Maternal Programming of Adult Rodent Integrative Phenotype by Prenatal Exposure to Predator Odour

by

Sophie St-Cyr

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Graduate Department of Cell & Systems Biology
University of Toronto

© Copyright by Sophie St-Cyr 2017

Maternal Programming of Adult Rodent Integrative Phenotype by Prenatal Exposure to Predator Odour

Sophie St-Cyr, M.Sc.
Doctor of Philosophy
Graduate Department of Cell & Systems Biology
University of Toronto
2017

Abstract

Prenatal stress mediated through the mother can program the long-term phenotype of the offspring. The capacity for adaptation to adversity in early life depends in part on the life history of the animal. It is therefore likely that an early life ethologically relevant psychogenic stressor that has been present over evolutionary times could prime responses to an environment containing this stress via epigenetic mechanisms such as DNA methylation modifications. Pregnant C57BL/6 mice and, separately, Long-Evans rats were exposed daily to unpredictable and inescapable predator odors or distilled water control over the second half of pregnancy. I examined the effect of the prenatal predator odour exposure on the integrative phenotype of adult offspring at the level of behaviour, physiology, endocrinology, transcription and DNA methylation. Prenatally predator odour-exposed offspring exhibited an overall increase in stress-related behaviours on a variety of commonly-used and semi-naturalistic assessments of the response to stress, as well as modifications of energy consumption at baseline and under stress. These changes were accompanied by a sex-specific increase in endocrine responses to stress and an increase in circulating thyroid hormone. Additionally, I observed modifications in stress-related transcript abundance at birth and in adulthood accompanied by DNA methylation modifications in adulthood. Overall, assessments of the integrative phenotype of prenatal predator odour-exposed animals indicate a persistent increase in stress responsiveness across a variety of experimental conditions and phenotypic levels in the two rodent species. This phenotype supports the hypothesis that maternal programming allows

developmental forecasting that shapes the individual developmental trajectory. Prenatal predator odour is therefore a potent programming stressor.

Acknowledgements

I would like to thank the Natural Sciences and Engineering Research Council of Canada and Ontario Graduate Scholarship for vastly funding my PhD and allowing me to take maternity leaves.

Many thanks also to the McGowan lab members that have listened, supported and helped me throughout my PhD, especially Pauline Pan, Will de Vega and Sophia Lavergne. I am very greatful to Sameera Abuaish who participated to a large part of my experiments, especially when I left for my second maternity leave. I want to acknowledge several undergraduate students for their work under my supervision including Atif Hussain, Vinusha Gunaseelan and Jing-Jing Lee with the maternal behavior coding, Raafay Syed Ali conducted some of the rat's behavioural testing and Shathveekan Sivanathan for the rat PN0 gene expression. Thanks to the vivarium staff, especially Andrew Gristok and Kim Hannon-Kovasci who took care of the animals and supported me with my very hands-on approach. I also have a special though for all the mice and rats that were used for this research, as they were crucial for the scientific advances presented in this thesis.

A big thank you to my supervisor, Dr. Patrick McGowan, for being supportive and understanding throughout my PhD and for his help in designing the paradigm and experiments. He was able to canalize my enthusiasm and helped me to become a better scientist. I also appreciated the intellectual challenges and guidance provided by my committee members, Dr. Rudy Boonstra (UTSC) and Dr. Malcolm Campbell (Guelph) who were present and available throughout my PhD and for Dr. Maydianne Andrade and Dr. Ian Brown (both UTSC) input during my proposal exam. I would also like to acknowledge Dr. Tara Perrot (Dalhousie) who agreed to be the external examiner for my defense.

Last but not least, I would like to thank my family for their love, support, trust and encouragements throughout the years. My mother, Louise, offered tremendous support by being an amazing and very present grandmother, as were my mother and father in law,

Sylvie and Alain. I could not have completed this amount of work without my wonderful husband who knows how to push me and boost my confidence in times of needs. Finally, I am extremely grateful for my two beautiful and intelligent boys, Arthur and Victor, who are my greatest PhD achievements and who motivate me everyday to become a better scientist, a better mother and a better person.

Table of contents

List of Abbreviations

18S: 18S ribosomal RNA

ACTH: Adrenocorticotropic Hormone

ACTB: Actin beta

AVP: Arginine Vasopressin

BDNF: Brain-derived neurotrophic factor

C: Control

CBG: Corticosteroid-Binding Globulin

C-F: Control female

C-M: Control male

CORT: Corticosterone

CpG: Cyosine-Guanine dinucleotide

CRF: Corticotropin-Releasing Factor

CRFR1: Corticotropin-Releasing Factor Receptor 1

CRFR2: Corticotropin-Releasing Factor Receptor 2

DNA: Deoxyribonucleic Acid

DOHaD: Developmental Origin of Health and Disease

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

FKBP5: FK506 Binding Protein 5

GUD: Giving-Up Density

GRE: Glucocorticoid Response Element

HPA axis: Hypothalamic-Pituitary-Adrenal axis

IGF: Insulin Growth Factor

LD: light-dark transition task

NR3C1: Glucocorticoid Receptor

NR3C2: Mineralocorticoid Receptor

OF: Open Field

PO-F: Prenatally predator Odour-exposed Female

PO-M: Prenatally predator Odour-exposed Male

PN: Postnatal Day

PVN: Paraventricular Nucleus of the hypothalamus

RNA: Ribonucleic Acid

RNA-Seq: RNA sequencing

SCT: Social Choice Test

SIT: Social Interaction Test

SRT: Social Recognition Test

T_4: Thyroxine

TMT: 2,3,5-trimethyl-3-thiazoline

UBC: Ubiquitin C

$\dot{V}_{O_2}$: Oxygen consumption rate

YWHAZ: 14-3-3 protein zeta/delta

List of Tables

List of Figures

Introduction

1.1 Publication

Part of the introduction is adapted in the following review article:
St-Cyr, Sophie and McGowan, Patrick O. Adaptation or pathology? The role of prenatal stressor type and intensity in the developmental programing of adult phenotype. *Invited review submitted to Neurotoxicology and Teratology*.

1.2 Early life influence on phenotype

The mother is the major interface between the offspring and its prenatal environment. Early life environment is known to be a significant factor in programming the developmental trajectory of animals. These early life decisions are therefore responsible for the long-term phenotype of an individual. These effects have been observed for 60 years now. The first evidence of early environment programming was reported in rats where pups handled every day before weaning possessed lighter adrenal glands, indicating a decreased stress response, in adulthood compared to non-handled control rats (Levine, 1957). Several hypotheses have been developed to explain the mechanism by which the early life milieu can lead to developmental and long-term phenotypic changes.

1.2.1 Description of Thrifty phenotype and Developmental Origin of Health and Disease hypotheses

The 'Thrifty phenotype' hypothesis was proposed in 1992 by Hales and Barker. This hypothesis states that predisposition to type 2 diabetes is determined through energetic availability in early human development. More broadly, energy restriction *in utero* leads to a growth restriction through an energy allocation trade-off in which vital organs development is prioritized over tissues development. This trade-off leads to an irreversible change in the developmental trajectory of the individual. This prenatal

condition can be detected through a low birth-weight and, later, by a raised blood pressure and increased insulin resistance in adulthood. Insulin growth factors (*IGF-I* and *II*) and their receptors have been hypothesized to mediate this phenotypic plasticity as they constitute key regulators of foetal growth that could be imprinted in prenatal life depending upon the nutritional resource availability (McMillen and Robinson, 2005).

These long-term modifications in low-birth weight offspring have later been described as the metabolic syndrome, encompassing changes in growth, metabolism and vasculature. Organ development alterations leads to metabolic and hormonal imprinting in the individuals which can lead to hypertension, insulin resistance and increased stress response (as detected through increased blood glucocorticoid and adrenocorticotropic hormone) (Hales and Barker, 2001; Phillips et al., 2000; Reynolds, 2001). Therefore, early life energy deprivation changes the structure and life-long functional capacity of the organs affecting the individuals' glucose homeostasis, insulin secretion and action, blood pressure regulation, renal and endothelial function, appetite and hypothalamic-pituitary-adrenal axis reactivity. The window of programming can be extended to the early postnatal environment as observed in rats from small litters exhibiting chronic hyperphagia, obesity, hyperleptinemia, hyperglycemia, hyperinsulinemia and insulin resistance (McMillen and Robinson, 2005). The metabolic syndrome crystallizes the impact of early life events on the developmental trajectory of the individual. This concept gave birth to the Developmental Origin of Health and Disease (DOHaD) hypothesis predicting inflammation and increased risk of obesity in preadolescent years (Gluckman and Hanson, 2006). Therefore, the foetus trade-off strategy of reducing growth when energy is limited promotes immediate survival against health in later life.

