp. 1118–1122). https://doi.org/10.1097/01.smj.0000184792.15407.51

Wong, S. H. Y., Knight, J. A., Hopfer, S. M., Zaharia, O., Leach, C. N., & Sunderman, F. W. (1987). Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clinical Chemistry*, *33*(2), 214–220. https://doi.org/10.1093/clinchem/33.2.214

Woods, L., Perez-Garcia, V., & Hemberger, M. (2018). Regulation of Placental Development and Its Impact on Fetal Growth—New Insights From Mouse Models. In *Frontiers in Endocrinology* (Vol. 9, p. 570). Frontiers Media S.A. https://doi.org/10.3389/fendo.2018.00570

CHAPTER 4

Oxidative stress as a shared mechanism underlying the long-term effects of different prenatal stressors on brain plasticity and emotional behavior of adolescent mice

Chiara Musillo[1,2], Kerstin C. Creutzberg[3], Barbara Collacchi[1], Maria Antonietta Ajmone-Cat[4], Roberta De Simone[4], Marcello Lepre[1], Irmgard Amrein[5], Lutz Slomianka[5], Marco A. Riva[3], Alessandra Berry[1], Francesca Cirulli[1]

[1] Center for Behavioral Sciences and Mental Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. [2] Ph.D. Program in Behavioral Neuroscience, Department of Psychology, Sapienza University of Rome, 00185 Rome, Italy. [3] Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy. [4] National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. [5] Functional Neuroanatomy, Institute of Anatomy, University of Zürich, Zurich, Switzerland.

In preparation

Abstract

The intrauterine milieu plays a pivotal role for fetal brain development and maternal stressful events can negatively shape developmental trajectories, increasing risks for neuropsychiatric diseases. Maternal obesity is emerging as a stressor capable to affect the developing fetal brain exerting long-term effects comparable to those resulting from maternal psychological stress. Here, we hypothesized that prenatal adversities as diverse as psychophysical or metabolic stress might affect neuronal structures involved in emotional regulation and stress responses to a very similar extent, triggering oxidative stress derangements, leading to a long-lasting vulnerability to neuropsychiatric diseases. We contrasted and compared adolescent male and female offspring of two mouse models of maternal psychophysical stress (restraint during pregnancy - PNS) and maternal obesity (high-fat diet before and during gestation - mHFD) by combining behavioral assays, evaluation of the hypothalamic-pituitary-adrenal (HPA) axis reactivity and a targeted gene expression analysis in the hippocampus. The prenatal administration of the antioxidant N-acetyl-cysteine (NAC) was used as a strategy to protect fetal neurodevelopment from the negative effects of PNS and mHFD. Our findings show that different stressors can have comparable effects, reducing brain anti-oxidant defenses, driving impairments in hippocampal *Bdnf* levels and leading to alterations in the emotional behavior and HPA axis functionality, in a sex-dependent fashion. Moreover, maternal NAC treatment, by restoring the redox balance, has long-term protective effects on brain development.

Keywords

Maternal obesity, maternal stress, fetal programming, brain, BDNF, adolescence, sex, oxidative stress, behavior, mouse model.

Introduction

Psychological stress or overt psychiatric disorders affect about 10-15% of pregnant women worldwide being issues of main concern not only for the mother but also for the mental health of the offspring (https://www.who.int/). Likewise, a variety of different modern-day life environmental challenges, such as infection, air pollution, smoking or dietary imbalance may contribute to disrupt the intrauterine environment and, in turn, affect fetal development (al-Haddad et al., 2019; Banderali et al., 2015; Block et al., 2022; Cirulli et al., 2020). Among the others, metabolic stress during pregnancy, due to unhealthy nutritional habits, is emerging as a pressing public health issue. While pioneering studies in this field of research were prompted by the sequelae of starvation and undernutrition on fetal development due to the second World War (Barker and Osmond, 1986; Roseboom et al., 2011), we are currently dealing with quite an opposite condition such as prenatal exposure to an "obesogenic environment" characterized by unlimited access to high-caloric "junk food" (Cirulli et al., 2020; Congdon, 2019). In this context, a growing body of evidence links maternal overnutrition or obesity to a higher risk for neurodevelopmental and psychiatric disorders in the offspring such as cognitive deficits, attention deficit hyperactivity, autism spectrum disorders and depression (Cirulli et al., 2020; Davis and Mire, 2021; Sanchez et al., 2018). Intriguingly, similar health outcomes have been also reported as a result of maternal psychological stress during pregnancy (Brannigan et al., 2019; Gustafsson et al., 2018; Lipner et al., 2019). This piece of evidence leads to hypothesize that, according to a "funnel effect" model, stressors of different nature might trigger similar stress-responsive pathways affecting neuroendocrine system, immune-inflammatory processes and energy metabolism regulation (Musillo et al., 2022). Indeed, taking advantage of mouse models of maternal psychophysical stress and obesity, we have previously provided evidence to support such a "funnel effect" model, showing that these two prenatal stressors exert similar detrimental effects at least during early developmental stages, weakening the placental barrier and affecting fetal brain development towards a pro-inflammatory/pro-oxidant phenotype (Bellisario et al., 2015; Panetta et al., 2017)Musillo et al. in preparation).

Adolescence represents a critical time window for the onset of neuropsychiatric disorders. In fact the adolescent brain is characterized by a high degree of plasticity and undergoes many physiological rearrangements including an overproduction of synapses - to increase the plasticity needed to adapt to the environmental stimuli - followed by synaptic pruning that refines neuronal connections shaping complex circuits and removing synapses with low activity (Katz and Shatz, 1996; Malave et al., 2022). Microglia, the resident immune cells in the brain, play a key role during pruning by phagocytosing targeted synapses (Schafer et al., 2012). Exposure to early life stressors (e.g. maternal stress, infection or obesity) can disrupt microglial function, ultimately resulting in permanent immature synapses characterized by weak transmission and altered connectivity causing impairments in the maturation of specific brain regions such as the hippocampus (Block et al., 2022; Bordeleau et al., 2020; Delpech et al., 2016; Paolicelli et al., 2011). In addition to the brain, the hypothalamic-pituitary-adrenal (HPA) axis also displays a great plasticity during adolescence, being characterized by a delayed and a more prolonged activation in response to stress, compared to adulthood, with

sex hormones driving its activity (McCormick and Mathews, 2007). Thus, the programming effects of early life stressors on the HPA axis functionality might be exacerbated in adolescent offspring, resulting in maladaptive coping strategies in response to stressful events.

While adolescence represents a window of vulnerability, at the same time it could also represent an important window of opportunity for early intervention. N-acetyl-cysteine (NAC) is receiving particular attention in the treatment of neuropsychiatric disorders due to its ability to interact with neurotransmitter systems, inflammatory processes and oxidative stress (OS) related pathways (Berk et al., 2013). We have previously shown beneficial effects of maternal NAC treatment in alleviating the short-term negative effects of prenatal psychophysical or metabolic stress on fetal neuroinflammation and redox balance (Berry et al., 2018) Musillo et al. in preparation). This evidence points to NAC as a promising candidate compound capable to modulate converging mechanisms activated by different stressors such as psychophysical stress and maternal obesity.

The aim of this study was to compare and contrast the potential negative health outcomes deriving from prenatal exposure to either psychophysical (maternal restraint stress during the last third of pregnancy) or metabolic stress (maternal high-fat diet - HFD - consumption before and during gestation) in adolescent male and female mouse offspring. Main focus has been given to the investigation of OS as a central mechanism put in place within the hypothesized "funnel effect" model. More in detail, in the adolescent male and female offspring we characterized HPA axis reactivity, emotional profile and sociability as well as coping strategies to an acute stressor, in addition to brain expression of genes involved in neuroendocrine, metabolic, inflammatory and OS regulation. Finally, we tested the hypothesis that maternal antioxidant (NAC) treatment, modulating redox mechanisms, would prevent the negative effects of prenatal stressors on the offspring.

Materials and Methods

This study was reported in conformity with ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) (du Sert et al., 2020).

Animal handling

One-month-old C57BL/6N mice, 190 females and 95 males, were purchased from a commercial breeder (Charles River, Italy) and housed sex-matched two/cage in transparent Plexiglas cages provided by Tecniplast, in an air-conditioned room (temperature 21 ± 1°C, relative humidity 60 ± 10%), under a reversed 12/12 h light/dark cycle with lights off from 07:00 a.m. to 07:00 p.m. Fresh tap-water and standard chow (energy 3.3 kcal/g, fat 17%, carbohydrate 60% and protein 23% provided by Mucedola, Italy) were constantly available. After three weeks of habituation, female mice were allocated into the experimental groups (see below for further details).

All experimental procedures were approved by the ethical body of the Istituto Superiore di Sanità for animal welfare and conducted in conformity with the European Directive 2010/63/EU and the Italian legislation on animal experimentation, D.Lgs. 26/2014. They were authorized by the Italian Ministry of Health.

Prenatal stressors

Female breeders were allocated into two models of maternal stress as previously described (see Musillo et al. 2022 *in preparation*), where the assignment of each animal to either model and experimental groups was based on a minimization approach avoiding body weight bias.

Psychophysical stress (maternal restraint - prenatal stress - PNS). In this cohort, 47 pregnant females were assigned to the control (CTRL = 27) or the PNS (PNS = 20) groups. Females assigned to the PNS group were individually restrained in a transparent Plexiglas cylinder (11.5 × 3 cm) and contextually exposed to bright light as an additional stressor (6.500 lux) for 30 minutes, three times daily, from gestational day (G) 12.5 until G18.5. Stress sessions were conducted during the dark phase (07:00 a.m. to 07:00 p.m.) at different times during the day in order to prevent habituation to the repeated procedure (Maccari et al., 1995). Females assigned to the CTRL group were left undisturbed during the entire pregnancy period.

Metabolic stress (maternal HFD - mHFD). Female breeders were assigned to the control diet (CD, n = 47) or the high-fat diet (HFD, n = 48) groups. Diets were administered *ad libitum* for 10 weeks before mating and throughout gestation (for a total of 13 weeks), until G16 when both HFD and CD were replaced with a standard chow to prevent cannibalistic behaviors and pups' mortality (Bellisario et al., 2015). HFD (D12331, energy 5.56 kcal/g, fat 58%, carbohydrate 25.5% and protein 16.4) and CD (D12328, energy 4.07 kcal/g, fat 10.5%, carbohydrate 73.1% and protein 16.4%) were provided by Research Diets Inc., New Brunswick, NJ, USA.

NAC administration and breeding procedure

Five weeks before mating, female breeders were divided in two groups receiving either the antioxidant treatment with NAC (n = 95: 47 for the PNS model and 48 for the mHFD model) or tap water as vehicle (n = 95: 47 for the PNS model and 48 for the mHFD model). Treatment was administered also during pregnancy until G16, for a total of 8 weeks. In order to minimize stress due to excessive handling procedure, NAC was daily administered in drinking water to yield an average dose of 1 g/kg body weight (Berry et al., 2018). NAC was purchased as powder by Sigma Aldrich.

After 5 weeks on NAC administration (and 10 weeks on diets for mHFD cohort), all females were bred (see Bellisario et al., 2015 for further details on the breeding procedure). Body weight gain was measured weekly before mating as well as during gestation, specifically at G7, at G14 and at G18 to monitor pregnancy. Females that did not get pregnant underwent a second 48 h mating with a different male breeder.

The final number of pregnant females was: CTRL-Vehicle = 14; CTRL-NAC = 11; PNS-Vehicle = 11; PNS-NAC = 11; CD-Vehicle = 13; CD-NAC = 13; HFD-Vehicle = 12; HFD-NAC = 12.

Experimental procedures on the adolescent offspring

Pups' birth was considered as post-natal day 0 (PND 0). At PND 30, offspring were weaned and body weight registered. One or two males and one or two females, when possible, were selected from each litter to perform the behavioral characterization. Litters with ≤3 pups were excluded from the study. Starting at PND 33, male and female offspring were tested to assess the emotional profile, the sociability and the neuroendocrine reactivity in response to an acute stress, resulting from prenatal exposure to PNS or mHFD and the role of NAC on these effects. The experimental design of this study is reported in Figure 1.

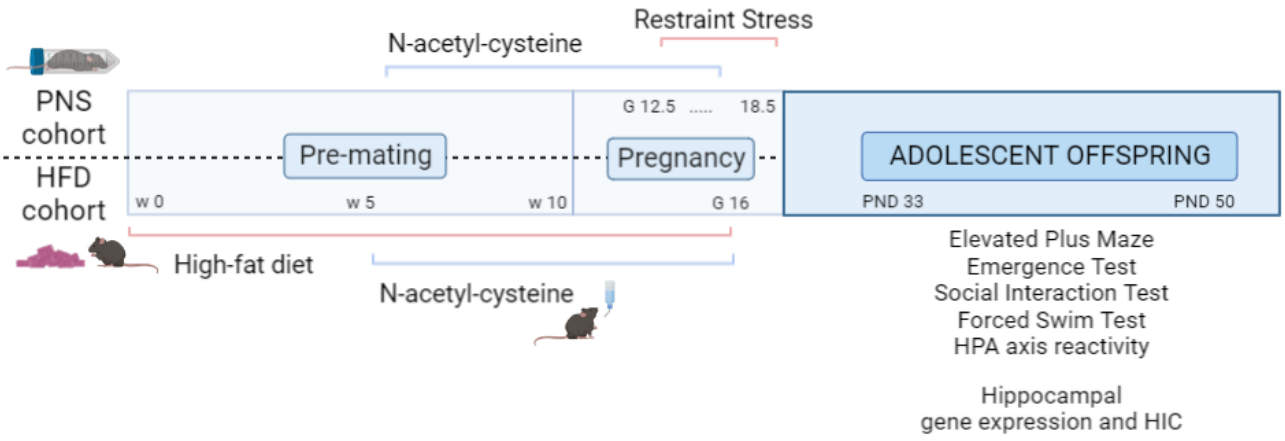


Figure 1 Experimental design.

Behavioral phenotype

All tests were video-recorded and behavioral analysis was performed by an observer blind to the experimental conditions using dedicated video tracking software: The Observer XT 15, Noldus (the Netherlands) and AnyMaze (Stoelting CO, Wood Dale, IL, USA). All apparatuses were cleaned by a cotton pad and 70% ethanol solution between each session.

Elevated Plus Maze - EPM. Emotional profile, exploration and risk-assessment behaviors were evaluated through the EPM. The apparatus was made of two open (30 x 5 cm) and two enclosed arms (30 x 5 x 15 cm) that extend from a common central platform (5 x 5 cm). The apparatus was made by Plexiglas (dim grey floor, transparent walls) and raised to a height of 60 cm above the floor level. Mice were individually placed on the central platform facing an open arm and were allowed to freely explore the maze for 5 minutes. The % time spent in the open vs the closed arms was assessed (Fernandes and File, 1996). In addition, the frequency of rearing, wall-rearing and head dipping were evaluated through a Z-score as a behavioral index of exploratory activity, while the frequency of stretched attend posture (SAP) was analyzed as a measurement of risk assessment. During the test, illumination level was maintained at 100 lux.

Emergence test. This test was used to further characterize the emotional profile. The apparatus was a cubic arena (40 x 40 x 40 cm) made of Plexiglas, ideally divided into 25 squares partitioned into a central zone (24 x 24 cm) and a peripheral zone (the remaining part of the arena). A shelter (black plastic cup - 15 cm diameter) in one corner of the arena provided a retreat possibility from the brightly lit arena (600 lux) (Lalonde and Strazielle, 2009). Each mouse started the test inside the shelter and the latency to emerge was evaluated as an index of emotionality or loss of inhibition. The test had a total duration of 20 min during which the mice were free to explore: distance travelled, mean speed and the time spent in the different zones of the arena were measured.

Social interaction test - SIT. To increase social behaviors, 24 hours before the test experimental subjects were individually housed. During the test, mice were placed in a novel cage, identical to the holding cage, with an unfamiliar conspecific, sex- and weight-matched that had been previously isolated (standard opponent with the tail marked by a nontoxic paint). During each 20 min session, the frequency of snout sniffing, body sniffing and ano-genital sniffing were evaluated through a composite Z-score as an index of social behaviors, while the durations of wall-rearing and rearing have been added together and analyzed as an index of vertical exploration.

Forced swim test - FST - and coping stress strategy. This test was used to evaluate the copying strategies in response to an acute stress. Mice were gently placed into a cylindrical transparent tank (30 cm h x 20 cm diameter) filled with water (26 ± 1°C) up to 25 cm, so mice were not able to touch the bottom of the tank. One session of 6 minutes was performed and only the last four minutes were analyzed (Can et al., 2011). The % time spent performing the following strategies was assessed: passive strategy (floating); active strategy (swimming and struggling).

Plasma corticosterone levels in response to forced swim test

The activation of the HPA axis was assessed in response to an unescapable stressful challenge. All mice underwent an acute unescapable forced swim test (6 min) and blood samples were collected by a tail nick at different time points: baseline (0 min) before the stress, 30 and 180 following the stress exposure, to measure plasma levels of corticosterone (CORT). Blood samples were collected in potassium EDTA coated tubes and plasma was separated by centrifugation at 3000 rpm for 15 min at +4°C and immediately stored at -80°C. CORT levels were determined using an enzyme-linked immunoassay based commercial kit (Enzo Life Sciences, NY, USA).

Tissues collection

At PND 50, male and female offspring were sacrificed by cervical dislocation and trunk blood was collected into either heparinized or standard tubes to obtain plasma or serum respectively. Tubes were centrifuged at 3000 rpm for 15 min at +4°C and immediately stored at -80°C until further analysis. Brains were removed from the skull, hippocampi were dissected from the left hemispheres and immediately frozen at -80°C until gene expression analysis. The right hemispheres were post-fixed in 4% paraformaldehyde (PFA) overnight at +4°C and then stored in Sodium Azide 0.05 solution until immunohistochemistry analysis.

RNA extraction and gene expression analysis

Total RNA was isolated from the left hippocampus using the RNeasy Micro Kit (Qiagen) according to the manufacturer's protocol. RNA concentration was measured at NanoDrop spectrophotometer (Thermo Fisher) and further used for quantitative real-time polymerase chain reaction (qRT-PCR) (CFX384 real-time system, Bio-Rad Laboratories). Samples were run in triplicate and beta-actin was used as housekeeping gene. Primers for Nuclear factor erythroid 2-related factor 2 (Nrf2, Mm00477784_m1); Kelch Like ECH Associated Protein 1 (Keap1, Mm00497268_m1); cluster of differentiation 68 (CD68, Mm03047343_m1), triggering receptor expressed on myeloid cells 2 (TREM 2, Mm04209424_g1), transmembrane protein 119 (TMEM 119, Mm00525305_m1) were purchased from Thermo Fisher Scientific while beta-actin (Fwd: ACCTTCTACAATGAGCTGCG, Rev: CTGGATGGCTACGTACATGG, probe: TCTGGGTCATCTTTTCACGGTTGGC), Bdnf total (Fwd: AAGTCTGCATTACATTCCTCGA, Rev: GTTTTCTGAAAGAGGGACAGTTTAT, probe: TGTGGTTTGTTGCCGTTGCCAAG), primers and probes were purchased from Eurofins Genomics. All analyses were performed following the deltadeltaCT method with the beta-actin as endogenous control. Data are presented as fold change % compared to the control group (set at 100%).

Immunohistochemistry (HIC)

Post-fixed right hemispheres were coded to conduct the HIC procedure and analysis. Brains were placed in 20% glycerol solution at 4 °C overnight for cryoprotection and then embedded in gelatin-egg-albumin blocks (4 brains in each block) (Smiley and Bleiwas, 2012). Each block was cut into 40 µm-thick coronal sections using a freezing microtome (HYRAX S 30), ten complete series were collected in cryoprotection solution and stored at -20°C until use. One complete series was mounted in the correct anatomical order (reference series) and Giemsa stained. Sections containing the hippocampus were selected based on the stereotaxic atlas of Paxinos and Franklin (4th edition) and used for HIC Iba-1 staining. For HIC staining, one complete series of free-floating sections were first rinsed in Tris-Triton and incubated in citrate buffer [0.1 M] at 95 °C for 40 min to retrieve antigens. After cooling down, the sections were treated with 0.6% peroxidase solution for 15 min and rinsed again in Tris-Triton. They were then placed in a blocking buffer of 2% normal goat serum (NGS) and 0.2% Triton in Tris-Triton for 1h. Afterwards, sections were incubated with anti-IBA1 antibody (ionized calcium-binding adapter molecule 1; anti-rabbit, 1:3000, Wako) at 4 °C overnight. The next day, sections were rinsed in Tris-buffered saline (TBS) and incubated with a goat-anti-rabbit secondary antibody (1:300, Reactolab) for 40 min; rinsed again in TBS and incubated with ABC solution (Reactolab) for 20 min. After additional rinses, sections were stained with DBA (Sigma-Aldrich) for 4 min, mounted, counterstained with Giemsa and embedded.

Quantitative analysis of Iba1-positive cells

Quantification of Iba1-positive cells in the hippocampal CA1 region was performed using the optical fractionator probe (Slomianka, 2021; West et al., 1991), with section sampling fraction of 10, step size of 250µm and frame size of 90µm. Delineation of the region was made based on the reference series and mouse brain atlas of Paxinos and Franklin (4th edition), on average 6 sections per animal contained the CA1 region. Iba1-positive cells were identified as ramified (multiple long processes) or intermediate (multiple short processes). Iba1-positive cell number estimates were performed with StereoInvestigator 10 software (MBF Bioscience, Williston, VT, USA) on a Zeiss microscope (Zeiss, Germany) using a 40x oil immersion lens. Sections were analyzed in the correct anatomical order, cell numbers from each section were then standardized into 2 virtual sections representing the dorsal (rostral) and ventral (intermediate/temporal) CA1 region (Amrein et al., 2015). Analysis was done blind to animal identity in 6-7 animals per sex and experimental group.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA). All the data were graphically presented as mean ± SEM. Since a main effect of sex was often found on the parameters related to behavioral tests and *ex vivo* analyses, male and female mice were analyzed separately

by means of a two-way ANOVA with prenatal condition (PNS/CTRL or HFD/CD) and prenatal treatment (NAC/Vehicle) as between-subject factors and repeated measure as within subject factors (i.e. time, zones of the apparatus, changes in CORT levels, microglia cell morphology). Post hoc comparisons among groups were performed using the Tukey's test. The cumulative incidence of latency to emerge from the shelter was analyzed using a log-rank (Mantel–Cox) test (Jahn-Eimermacher et al., 2011) with the application of Bonferroni correction. A composite Z-score was calculated using the three main behaviors used for measuring the exploration of the environment in the EPM test (Kestering-Ferreira et al., 2021). We included the frequency of rearing, wall-rearing and head dipping and refer to this score as "Exploration" Z-score. Moreover, we also calculated a composite Z-score for social behaviors evaluated in the SIT test, including the frequency of those behaviors directed to the conspecific such as snout sniffing, body sniffing and ano-genital sniffing. Binary data such as the number of animals performing (or not) self-grooming within the same experimental group were analyzed by Fisher's exact test. Grubb's test, using 5% significance level critical values, was used to detect outliers (Grubbs, 1950). A level of probability set a $p<0.05$ was used as statistically significant. Statistical tendency was set at $p<0.1$ and for interactions at $p<0.1$ also lower order effects were evaluated.

Results

Elevated plus maze - EPM

PNS cohort. In general, all adolescent mice showed a preference for the open arms of the apparatus rather than the closed arms (Main effect of zone - females: $F(1,37)=9.401$, $p=0.0040$; males: $F(1,43)=3.612$, $p=0.0641$), a behavior often observed during adolescence. In particular, PNS females spent more time in the open arms compared to the controls (interaction between stress and zone: $F(1,37)=7.867$, $p=0.0080$; post hoc comparisons: $p<0.05$ PNS vs CTRL), see Figure 2A. When we assessed the composite exploration Z-score, we found that PNS increased exploratory activity in both sexes (females: $F(1,37)=5.136$, $p=0.0294$; males: $F(1,43)=9.290$, $p=0.0039$) and reduced the frequency of risk assessment behaviors (females: $F(1,37)=4.947$, $p=0.0323$; males: $F(1,43)=5.015$, $p=0.0304$), see Figure 2B. In addition, the number of PNS females performing self-grooming was greater when compared to controls (Fisher's test $p=0.0094$, PNS-Veh vs CTRL-Veh). In this specific test, maternal administration of NAC did not affect the parameters assessed.

mHFD cohort. Also in this cohort, all adolescent mice preferred the open arms (Main effect of zone - females: $F(1,43)=12.891$, $p=0.0008$; males: $F(1,49)=12.848$, $p=0.0008$). Similarly to PNS, prenatal exposure to the mHFD increased the time spent by female subjects in the open arms compared to the controls (interaction between diet and zone: $F(1,43)=5.827$, $p=0.0201$; post hoc comparisons: $p<0.05$ HFD vs CD), see Figure 2A. However, differently from what was observed in the PNS cohort, mHFD was associated with a decreased composite exploration Z-score specifically in female mice ($F(1,43)=4.611$, $p=0.0374$), while no difference between HFD and CD groups was observed in the frequency of risk assessment behaviors (Figure 2B). In this specific test, maternal administration of NAC did not affect the parameters assessed.

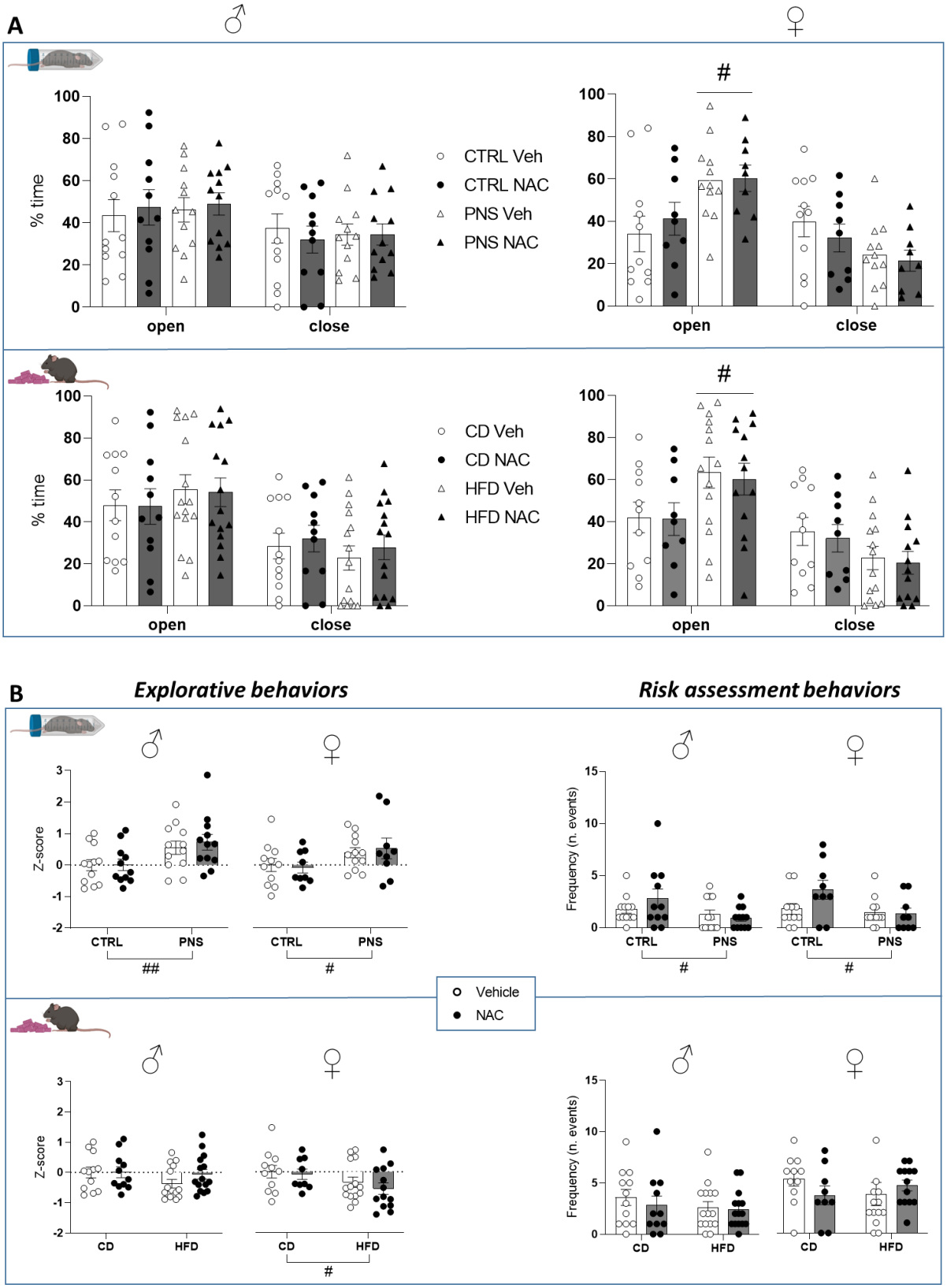


Figure 2 Emotional and explorative behaviors in the EPM. A) The prenatal exposure to either PNS or mHFD resulted in similar effects on the emotional profile of female offspring increasing the time spent in the open arms. **B)** Overall, PNS increased exploration and reduced risk assessment behaviors while mHFD reduced exploratory behaviors only in females

and did not affect risk assessment. # $p<0.05$, ## $p<0.01$ main effect of prenatal stress/diet. Data are mean ± SEM. Number of subjects: 9-15 within each experimental group.

Emergence test

PNS cohort. Latency to first emergence from the shelter showed that PNS affected female offspring only, reducing the latency to emerge compared to their CTRL counterpart (χ^2=8.364, p=0.0038), see Figure 3A. Sex-dependent effects were also found when the time spent in the different zones of the apparatus was evaluated. More in detail, an interaction between stress and zones revealed that PNS females reduced the time spent inside the shelter while increased the time spent in the periphery of the arena compared to control subjects (F(3,126)=9.585, $p<0.0001$; post hoc comparisons: $p<0.01$ PNS vs CTRL), see Figure 3B. Furthermore, PNS females were characterized by enhanced speed and increased distance travelled when compared to CTRL females (Main effect of stress - mean speed: F(1,42)=5.675, p=0.0218; distance: F(1,42)=5.485, p=0.0240. See Table1). In this specific test, maternal administration of NAC did not affect the parameters assessed.

mHFD cohort. No difference between the groups was observed when assessing the latency to emerge from the shelter. A general preference for the peripheral zone of the apparatus was observed in all experimental subjects (Main effect of zones - males: F(3,93)=519.339, $p<0.0001$; females: F(3,87)=374.592, $p<0.0001$), see Figure 3A. Overall, neither the prenatal exposure to mHFD nor NAC affected the behaviors assessed in the Emergence test in both sexes (Figure 3B and Table1).

Table 1 Locomotor activity in the Emergence test

	Distance (m)		**Speed (cm/s)**	
	Males	**Females**	**Males**	**Females**
CTRL-Vehicle	32.43 ± 2.84	32.06 ± 1.48	2.69 ± 0.24	2.67 ± 0.13
CTRL-NAC	30.77 ± 2.51	34.33 ± 2.66	2.56 ± 0.21	2.85 ± 0.22
PNS-Vehicle	33.09 ± 1.94	37.45 ± 2.40	2.75 ± 0.16	3.12 ± 0.20
PNS-NAC	29.76 ± 2.54	41.73 ± 3.79	2.49 ± 0.21	3.48 ± 0.31
CD-Vehicle	38.20 ± 1.35	35.63 ± 1.47	3.18 ± 0.11	2.96 ± 0.13
CD-NAC	36.41 ± 2.92	39.76 ± 2.70	3.03 ± 0.24	3.30 ± 0.22
HFD-Vehicle	33.67 ± 2.33	37.14 ± 2.48	2.82 ± 0.19	3.09 ± 0.21
HFD-NAC	39.86 ± 2.34	41.90 ± 4.06	3.31 ± 0.19	3.50 ± 0.33

Data are mean ± SEM. Number of subjects: 6-12 within each experimental group

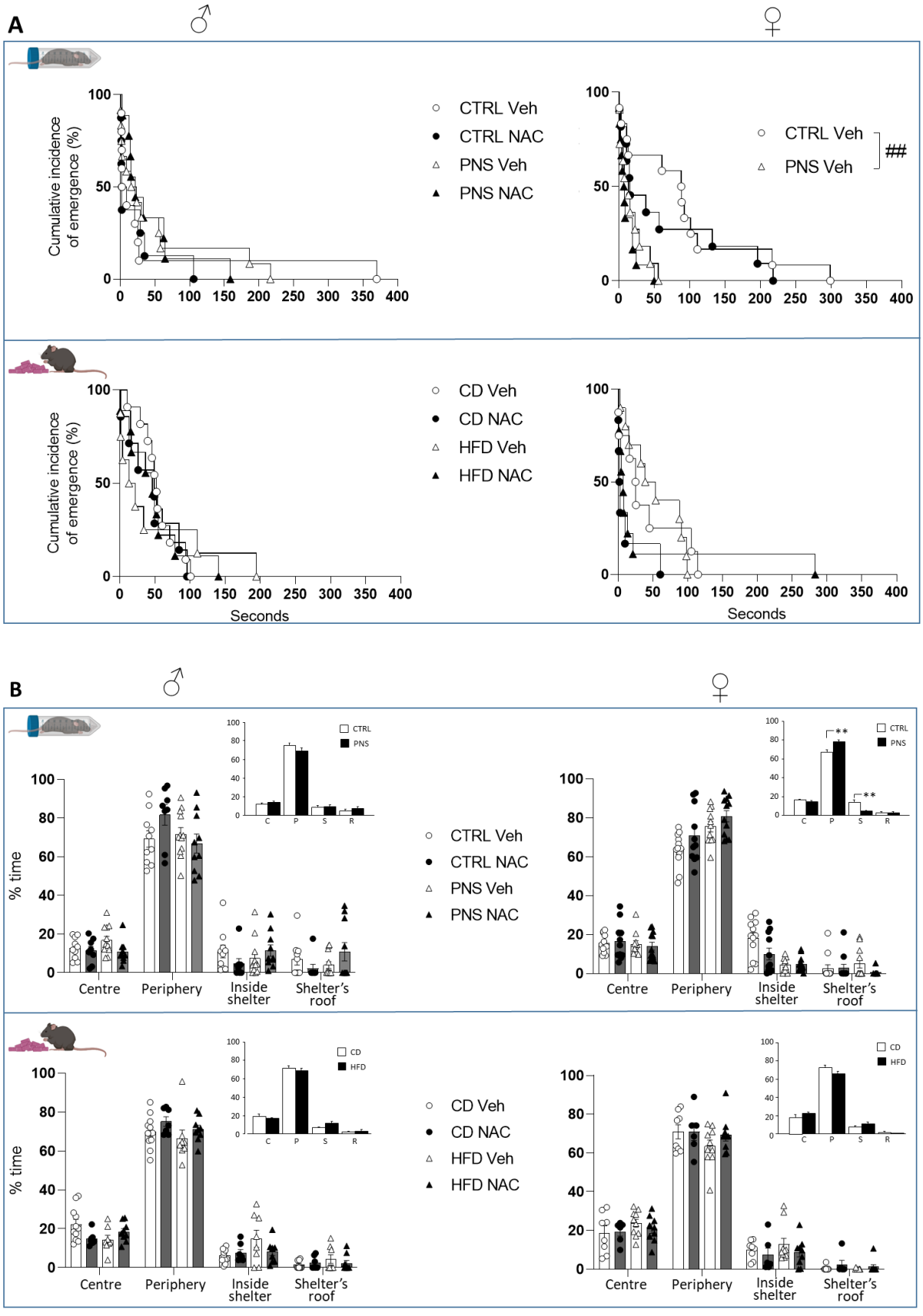


Figure 3 Emotional behavior in the Emergence test. A) PNS reduced the latency to emerge from the shelter specifically in female offspring, while no effects were observed upon mHFD. **B)** PNS reduced total time spent in the shelter and increased time in the periphery, while no effects were observed in mHFD. ##p<0.01 log-rank (Mantel-Cox) test with

Bonferroni correction; **p<0.01 Tukey's test PNS vs CTRL. Data are mean ± SEM. Number of subjects: 6-12 within each experimental group. C=centre; P=periphery; S=inside shelter; R=shelter's roof.

Social interaction test - SIT

PNS cohort. When the sociability of adolescent offspring exposed to PNS was evaluated, similar effects were observed in both sexes, with a greater impact on female subjects. In particular, PNS decreased the composite Z-score of social behaviors towards a conspecific in female subjects (interaction between stress and treatment: $F(1,38)=22.67$, $p<0.0001$; post hoc comparisons: *p<0.05 Tukey's test PNS-Veh vs CTRL-Veh). Moreover, maternal NAC administration led to decreased social behaviors in female mice (*p<0.05 Tukey's test CTRL-NAC vs CTRL-Veh) in favor of increased exploration of the environment (Main effect of treatment: $F(1,38)=4.216$, $p=0.0470$; data not shown). As for male offspring, a similar trend of reduced Z-score of social behaviors in CTRL-NAC and PNS-Veh groups was observed ($F(1,40)=3.920$, $p=0.0546$), in addition to a significant increase in exploratory behaviors upon the prenatal exposure to NAC (Main effect of treatment: $F(1,40)=6.499$, $p=0.0147$).

mHFD cohort. Similarly to the findings observed in the PNS cohort, also in the mHFD cohort we found that maternal NAC administration *per se* decreased Z-score of social behaviors in female control offspring (interaction between diet and treatment: $F(1,45)=3.904$, $p=0.0543$; *p<0.05 Tukey's test CD-NAC vs CD-Veh). A significant interaction between diet and treatment revealed that mHFD males were characterized by a slight decrease of social behaviors when placed in direct contact with a conspecific ($F(1,39)=6.962$, $p=0.0119$, although post hoc analyses did not reach statistical significance). Interestingly, NAC treatment was able to boost social behaviors in these same subjects (post hoc comparisons: *p<0.05 Tukey's test HFD-NAC vs HFD-Veh; data not shown). No significant changes were observed in the exploratory behaviors of mice prenatally exposed to the metabolic stress.