New evidence indicates that early energetic deprivation confers an advantage later in life by increasing energy conservation and reducing somatic growth in poor nutrition environments (Youngson and Whitelaw, 2008), which can confer increased longevity (Ozanne and Hales, 2004) in this type of environment. Incidentally, in human populations exposed to famine, increased birth weight increased 9-fold the risk of rickets, a disease associated to severe malnutrition (Chali et al., 1998) as large babies are more likely to

exceed their mother energetic resources and suffer from energetic insufficiency. These observations suggest that the thrifty phenotype could be adaptive in a deprived thrifty postnatal environment and maximize the immediate offspring fitness. On the contrary, a thrifty phenotype would be inappropriate in an environment where energy is abundant and available and would induce obesity or insulin resistance. This observation introduced the concept of the importance of a similar and stable prenatal and postnatal environment for efficient maternal programming, through reliable maternal cues.

1.2.2 Description of Mismatch and Predictive Adaptive Response hypotheses

The Predictive Adaptive Response hypothesis is an extension of the mismatch hypothesis by Bateson and Gluckman (2011) stating that developmental forecasting determines the developmental trajectory of the individual. Cues from the early life would therefore influence the development of a phenotype appropriate to the predicted later life environment. However, a mismatch from the predicted environment would lead to adverse health and fitness effects. The prenatal cues can be the nutrition, body composition or stress level of the mother. This hypothesis also predicts that developmental plasticity happens in response to an adaptive range of cues and signals, while producing non-adaptive outcomes in response to a novel or extreme insult (Bateson et al., 2014). Contrary to the Developmental Origin of Health and Disease hypothesis, which produces two distinct phenotypes, the Predictive Adaptive Response hypothesis predicts graded phenotypic changes. The Predictive Adaptive Response hypothesis states that maternal effects can maximize the offspring fitness by preparing the offspring to future environmental conditions. Further, the Predictive Adaptive Response hypothesis explains the adverse effects described in the thrifty hypothesis as by-products of the evolutionary recent raise in human longevity and emergence of the westernized diet rich in carbohydrate and fat (Bateson et al., 2014), therefore increasing the probability of long-term mismatched environments. The major implication of this mismatch is that the ‘adaptive’ trade-off traits triggered prenatally by maternal cues leads to adverse outcomes in the long run.

1.2.3 Epigenetics as the mechanism of maternal programming

Epigenetics refers to molecular processes leading to stable changes in gene expression that can last over cell divisions without altering the underlying deoxyribonucleic acid (DNA) sequence (Allis et al., 2007). The most studied epigenetic marks are DNA methylation and histone modifications including methylation and acetylation amongst other. DNA methylation produces a long-lasting addition of a methyl group by a DNA methyltransferase on a carbon of the cytosine nucleotide ring, most often in the context of a Cytosine-Guanine dinucleotide. DNA methylation marks are long lasting, especially in post-mitotic cells such as neurons. This epigenetic mark is linked to a decreased access to the DNA, and concurrently a decrease in the associated gene expression. For example, highly methylated Cytosine-Guanine dinucleotide sites in a promoter region leads to the inhibition of transcription factor binding at this site, reducing gene expression. On the contrary, a methylated repressive binding site would increase the gene expression. At a higher level, histone modifications (e.g. acetylation, methylation, ubiquitination) change the chromatin structure, making nucleosomes more or less accessible for transcription. The most studied histone modification is the acetylation of positively charged amino acids on histone tails through histone acetyltransferases (HATs). Acetylation generally opens the chromatin, allowing transcription factors to bind to the DNA. Histone deacetylase (HDACs) have the opposite effect (Allis et al., 2007). Therefore, permanent programming of the foetus through maternal influence can happen through long-lasting epigenetic alterations of the foetus, a phenomenon called maternal programming.

Epigenetic programming has been suggested to mediate offspring phenotypic plasticity described in the Developmental Origin of Health and Disease and Predictive Adaptive Response hypotheses. Epigenetic inheritance could optimally transmit Predictive Adaptive Responses by propagating a well-adapted phenotype in a specific environment within a population until genetic fixation occurs (Bateson et al., 2014). Epigenetic inheritance would also allow species to survive short-term environmental challenges while preserving maximal genotypic variation.

1.3 Stress affects bodily functions

1.3.1 Stress is a brake in the body equilibrium

The stress response has been described by Hans Selye as the physiological consequences to a wide variety of noxious stimulus (Herman, 2010). These physiological changes are triggered to restore homeostasis in cases of real or anticipated threat to homeostasis (physical or psychological stressors). The adaptive way to cope with stress is through the 'fight-or-flight' response involving active problem-solving in response to an escapable stress, or passively through immobility or reduction of harm when exposed to an inescapable stress (Homberg, 2012). Generally, acute stress leads to the mobilization of energy while chronic stress generally suppresses growth, reproduction, digestion and immunity. The autonomic nervous system and Hypothalamic-Pituitary Adrenal axis are the main components of the stress response mediating this response (Tsigos and Chrousos, 2002). The initial fast response to stress happens within seconds through the activation of the autonomic sympathetic activation of the locus coeruleus and noradrenergic cell groups of the medulla and pons. This fast response changes the function of several target organs through epinephrine and norepinephrine signalling (Chrousos, 1998; Tsigos and Chrousos, 2002). This initial stress response has not been shown to be 'programmable'; therefore it will not further be discussed in the context of this thesis.

1.3.2 Stress response through the Hypothalamic-Pituitary Adrenal axis

The endocrine response to stress is mediated through the hypothalamic-pituitary adrenal (HPA) axis from minutes to hours following the stress onset through glucocorticoids. The corticotropin-releasing factor and arginine vasopressin are released by the paraventricular nucleus of the hypothalamus and, acting synergistically, lead the pituitary to synthesize and secrete adrenocorticotropic hormone. The adrenocorticotropic hormone is derived from proopiomelanocortin that is produced by the arcuate nucleus of the hypothalamus and the pituitary gland (Chrousos, 1998). The adrenocorticotropic hormone in turn

induces the downstream release of glucocorticoids, such as cortisol and corticosterone, their amount depending on the species, by the adrenal cortex and into the circulatory system. The adrenal cortex produces and releases mineralocorticoids, mainly aldosterone, glucocorticoids and androgens (Marieb and Hoehn, 2012). The main glucocorticoid in fish and humans is cortisol while corticosterone is found in rodents, birds, amphibians and reptiles (Nelson, 2000). When glucocorticoids reach the glucocorticoid receptors in the hippocampus, negative feedback prevents further Adrenocorticotropic hormone release. Glucocorticoid receptors include the type-2 high affinity mineralocorticoid receptor (*NR3C2*) binding low levels of cortisone and cortisol or corticosterone and the type-1 low affinity glucocorticoid receptor (*NR3C1*) mediating the response to higher glucocorticoid levels (O'Connor et al., 2000), including during the stress response (Figure 1). Mineralocorticoid receptors show a ten times greater affinity to glucocorticoids than glucocorticoid receptors (O'Connor et al., 2000). Glucocorticoid receptors and mineralocorticoid receptors are present in cell cytoplasm until activation, bound to the co-chaperone *FKBP5* (FK506 binding protein 5) and heat-shock protein 90 (*Hsp90*). *FKBP5* is the co-chaperone protein of multiple steroid receptors and is essential for mediating the effect of those steroid hormones on gene expression via transactivation. Once in the nucleus, glucocorticoid receptors act as a transcription factor binding to genomic glucocorticoid receptor elements by altering the transcription of multiple genes (Jääskeläinen et al., 2011).