Forced swim test and coping stress strategy

PNS cohort. When the ability to deal with an acute unescapable stress (forced swim test) was evaluated, control male offspring showed a clear preference towards an active coping strategy (interaction between strategy and stress: $F(1,36)=5.341$, $p=0.0267$; post hoc comparisons: *p<0.05 Tukey's test active CTRL-Veh vs passive CTRL-Veh). By contrast, PNS males did not specifically choose an active strategy to cope with stress but they spent an equal amount of time displaying active as well as passive strategies (Figure 4). No significant differences were observed in coping stress strategy adopted by female offspring exposed to different prenatal conditions.

mHFD cohort. Prenatal exposure to mHFD increased the time spent performing an active coping strategy at the expense of a passive strategy in both sexes, exacerbating the phenotype observed in CD subjects

(interaction between strategy and stress - males: F(1,33)=3.901, p=0.0567; females: F(1,31)=3.997, p=0.0544; post hoc comparisons: **p<0.01 Tukey's test males active HFD-Veh vs passive HFD-Veh; females active HFD-Veh vs passive HFD-Veh), see Figure 4. No significant changes were observed as a result of maternal NAC administration.

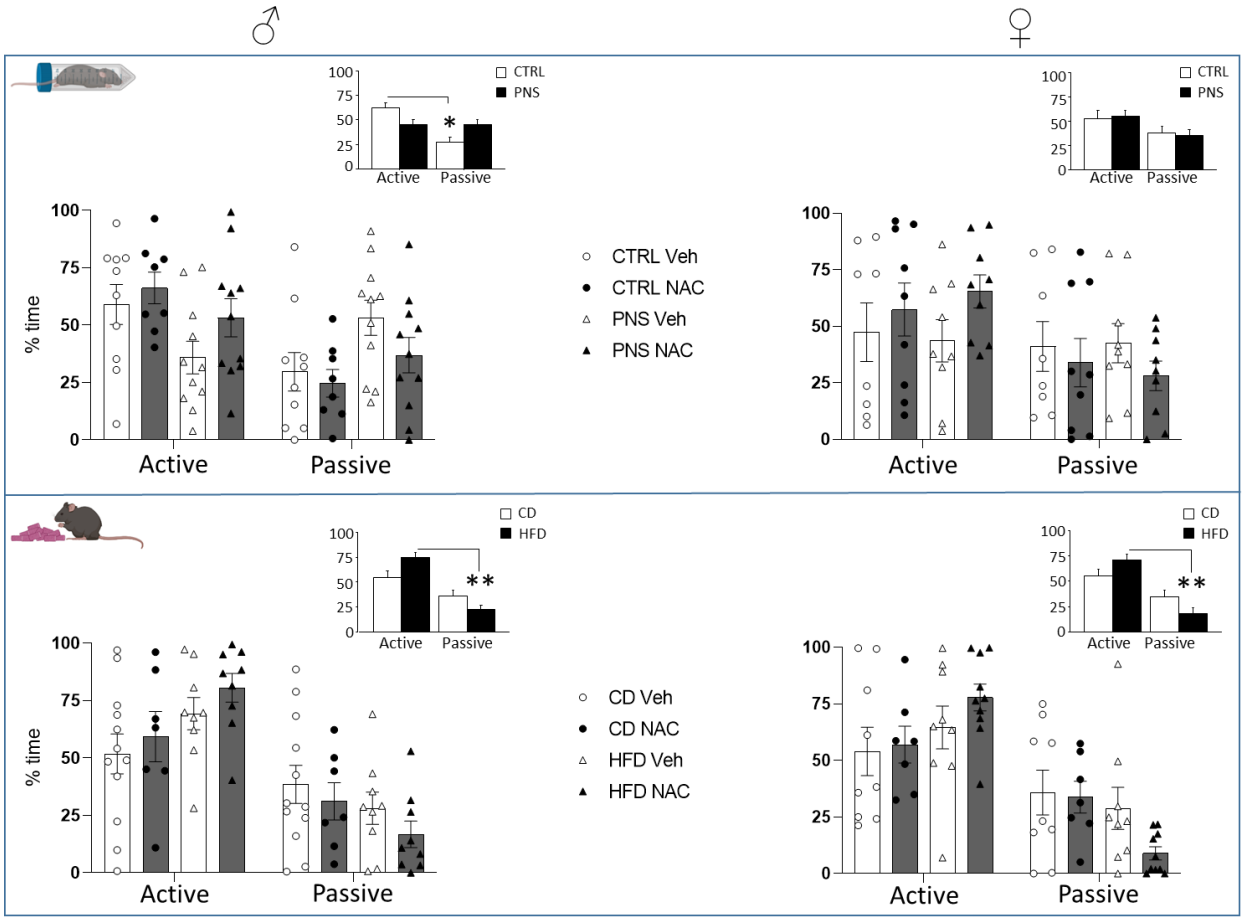


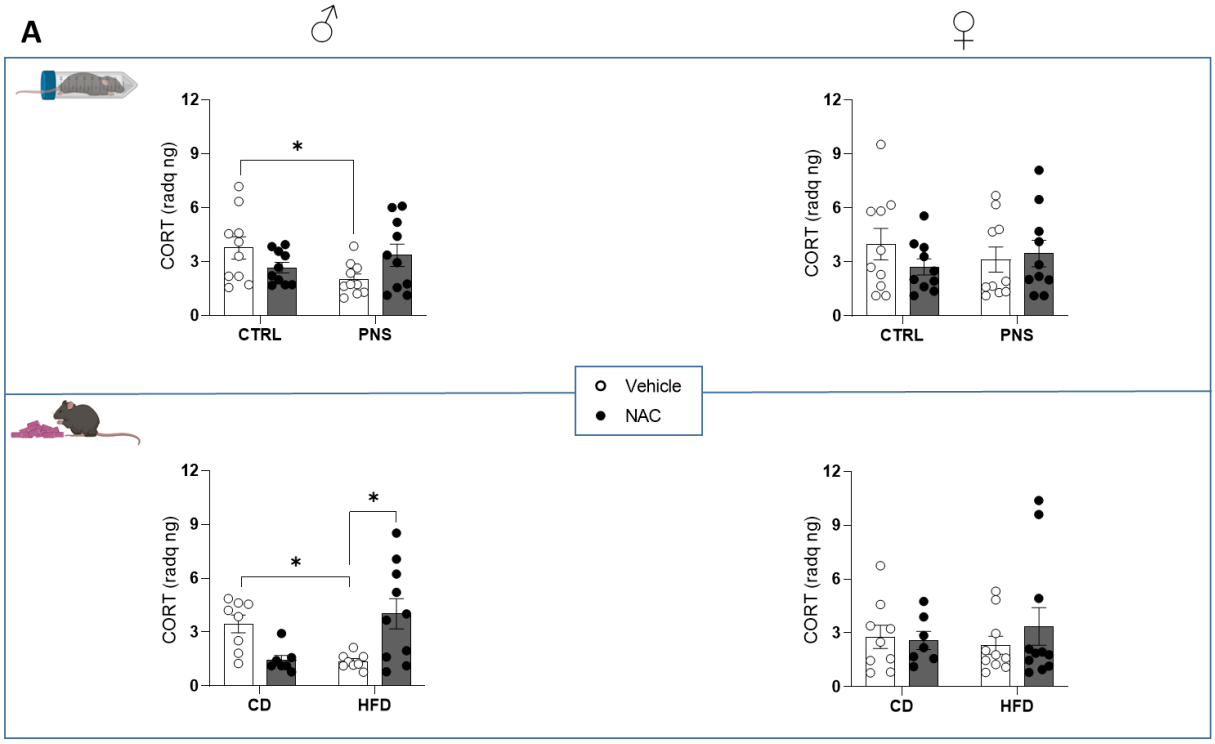
Figure 4 Coping strategies in response to an acute stress. PNS males failed to show a clear preference for a coping strategy towards stress when compared to the CTRL group. mHFD exacerbated the preference for adopting an active coping strategy towards stress in both sexes. *p<0.05 Tukey's test active CTRL-Veh vs passive CTRL-Veh; **p<0.01 males active HFD-Veh vs passive HFD-Veh; females active HFD-Veh vs passive HFD-Veh. Data are mean ± SEM. Number of subjects: 7-11 within each experimental group.

HPA axis reactivity

PNS cohort. When the functionality of the HPA axis under basal conditions was assessed, we found sex-dependent effects indicating that PNS reduced basal CORT levels in male offspring only (interaction between stress and treatment: F(1,36)=6.353, p=0.0163; post hoc comparisons: *p<0.05 Tukey's test PNS-Veh vs CTRL-Veh), see Figure 5A. Subsequently, the reactivity of the HPA axis was evaluated 30 and 180 minutes following an acute stress (6 min of forced swim test). Overall, PNS males reacted to an acute stress with enhanced CORT release after 30 minutes and they still showed higher circulating levels after 180 minutes from

the stress, when usually CORT is expected to return to a baseline (interaction between stress and treatment: F(1,36)=5.585, p=0.0236; post hoc comparisons: *p<0.05 Tukey's test PNS-Veh vs CTRL-Veh), see Figure 5B. Interestingly, the prenatal NAC treatment was effective in buffering CORT rise in PNS males, leading to levels comparable to those observed in the CTRL group (*p<0.05 PNS-NAC vs PNS-Veh), see Figure 5B. In general, we did not observe significant statistical differences in corticosterone levels of female offspring.

mHFD cohort. Similarly to the what observed in the PNS cohort, exposure to mHFD led to reduced CORT levels under basal conditions specifically in male mice (interaction between diet and treatment: F(1,29)=15.49, p=0.0005; post hoc comparisons: *p<0.05 Tukey's test HFD-Veh vs CD-Veh), see Figure 5A. In addition, the maternal administration with NAC was able to prevent this reduction, restoring basal levels of CORT (*p<0.05 PNS-NAC vs PNS-Veh), see Figure 5A. As for the HPA axis reactivity after an acute stress, no significant changes were observed among the different prenatal conditions in both male and female offspring (Figure 5B).



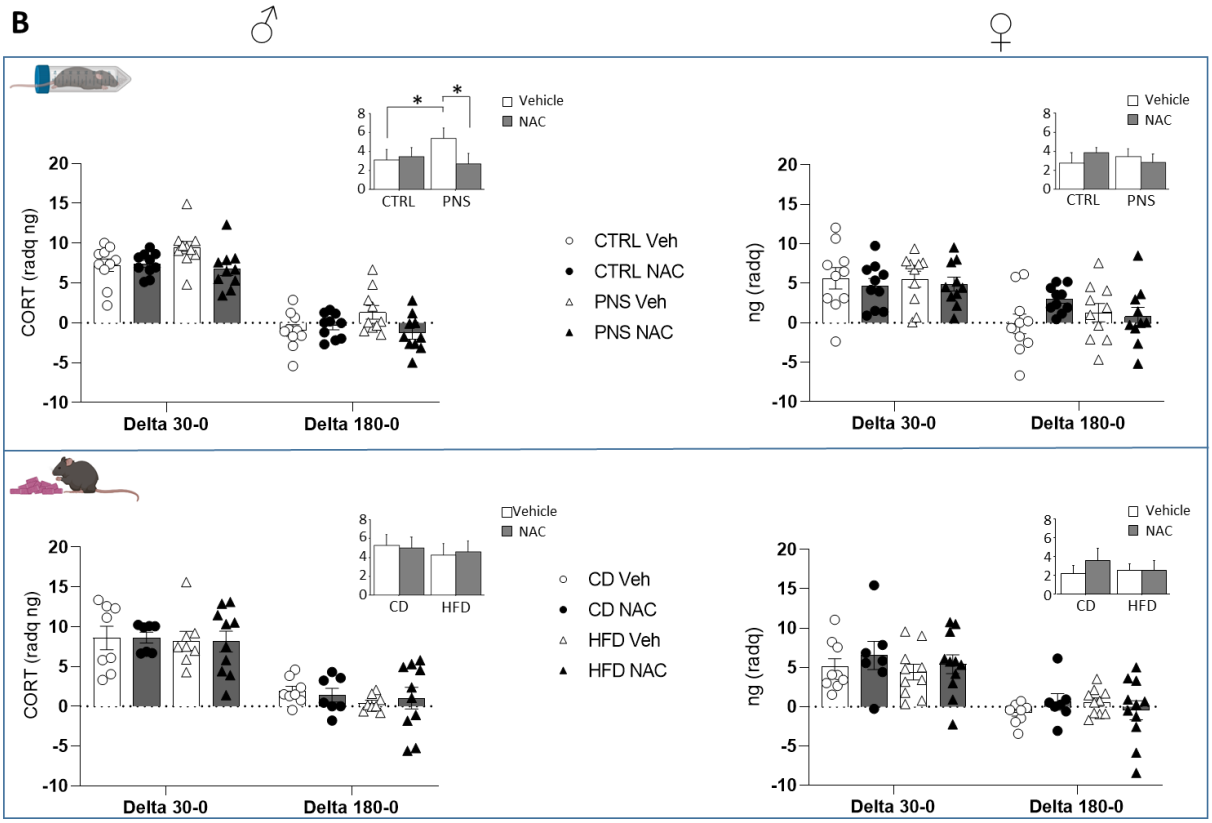


Figure 5 HPA axis under basal conditions (A) and its reactivity in response to an acute stress (B). A) Both PNS and HFD resulted in similar lower basal CORT levels when compared to their respective control groups. Prenatal NAC administration was able to prevent this effect only in HFD males. **B)** When the reactivity of the HPA axis was evaluated 30 and 180 minutes after the exposure to an acute stress, PNS males were characterized by overall higher CORT levels and prenatal NAC was effective in buffering the CORT rise. *p<0.05 Tukey's test males PNS-Vehicle vs CTRL-Vehicle; PNS-NAC vs PNS-Vehicle; HFD-Vehicle vs CD-Vehicle; HFD-NAC vs HFD-Vehicle. Data are mean ± SEM. Number of subjects: 7-11 within each experimental group.

Gene expression on the hippocampus

To investigate the molecular changes underlying prenatal psychophysical and metabolic stress, we first analyzed gene expression of the neurotrophin *Bdnf* in the hippocampus of adolescent male and female offspring. Then, in order to investigate the mutual regulation between Bdnf and redox balance, we assessed the gene expression of *Nrf2*, which is a transcriptional factor that in response to OS quickly translocates into the nucleus to activate the expression of antioxidant genes, and its main inhibitory regulator *Keap1*. Finally, in order to assess the effect of PNS and mHFD on microglia homeostasis, we investigated microglia/macrophage markers CD68, TMEM119 and TREM2.

PNS cohort. Hippocampal gene expression profile revealed that levels of total *Bdnf* were significantly decreased in PNS female offspring compared to the control group (interaction between stress and treatment: $F(1,40)=24.36$, $p<0.0001$; post hoc comparisons: **p<0.01 Tukey's test PNS-Veh vs CTRL-Veh).

Interestingly, the prenatal administration of NAC was able to prevent this effect, restoring *Bdnf* levels in PNS females (**p<0.01 Tukey's test PNS-NAC vs PNS-Veh), see Figure 6A. Less pronounced effects were observed in male offspring, showing an effect of prenatal NAC in increasing total *Bdnf* levels in PNS subjects only (interaction between stress and treatment: F(1,43)=5.240, p=0.027; post hoc comparisons: *p<0.05 Tukey's test PNS-NAC vs PNS-Veh). As for the redox regulations, we found that PNS greatly decreased hippocampal *Nrf2* expression in the offspring, regardless of sex (main effect of stress - females: F(1,42)=46.05, p<0.0001; males: F(1,43)=10.10, p=0.0027), see Figure 6A. In female mice, a main effect of prenatal treatment was found showing a booster effect of NAC in enhancing *Nrf2* expression (F(1,42)=4.700, p=0.0359), while in male offspring only a tendency toward a similar effect was observed. When we evaluated the expression levels of *Keap1*, we founded no difference in females but a significant interaction between stress and treatment in male offspring, revealing an increase in PNS-NAC group compared to PNS-Vehicle (F(1,44)=6.450, p=0.0147; post hoc comparisons: *p<0.05 Tukey's test PNS-NAC vs PNS-Veh), see Figure 6A.

When assessing markers of microglia, we found that hippocampal levels of *Cd68* and *Tmem119* were decreased by PNS specifically in female offspring, while no effects were observed in males (main effect of stress in females - Cd68: F(1,26)=8.349, p=0.0077; Tmem119: F(1,24)=31.46, p<0.0001), see Figure 6B. Prenatal NAC did not affect these markers, but it increased *Trem2* levels in female offspring only (main effect of treatment: F(1,26)=8.520, p=0.0072).

mHFD cohort. The evaluation of the hippocampal transcript profile performed on this experimental cohort unmasked very similar effects between the prenatal exposure to PNS or mHFD. More in detail, a significant interaction between diet and treatment showed decreased *Bdnf* total levels upon the exposure to mHFD, specifically in female brains (F(1,41)=34.69, p<0.0001; post hoc comparisons: **p<0.01 Tukey's test HFD-Veh vs CTRL-Veh), while no significant changes were observed in male mice. Also in this case, the maternal treatment with NAC, in addition to mHFD, led to restored expression levels of the neurotrophin in female offspring (**p<0.01 Tukey's test HFD-NAC vs HFD-Veh), see Figure 6A. When *Nrf2* mRNA expression was measured, similarly to PNS females, also female offspring exposed to mHFD were characterized by lower levels of *Nrf2* compared to CD group (main effect of diet: F(1,47)=10.99, p=0.0018); overall, a main effect of treatment revealed that *Nrf2* levels resulted increased by the maternal administration with NAC (F(1,47)=27.04, p<0.0001), see Figure 6A. A general increase of *Nrf2* mRNA levels were observed in male offspring upon the prenatal exposure to NAC (main effect of treatment: F(1,51)=7.715, p=0.0076), see Figure 6A. The assessment of *Keap1* levels revealed a main effect of diet showing reduced *Keap1* expression specifically in male subjects (F(1,45)=4.673, p=0.0360), while no changes were observed in females.

When microglia markers were measured, we found pervasive effects of mHFD in increasing *Cd68* levels both in males and females (main effect of diet: F(1,20)=20.27, p=0.0002); F(1,20)=6.699, p=0.0176, respectively for males and females), see Figure 6B. Prenatal treatment with NAC was able to overall reduce this parameter only in females (main effect of treatment: F(1,20)=6.004, p=0.0236). No significant changes were observed in mRNA levels of *Tmem119* or *Trem2* between groups.

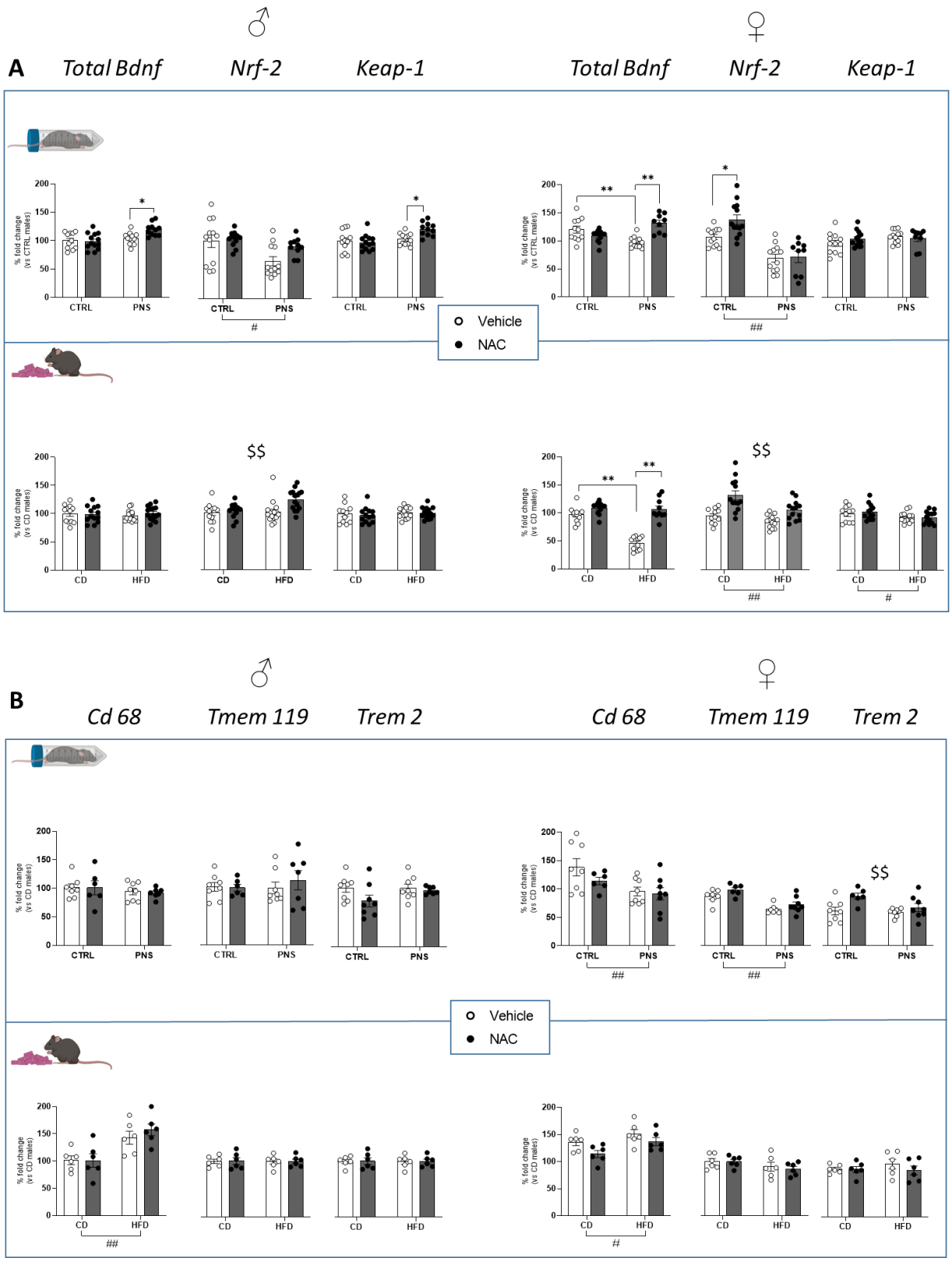


Figure 6 Hippocampal expression of genes involved in plasticity and OS (A) and microglia-related markers (B). **A)** Both PNS and HFD resulted in a similar reduction of hippocampal *Bdnf* and *Nrf-2*, with greater effects on females. **B)** While PNS reduced Cd68 and Tmem119 levels specifically in females, mHFD overall increased Cd68 both in males and females. #p<0.05, ## p<0.01 main effect of prenatal stress/diet; $$p<0.01 main effect of NAC; *p<0.05, **p<0.01 Tukey's test. Data are mean ± SEM. Number of subjects: 6-12 within each experimental group.

Stereological counting and morphological analysis of Iba1-positive cells in the hippocampal CA1

We estimated the number of microglia in the CA1 region of the hippocampus and analyzed their cellular morphology as well as dorso-ventral distribution. The analysis was done in the adolescent offspring, performing an IHC using antibodies against Iba1.

PNS cohort. There were no significant differences in the total number of microglial cells in the CA1 between CTRL and PNS groups, neither in male nor in female offspring (data not shown). However, when the morphological analysis was performed, evaluating both the ventral and the dorsal sub-region of the CA1, a significant interaction among cell morphology, stress and treatment was found in the ventral part of male offspring ($F(1,21)=4.680$, $p=0.0422$). More in detail, the exposure to PNS combined with the NAC treatment reduced the number of ramified microglia cells, displaying a so-called "surveillance" phenotype usually characterizing physiological conditions, compared to prenatal exposure to NAC alone (*$p<0.05$ Tukey's test PNS-NAC vs CTRL-NAC, see Figure 7). Overall, in the dorsal part of the CA1 a similar trend in PNS-NAC male mice was observed although no significant changes were detected.

mHFD cohort. Similarly to what observed in the PNS cohort, also in the mHFD cohort no significant changes were found in the total number of microglial cells in the CA1, either in male or in female offspring (data not shown). In this cohort, the morphological analysis revealed a significant interaction among cell morphology, diet and treatment in the dorsal part of male offspring. In particular, also in this case, the exposure to mHFD combined with the NAC treatment reduced the number of ramified "surveillance" microglia cells compared to prenatal exposure to NAC alone (**$p<0.01$ Tukey's test HFD-NAC vs CD-NAC, see Figure 8). No significant differences in the morphology of microglia in female offspring were observed.

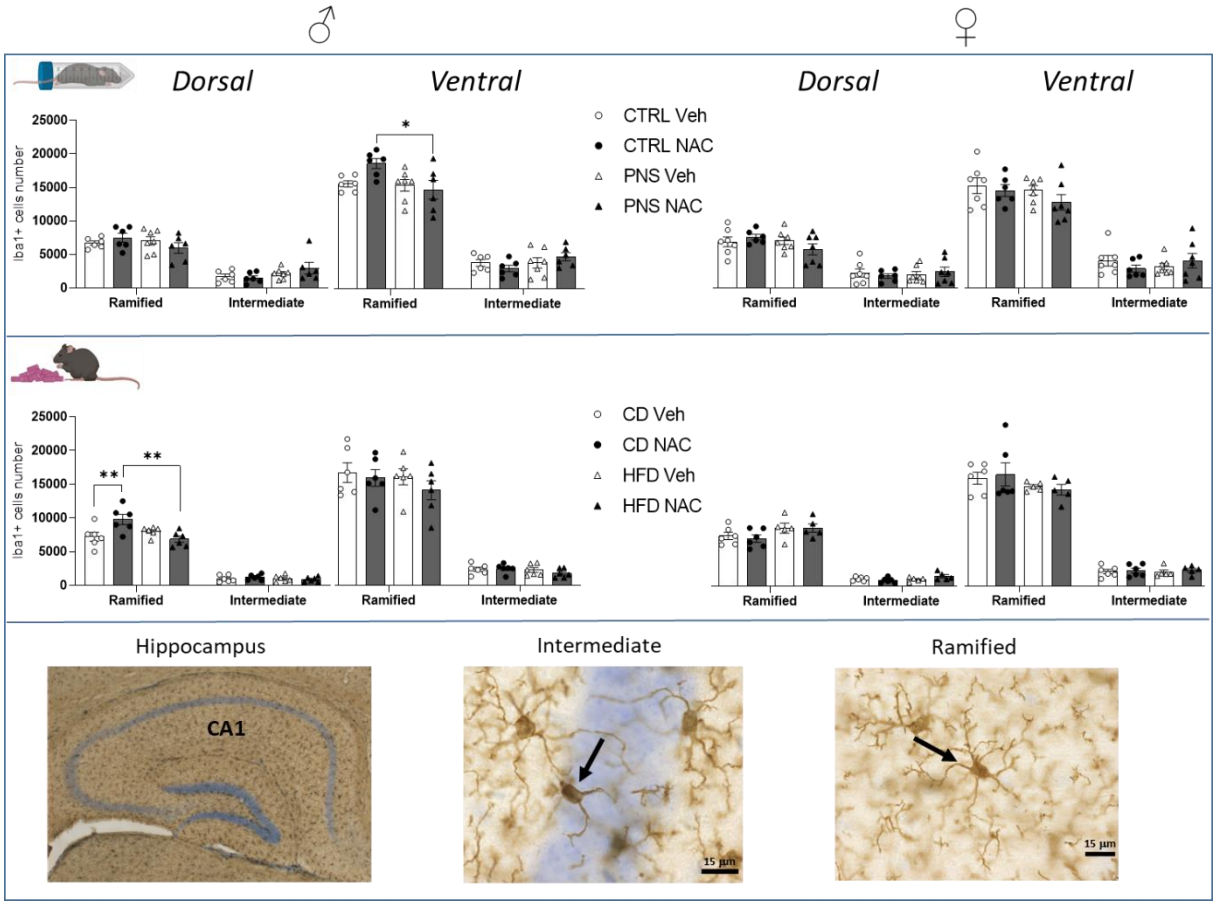


Figure 7 Stereological counting and morphological analysis of Iba1-positive cells in the CA1. Similarly, exposure to PNS or mHFD, combined with NAC treatment, reduced the number of ramified microglia cells compared to prenatal exposure to NAC alone. *p<0.05, **p<0.01 Tukey's test. Data are mean ± SEM. Number of subjects: 5-6 within each experimental group.

Discussion

In the present study, we provide evidence supporting the hypothesis that psychophysical or metabolic stress act through a "funnel effect" model, triggering shared conserved pathways and redirecting fetal developmental trajectories, potentially leading to increased vulnerability to negative mental health outcomes in a sex-dependent fashion (Musillo et al., 2022). NAC administration was able to prevent some of these effects, suggesting that the modulation of OS-related pathways might be effective in targeting common shared mechanisms between different adverse prenatal conditions.

The first evidence supporting the hypothesis of a "funnel effect" of different prenatal stressors comes from the assessment of the hippocampal transcript profile of genes involved in brain plasticity and OS regulation in the adolescent offspring exposed to PNS or mHFD. BDNF was assessed as it plays a pivotal role during brain development, being involved in neuronal differentiation, survival and neuroplasticity as well as in the integration of neuroendocrine and metabolic pathways in response to stressful challenges (Cirulli and Alleva, 2009). PNS and mHFD were both able to reduce hippocampal mRNA levels of this neurotrophin in adolescent females, suggesting that, at least at this age, female mice are more vulnerable to prenatal stressors, confirming and expanding our previous findings showing a reduction of *Bdnf* upon PNS in female rats during adolescence (Berry et al., 2015). Parallel changes were also found in hippocampal levels of *Nrf2* that is a master regulator of antioxidant defenses. (Bellezza et al., 2018; Fuse and Kobayashi, 2017). This is in line with the fact that a BDNF-NRF2 mutual crosstalk in mood disorders has been previously shown (Bouvier et al., 2017; Bruna et al., 2018; Hashimoto, 2018). For example, Bouvier and colleagues found that vulnerability to depressive-like behavior in a rat model of social defeat stress resulted in a persistent state of OS due to a failure of BDNF to activate the NRF2-KEAP1 complex, a condition that was reversed by treatment with antioxidants (Bouvier et al., 2017). Here we show that the exposure to PNS or mHFD reduces hippocampal levels of *Nrf2* in female subjects, mirroring *Bdnf* changes. This effect was also associated with a decrease in *Keap1* specifically in the mHFD group. We have previously shown that both these prenatal stressors increase circulating OS markers in the mothers possibly providing a pro-oxidant intrauterine milieu (Musillo et al. 2022 in preparation). Thus, it is possible to hypothesize that increased fetal OS, due to maternal stress or obesity, might disrupt the NRF2-BDNF regulation, ultimately leading to reduced *Bdnf* expression in the offspring brain at adolescence. This hypothesis is corroborated by the fact that supplementation with an antioxidant compound, NAC, early on, by rebalancing the redox status, is capable to restore expression of both *Bdnf* and *Nrf2*.

The emotional behavior of the adolescent offspring also showed strong, sex-specific differences, with larger effects, also in this case, in female mice. In particular, females prenatally exposed to PNS or mHFD, when tested in the EPM, spent more time in the open arms compared to their control groups, suggesting a more pronounced behavioral disinhibition as they were also characterized by increased exploration and decreased risk-assessment behaviors. These same subjects, when assessed in the Emergence test, were also characterized by reduced latency to emerge from the shelter and less total time spent inside the shelter, accompanied to increased locomotor activity. All these features overall describe a disinhibited phenotype that might be

considered as an adaptive behavioral strategy to meet immediate emotional demands in a novel unfamiliar context. However, such a disinhibition, generally including a broad spectrum of behavioral traits such as impulsiveness, low levels of control and increased novelty seeking, might have severe long-term consequences, setting the stage for later adverse functional outcomes and psychopathology (Clark et al., 2019, 2016; Moffitt et al., 2011; Nigg, 2017). Our behavioral results, showing an increased behavioral disinhibition associated with a decrease in hippocampal Bdnf levels of female offspring exposed to PNS or mHFD, might be explained through the intriguing "stress acceleration hypothesis" (Callaghan and Tottenham, 2016). According to this theory, early adversities might prematurely activate neuronal structures involved in emotional regulation and stress response (e.g. hippocampus and prefrontal cortex), causing an overall premature development of these circuits (Callaghan and Tottenham, 2016). In an evolutionary framework, this accelerated maturation would confer advantageous emotional adaptation and behavioral flexibility characterized by a rapid response to new conditions and less anxiety in the short-term, also allowing a higher reproductive success and fitness in adverse conditions. Nevertheless, such a faster neurodevelopment has been associated with permanent impairment of developmental plasticity, increasing the risk for poor cognitive abilities, anxiety and psychopathology in the long-term (Fragale et al., 2016; Frankenhuis and de Weerth, 2013; Zehr et al., 2007). Our findings of reduced *Bdnf* levels in the hippocampus might indirectly indicate that a reduction in neuroplasticity is already underway during late adolescence and the negative behavioral effects may become manifest in adulthood.

Stress occurring during pregnancy has been linked to altered HPA axis activity in the offspring, although the findings appear to be very heterogeneous. While some preclinical and clinical studies have reported that early life stress leads to a hyper-activation of the HPA axis in the offspring, other evidence indicates lower basal cortisol levels or a blunted cortisol response to an acute stress (Osborne et al., 2018; Send et al., 2019; Simons et al., 2015; Van Der Voorn et al., 2019). One possible explanation is based on the concept that disruptions of offspring HPA axis, due to maternal stress, can change throughout life, as shown by the ALSPAC cohort reporting higher cortisol awakening response in 10-years-old children but lower levels of the same parameter at 15-years-old (O'Connor et al., 2005; O'Donnell et al., 2013). In our study, when we measured the functionality of the HPA axis, we found that both PNS as well as mHFD were associated to reduced baseline CORT levels in adolescent male offspring and that NAC administration prevented this reduction. This effect, which corroborates our "funnel effect" hypothesis, was specific to male subjects providing a further evidence that these two different prenatal stressors converge on common mechanisms in a sex-dependent fashion. We also found that exposure to PNS led to an overall increased response to a stressful challenge, an effect that was maintained three hours following the end of stress when the HPA axis is expected to return to a steady state. Interestingly in this same group of PNS subjects, NAC administration prevented the increased adrenocortical output rendering PNS males similar to controls.

As for the potential mechanisms, our group has previously shown that both PNS and mHFD are able to weaken the placental barrier by reducing the enzymatic activity of 11β-Hydroxysteroid dehydrogenase 2 (11β-HSD 2)

allowing excessive amount of glucocorticoids (GCs) to reach the fetus and to affect neuroendocrine development (Bellisario et al., 2015; Panetta et al., 2017). A very similar effect of reduction in the placental levels of 11β-HSD2 has been observed by Shi and co-workers as a result of prenatal exposure to OS due to environmental pollution, an effect that was reverted by NAC administration acting through inhibition of the ROS-induced PERK/p-eIF2α signaling cascade in the placenta (Shi et al., 2020; Zhang et al., 2019). Given our recent data showing increased markers of OS in maternal blood as a result of both PNS and mHFD (Musillo et al 2022, in preparation), we can hypothesize that the activation of the placental signaling cascade PERK/p-eIF2 α might represent a specific common mechanism underlying the male-specific derangement in HPA axis function and that NAC may target it, preventing HPA axis dysfunctions in male offspring.

Physiological and behavioral responses to acute stressors are, at least in part, driven by the HPA axis activity and adverse early life experiences might affect fetal development to shape the HPA axis functionality and the related coping strategies (Maccari et al., 1995; Veenema et al., 2006). When PNS males were testes in the FST, they failed to show a clear preference for a coping strategy, with overall higher CORT levels following this stressful challenge, suggesting an inability to properly react to a situation potentially harmful. This effect was sex-specific and was not observed in adolescent PNS females. By contrast, in the mHFD cohort, both males and females were characterized by a strong preference for the active coping strategy. To this regard, it is interesting to note that in a recent review Molendijk and de Kloet have provided a novel key to interpret data deriving from FST, pointing to behavioral coping strategies rather than to depressive-like behaviors (Molendijk and de Kloet, 2019). In particular, subjects characterized by low stress-induced GCs secretion together with increased pro-inflammatory profile might be defined as proactive copers, while an opposite pattern holds true for reactive (passive) copers (elevated GCs secretion upon stress and reduced inflammation) (de Boer et al., 2017; Koolhaas et al., 2010). Thus, it is possible to hypothesize that PNS and mHFD might affect coping strategies towards stress and the associated neuroendocrine and immune profile in a sex-dependent fashion. In particular, while PNS males might be characterized by a reactive/passive profile, mHFD males and females show a proactive profile when facing an unescapable stress.

We have previously shown that the exposure to PNS or mHFD affects the brain inflammatory state already during embryonal stages (E18), in a sex dimorphic manner (see Musillo et al. 2022 in preparation). Given the embryonic origins of microglia and their role in brain development and homeostasis, in the current study, we assessed whether the fetal microglial pro-inflammatory phenotype is maintained across critical developmental windows such as adolescence. Our results overall indicate only limited effects of PNS or mHFD on microglial markers at adolescence, with the exposure to mHFD increasing the levels of hippocampal *Cd68*, a marker of microglia activation, in both male and female adolescent offspring in line with previous data (Bilbo and Tsang, 2010). When the same marker (Cd68) was assessed in the PNS cohort, we observed a specific decrease in females, suggesting a blunted priming of microglia cells, a condition that, at this age, might affect proper brain development. Overall, the limited effects found at this age suggest that long-term effects of these prenatal conditions might be revealed only following an immune challenge to the system and may be difficult to

ascertain at baseline. To further investigate the effects of early life stressors on microglial function, we have performed a stereological counting accompanied by a morphological characterization of Iba1+ cells, specifically in the CA1 of the hippocampus. We found sex-specific effects revealing that prenatal NAC treatment increased the number of ramified cells - "surveillance" phenotype -, while NAC treatment in combination with the exposure to PNS or mHFD reduced this cell population specifically in male offspring. An unbalanced number of ramified cells generally mirrors complementary changes in the number of the second most abundant microglia population characterized by an "intermediate" phenotype with fewer, thicker and shorter processes, overall polarized towards a pro-inflammatory profile. In fact, together with the decreased number of ramified cells, we observed a slight shift through increased number of intermediate cells in PNS-NAC and HFD-NAC groups. It is worth to note that microglia morphology is tightly regulated by redox balance, usually displaying a "surveillance" state when ROS and antioxidant defenses are counterbalanced between each other; when the OS increases, microglia shift to a pro-inflammatory morphology that is accompanied by the activation of NF-kB pathway and the transcription of pro-inflammatory genes (Rojo et al., 2014). Finally, when the antioxidant machinery restores the redox balance also the microglia remodel its morphology towards a physiological ramified state. Given this evidence, it is possible to hypothesize that the boost in the antioxidant defenses that we observed in our models (increased *Nrf2* levels) as a result of NAC treatment alone, by rebalancing the redox status of the brain, might mediate the shift of microglia from an activated morphology towards a "surveillance" state (increased ramified cells).

Conclusions

In this study, we have provided preclinical evidence to support the hypothesis of a "funnel effect" by which different adverse prenatal conditions can affect fetal brain development to a very similar extent, setting the stage for an increased vulnerability for negative health outcomes. In particular, we found comparable changes in offspring prenatally exposed to PNS or mHFD, when assessing stress-responsive pathways. A main role of brain redox mechanisms was highlighted in orchestrating a synchronized response of different systems, including the neuroendocrine and the immune system, to early adversity. While differences emerge as a result of prenatal stressors, indicating differential vulnerability in females and males, buffering OS early on appears an ever effective mean to prevent at least some of the long-term effects of adverse prenatal conditions. Ultimately, these studies may lead to future investigations assessing the potential role of redox regulations as basic mechanisms involved the co-morbidity characterizing metabolic and psychiatric conditions.

Acknowledgment

We are thankful to Marianna Samà, Letizia Giona and Chiara D'Amelio for assistance with animal behavioral characterization; Jovana Maliković and Sonia Matos for support in the HIC technique; Enrico Cardarelli, Andrea Giovannelli and Antonio Di Virgilio for animal care.

This work was supported by ERANET NEURON JTC 2018 (Mental Disorders) Project ''EMBED" to F.C. HDHL-INTIMIC-085-GUTMOM project (co-PI F.C.) within the ERA-NET Co-fund HDHL-INTIMIC (INtesTInal MICrobiomics) programme; "Avvio alla Ricerca - Tipo 1" 2021 "Sapienza" University of Rome funding to C.M. and funding from the Italian Ministry of Health (Ricerca Corrente 2021 to F.C.). ISS Project MOMINFLAM - Unique signatures underlying placental-fetal brain crosstalk in maternal obesity. Proj code ISS20-88bc10d8839b to F.C.

Conflict of interest

The authors declare no conflict of interest

References

al-Haddad, B.J.S., Oler, E., Armistead, B., Elsayed, N.A., Weinberger, D.R., Bernier, R., Burd, I., Kapur, R., Jacobsson, B., Wang, C., Mysorekar, I., Rajagopal, L., Adams Waldorf, K.M., 2019. The fetal origins of mental illness. Am. J. Obstet. Gynecol. https://doi.org/10.1016/j.ajog.2019.06.013

Amrein, I., Nosswitz, M., Slomianka, L., van Dijk, R.M., Engler, S., Klaus, F., Raineteau, O., Azim, K., 2015. Septo-temporal distribution and lineage progression of hippocampal neurogenesis in a primate (Callithrix jacchus) in comparison to mice. Front. Neuroanat. 9, 85. https://doi.org/10.3389/fnana.2015.00085

Banderali, G., Martelli, A., Landi, M., Moretti, F., Betti, F., Radaelli, G., Lassandro, C., Verduci, E., 2015. Short and long term health effects of parental tobacco smoking during pregnancy and lactation: A descriptive review. J. Transl. Med. https://doi.org/10.1186/s12967-015-0690-y

Barker, D.J.P., Osmond, C., 1986. Infant mortality, childhood nutrition, and ischaemic heart disease in England and wales. Lancet 327, 1077–1081. https://doi.org/10.1016/S0140-6736(86)91340-1

Bellezza, I., Giambanco, I., Minelli, A., Donato, R., 2018. Nrf2-Keap1 signaling in oxidative and reductive stress. Biochim. Biophys. Acta - Mol. Cell Res. https://doi.org/10.1016/j.bbamcr.2018.02.010

Bellisario, V., Panetta, P., Balsevich, G., Baumann, V., Noble, J., Raggi, C., Nathan, O., Berry, A., Seckl, J., Schmidt, M., Holmes, M., Cirulli, F., 2015. Maternal high-fat diet acts as a stressor increasing maternal glucocorticoids' signaling to the fetus and disrupting maternal behavior and brain activation in C57BL/6J mice. Psychoneuroendocrinology 60, 138–150. https://doi.org/10.1016/j.psyneuen.2015.06.012

Berk, M., Malhi, G.S., Gray, L.J., Dean, O.M., 2013. The promise of N-acetylcysteine in neuropsychiatry. Trends Pharmacol. Sci. https://doi.org/10.1016/j.tips.2013.01.001

Berry, A., Bellisario, V., Panetta, P., Raggi, C., Magnifico, M.C., Arese, M., Cirulli, F., 2018. Administration of the antioxidant n-acetyl-cysteine in pregnant mice has long-term positive effects on metabolic and behavioral endpoints of male and female offspring prenatally exposed to a high-fat diet. Front. Behav. Neurosci. 12. https://doi.org/10.3389/fnbeh.2018.00048

Berry, A., Panetta, P., Luoni, A., Bellisario, V., Capoccia, S., Andrea Riva, M., Cirulli, F., 2015. Decreased Bdnf expression and reduced social behavior in periadolescent rats following prenatal stress. Dev. Psychobiol. 57, 365–373. https://doi.org/10.1002/dev.21297

Bilbo, S.D., Tsang, V., 2010. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. FASEB J. 24, 2104–2115. https://doi.org/10.1096/fj.09-144014

Block, C.L., Eroglu, O., Mague, S.D., Smith, C.J., Ceasrine, A.M., Sriworarat, C., Blount, C., Beben, K.A., Malacon, K.E., Ndubuizu, N., Talbot, A., Gallagher, N.M., Chan Jo, Y., Nyangacha, T., Carlson, D.E., Dzirasa, K., Eroglu, C., Bilbo, S.D., 2022. Prenatal environmental stressors impair postnatal microglia function and adult behavior in males. Cell Rep. 40. https://doi.org/10.1016/j.celrep.2022.111161

Bordeleau, M., Lacabanne, C., Fernández De Cossío, L., Vernoux, N., Savage, J.C., González-Ibáñez, F., Tremblay, M.È., 2020. Microglial and peripheral immune priming is partially sexually dimorphic in adolescent mouse offspring exposed to maternal high-fat diet. J. Neuroinflammation 17. https://doi.org/10.1186/s12974-020-01914-1

Bouvier, E., Brouillard, F., Molet, J., Claverie, D., Cabungcal, J.H., Cresto, N., Doligez, N., Rivat, C., Do, K.Q., Bernard, C., Benoliel, J.J., Becker, C., 2017. Nrf2-dependent persistent oxidative stress results in stress-induced vulnerability to depression. Mol. Psychiatry 22, 1701–1713. https://doi.org/10.1038/mp.2016.144

Brannigan, R., Cannon, M., Tanskanen, A., Huttunen, M.O., Leacy, F.P., Clarke, M.C., 2019. The association between subjective maternal stress during pregnancy and offspring clinically diagnosed psychiatric disorders. Acta Psychiatr. Scand. 139, 304–310. https://doi.org/10.1111/acps.12996

Bruna, B., Lobos, P., Herrera-Molina, R., Hidalgo, C., Paula-Lima, A., Adasme, T., 2018. The signaling pathways underlying BDNF-induced Nrf2 hippocampal nuclear translocation involve ROS, RyR-

Mediated Ca2+ signals, ERK and PI3K. Biochem. Biophys. Res. Commun. 505, 201–207. https://doi.org/10.1016/j.bbrc.2018.09.080

Callaghan, B.L., Tottenham, N., 2016. The Stress Acceleration Hypothesis: Effects of early-life adversity on emotion circuits and behavior. Curr. Opin. Behav. Sci. https://doi.org/10.1016/j.cobeha.2015.11.018

Can, A., Dao, D.T., Arad, M., Terrillion, C.E., Piantadosi, S.C., Gould, T.D., 2011. The mouse forced swim test. J. Vis. Exp. e3638. https://doi.org/10.3791/3638

Cirulli, F., Alleva, E., 2009. The NGF saga: From animal models of psychosocial stress to stress-related psychopathology. Front. Neuroendocrinol. https://doi.org/10.1016/j.yfrne.2009.05.002

Cirulli, F., Musillo, C., Berry, A., 2020. Maternal Obesity as a Risk Factor for Brain Development and Mental Health in the Offspring. Neuroscience. https://doi.org/10.1016/j.neuroscience.2020.01.023

Clark, C.A.C., Espy, K.A., Wakschlag, L., 2016. Developmental pathways from prenatal tobacco and stress exposure to behavioral disinhibition. Neurotoxicol. Teratol. 53, 64–74. https://doi.org/10.1016/j.ntt.2015.11.009

Clark, C.A.C., Massey, S.H., Wiebe, S.A., Espy, K.A., Wakschlag, L.S., 2019. Does early maternal responsiveness buffer prenatal tobacco exposure effects on young children's behavioral disinhibition? Dev. Psychopathol. 31, 1285–1298. https://doi.org/10.1017/S0954579418000706

Congdon, P., 2019. Obesity and urban environments. Int. J. Environ. Res. Public Health. https://doi.org/10.3390/ijerph16030464

Davis, J., Mire, E., 2021. Maternal obesity and developmental programming of neuropsychiatric disorders: An inflammatory hypothesis. Brain Neurosci. Adv. 5, 239821282110034. https://doi.org/10.1177/23982128211003484

de Boer, S.F., Buwalda, B., Koolhaas, J.M., 2017. Untangling the neurobiology of coping styles in rodents: Towards neural mechanisms underlying individual differences in disease susceptibility. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2016.07.008

Delpech, J.C., Wei, L., Hao, J., Yu, X., Madore, C., Butovsky, O., Kaffman, A., 2016. Early life stress perturbs the maturation of microglia in the developing hippocampus. Brain. Behav. Immun. 57, 79–93. https://doi.org/10.1016/j.bbi.2016.06.006

du Sert, N.P., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Hurst, V., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T., Würbel, H., 2020. Reporting animal research: Explanation and elaboration for the arrive guidelines 2.0. PLoS Biol. https://doi.org/10.1371/journal.pbio.3000411

Fernandes, C., File, S.E., 1996. The influence of open arm ledges and maze experience in the elevated plus-maze, in: Pharmacology Biochemistry and Behavior. Pharmacol Biochem Behav, pp. 31–40. https://doi.org/10.1016/0091-3057(95)02171-X

Fragale, J.E.C., Khariv, V., Gregor, D.M., Smith, I.M., Jiao, X., Elkabes, S., Servatius, R.J., Pang, K.C.H., Beck, K.D., 2016. Dysfunction in amygdala-prefrontal plasticity and extinction-resistant avoidance: A model for anxiety disorder vulnerability. Exp. Neurol. 275, 59–68. https://doi.org/10.1016/j.expneurol.2015.11.002

Frankenhuis, W.E., de Weerth, C., 2013. Does Early-Life Exposure to Stress Shape or Impair Cognition? Curr. Dir. Psychol. Sci. 22, 407–412. https://doi.org/10.1177/0963721413484324

Fuse, Y., Kobayashi, M., 2017. Conservation of the Keap1-Nrf2 system: An evolutionary journey through stressful space and time. Molecules. https://doi.org/10.3390/molecules22030436

Grubbs, F.E., 1950. Sample Criteria for Testing Outlying Observations. Ann. Math. Stat. 21, 27–58. https://doi.org/10.1214/aoms/1177729885

Gustafsson, H.C., Sullivan, E.L., Nousen, E.K., Sullivan, C.A., Huang, E., Rincon, M., Nigg, J.T., Loftis, J.M., 2018. Maternal prenatal depression predicts infant negative affect via maternal inflammatory cytokine levels. Brain. Behav. Immun. 73, 470–481. https://doi.org/10.1016/j.bbi.2018.06.011

Hashimoto, K., 2018. Essential role of Keap1-Nrf2 signaling in mood disorders: Overview and future perspective. Front. Pharmacol. https://doi.org/10.3389/fphar.2018.01182

Jahn-Eimermacher, A., Lasarzik, I., Raber, J., 2011. Statistical analysis of latency outcomes in behavioral experiments. Behav. Brain Res. 221, 271–275. https://doi.org/10.1016/j.bbr.2011.03.007

Katz, L.C., Shatz, C.J., 1996. Synaptic activity and the construction of cortical circuits. Science (80-.). https://doi.org/10.1126/science.274.5290.1133

Kestering-Ferreira, E., Tractenberg, S.G., Lumertz, F.S., Orso, R., Creutzberg, K.C., Wearick-Silva, L.E., Viola, T.W., Grassi-Oliveira, R., 2021. Long-term Effects of Maternal Separation on Anxiety-Like Behavior and Neuroendocrine Parameters in Adult Balb/c Mice. Chronic Stress 5. https://doi.org/10.1177/24705470211067181

Koolhaas, J.M., de Boer, S.F., Coppens, C.M., Buwalda, B., 2010. Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. Front. Neuroendocrinol. https://doi.org/10.1016/j.yfrne.2010.04.001

Lalonde, R., Strazielle, C., 2009. The relation between open-field and emergence tests in a hyperactive mouse model. Neuropharmacology 57, 722–724. https://doi.org/10.1016/j.neuropharm.2009.07.010

Lipner, E., Murphy, S.K., Ellman, L.M., 2019. Prenatal Maternal Stress and the Cascade of Risk to Schizophrenia Spectrum Disorders in Offspring. Curr. Psychiatry Rep. https://doi.org/10.1007/s11920-019-1085-1

Maccari, S., Piazza, P.V., Kabbaj, M., Barbazanges, A., Simon, H., Le Moal, M., 1995. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. J. Neurosci. 15, 110–116. https://doi.org/10.1523/jneurosci.15-01-00110.1995

Malave, L., van Dijk, M.T., Anacker, C., 2022. Early life adversity shapes neural circuit function during sensitive postnatal developmental periods. Transl. Psychiatry. https://doi.org/10.1038/s41398-022-02092-9

McCormick, C.M., Mathews, I.Z., 2007. HPA function in adolescence: Role of sex hormones in its regulation and the enduring consequences of exposure to stressors. Pharmacol. Biochem. Behav. https://doi.org/10.1016/j.pbb.2006.07.012

Moffitt, T.E., Arseneault, L., Belsky, D., Dickson, N., Hancox, R.J., Harrington, H.L., Houts, R., Poulton, R., Roberts, B.W., Ross, S., Sears, M.R., Thomson, W.M., Caspi, A., 2011. A gradient of childhood self-control predicts health, wealth, and public safety. Proc. Natl. Acad. Sci. U. S. A. 108, 2693–2698. https://doi.org/10.1073/pnas.1010076108

Molendijk, M.L., de Kloet, E.R., 2019. Coping with the forced swim stressor: Current state-of-the-art. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2019.02.005

Musillo, C., Berry, A., Cirulli, F., 2022. Prenatal psychological or metabolic stress increases the risk for psychiatric disorders: the "funnel effect" model. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2022.104624

Nigg, J.T., 2017. Annual Research Review: On the relations among self-regulation, self-control, executive functioning, effortful control, cognitive control, impulsivity, risk-taking, and inhibition for developmental psychopathology. J. Child Psychol. Psychiatry Allied Discip. https://doi.org/10.1111/jcpp.12675

O'Connor, T.G., Ben-Shlomo, Y., Heron, J., Golding, J., Adams, D., Glover, V., 2005. Prenatal anxiety predicts individual differences in cortisol in pre-adolescent children. Biol. Psychiatry 58, 211–217. https://doi.org/10.1016/j.biopsych.2005.03.032

O'Donnell, K.J., Glover, V., Jenkins, J., Browne, D., Ben-Shlomo, Y., Golding, J., O'Connor, T.G., 2013. Prenatal maternal mood is associated with altered diurnal cortisol in adolescence. Psychoneuroendocrinology 38, 1630–1638. https://doi.org/10.1016/j.psyneuen.2013.01.008

Osborne, S., Biaggi, A., Chua, T.E., Du Preez, A., Hazelgrove, K., Nikkheslat, N., Previti, G., Zunszain, P.A., Conroy, S., Pariante, C.M., 2018. Antenatal depression programs cortisol stress reactivity in offspring through increased maternal inflammation and cortisol in pregnancy: The Psychiatry Research and Motherhood – Depression (PRAM-D) Study. Psychoneuroendocrinology 98, 211–221. https://doi.org/10.1016/j.psyneuen.2018.06.017

Panetta, P., Berry, A., Bellisario, V., Capoccia, S., Raggi, C., Luoni, A., Longo, L., Riva, M.A., Cirulli, F., 2017. Long-term sex-dependent vulnerability to metabolic challenges in prenatally stressed rats. Front. Behav. Neurosci. 11. https://doi.org/10.3389/fnbeh.2017.00113

Paolicelli, R.C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T.A., Guiducci, E., Dumas, L., Ragozzino, D., Gross, C.T., 2011. Synaptic pruning by microglia is necessary for normal brain development. Science (80-.). 333, 1456–1458. https://doi.org/10.1126/science.1202529

Rojo, A.I., McBean, G., Cindric, M., Egea, J., López, M.G., Rada, P., Zarkovic, N., Cuadrado, A., 2014. Redox control of microglial function: Molecular mechanisms and functional significance. Antioxidants Redox Signal. https://doi.org/10.1089/ars.2013.5745

Roseboom, T.J., Painter, R.C., Van Abeelen, A.F.M., Veenendaal, M.V.E., De Rooij, S.R., 2011. Hungry in the womb: What are the consequences? Lessons from the Dutch famine. Maturitas. https://doi.org/10.1016/j.maturitas.2011.06.017

Sanchez, C.E., Barry, C., Sabhlok, A., Russell, K., Majors, A., Kollins, S.H., Fuemmeler, B.F., 2018. Maternal pre-pregnancy obesity and child neurodevelopmental outcomes: a meta-analysis. Obes. Rev. https://doi.org/10.1111/obr.12643

Schafer, D.P., Lehrman, E.K., Kautzman, A.G., Koyama, R., Mardinly, A.R., Yamasaki, R., Ransohoff, R.M., Greenberg, M.E., Barres, B.A., Stevens, B., 2012. Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner. Neuron 74, 691–705. https://doi.org/10.1016/j.neuron.2012.03.026

Send, T.S., Bardtke, S., Gilles, M., Wolf, I.A.C., Sütterlin, M.W., Wudy, S.A., Wang, R., Laucht, M., Witt, S.H., Rietschel, M., Streit, F., Deuschle, M., 2019. Prenatal maternal stress is associated with lower cortisol and cortisone levels in the first morning urine of 45-month-old children. Psychoneuroendocrinology 103, 219–224. https://doi.org/10.1016/j.psyneuen.2019.01.017

Shi, X.T., Zhu, H.L., Xiong, Y.W., Liu, W.B., Zhou, G.X., Cao, X.L., Yi, S.J., Dai, L.M., Zhang, C., Gao, L., Xu, D.X., Wang, H., 2020. Cadmium down-regulates 11β-HSD2 expression and elevates active glucocorticoid level via PERK/p-eIF2α pathway in placental trophoblasts. Chemosphere 254. https://doi.org/10.1016/j.chemosphere.2020.126785

Simons, S.S.H., Beijers, R., Cillessen, A.H.N., Weerth, C. de, 2015. Development of the cortisol circadian rhythm in the light of stress early in life. Psychoneuroendocrinology 62, 292–300. https://doi.org/10.1016/j.psyneuen.2015.08.024

Slomianka, L., 2021. Basic quantitative morphological methods applied to the central nervous system. J. Comp. Neurol. https://doi.org/10.1002/cne.24976

Smiley, J.F., Bleiwas, C., 2012. Embedding matrix for simultaneous processing of multiple histological samples. J. Neurosci. Methods 209, 195–198. https://doi.org/10.1016/j.jneumeth.2012.06.005

Van Der Voorn, B., Hollanders, J.J., Kieviet, N., Dolman, K.M., De Rijke, Y.B., Van Rossum, E.F.C., Rotteveel, J., Honig, A., Finken, M.J.J., 2019. Maternal Stress during Pregnancy Is Associated with Decreased Cortisol and Cortisone Levels in Neonatal Hair. Horm. Res. Paediatr. 90, 299–307. https://doi.org/10.1159/000495007

Veenema, A.H., Blume, A., Niederle, D., Buwalda, B., Neumann, I.D., 2006. Effects of early life stress on

adult male aggression and hypothalamic vasopressin and serotonin. Eur. J. Neurosci. 24, 1711–1720. https://doi.org/10.1111/j.1460-9568.2006.05045.x

West, M.J., Slomianka, L., Gundersen, H.J.G., 1991. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anat. Rec. 231, 482–497. https://doi.org/10.1002/ar.1092310411

Zehr, J.L., Culbert, K.M., Sisk, C.L., Klump, K.L., 2007. An association of early puberty with disordered eating and anxiety in a population of undergraduate women and men. Horm. Behav. 52, 427–435. https://doi.org/10.1016/j.yhbeh.2007.06.005

Zhang, Bingbing, Li, M., Zou, Y., Guo, H., Zhang, Bingdong, Xia, C., Zhang, H., Yang, W., Xu, C., 2019. Corrigendum: Nfkb/orai1 facilitates endoplasmic reticulum stress by oxidative stress in the pathogenesis of non-alcoholic fatty liver disease (Front. Cell Dev. Biol. (2019) 7: 202. 10.3389/fcell.2019.00202). Front. Cell Dev. Biol. https://doi.org/10.3389/fcell.2019.0029

GENERAL CONCLUSIONS

Stress is a main risk factor for psychopathology. Extensive research on the biology of stress now shows that healthy development can be derailed by excessive or prolonged activation of the stress response in the brain already during fetal life. Such toxic stress exposure can have damaging effects on mental health across the lifespan. Overall, identifying shared biological mechanisms underlying different stressors can be crucial to unveil early risk biomarkers of mental disorders and open up novel early diagnostic and treatment avenues.

We identified the involvement of two main shared pathways that result similarly disrupted by both PNS and mHFD in a fine sex-dimorphic manner: metabolic-neuroendocrine regulations in males and inflammatory-redox balance in females. We can speculate that the effects of prenatal stressors observed in male offspring might result from specific sex-dependent adaptations in placental functionality. More in detail, we found structural alterations in PNS and mHFD male placentas that might underlie an insufficient supply of nutrients to these fetuses (**Chapter 3**). A possible explanation for this phenomenon is that male fetuses invest more energy on brain growth, compared to placental development, which gives them less reserve capacity, with a consequent higher risk for undernourishment when facing a stressful challenge, compared to females. Such lack of nourishment could prime the metabolic and neuroendocrine function (HPA axis) towards a more vulnerable phenotype later in life. In line with this interpretation, we found that, despite the same reduction of 11β-HSD 2 placental activity observed in both sexes, a specific baseline ipo-functionality of the HPA axis characterized adolescent males, being associated with changes in coping strategies towards stress (**Chapter 4**). Previous data from our research group support this sex-dependent programming effect of prenatal stress on the metabolic-neuroendocrine regulations, showing a greater vulnerability of males towards metabolic insults experienced later in life.

With regard to females, despite the apparent preserved functionality of the placenta, we clearly observed short-term disruption in fetal brains with increased pro-inflammatory and pro-oxidant markers (**Chapter 3**). Intriguingly, similar derangements in these patterns were maintained throughout adolescence, in addition to a reduction in markers of brain plasticity and to a more disinhibited phenotype **(Chapter 4)**. Since female mammals are characterized by more efficient antioxidant defenses compared to males - an evolutionary conserved feature dealing with the cost of reproduction - it is possible to hypothesize that prenatal stressors, by specifically targeting the antioxidant machinery, might lead to a sex-specific vulnerability. The preventive effect shown by NAC on many of the outcomes measured supports a main role of oxidative stress as a converging mechanism between metabolic and psychological stressors.

Unraveling further time- and sex-specific mechanisms will be crucial to develop personalized diagnostic tools and strategies for early intervention in order to buffer the harmful impact of prenatal adverse conditions on mental disorders.

APPENDIX

High-fat diet during adulthood interacts with prenatal stress, affecting both brain inflammatory and neuroendocrine markers in male rats

Alessandra Berry [#1], Monica Mazzelli [#2,3], Chiara Musillo [1,4], Marco Andrea Riva [2,3], Annamaria Cattaneo [2,3], Francesca Cirulli [1]

[1]Center for Behavioral Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy. [2]Biological Psychiatry Laboratory, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy. [3]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy. [4]PhD Program in Behavioral Neuroscience, Department of Psychology, Sapienza University of Rome, Rome, Italy. #Contributed equally.

The European Journal of Neuroscience (2022) 9-10:2326-2340. doi: 10.1111/ejn.15181

Abstract

Prenatal stress (PNS) affects foetal programming and, through an interaction with subsequent challenges, can increase vulnerability to mood and metabolic disorders. We have previously shown that, following PNS, adult male rats are characterized by increased vulnerability to a metabolic stressor experienced at adulthood (8-week-high-fat diet-HFD). In this study, we specifically assessed whether PNS might interact with an adult metabolic challenge to induce an inflammatory phenotype. Changes in the expression levels of inflammatory (Il-1β, Tnf-α, Il-6) and of stress response mediators (Nr3c1, Fkbp5) as well as of mood and metabolic regulators (Bdnf, Ghs-R) were investigated in the hippocampus, prefrontal cortex and hypothalamus, brain regions involved in the pathogenesis of depression and prone to inflammation in response to stress. Overall, PNS reduced the expression of Bdnf and Tnf-α, while HFD administered at adulthood counteracted this effect suggesting that PNS impinges upon the same pathways regulating responses to a metabolic challenge at adulthood. Furthermore, HFD and PNS affected the expression of both Nr3c1 and Fkbp5, two neuroendocrine mediators involved in the response to stress, metabolic challenges and in the modulation of the emotional profile (as shown by the correlation between Fkbp5 and the time spent in the open arms of the elevated plus-maze). Overall, these results indicate that the same metabolic and neuroendocrine effectors engaged by PNS are affected by metabolic challenges at adulthood, providing some mechanistic insight into the well-known comorbidity between mood and metabolic disorders.

Keywords

Co-morbidity; animal model; high-fat diet; mood disorders; neuroinflammation; prenatal stress.

Introduction

Mood disorders are common conditions with major public health implications. It is estimated that each year around 40% of the EU population suffers from a mental disorder. Adjusted for age and comorbidity, this corresponds to 164.8 million people affected (Health Organization Regional Office for Europe, 2015). Among the risk factors that might trigger the onset of psychiatric conditions, or an exacerbation of symptoms, stressful life events play a pivotal role. In fact, according to a "two-hit model" of vulnerability to diseases, stress experienced during early life phases, might affect brain development leading to a reduced ability to cope with further stressors during life (Daskalakis et al., 2013). To this regard, a suboptimal intrauterine environment, prompted by maternal stress (ranging from poor socio-economic status and maternal obesity to depression or maltreatments) may predispose the offspring to lifelong negative health outcomes, including neuropsychiatric disorders as well as metabolic and immune dysregulations (Barker, 1995; Berry et al., 2015; Boersma et al., 2013; Cattane et al., 2020; Cirulli et al., 2020; Krontira et al., 2020).

Consistent evidence suggests that depressed patients are characterised by alterations in the functional activity of the immune system, affecting both peripheral and central tissues and that early life stress represents an important factor influencing the inflammatory status (Cattaneo et al., 2015; Dantzer et al., 2008; Dowlati et al., 2010; Liu et al., 2012; Valkanova et al., 2013). As an example, Danese and colleagues reported increased blood C-reactive protein (CRP) levels in maltreated children, an effect that was larger in those subjects that also developed depression later in life (Danese et al., 2009, 2008). Likewise, Slopen and co-workers reported increased CRP and interleukin-6 (IL-6) in adolescents who experienced early life adversities (Slopen et al., 2014). Offspring of women who experienced stressful life events during pregnancy show higher levels of IL-1β, IL-4, IL-5, IL-6, and IL-8 in umbilical cord blood at delivery (Andersson et al., 2016). Interestingly, it has been recently proposed that inflammation may act as a key mediator linking exposure to prenatal stress (PNS) and enhanced vulnerability to psychopathology in the offspring (Hantsoo et al., 2019).

Preclinical studies in rodent models provide strong support to the above mentioned clinical findings, showing for example that PNS exposure causes extended inflammation in the foetal brain (Ślusarczyk et al., 2015). To this regard, Gur and colleagues found increased levels of IL-1β in the placenta of mouse dams undergoing stress during pregnancy and in the foetal brain of their offspring; this was also associated with decreased levels of the neurotrophin Brain-Derived Neurotrophic Factor (BDNF) specifically in the amygdala (Chen et al., 2020; Gur et al., 2017) suggesting that increased expression of placental immune responsive genes upon maternal stress might be considered as a potential mechanism (Bronson and Bale, 2014; Mueller and Bale, 2008).

Despite the large body of clinical and preclinical evidence showing associations among PNS, the hyperactivation of the immune system and an enhanced vulnerability to mood disorders, cause-effect mechanisms are still largely unknown, and the prenatal environment may not act alone to set the stage for long-term onset of mood disorders. Interestingly, psychiatric conditions and metabolic pathologies are often found

to co-occur within the same individual showing a feed-forward pattern overall suggesting the activation of shared mechanisms/pathways (Cattane et al., 2020; Cirulli et al., 2020; Milaneschi et al., 2019). Among these, BDNF and glucocorticoids (GC) hormones have been identified as main effectors of brain plasticity and metabolic function in response to stressful events. Thus, it is possible that these actors may represent both effectors as well as targets of stress - during sensitive developmental periods - leading, in the developing organism, to a remodelling of the mechanisms associated with stress responsiveness (Cattaneo et al., 2015; Cirulli and Alleva, 2009; McEwen, 2000). The neurotrophin BDNF plays a pivotal role in brain and behavioural plasticity as well as in the control of energy homeostasis (Cirulli and Alleva, 2009; Marosi and Mattson, 2014); its expression is finely tuned by GC in response to acute or chronic stressful stimuli (Cirulli, 2017;Cirulli & Alleva, 2009) and it has been also suggested to be negatively regulated by pro-inflammatory cytokines (Barrientos et al., 2004; Bilbo et al., 2008). Thus, BDNF appears as a sensitive target as well as a biomarker of stress response as it could actively contribute to maintain brain health/homeostasis in different challenging conditions. To this regard, the interplay between the activation of stress-related pathways by adverse experiences during development and adult metabolic challenges might be crucial in shaping individual vulnerability to stress (Nederhof and Schmidt, 2012).

A growing body of evidence suggests that the effects of PNS are sex-specific and that males' foetal brain might be more vulnerable to changes in the inflammatory mediators overall showing learning deficits and decreased brain plasticity particularly with regard to the hippocampus prefrontal cortex (McCarthy, 2019; Weinstock, 2007). Indeed, in a previous study we have shown that male rats who underwent PNS were more vulnerable than females to the effects of a metabolic challenge experienced at adulthood (high-fat diet feeding - HFD), while females showed an overall greater plasticity, possibly mediated by increased total Bdnf mRNA expression levels both in the hippocampus and in the hypothalamus. Based on the "two hit model" of diseases, we hypothesized that PNS might disrupt the intrauterine environment affecting brain developmental trajectories leading, in turn, to increased brain inflammation and reduced plasticity in the offspring (first hit). We also hypothesized that a metabolic challenge such as a HFD, experienced at adulthood, might interact with inflammatory pathways to exacerbate the PNS-induced pro-inflammatory phenotype. Thus, the aim of this study was to investigate the effect of a second hit - represented by an HFD - specifically in male rats. We focused on selected brain regions - characterised by high degree of sexual dimorphism (Handa et al., 1994) - such as the dorsal and the ventral hippocampus, the prefrontal cortex and the hypothalamus known to be involved in the pathogenesis of depression and to be prone to inflammatory status in response to external challenges. We specifically assessed in these brain regions levels of Bdnf, Tnf-□, Il-6 and Il-□.

Stress powerfully affects both mood and energy homeostasis. Such effects are achieved not only through the interaction between GC hormones and their receptors (that regulate HPA axis function in addition to glucose metabolism) but also by triggering a multitude of signalling cascades reciprocally modulating one another in a regional-, temporal, and functional-dependent manner (Balsevich et al., 2019). Among these, ghrelin is a peptide hormone produced in the stomach and involved in the signalling of meal initiation. Its action is

mediated through the growth hormone secretagogue receptor (Ghs-R) that is found to be highly expressed in several brain regions including the hypothalamus, pituitary gland and hippocampus (see Zarouna, 2015 and references therein). Both Ghrelin and Ghs-R are modulated by stress (Patterson et al., 2010); there is evidence that ghrelin may decrease anxiety-like and depressive-like behaviours in mice (Lutter et al., 2008) moreover, it appears to be involved in in food anticipatory activity and consumption (Blum et al., 2009; Verhagen et al., 2011) as well as in mood disorders (Zarouna, 2015). Interestingly, both genetic variants within ghrelin and GC signalling pathways have been associated with obesity, stress-related mental disorders, or both (see Balsevich et al., 2019 and references therein). To this regard, we also investigated changes in the expression levels of Ghs-R as well as of Nr3c1 (encoding for the glucocorticoid receptors - GR) and the GC co-chaperon Fkbp5 (encoding for the FK506 binding protein 51) in all the above-mentioned brain regions.

Materials and methods

Animals and experimental design

Twelve adult nulliparous female (230–260 g) and 6 male Sprague-Dawley rats (400 g) were purchased from a commercial breeder (Charles River, Calco, Italy). Upon arrival, animals were pair-housed with same sex conspecifics under standard laboratory conditions (see Panetta et al. 2015 for further details). Pellet food (Altromin-R, Rieper, Italy) and tap water were continuously available. Following one week of adaptation, two females and one male were mated for 24 hours. To assess pregnancy, changes in body weight were monitored. Pregnant females were randomly assigned to either the control (CTRL) or prenatal stress (PNS) groups. CTRL rats were left undisturbed throughout gestation, while PNS females underwent a repeated restraint stress procedure during the last third of gestation until the day delivery day (postnatal day 0 - PND-0). On PND-1 all pups were weighted and litters culled to an average of 5 male and 5 female pups. On PND-21 pups were weaned and housed in groups of 2 or 3 same-sex littermates until 2 months of age. By this time, n=20 CTRL and n=21 PNS males were assigned either to high-fat diet (HFD) or control diet (CD) regimen for 8 weeks. The final number of animals per group was: n=10 CTRL-CD, n=10 CTRL -HFD, n=10 PNS-CD and n=11 PNS-HFD. Groups were composed by 1 pup/litter (CTRL-CD; CTRL-HFD; PNS-CD; PNS-HFD. After 4 weeks on the respective diets, all animals underwent a number of metabolic and behavioural assessments; they were successively sacrificed and brains dissected out to investigate the expression levels of Il-6, Il-1β, Tnf-α, Nr3c1, Fkbp5, Ghs-R, in the dorsal hippocampus, ventral hippocampus, hypothalamus and prefrontal cortex; total Bdnf was investigated only in the dorsal hippocampus and prefrontal cortex (since it was already assessed in the other two areas, see Panetta et al., 2017).