The regulation of the hypothalamic-pituitary adrenal axis is complex and takes place at all the levels of the axis including extra-hypothalamic brain regions such as the medial prefrontal cortex, the hippocampus and amygdala. The glucocorticoid receptor activation in the pituitary, hypothalamus and hippocampus inhibits the hypothalamic-pituitary adrenal axis activity. Corticotropin-releasing hormone neurons are also subject to an ultra short inhibitory corticotropin-releasing hormone loop and an hypothalamic inhibitory Proopiomelanocortin loop (Calogero et al., 1988). The hippocampus and amygdala, which are inter-connected and mediate anxiety behaviour, can also modulate the hypothalamus-pituitary adrenal axis activity (Davis, 1992; McHugh et al., 2004; Snyder et al., 2011). Hippocampal projections inhibit the hypothalamus-pituitary adrenal axis

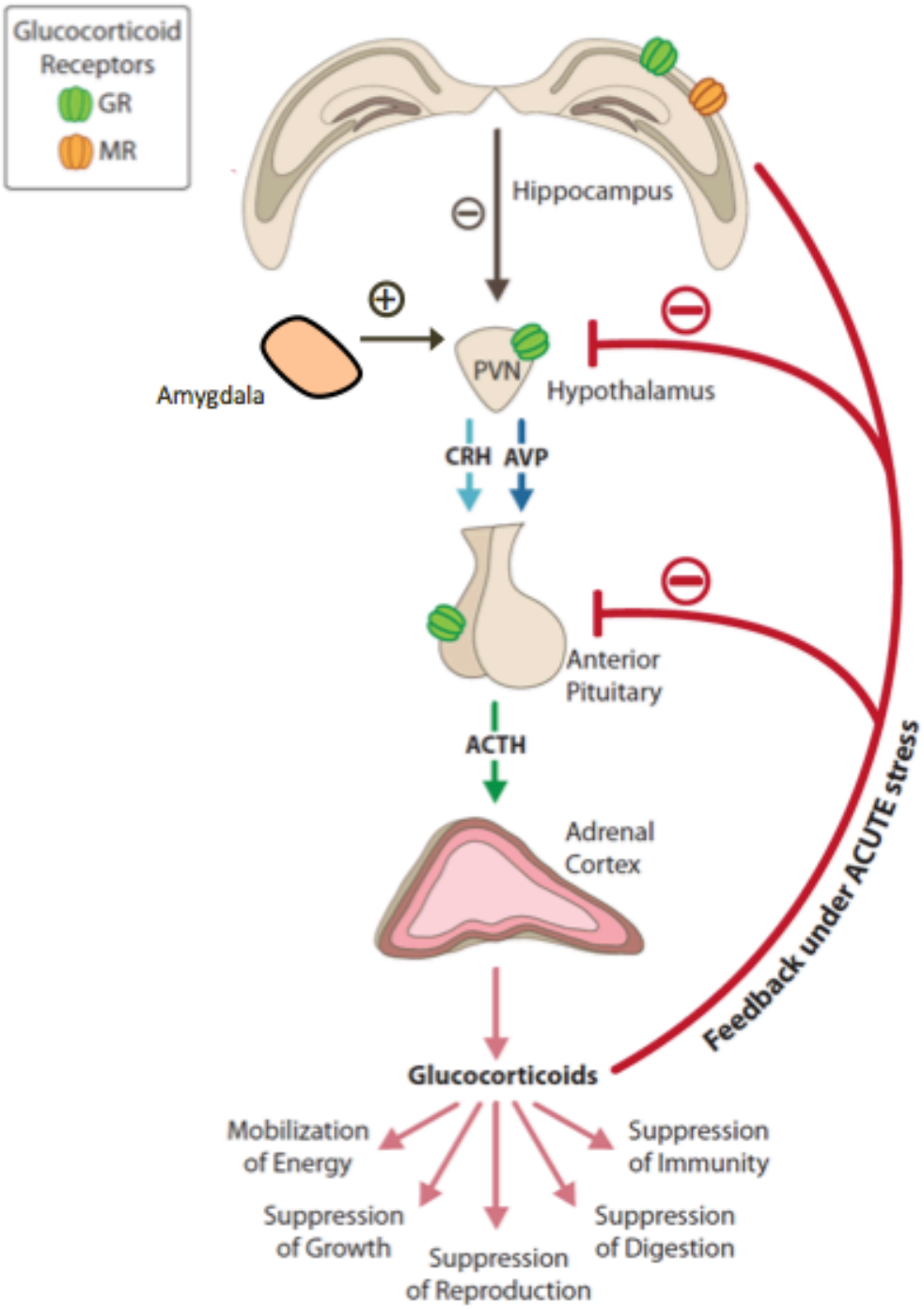


Figure 1. Hypothalamic-Pituitary Adrenal axis

Adapted from Boonstra et al. (2014). GR: Glucocorticoid Receptor; MR: Mineralocorticoid Receptor; PVN: Paraventricular Nucleus of the hypothalamus; CRH: Corticotropin-Releasing Factor; AVP: Arginine Vasopressin; ACTH: Adrenocorticotropic Hormone.

through projections to the hypothalamus, bed nucleus of the stria terminalis and septum to the medial prefrontal cortex. On the contrary, amygdala projections to areas adjacent to the paraventricular nucleus of the hypothalamus, bed nucleus of the stria terminalis, medial prefrontal cortex potentiate the hypothalamus-pituitary adrenal axis activation (Herman et al., 2003; Schulkin et al., 2005). Finally, the medial prefrontal cortex can also mediate negative feedback of the hypothalamus-pituitary adrenal axis through glucocorticoid receptor binding (Herman et al., 2005).

On average, only 5% of the circulating glucocorticoids are biologically active while 90% is bound to corticosteroid-binding globulin (or transcortin) along with residual albumin binding (Qian et al., 2011). Bound cortisol and corticosterone are physiologically inactive. In situations of stress, the glucocorticoid reservoir in the blood becomes more readily available through decreased corticosteroid-binding globulin-binding. Further, glucocorticoids are short-lived molecules with the half-life of corticosterone bound and unbound in rodents being under 30 minutes (Sainio et al., 1988).

Glucocorticoids naturally show a circadian rhythm under the control of the suprachiasmatic nucleus of the hypothalamus (Moore and Eichler, 1972) with levels peaking at the onset of the active phase, which is the dark phase in rodents (Qian et al., 2012). The circadian rhythm of the hypothalamic-pituitary adrenal axis is driven by mineralocorticoid receptor glucocorticoid binding (Dallman et al., 1989). Elevated corticosterone at arousal time is thought to have evolved as an adaptation to match the predictable rise in energy needed to support an increase in locomotor activity at that moment (Malisch et al., 2008). The circadian rhythm can be altered by changes in lighting and feeding schedules (Moore and Eichler, 1972) as well as chronic stress which can blunt the corticosterone circadian rhythm (Yuan et al., 2016).

Multiple tissues including the liver and placenta express the enzyme 11β-hydroxysteroid dehydrogenase. This key enzyme presents two isoforms: Type 1 catalyzes the reduction of 11-dehydrocorticosterone to corticosterone while, inversely, 11β-hydroxysteroid dehydrogenase dehydrogenases corticosterone into the inactive 11-dehydrocorticosterone

metabolite. 11β-hydroxysteroid dehydrogenase is dynamically expressed in the placenta and regulates the amount of maternal glucocorticoids received by the foetus, which can in turn mediate modifications in the offspring phenotype (Bellisario et al., 2015; Welberg et al., 2000).

1.3.3 Mechanisms and effects of acute and chronic stress

The acute stress response is responsible for behavioural and physiological adaptations. The stress-induced 'fight-or-flight' response is associated with the facilitation of neural pathways mediating arousal, alertness, vigilance, cognition, focused attention and aggression while inhibiting feeding and reproduction through a redirection of energy allocation (Homberg, 2012; Wingfield and Ramenofsky, 1999). These behavioural changes are accompanied by physiological adaptations such as an increase in heart rate, blood pressure, respiratory rate, temperature, analgesia, hepatic energy production gluconeogenesis and lipolysis. While energy is being allocated to the 'fight-or-flight' adaptive response, there is an associated decrease of digestion, growth, reproductive investment, inflammation and immunity (Chrousos, 1998) (Figure 1) (Table 1). Growth suppression is mediated mostly through a glucocorticoid inhibition of the release of growth hormone, thyroid-stimulating hormone and conversion of thyroxine to the biologically active triiodothyronine. Reproductive investment is limited mainly through the inhibition of luteinizing hormone, follicle-stimulating hormone, testosterone and estradiol by glucocorticoids. Inhibition of inflammation and immune response is mediated through glucocorticoids and induced nuclear factor-kappa B which inhibits the release of cytokines (interleukin-1 and 6, tumor necrosis factor) and mediators of inflammation such as serotonin (Chrousos, 1998; Munhoz et al., 2010). However, new theories suggest that acute stress might mediate an increase in innate immune response while suppressing the adaptive immune response (Cruz-Topete and Cidlowski, 2014). Finally, analgesia is mediated by the increased transcript abundance of β-endorphin (Chrousos and Gold, 1992; O'Connor et al., 2000). The genes reported here, expression of which is modified under stress, are interesting potential programming targets during prenatal stress exposure.

Table 1. Behavioural and physiological adaptations during acute and chronic stress

Behavioural adaptations	Physiological adaptations
↑ Arousal/Alertness	↑ Heart rate/Blood pressure
↑ Anxiety	↑ Respiratory rate
↑ Cognition/Vigilance	↑ Temperature
↓ Appetite/Feeding	↑ Gluconeogenesis/Lipolysis
↓ Reproductive behaviour	↓ Growth
	↓ Reproductive system investment
	↓ Digestion
	↓ Inflammation/Immunity

Adapted from Chrousos and Gold (1992).