To better characterise the effects of possible changes in gene expression levels, regression analyses were carried out by taking into account specific outcomes deriving from the Elevated Plus-Maze (EPM), as previously performed in (Panetta et al., 2017). We focused on immobility time since this is a well-known proxy for emotionality and reactivity to novel environment (Fernandes and File, 1996) and correlated it with Bdnf

and Il-1β since both these mediators are involved in behavioural plasticity (Bourgognon and Cavanagh, 2020; Cirulli and Alleva, 2009; Goshen et al., 2007). Moreover, we also assessed the ability of Fkbp5 to affect emotionality in the EPM by correlating expression levels of this gene in the dorsal hippocampus to the time spent in the open arms of the maze. As for the EPM protocol used, briefly, the apparatus was made of Plexiglas and consisted of two opposite open arms and two arms closed by transparent walls (50×10×40 cm). Each rat was placed in the center of the maze and video-recorded for 5 minutes under dim light conditions. Each session was recorded and behavioral analysis was carried out using a commercial software (''The Observer 3.0'', Noldus, The Netherlands) (see (Panetta et al., 2017) for further details).

All experimental procedures were reviewed by the ethical body of the Istituto Superiore di Sanità for animal welfare and conducted in conformity with the European Directive 2010/63/EU and the Italian legislation on animal experimentation, D.Lgs. 26/2014. They were authorized by the Italian Ministry of Health.

Dams' stress procedure

Pregnant females (at gestational day - GD - 14) were restrained in a transparent Plexiglas cylinder (7.5×19 cm) under a bright light (6.500 lux) for 45 minutes three times daily at random times (between 9:00 a.m.-5:00 p.m.) during the dark phase until the expected delivery day (GD-21, see Maccari et al., 1995 for further details). This stress has been selected since it is one of the most well characterised prenatal stressors with well-known effects on developmental trajectories of the offspring.

High-fat diet administration

CTRL and PNS offspring were fed ad libitum either with HFD (energy: 5.24 kcal/g; composition: fat 60%, carbohydrate 20% and protein 20%) or CD (energy: 3.3 kcal/g; composition: fat 17%, carbohydrate 60% and protein 23%) starting from two months of age. The HFD diet (D12492) was purchased from Research Diets, Inc., New Brunswick, NJ, USA; the CD was purchased from Altromin-R, Rieper, Italy.

Tissue collection

At 4 months of age offspring was weighted and sacrificed. Brains were removed and the dorsal and ventral hippocampus, hypothalamus and prefrontal cortex were dissected out and immediately frozen at -80°C (Panetta et al., 2017).

Molecular analysis

RNA isolation and Real Time PCR Analyses

Total RNA was isolated using PureZol RNA isolation reagent (Bio–Rad Laboratories, Italy), treated with DNase to avoid DNA contamination and quantified by spectrophotometric analysis. The quantified RNA was analyzed by TaqMan q-RT PCR Instrument (CFX384 real time system, Bio-Rad Laboratories) using the iScriptTM one-step RT-PCR kit for probes (Bio-Rad Laboratories) and Applied BioSystem Assays (Gene Expression Assays: Il6, Il-β, Tnf-□, Bdnf, Nr3c1, Fkbp5, and Ghs-R). Samples were run in triplicate and each

target gene analyzed has been normalized to the expression of the housekeeping (HK) gene β-actin (ActB). The expression of target genes was calculated by using to the Ct method (-ΔΔCt method) (Schmittgen and Livak, 2008), where CTRL-CD rats have been used as a reference group.

Statistical analysis

Data were evaluated by a two-way ANOVA with Diet (HFD vs CD) and Prenatal condition (PNS vs CTRL) as between-subject factors. Post-hoc comparisons between groups were performed using the Tukey's test. A linear regression model was used to assess the ability of Bdnf levels and of Il-1 in the dorsal hippocampus to affect immobility duration (a parameter indicative of increased emotionality) in the EPM test. A level of probability set at $p<0.05$ was used as statistically significant. Statistical tendency was set at $p<0.1$. For interactions at $p<0.1$, we also examined lower order effects. Data are presented graphically as means ± SEM box plot (observations outside the ranges are represented with dots outside the boxes). The raw data supporting the conclusions of this article will be made available by the authors upon request without undue reservation.

Results

Inflammatory mediators *Il-1β*, *Tnf-α* and *Il-6* mRNA expression

Il-1β - We first analyzed the expression of *Il-1β*, a prototype pro-inflammatory cytokine. We observed that PNS resulted in a nearly significant decrease in the dorsal hippocampus while neither a main effect of HFD nor a PNS x HFD interaction effect were found (F (1,35)=3.818, 0.004, 2.184; p= 0.0587, 0.9518, 0.1484).

No main effects of PNS nor of HFD were observed in the ventral hippocampus (F(1,34)=0.152, 0.335; p=0.6995, 0.5665). A strong PNS x HFD interaction was found (F(1,34)=4.655; p=0.0381). More in detail, PNS exposed animals were characterized by decreased levels of this cytokine, while the administration of postnatal HFD counteracted the effects of the PNS (post-hoc comparisons just missed statistical significance).

In the prefrontal cortex while no main effects of PNS was observed (F(1,33)=0.969; p=0.3321) postnatal HFD overall increased levels of *Il-1β* (main effect of HFD: F(1,33)=5.112, p=0.0305). Similarly to what was observed in the ventral hippocampus, a nearly significant PNS x HFD interaction was found with decreased levels of *Il-1β* in PNS animals, this effect being reverted by postnatal administration of HFD (F(1,33)=3.858; p=0.0580, post hoc comparisons: p<0.01, PNS vs. PNS-HFD); (see Figure 1A).

In the hypothalamus, levels of *Il-1β* were not affected by PNS, HFD nor an interaction was found (F(1,36)=0.042, 0.0001633, 0.070; p=0.8386, 0.9899, 0.7934 respectively for PNS, HFD and PNS x HFD, see Figure 1A).

Tnf-α - In the dorsal hippocampus no main effects of PNS nor of HFD were observed (F(1,37)=1.288, 0.209; p=0.2638, 0.6506). By contrast, a significant interaction was found between PNS and HFD (F(1,37)=5.772; p=0.0214). In particular, PNS animals were characterised by decreased levels of *Tnf-α,* although this effect was no longer observed in PNS-HFD animals that showed levels of this cytokine comparable to those observed in CTRL animals (post hoc comparisons: p<0.05, PNS vs. CTRL).

No main effects of PNS nor of HFD were found in the ventral hippocampus (F(1,34)=0.033, 2.941; p=0.8573, 0.0955, respectively for PNS and HFD), while a PNS x HFD interaction was found showing that administration of postnatal HFD in the PNS group selectively increased levels of this cytokine ((F(1,34)=6.747; p=0.0138); post hoc comparisons: PNS vs. HFD-PNS, p<0.01).

No main effects of PNS nor of HFD were observed in the prefrontal cortex (F(1,33)= 0.079, 1.648; p=0.7809, 0.2082 respectively for PNS and HFD) however, as also observed for the ventral hippocampus a PNS x HFD interaction effect was found showing that the administration of postnatal HFD in the PNS group selectively increased levels of this cytokine (F(1,33)=7.946; p=0.0081); post hoc comparisons: PNS vs. HFD-PNS, p<0.01); (see Figure 1B).

Levels of *Tnf-α* in the hypothalamus (see Figure 1B) were not affected neither by PNS nor by postnatal HFD (F(1, 35)=0.286, 0.702, 2.111; p= 0.5964; 0.4079; 0.1551 respectively for PNS, HFD and PNS x HFD).

Il-6 - Postnatal HFD decreased levels of *Il-6* in the dorsal hippocampus (main effect of HFD: F(1,37=0.814; p=0.0066), whereas no effect of the PNS nor of the PNS x HFD interaction effect were found (PNS main effect: F(1,37)=1.387; p=0.2465; PNS x HFD interaction: F(1,37)=0.164; p=0.6881).

Levels of *Il-6* did not change as a result of PNS, HFD or their interaction neither in the ventral hippocampus (F(1,36)=0.759, 2.631, 0.029; p=0.3893, 0.1135, 0.8647 respectively for PNS, HFD and PNS x HFD) nor in the prefrontal cortex (F(1,35)=0.144, 0.053, 0.202 respectively for PNS, HFD and PNS x HFD); (see Figure 1C). PNS decreased nearly significantly the levels of *Il-6* also in the hypothalamus (F(1,36)=3.889; p=0.0563), but no effect of HFD nor an interaction PNS x HFD were found to be significant (F(1,36)=0.903, 0.757; p=0.3482, 0.3899, respectively for HFD and PNS x HFD).

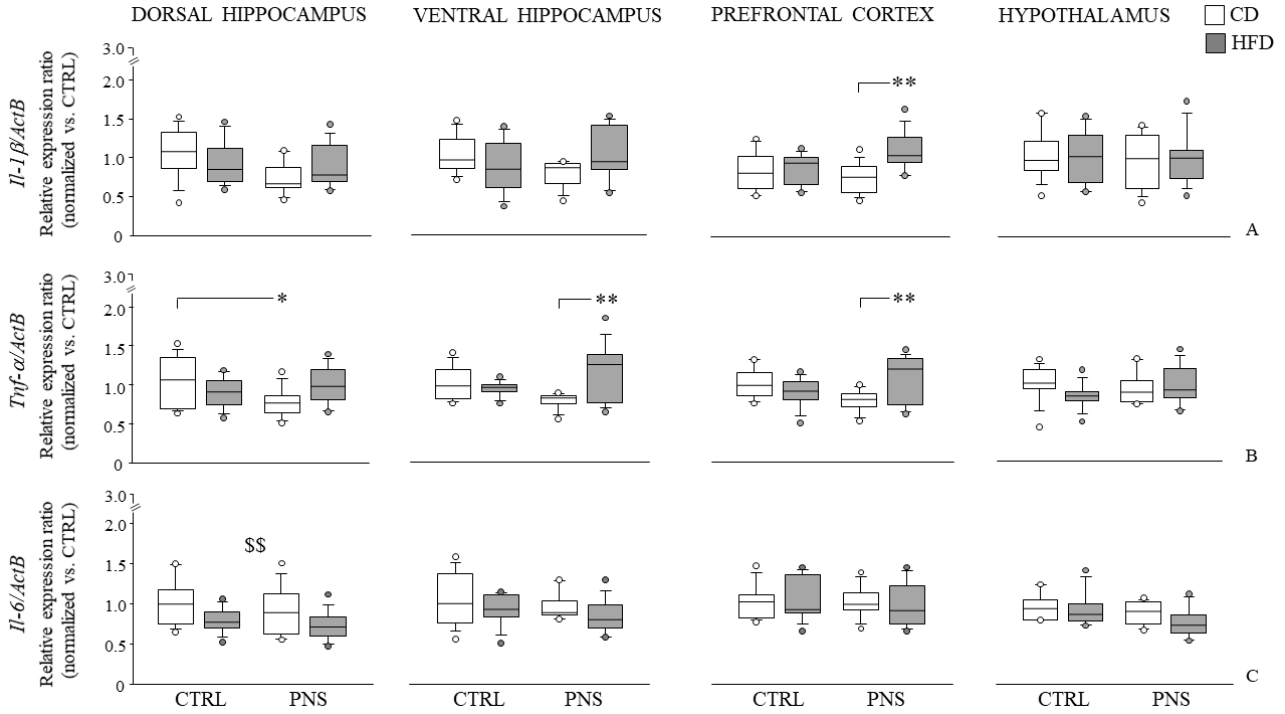


Figure 1. Overall subjects who experienced prenatal stress (PNS-CD) were characterized by decreased m-RNA levels of both *Il-1β* (panel A) and *Tnf-α* (panel B) in almost all the brain regions investigated; *Il-6* showed a decrease in its expression levels upon HFD in the dorsal hippocampus (panel C). *Il-1β* showed statistical significance only in the prefrontal cortex while *Tnf-α* was tightly modulated upon pre- and postnatal stressors. Data are presented graphically as means ± SEM box plot (observations outside the ranges are represented with dots outside the boxes). Post hoc comparisons: $$p<0.01, main effect of diet (dorsal hippocampus, *Il-6*); post hoc comparisons: *p<0.05, interaction effect (dorsal hippocampus, *Tnf-α*: PNS-CD vs. CTRL-CD); **p<0.01, interaction effect (ventral hippocampus, *Tnf-α*: PNS-CD vs. PNS-HFD; Prefrontal cortex, *Tnf-α* and *Il1-β*: PNS-CD vs. PNS-HFD). Number of subjects:8-10 within each experimental group.

Interestingly, when expression levels of *Il-1β* in the dorsal hippocampus were related to immobility duration in the EPM (a test run in a companion paper on the same experimental animals, see (Panetta et al., 2017)), we observed a significant strong negative association linking these two parameters only in the PNS-CD animals,

overall suggesting that reduced levels of this cytokine can be related with reduced behavioral plasticity (see discussion; CTRL-CD: F(1,8)=0.007; p=0.9367; R^2=0.01; CTRL-HFD: F(1,8)=3.461; p=0.1051; R^2=33.1; PNS-CD: F81,8)= 7.538; p=0.0287; R^2=51.8; PNS-HFD: F(1,9)= 1.739; p=0.2237, R^2=17.9; see Figure 2). By contrast, regression plot between *Il-1β* and time spent in the open arms of the maze was not significant in neither of the four groups considered (CTRL-CD F(1,9)= 1.387; p=0.2742; R^2= 0.147; CTRL-HFD: F(1,9)=0.91; p=0.7708; R^2= 0.011; PNS-CD: F(1,8)=2.875; p=0.1338; R^2= 0.291; PNS-HFD: F(1,8)=1.040; p=0.3417;R^2= 0.129).

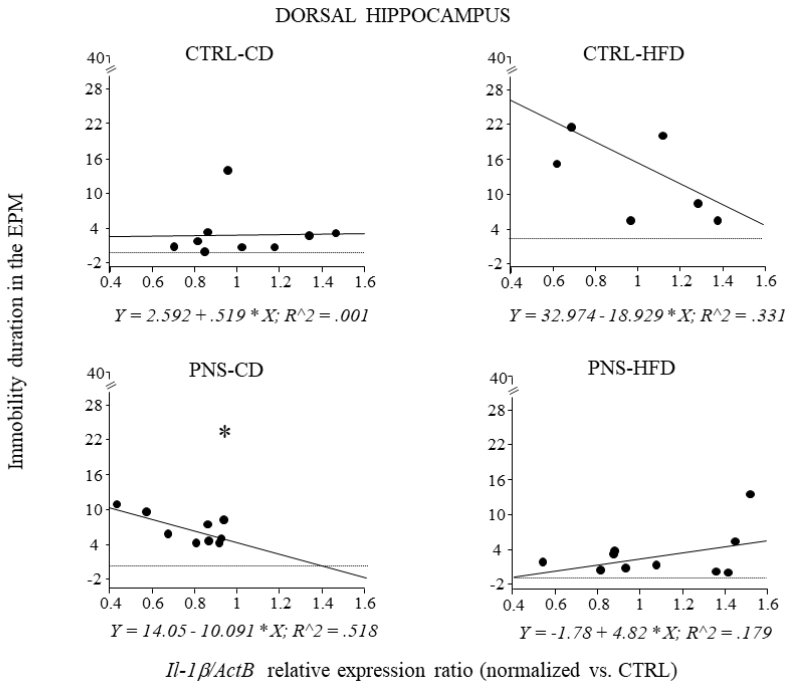


Figure 2. Male rats exposed to prenatal stress (PNS) were characterised by an inverse relation between levels of Il-1β in the dorsal hippocampus and immobility duration as assessed in the elevated plus maze test (EPM). *p<0.05. Number of subjects: 6-10 within each experimental group.

Neuronal plasticity marker - *Bdnf* mRNA expression

We first investigated the expression of *Bdnf*, a neurotrophin that is considered a prototype marker of plasticity. Within the dorsal hippocampus, overall PNS and postnatal HFD did not affect levels of *Bdnf* (F(1, 36)= 3.678; 1.964;p= 0.0631; 0.1696), main effect of PNS and HFD respectively), although we observed a significant interaction between PNS and postnatal HFD (F(1, 36)=5.606; p=0.0234). More in detail, PNS decreased *Bdnf* expression, while the postnatal administration of HFD was able to counteract this effect (PNS vs. CTRL, p<0.05; PNS vs. PNS-HFD, p<0.05). In the prefrontal cortex no effect of PNS, HFD or their interaction was observed (F(1, 36)=0.200, 1.377, 0.174; p=0.6572; 0.2482; 0.6794, respectively for PNS, HFD and PNS x HFD); (see Figure 3).

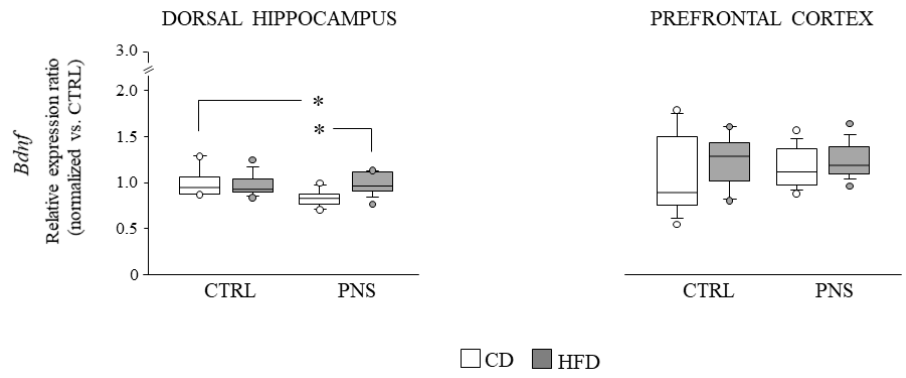


Figure 3. Prenatal stress decreased *Bdnf* m-RNA levels specifically in the dorsal hippocampus suggesting a reduced neuronal plasticity. This effect is reversed by HFD administration at adulthood. Data are presented graphically as means ± SEM box plot (observations outside the ranges are represented with dots outside the boxes). Post hoc comparisons *p<0.05, interaction effect in the dorsal hippocampus, PNS-CD vs. CTR-CD and PNS-HFD. Number of subjects is between 9-11 within each experimental group.

Interestingly, levels of *Bdnf* in the dorsal hippocampus were negatively associated with the time spent immobile in the EPM (a test run in a companion paper on same experimental animals, see (Panetta et al., 2017)) only in the PNS-CD group (CTRL-CD: $F(1,9)=0.730$; $p=0.4261$; $R^2= 0.081$; CTRL-HFD: $F(1,9)= 0.090$; $p=0.7713$; $R^2=0.011$; PNS-CD: $F(1,8)=11.084$; $p=0.0127$; $R^2=0.612$; PNS-HFD: $F(1,9)=0.716$; $p=0.4221$; $R^2= 0.082$), supporting a role of this neurotrophin in the emotional phenotype (see Figure 4).

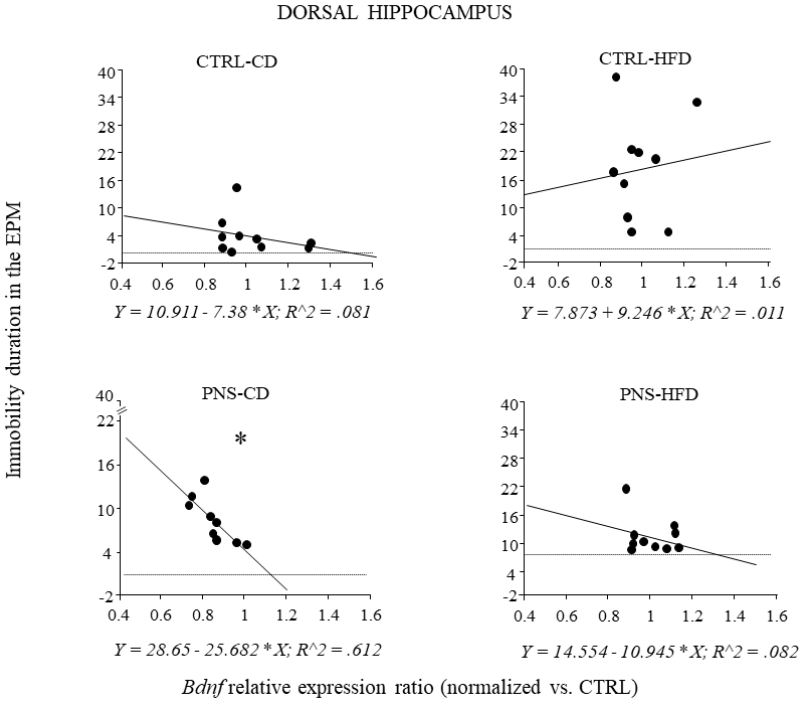


Figure 4. Male rats exposed to prenatal stress (PNS) were characterised by an inverse relation between levels of *Bdnf* in the dorsal hippocampus and immobility duration as assessed in the elevated plus maze test (EPM). Post hoc comparisons *p<0.05. Number of subjects: 9-10 within each experimental group.

Stress response mediators - *Nr3c1, Fkbp5* mRNA expression

Nr3c1 - In the dorsal hippocampus the postnatal HFD decreased the levels of *Nr3c1* when compared to controls and a similar nearly significant effect was observed for PNS (main effect: F(1,36)=3.827, 7.063, p=0.0582; 0.0117 respectively for PNS, HFD). Moreover, a significant PNS x HFD interaction was found showing that both PNS-CD and CTRL-HFD as well as the combination PNS-HFD were characterised by a decrease of GR mRNA levels (F(1,36)=6.228; p=0.0173 and PNS x HFD; post hoc comparisons p<0.05).

In the ventral hippocampus levels of *Nr3c1* were increased a result of postnatal HFD (main effect: F(1,36)=10.405; p=0.0027), while no effects of PNS nor of the PNS x HFD interaction were found (F(1, 36)=0.011, 0.258; p= 0.9167; 6146). In the prefrontal cortex the postnatal HFD overall increased the levels of *Nr3c1* (main effect of HFD: F(1, 37)=12.453; p=0.0011); no effects of PNS nor of the PNS x HFD interaction were observed ((F(1, 37)=1.001, 0.702; p=0.3236; 0.4076). No effects of PNS, HFD nor of their interaction were found in the hypothalamus (F(1, 37)=0.157, 0.009, 1.067; p= 0.6943; 0.9257; 0.3084); (see Figure 5).

Fkbp5 - In the dorsal hippocampus the postnatal HFD increased the levels of *Fkbp5* (F(1,35)=10.331;p=0.0028), while no effect of PNS nor of the PNS x HFD interaction was observed (F(1,35)=1.502, 0.958; p= 0.2285; 0.3343). No effect of PNS, HFD or their interaction was found in the ventral hippocampus (F(1,36)=0.781, 3.222, 0.007; p=0.3828; 0.0811; 0.9359). In the prefrontal cortex, PNS increased the levels of *Fkbp5* (F(1,37)=5.244; p=0.0278), while no effect of HFD nor of the PNS x HFD interaction was observed (F(1,37)=1.219, 2.019; p=0.2767; 0.1637). A main effect of the postnatal HFD was also observed In the hypothalamus, the Postnatal HFD decreased levels of *Fkbp5* (F(1,37)=9.156; p=0.0045); no effect of PNS nor of the PNS x HFD interaction was observed (F(1,37)=0.094, 3.353; p= 0.7608; 0.0751); (see Figure 5).

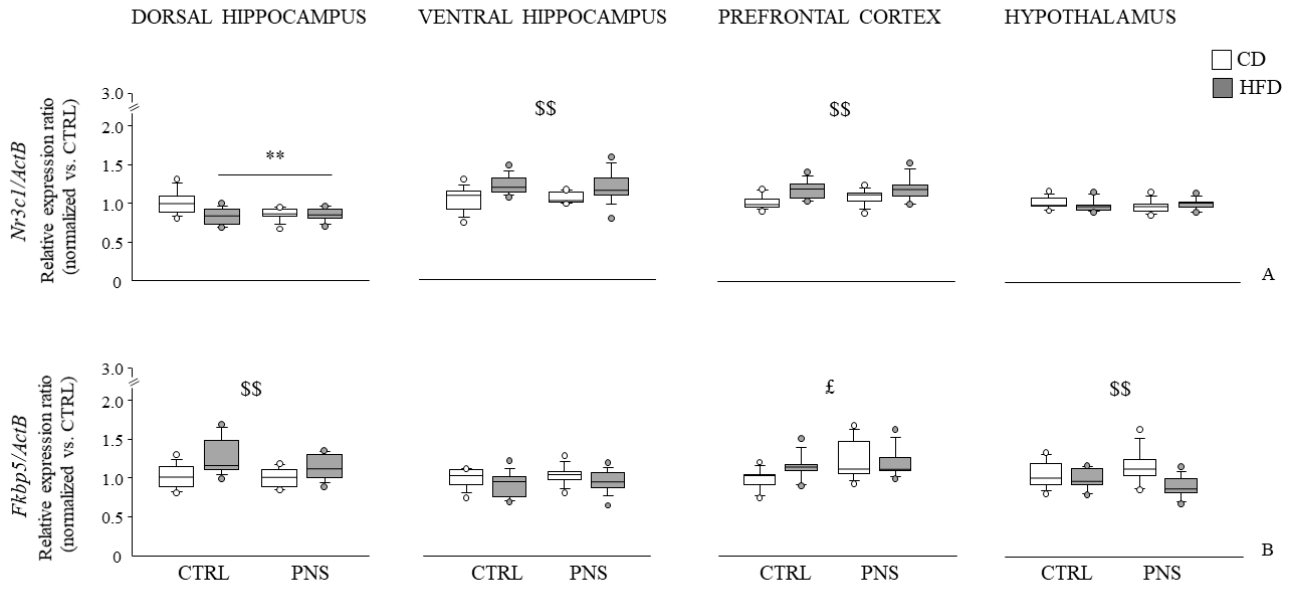


Figure 5. Independently from the prenatal condition, post-natal HFD administration increases levels of *Nr3c1* in the ventral hippocampus and prefrontal cortex. By contrast, in the dorsal hippocampus a significant interaction between PNS and HFD results in decreased levels of this gene both as a result of pre- and post-natal stressors. No change in *Nr3c1* was observed in the hypothalamus as a result of PNS nor of HFD (see panel A). As for *Fkbp5*, HFD independently from the prenatal condition, increased its expression levels in the dorsal hippocampus while a decrease was observed in the hypothalamus; no change was observed in the ventral hippocampus. PNS overall increased levels of Fkbp5 specifically in the prefrontal cortex. $$p<0.01, main effect of diet (*Nr3c1*: ventral hippocampus and prefrontal cortex; Fkbp5: dorsal hippocampus and hypothalamus; *Fkbp5*: dorsal hippocampus; hypothalamus); £p<0.05, main effect of PNS (*Fkbp5*: prefrontal cortex); post hoc comparisons: **p<0.01, interaction effect (*Nr3c1*: CTRL-CD vs. CTRL-HFD, PNS-CD and PNS-HFD); Number of subjects:8-10 within each experimental group.

Interestingly, levels of *Fkbp5* in the dorsal hippocampus were positively related with increased time spent in the open arm of the EPM (a test run in a companion paper on same experimental animals, see (Panetta et al., 2017)) only in the CTRL-CD group (CTRL-CD: $F(1,8)=26.617$; $p=0.0013$; $R^2= 0.792$; CTRL-HFD: $F(1,9)= 0.104$; $p=0.7713$; $R^2=0.013$; PNS-CD: $F(1,8)=0.253$; $p=0.6307$; $R^2=0.035$; PNS-HFD: $F(1,9)=0.503$; $p=0.6307$; $R^2= 0.4982$). This result might suggest that *Fkbp5* is physiologically required to engage animals in the proper exploration of novel environments through a fine modulation of GR receptors (see Figure 6). By contrast, regression plot between *Fkbp5* and time spent immobile in this same brain area was not significant in neither of the four groups considered (CTRL-CD $F(1,8)= 0.718$; $p=0.4249$; $R^2= 0.093$; CTRL-HFD: $F(1,9)=0.325$; $p=0.5841$; $R^2= 0.039$; PNS-CD: $F(1,8)=4.195$; $p=0.798$; $R^2= 0.375$; PNS-HFD: $F(1,9)= 0.983$; $p=0.3504$; $R^2= 0.109$).

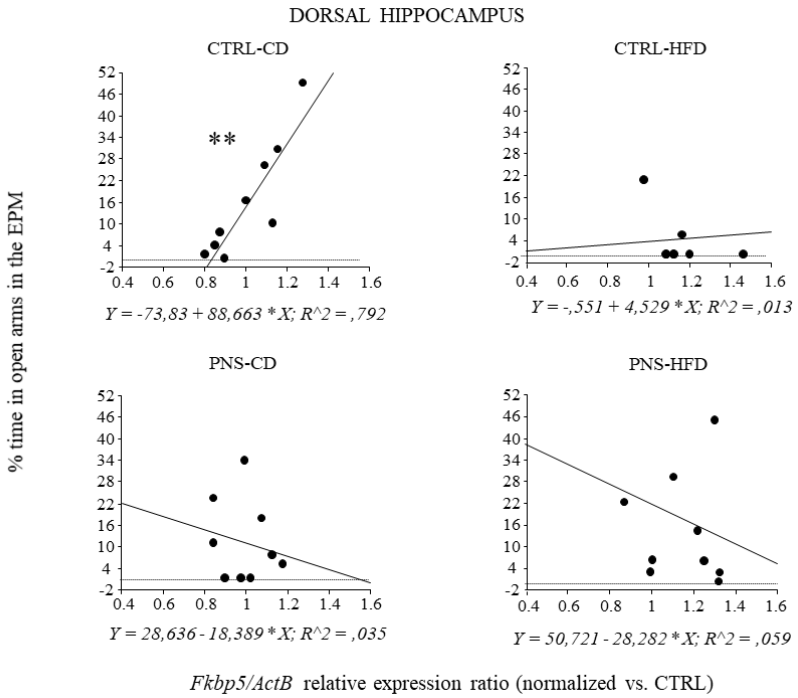


Figure 6. Control subjects are characterized by a direct relationship between expression levels of *Fkbp5* in the dorsal hippocampus and the time spent in the open arms of the Elevated Plus Maze, overall suggesting a permissive role for explorative behavior of this GC-related genes under physiological conditions. Post hoc comparisons **p<0.01. Number of subjects: 6-9 within each experimental group.

Metabolic/mood regulator marker - *Ghs-R* mRNA expression

Ghs-R - In the dorsal hippocampus the postnatal HFD overall decreased levels of *Ghs-R* (F(1,33)=1,948; p=0.0007). By contrast, PNS increased *Ghs-R* levels (F(1,33)=5.192; p=0.0293), but no interaction between PNS x HFD was observed (F(1,33)=0.290; p= 0.5941). In the ventral hippocampus HFD overall increased the levels of *Ghs-R* (F(1,32)=6.278; p=0.0175), while no main effect of PNS was observed (F(1,32)=0.593; p=0.4468). However, *Ghs-R* was increased in the CTRL-HFD and in the PNS-CD groups (interaction between PNS and HFD: F(1,32)=14.778; p=0.0005, post hoc: CTRL vs. CTRL-HFD, p<0.01; CTRL vs. PNS, p<0.01). In the prefrontal cortex, PNS increased the levels of *Ghs-R* (F(1,29)=5.865; p=0.0219) and no effect of HFD was observed (F(1,29)=2.191; p=9.1496). However, in this brain area, *Ghs-R* was increased in the CTRL-HFD and in the PNS-CD groups (interaction between PNS and HFD: F(1,29)=6.870; p= 0.0138, post hoc: CTRL vs. CTRL-HFD, p<0.05; CTRL vs. PNS, p<0.01); (see Figure 7). As for the hypothalamus, neither PNS nor HFD affected levels of *Ghs-R* (F(1,34)=3.155, 3.033, 0.907; p=0.0846; 0.0906; 0.3477).

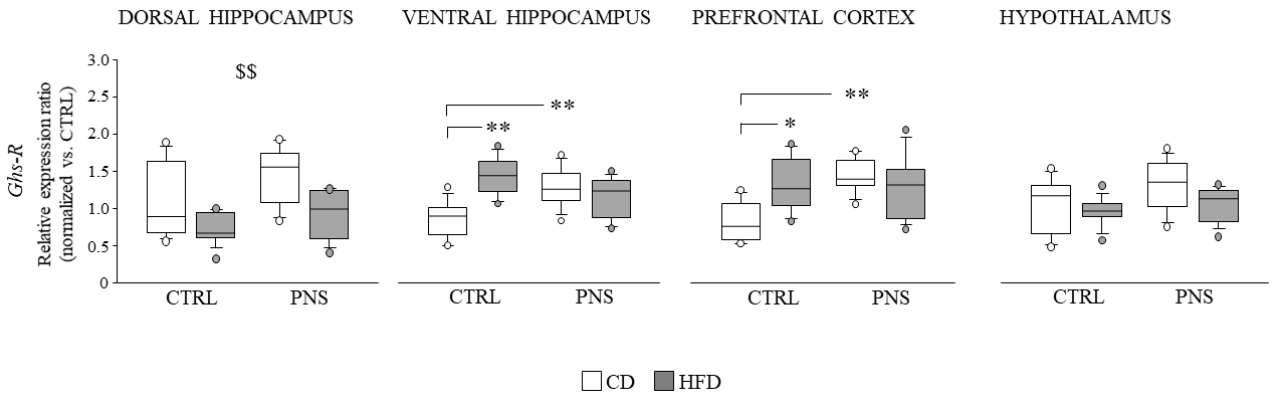


Figure 7. Levels of *Ghs-R* were found to be specifically decreased upon HFD in the dorsal hippocampus while an increase was observed in both the ventral hippocampus and the prefrontal cortex in CTRL-HFD and PNS-CD animals. No change was observed in hypothalamus. Main effect of HFD: main effect of diet, $$ p<0.01; post hoc comparisons for the interaction effects: *p<0.05: prefrontal cortex, CTRL-CD vs. CTRL-HFD; **p<0.01: ventral hippocampus, CTRL-CD vs. CTRL-HFD and PNS-CD, prefrontal cortex, CTRL-CD vs. PNS-CD. Number of subjects: 8-10 within each experimental group.

Discussion

In the present study we assessed the long-term effects of exposure to PNS (a first hit) on foetal brain programming with regard to neuronal plasticity and brain inflammation. Moreover, we used a metabolic challenge (HFD) at adulthood (second hit) to assess potential interaction effects with the PNS-induced phenotype. We have previously shown (Panetta et al., 2017) that although PNS resulted in a strong metabolic liability in male rats fed with HFD at adulthood it also dampened the negative effects driven by such a metabolic challenge on the emotional phenotype as assessed in the EPM. Here we extended such body of evidence by providing information on the possible mechanisms underlying this interaction. PNS-CD rats were characterised by reduced brain plasticity showing decreased expression levels of the neurotrophin Bdnf and of pro-inflammatory cytokines in a number of brain regions; moreover, HFD feeding, experienced at adulthood, induced an increase in pro-inflammatory cytokines in those animals that had experienced PNS. This suggests that PNS might impinge upon the same mechanisms underlying vulnerability to metabolic challenges at adulthood. Furthermore, HFD greatly affected the expression levels of the main effectors of HPA axis function (the glucocorticoid receptor - GR - gene Nr3c1 and of the GR's co-chaperone Fkbp5) an effect possibly underlying the metabolic vulnerability previously observed in male rats.

In our study, we found that PNS animals were characterised by differential expression of mRNA levels of Il-1β and Tnf-α in almost all the brain regions investigated except for the hypothalamus, an area that, in our experimental condition, appeared to be resilient to inflammatory changes driven by both pre- and postnatal stressors. While Tnf-α was tightly modulated upon pre- and postnatal stressors, changes in Il-1β were observed only when considering the interaction with the second hit (HFD), suggesting that the former cytokine (Tnf-α) might be considered as a reliable marker of stress adaptation in response to a prenatal challenge. As for Il-6, no effect was observed upon PNS in neither of the brain areas considered, though HFD overall decreased its expression levels in the dorsal hippocampus. TNF-α, IL-1β and IL-6 are the main activators of the HPA and are in turn modulated (inhibited) by GC hormones. As described by O'Connors and colleagues these cytokines hold differential sensitivity for adrenal steroids with TNF-α being the most sensitive to such inhibition in the range of physiological levels, IL-1β being second and IL-6 being the most resistant (O'Connor et al., 2000).

There is general consensus that elevated inflammation can exacerbate or even give rise to depressive symptoms or may be associated to other psychiatric conditions (Dantzer et al., 2018, 2008). However, cytokines are also constitutively released in the healthy brain by resident myeloid cells to keep proper synaptic plasticity. As an example, the modulation of both IL-1β and TNF-α plays an important role in the processes of LTP and synaptic scaling (a form of homeostatic plasticity) (Rizzo et al., 2018; Salim et al., 2012). Here we found that lower mRNA levels of Il-1β in the dorsal hippocampus of PNS rats were associated to increased time spent immobile in the EPM (as previously assessed in (Panetta et al., 2017)). Moreover, and in line with our previous work (Panetta et al., 2017), PNS rats were characterised by decreased levels of the neurotrophin Bdnf (in the dorsal hippocampus); this decrease was also associated to an increase in the time spent immobile in the EPM, suggesting that PNS might affect foetal brain programming by reducing neuronal plasticity. Interestingly, the

interaction between PNS and adult HFD (PNS-HFD group) resulted in increased levels of Il-1β and Tnf-α mRNA. Alboni and colleagues have recently provided evidence for increased rather than decreased levels of pro-inflammatory cytokines in the brain of stressed mice treated with the antidepressant fluoxetine. Such an effect was associated to increased BDNF levels and stronger LTP in the hippocampus (Alboni et al., 2017, 2016), suggesting that brain plasticity may also be related to the activation of basal metabolism that in turn is positively associated to the ability to properly mount and control inflammatory responses. To this regard, we cannot exclude that prolonged exposure to the HFD or to a stronger metabolic insult, such as a western pattern diet (rich in fats and sugar), should lead to an overall excessive brain inflammation (not observed in this study) with main consequences on emotional/cognitive behaviour. Worth to notice, we have previously shown that upon PNS, male rats were characterized by increased corticosterone levels under basal conditions, this effect was associated with a decrease in reactive oxygen species (ROS) as well as a with decreased NF-kB signalling in the hippocampus suggesting a lower set-point under basal conditions in PNS male rats (Anacker et al., 2013). Here we observed a similar blunted activation upon PNS with HFD triggering a response only in PNS subjects (increased Il-1β and Tnf-α in the prefrontal cortex and Tnf-α in the dorsal and ventral hippocampus). To our knowledge this is one of the few instances in which a reduction in inflammatory mediators is described as a result of PNS. Because cytokines have been shown to modulate hippocampal development and plasticity (Bourgognon and Cavanagh, 2020; Goshen et al., 2007) a decreased expression profile as a result of a "first hit" (PNS) might set the stage for an increased response to a "second hit" (metabolic challenge). Obesity is characterised by low-grade systemic and central inflammation and TNF-α and IL-1β appear to be key players in this condition and in the associated pathologies (Mighiu et al., 2012). Although the hypothalamus is a well-known area playing a main role in the crosstalk between brain and periphery in metabolic pathologies associated to obesity, other brain regions have been shown to be affected by high-fat diet- or obesity-induced inflammation such as the hippocampus, cortex, brainstem, or amygdala (Guillemot-Legris and Muccioli, 2017). There are data to suggest that the effects of a metabolic challenge may be different depending on the region of interest dealing with brain plasticity (hippocampus) or metabolic regulation and homeostasis (hypothalamus) (Rasgon and McEwen, 2016). Thus, our data also indicate that PNS might set the stage for a differential regulation of central inflammatory mediators in specific brain regions, an effect that can be unmasked only upon the occurrence of a second hit, such as the HFD at adult age.

Hyperactivity of the HPA axis and increased circulating GC have been implicated in the pathogenesis of mood and anxiety disorders suggesting an impairment in the ability of the HPA axis to self-regulate its function by shutting down the system (Binder, 2009; Holsboer, 2000; Pariante and Miller, 2001). Glucocorticoids have also an important function in metabolic regulations as they mobilize glucose to fuel the energy demands of the stress response and furthermore promote energy storage, feeding, and weight gain. Thus, it is possible to hypothesize that both psychological as well as metabolic stress might be able to affect HPA axis functionality (Balsevich et al., 2019). A growing body of evidence suggests that levels of Fkbp5 mRNA GR co-chaperone have been associated with higher levels of circulating cortisol and reduced negative feedback inhibition of the stress response associated with a depressive phenotype (Binder, 2009). We have previously shown that,

following PNS, male rats were characterised by elevated GC levels when compared to controls (Anacker et al., 2013); in this study we show elevated levels of Fkbp5 upon PNS in the prefrontal cortex and upon HFD administration in the dorsal hippocampus, suggesting that both psychological and metabolic stressors affect this mediator. When we looked at the hypothalamus, Fkbp5 expression levels were reduced in the HFD group, possibly accounting for a compensatory mechanism. Interestingly, we also showed that levels of Fkbp5 in the dorsal hippocampus were positively related to the time spent in the open arm of the EPM and that this positive correlation is lost as a result both of PNS as well as of HFD. This result might suggest that under physiological conditions Fkbp5 is required to engage animals in the proper exploration of novel environments through a fine modulation of the GR receptors and that this balance can be greatly affected by both early life stressors as well as by metabolic challenges.

The prefrontal cortex is one of the most important cortical areas among the network of regions being involved in the pathogenesis of depression that plays a main role in the ability to process positive and negative emotions (Harms et al., 2017; Kaya and McCabe, 2019). Thus, the observed increase in levels of Fkbp5 in this brain area should not be surprising and may underlie a condition of potential increased vulnerability to stress.

Changes in Nr3c1 and Fkbp5 in the hippocampus, a result of HFD, indicate a cross-regulation between a metabolic challenge and Nr3c1/Fkbp5 balance, strengthening the notion that indeed these molecules are involved in "sensing" environmental challenges, be these of psychological or metabolic nature. We also found that HFD increased the levels of Nr3c1 in the prefrontal cortex; by contrast, changes in Fkbp5 in this brain area were observed only as a result of PNS. These results possibly suggest a fine and complex multiple level regulation of the GR-negative feedback in this specific brain region highlighting the role of this brain area in mood disorders such as depression. Transcriptional control of GR relies in part upon the DNA methylation status at multiple alternative initiation sites that are tissue specific. In fact, Nr3c1 gene is characterised by an unusually complex promoter structure (Turner et al., 2010). This might possibly explain the unexpected decrease in the dorsal hippocampus observed as a result of HFD or the lack of effects observed in the hypothalamus (see also concluding remarks).

When we investigated levels of Ghs-R we found a specific decrease upon HFD in the dorsal hippocampus while an increase was observed in both the ventral hippocampus and the prefrontal cortex in CTRL-HFD and PNS-CD animals. It has become increasingly apparent that the dorsal and the ventral portions of the hippocampus are preferentially involved in different physiological functions the first playing a role in the regulation of cognitive processes (spatial memory) the second in the modulation of emotions - such that they have been defined the cold and the hot hippocampus respectively - (Fanselow and Dong, 2010). Chen and co-workers provided evidence that infusion of ghrelin in the dorsal hippocampus enhanced synaptic plasticity and spatial memory (Chen et al., 2020). By contrast, Kanoski and colleagues showed that ghrelin delivery to the ventral but not the dorsal hippocampus increased the ability of environmental food-related cues to stimulate meal initiation and to enhance motivation to obtain it by increasing food intake frequency (Kanoski et al., 2013) confirming that this hippocampal sub-region might be of importance in feeding/appetitive rewarding behaviours. To this regard it is interesting to note that we have previously found an increase in caloric intake

upon HFD (Panetta et al., 2017). Recently Guo and colleagues have shown that GHS-R knock-out mice were characterised by improved abilities to cope to social stressors, decreased emotionality and depressive-like behaviours (Guo et al., 2019). Thus, we can hypothesize that both the pre-natal and the postnatal stressors might act by increasing the ghrelin signal in two brain regions of main importance for the ability to process emotions (the ventral hippocampus and the prefrontal cortex) setting the stage for increased vulnerability to mood disorders.

Concluding remarks and future directions

We have here provided evidence that PNS can affect brain development (first hit) setting the stage for increased vulnerability to further insults during life (HFD, second hit). In particular, we observed important changes in the expression levels of GR, their chaperones and *Ghs-R* in response to both PNS and HFD, confirming that the there is an important overlap between pathways and effectors involved in the regulation of emotions and in food intake and metabolic balance. Further investigations are warranted to assess more in detail the role played by these molecules in setting the stage for co-morbidity between metabolic and psychiatric disorders. In addition, the role of additional co-regulators, such as endocannabinoids should be tested (Balsevich et al., 2019).

There are some limitations of the current study that should be mentioned. First, we examined here the expression level of selected genes of interest but did not complement this with the levels of the corresponding proteins, the functional end products of the genes. Secondly, we have used real-time PCR in combination with biopsy punching as a quantitative approach instead of in situ hybridization. Due to this approach we had to make a selection of brain areas and lose anatomical resolution. Thirdly, genomic and epigenomic regulation of glucocorticoids in the brain may affect mood and metabolism but is based upon the coordinated activity of many chaperone proteins, in addition to proper circadian and ultradian fluctuation of hormone release (Gray et al., 2017). Future studies should assess the interrelationship between psychological and metabolic stressors. In addition, given the observed changes of *Ghs-R* and *Nr3c1* in the dorsal hippocampus following a HFD, further research should be devoted to explore more in depth the effects of HFD and of PNS, and their interaction, on brain plasticity.

Conflict of Interest Statement

The authors have nothing to disclose.

Data sharing statement

The raw data supporting the conclusions of this article will be made available by the authors upon request without undue reservation.

Author's contribution

AB and CM analysed, interpreted data and wrote the manuscript; MM and AC collected all gene expression data; FC and MR designed the experiment and provided data interpretation.

Funding

This work was supported by ERANET- NEURON JTC 2018 (Mental Disorders) Project "EMBED", JPI HDHL-INTIMIC-085-GUTMOM project within the ERA-NET Co-fund HDHL-INTIMIC (INtesTInal MICrobiomics) programme and HDHL-INTIMIC Knowledge Platform on Food, Diet, Intestinal Microbiomics and Human Health to F.C., as well as by grants from the Italian Ministry of University and Research to M.A.R. (PRIN – grants number 2017AY8BP4 and PON "Ricerca e Innovazione" PerMedNet - project ARS01_01226). A.C. and F.C. received funding from the Italian Ministry of Health (Ricerca Corrente).

Acknowledgements

We thank Enrico Cardarelli, Andrea Sagrestani and Andrea Giovannelli for her technical skills with animal handling procedures; Marcello Lepre, Maria Eleonora Pieroni for their help in retrieving and selecting bibliographic entries; Paola Rossolino for management of external funding.

References

Alboni, S., Poggini, S., Garofalo, S., Milior, G., El Hajj, H., Lecours, C., Girard, I., Gagnon, S., Boisjoly-Villeneuve, S., Brunello, N., Wolfer, D.P., Limatola, C., Tremblay, M.È., Maggi, L., Branchi, I., 2016. Fluoxetine treatment affects the inflammatory response and microglial function according to the quality of the living environment. Brain. Behav. Immun. 58, 261–271. https://doi.org/10.1016/j.bbi.2016.07.155

Alboni, S., Van DIjk, R.M., Poggini, S., Milior, G., Perrotta, M., Drenth, T., Brunello, N., Wolfer, D.P., Limatola, C., Amrein, I., Cirulli, F., Maggi, L., Branchi, I., 2017. Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment. Mol. Psychiatry 22, 552–561. https://doi.org/10.1038/mp.2015.142

Anacker, C., Cattaneo, A., Luoni, A., Musaelyan, K., Zunszain, P.A., Milanesi, E., Rybka, J., Berry, A., Cirulli, F., Thuret, S., Price, J., Riva, M.A., Gennarelli, M., Pariante, C.M., 2013. Glucocorticoid-related molecular signaling pathways regulating hippocampal neurogenesis. Neuropsychopharmacology 38, 872–883. https://doi.org/10.1038/npp.2012.253

Andersson, N.W., Li, Q., Mills, C.W., Ly, J., Nomura, Y., Chen, J., 2016. Influence of prenatal maternal stress on umbilical cord blood cytokine levels. Arch. Womens. Ment. Health 19, 761–767. https://doi.org/10.1007/s00737-016-0607-7

Balsevich, G., Abizaid, A., Chen, A., Karatsoreos, I.N., Schmidt, M. V., 2019. Stress and glucocorticoid modulation of feeding and metabolism. Neurobiol. Stress. https://doi.org/10.1016/j.ynstr.2019.100171

Barker, D.J.P., 1995. Intrauterine programming of adult disease. Mol. Med. Today. https://doi.org/10.1016/S1357-4310(95)90793-9

Barrientos, R.M., Sprunger, D.B., Campeau, S., Watkins, L.R., Rudy, J.W., Maier, S.F., 2004. BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1β administration. J. Neuroimmunol. 155, 119–126. https://doi.org/10.1016/j.jneuroim.2004.06.009

Berry, A., Panetta, P., Luoni, A., Bellisario, V., Capoccia, S., Andrea Riva, M., Cirulli, F., 2015. Decreased Bdnf expression and reduced social behavior in periadolescent rats following prenatal stress. Dev. Psychobiol. 57, 365–373. https://doi.org/10.1002/dev.21297

Bilbo, S.D., Barrientos, R.M., Eads, A.S., Northcutt, A., Watkins, L.R., Rudy, J.W., Maier, S.F., 2008. Early-life infection leads to altered BDNF and IL-1β mRNA expression in rat hippocampus following learning in adulthood. Brain. Behav. Immun. 22, 451–455. https://doi.org/10.1016/j.bbi.2007.10.003

Binder, E.B., 2009. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology. https://doi.org/10.1016/j.psyneuen.2009.05.021

Blum, I.D., Patterson, Z., Khazall, R., Lamont, E.W., Sleeman, M.W., Horvath, T.L., Abizaid, A., 2009. Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice. Neuroscience 164, 351–359. https://doi.org/10.1016/j.neuroscience.2009.08.009

Boersma, G.J., Lee, R.S., Cordner, Z.A., Ewald, E.R., Purcell, R.H., Moghadam, A.A., Tamashiro, K.L., 2013. Prenatal stress decreases Bdnf expression and increases methylation of Bdnf exon IV in rats. Epigenetics 9, 437–447. https://doi.org/10.4161/epi.27558

Bourgognon, J.-M., Cavanagh, J., 2020. The role of cytokines in modulating learning and memory and brain plasticity. Brain Neurosci. Adv. 4, 239821282097980. https://doi.org/10.1177/2398212820979802

Bronson, S.L., Bale, T.L., 2014. Prenatal stress-induced increases in placental inflammation and offspring hyperactivity are male-specific and ameliorated by maternal antiinflammatory treatment. Endocrinology 155, 2635–2646. https://doi.org/10.1210/en.2014-1040

Cattane, N., Räikkönen, K., Anniverno, R., Mencacci, C., Riva, M.A., Pariante, C.M., Cattaneo, A., 2020. Depression, obesity and their comorbidity during pregnancy: effects on the offspring's mental and physical health. Mol. Psychiatry. https://doi.org/10.1038/s41380-020-0813-6

Cattaneo, A., Macchi, F., Plazzotta, G., Veronica, B., Bocchio-Chiavetto, L., Riva, M.A., Pariante, C.M., 2015. Inflammation and neuronal plasticity: A link between childhood trauma and depression pathogenesis. Front. Cell. Neurosci. https://doi.org/10.3389/fncel.2015.00040

Chen, H.J., Antonson, A.M., Rajasekera, T.A., Patterson, J.M., Bailey, M.T., Gur, T.L., 2020. Prenatal stress causes intrauterine inflammation and serotonergic dysfunction, and long-term behavioral deficits through microbe- and CCL2-dependent mechanisms. Transl. Psychiatry 10, 1–12. https://doi.org/10.1038/s41398-020-00876-5

Cirulli, F., 2017. Interactions between early life stress and metabolic stress in programming of mental and metabolic health. Curr. Opin. Behav. Sci. https://doi.org/10.1016/j.cobeha.2016.12.009

Cirulli, F., Alleva, E., 2009. The NGF saga: From animal models of psychosocial stress to stress-related psychopathology. Front. Neuroendocrinol. https://doi.org/10.1016/j.yfrne.2009.05.002

Cirulli, F., Musillo, C., Berry, A., 2020. Maternal Obesity as a Risk Factor for Brain Development and Mental Health in the Offspring. Neuroscience. https://doi.org/10.1016/j.neuroscience.2020.01.023

Danese, A., Moffitt, T.E., Harrington, H.L., Milne, B.J., Polanczyk, G., Pariante, C.M., Poulton, R., Caspi, A., 2009. Adverse childhood experiences and adult risk factors for age-related disease: Depression, inflammation, and clustering of metabolic risk markers. Arch. Pediatr. Adolesc. Med. 163, 1135–1143. https://doi.org/10.1001/archpediatrics.2009.214

Danese, A., Moffitt, T.E., Pariante, C.M., Ambler, A., Poulton, R., Caspi, A., 2008. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. Arch. Gen. Psychiatry 65, 409–416. https://doi.org/10.1001/archpsyc.65.4.409

Dantzer, R., Cohen, S., Russo, S.J., Dinan, T.G., 2018. Resilience and immunity. Brain. Behav. Immun. 74, 28–42. https://doi.org/10.1016/j.bbi.2018.08.010

Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: When the immune system subjugates the brain. Nat. Rev. Neurosci. https://doi.org/10.1038/nrn2297

Daskalakis, N.P., Bagot, R.C., Parker, K.J., Vinkers, C.H., de Kloet, E.R., 2013. The three-hit concept of vulnerability and resilience: Toward understanding adaptation to early-life adversity outcome. Psychoneuroendocrinology 38, 1858–1873. https://doi.org/10.1016/j.psyneuen.2013.06.008

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., Lanctôt, K.L., 2010. A Meta-Analysis of Cytokines in Major Depression. Biol. Psychiatry 67, 446–457. https://doi.org/10.1016/j.biopsych.2009.09.033

Fanselow, M.S., Dong, H.W., 2010. Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? Neuron. https://doi.org/10.1016/j.neuron.2009.11.031

Fernandes, C., File, S.E., 1996. The influence of open arm ledges and maze experience in the elevated plus-maze, in: Pharmacology Biochemistry and Behavior. Elsevier Inc., pp. 31–40. https://doi.org/10.1016/0091-3057(95)02171-X

Goshen, I., Kreisel, T., Ounallah-Saad, H., Renbaum, P., Zalzstein, Y., Ben-Hur, T., Levy-Lahad, E., Yirmiya, R., 2007. A dual role for interleukin-1 in hippocampal-dependent memory processes. Psychoneuroendocrinology 32, 1106–1115. https://doi.org/10.1016/j.psyneuen.2007.09.004

Gray, J.D., Kogan, J.F., Marrocco, J., McEwen, B.S., 2017. Genomic and epigenomic mechanisms of glucocorticoids in the brain. Nat. Rev. Endocrinol. https://doi.org/10.1038/nrendo.2017.97

Guillemot-Legris, O., Muccioli, G.G., 2017. Obesity-Induced Neuroinflammation: Beyond the Hypothalamus. Trends Neurosci. https://doi.org/10.1016/j.tins.2017.02.005

Guo, L., Niu, M., Yang, J., Li, L., Liu, S., Sun, Y., Zhou, Z., Zhou, Y., 2019. GHS-R1a Deficiency Alleviates Depression-Related Behaviors After Chronic Social Defeat Stress. Front. Neurosci. 13. https://doi.org/10.3389/fnins.2019.00364

Gur, T.L., Shay, L., Palkar, A.V., Fisher, S., Varaljay, V.A., Dowd, S., Bailey, M.T., 2017. Prenatal stress affects placental cytokines and neurotrophins, commensal microbes, and anxiety-like behavior in adult female offspring. Brain. Behav. Immun. 64, 50–58. https://doi.org/10.1016/j.bbi.2016.12.021

Handa, R.J., Burgess, L.H., Kerr, J.E., O'keefe, J.A., 1994. Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. Horm. Behav. 28, 464–476. https://doi.org/10.1006/hbeh.1994.1044

Hantsoo, L., Kornfield, S., Anguera, M.C., Epperson, C.N., 2019. Inflammation: A Proposed Intermediary Between Maternal Stress and Offspring Neuropsychiatric Risk. Biol. Psychiatry. https://doi.org/10.1016/j.biopsych.2018.08.018

Harms, M.B., Birn, R., Provencal, N., Wiechmann, T., Binder, E.B., Giakas, S.W., Roeber, B.J., Pollak, S.D., 2017. Early life stress, FK506 binding protein 5 gene (FKBP5) methylation, and inhibition-related prefrontal function: A prospective longitudinal study. Dev. Psychopathol. 29, 1895–1903. https://doi.org/10.1017/S095457941700147X

Health Organization Regional Office for Europe, W., 2015. The European Mental Health Action Plan 2013-2020.

Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23, 477–501. https://doi.org/10.1016/S0893-133X(00)00159-7

Kanoski, S.E., Fortin, S.M., Ricks, K.M., Grill, H.J., 2013. Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling. Biol. Psychiatry 73, 915–923. https://doi.org/10.1016/j.biopsych.2012.07.002

Kaya, S., McCabe, C., 2019. What role does the prefrontal cortex play in the processing of negative and positive stimuli in adolescent depression? Brain Sci. 9. https://doi.org/10.3390/brainsci9050104

Krontira, A.C., Cruceanu, C., Binder, E.B., 2020. Glucocorticoids as Mediators of Adverse Outcomes of Prenatal Stress. Trends Neurosci. https://doi.org/10.1016/j.tins.2020.03.008

Liu, Y., Ho, R.C.M., Mak, A., 2012. Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: A meta-analysis and meta-regression. J. Affect. Disord. https://doi.org/10.1016/j.jad.2011.08.003

Lutter, M., Sakata, I., Osborne-Lawrence, S., Rovinsky, S.A., Anderson, J.G., Jung, S., Birnbaum, S., Yanagisawa, M., Elmquist, J.K., Nestler, E.J., Zigman, J.M., 2008. The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. Nat. Neurosci. 11, 752–753. https://doi.org/10.1038/nn.2139

Maccari, S., Piazza, P.V., Kabbaj, M., Barbazanges, A., Simon, H., Le Moal, M., 1995. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. J. Neurosci. 15, 110–116. https://doi.org/10.1523/jneurosci.15-01-00110.1995

Marosi, K., Mattson, M.P., 2014. BDNF mediates adaptive brain and body responses to energetic challenges. Trends Endocrinol. Metab. https://doi.org/10.1016/j.tem.2013.10.006

McCarthy, M.M., 2019. Stress during pregnancy: Fetal males pay the price. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.1916909116

McEwen, B.S., 2000. Effects of adverse experiences for brain structure and function, in: Biological Psychiatry. Elsevier, pp. 721–731. https://doi.org/10.1016/S0006-3223(00)00964-1

Mighiu, P.I., Filippi, B.M., Lam, T.K.T., 2012. Linking inflammation to the brain-liver axis. Diabetes. https://doi.org/10.2337/db12-0330

Milaneschi, Y., Simmons, W.K., van Rossum, E.F.C., Penninx, B.W., 2019. Depression and obesity: evidence of shared biological mechanisms. Mol. Psychiatry. https://doi.org/10.1038/s41380-018-0017-5

Mueller, B.R., Bale, T.L., 2008. Sex-specific programming of offspring emotionality after stress early in pregnancy. J. Neurosci. 28, 9055–9065. https://doi.org/10.1523/JNEUROSCI.1424-08.2008

Nederhof, E., Schmidt, M. V., 2012. Mismatch or cumulative stress: Toward an integrated hypothesis of programming effects. Physiol. Behav. https://doi.org/10.1016/j.physbeh.2011.12.008

O'Connor, T.M., O'Halloran, D.J., Shanahan, F., 2000. The stress response and the hypothalamic-pituitary-adrenal axis: From molecule to melancholia. QJM - Mon. J. Assoc. Physicians. https://doi.org/10.1093/qjmed/93.6.323

Panetta, P., Berry, A., Bellisario, V., Capoccia, S., Raggi, C., Luoni, A., Longo, L., Riva, M.A., Cirulli, F., 2017. Long-term sex-dependent vulnerability to metabolic challenges in prenatally stressed rats. Front. Behav. Neurosci. 11. https://doi.org/10.3389/fnbeh.2017.00113

Pariante, C.M., Miller, A.H., 2001. Glucocorticoid receptors in major depression: Relevance to pathophysiology and treatment. Biol. Psychiatry. https://doi.org/10.1016/S0006-3223(00)01088-X

Patterson, Z.R., Ducharme, R., Anisman, H., Abizaid, A., 2010. Altered metabolic and neurochemical responses to chronic unpredictable stressors in ghrelin receptor-deficient mice. Eur. J. Neurosci. 32, 632–639. https://doi.org/10.1111/j.1460-9568.2010.07310.x

Rasgon, N.L., McEwen, B.S., 2016. Insulin resistance - A missing link no more. Mol. Psychiatry. https://doi.org/10.1038/mp.2016.162

Rizzo, F.R., Musella, A., De Vito, F., Fresegna, D., Bullitta, S., Vanni, V., Guadalupi, L., Stampanoni Bassi, M., Buttari, F., Mandolesi, G., Centonze, D., Gentile, A., 2018. Tumor Necrosis Factor and Interleukin-1β Modulate Synaptic Plasticity during Neuroinflammation. Neural Plast. https://doi.org/10.1155/2018/8430123

Salim, S., Chugh, G., Asghar, M., 2012. Inflammation in anxiety, in: Advances in Protein Chemistry and Structural Biology. Academic Press Inc., pp. 1–25. https://doi.org/10.1016/B978-0-12-398314-5.00001-5

Slopen, N., Koenen, K.C., Kubzansky, L.D., 2014. Cumulative adversity in childhood and emergent risk factors for long-term health. J. Pediatr. 164, 631-638.e2. https://doi.org/10.1016/j.jpeds.2013.11.003

Ślusarczyk, J., Trojan, E., Głombik, K., Budziszewska, B., Kubera, M., Lasoń, W., Popiołek-Barczyk, K., Mika, J., Wędzony, K., Basta-Kaim, A., 2015. Prenatal stress is a vulnerability factor for altered morphology and biological activity of microglia cells. Front. Cell. Neurosci. 9. https://doi.org/10.3389/fncel.2015.00082

Turner, J.D., Alt, S.R., Cao, L., Vernocchi, S., Trifonova, S., Battello, N., Muller, C.P., 2010. Transcriptional control of the glucocorticoid receptor: CpG islands, epigenetics and more. Biochem. Pharmacol. 80, 1860–8. https://doi.org/10.1016/j.bcp.2010.06.037

Valkanova, V., Ebmeier, K.P., Allan, C.L., 2013. CRP, IL-6 and depression: A systematic review and meta-analysis of longitudinal studies. J. Affect. Disord. https://doi.org/10.1016/j.jad.2013.06.004

Verhagen, L.A.W., Egecioglu, E., Luijendijk, M.C.M., Hillebrand, J.J.G., Adan, R.A.H., Dickson, S.L., 2011. Acute and chronic suppression of the central ghrelin signaling system reveals a role in food anticipatory activity. Eur. Neuropsychopharmacol. 21, 384–392. https://doi.org/10.1016/j.euroneuro.2010.06.005

Weinstock, M., 2007. Gender differences in the effects of prenatal stress on brain development and behaviour. Neurochem. Res. https://doi.org/10.1007/s11064-007-9339-4

Zarouna, S., 2015. Mood disorders: A potential link between ghrelin and leptin on human body? World J. Exp. Med. 5, 103. https://doi.org/10.5493/wjem.v5.i2.103

Inflammatory signatures of maternal obesity as risk factors for neurodevelopmental disorders: role of maternal microbiota and nutritional intervention strategies

Francesca Cirulli[1], Roberta De Simone[2], Chiara Musillo[1,3], Maria Antonietta Ajmone-Cat[2], Alessandra Berry[1]

[1]Center for Behavioral Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy. [2]National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. [3]PhD Program in Behavioral Neuroscience, Department of Psychology, Sapienza University of Rome, Rome, Italy.

Nutrients (2022) 15:3150. doi: 10.3390/nu14153150

Abstract

Obesity is a main risk factor for the onset and the precipitation of many non-communicable diseases. This condition, which is associated with low-grade chronic systemic inflammation, is of main concern during pregnancy leading to very serious consequences for the new generations. In addition to the prominent role played by the adipose tissue, dysbiosis of the maternal gut may also sustain the obesity-related inflammatory milieu contributing to create an overall suboptimal intrauterine environment. Such a condition here generically defined as "inflamed womb" may hold long-term detrimental effects on fetal brain development, increasing the vulnerability to mental disorders. In this review, we will examine the hypothesis that maternal obesity-related gut dysbiosis and the associated inflammation might specifically target fetal brain microglia, the resident brain immune macrophages, altering neurodevelopmental trajectories in a sex-dependent fashion. We will also review some of the most promising nutritional strategies capable to prevent or counteract the effects of maternal obesity through the modulation of inflammation and oxidative stress or by targeting the maternal microbiota.

Keywords

gut microbiota; high-fat diet; inflammation; maternal obesity; microglia; nutritional intervention strategies; oxidative stress.

1 Introduction

The perinatal environment plays a pivotal role for the developing mammal and, as postulated by the Developmental Origin of Health and Disease (DOHaD) theory, negative experiences, such as a suboptimal intrauterine environment, may set the stage for later life vulnerability to diseases [1]. To this regard, malnourishment during pregnancy, either deriving from the lack or from the excess of nutrients, can be considered as a maternal metabolic stress that can negatively affect fetal programming increasing the risk for metabolic disorders and cardiovascular diseases in the offspring [2,3,4,5]. Interestingly, while the pioneering studies in this field of research, for historical reasons, were framed in the context of hunger and starvation nowadays we have to face the sequelae of malnourishment due to the global spread of obesity. In fact, globalization and urbanization have gradually led to the so-called nutrition transition namely a reduction of physical activity associated with the increase in the consumption of "junk food" (i.e., low-cost food easily accessible, ultra-processed, hypercaloric and poor in nutrient) [6,7]. Worth to mention, a number of long-term longitudinal studies have provided evidence for an association between maternal obesity and an increased risk to develop cognitive disabilities, autism spectrum disorders, attention deficit hyperactivity disorder, anxiety and depression in the offspring [6,8,9,10]. In this context, it is important to highlight that obesity and the consumption of "junk food" most often co-occur in humans making difficult to discriminate the effects of the nutritional aspects per se from those related to maternal metabolic profile in the offspring. Thus, it is possible to hypothesize that overall derangements in the prenatal metabolic environment hold similar effects as those observed as a result of maternal psychological stressors, profoundly affecting fetal development and future life liability to mental health [6,8,11,12].

Despite the increasing body of evidence linking maternal obesity or stress to offspring mental health problems, the mechanisms underpinning these effects remain poorly understood.

Inflammation has recently obtained widespread attention as a likely mediating mechanism for its involvement in the pathophysiology of both obesity and mental disorders [13] and for the emerging role of inflammatory mediators as regulators of brain homeostasis and plasticity [14,15,16]. As a general mechanism, the expansion of adipose tissue observed in obese subjects is associated with increased size and number of adipocytes leading to adipocyte hypoxia, infiltration of leukocytes, inflammation and insulin resistance [17,18,19]. Preclinical and clinical studies clearly indicate that these conditions, which set a chronic state of low-grade systemic inflammation, are magnified during gestation [20,21,22], a time when tightly balanced immune changes naturally occur in non-obese pregnant women [23]. This chronic low-grade systemic inflammation is also maintained by the adipose tissue that, acting as a metabolic and endocrine organ, greatly affects the physiology of pregnancy, as well as fetal development [24] providing an overall suboptimal intrauterine environment here generically defined as "inflamed womb" [17,25,26]. As an example, in physiological pregnancies, the placenta is fairly protected from peripheral inflammation, with macrophages of both the decidua and placental typically observed in an anti-inflammatory condition to allow the immune tolerance of the growing fetus. However, in the setting of maternal obesity, histopathological evidence of placental inflammation is described suggesting

that such immune tolerance may be disrupted [20,27,28]. Moreover, Melekoglu and colleagues have provided evidence for increased levels of inflammatory cytokines in the amniotic fluid of women with a body mass index (BMI) ≥35 [29]. Notwithstanding the prominent role played by the adipose tissue, other players may also greatly contribute to create an inflammatory state: one above all is the maternal gut microbiota. This population of trillions of symbiotic bacteria participates in the metabolic, biochemical, and immunological balance of the host organism, hence playing a central role for human health [30]. The composition and function of this bacterial community is tightly modulated by the environment. To this regard, treatment with antibiotic, consumption of "junk food" on a regular base, obesity, and chronic stress are all factors that may trigger a condition of dysbiosis within the gut bacterial community, i.e., a dramatic loss of the homeostatic balance between the beneficial and potentially pathogenic bacteria that has been associated with a great variety of pathological conditions (metabolic, autoimmune, psychiatric, neurodegenerative) related to systemic inflammation (see [31] and references therein). A growing number of clinical evidence suggests that the quality and patterns of diets by affecting gut microbiota may modulate mood and behavior [32,33,34]. Changes in the inflammatory profile characterize healthy pregnancies—often associated to changes in the redox balance—and are aimed at promoting embryo implantation and parturition [23]; such changes are accompanied by time-specific adaptations of the gut microbiota composition. This tightly regulated process when deranged by metabolic stressors results in a pro-inflammatory condition [8,35,36,37].

However, how do we go from an "inflamed womb", such as that characterizing maternal obesity, to long-term changes in brain function in the offspring? A number of preclinical studies is starting to answer this question, showing consistent findings indicating that maternal obesity during pregnancy might specifically affect brain development by targeting microglia, the resident brain immune macrophages. These cells are of unique origin and function compared to their peripheral counterpart and, besides immune defense, they play a pivotal role in brain development, homeostasis and plasticity; their homeostatic functions as well as their response to local or systemic challenges are sex-dimorphic and age-specific [38,39,40,41,42,43,44,45]. Alterations in microglial function in the offspring of obese dams appear to be long-lasting and associated to inflammation and changes in the architecture of specific brain areas overall resulting in decreased cognitive abilities [41]. Interestingly, a direct link between maternal microbiota and the development of embryonal microglia has been recently reported providing evidence for microglia derived from germ free (GF) mice to show an altered expression of those genes involved in the immune response and to be characterized by an overall immature or hypoactive immune state [46]. Although microglia might be only one of the many players, in this review we have taken into account also the hypothesis that maternal obesity-related dysbiosis and the associated inflammation might target this specific cell type altering neurodevelopmental trajectories in a sex dimorphic fashion. Moreover, we have reviewed some of the most promising nutritional strategies to prevent or counteract the effects of maternal obesity both in terms of their efficacy in modulating oxidative stress (OS) and inflammation, and their ability to target the maternal microbiota. Relevant literature to the main topic of this review has been retrieved by searching on PubMed.

2. Microglia as a main target of maternal obesity-derived inflammation

The chronic low-grade systemic inflammation characterizing maternal obesity is similar to that observed as a result of an early-life bacterial infection and suggests that common molecular and cellular mechanisms are activated by these different prenatal conditions [13,47,48]. The mechanisms mediating the propagation of maternal inflammation to the developing fetal brain are poorly elucidated, although the microenvironment generated by the placenta at the maternal/fetal interface is emerging as central in this phenomenon [13,49]. Altered ratios of innate and adaptive immune cell subsets and increased expression of placental pro-inflammatory and oxidative factors interfere with fetal development through several mechanisms, including maternal delivery of metabolites, nutrients, hormones, antibodies, and also cells, to the fetus [50]. Fetal brain microglia originate from a common erythromyeloid precursor in the extra-embryonic yolk sac, in which they are exposed to mother-derived signals since early developmental stages. Microglial progenitors colonize the developing brain as early as embryonic day 9 in rodents [51] and at the 4th–5th month and beyond in humans [52] where they persist and self-sustain throughout adulthood by local self-proliferation under steady state conditions. The estimated median lifespan of microglia is about 15 months in the mouse cortex and around 4 up to 20-years in humans. Their longevity and their remarkable capacity of long-lasting immune memory responses holds main implications for the quality and extent of the brain's responses to new threats following a previous experience [53]. Microglia are key players in brain development, maturation, function and plasticity in healthy and disease conditions. By acquiring distinct phenotypes, with unique gene expression profiles, during the different phases of development they shape neuronal circuits and neuronal apoptosis to support and regulate neurogenesis and synapse plasticity [38,39]. Being the primary sensors of environmental changes in the central nervous system (CNS) they are key actors in mediating the effects of maternal obesity.

In the last decade, several preclinical studies of maternal obesity have shown evidence of how HFD-induced neuroinflammation might contribute to impaired social behavior in offspring of overfed dams. One of the first evidence demonstrating that the peripheral inflammation associated with maternal obesity is capable of programming microglial reactivity and inflammation within the offspring brain comes from the study of Bilbo and Tsang [40]. These authors found increased levels of the microglial activation marker cluster of differentiation molecule 11b (CD11b) within the hippocampus of newborn pups from HFD dams, as well as higher density of Ionized calcium-binding adaptor molecule 1 (Iba1) positive microglia in the hippocampus (CA1, CA3 and dentate gyrus) of male and female rat offspring at adulthood. They also found that a peripheral immune challenge at weaning and adulthood, despite the fact that the offspring themselves were maintained on a low-fat diet after weaning, induced a significant increase of the pro-inflammatory cytokine Inteleukin-1 beta (IL-1 beta) in the hippocampus of HFD offspring compared to controls. These alterations were accompanied by marked changes in anxiety-like behaviors and spatial learning. This evidence strongly supports that maternal obesity could prime offspring microglia towards a pro-inflammatory phenotype that is retained postnatally; although the underlying mechanisms have not been elucidated in this study, they are very

likely to involve mechanisms of epigenetic reprogramming, as described in other phenomena of microglial immune memory [53].