However, in the case of chronic stress, the prolonged activation of the adaptive stress responses triggers negative outcomes including hyper-arousal, hyper-vigilance, prolonged anorexia, hypogonadism and decreased libido. A lot of these effects are associated to symptoms of depression and facilitate the development of addictive behaviours such as alcoholism (Chrousos and Gold, 1992).

1.3.4 Targets and timing of maternal programming during the hypothalamic-pituitary adrenal axis development and maturation

The hypothalamus, pituitary and adrenal glands are active endocrine organs during foetal development (Ng, 2000). The hypothalamic-pituitary adrenal axis development is related to periods of rapid brain growth associated to species-specific gestation times (Clancy et al., 2001). In this section, I will review the maternal programming during the hypothalamic-pituitary adrenal axis development and maturation in laboratory rodents. In mice and rats, the gestation time is respectively of 19 and 21 days and the hypothalamic-pituitary adrenal axis maturation starts around gestational day 14. During development, maternal glucocorticoids modulate and can inhibit the activation of the foetal hypothalamic-pituitary adrenal axis. Further, intrauterine homeostasis and vital organ maturation including lung, liver and central nervous system requires neural development of the hypothalamic-pituitary adrenal axis (O'Connor et al., 2000).

In rodent foetus, although glucocorticoid receptors are expressed as early as gestational day 12.5 in the neuroepithelium, negative feedback of the hypothalamic-pituitary adrenal axis is mediated by neural transcription of glucocorticoid receptors in the foetus happening from the gestational day 16.5 onward (Diaz et al., 1998; Reichardt and Schütz, 1996). Glucocorticoid receptors are not detected in the foetal brain before the gestational day 15.5 (Diaz et al., 1998). This period coincides with sex-specific transcript abundance difference in placental 11β-hydroxysteroid dehydrogenase, with female placentas showing reduced transcript abundance and thus reduced maternal glucocorticoid buffering (Pankevich et al., 2009). As effects of prenatal stress on placental 11β-hydroxysteroid dehydrogenase transcript abundance have been observed until parturition,

differential glucocorticoid exposure in utero could explain the sex-specific glucocorticoid receptor transcript abundance in offspring at birth (Peña et al., 2012).

In rats, the proopiomelanocortin transcript, from which adrenocorticotropic hormone is derived, is detected in the pituitary as early as gestational day 13.5 and it's abundance is responsive to physiological mediators (Lugo and Pintar, 1996). Arginine-vasopressin transcription starts later, in the hypothalamus on the gestational day 15 and on gestational day 17 and 18 in the pituitary and hypothalamus (Rundle and Funder, 1988; Tribollet et al., 1991). The corticotropin-releasing hormone regulates the growth of pituitary corticotrophs, adrenocortical differentiation and the maturation of the foetal hypothalamic-pituitary adrenal axis during the late foetal development. Foetal corticotropin-releasing factor rise in maternal and foetal circulation triggers parturition as well as a surge in foetal glucocorticoids (O'Connor et al., 2000). Corticotropin-releasing factor receptors are expressed in the rat foetal hypothalamus from the gestational day 16 and in the foetal amygdala and cortex on the gestational day 17 while hippocampal transcription is detected postnatally only (Eghbal-Ahmadi et al., 1998). Foetal corticosterone-binding globulin concentration and binding capacity increases up to the gestational day 20 and lead to a 3-fold increase in bioactive glucocorticoids in late pregnancy (gestational day 18 to term) up to postnatal day 28 (Gewolb and Warshaw, 1986).

Early postnatally, between postnatal day 4 to 14, the hypothalamic-pituitary adrenal axis is hyporesponsive (stress hyporesponsive period, Lupien et al., 2009). This hyporesponsive period corresponds to a low baseline blood glucocorticoid level with a minimal hypothalamic-pituitary adrenal axis reactivity in response to noxious stressors. The stress hyperesponsive period could protect the rapidly developing brain from glucocorticoids and is maintained by maternal care (Lupien et al., 2009). The stress hyperesponsive period is accompanied by decreased hypothalamic glucocorticoid receptor and corticotropin-releasing factor transcription (Halasz et al., 1997). Hippocampal glucocorticoid receptor and mineralocorticoid receptor transcript abundance increases from postnatal day 9 to 15 (Meaney et al., 1985). Similarly, lactating

dams show significantly reduced diurnal peaks of corticosterone and hypothalamic-pituitary adrenal axis reactivity in response to an acute stressor. This stable circadian corticosterone level is probably important to normalize and maintain metabolic activity in lactating mothers while decreasing their anxiety and stress responsiveness to enable normal postnatal development in the offspring (Hillerer et al., 2011; Windle et al., 2013). The normal hypothalamic-pituitary adrenal axis activity of the mother is resumed within two days of pups weaning (Windle et al., 2013). Stress during pregnancy can lead to a shift in the circadian pattern of corticosteroid release in the dam, preventing the normal hypocorticism of the lactating dam (Malisch et al., 2008).

Therefore, the foetal maturation of the hypothalamic-pituitary adrenal axis in rodents happens well into the second half of pregnancy. This period is likely the most responsive to maternal programming. Further, as maternal influence on the offspring's hypothalamic-pituitary adrenal axis remains over the first two postnatal weeks, this period is likely responsible for further programming or 'rescue' of prenatal programming.

1.4 Traditionally studied prenatal stress paradigms on the long-term individual phenotype

The basis of the prenatal and early postnatal paradigms leading to long-term phenotypic modifications is to provide an unpredictable and uncontrollable (unavoidable) stress of a physical, psychological or psychosocial nature. Some examples of early life stressors applied to laboratory rodent models include saline injections, chemical exposure, forced swimming, crowding, electric shocks, restraint or immobilization combined or not with heat or bright light, acoustic startle, forced swimming, crowding (high conspecific density) or combinations of stressors in chronic variable stress paradigms (Charil et al., 2010; Crews et al., 2007; Darnaudéry and Maccari, 2008; Mueller and Bale, 2006; Weinstock, 1997). More directly, some females were supplemented with glucocorticoids or synthetic glucocorticoids during pregnancy (Muneoka et al., 1997). Equivalent

stressors in humans are thought to be marital discord, threat of war, death of husband or unpredictable aircraft noise among others (Weinstock, 1997).

To report the overall impacts associated to prenatal stress, I will focus on two of the most traditional and studied paradigms: prenatal restraint stress and prenatal chronic variable stress. Postnatal stressors applied to the mother and maternal separation stress are not included here as, in these cases, pups are directly stressed. In these cases, maternal programming is mitigated between the maternal stress and direct exposure to the stressor components.

1.4.1 General impacts of prenatal restraint stress

Prenatal restraint stress consists of rodent dams being restrained up to three times a day for an hour during the last week of pregnancy. Prenatal restraint stress during the second half of pregnancy leads to a decrease in 11β-hydroxysteroid dehydrogenase placental transcription and enzymatic activity. This change is accompanied by an increase in maternal circulating corticosterone and a decreased corticosterone-binding globulin level in rats (Koehl et al., 1999; Morley-Fletcher et al., 2003). Taken together, prenatal restraint stress therefore leads to an increase in corticosterone reaching the foetus during the prenatal stress period.

Prenatal restraint stress leads to several long-term behavioural alterations including an increase in stress-related behaviour and activity during novel environment exploration in adulthood compared to non-prenatally stressed rodents (Darnaudéry and Maccari, 2008; De Souza et al., 2013; Dong et al., 2015; Morley-Fletcher et al., 2003; Vallée et al., 1997; Xu et al., 2014). Further, prenatal restraint stress is associated to a stronger startle response to an unpredictable loud noise paired with a foot shock, that does not fade with time when compared to control mice (Matrisciano et al., 2013). Prenatally restrained adult rodents of both sexes display increased stereotypic behaviours (Matrisciano et al., 2013) and slower learning in novel environments (Darnaudéry et al., 2006; Vallée et al., 1999). Finally, a general decrease in social interaction, investigation, memory and social

play behaviour was detected following prenatal restraint stress in rodents (De Souza et al., 2013; Dong et al., 2015; Matrisciano et al., 2013; Morley-Fletcher et al., 2003).