The alterations in offspring's behavior and CNS inflammation induced by maternal HFD have been shown to be sex, age and brain region specific. Exposure to a maternal HFD during gestation and or lactation in mice results in increased anxiety behavior in females and hyperactivity in male offspring while alterations in sociability are observed only in female offspring [54]. Interestingly, these behavioral changes are accompanied by increased mRNA expression levels of the pro-inflammatory genes IL-1 beta, Tumor Necrosis Factor-alpha (TNF-alpha) and Iba1, and by increased Iba1 staining intensity at 6 weeks of age in the amygdala—a region associated with anxiety and social behaviors—only in female offspring from HFD-fed dams. Switching maternal diet from HFD to control diet during lactation results in a significant decrease in Iba1 transcripts in the brain suggesting that dietary intervention may be able to alleviate the impact of maternal HFD on offspring brain inflammation [54]. HFD was reported to induce distinct behavioral and inflammatory response profiles between adolescent and adult animals with also sex- and region- specific changes [55,56]. Adolescent animals perinatally exposed to maternal HFD, showed a decreased anxiety behavior associated with increased levels of glucocorticoid receptor transcripts in the hippocampus, but not in the amygdala, and by a dysregulation of inflammatory gene expression in both hippocampus and amygdala. Male and female adult rats showed instead increased anxiety behavior, concomitantly to a selective alteration in the expression of corticosteroid receptors in the amygdala in both sexes, and by higher levels of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and Interleukin-6 (IL-6) transcripts, two important inflammatory genes downstream to the glucocorticoid signaling, only in females.Winther and colleagues [57] found that the anxiogenic phenotype of the adult offspring born to HFD dams was correlated to an increase of the hippocampal mRNA levels of the pro-inflammatory cytokine TNF-alpha and of monocyte chemoattractant protein-1 (mcp-1), an important chemokine that regulates microglial recruitment and activation. In addition, maternal HFD increased the offspring's levels of hippocampal corticosteroid releasing hormone receptor 2 (crhr2) and kynurenine mono oxygenase (kmo), whereas kynurenine aminotransferase I (kat1) mRNA levels were decreased. These observations strongly support that neuroinflammatory and stress-axis pathways may contribute to anxiogenic effects of maternal HFD [57].

More recently, Wijenayake and co-workers investigated the susceptibility of the adult offspring exposed to maternal HFD during perinatal life to an immune (lipopolysaccharide, LPS) and a stress (corticosterone, CORT) challenge or to their combination [58]. They observed that in response to CORT alone, male HFD offspring were more affected than females, showing increased levels of anti-inflammatory transcripts in the hippocampus and amygdala, whereas in response to LPS alone, female HFD offspring showed, in the same brain regions, increased levels of pro-inflammatory transcripts. Furthermore, in response to a CORT and LPS combination male HFD offspring showed greater pro-inflammatory transcriptional response in the cerebral cortex while female HFD offspring exhibited increased anti-inflammatory gene expression in the amygdala and cortex [58].

In another recent study, Bordeleau and colleagues found sex-dimorphic ultrastructural changes of microglia in maternal HFD-exposed offspring in the dorsal CA1 region of the hippocampus. They reported the presence of morphological microglial alterations and a reduction in microglia-associated pockets of extracellular space that may indicate diminished capacities to remove extracellular debris. While these changes were observed in both sexes, male offspring also showed increased microglia-astrocyte interactions as well as a reduction of mRNA expression of the inflammatory-regulating mediators NF-kB and Transforming growth factor-beta (TGF-beta), and the homeostatic microglial receptors Transmembrane Protein 119 (Tmem119), Triggering receptor expressed on myeloid cells 2 (TREM2) and CX3C chemokine receptor 1 (CX3CR1), all key factors in the synaptic remodeling and inflammatory response [42].

Interestingly, a few studies in rodent models explored the combined effect of prenatal exposure to LPS with pre- and postnatal HFD on the immune and behavioral profile of the offspring [59,60,61]. Repeated LPS-stimulation of HFD dams during pregnancy affected the inflammatory profile in the offspring's brain in a different way compared to a post-natal LPS exposure of maternal HFD offspring. The combined prenatal exposure (maternal HFD+LPS) lowered the IL-6/IL-10 ratio in the amygdala, hippocampus and prefrontal cortex, and reduced anxiety-like behaviors and short-term memory impairment at adolescence suggesting an unexpected protective effect of LPS against maternal HFD. Although in these studies microglial activation markers have not been evaluated, is reasonable that these cells play a key role in the protection induced by LPS, likely through the well-described mechanism of tolerance/sensitization, in which repeated exposure to an activating stimulus reduces macrophages/microglia responses to subsequent stimuli of similar or different nature and polarize them to anti-inflammatory functions [53,62]. These studies unveil poorly explored compensatory mechanisms of adaptation in the offspring brain.

The inflammatory response induced in HFD-fed dams and their pups by an excessive intake of dietary fats during pregnancy also affects myelination within the offspring's cortex. Reduced levels of myelin basic protein (MBP), a mature oligodendrocyte marker, were revealed in the medial cortex of male offspring [63]. These changes were accompanied by a dysregulated brain iron metabolism and neurocognitive behavioral alterations, suggesting that the neuroinflammatory response induced in utero by maternal HFD may be correlated with a dysregulation of iron homeostasis and a reduction in myelination in male offspring [63].

Myelination alterations were also found within the corpus callosum—the region containing the most of the myelinated fiber projections within the brain—of HFD-fed dams' offspring at adolescent stage [64]. Specifically, male adolescent offspring showed a reduction in the area of cytosolic myelin channels and of myelination-associated transcripts levels in the hippocampus, the projecting region of the corpus callosum. In addition, in both sexes, maternal HFD-exposed microglia exhibited increased numbers of contacts with synapses within the corpus callosum and reduced numbers of mature tertiary lysosomes suggesting an impaired phagolysosomal pathway [64]. Associated to these alterations, both male and female maternal HFD-adolescent offspring presented loss of social memory and sensorimotor gating deficits [64].

Collectively, these findings highlight how dysfunctional microglia and their crosstalk with neighboring glia may contribute to the adverse neurodevelopmental outcomes resulting from maternal HFD. Several other preclinical studies report how maternal HFD affects also hypothalamic inflammation, and microglial reactivity [65,66], as well as astrocyte proliferation [67]. It was shown that the proportion of proliferating astrocytes was significantly higher in the arcuate nucleus (ARC) and the supraoptic nucleus (SON) of the hypothalamus of maternal HFD offspring compared to control offspring from normal weight pregnancies. In addition, cultured fetal hypothalamic astrocytes proliferated significantly in response to IL-6, one of the cytokines significantly elevated in fetuses of obese dams, via the JAK/STAT3 signaling pathway [67] indicating that pathological exposure of developing hypothalamic astrocytes to cytokines would alter their development with repercussions for the developing brain.

The mechanism linking maternal obesity to long-term changes in brain function may depend upon complex interactions with the gut microbiome as microglia appear to be uniquely responsive to signals from the gut microbiome [30,42,46,68,69,70].

3. Obesity, maternal microbiota dysbiosis and its sequelae on the neurodevelopment of the offspring

3.1 Role of the microbiota in human health: the forgotten organ

The gut is the main site of interaction between the host immune system, commensals as well as pathogenic microbes in both mother and offspring. In fact, the gut microbiota is made up of a variety of microorganisms—mainly bacteria—playing a pivotal role in human health, so much so that it has been defined as the "forgotten organ" [71]. It is involved in the maintenance of the immunologic, hormonal, and metabolic homeostasis of the host and provides protection against pathogens; during the digestive process, it supports the extraction of energy and nutrients from foods serving as a source of metabolites, vitamins and essential nutrients. It synthesizes and releases neurotransmitters and neuromodulators, such as short-chain fatty acids (SCFAs), biogenic amines and other amino acid-derived metabolites playing a pivotal role in brain development, function and plasticity via a bidirectional exchange of signals between the gut and the brain occurring through the microbiota-gut-brain axis [30].

Physiological changes in the microbiota are observed throughout life, with life extremes (i.e., birth and senescence) being characterized by overt differences from the typical adult gut microbiota in terms of diversity, as well as in the representation of specific taxa [30]. Significant alterations in the microbiota composition are found in response to diet, BMI and stressful life events (among many conditions), greatly compromising its proper functioning leading to increased vulnerability to the onset of a variety of pathological conditions [59]. Alterations in the gut microbiota and inflammation may involve a bidirectional connection. In fact, while dysbiosis may promote the onset of pathological conditions such as metabolic syndrome, type 2 diabetes and inflammatory bowel disease (IBD), on the other hand, changes in microbiota composition may result from

these same pathological states (see [32] and references therein). A similar bidirectional effect may apply also to neurodevelopmental and psychiatric disorders characterized by neuroinflammation through the microbiota-gut-brain axis [30,68,69,70] Changes in microbiota composition have been suggested to contribute to the endocrine, neurochemical and inflammatory alterations underlying obesity and the often-associated psychiatric disorders, a role that might be already at play during fetal life [33,34,65,72].

3.2 Microbiota dysbiosis in maternal obesity

Clinical and epidemiological studies are consistently showing that the rise in the prevalence of obesity and metabolic diseases among women of the reproductive age has been paralleled by an increase in the occurrence of neurodevelopmental disorders in the offspring. According to the DOHaD theory, it is possible to hypothesize a direct relationship between metabolic derangements in the mother and neurodevelopmental outcomes in the offspring [73,74]. In this scenario, maternal microbiota is emerging as a key player providing a mechanistic link for the intergenerational negative effects observed as a result of metabolic maternal derangements. Specifically, and most importantly, maternal microbiota might contribute to the process of offspring brain development through different mechanisms, not mutually exclusive. One of the proposed mechanisms relies upon the phenomenon of the "leaky gut" a condition resulting in increased systemic and intrauterine inflammation due to obesity-related maternal dysbiosis. In particular, excessive BMI during pregnancy has been related to reduced alpha diversity and higher abundance of bacterial species such as Staphylococcus aureus, Escherichia coli, and in general of Proteobacteria, and lower counts of species such as Bifidobacterium longum and Bacteroides fragilis [75,76]. This abundance in Proteobacteria may lead to pro-inflammatory changes enhancing maternal gut permeability favoring the translocation of maternal intestinal bacteria to the fetus, eventually priming immune activation through the toll-like receptors (TLRs) [77,78]. Besides alive bacteria, many microbiota-derived microbial compounds such as LPS or flagellin may increase the production of pro-inflammatory cytokines (e.g., TNF-alpha, IL-6 and IL-1beta) activating the TLRs and affecting fetal development [78,79]. To this regard, studies performed in non-human primates and rodents have provided evidence that microbial maternal dysbiosis, deriving from HFD consumption, a model of maternal obesity, is associated with increased inflammation and is accompanied by elevated anxiety levels and reduced social behavior in the offspring [40,80].

As previously mentioned, bacterial metabolism is characterized by the production of SCFAs, macromolecules resulting from gut microbial anaerobic fermentation, that play a pivotal role in brain development, function, and plasticity. A growing body of evidence suggests that SCFAs play a pivotal role in linking maternal diet during pregnancy (and/or lactation) to inflammatory diseases (e.g., asthma) and obesity in offspring. In fact, being potent epigenetic modifiers and holding anti-obesity effects SCFAs have the potential to modulate host energy metabolism and the infant immune system through the interactions of leptin and insulin signaling in hypothalamic neurons, affecting food intake and energy expenditure (see [81,82,83] and references therein).

SCFAs concentrations increase significantly in maternal gut throughout pregnancy and recent preclinical studies have provided evidence for their beneficial effects on embryonal development acting as signaling molecules through G-protein receptors (GPR) GPR41 and GPR43 [84,85]. In particular, SCFAs have a major impact on fetal immune system development affecting cytokine synthesis and anti-inflammatory activities (see [78] and references therein).

Microglia are sensitive to intestinal microbiome changes and receive signals from the vagus nerve to regulate neuroimmune activity and function. Importantly, recent evidence suggests that microglia is developmentally regulated by the host microbiome and have been shown to dynamically respond to metabolic products from gut microbiota (SCFAs) in a sex-dependent manner [46,86]. Thion and collaborators [46] found that offspring born from GF mouse dams expressed changes in genes regulating LPS recognition and processing in utero and continued to exhibit sex-specific alterations in microglial-related gene expression postnatally [87]. Furthermore, altered microglial morphology and density, as well as attenuated inflammatory responses, were also observed right after birth, that is when microglia exhibit an activated phenotype [88]. It is important to note that the timeframe when the human gestational microbiota begins to stabilize resembling an adult composition - i.e., around three years of age - also overlaps with critical periods of CNS development, synaptic pruning, and neural remodeling. These observations support the hypothesis that a complex commensal microbiota ecosystem and their metabolites are integral to the early programming of key host physiological systems [87].

A further - though debated - mechanism through which changes in microbiota might contribute to modulate fetal brain development refers to the hypothesis of an intrauterine origin of fetal microbiota [77,78]. Given the importance of the microbiota-gut-brain axis for (neuro)development, gastrointestinal tract colonization represents indeed a milestone [89]. Although for about a century it has been hypothesized that the fetus is sterile, and that vaginal delivery is the first opportunity for large-scale bacterial colonization, recent studies are challenging the dogma of the “sterile womb” providing evidence for microbes to colonize the amniotic fluid, the umbilical blood cord and the placenta. In particular, the presence of an established microbiota in healthy and term infants supports the hypothesis that microbial colonization, occurring before birth, may play a role in the physiological development of the fetus [76,77]. See Figure 1.

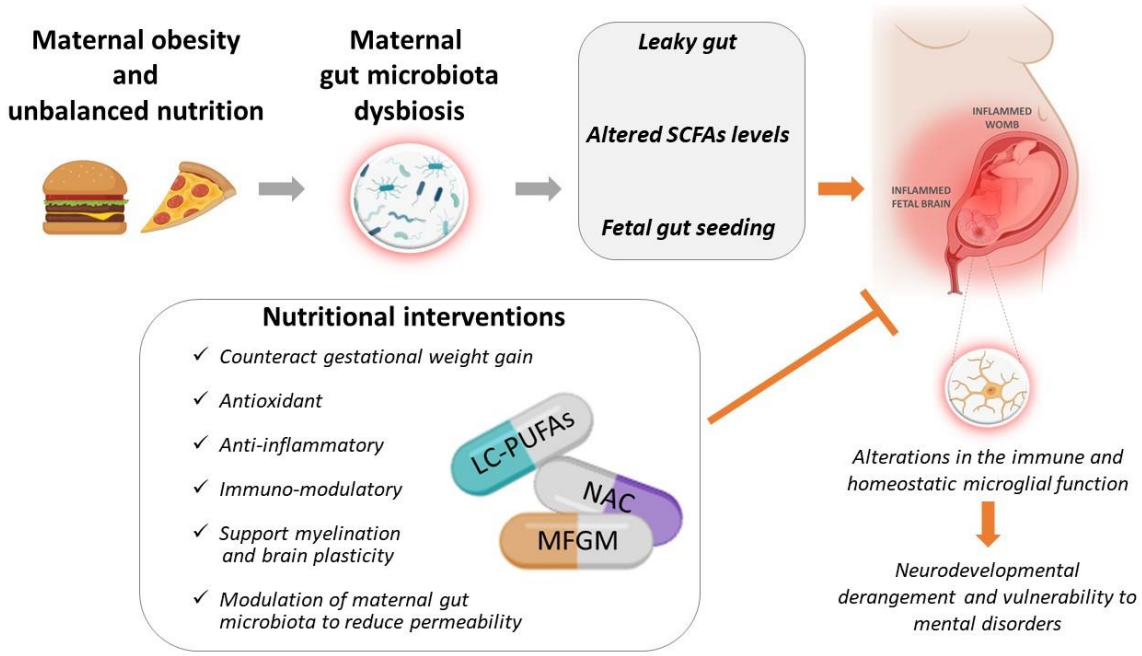


Figure 1 Obesogenic diets before and during pregnancy may trigger a dramatic loss of the homeostatic balance between the beneficial and potentially pathogenic bacteria in maternal gut leading to a condition of dysbiosis. This enhances gut inflammation that weakens the intestinal barrier (leaky gut) eventually resulting in the transplacental passage of bacteria and microbial compounds in the womb providing an immunogenic challenge to the fetus. SCFAs the main end-product of bacterial metabolism hold anti-inflammatory properties; they are tightly regulated during pregnancy to provide optimal fetal development. Obesity-related dysbiosis and inflammation may alter levels of SCFAs reaching the fetus. Finally, although still debated, the colonization of the fetal gut by maternally-derived bacteria (fetal gut seeding) in a condition of maternal dysbiosis might negatively affect the development of the gut-brain axis. The above-mentioned mechanisms may all contribute to provide a suboptimal intrauterine environment characterized by elevated systemic inflammation (inflamed womb) in turn affecting homeostasis in the developing fetal brain (inflamed brain) with microglia being a preferential target. Developing nutraceutical strategies, based on safe and feasible compounds (NAC, MFGM, LC-PUFA), aimed at counteracting gestational weight gain, reducing inflammation and oxidative stress is of paramount importance to support optimal brain development and to promote mental health throughout life. This image is original and has been created with BioRender.

4. Nutritional strategies to counteract maternal obesity

The sequelae of maternal obesity might become manifest early during development but also in the long run [90]. Excessive BMI before and during pregnancy has been associated with insulin resistance and gestational diabetes and is more likely to result in life-threatening conditions (both for the mother and the offspring) such as pre-eclampsia [91]. Moreover, it has been related to perinatal morbidity and to an overall increased chance for the offspring to develop metabolic, cardiovascular, and mental health disorders throughout life [8,14,92]. To this regard, an interesting meta-analysis performed by Thangaratinam and co-workers suggests that dietary interventions are the most effective way to contrast maternal obesity. Based on 34 different randomized trials,

the mean reduction in maternal gestational weight gain due to the dietary intervention was −3.84 kg vs.−1.42 kg obtained through interventions such as moderate physical exercise [93]. Notwithstanding the great efforts of clinical practitioners to inform the general population about the risks related to excessive BMI, particularly during pregnancy, and the recommendation to reduce or contain gestational weight gain, compliance to diet regimens and to moderate physical exercise during pregnancy is very low and women are likely to maintain pre-pregnancy lifestyle until parturition (and even to gain weight during lactation) [94]. In this context safe and effective nutritional strategies, with limited side-effects, are becoming of utmost importance as rates of obesity continue to increase and the long-term negative effects on the offspring health is becoming more and more apparent [95]. Thus, addressing proper dietary supplementation might provide a broad-spectrum promising and feasible strategy to prevent or counteract the disruptive effects of the "inflamed womb". In the following paragraphs, we will review nutritional strategies and dietary supplementation that have received recent attention and that appear to be promising based on the results obtained on preclinical studies and in clinical trials.

These intervention strategies include Long Chain Polyunsaturated Fatty Acids (LC-PUFA), Milk fat globule membrane (MFGM) and N-Acetyl-Cysteine (NAC) supplementation, all of which hold the potential to modulate inflammatory/oxidant processes and to target microbiota functions by multiple mechanisms, as described in the following paragraphs.

4.1 LC-PUFA supplementation as anti-inflammatory and protective strategy

Obesity is associated with an altered profile of circulating fatty acids (FAs), including LC-PUFAs of the omega-3 and omega-6 series which is exacerbated by pregnancy-related adaptations. In both humans and animal models of maternal HFD, increased circulating levels of n-6 PUFAs, mainly arachidonic acid AA (AA, C20:4n-6) and linoleic acid (LA, C18:2n-6), and decreased levels of n-3 PUFAs, mainly docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) were found. These alterations in maternal circulating levels are mirrored in breast milk and fetal circulation [96,97]. As the LC-PUFA supply to the fetus and the newborn relies mainly on their transfer from the maternal circulation across the placenta and from the breast milk, the dysregulation of maternal lipid metabolism profoundly affects the lipid profile of the fetus in different organs, including the brain [98,99].

In addition, the placental uptake and metabolism of essential FAs, particularly of DHA, is impaired by maternal obesity [96,97] and this occurs in a sex-specific manner, reflecting the differential expression, content, and activity of placental FAs transport and translocator proteins, as well as metabolizing enzymes [100,101]. Males' offspring placentas showed reduced DHA-transfer capacity and the umbilical cord plasma had lower DHA-phospholipids than in females, which could lead to a lower DHA supply for proper fetal development in males under metabolic stress conditions [100].

The elevated n-6/n-3 PUFA ratio in the mother is associated with adverse pregnancy outcomes, shorter length of gestation and impaired fetal growth [102] being a key factor in the onset of low-grade, chronic inflammation associated with obesity in the mother-fetus dyad

Long-chain fatty acids are indeed crucial regulators of the functional polarization of macrophages, through a multiplicity of mechanisms yet to be fully elucidated. These include the modulation of membrane fluidity—and hence of membrane-associated enzymes, receptors and signaling pathways—and the direct agonism to extracellular or intracellular receptors and transcription factors, such as G protein-coupled receptor 120 (GPR120), retinoid X receptors (RXR) and Peroxisome Proliferator-activated Receptor-gamma (PPAR-gamma), involved in the regulation of inflammation. AA and AA-derived eicosanoids act mainly, though not univocally, as pro-inflammatory mediators [103,104,105], while EPA, DHA and the DHA-derivatives docosanoids are recognized anti-inflammatory pro-resolving mediators (see [103,104,105] and references therein).

A recent study from Belcastro and colleagues found that placentas from women with pre-gestational obesity contain overall higher contents of PUFAs and a higher ratio of AA-derived isoprostanes vs. DHA-derived neuroprostanes, which are pro- and anti-inflammatory bioactive molecules, respectively [106]. Neuroprostanes were negatively correlated with inflammation markers, while isoprostanes were positively associated with maternal pre-gestational BMI [107], suggesting the importance of rebalancing PUFA levels in obese pregnant women as an anti-inflammatory/protective strategy for the progeny.

In the Fat-1 transgenic mouse model, capable of producing n-3 fatty acids from the n-6 type, Heerwagen and colleagues demonstrated that high maternal n-3/n-6 ratio prevents the consequences of maternal HFD-associated inflammation on weight gain, body and liver fat, and insulin resistance, and limits the development of adverse metabolic outcomes in adult offspring [108]. In line with these findings, DHA supplementation or higher fish consumption as a dietary omega-3 source in obese pregnant women decrease placental inflammation [109,110] further supporting that re-balancing n-3/n-6 LC-PUFA ratio represent a promising interventional approach to counteract the negative effects of maternal obesity and related inflammation.

The re-balancing of the n-3/n-6 LC-PUFA ratio could be crucial also for the proper fetal brain development. Notably, maternal obesity and maternal dietary n-3 PUFA deficiency leads to similar defects in prenatal brain development, immune status and behavioral impairments in animal models. Both maternal HFD and maternal low n-3 PUFA studies reported long-lasting changes in basal microglial reactivity and functions in the progeny, and higher susceptibility to subsequent inflammatory stimuli, as well as marked changes in anxiety and spatial learning behavioral patterns, as also mentioned in the previous section [40,42,111,112,113].

In mice, n-3 PUFA deficient maternal diet leads to increased n-6 PUFA levels in microglial membranes, while maternal n-3 PUFA dietary supply increases microglial DHA membrane content [113], demonstrating the strict correlation between maternal n-3 PUFA intake and microglial lipid profile in the developing brain, with likely repercussion for microglial reactivity and brain development.

Indeed, in activated brain microglia as in peripheral macrophages, DHA inhibits TLR inflammatory signaling, enhance fatty acid metabolism-related genes, reduces the synthesis of pro-inflammatory and anti-neurogenic molecules, and favors the shift to a phenotype that promotes the survival and differentiation of neural precursor cells [114,115,116,117,118,119,120,121]. Madore and colleagues recently reported that DHA is also crucial in maintaining the homeostatic molecular signature of microglia in physiological conditions and in tuning microglia-mediated phagocytosis of synaptic elements in the rodent developing hippocampus. In particular, a maternal n-3 PUFA deficient diet dysregulated microglial homeostasis in the progeny, increasing microglial immune and inflammatory pathways, and enhancing the phagocytosis of synaptic elements leading to their excessive removal [122].

Besides their immunomodulatory properties, n-6 (particularly AA) and n-3 (particularly DHA) PUFAs play essential roles for neural and visual development, as structural and functional components of neural membranes [123]. Incorporated into membrane phospholipids in uniquely high levels in the CNS, with the highest demand during the third trimester of pregnancy and the first two years of postnatal life, they promote neural and glial cell growth and differentiation [124,125,126]. For example, DHA was shown to promote oligodendrocyte progenitor cell maturation through its PPAR-gamma agonistic activity and the activation of extracellular signal-regulated-kinase (ERK)-1/2 [127]. Consistently, Leyrolle et al., 2021 recently demonstrated that maternal dietary n-3 PUFA deficiency impairs oligodendrocyte maturation and myelination during the postnatal phase, with long-term consequences on white matter structure, brain functional connectivity, mood, and cognition in adult mice. Other studies, in experimental models and humans, illustrated that LC-PUFAs are essential to astrogliogenesis, neurogenesis, synaptic plasticity, and neuronal wiring [128,129,130,131,132,133,134]. Collectively, these studies highlight the importance of an adequate dietary intake of LC-PUFAs, especially in highly sensitive windows of development since conception.

A recent study in mice fed a HFD suggested that high-DHA tuna oil significantly ameliorated obesity and metabolic dysfunctions in offspring by the regulation of taxonomic compositions of gut microbiota and their functional profiling, as the intestinal arginine and proline metabolism was restored by DHA supplementation [134]. Accumulating evidence from case reports and from animal studies suggests that DHA and other n-3 PUFAs can exert positive effects on gut microbiome signatures and composition [135,136,137]. More recently, it was shown that DHA supplementation repaired the lipids metabolism shifts in a mouse model of antibiotic-mediated gut dysbiosis [138]; similarly DHA significantly improved dyslipidemia and anxiety-like behaviors induced by long-term azithromycin (AZI) treatment in mice [139].

In the last decade several prenatal and postnatal clinical trials of n-3 PUFA supplementation have been conducted varying in sample size, dose and composition of supplements, timing and duration of intervention and demographic characteristics. The results regarding beneficial effects on offspring cognitive and/or metabolic outcomes remain controversial (see [140] and references therein). In addition, studies evaluating the role of n-3 PUFA supplementation specifically in the higher risk cohort of women with higher baseline metabolic dysregulation and with low n-3 status are limited to a pilot study from Monthé-Drèze and colleagues

[141]. Supplementation with n-3 PUFA in women with obesity during pregnancy was associated with increased fetal fat-free mass accrual, improved fetal growth, and increased length of gestation. Larger adequately powered trials of n-3 supplementation or dietary intervention, starting before conception or early in pregnancy, specifically in women with excessive overweight should be conducted to confirm these findings.

4.2 Milk fat globule membrane

The milk fat globule membrane (MFGM) is a unique, complex structure surrounding milk fat globule. It is essentially an oil droplet enclosed in plasma membrane that is secreted from the milk-producing cells in the epithelium of the mammary gland (lactocytes) and is physiologically delivered to the newborn through breastfeeding. It is a rich source of bioactive compounds including phospholipids, sphingolipids, gangliosides—and polar lipids in general—that hold a main functional role for the proper development of both the brain and the gut, two organs that communicate bidirectionally through the gut-brain axis system [142]. Moreover, MFGM has also the potential to synergize with probiotic bacteria—through direct interactions with proteins of the bacterial surface—overall increasing the survival and adhesion of probiotic bacteria during the gastrointestinal transit, improving mucosal immunity, and neurodevelopment and cognitive abilities in developing infants [143].

For the sake of clarity, in this paragraph, we will first review the beneficial effects of MFGM in postnatal development and adulthood and only successively we will hypothesize about the potential use of this compound in obese mothers during pregnancy to counteract the “inflamed womb” and its sequelae. Supplementation with dietary MFGM or with selected components thereof has shown beneficial effects on brain function, microbiome composition and immune system in several clinical and preclinical studies [142,144,145,146].

Breastfeeding appears to be positively related to cognitive and immune outcomes and to be overall associated with a decreased incidence of all-cause infection-related mortality in infants [142]. Recent preclinical evidence clearly suggests that MFGM-based interventions targeting the microbiota-gut-brain axis are able to buffer stress-induced dysfunctions of physiological processes and brain development [147,148]. Given the complex nature of this compound the pathways affected by MFGM are multiple and non-mutually exclusive and therefore quite a number of mechanisms are currently under investigation to clarify its positive effects on health outcomes during development and at adult age. Among all, MFGM has been shown to hold anti-inflammatory properties. Preclinical studies have provided evidence for laboratory rodents supplemented with MFGM to be characterized by a greater ability to counteract inflammation upon an immunogenic challenge. As an example, Snow and co-workers showed that MFGM-treated weanling mice displayed lower levels of inflammatory cytokines and reduced intestinal permeability upon LPS injection [149]; Huang and colleagues provided evidence for low-birth-weight mouse pups supplemented with MFGM during the suckling period to

be characterized by reduced cytokines expression levels in the ileum (TNF-alpha, IL-6, IFN-gamma, IL-1beta) upon LPS treatment [150].

Recent evidence by Arnoldussen and co-workers has shown that MFGM was able to attenuate neuroinflammation in a mouse-model of HFD by reducing microglia activation resulting in better spatial memory performance and functional connectivity in the hippocampus, an effect possibly resulting from the modulation of phospholipid and metabolite composition in brain tissue or by increasing the level of gangliosides [151]. Besides their anti-inflammatory effects, gangliosides are known to be involved in neuronal transmission and are believed to support myelination, neuronal and synaptic plasticity during postnatal development. However, the rate of gangliosides accretion in the developing brain is highest in utero and in very early postnatal phases. Preclinical research has shown that complex milk lipids (also contained in MFGM) are transferred across the placenta and as such, an increased maternal intake during pregnancy has been associated with increases in fetal gangliosides levels [152,153]. Interestingly, a recent multicenter randomized controlled trial—the CLIMB (Complex Lipids In Mothers and Babies) study—was aimed to investigate the effects of supplementation of complex lipids in pregnancy, on maternal ganglioside status and subsequent cognitive outcomes in the offspring [154]; the authors report the absence of any adverse outcome but no effect of MFGM was reported on fetal growth, at least soon after birth, and for the parameters investigated in a population of healthy women [155]. Despite this inconclusive result, preclinical evidence rather suggests MFGM to be very promising to improve the health of the offspring. As for example, Yuan and co-workers report that supplementation of MFGM to obese rat dams during pregnancy and lactation improves neurodevelopment and cognitive function in male offspring. In particular, these authors reported that while maternal obesity induced insulin resistance and aberrant brain-derived neurotrophic factor (BDNF) signaling in the hippocampus of neonatal and adult offspring, these effects were counteracted by maternal MFGM administration [156]. Moreover, Li and colleagues provided evidence for the supplementation during pregnancy of MFGM to promote brown/beige adipocyte development and to prevent obesity in male offspring born from HFD-dams rats [157]. These same authors showed that MFGM may act through the modulation of gut microbiota to alleviate obesity-induced glucose metabolism disorders in peripheral tissues in rat dams [158]. Indeed, as mentioned above, MFGM is able to decrease gut permeability, which could attenuate gut-derived endotoxin translocation and the associated inflammatory responses supporting its use during pregnancies characterized by a general inflammatory condition such as those associated to obesity or hypercaloric unbalanced diets. Very interestingly, recent preclinical evidence shows that MFGM supplementation could reduce high-fat diet (HFD)-induced body weight gain and may control gut physiology and the amount of bacteria of metabolic interest [159]. Moreover, a clinical study showed that in overweight/obese adult subject, MFGM supplementation was able to reduce postprandial inflammation in a population characterized by a chronic inflammatory state due to obesity. In particular, MFGM lowered fasting plasma cholesterol, inflammatory markers as well as postprandial insulin response [160]. Thus, MFGM appears to be a safe and promising compound with the great potential to counteract the negative effect of maternal obesity promoting health outcomes of both the mother and the offspring.

4.3 The antioxidant n-acetyl-cysteine as an anti-inflammatory and protective strategy in the maternal-fetal crosstalk

OS and inflammation are main features of obesity and play a pivotal role in mediating the negative effects of this condition during pregnancy for both the mother and the offspring [8,92,161]. Indeed, a tight regulation of the redox balance and inflammatory processes, particularly during pregnancy, is crucial for a proper fetal development [14,162]. Moreover, OS has been reported to impair human placental amino acid uptake and increase Na+ permeability, directly affecting amino acid transporters [163].

To this regard, antioxidant compounds are emerging as a promising strategy for the treatment of diabetes and other metabolism-related pathologies [164]. Moreover, given the role played by OS and specific reactive oxygen species (ROS) in mediating fat accumulation, the reduction of OS through antioxidant compounds administration might mimic caloric restriction contributing to limit body weight gain [165,166,167,168]. Among the antioxidants, N-Acetyl-Cysteine (NAC) is receiving growing attention as a feasible pharmacological strategy in counteracting the detrimental effects related to maternal obesity [162,169]. NAC is the rate-limiting substrate in the biosynthesis of glutathione (GSH) and holds a great efficacy as a ROS scavenger [164]. We have previously shown that administration of NAC during pregnancy in a mouse model of maternal obesity resulted in improved glucose tolerance, in addition to a reduced activation of the HPA axis, when exposed to stress. Moreover, while HFD reduced antioxidant defenses (glutathione GSH levels) in the hypothalamus of male offspring, this effect was prevented by prenatal NAC administration. Since the hypothalamus plays a key role in both metabolic regulation and emotional behaviors, our data support a role for OS in the long-term effects of maternal obesity [162]. Interestingly, the above-mentioned results on NAC supplementation appear to mirror the protective effects observed in a genetically modified mouse model of reduced OS (p66Shc-/- mice) confirming the tight relationship existing among prenatal metabolic stress (maternal obesity), fetal programming and OS pathways [92,162,166,170]. NAC also holds anti-inflammatory capacity, being able to reduce the expression of pro-inflammatory mediators by suppressing the activation of the transcription factor NF-kB, whose regulation is redox sensitive [171,172,173] As such, NAC has been reported to reduce oxidative and inflammatory responses at the maternal-fetal interface and to improve placental efficiency in a number of clinical and preclinical studies. As an example, Williams and co-workers reported that NAC administration improved HFD-induced decidual vasculopathy in mouse placenta by reducing mRNA and immunostaining of IL-1beta and monocyte chemoattractant protein-1 and increasing Vegf m-RNA [174]. Paintlia and colleagues showed that NAC pretreatment was able to prevent LPS-induced preterm labor as well as inflammation and OS at the placental level and in the amniotic fluid and to inhibit LPS-induced up-regulation of phospholipids metabolism in placenta [175]. In the same rat model, these authors report that NAC was able to provide neuroprotection and to attenuate the degeneration of oligodendrocytes progenitor cells and white matter injury in the developing rat brain [176].

A number of severe pathological conditions of the newborns can be observed as a result of pre-eclampsia, a pregnancy-specific disorder characterized by new-onset hypertension and proteinuria that poses at great life-

risk the mother and the newborn [177,178]. Pre-eclampsia shows a strong direct correlation with BMI and both inflammation and OS are involved in its etiopathogenesis. Although there is still no consensus in the literature on the best strategy for the prevention and the treatment of this disease, both anti-oxidant and anti-inflammatory approaches seem to be feasible [179]. To this regard, Motawei and colleagues observed attenuation of pre-eclampsia severity (improved liver and kidney function, decreased blood pressure, decreased proteinuria) and improved pregnancy outcomes among patients with pre-eclampsia who received NAC as they reported an increase in birth weight as well as a higher Apgar score [169].

As previously-mentioned one possible mechanism through which maternal obesity and maternal over-nutrition may trigger intrauterine inflammation—eventually affecting fetal brain development—is by inducing a condition of gut dysbiosis. To this regard, very interestingly, Luo and co-workers [180] showed that NAC supplementation in pigs during late gestation alleviated maternal-placental OS and inflammatory response, improved placental function, and altered fecal microbial communities. More in detail, NAC increased the relative abundance of fecal Prevotella that was positively related with the increased propionate and butyrate concentrations (two SCFAs able to reduce inflammation and OS). In addition, the relative abundances of Clostridium cluster XIVa, Prevotella, and Roseburial/Eubacterium rectale were related negatively to the gene expression levels of the NLRP3 and positively to that of Slc7a8 (that encodes for a transporter that mediates the uptake of amino acids in the placenta [181]. Overall evidence provided by Luo and co-workers indicates strong interactions between gut microbiota, placental NLRP3 inflammasome and nutrients delivery [180] and support the use of NAC as a promising compound to counteract the negative effect of maternal obesity. However, OS and inflammatory pathways need to be tightly regulated during pregnancy to ensure proper fetal development and a general consensus on the use NAC (and other antioxidants) during pregnancy in the clinical practice is still lacking [14,162].

5. Conclusions

A number of studies have clearly demonstrated an association between prenatal stress and neurodevelopmental disorders. Metabolic stressors, such as maternal obesity or malnutrition, due for example to the exposure to unbalanced diets rich in fats and sugars, can have similar long-term effects disrupting fetal programming. The role of the maternal gut microbiota in this context is still under study. However, being finely tuned throughout pregnancy and being very responsive to metabolic stressors, it could clearly represent an intriguing mediator capable to transmit the negative effects of maternal obesity/diet to the growing organism through multiple and non-exclusive pathways, leading to long-lasting inflammatory signatures.