Prenatal restraint stress animals also exhibit an altered physiological and endocrine phenotype. Prenatally restrained rats are lighter at birth with males remaining lighter up to adulthood (Lesage et al., 2004; Vallee et al., 1996). Prenatally restrained rat males also consumed less food daily at baseline (Vallee et al., 1996) but more after a day of fasting (Lesage et al., 2004). Prenatally restrained rat males also displayed higher blood glucose at baseline and after feeding (Lesage et al., 2004; Vallee et al., 1996). Baseline corticosterone levels were also decreased at birth in prenatally restrained male rats (Koehl et al., 1999, 1997; Lesage et al., 2004). Furthermore, prenatally restrained rats display a stress response (corticosterone increase) to a novel environment stress at a younger age, even during the stress hyporesponsive period compared to non-stressed rats (Henry et al., 1994). Furthermore, from weaning to adulthood, prenatally restrained rats showed higher corticosterone release and slower recovery in response to restraint stress (Henry et al., 1994; Morley-Fletcher et al., 2003; Vallée et al., 1999; Xu et al., 2014). Surprisingly, prenatally restrained rats have a decreased baseline circulating corticosterone level while being increased toward the end of the male light phase and throughout the entire circadian cycle in females compared to non-prenatally stressed rats (Koehl et al., 1999, 1997; Lesage et al., 2004). These changes were accompanied by a lower adrenal weight at birth in males and higher adrenal weight in both rat sexes in adulthood (Darnaudéry et al., 2006; Lesage et al., 2004).

Long-term transcription levels and neuropeptide metabolism are affected by prenatal restraint stress. From weaning to adulthood, prenatally restrained rats exhibit decreased glucocorticoid and mineralocorticoid receptor transcript abundance and binding in the hippocampus. Further, immediate early gene transcription, indicating brain region activation, is higher in the prenatally restrained rats hippocampus compared to non-prenatally stressed rats. These results indicate a potentiation of the hypothalamic-pituitary adrenal axis reactivity along with a reduction in the associated negative feedback in prenatally restrained rats when compared to control rats (Henry et al., 1994; Koehl et al.,

1999). Neuronal activity is also showing sex- and prenatally restrained-treatment specific prefrontal cortex monoaminergic, dopaminergic and serotonergic activity and metabolism (Bowman et al., 2004).

Finally, prenatally restrained-specific epigenetic states were investigated. For example, Cytosine-Guanine dinucleotide methylation levels were different at several sites within the prenatally restrained rat foetal hypothalamus 11β-hydroxysteroid dehydrogenase with no associated transcript abundance change (Darnaudéry and Maccari, 2008; Peña et al., 2012). Furthermore, associated increase in DNA methyltransferases and micro ribonucleic acid (RNA) transcripts were detected in the placenta and the foetal hippocampus and cortex up to adulthood in prenatally restrained rats (Matrisciano et al., 2013; Monteleone et al., 2014; Peña et al., 2012). Prenatally restrained rodents also show decreased brain-derived neurotrophic factor (*BDNF*) and Glycoprotein M6A transcript abundance with an associated increase in DNA methylation in the prefrontal cortex and hippocampus up to adulthood (Dong et al., 2015; Monteleone et al., 2014). The brain-derived neurotrophic factor is associated to synaptic plasticity and neuronal survival including normal neuronal development and is implicated in several psychiatric disorders such as depression (Boulle et al., 2012). Both of these genes are linked to alterations in neuronal connectivity and plasticity and have been suggested to counteract stress damage in neuronal cells. More broadly, sex-specific histone acetylation (H3K9) and methylation marks were detected in the prenatally restrained mice frontal cortex at birth (Schneider et al., 2016).

Therefore, prenatally restrained rodents show stress-associated behavioural, physiological, endocrine, transcript abundance and epigenetic modifications compared to control rodents from birth to adulthood with hints for a prenatal restraint stress-induced acceleration in development (Henry et al., 1994). Taken together, these results indicate a potentiation of the hypothalamic-pituitary adrenal axis reactivity along with a reduction in the associated negative feedback. These modifications potentially mediate long-term detrimental effects such as stress-associated body weight reduction and neuronal damage. Prenatal restraint stress-associated phenotypic modifications have been associated to

increased susceptibility to develop neuropsychiatric disorders such as major depression, drug addiction, metabolic syndrome or anxiety (Darnaudéry and Maccari, 2008). Although the layers of phenotypic differences have not yet been tied in a coherent contextual framework, the phenotypic impacts of prenatal restraint stress are profound and long-lasting and exhibit strong sex differences (Bowman et al., 2004; Lesage et al., 2004; Zuena et al., 2008). Results from this section are summarized in Table 2.

1.4.2 General impacts of prenatal chronic variable stress

Chronic variable stress involves dams being exposed to an unpredictable mild daily stressor. Stressors are applied one to three times a day and range from exposure to 2,3,5-trimethyl-3-thiazoline (TMT, a purified fox feces component) or a new object, white noise, saturated bedding, restraint, multiple cage change, forced swimming, cold exposure, social crowding or light-dark reversal (Koenig et al., 2005; Mueller and Bale, 2006; Tamashiro et al., 2009). Some of those stressors are applied overnight. Chronic variable stress has been applied over different foetal periods but I will report here chronic variable stress applied mainly during the second half of pregnancy. Dams submitted to chronic variable stress show higher circulating corticosterone at the end of pregnancy.

Behavioural impacts of prenatal chronic variable stress includes increased locomotor activity and aggression accompanied by decreased social interaction in both sexes compared to control rats (Koenig et al., 2005; Lee et al., 2007; Wilson and Terry, 2013). Decreased object recognition memory was detected in prenatally chronically variably stressed rats of both sexes (Wilson and Terry, 2013) while spatial learning was slower in prenatally chronically variably stressed males and faster in prenatally chronically variably stressed females compared to control unstressed mice (Mueller and Bale, 2007). Finally, prenatally chronically variably stressed rats showed decreased sensorimotor gating through an increased startle response to a stress-associated cue (Koenig et al., 2005).

Physiologically, prenatally chronically variably stressed mice are heavier at birth (Mueller and Bale, 2006). Prenatally chronically variably stressed rats also show

Table 2. Effects of traditional prenatal stress paradigms on the animal phenotype

Paradigm	Offspring behavioral traits								Offspring physiological traits		
	Stress-related behavior	Activity	Immobility	Social interaction	Startle response	Learning	Stereotypic behavior	Food consumption	Weight	Glucose/Insulin to challenge	Adrenal weight
PRS	↑[4,5,24,27]	↑[4,6,24]	↑[16]	↓[5,6,13,16]	↑[13]	↓[3,6,25]	↑[13]	↓M [23]	↓M [12,23]	↑[12,23]	B↓[12]A↑[3]
CVS	↑[19]	↑[10,26]	↑[19]	↓[11] agg.↑[26]	↑[10]=[26]	↓[18,26] ↑F[18]			B↑[17]	↑[22]	

Paradigm	Offspring endocrine traits		Offspring gene transcript abundance and epigenetic modifications traits									
	Baseline CORT	Glucocorticoid stress response	NR3C1 expression	CRF expression	BDNF expression	DNMT expression	NR3C1 DNA methylation	CRF DNA methylation	BDNF DNA methylation	11β-HSD2 DNA methylation	Histone modifications	Transcriptome
PRS	↓[12]	↑[7,16,24,25,27]	↓[7,9]		↓[6,14]	↑[13,14,20]			↑[6,14]	✔[20,21]	✔[21]	
CVS	B↓[22]	↑[10,19]	↓M[19]	↑M [19]	↓[1]	↑[1]	↑M[19]	↓M[19]	↑[1]M=[19]			✔[15]

PRS: Prenatal Restraint Stress
CVS: Chronic variable Stress
Agg.: Aggression
F: Female
M: Male
B: Birth
A: Adult
✔: Induced trait

[1]Boersma et al. 2014
[2]Bowman et al. 2004
[3]Darnaudéry et al. 2006
[4]Darnaudéry and Maccari 2008
[5]De Souza et al. 2013
[6]Dong et al. 2015
[7]Henry et al. 1994
[8]Koehl et al. 1997
[9]Koehl et al. 1999
[10]Koenig et al. 2005
[11]Lee et al. 2007
[12]Lesage et al. 2004
[13]Matrisciano et al. 2013
[14]Monteleone et al. 2014
[15]Morgan and Bale 2011
[16]Morley-Fletcher et al. 2003
[17]Mueller and Bale 2006
[18]Mueller and Bale 2007
[19]Mueller and Bale 2008
[20]Pena et al. 2012
[21]Schneider et al. 2016
[22]Tamashiro et al. 2009
[23]Vallée et al. 1996
[24]Vallée et al. 1997
[25]Vallée et al. 1999
[26]Wilson and Terry 2013
[27]Xu et al. 2014

decreased glucose tolerance from weaning to adulthood (Tamashiro et al., 2009). Prenatally chronically variably stressed rodent males also show endocrinological modifications through a decrease in circulating corticosterone at birth but increased hypothalamic-pituitary adrenal axis reactivity in adulthood (Koenig et al., 2005; Mueller and Bale, 2008; Tamashiro et al., 2009).

At the epigenetic level, the main differences were found in adult males exposed to prenatal chronic variable stress during the first week of pregnancy. DNA methylation was altered in adult prenatally chronically variably stressed male mice amygdala corticotropin-releasing factor and hippocampal glucocorticoid receptor genes while the brain-derived neurotrophic factor gene DNA methylation was not affected (Mueller and Bale, 2008) when compared to control male mice. Increased hippocampal glucocorticoid receptor methylation was associated to decreased transcript abundance and the opposite was observed in the amygdala corticotropin-releasing factor. Taken together, these results are in accordance with an increase in stress reactivity in prenatally chronically variably stressed male mice. Further, early prenatal chronic variable stress is associated with male dysmasculinization through changes in steroid metabolism and microRNA transcript abundance as detected through the comparison of postnatal day 1, second generation brain transcriptome of mice of both sexes (Morgan and Bale, 2011). Female epigenetic state has not been assessed in response to chronic variable stress applied on dams during the first week of pregnancy. In a very thorough study, chronic variable stress applied on dams during the last week of pregnancy lead to decreased brain-derived neurotrophic factor transcript abundance in the hippocampus and amygdala after weaning up to adulthood associated to an increase in DNA methyltransferase transcript abundance and brain-derived neurotrophic factor DNA methylation within the exon *IV* promoter (Boersma et al., 2014).

Therefore, prenatally restrained animals show stress-associated behavioural, physiological, endocrine, gene transcript abundance and epigenetic modifications somewhat similarly to the phenotypic differences found in response to the prenatal restraint stress (see Table 2). Further, the phenotypic impacts of prenatal chronic variable

stress are profound and long-lasting and exhibit sex differences when both sexes were investigated (Morgan and Bale, 2011; Mueller and Bale, 2007).

1.4.3 Caveats of traditionally studied prenatal stress paradigms

Although phenotypic modifications triggered by prenatal restraint stress and prenatal chronic variable stress are widespread and interesting, some caveats should be considered. Indeed, the prenatal restraint stress-induced phenotype has been exhaustively studied; this physical stressor is artificial and lacks a proper ecological context to interpret the impact of the elicited phenotypic modifications. Similarly, the chronic variable stressors are combining physical and psychogenic stressors that are mitigated by the presence of side effects such as the heat or cold stress initiated by restraint, cold exposure, wet bedding or forced swimming. These stressors have a physical component to them which underlies endocrine modifications, such as thyroid hormone release in response to cold stress (Gaskill et al., 2013), beyond the glucocorticoid release elicited. Subsequently, the programming effects are more difficult to disentangle and interpret. These complications are further added to the different chronic variable stressors combinations and frequency applied by different research groups.

Therefore, an optimal prenatally applied stressor to study the broad phenotypic impacts and mechanisms underlying maternal programming should be psychological and mild. Additionally, this stressor should offer a context to evaluate the fitness consequences of the expressed programmed phenotype.

1.5 Predator and predator cues presence lead to modifications of the prey phenotype

Predation cues constitute an optimal naturally occurring stressor to elicit maternal programming as it provides a context to evaluate the consequent long-term survival and

health phenotypic impacts. Further, this type of stress has been present over evolutionary times.

In this section, I will describe how individual mammalian phenotypes are modified by non-lethal predator or predator cue presence at the behavioural, physiological, endocrine, neural circuits activation and transcript abundance level. However, most of the impacts described here are also widely found in non-mammalian vertebrate species and several invertebrate species (Lima, 1998). Finally, I will report direct fitness impacts of the induced phenotypic changes as well as the programming potential of this type of stress.

This review is not exhaustive but rather conveys the breadth of the phenotypic alterations following the predation stress. I report studies conducted on wild or laboratory animals in wild, semi-natural or laboratory settings using live predators or predator cues. The studies reported also covers mammals of all developmental ages.

1.5.1 Behavioural changes elicited by predator or predator cue presence in mammalian prey species

1.5.1.1 Behavioural changes elicited by predator or predator cue presence in mammalian prey species in a wild or semi-natural context

One of the common behavioural trade-offs identified in natural populations and in semi-natural settings following changes in the risk of predation is to leave a safe habitat to forage. Several rodent species including house mice and white-footed mice exposed to mammalian feces or raptor predation cues favoured foraging under a vegetation cover instead of an open habitat, while simultaneously reducing their general activity level (Bowers et al., 1993; Dickman, 1992). Similarly, the introduction of wolves in elk habitats lead to the displacement of the elks from an optimal grassland meadow habitat to a protected wooded area (Creel et al., 2005). This displacement was associated to a reduction in their food intake (Christianson and Creel, 2010). Further, elks doubled the proportion of daylight hours they spent vigilant (Creel et al., 2008).

A general decrease in activity accompanied by increased hiding in refuges has been detected following increased predation risk. Indeed, gerbils and desert rodent species decreased their activity under owl presence cues (Abramsky et al., 1996; Longland and Price, 1991). Similarly, rats exposed to a cat predator in a visible burrow system reduced their activity level while increasing their flight response and time to emerge from the burrow (Blanchard and Blanchard, 1989). Bank voles exposed to predator scents also decreased their activity in response to weasels, stoats, stone martens or polecats predators (Jedrzejewski et al., 1993). In addition, field voles exposed to a falcon threat decreased their activity level and favoured less risky areas of the environment to avoid predation (Korpimaki et al., 1996). Finally, wild rat populations that are normally nocturnal became diurnal in response to a nocturnal fox threat. This behaviour was inducible and reversed back after the removal of the predatory threat (Fenn and MacDonald, 1995).

1.5.1.2 Behavioural changes elicited by predator or predator cue presence in mammalian prey species in a laboratory context

Studies conducted in the laboratory complement and confirm a lot of the wild population observations. The bulk of the research in laboratory settings has been done on laboratory mice and rat strains.

Few studies have looked at the impact of predation on foraging in a laboratory setting. Rats exposed to a predator-simulating robot reduced their food consumption in its presence while hiding longer in their nest (Choi and Kim, 2010). However, foraging in the presence of uniform predator odour did not change the use of exposed or covered food patches in mice (Troxell-Smith et al., 2016).

Avoidance of predator or predator cues is widely observed in laboratory rodents. CD-1 mice avoided fox feces scent more than an aversive odour not associated to predation threat (butyric acid, Sotnikov et al., 2011). Rodents exposed to a purified fox feces scent (2,3,5-trimethyl-3-thiazoline), to a live or feces from a cat or ferret increased their startle

response, avoidance, emergence time form a shelter and spontaneous defecation and urination while decreasing their motor activity and visits of the exposed portions of a novel environment (open field) (Belzung et al., 2001; Dielenberg et al., 2001; Fendt et al., 2005; Plata-Salaman et al., 2000). Male mice and rats exposed to a cat or a ferret acutely increased their frequency of risk assessment behaviours in a stressful elevated novel environment (elevated-plus maze). Further, this effect lasted at least a week and potentiated the long-term startle response of the prey (Adamec et al., 2004b, 2003; Plata-Salaman et al., 2000). Lastly, predator odour exposure is sufficient to elicit increased stress-induced behaviours in rodents, including decreased activity in the elevated-plus maze (Armario et al., 2008).

Social affiliation with conspecifics can be modulated with predation risk. Long-Evans rats exposed to a cat subsequently decreased the time they spend investigating a conspecific (Adamec et al., 2003; Blundell et al., 2005). However, in other studies, predation stress did not modify the amount of social interaction in mice (Adamec et al., 2006, 2005). Male striped field mice exposed chronically over 21 days to weasel male anal gland odour exhibited a transient increase in aggressive behaviour in staged encounters during the first week of predator odour exposure (Wang et al., 2011).

Predation stress can also enhance learning, an influence best tested in a laboratory setting. CD-1 mice learned faster the localization of a platform in a Morris water maze when they are exposed to a fox feces scent beforehand, a phenomenon mediated through the amygdala and hippocampus (Galliot et al., 2010). On the contrary, Swiss mice of both sexes trained in a Morris water maze learned slower when simultaneously presented with a rat odour (Banik and Anand, 2011). Although the effects of predator odour are contradictory, the presence of a predator cue before or during the learning task likely affects the end result.

Furthermore, the behavioural response to predatory stimuli changed at different developmental stages. Rat weanlings at 26 days of age or near weaning (postnatal day 20) display increased immobility and analgesia in response to a cat odour or a live cat, while

it is not the case during their stress hyporesponsive period on postnatal day 14. This progression indicates potentially age-specific selection pressure around the weaning age to express anti-predatory behaviour (Wiedenmayer and Barr, 2001).