In general, it is clear that the immune priming occurring in the fetal/newborn gut plays a pivotal role in brain development and in the health outcomes of the individual. This has been confirmed by a number of different experimental approaches ranging from GF animal (suggesting that microbiota plays a pivotal role in shaping both innate and adaptive immunity) to the manipulation of microbiota (either with antibiotic treatment or

microbiota reconstitution providing evidence for the main role of the microbiota in immune homeostasis) [182]. Whether this priming occurs primarily in uterus or after birth is still a matter of debate however, it is also clear that the development of the immune system and the modulation of inflammatory responses both play a pivotal role in brain and metabolic health. This is also suggested by the growing number of studies reporting a comorbidity among autoimmune, psychiatric and metabolic disorders also in relation with changes in microbiota composition (see for example [183,184] and references therein).

In fetal brain, one important target of maternal microbiota is represented by microglia. Given the fundamental role played by this cell type on brain development, effective intervention strategies are needed in the future to break the vicious cycle that links maternal gut dysbiosis and inflammation to derangements in proper microglia development and function.

Overall, better understanding of the role of gut microbiota in maternal obesity might help us to promote healthy fetal brain and to prevent neurodevelopmental disorders.

Acknowledgments

We are thankful to Marianna Samà and Letizia Giona for bibliographical retrieving of literature and text formatting; Antonio Maione, Sabrina Alviti, Paola Rossolino, Valerio Occhiodoro, Monica Bolli, Romano Arcieri and Anna Ceccarelli for administrative and technical support.

Funding Statement

This work was supported by HDHL INTIMIC: Knowledge Platform on Food, Diet, Intestinal Microbiomics and Human Health (partner ISS-RDS, FC); ERANETNEURON JTC 2018 (Mental Disorders) Project ‘‘EMBED” to F.C. HDHL-INTIMIC-085-GUTMOM project (co-PI FC) within the ERA-NET Co-fund HDHL-INTIMIC (INtesTInal MICrobiomics) programme; “Avvio alla Ricerca—Tipo 1” 2021 “Sapienza” University of Rome funding to CM; funding from the Italian Ministry of Health (Ricerca Corrente 2021 to FC); ISS Project MOMINFLAM - Unique signatures underlying placental-fetal brain crosstalk in maternal obesity. Proj code ISS20-88bc10d8839b to FC.

Author Contributions

A.B. and F.C. conceptualized the review, A.B., F.C., M.A.A.-C., R.D.S. and C.M. wrote the review; A.B. conceptualized the figure. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

A.B., F.C. and C.M. are currently running experimental activities dealing with the administration of MFGM (provided by Reckitt|Mead Johnson Nutrition Institute) in animal models. M.A.A.-C. and R.D.S. declare no conflict of interest.

References

1. Barker, D.J.P.; Osmond, C. INFANT MORTALITY, CHILDHOOD NUTRITION, AND ISCHAEMIC HEART DISEASE IN ENGLAND AND WALES. *Lancet* **1986**, *327*, 1077–1081, doi:10.1016/S0140-6736(86)91340-1.

2. Spencer, S.J.; Korosi, A.; Layé, S.; Shukitt-Hale, B.; Barrientos, R.M. Food for thought: how nutrition impacts cognition and emotion. *npj Sci. Food* **2017**, *1*, doi:10.1038/s41538-017-0008-y.

3. Reemst, K.; Tims, S.; Yam, K.-Y.; Mischke, M.; Knol, J.; Brul, S.; Schipper, L.; Korosi, A. The Role of the Gut Microbiota in the Effects of Early-Life Stress and Dietary Fatty Acids on Later-Life Central and Metabolic Outcomes in Mice. *mSystems* **2022**, *7*, e0018022, doi:10.1128/msystems.00180-22.

4. Gomes, D.; Le, L.; Perschbacher, S.; Haas, N.A.; Netz, H.; Hasbargen, U.; Delius, M.; Lange, K.; Nennstiel, U.; Roscher, A.A.; et al. Predicting the earliest deviation in weight gain in the course towards manifest overweight in offspring exposed to obesity in pregnancy: a longitudinal cohort study. *BMC Med.* **2022**, *20*, 1–18, doi:10.1186/s12916-022-02318-z.

5. Kereliuk, S.M.; Dolinsky, V.W. Recent Experimental Studies of Maternal Obesity, Diabetes during Pregnancy and the Developmental Origins of Cardiovascular Disease. *Int. J. Mol. Sci.* 2022, *23*, 4467.

6. Cirulli, F.; Musillo, C.; Berry, A. Maternal Obesity as a Risk Factor for Brain Development and Mental Health in the Offspring. *Neuroscience* **2020**, *447*, 122–135, doi:10.1016/J.NEUROSCIENCE.2020.01.023.

7. Congdon, P. Obesity and Urban Environments. *Int. J. Environ. Res. Public Health* **2019**, *16*, doi:10.3390/IJERPH16030464.

8. Musillo, C.; Berry, A.; Cirulli, F. Prenatal psychological or metabolic stress increases the risk for psychiatric disorders: the "funnel effect" model. *Neurosci. Biobehav. Rev.* 2022, *136*, 104624.

9. Sullivan, E.L.; Nousen, E.K.; Chamlou, K.A. Maternal high fat diet consumption during the perinatal period programs offspring behavior. *Physiol. Behav.* 2014, *123*, 236–242.

10. Rivera, H.M.; Christiansen, K.J.; Sullivan, E.L. The role of maternal obesity in the risk of neuropsychiatric disorders. *Front. Neurosci.* 2015, *9*, 194.

11. Bellisario, V.; Panetta, P.; Balsevich, G.; Baumann, V.; Noble, J.; Raggi, C.; Nathan, O.; Berry, A.; Seckl, J.; Schmidt, M.; et al. Maternal high-fat diet acts as a stressor increasing maternal glucocorticoids' signaling to the fetus and disrupting maternal behavior and brain activation in C57BL/6J mice. *Psychoneuroendocrinology* **2015**, *60*, 138–150, doi:10.1016/j.psyneuen.2015.06.012.

12. Iozzo, P.; Holmes, M.; Schmidt, M. V.; Cirulli, F.; Guzzardi, M.A.; Berry, A.; Balsevich, G.; Andreassi, M.G.; Wesselink, J.J.; Liistro, T.; et al. Developmental ORIgins of healthy and unhealthy AgeiNg: The role of maternal obesity - Introduction to DORIAN. *Obes. Facts* **2014**, *7*, 130–151, doi:10.1159/000362656.

13. Davis, J.; Mire, E. Maternal obesity and developmental programming of neuropsychiatric disorders: An inflammatory hypothesis. *Brain Neurosci. Adv.* **2021**, *5*, 239821282110034, doi:10.1177/23982128211003484.

14. Berry, A.; Mazzelli, M.; Musillo, C.; Riva, M.A.; Cattaneo, A.; Cirulli, F. High-fat diet during adulthood interacts with prenatal stress, affecting both brain inflammatory and neuroendocrine markers in male rats. *Eur. J. Neurosci.* **2022**, *55*, 2326–2340, doi:10.1111/ejn.15181.

15. Dantzer, R.; O'Connor, J.C.; Freund, G.G.; Johnson, R.W.; Kelley, K.W. From inflammation to sickness and depression: When the immune system subjugates the brain. *Nat. Rev. Neurosci.* 2008, *9*, 46–56.

16. Rizzo, F.R.; Musella, A.; De Vito, F.; Fresegna, D.; Bullitta, S.; Vanni, V.; Guadalupi, L.; Stampanoni Bassi, M.; Buttari, F.; Mandolesi, G.; et al. Tumor Necrosis Factor and Interleukin-1 β Modulate Synaptic Plasticity during Neuroinflammation. *Neural Plast.* 2018, *2018*.

17. Sun, K.; Kusminski, C.M.; Scherer, P.E. Adipose tissue remodeling and obesity. *J. Clin. Invest.* 2011, *121*, 2094–2101.

18. Longo, M.; Zatterale, F.; Naderi, J.; Parrillo, L.; Formisano, P.; Raciti, G.A.; Beguinot, F.; Miele, C. Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. *Int. J. Mol. Sci.* **2019**, *20*, doi:10.3390/ijms20092358.

19. Fuster, J.J.; Ouchi, N.; Gokce, N.; Walsh, K. Obesity-induced changes in adipose tissue microenvironment and

their impact on cardiovascular disease. *Circ. Res.* 2016, *118*, 1786–1807.

20. Challier, J.C.; Basu, S.; Bintein, T.; Minium, J.; Hotmire, K.; Catalano, P.M.; Hauguel-de Mouzon, S. Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. *Placenta* **2008**, *29*, 274, doi:10.1016/J.PLACENTA.2007.12.010.

21. Basu, S.; Haghiac, M.; Surace, P.; Challier, J.C.; Guerre-Millo, M.; Singh, K.; Waters, T.; Minium, J.; Presley, L.; Catalano, P.M.; et al. Pregravid obesity associates with increased maternal endotoxemia and metabolic inflammation. *Obesity* **2011**, *19*, 476–482, doi:10.1038/oby.2010.215.

22. Parisi, F.; Milazzo, R.; Savasi, V.M.; Cetin, I. Maternal low-grade chronic inflammation and intrauterine programming of health and disease. *Int. J. Mol. Sci.* 2021, *22*, 1–16.

23. Edwards, S.M.; Cunningham, S.A.; Dunlop, A.L.; Corwin, E.J. The Maternal Gut Microbiome during Pregnancy. *MCN Am. J. Matern. Nurs.* **2017**, *42*, 310–316, doi:10.1097/NMC.0000000000000372.

24. Bolton, J.L.; Bilbo, S.D. Developmental programming of brain and behavior by perinatal diet: Focus on inflammatory mechanisms. *Dialogues Clin. Neurosci.* **2014**, *16*, 307–320, doi:10.31887/dcns.2014.16.3/jbolton.

25. Segovia, S.A.; Vickers, M.H.; Gray, C.; Reynolds, C.M. Maternal obesity, inflammation, and developmental programming. *Biomed Res. Int.* 2014, *2014*.

26. Salans, L.B.; Cushman, S.W.; Weismann, R.E. Studies of human adipose tissue. Adipose cell size and number in nonobese and obese patients. *J. Clin. Invest.* **1973**, *52*, 929–941, doi:10.1172/JCI107258.

27. Challier, J.C.; Basu, S.; Bintein, T.; Minium, J.; Hotmire, K.; Catalano, P.M.; Hauguel-de Mouzon, S. Obesity in Pregnancy Stimulates Macrophage Accumulation and Inflammation in the Placenta. *Placenta* **2008**, *29*, 274–281, doi:10.1016/j.placenta.2007.12.010.

28. Aye, I.L.M.H.; Lager, S.; Ramirez, V.I.; Gaccioli, F.; Dudley, D.J.; Jansson, T.; Powell, T.L. Increasing maternal body mass index is associated with systemic inflammation in the mother and the activation of distinct placental inflammatory pathways. *Biol. Reprod.* **2014**, *90*, doi:10.1095/biolreprod.113.116186.

29. Edlow, A.G.; Glass, R.M.; Smith, C.J.; Tran, P.K.; James, K.; Bilbo, S. Placental Macrophages: A Window Into Fetal Microglial Function in Maternal Obesity. *Int. J. Dev. Neurosci.* **2019**, *77*, 60–68, doi:10.1016/j.ijdevneu.2018.11.004.

30. Melekoglu, R.; Ciftci, O.; Eraslan, S.; Basak, N.; Celik, E. The Effects of Body Mass Index on Second-Trimester Amniotic Fluid Cytokine and Matrix Metalloproteinase Levels. *Gynecol. Obstet. Invest.* **2018**, *83*, 70–75, doi:10.1159/000455192.

31. Cryan, J.F.; O'riordan, K.J.; Cowan, C.S.M.; Sandhu, K. V.; Bastiaanssen, T.F.S.; Boehme, M.; Codagnone, M.G.; Cussotto, S.; Fulling, C.; Golubeva, A. V.; et al. The microbiota-gut-brain axis. *Physiol. Rev.* **2019**, *99*, 1877–2013, doi:10.1152/physrev.00018.2018.

32. Álvarez, D.; Muñoz, Y.; Ortiz, M.; Maliqueo, M.; Chouinard-Watkins, R.; Valenzuela, R. Impact of Maternal Obesity on the Metabolism and Bioavailability of Polyunsaturated Fatty Acids during Pregnancy and Breastfeeding. *Nutrients* **2020**, *13*, 1–24, doi:10.3390/NU13010019.

33. Bander, Z. Al; Nitert, M.D.; Mousa, A.; Naderpoor, N. The gut microbiota and inflammation: An overview. *Int. J. Environ. Res. Public Health* 2020, *17*, 1–22.

34. Agustí, A.; García-Pardo, M.P.; López-Almela, I.; Campillo, I.; Maes, M.; Romaní-Pérez, M.; Sanz, Y. Interplay Between the Gut-Brain Axis, Obesity and Cognitive Function. *Front. Neurosci.* **2018**, *12*, doi:10.3389/FNINS.2018.00155.

35. Carlessi, A.S.; Borba, L.A.; Zugno, A.I.; Quevedo, J.; Réus, G.Z. Gut microbiota–brain axis in depression: The role of neuroinflammation. *Eur. J. Neurosci.* 2021, *53*, 222–235.

36. Cirulli, F. Interactions between early life stress and metabolic stress in programming of mental and metabolic health. *Curr. Opin. Behav. Sci.* **2017**, *14*, 65–71, doi:10.1016/J.COBEHA.2016.12.009.

37. Gohir, W.; Ratcliffe, E.M.; Sloboda, D.M. Of the bugs that shape us: maternal obesity, the gut microbiome, and long-term disease risk. *Pediatr. Res. 2015 771* **2014**, *77*, 196–204, doi:10.1038/pr.2014.169.

38. Bardos, J.; Fiorentino, D.; Longman, R.E.; Paidas, M. Immunological Role of the Maternal Uterine Microbiome in Pregnancy: Pregnancies Pathologies and Alterated Microbiota. *Front. Immunol.* 2020, *10*, 2823.

39. Lenz, K.M.; Nelson, L.H. Microglia and beyond: Innate immune cells as regulators of brain development and behavioral function. *Front. Immunol.* 2018, *9*.

40. Hammond, B.P.; Manek, R.; Kerr, B.J.; Macauley, M.S.; Plemel, J.R. Regulation of microglia population dynamics throughout development, health, and disease. *Glia* 2021, *69*, 2771–2797.

41. Bilbo, S.D.; Tsang, V. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. *FASEB J.* **2010**, *24*, 2104–2115, doi:10.1096/fj.09-144014.

42. Bordeleau, M.; Fernández de Cossío, L.; Chakravarty, M.M.; Tremblay, M.È. From Maternal Diet to Neurodevelopmental Disorders: A Story of Neuroinflammation. *Front. Cell. Neurosci.* 2021, *14*.

43. Bordeleau, M.; Lacabanne, C.; Fernández De Cossío, L.; Vernoux, N.; Savage, J.C.; González-Ibáñez, F.; Tremblay, M.È. Microglial and peripheral immune priming is partially sexually dimorphic in adolescent mouse offspring exposed to maternal high-fat diet. *J. Neuroinflammation* **2020**, *17*, doi:10.1186/s12974-020-01914-1.

44. Butovsky, O.; Weiner, H.L. Microglial signatures and their role in health and disease. *Nat. Rev. Neurosci.* 2018, *19*, 622–635.

45. Zhang, X.; Kracht, L.; Lerario, A.M.; Dubbelaar, M.L.; Brouwer, N.; Wesseling, E.M.; Boddeke, E.W.G.M.; Eggen, B.J.L.; Kooistra, S.M. Epigenetic regulation of innate immune memory in microglia. *J. Neuroinflammation* **2022**, *19*, doi:10.1186/s12974-022-02463-5.

46. Schaafsma, W.; Basterra, L.B.; Jacobs, S.; Brouwer, N.; Meerlo, P.; Schaafsma, A.; Boddeke, E.W.G.M.; Eggen, B.J.L. Maternal inflammation induces immune activation of fetal microglia and leads to disrupted microglia immune responses, behavior, and learning performance in adulthood. *Neurobiol. Dis.* **2017**, *106*, 291–300, doi:10.1016/j.nbd.2017.07.017.

47. Thion, M.S.; Low, D.; Silvin, A.; Chen, J.; Grisel, P.; Schulte-Schrepping, J.; Blecher, R.; Ulas, T.; Squarzoni, P.; Hoeffel, G.; et al. Microbiome Influences Prenatal and Adult Microglia in a Sex-Specific Manner. *Cell* **2018**, *172*, 500-516.e16, doi:10.1016/j.cell.2017.11.042.

48. Williamson, L.L.; Sholar, P.W.; Mistry, R.S.; Smith, S.H.; Bilbo, S.D. Microglia and memory: Modulation by early-life infection. *J. Neurosci.* **2011**, *31*, 15511–15521, doi:10.1523/JNEUROSCI.3688-11.2011.

49. Han, V.X.; Patel, S.; Jones, H.F.; Dale, R.C. Maternal immune activation and neuroinflammation in human neurodevelopmental disorders. *Nat. Rev. Neurol.* 2021, *17*, 564–579.

50. Hunter, S.K.; Hoffman, M.C.; D'Alessandro, A.; Noonan, K.; Wyrwa, A.; Freedman, R.; Law, A.J. Male fetus susceptibility to maternal inflammation: C-reactive protein and brain development. *Psychol. Med.* **2021**, *51*, 450–459, doi:10.1017/S0033291719003313.

51. Monaco-Brown, M.; Lawrence, D.A. Obesity and Maternal-Placental-Fetal Immunology and Health. *Front. Pediatr.* **2022**, *10*, 591, doi:10.3389/fped.2022.859885.

52. Ginhoux, F.; Greter, M.; Leboeuf, M.; Nandi, S.; See, P.; Gokhan, S.; Mehler, M.F.; Conway, S.J.; Ng, L.G.; Stanley, E.R.; et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **2010**, *330*, 841–845, doi:10.1126/SCIENCE.1194637.

53. Rezaie, P.; Male, D. Colonisation of the developing human brain and spinal cord by microglia: A review. *Microsc. Res. Tech.* 1999, *45*, 359–382.

54. Neher, J.J.; Cunningham, C. Priming Microglia for Innate Immune Memory in the Brain. *Trends Immunol.* 2019, *40*, 358–374.

55. Kang, S.S.; Kurti, A.; Fair, D.A.; Fryer, J.D. Dietary intervention rescues maternal obesity induced behavior deficits and neuroinflammation in offspring. *J. Neuroinflammation* **2014**, *11*, doi:10.1186/s12974-014-0156-9.

56. Sasaki, A.; de Vega, W.C.; St-Cyr, S.; Pan, P.; McGowan, P.O. Perinatal high fat diet alters glucocorticoid signaling and anxiety behavior in adulthood. *Neuroscience* **2013**, *240*, 1–12, doi:10.1016/j.neuroscience.2013.02.044.

57. Sasaki, A.; de Vega, W.; Sivanathan, S.; St-Cyr, S.; McGowan, P. Maternal high-fat diet alters anxiety behavior and glucocorticoid signaling in adolescent offspring. *Neuroscience* **2014**, *272*, 92–101, doi:10.1016/j.neuroscience.2014.04.012.

58. Winther, G.; Elfving, B.; Müller, H.K.; Lund, S.; Wegener, G. Maternal High-fat Diet Programs Offspring

Emotional Behavior in Adulthood. *Neuroscience* **2018**, *388*, 87–101, doi:10.1016/j.neuroscience.2018.07.014.

59. Wijenayake, S.; Rahman, M.F.; Lum, C.M.W.; De Vega, W.C.; Sasaki, A.; McGowan, P.O. Maternal high-fat diet induces sex-specific changes to glucocorticoid and inflammatory signaling in response to corticosterone and lipopolysaccharide challenge in adult rat offspring. *J. Neuroinflammation* **2020**, *17*, doi:10.1186/s12974-020-01798-1.

60. Rinninella, E.; Raoul, P.; Cintoni, M.; Franceschi, F.; Miggiano, G.A.D.; Gasbarrini, A.; Mele, M.C. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms* **2019**, *7*, 14, doi:10.3390/microorganisms7010014.

61. Rocha-Gomes, A.; Teixeira, A.E.; de Oliveira, D.G.; Santiago, C.M.O.; da Silva, A.A.; Riul, T.R.; Lacerda, A.C.R.; Mendonça, V.A.; Rocha-Vieira, E.; Leite, H.R. LPS tolerance prevents anxiety-like behavior and amygdala inflammation of high-fat-fed dams' adolescent offspring. *Behav. Brain Res.* **2021**, *411*, doi:10.1016/j.bbr.2021.113371.

62. Huang, C.F.; Du, J.X.; Deng, W.; Cheng, X.C.; Zhang, S.Y.; Zhao, S.J.; Tao, M.J.; Chen, G.Z.; Hao, X.Q. Effect of prenatal exposure to LPS combined with pre- and post-natal high-fat diet on hippocampus in rat offspring. *Neuroscience* **2015**, *286*, 364–370, doi:10.1016/j.neuroscience.2014.12.002.

63. Ajmone-Cat, M.A.; D'Urso, M.C.; di Blasio, G.; Brignone, M.S.; De Simone, R.; Minghetti, L. Glycogen synthase kinase 3 is part of the molecular machinery regulating the adaptive response to LPS stimulation in microglial cells. *Brain. Behav. Immun.* **2016**, *55*, 225–235, doi:10.1016/J.BBI.2015.11.012.

64. Graf, A.E.; Lallier, S.W.; Waidyaratne, G.; Thompson, M.D.; Tipple, T.E.; Hester, M.E.; Trask, A.J.; Rogers, L.K. Maternal high fat diet exposure is associated with increased hepcidin levels, decreased myelination, and neurobehavioral changes in male offspring. *Brain. Behav. Immun.* **2016**, *58*, 369–378, doi:10.1016/j.bbi.2016.08.005.

65. Bordeleau, M.; Fernández de Cossío, L.; Lacabanne, C.; Savage, J.C.; Vernoux, N.; Chakravarty, M.; Tremblay, M.-È. Maternal high-fat diet modifies myelin organization, microglial interactions, and results in social memory and sensorimotor gating deficits in adolescent mouse offspring. *Brain, Behav. Immun. - Heal.* **2021**, *15*, 100281, doi:10.1016/j.bbih.2021.100281.

66. Hasebe, K.; Kendig, M.D.; Morris, M.J. Mechanisms underlying the cognitive and behavioural effects of maternal obesity. *Nutrients* 2021, *13*, 1–24.

67. Maldonado-Ruiz, R.; Garza-Ocañas, L.; Camacho, A. Inflammatory domains modulate autism spectrum disorder susceptibility during maternal nutritional programming. *Neurochem. Int.* 2019, *126*, 109–117.

68. Kim, D.W.; Glendining, K.A.; Grattan, D.R.; Jasoni, C.L. Maternal obesity leads to increased proliferation and numbers of astrocytes in the developing fetal and neonatal mouse hypothalamus. *Int. J. Dev. Neurosci.* **2016**, *53*, 18–25, doi:10.1016/j.ijdevneu.2016.06.005.

69. Hughes, H.K.; Rose, D.; Ashwood, P. The Gut Microbiota and Dysbiosis in Autism Spectrum Disorders. *Curr. Neurol. Neurosci. Rep.* **2018**, *18*, doi:10.1007/S11910-018-0887-6.

70. Yu, L.W.; Agirman, G.; Hsiao, E.Y. The Gut Microbiome as a Regulator of the Neuroimmune Landscape. *Annu. Rev. Immunol.* 2022, *40*, 143–167.

71. Angelucci, F.; Cechova, K.; Amlerova, J.; Hort, J. Antibiotics, gut microbiota, and Alzheimer's disease. *J. Neuroinflammation 2019 161* **2019**, *16*, 1–10, doi:10.1186/S12974-019-1494-4.

72. O'Hara, A.M.; Shanahan, F. The gut flora as a forgotten organ. *EMBO Rep.* 2006, *7*, 688–693.

73. Vuong, H.E.; Pronovost, G.N.; Williams, D.W.; Coley, E.J.L.; Siegler, E.L.; Qiu, A.; Kazantsev, M.; Wilson, C.J.; Rendon, T.; Hsiao, E.Y. The maternal microbiome modulates fetal neurodevelopment in mice. *Nature* **2020**, *586*, 281–286, doi:10.1038/S41586-020-2745-3.

74. Codagnone, M.G.; Spichak, S.; O'Mahony, S.M.; O'Leary, O.F.; Clarke, G.; Stanton, C.; Dinan, T.G.; Cryan, J.F. Programming Bugs: Microbiota and the Developmental Origins of Brain Health and Disease. *Biol. Psychiatry* 2019, *85*, 150–163.

75. Daliry, A.; Pereira, E.N.G. da S. Role of maternal microbiota and nutrition in early-life neurodevelopmental disorders. *Nutrients* 2021, *13*.

76. Aatsinki, A.-K.; Uusitupa, H.-M.; Munukka, E.; Pesonen, H.; Rintala, A.; Pietilä, S.; Lahti, L.; Eerola, E.;

Karlsson, L.; Karlsson, H. Gut Microbiota Composition in Mid-Pregnancy Is Associated with Gestational Weight Gain but Not Prepregnancy Body Mass Index. **2018**, doi:10.1089/jwh.2017.6488.

77. Turjeman, S.; Collado, M.C.; Koren, O. The gut microbiome in pregnancy and pregnancy complications. *Curr. Opin. Endocr. Metab. Res.* 2021, *18*, 133–138.

78. D'Argenio, V. The Prenatal Microbiome: A New Player for Human Health. *High-throughput* **2018**, *7*, doi:10.3390/HT7040038.

79. Miko, E.; Csaszar, A.; Bodis, J.; Kovacs, K. The Maternal–Fetal Gut Microbiota Axis: Physiological Changes, Dietary Influence, and Modulation Possibilities. *Life* 2022, *12*, 424.

80. Bodden, C.; Hannan, A.J.; Reichelt, A.C. Of 'junk food' and 'brain food': how parental diet influences offspring neurobiology and behaviour. *Trends Endocrinol. Metab.* 2021, *32*, 566–578.

81. Buffington, S.A.; Di Prisco, G.V.; Auchtung, T.A.; Ajami, N.J.; Petrosino, J.F.; Costa-Mattioli, M. Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell* **2016**, *165*, 1762–1775, doi:10.1016/j.cell.2016.06.001.

82. Alsharairi, N.A. The role of short-chain fatty acids in the interplay between a very low-calorie ketogenic diet and the infant gut microbiota and its therapeutic implications for reducing asthma. *Int. J. Mol. Sci.* 2020, *21*, 1–21.

83. Alsharairi, N.A. The role of short-chain fatty acids in mediating very low-calorie ketogenic diet-infant gut microbiota relationships and its therapeutic potential in obesity. *Nutrients* 2021, *13*.

84. Al Rubaye, H.; Adamson, C.C.; Jadavji, N.M. The role of maternal diet on offspring gut microbiota development: A review. *J. Neurosci. Res.* 2021, *99*, 284–293.

85. Ferguson, J. Maternal microbial molecules affect offspring health. *Science (80-.).* 2020, *367*, 978–979.

86. Kimura, I.; Miyamoto, J.; Ohue-Kitano, R.; Watanabe, K.; Yamada, T.; Onuki, M.; Aoki, R.; Isobe, Y.; Kashihara, D.; Inoue, D.; et al. Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. *Science (80-.).* **2020**, *367*, doi:10.1126/science.aaw8429.

87. Cowan, M.; Petri, W.A. Microglia: Immune regulators of neurodevelopment. *Front. Immunol.* 2018, *9*, 2576.

88. Abdel-Haq, R.; Schlachetzki, J.C.M.; Glass, C.K.; Mazmanian, S.K. Microbiome-microglia connections via the gut-brain axis. *J. Exp. Med.* **2019**, *216*, 41–59, doi:10.1084/JEM.20180794.

89. Castillo-Ruiz, A.; Mosley, M.; George, A.J.; Mussaji, L.F.; Fullerton, E.F.; Ruszkowski, E.M.; Jacobs, A.J.; Gewirtz, A.T.; Chassaing, B.; Forger, N.G. The microbiota influences cell death and microglial colonization in the perinatal mouse brain. *Brain. Behav. Immun.* **2018**, *67*, 218, doi:10.1016/J.BBI.2017.08.027.

90. Dominguez-Bello, M.G.; Costello, E.K.; Contreras, M.; Magris, M.; Hidalgo, G.; Fierer, N.; Knight, R. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 11971–11975, doi:10.1073/pnas.1002601107.

91. Tenenbaum-Gavish, K.; Hod, M. Impact of maternal obesity on fetal health. *Fetal Diagn. Ther.* 2013, *34*, 1–7.

92. Jeyabalan, A. Epidemiology of preeclampsia: Impact of obesity. *Nutr. Rev.* **2013**, *71*, doi:10.1111/nure.12055.

93. Berry, A.; Cirulli, F. High-Fat Diet and Foetal Programming: Use of P66Shc Knockouts and Implications for Human Kind. In *Diet, Nutrition, and Fetal Programming*; Humana Press, Cham, 2017; pp. 557–568.

94. Thangaratinam, S.; Rogozińska, E.; Jolly, K.; Glinkowski, S.; Roseboom, T.; Tomlinson, J.W.; Kunz, R.; Mol, B.W.; Coomarasamy, A.; Khan, K.S. Effects of interventions in pregnancy on maternal weight and obstetric outcomes: Meta-analysis of randomised evidence. *BMJ* **2012**, *344*, doi:10.1136/bmj.e2088.

95. Bruno, R.; Petrella, E.; Bertarini, V.; Pedrielli, G.; Neri, I.; Facchinetti, F. Adherence to a lifestyle programme in overweight/obese pregnant women and effect on gestational diabetes mellitus: a randomized controlled trial. *Matern. Child Nutr.* **2017**, *13*, 1–11, doi:10.1111/mcn.12333.

96. Shrestha, A.; Prowak, M.; Berlandi-Short, V.-M.; Garay, J.; Ramalingam, L. Maternal Obesity: A Focus on Maternal Interventions to Improve Health of Offspring. *Front. Cardiovasc. Med.* **2021**, *8*, 696812, doi:10.3389/fcvm.2021.696812.

97. Vidakovic, A.J.; Jaddoe, V.W.V.; Gishti, O.; Felix, J.F.; Williams, M.A.; Hofman, A.; Demmelmair, H.; Koletzko, B.; Tiemeier, H.; Gaillard, R. Body mass index, gestational weight gain and fatty acid concentrations

during pregnancy: the Generation R Study. *Eur. J. Epidemiol.* **2015**, *30*, 1175–1185, doi:10.1007/s10654-015-0106-6.

98. Benaim, C.; Freitas-Vilela, A.A.; Pinto, T. de J.P.; Lepsch, J.; Farias, D.R.; dos Santos Vaz, J.; El-Bacha, T.; Kac, G. Early pregnancy body mass index modifies the association of pre-pregnancy dietary patterns with serum polyunsaturated fatty acid concentrations throughout pregnancy in Brazilian women. *Matern. Child Nutr.* **2018**, *14*, doi:10.1111/mcn.12480.

99. Joffre, C.; Grégoire, S.; De Smedt, V.; Acar, N.; Bretillon, L.; Nadjar, A.; Layé, S. Modulation of brain PUFA content in different experimental models of mice. *Prostaglandins Leukot. Essent. Fat. Acids* **2016**, *114*, 1–10, doi:10.1016/j.plefa.2016.09.003.

100. Sakayori, N.; Kikkawa, T.; Tokuda, H.; Kiryu, E.; Yoshizaki, K.; Kawashima, H.; Yamada, T.; Arai, H.; Kang, J.X.; Katagiri, H.; et al. Maternal dietary imbalance between omega-6 and omega-3 polyunsaturated fatty acids impairs neocortical development via epoxy metabolites. *Stem Cells* **2016**, *34*, 470–482, doi:10.1002/stem.2246.

101. Powell, T.L.; Barner, K.; Madi, L.; Armstrong, M.; Manke, J.; Uhlson, C.; Jansson, T.; Ferchaud-Roucher, V. Sex-specific responses in placental fatty acid oxidation, esterification and transfer capacity to maternal obesity. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **2021**, *1866*, doi:10.1016/j.bbalip.2020.158861.

102. Mao, J.; Zhang, X.; Sieli, P.T.; Falduto, M.T.; Torres, K.E.; Rosenfeld, C.S. Contrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 5557–5562, doi:10.1073/pnas.1000440107.

103. Penfield-Cyr, A.; Monthe-Dreze, C.; Smid, M.C.; Sen, S. Maternal BMI, Mid-pregnancy Fatty Acid Concentrations, and Perinatal Outcomes. *Clin. Ther.* **2018**, *40*, 1659-1667.e1, doi:10.1016/j.clinthera.2018.08.011.

104. Tylek, K.; Trojan, E.; Leśkiewicz, M.; Regulska, M.; Bryniarska, N.; Curzytek, K.; Lacivita, E.; Leopoldo, M.; Basta-Kaim, A. Time-dependent protective and pro-resolving effects of fpr2 agonists on lipopolysaccharide-exposed microglia cells involve inhibition of nf-κb and mapks pathways. *Cells* **2021**, *10*, doi:10.3390/cells10092373.

105. Gorica, E.; Calderone, V. Arachidonic Acid Derivatives and Neuroinflammation. *CNS Neurol. Disord. - Drug Targets* **2021**, *21*, 118–129, doi:10.2174/1871527320666210208130412.

106. Wang, B.; Wu, L.; Chen, J.; Dong, L.; Chen, C.; Wen, Z.; Hu, J.; Fleming, I.; Wang, D.W. Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets. *Signal Transduct. Target. Ther.* 2021, *6*.

107. Ahme, O.S.; Galano, J.M.; Pavlickova, T.; Revol-Cavalier, J.; Vigor, C.; Lee, J.C.Y.; Oger, C.; Durand, T. Moving forward with isoprostanes, neuroprostanes and phytoprostanes: Where are we now? *Essays Biochem.* 2020, *64*, 463–484.

108. Belcastro, L.; Ferreira, C.S.; Saraiva, M.A.; Mucci, D.B.; Murgia, A.; Lai, C.; Vigor, C.; Oger, C.; Galano, J.M.; Pinto, G.D.A.; et al. Decreased fatty acid transporter fabp1 and increased isoprostanes and neuroprostanes in the human term placenta: Implications for inflammation and birth weight in maternal pre-gestational obesity. *Nutrients* **2021**, *13*, doi:10.3390/nu13082768.

109. Heerwagen, M.J.R.; Stewart, M.S.; de la Houssaye, B.A.; Janssen, R.C.; Friedman, J.E. Transgenic Increase in N-3/N-6 Fatty Acid Ratio Reduces Maternal Obesity-Associated Inflammation and Limits Adverse Developmental Programming in Mice. *PLoS One* **2013**, *8*, doi:10.1371/journal.pone.0067791.

110. Alvarado, F.L.; Calabuig-Navarro, V.; Haghiac, M.; Puchowicz, M.; Tsai, P.J.S.; O'Tierney-Ginn, P. Maternal obesity is not associated with placental lipid accumulation in women with high omega-3 fatty acid levels. *Placenta* **2018**, *69*, 96–101, doi:10.1016/J.PLACENTA.2018.07.016.

111. Lager, S.; Ramirez, V.I.; Acosta, O.; Meireles, C.; Miller, E.; Gaccioli, F.; Rosario, F.J.; Gelfond, J.A.L.; Hakala, K.; Weintraub, S.T.; et al. Docosahexaenoic acid supplementation in pregnancy modulates placental cellular signaling and nutrient transport capacity in obese women. *J. Clin. Endocrinol. Metab.* **2017**, *102*, 4557–4567, doi:10.1210/jc.2017-01384.

112. Labrousse, V.F.; Leyrolle, Q.; Amadieu, C.; Aubert, A.; Sere, A.; Coutureau, E.; Grégoire, S.; Bretillon, L.; Pallet, V.; Gressens, P.; et al. Dietary omega-3 deficiency exacerbates inflammation and reveals spatial memory deficits in mice exposed to lipopolysaccharide during gestation. *Brain. Behav. Immun.* **2018**, *73*, 427–440, doi:10.1016/j.bbi.2018.06.004.

113. Madore, C.; Nadjar, A.; Delpech, J.C.; Sere, A.; Aubert, A.; Portal, C.; Joffre, C.; Layé, S. Nutritional n-3 PUFAs deficiency during perinatal periods alters brain innate immune system and neuronal plasticity-associated genes. *Brain. Behav. Immun.* **2014**, *41*, 22–31, doi:10.1016/j.bbi.2014.03.021.

114. Rey, C.; Delpech, J.C.; Madore, C.; Nadjar, A.; Greenhalgh, A.D.; Amadieu, C.; Aubert, A.; Pallet, V.; Vaysse, C.; Layé, S.; et al. Dietary n-3 long chain PUFA supplementation promotes a pro-resolving oxylipin profile in the brain. *Brain. Behav. Immun.* **2019**, *76*, 17–27, doi:10.1016/j.bbi.2018.07.025.