1.5.1.3 Summary of behavioural modifications elicited by predator or predator cue presence in mammalian prey species

As reported here, anti-predatory behaviours have been widely observed in mammals. The most broadly expressed anti-predatory behaviours include avoidance through increased refuging and vigilance associated to decreased activity and foraging. Additionally, some prey studies report broad habitat and circadian activity shifts to avoid predators. Finally, predator or predator cue exposure can lead to a reduction in social affiliation and increase in competition-based aggressive behaviours. These behaviours are in accordance with the stress-induced 'fight-or-flight' response associated to trade-offs such as reduced energy intake through foraging. Results from this section are summarized in Table 3.

1.5.2 Physiological changes in response to predator or predator cue presence in mammalian prey species

1.5.2.1 Physiological changes in response to predator or predator cue presence in mammalian prey species in a wild or semi-natural context

A well-documented physiological effect of stress and predation is stress-induced hyperthermia. Stress-induced hyperthermia is defined as an increase in body temperature in response to stress through a temporary elevation of the metabolic rate (Bouwknecht et al., 2007). In the wild, kangaroo rats investigating a predatory rattlesnake dynamically altered their body heat temperature signature with their head and snout showing a decrease in temperature while their leg temperature significantly increased

Table 3. Effects of predator and predator cue presence on prey phenotype

Behavior		Physiology		Endocrine		Neural circuit and genes transcription	
Trait	Type of change	Trait	Type of change	Trait	Type of change	Trait	Type of change
Foraging	↓[14,17,18,22] =[42]	SIH	✔[15,36,38]	Glucocorticoid	↑[12,15,33,35,39,43]	HPA axis brain regions IEG*	↑[7,22,26,32,37,40]
Refuging/ Avoidance	↑[10,17,19,23,35,39]	Metabolic rate**	↑[15,16]	ACTH	↑[15,32,33]	Transcriptome	HP✔[30]
Activity	↓[1,6,9,10,13,22,28,29,31]	Body condition	↓[12]	CBG binding	↓[12]	Stress-related genes	↑[26,37,41]
Vigilance/ Immobility	↑[3,13,21,24,44]	Weight	↓[12,43]	Testosterone	↓[12]	Thyroid-hormone related genes	↑[30] ↓[34]
Habitat shift	✔[19,29]	Immunity	↓[12]	Progesterone	↓[20]	Energy mobilization related genes	↑[32]
Circadian activity shift	✔[25]	Adrenal gland size	↑[43]			Steroid metabolism related genes	↑[32]
Social behavior	↓int.[2,11] ↑agg.[43] =[4,5]					Monoamine turnover	↑[8]
Startle response	↑[35]						
Analgesia	↑[44]						
Learning	↑[27] ↓[8]						

SIH: Stress Induced Hyperthermia

✔: Induced trait

**Including oxygen consumption and heart rate

CBG: Corticosteroid-binding globulin

IEG: Immediate Early Gene

HP: Hippocampus

[1]Abramsky et al. 1996
[2]Adamec et al. 2003
[3]Adamec et al. 2004
[4]Adamec et al. 2005
[5]Adamec et al. 2006
[6]Armario et al. 2008
[7]Asok et al. 2013
[8]Banik and Anand 2011
[9]Belzung et al. 2001
[10]Blanchard and Blanchard 1989
[11]Blundell et al. 2005
[12]Boonstra et al. 1998
[13]Bowers et al. 1993
[14]Brabrand and Faafeng 1993
[15]Campeau et al. 2008
[16]Chabot et al. 1996
[17]Choi and Kim, 2010
[18]Christianson and Creel 2010
[19]Creel et al. 2005
[20]Creel et al. 2007
[21]Creel et al. 2008
[22]Dickman 1992
[23]Dielenberg et al. 2001
[24]Fendt et al 2005
[25]Fenn and MacDonald 1995
[26]Figueiredo et al. 2003
[27]Galliot et al. 2010
[28]Jedrzejewski et al. 1993
[29]Korpimaki et al. 1996
[30]Lavergne et al. 2014
[31]Longland et al. 1991
[32]Masini et al. 2005
[33]Masini et al. 2009
[34]Nanda et al. 2008
[35]Plata-Samalan et al. 2000
[36]Rorick-Kehn et al. 2005
[37]Roseboom et al. 2007
[38]Shraft and Clark 2017
[39]Sotnikov et al. 2011
[40]Staples et al. 2009
[41]Thomson et al. 2012
[42]Troxell-Smith et al. 2016
[43]Wang et al. 2011
[44]Wiedenmayer and Barr 2001

(Schraft and Clark, 2017). Other metabolic impacts reported include increased heart rate in elk exposed to coyote predator scent (Chabot et al., 1996).

Weight maintenance is also largely affected by predatory threat. Snowshoe hares under high predation stress (e.g. lynx and coyotes) show increased overwinter body mass loss compared to hares from the same population under low predation stress. This weight loss is accompanied by a decrease in body condition and immunity, demonstrated in part through lower leucocyte blood counts (Boonstra et al., 1998).

1.5.2.2 Physiological changes in response to predator or predator cue presence in mammalian prey species in a laboratory context

Important physiological impacts of predation are also reported in laboratory settings. The stress-induced hyperthermic response was detected in rats exposed to ferret skin odour exposure along with an increased heart rate as compared with the exposure to an unknown strawberry odour (Campeau et al., 2008).

Regarding weight maintenance, female striped field mice exposed chronically over 21 days to weasel male anal gland odour lost weight while their adrenal gland relative size increased (Wang et al., 2011).

1.5.2.3 Summary of physiological modifications in response to predator or predator cue presence in mammalian prey species

Physiological changes in prey species in response to predator or predator cue presence has been observed in response to predation stress. Acute physiological changes include an increase in metabolic rate detected through a rise in body temperature and faster heart rate. Chronic predation stress can also lead to weight loss and a decrease in body condition. Some of these effects are likely downstream consequences from the predator-induced decrease in foraging reported previously. Results from this section are summarized in Table 3.

1.5.3 Endocrine changes in response to predation or predation cue presence in mammalian prey species

1.5.3.1 Endocrine changes in response to predation or predation cue presence in mammalian prey species in a wild or semi-natural context

Glucocorticoids widely mediate the stress response to predation. Female snowshoe hares exposed to a high predation stress displayed higher blood free cortisol level and reduced maximum cortisol-binding globulin binding capacity than hares from the same population exposed to a low predation risk (Sheriff et al., 2010).

1.5.3.2 Endocrine changes in response to predation or predation cue presence in mammalian prey species in a laboratory context

Female striped field mice chronically exposed to male weasel anal gland scent overnight showed an increased baseline corticosterone concentration as measured in the feces over the entire three week exposure while males and control mice did not show this elevated corticosterone level (Wang et al., 2011). Similarly, rats acutely and chronically exposed to ferrets exhibited an increase in circulating corticosterone when compared to unexposed animals (Plata-Salaman et al., 2000). This increase in corticosterone and adrenocorticotropic hormone is specific to the predator odour and does not happen in response to a novel odour such as a strawberry scent (Campeau et al., 2008; Masini et al., 2009). Finally, CD-1 mice exposed acutely to 2,3,5-trimethyl-3-thiazoline show increased circulating corticosterone as compared to the exposure to a solely aversive odour (butyric acid) or cat odour (Sotnikov et al., 2011).

1.5.3.3 Summary of endocrine modifications in response to predation or predation cue presence in mammalian prey species

Glucocorticoid release in prey species in response to predator or predator cue presence is the hallmark indicator that predation elicits stress in prey species. This induction is robust, stable and long lasting with no signs of habituation over time. Further, this induction is specific to predator odours, as other novel odours do not elicit a similar strong increase in circulating glucocorticoids. Results from this section are summarized in Table 3.

1.5.4 Neural circuits activation and transcript abundance changes in response to predator or predator cue presence in mammalian prey species

1.5.4.1 Neural circuits activation and transcript abundance changes in response to predator or predator cue presence in mammalian prey species in a wild and semi-natural context

There are only a few studies that examine the impact of differential predation stress on transcript abundance in wild populations. Lavergne et al. (2014) compared the hippocampal transcriptome of wild adult male hares from the same population during years of high and low predation stress. Predation stress lead to the differential regulation of 106 genes, with high predation stress leading to the up-regulation of genes implicated in metabolic processes and hormonal response concomitantly to the down-regulation of genes playing a role in immune response and blood cell production. Interestingly, the strongest down-regulated gene was transthyretin, implicated in thyroid hormone transport regulating upon other the growth of the animal. Furthermore, transthyretin contains a glucocorticoid-response element making it stress-sensitive. The pathways and differentially expressed genes found in this study are therefore in accordance with the physiological impacts found in hares under different predation stress such as the difference in hormonal levels, increased weight loss and decreased body condition and immunity found in this species (Boonstra et al., 1998).