115. Yang, B.; Yang, B.; Li, R.; Liu, P.N.; Geng, X.; Mooney, B.P.; Mooney, B.P.; Chen, C.; Cheng, J.; Fritsche, K.L.; et al. Quantitative Proteomics Reveals Docosahexaenoic Acid-Mediated Neuroprotective Effects in Lipopolysaccharide-Stimulated Microglial Cells. *J. Proteome Res.* **2020**, *19*, 2236–2246, doi:10.1021/acs.jproteome.9b00792.

116. Layé, S.; Nadjar, A.; Joffre, C.; Bazinet, R.P. Anti-inflammatory effects of omega-3 fatty acids in the brain: Physiological mechanisms and relevance to pharmacology. *Pharmacol. Rev.* **2018**, *70*, 12–38, doi:10.1124/pr.117.014092.

117. Fourrier, C.; Remus-Borel, J.; Greenhalgh, A.D.; Guichardant, M.; Bernoud-Hubac, N.; Lagarde, M.; Joffre, C.; Layé, S. Docosahexaenoic acid-containing choline phospholipid modulates LPS-induced neuroinflammation in vivo and in microglia in vitro. *J. Neuroinflammation* **2017**, *14*, doi:10.1186/s12974-017-0939-x.

118. Hao, S.; Dey, A.; Yu, X.; Stranahan, A.M. Dietary obesity reversibly induces synaptic stripping by microglia and impairs hippocampal plasticity. *Brain. Behav. Immun.* **2016**, *51*, 230–239, doi:10.1016/j.bbi.2015.08.023.

119. Tremblay, M.E.; Zhang, I.; Bisht, K.; Savage, J.C.; Lecours, C.; Parent, M.; Titorenko, V.; Maysinger, D. Remodeling of lipid bodies by docosahexaenoic acid in activated microglial cells. *J. Neuroinflammation* **2016**, *13*, doi:10.1186/s12974-016-0580-0.

120. Chang, P.K.Y.; Khatchadourian, A.; McKinney, A.A.; Maysinger, D. Docosahexaenoic acid (DHA): a modulator of microglia activity and dendritic spine morphology. *J. Neuroinflammation* **2015**, *12*, doi:10.1186/S12974-015-0244-5.

121. Ajmone-Cat, M.A.; Salvatori, M.L.; de Simone, R.; Mancini, M.; Biagioni, S.; Bernardo, A.; Cacci, E.; Minghetti, L. Docosahexaenoic acid modulates inflammatory and antineurogenic functions of activated microglial cells. *J. Neurosci. Res.* **2012**, *90*, 575–587, doi:10.1002/JNR.22783.

122. De Smedt-Peyrusse, V.; Sargueil, F.; Moranis, A.; Harizi, H.; Mongrand, S.; Layé, S. Docosahexaenoic acid prevents lipopolysaccharide-induced cytokine production in microglial cells by inhibiting lipopolysaccharide receptor presentation but not its membrane subdomain localization. *J. Neurochem.* **2008**, *105*, 296–307, doi:10.1111/j.1471-4159.2007.05129.x.

123. Madore, C.; Leyrolle, Q.; Morel, L.; Rossitto, M.; Greenhalgh, A.D.; Delpech, J.C.; Martinat, M.; Bosch-Bouju, C.; Bourel, J.; Rani, B.; et al. Essential omega-3 fatty acids tune microglial phagocytosis of synaptic elements in the mouse developing brain. *Nat. Commun.* **2020**, *11*, doi:10.1038/s41467-020-19861-z.

124. Basak, S.; Mallick, R.; Banerjee, A.; Pathak, S.; Duttaroy, A.K. Maternal supply of both arachidonic and docosahexaenoic acids is required for optimal neurodevelopment. *Nutrients* 2021, *13*.

125. Lauritzen, L.; Brambilla, P.; Mazzocchi, A.; Harsløf, L.B.S.; Ciappolino, V.; Agostoni, C. DHA effects in brain development and function. *Nutrients* 2016, *8*.

126. Hadley, K.B.; Ryan, A.S.; Forsyth, S.; Gautier, S.; Salem, N. The essentiality of arachidonic acid in infant development. *Nutrients* 2016, *8*.

127. Swanson, D.; Block, R.; Mousa, S.A. Omega-3 fatty acids EPA and DHA: Health benefits throughout life. *Adv. Nutr.* 2012, *3*, 1–7.

128. Bernardo, A.; Giammarco, M.L.; De Nuccio, C.; Ajmone-Cat, M.A.; Visentin, S.; De Simone, R.; Minghetti, L. Docosahexaenoic acid promotes oligodendrocyte differentiation via PPAR-γ signalling and prevents tumor necrosis factor-α-dependent maturational arrest. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **2017**, *1862*, 1013–1023, doi:10.1016/j.bbalip.2017.06.014.

129. Belayev, L.; Hong, S.H.; Freitas, R.S.; Menghani, H.; Marcell, S.J.; Khoutorova, L.; Mukherjee, P.K.; Reid, M.M.; Oria, R.B.; Bazan, N.G. DHA modulates MANF and TREM2 abundance, enhances neurogenesis, reduces infarct size, and improves neurological function after experimental ischemic stroke. *CNS Neurosci. Ther.* **2020**, *26*, 1155–1167, doi:10.1111/cns.13444.

130. Darios, F.; Davletov, B. Omega-3 and omega-6 fatty acids stimulate cell membrane expansion by acting on syntaxin 3. *Nature* **2006**, *440*, 813–817, doi:10.1038/nature04598.

131. Das, M.; Das, S. Docosahexaenoic Acid (DHA) Induced Morphological Differentiation of Astrocytes Is Associated with Transcriptional Upregulation and Endocytosis of β 2 -AR. *Mol. Neurobiol.* **2019**, *56*, 2685–2702, doi:10.1007/s12035-018-1260-0.

132. Hamazaki, K.; Itomura, M.; Huan, M.; Nishizawa, H.; Sawazaki, S.; Tanouchi, M.; Watanabe, S.; Hamazaki, T.; Terasawa, K.; Yazawa, K. Effect of ω-3 fatty acid-containing phospholipids on blood catecholamine concentrations in healthy volunteers: A randomized, placebo-controlled, double-blind trial. *Nutrition* **2005**, *21*, 705–710, doi:10.1016/j.nut.2004.07.020.

133. Kawakita, E.; Hashimoto, M.; Shido, O. Docosahexaenoic acid promotes neurogenesis in vitro and in vivo. *Neuroscience* **2006**, *139*, 991–997, doi:10.1016/j.neuroscience.2006.01.021.

134. Tokuda, H.; Kontani, M.; Kawashima, H.; Kiso, Y.; Shibata, H.; Osumi, N. Differential effect of arachidonic acid and docosahexaenoic acid on age-related decreases in hippocampal neurogenesis. *Neurosci. Res.* **2014**, *88*, 58–66, doi:10.1016/j.neures.2014.08.002.

135. Zhang, J.; Yi, C.; Han, J.; Ming, T.; Zhou, J.; Lu, C.; Li, Y.; Su, X. Novel high-docosahexaenoic-acid tuna oil supplementation modulates gut microbiota and alleviates obesity in high-fat diet mice. *Food Sci. Nutr.* **2020**, *8*, 6513–6527, doi:10.1002/fsn3.1941.

136. Noriega, B.S.; Sanchez-Gonzalez, M.A.; Salyakina, D.; Coffman, J. Understanding the Impact of Omega-3 Rich Diet on the Gut Microbiota. *Case Rep. Med.* **2016**, *2016*, doi:10.1155/2016/3089303.

137. Kaliannan, K.; Wang, B.; Li, X.Y.; Kim, K.J.; Kang, J.X. A host-microbiome interaction mediates the opposing effects of omega-6 and omega-3 fatty acids on metabolic endotoxemia. *Sci. Rep.* **2015**, *5*, 1–17, doi:10.1038/srep11276.

138. Yu, H.N.; Zhu, J.; Pan, W. sheng; Shen, S.R.; Shan, W.G.; Das, U.N. Effects of Fish Oil with a High Content of n-3 Polyunsaturated Fatty Acids on Mouse Gut Microbiota. *Arch. Med. Res.* **2014**, *45*, 195–202, doi:10.1016/j.arcmed.2014.03.008.

139. Yu, H.; Fang, C.; Li, P.; Wu, M.; Shen, S. The relevance of DHA with modulating of host-gut microbiome signatures alterations and repairing of lipids metabolism shifts. *Eur. J. Pharmacol.* **2021**, *895*, 173885, doi:10.1016/J.EJPHAR.2021.173885.

140. Tang, Q.; Li, S.; Fang, C.; Yu, H. Evaluating the reparative effects and the mechanism of action of docosahexaenoic acid on azithromycin-induced lipid metabolism dysfunction. *Food Chem. Toxicol.* **2022**, *159*, 112699, doi:10.1016/j.fct.2021.112699.

141. Vahdaninia, M.; Mackenzie, H.; Dean, T.; Helps, S. The effectiveness of ω-3 polyunsaturated fatty acid interventions during pregnancy on obesity measures in the offspring: an up-to-date systematic review and meta-analysis. *Eur. J. Nutr.* 2019, *58*, 2597–2613.

142. Monthé-Drèze, C.; Sen, S.; Mouzon, S.H. De; Catalano, P.M. Effect of omega-3 supplementation in pregnant women with obesity on newborn body composition, growth and length of gestation: A randomized controlled pilot study. *Nutrients* **2021**, *13*, 1–19, doi:10.3390/nu13020578.

143. Brink, L.R.; Lönnerdal, B. Milk fat globule membrane: the role of its various components in infant health and development. *J. Nutr. Biochem.* **2020**, *85*, 1–14, doi:10.1016/j.jnutbio.2020.108465.

144. Kosmerl, E.; Rocha-Mendoza, D.; Ortega-Anaya, J.; Jiménez-Flores, R.; García-Cano, I. Improving human health with milk fat globule membrane, lactic acid bacteria, and bifidobacteria. *Microorganisms* 2021, *9*, 1–22.

145. Mudd, A.T.; Alexander, L.S.; Berding, K.; Waworuntu, R. V.; Berg, B.M.; Donovan, S.M.; Dilger, R.N. Dietary prebiotics, milk fat globule membrane, and lactoferrin affects structural neurodevelopment in the young piglet. *Front. Pediatr.* **2016**, *4*, doi:10.3389/fped.2016.00004.

146. Vickers, M.H.; Guan, J.; Gustavsson, M.; Krägeloh, C.U.; Breier, B.H.; Davison, M.; Fong, B.; Norris, C.; McJarrow, P.; Hodgkinson, S.C. Supplementation with a mixture of complex lipids derived from milk to growing rats results in improvements in parameters related to growth and cognition. *Nutr. Res.* **2009**, *29*, 426–435, doi:10.1016/j.nutres.2009.06.001.

147. Moukarzel, S.; Dyer, R.A.; Garcia, C.; Wiedeman, A.M.; Boyce, G.; Weinberg, J.; Keller, B.O.; Elango, R.; Innis,

S.M. Milk Fat Globule Membrane Supplementation in Formula-fed Rat Pups Improves Reflex Development and May Alter Brain Lipid Composition. *Sci. Rep.* **2018**, *8*, doi:10.1038/s41598-018-33603-8.

148. O'Mahony, S.M.; McVey Neufeld, K.A.; Waworuntu, R. V.; Pusceddu, M.M.; Manurung, S.; Murphy, K.; Strain, C.; Laguna, M.C.; Peterson, V.L.; Stanton, C.; et al. The enduring effects of early-life stress on the microbiota–gut–brain axis are buffered by dietary supplementation with milk fat globule membrane and a prebiotic blend. *Eur. J. Neurosci.* **2020**, *51*, 1042–1058, doi:10.1111/ejn.14514.

149. Collins, J.M.; Caputi, V.; Manurung, S.; Gross, G.; Fitzgerald, P.; Golubeva, A. V.; Popov, J.; Deady, C.; Dinan, T.G.; Cryan, J.F.; et al. Supplementation with milk fat globule membrane from early life reduces maternal separation-induced visceral pain independent of enteric nervous system or intestinal permeability changes in the rat. *Neuropharmacology* **2022**, *210*, doi:10.1016/j.neuropharm.2022.109026.

150. Snow, D.R.; Ward, R.E.; Olsen, A.; Jimenez-Flores, R.; Hintze, K.J. Membrane-rich milk fat diet provides protection against gastrointestinal leakiness in mice treated with lipopolysaccharide. *J. Dairy Sci.* **2011**, *94*, 2201–2212, doi:10.3168/jds.2010-3886.

151. Huang, S.; Wu, Z.; Liu, C.; Han, D.; Feng, C.; Wang, S.; Wang, J.; Song, D. Milk fat globule membrane supplementation promotes neonatal growth and alleviates inflammation in low-birth-weight mice treated with lipopolysaccharide. *Biomed Res. Int.* **2019**, *2019*, doi:10.1155/2019/4876078.

152. Arnoldussen, I.A.C.; Morrison, M.C.; Wiesmann, M.; van Diepen, J.A.; Worms, N.; Voskuilen, M.; Verweij, V.; Geenen, B.; Gualdo, N.P.; van der Logt, L.; et al. Milk fat globule membrane attenuates high fat diet-induced neuropathological changes in obese Ldlr−/−.Leiden mice. *Int. J. Obes.* **2022**, *46*, 342–349, doi:10.1038/s41366-021-00998-w.

153. Gustavsson, M.; Hodgkinson, S.C.; Fong, B.; Norris, C.; Guan, J.; Krageloh, C.U.; Breier, B.H.; Davison, M.; McJarrow, P.; Vickers, M.H. Maternal supplementation with a complex milk lipid mixture during pregnancy and lactation alters neonatal brain lipid composition but lacks effect on cognitive function in rats. *Nutr. Res.* **2010**, *30*, 279–289, doi:10.1016/j.nutres.2010.04.005.

154. Mitchell, M.D.; Henare, K.; Balakrishnan, B.; Lowe, E.; Fong, B.Y.; McJarrow, P. Transfer of gangliosides across the human placenta. *Placenta* **2012**, *33*, 312–316, doi:10.1016/j.placenta.2011.12.018.

155. Huang, S.; Mo, T.T.; Norris, T.; Sun, S.; Zhang, T.; Han, T.L.; Rowan, A.; Xia, Y.Y.; Zhang, H.; Qi, H.B.; et al. The CLIMB (Complex Lipids in Mothers and Babies) study: Protocol for a multicentre, three-group, parallel randomised controlled trial to investigate the effect of supplementation of complex lipids in pregnancy, on maternal ganglioside status and subsequen. *BMJ Open* **2017**, *7*, doi:10.1136/bmjopen-2017-016637.

156. Norris, T.; Souza, R.; Xia, Y.; Zhang, T.; Rowan, A.; Gallier, S.; Zhang, H.; Qi, H.; Baker, P. Effect of supplementation of complex milk lipids in pregnancy on fetal growth: results from the Complex Lipids in Mothers and Babies (CLIMB) randomized controlled trial. *J. Matern. Neonatal Med.* **2021**, *34*, 3313–3322, doi:10.1080/14767058.2019.1683539.

157. Yuan, Q. chen; Gong, H.; Du, M.; Mao, X. ying Supplementation of milk polar lipids to obese dams improves neurodevelopment and cognitive function in male offspring. *FASEB J.* **2021**, *35*, doi:10.1096/fj.202001974RRR.

158. Li, T.; Gong, H.; Yuan, Q.; Du, M.; Ren, F.; Mao, X. Supplementation of polar lipids-enriched milk fat globule membrane in high-fat diet-fed rats during pregnancy and lactation promotes brown/beige adipocyte development and prevents obesity in male offspring. *FASEB J.* **2020**, *34*, 4619–4634, doi:10.1096/fj.201901867RRR.

159. Li, T.; Yuan, Q.; Gong, H.; Du, M.; Mao, X. Gut microbiota mediates the alleviative effect of polar lipids-enriched milk fat globule membrane on obesity-induced glucose metabolism disorders in peripheral tissues in rat dams. *Int. J. Obes.* **2022**, *46*, 793–801, doi:10.1038/s41366-021-01029-4.

160. Milard, M.; Laugerette, F.; Durand, A.; Buisson, C.; Meugnier, E.; Loizon, E.; Louche-Pelissier, C.; Sauvinet, V.; Garnier, L.; Viel, S.; et al. Milk Polar Lipids in a High-Fat Diet Can Prevent Body Weight Gain: Modulated Abundance of Gut Bacteria in Relation with Fecal Loss of Specific Fatty Acids. *Mol. Nutr. Food Res.* **2019**, *63*, doi:10.1002/mnfr.201801078.

161. Demmer, E.; Van Loan, M.D.; Rivera, N.; Rogers, T.S.; Gertz, E.R.; Bruce German, J.; Smilowitz, J.T.; Zivkovic, A.M. Addition of a dairy fraction rich in milk fat globule membrane to a high-saturated fat meal reduces the postprandial insulinaemic and inflammatory response in overweight and obese adults. *J. Nutr. Sci.* **2016**, *5*, doi:10.1017/jns.2015.42.

162. Cirulli, F.; Berry, A. Early Developmental Trajectories of Brain Development: New Directions in the Search for

Early Determinants of Health and Longevity. *Adapt. Mal. Asp. Dev. Stress* **2013**, 211–227, doi:10.1007/978-1-4614-5605-6_10.

163. Berry, A.; Bellisario, V.; Panetta, P.; Raggi, C.; Magnifico, M.C.; Arese, M.; Cirulli, F. Administration of the antioxidant n-acetyl-cysteine in pregnant mice has long-term positive effects on metabolic and behavioral endpoints of male and female offspring prenatally exposed to a high-fat diet. *Front. Behav. Neurosci.* **2018**, *12*, doi:10.3389/fnbeh.2018.00048.

164. Khullar, S.; Greenwood, S.L.; McCord, N.; Glazier, J.D.; Ayuk, P.T.Y. Nitric oxide and superoxide impair human placental amino acid uptake and increase Na+ permeability: Implications for fetal growth. *Free Radic. Biol. Med.* **2004**, *36*, 271–277, doi:10.1016/j.freeradbiomed.2003.11.007.

165. Mokhtari, V.; Afsharian, P.; Shahhoseini, M.; Kalantar, S.M.; Moini, A. A review on various uses of N-acetyl cysteine. *Cell J.* 2017, *19*, 11–17.

166. Oh, S. Il; Park, S.K. N-acetyl-l-cysteine mimics the effect of dietary restriction on lifespan and reduces amyloid beta-induced toxicity in Caenorhabditis elegans. *Food Sci. Biotechnol.* **2017**, *26*, 783–790, doi:10.1007/s10068-017-0079-1.

167. Berry, A.; Cirulli, F. The p66Shc gene paves the way for healthspan: Evolutionary and mechanistic perspectives. *Neurosci. Biobehav. Rev.* 2013, *37*, 790–802.

168. Masschelin, P.M.; Cox, A.R.; Chernis, N.; Hartig, S.M. The Impact of Oxidative Stress on Adipose Tissue Energy Balance. *Front. Physiol.* 2020, *10*.

169. Berniakovich, I.; Trinei, M.; Stendardo, M.; Migliaccio, E.; Minucci, S.; Bernardi, P.; Pelicci, P.G.; Giorgio, M. p66Shc-generated oxidative signal promotes fat accumulation. *J. Biol. Chem.* **2008**, *283*, 34283–34293, doi:10.1074/jbc.M804362200.

170. Motawei, S.M.; Attalla, S.M.; Gouda, H.E.; Harouny, M.A.; Elmansoury, A.M. The effects of N-acetyl cysteine on oxidative stress among patients with pre-eclampsia. *Int. J. Gynecol. Obstet.* **2016**, *135*, 226–227, doi:10.1016/j.ijgo.2016.07.002.

171. Giorgio, M.; Berry, A.; Berniakovich, I.; Poletaeva, I.; Trinei, M.; Stendardo, M.; Hagopian, K.; Ramsey, J.J.; Cortopassi, G.; Migliaccio, E.; et al. The p66Shc knocked out mice are short lived under natural condition. *Aging Cell* **2012**, *11*, 162–168, doi:10.1111/J.1474-9726.2011.00770.X.

172. Kabe, Y.; Ando, K.; Hirao, S.; Yoshida, M.; Handa, H. Redox Regulation of NF-κB Activation: Distinct Redox Regulation Between the Cytoplasm and the Nucleus. *https://home.liebertpub.com/ars* **2005**, *7*, 395–403, doi:10.1089/ARS.2005.7.395.

173. Oka, S.I.; Kamata, H.; Kamata, K.; Yagisawa, H.; Hirata, H. N-Acetylcysteine suppresses TNF-induced NF-κB activation through inhibition of IκB kinases. *FEBS Lett.* **2000**, *472*, 196–202, doi:10.1016/S0014-5793(00)01464-2.

174. Tenório, M.C.D.S.; Graciliano, N.G.; Moura, F.A.; de Oliveira, A.C.M.; Goulart, M.O.F. N-acetylcysteine (Nac): Impacts on human health. *Antioxidants* 2021, *10*.

175. Williams, L.; Burgos, E.S.; Vuguin, P.M.; Manuel, C.R.; Pekson, R.; Munnangi, S.; Reznik, S.E.; Charron, M.J. N-Acetylcysteine Resolves Placental Inflammatory-Vasculopathic Changes in Mice Consuming a High-Fat Diet. *Am. J. Pathol.* **2019**, *189*, 2246–2257, doi:10.1016/j.ajpath.2019.07.010.

176. Paintlia, M.K.; Paintlia, A.S.; Singh, A.K.; Singh, I. Attenuation of lipopolysaccharide-induced inflammatory response and phospholipids metabolism at the feto-maternal interface by N-acetyl cysteine. *Pediatr. Res.* **2008**, *64*, 334–339, doi:10.1203/PDR.0b013e318181e07c.

177. Paintlia, M.K.; Paintlia, A.S.; Barbosa, E.; Singh, I.; Singh, A.K. N-acetylcysteine prevents endotoxin-induced degeneration of oligodendrocyte progenitors and hypomyelination in developing rat brain. *J. Neurosci. Res.* **2004**, *78*, 347–361, doi:10.1002/jnr.20261.

178. López Jaramillo, P.; Arenas, W.D.; García, R.G.; Rincon, M.Y.; López, M. The role of the L-arginine-nitric oxide pathway in preeclampsia. *Ther. Adv. Cardiovasc. Dis.* 2008, *2*, 261–275.

179. Lopez-Jaramillo, P.; Barajas, J.; Rueda-Quijano, S.M.; Lopez-Lopez, C.; Felix, C. Obesity and Preeclampsia: Common Pathophysiological Mechanisms. *Front. Physiol.* 2018, *9*.

180. Tenório, M.B.; Ferreira, R.C.; Moura, F.A.; Bueno, N.B.; De Oliveira, A.C.M.; Goulart, M.O.F. Cross-Talk

between Oxidative Stress and Inflammation in Preeclampsia. *Oxid. Med. Cell. Longev.* 2019, *2019*.

181. Luo, Z.; Xu, X.; Sho, T.; Luo, W.; Zhang, J.; Xu, W.; Yao, J.; Xu, J. Effects of n-acetyl-cysteine supplementation in late gestational diet on maternal-placental redox status, placental NLRP3 inflammasome, and fecal microbiota in sows. *J. Anim. Sci.* **2019**, *97*, 1757–1771, doi:10.1093/jas/skz058.

182. Gaccioli, F.; Aye, I.L.M.H.; Roos, S.; Lager, S.; Ramirez, V.I.; Kanai, Y.; Powell, T.L.; Jansson, T. Expression and functional characterisation of System L amino acid transporters in the human term placenta. *Reprod. Biol. Endocrinol.* **2015**, *13*, doi:10.1186/S12958-015-0054-8.

183. Wu, H.J.; Wu, E. The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes* **2012**, *3*, doi:10.4161/GMIC.19320.

184. Rosenblat, J.D.; McIntyre, R.S. Bipolar Disorder and Immune Dysfunction: Epidemiological Findings, Proposed Pathophysiology and Clinical Implications. *Brain Sci.* **2017**, *7*, doi:10.3390/BRAINSCI7110144.

185. Severance, E.G.; Yolken, R.H.; Eaton, W.W. Autoimmune diseases, gastrointestinal disorders and the microbiome in schizophrenia: more than a gut feeling. *Schizophr. Res.* **2016**, *176*, 23–35, doi:10.1016/J.SCHRES.2014.06.027.

CURRICULUM VITAE

EDUCATION AND TRAINING

Jun 2022 Ph.D. stage at the INRAE, Neurocampus, Bordeaux, France, supported by the "Young Investigator Training Programme" of the Federation of European Neuroscience Societies (FENS).

Apr 2021 - Aug 2021 Ph.D. stage at the University of Zurich, Switzerland, supported by the "NENS Exchange Grant" of the Federation of European Neuroscience Societies (FENS).

Dec 2020 Licensed Professional Biologist, Section A, Università degli Studi della Tuscia, Viterbo, Italy.

Nov 2019 - Jan 2023 Ph.D. student in Behavioural Neuroscience, Department of Psychology, Sapienza University of Rome, in collaboration with the Center for Behavioural Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy.

Oct 2018 Master degree in Neurobiology (110/110 *summa cum laude*), Sapienza University of Rome, Italy.

Oct 2016 Bachelor degree in Biological Sciences (110/110 *summa cum laude*), Sapienza University of Rome, Italy.

GRANTS, AWARDS, FELLOWSHIPS

Jul 2022 Travel Award to attend the FENS Forum 2022, funded by Atlas Antibodies.

Jun 2022 Fellowship "Young Investigator Training Programme" - funded by the Federation of European Neuroscience Societies (FENS).

2022 - 2023 Starting Grant for Outreach - Collaborator - funded by Sapienza University of Rome. Title project: AllenaMente: potenziare la memoria tramite l'attività sportiva

2021 - 2022 Starting Grant for Research - Principal Investigator - funded by Sapienza University of Rome. Title project: Alterations in the insulin/IGF signal in placenta and fetal brain as a risk factor for neurodevelopmental disorders: a targeted transcriptomic approach in a mouse model of maternal obesity.

2021 Travel Award to attend the 49th Meeting of the European Brain and Behaviour Society (EBBS), funded by EBBS.

Oct 2020 NENS Exchange Grant - funded by the Federation of European Neuroscience Societies (FENS).

Nov 2019 Ph.D. Fellowship, Behavioural Neuroscience Ph.D. Program, Department of Psychology, Sapienza University of Rome.

2019 Travel Award to attend the FENS Forum 2020, funded by EBBS.

2019 Best poster award SINS meeting of Ph.D. students in Neuroscience.

2014 Collaboration Scholarship, Department of Environmental Biology, Sapienza University of Rome.

PUBLICATIONS

2022 Cirulli F., De Simone R., **Musillo C.**, Ajmone-Cat M. A., Berry A. Inflammatory Signatures of Maternal Obesity as Risk Factors for Neurodevelopmental Disorders: Role of Maternal Microbiota and Nutritional Intervention Strategies. Nutrients, 14(15):3150.

2022 Sagi-Kiss V., Li Y., Carey M.R., Grover S.J., Siems K., Cirulli F., Berry A., **Musillo C.**, Wilson I.D., Want E.J., Bundy J.G. Ion-Pairing Chromatography and Amine Derivatization Provide Complementary Approaches for the Targeted LC-MS Analysis of the Polar Metabolome. J Proteome Res 2022, vol. 21, pp 1428–1437.

2022 Musillo C., Berry A., Cirulli F. Prenatal psychological or metabolic stress increases the risk for psychiatric disorders: the "funnel effect" model. Neurosci Biobehav Rev 2022, vol. 136, 104624.

2022 Musillo C., Berry A., Ajmone-Cat M.A., De Simone R., Creutzberg K.C., Collacchi B., Begni V., Riva M.A., Cirulli F. N-acetyl-cysteine prevents sex-dependent effects of HFD on sociability and inflammatory-plasticity genes in young mice. Neuroscience Applied 2022, vol. 1, Supplement 1, pp 100048.

2021 Berry A., Mazzelli M., **Musillo C.**, Riva M.A., Cattaneo A., Cirulli F. High-fat diet during adulthood interacts with prenatal stress, affecting both brain inflammatory and neuroendocrine markers in male rats. Eur J Neurosci. 2021, 1-15.

2021 Musillo C., Berry A., Collacchi B., Lepre M., Creutzberg K., Begni V., Riva M.A., Cirulli F. Prenatal N-acetyl-cysteine administration alleviates the long-term effects of maternal obesity of adolescent male and female mouse offspring. European Neuropsychopharmacology 2021, vol. 53, Supplement 1, pp S49-S50.

2021 Musillo C., Borgi M., Saul N., Möller S., Luytene W., Berry A., Cirulli F. Natural products improve healthspan in aged mice and rats: A systematic review and meta-analysis. Neurosci. Biobehav. Rev. 2020, vol. 121, 89-105.

2020 Cirulli F., **Musillo C.**, Berry A. Maternal obesity as a risk factor for brain development and mental health in the offspring. Neuroscience. 2020, vol. 447, 122-135.

2020 Berry A., Marconi M., **Musillo C.**, Chiarotti F., Bellisario V., Matarrese P., Gambardella L., Vona R., Foglieni C., Lombardi M., Cirulli F. Trehalose administration in C57BL/6N old mice affects healthspan improving motor learning and brain anti-oxidant defences in a sex-dependent fashion: a pilot study. Exp Gerontol. 2020, vol. 129, 110755.

ABSTRACTS PRESENTED AT NATIONAL AND INTERNATIONAL MEETINGS

Musillo C., Berry A., Creutzberg K.C., Begni V., Riva M.A., Cirulli F. Increased oxidative stress as a common mechanism for different prenatal stressors: long-term effects on adolescent male and female mice. Federation of European Neuroscience Societies Forum - FENS meeting, 9-13 july 2022, Paris, France.

Berry A., **Musillo C.**, Tassinari R., Tait S., Maranghi F., Cirulli F. Sex-dependent neurodevelopmental vulnerability in prenatally- stressed mouse offspring is mediated by oxidative stress and placental immune activation. Federation of European Neuroscience Societies Forum - FENS meeting, 9-13 july 2022, Paris, France.

Musillo C., Berry A., Ajmone-Cat M.A., De Simone R., Creutzberg K.C., Collacchi B., Begni V., Riva M.A., Cirulli F. Prenatal N-acetyl-cysteine prevents sex-dependent effects of maternal high-fat-

diet on social anxiety and hippocampal inflammatory and plasticity genes in adolescent mice. ECNP Workshop for Early Career Scientists in Europe 2022, 17-20 march, Nice, France.

Musillo C., Berry A., Ajmone-Cat M.A., De Simone R., Creutzberg K.C., Collacchi B., Begni V., Riva M.A., Cirulli F. Prenatal N-acetyl-cysteine prevents social anxiety and modulates hippocampal inflammatory-and plasticity-related genes in adolescent mice prenatally exposed to a high-fat diet. 34th ECNP Congress Hybrid, 2-5 october 2021, Lisbon, Portugal.

Musillo C., Berry A., Ajmone-Cat M.A., De Simone R., Creutzberg K.C., Collacchi B., Begni V., Riva M.A., Cirulli F. Prenatal N-acetyl-cysteine administration prevents social anxiety and modulates brain immune- and plasticity-related genes in adolescent offspring born from high-fat diet C57Bl6/N mouse dams. 49th European Brain and Behaviour Society (EBBS) meeting, 4-7 september 2021, Lousanne, Switzerland.

Berry A., **Musillo C.**, Tassinari R., Tait S., Maranghi F., Cirulli F. Oxidative stress and placental immune activation mediate sex-dependent vulnerability in prenatally- stressed mouse offspring. 49th European Brain and Behaviour Society (EBBS) meeting, 4-7 september 2021, Lousanne, Switzerland.

Musillo C., Berry A., Collacchi B., Pieroni E., Lepre M., Creutzberg K.C., Begni V., Riva M.A., Cirulli F. N-acetyl-cysteine administration during foetal life improves social behaviour and restores hippocampal BDNF levels in adolescent mice prenatally exposed to a high-fat diet. 29th European Congress of Psychiatry (EPA), 10-13 april 2021, Virtual.

Musillo C., Berry A., Collacchi B., Lepre M., Creutzberg K., Begni V., Riva M.A., Cirulli F. Prenatal N-acetyl-cysteine administration moderates the long-term negative effects of maternal obesity in adolescent male and female mouse offspring. 12th Annual General Meeting Federation of European Neuroscience Societies - FENS meeting, 11-15 july 2020, Virtual Forum.

Berry A., **Musillo C.**, Zarse K., Bundy J.G., Sagi-Kiss V., Siems K., Luyten W., Ristow M., Cirulli F. Rosmarinic acid administration improves cognitive and emotional behaviour and brain anti-oxidant defences in a sex-dependent manner in C57BL/6N aged mice fed a Western diet. 12th Annual General Meeting Federation of European Neuroscience Societies - FENS meeting, 11-15 july 2020, Virtual Forum.

Musillo C., Poggini S., Cristofaro G., Zarse K., Ristow M., Bundy J.G., Sagi-Kiss V., Siems K., Luyten W., Berry A., Cirulli F. "Western diet combined with rosmarinic acid in old mice exerts negative effects on metabolism but improves cognitive and emotional behaviour in a sex-dependent fashion". 48th European Brain and Behaviour Society (EBBS) meeting, 21-24 september 2019, Prague, Czech Republic.

Berry A., **Musillo C.**, Saul N., Borgi M., Möller S., Fuellen G., Luyten W., Cirulli F. Nutraceuticals counteract memory decline in murine models of aging. 48th European Brain and Behaviour Society (EBBS) meeting, 21-24 september 2019, Prague, Czech Republic.

Musillo C., Berry A., De Cristofaro G., Cirulli F. "Nutraceuticals have sex-dependent positive effects on healthy ageing in murine models". National Meeting of PhD Students in Neuroscience: New perspectives in neuroscience: research results of young Italian neuroscientists - SINS meeting, 1 march 2019, Naples, Italy.

Acknowledgments

"*A journey of a thousand miles begins with a single step*" disse una volta un saggio.

Ed è proprio così che è iniziato questo meraviglioso viaggio della mia vita: con un singolo passo che ha spostato il mio cammino dalle familiari lettere antiche e filosofia verso un mondo inaspettato: la Biologia. Questo cammino, col tempo, si è tinto di infinite sfumature di diversi colori provenienti da luoghi, esperienze, città ma soprattutto persone. Sono infatti le persone che hanno segnato questo percorso ad averlo reso unico.

Le prime tra tante, Francesca ed Alessandra, con le quali ho mosso i primi passi nel mondo della ricerca. A Francesca, mia mentore, un grande grazie per avermi sempre dato un'ampia visione del mondo, ben oltre le porte del nostro laboratorio. La sua professionalità e la sua determinazione sono da sempre un modello per me. Un immenso grazie ad Alessandra, che durante questi anni mi ha donato la cosa più preziosa: il tempo. Tempo per crescere, per imparare, per rendersi indipendente, per conoscere i propri limiti ed andare oltre ad essi. Con la sua costante disponibilità e passione per questo lavoro è stata molto più di un supervisor. Gli interminabili weekend in stabulario e le ore di lavoro insieme, troppe per essere contate, sono state le fondamenta su cui sono cresciuta professionalmente e personalmente.

Se oggi amo questo lavoro è anche grazie a voi.

Grazie a Francesca e Marta con le quali, nella nostra stanza sempre più verde, anche le giornate più lunghe e impegnative trovano sempre preziosi momenti di condivisione e svago. Grazie anche a Barbara per la sua sensibilità e la sua visione del mondo.

Un grazie va a tutti gli studenti che sono stati coinvolti nel mio progetto di Dottorato: ognuno di loro, con il proprio carattere, mi ha dimostrato la bellezza dell'essere diversi, che la diversità non toglie nulla ma anzi ti arricchisce dentro; mi hanno insegnato ad essere più paziente e comprensiva e ricordato che più spesso dovremmo guardare il mondo con occhi altrui. Quindi grazie a Eleonora, Marcello, Chiara, Massimo ma soprattutto a Marianna e Letizia per essere state fin da subito curiose e capaci; a loro un grazie particolare in quanto la loro gestione dell'attività sperimentale degli ultimi mesi mi ha permesso di concentrarmi a pieno sulla stesura di questa tesi.

Grazie infinite ai miei genitori, Roberto e Brunella. Questo traguardo è dedicato a voi, per il vostro costante sostegno, per aver creduto in me e nelle mie scelte. Grazie a mio fratello Stefano, mio grande sostenitore, che essendo da sempre il mio estremo opposto è anche il mio complemento perfetto. Non può mancare ovviamente un grazie a Kira che con la sua dolcezza e la sua tranquillità, essendo semplicemente lei, è sempre stata capace di riportare in me l'equilibrio e la serenità.

Grazie a tutti gli amici che hanno colorato e reso più leggero questo viaggio. Nonostante qualcuno si sia stabilito in terre lontane per seguire i propri sogni, la vostra vicinanza è sempre presente. Tra i tanti, Vanessa, Marta ed Elena: questo percorso nasce insieme voi e senza dubbio lo continuerà, nulla sarebbe stato uguale senza di voi.

Un grazie a Margherita, per l'amicizia che ha colorato più della metà della nostra esistenza: ogni evento importante della mia vita è legato ad un ricordo con te.

Infine, un immenso grazie ad Edoardo per essere il mio posto sicuro nel mondo, *my lighthouse*.