1.5.4.2 Neural circuits activation and transcript abundance changes in response to predator or predator cue presence in mammalian prey species in a laboratory context

Immediate early gene induction (e.g. *Egr1*, *C-fos*, *ΔFosB*), indicating general activation of a brain region, has been observed on several instances in laboratory rodents. Adult male rats exposed to a cat acutely or chronically exhibited an increase in the immediate early gene *C-fos* transcript abundance in several brain region including the medial amygdala, the medial hypothalamus and the bed nucleus of the stria terminalis, which are brain structures that are associated to the fear-induced 'defense circuit' (Dielenberg et al., 2001; Figueiredo et al., 2003). Additionally, an increase in the corticosterone-releasing factor transcript abundance in the paraventricular nucleus of the hypothalamus was detected (Figueiredo et al., 2003). Similarly, chronic exposure of adult male rats to a cat skin odour over a week lead to the up-regulation of the immediate early genes *FosB* and *ΔFosB* for a week in the nucleus accumbens and in the hypothalamus (Staples et al., 2009). Rats exposed to 2,3,5-trimethyl-3-thiazoline acutely showed a specific activation of early genes (*C-fos*, *Enk*, *Egr-1*) in the bed nucleus of the stria terminalis, the paraventricular nucleus of the hypothalamus, the central amygdala but not the medial prefrontal cortex as compared to the aversive butyric acid or neutral water odours (Asok et al., 2013). Adult male rats exposed acutely to a live ferret or its fur and skin odour induced an increase in *C-fos* transcript abundance in several brain regions including the cortex, the nucleus of the stria terminalis, paraventricular nucleus of the hypothalamus, basolateral nucleus of the amygdala and hippocampal cornu ammonis 3 (Masini et al., 2005; Roseboom et al., 2007). Further, an increase in the transcript abundance of corticosterone-releasing hormone-binding protein, corticosterone-releasing hormone receptor 1 and somatostatin receptor 2 was detected in the amygdala (Nanda et al., 2008; Roseboom et al., 2007). Corticosterone-releasing hormone-binding protein binds the corticosterone-releasing factor and buffers it's action by preventing its binding to the receptor, while possibly targeting the corticosterone-releasing factor peptide for degradation (Roseboom et al., 2007). Somatostatin inhibits the growth hormone and thyroid hormone secretion and has been associated to neuropsychiatric disorders such as

depression (Nanda et al., 2008). Finally, the medial amygdala is necessary for this specific predator stress response (corticosterone and adrenocorticotropic hormone release) as a bilateral lesion in this region invalidated this typical response (Masini et al., 2009).

Regarding neuropeptides, adult male Swiss mice exposed to a cat showed an increase in the turnover rates of several monoamines in the hippocampus (noradrenaline and serotonin) and hypothalamus (dopamine) as measured by liquid chromatography (Belzung et al., 2001). Furthermore, when serotonin reuptake was prevented, Swiss mice did not exhibit the normal avoidance to cat feces.

Although epigenetic modifications initiated by predator stress has never been reported to my knowledge, two studies in non-mammalian species are worth reporting. *Daphnia* juvenile exposed to a predatory Dipteran insect showed several transcriptome alterations when compared to unexposed *Daphnia* (Rozenberg et al., 2015). Similarly, Mori et al. (2015) found differences in the frog tadpole transcriptome in relation to the presence of larval dragonfly or salamander predators. Interestingly, in both cases, some of the up-regulated genes were associated to chromatin reorganization and regulation (Mori et al., 2015; Rozenberg et al., 2015). These genes could be associated to epigenetic modifications controlling predator-induced phenotypic plasticity.

1.5.4.3 Summary of neural circuits activation and transcript abundance changes in response to predator or predator cue presence in mammalian prey species

The bulk of the research done on predation stress-induced neural circuit activation and transcript abundance has been done in rodent laboratory experiments. In several studies, immediate early gene transcription was induced in brain regions associated to the 'defense circuit' including the hypothalamus, amygdala, hippocampus and bed nucleus of the stria terminalis among others. Taken together, this sustained activation links these brain regions to predator-induced defensive behaviours. Further, some candidate

transcript abundance including the corticosterone-releasing factor and some of its family members (corticosterone-releasing factor binding protein and corticosterone-releasing factor receptor 1) in the paraventricular nucleus of the hypothalamus and the amygdala are the most sustainably and widely activated in response to predation stress. Additionally, downstream effects detected target a reduction of growth and thyroid hormone secretion. Finally, non-mammalian species suggest, although not directly reported, an implication for epigenetic mechanisms in the adaptive response to predation stress. Results from this section are summarized in Table 3.

1.5.5 Phenotypic impacts of prenatal exposure to predator or predator cue presence

Interestingly, some studies have explored the impact of prenatal predator or predator cue presence on the long-term phenotype of an individual. I report non-mammalian species impacts of prenatal predator or predator cues exposure as few mammalian studies have been conducted to date.

1.5.5.1 Phenotypic impacts of prenatal exposure to predator or predator cue presence in a wild and semi-natural context

Injecting corticosterone within a physiological range in lizard eggs (*Lacerta vivipara*), mimicking maternal stress in the mother that could be elicited through predation stress, produced juvenile offspring with no obvious morphological changes at birth but expressing increased anti-predatory behaviours. The resulting juvenile lizards took longer to emerge from a shelter after a simulated predator attack with a paintbrush compared to control unstressed animals (Uller and Olsson, 2006). Further, gravid female common lizard exposed to predatory snake scents produced offspring which were heavier, grew longer tails, and, as juveniles, selected colder habitats and dispersed twice more frequently than the offspring of unexposed mothers (Bestion et al., 2014). Additionally, prenatally predator odour-exposed juvenile lizards exhibited greater sensitivity to the odour of the snake predator as detected through a higher tong-flicking rate when exposed

to the predator odour (Shine and Downes, 1999). These changes are in accordance with anti-predatory tactics detected in this species such as autotomy and increased risk avoidance by dispersal and selecting parts of the habitat under colder temperature while being increasingly vigilant to a predator odour to which they were never directly encountered.

In a song sparrow wild population, higher predation stress, through the presence of a greater variety of predators, decreased nestling quality compared to sparrows under lower predation pressure (Clinchy et al., 2004). Furthermore, twelve coexisting passerine species from which nest predation was prevented lead the parents to invest more in youth production through increased egg size, clutch mass and rate of nestling feeding (Fontaine and Martin, 2006). Similarly, a song-sparrow population for which the perceived predation pressure was increased through playbacks of predator calls display a reduction in the amount of offspring produced. Additionally, these offspring were more susceptible to thermoregulatory stress and death prior to fledging (Zanette et al., 2011)

Finally, in a semi-natural environment, pregnant hares exposed to a live predator (a trained dog) showed elevated fecal cortisol metabolites correlated with the average juvenile increased fecal cortisol metabolites (Sheriff et al., 2010). Furthermore, the offspring were also lighter and shorter at birth (Sheriff et al., 2009).

1.5.5.2 Phenotypic impacts of prenatal exposure to predator or predator cue presence in a laboratory context

Live predator cues (*Chaoborus* kairomones) in the rearing water of gravid *Daphnia* induced morphological defense (helmet) in the offspring up to the second generation downstream (F2) of offspring. The helmet makes these *Daphnia* less palatable to predators (Agrawal et al., 1999). Furthermore, offspring of gravid crickets exposed to a wolf spider show increased anti-predatory behaviours and survival to that same predator (Storm and Lima, 2010).

In threespine sticklebacks (*Gasterosteus aculeatus*), gravid females exposed to predation threat (chased by a northern pike model daily) produced eggs containing more corticosterone and displaying higher metabolic rates (consuming more oxygen) while staying longer in the embryonic stage than control animals (Giesing et al., 2011; Mommer and Bell, 2014). Furthermore, these embryos showed massive transcriptomic changes including several non-coding RNAs (Mommer and Bell, 2014). These altered transcripts are associated to metabolism of steroid and sex differentiation, epigenetic modifications (DNA methyltransferases and histones) and acceleration of the embryonic development. Furthermore, juvenile stickleback fish produced from a gravid female exposed to a clay predator formed tighter shoals (Giesing et al., 2011).

In accordance with this observation, song sparrows exposed to acute corticosterone supplementation mimicking an acute stressor (such as frequent encounters with predators) during development and growth displayed an increase in standard metabolic rate and diurnal metabolism, especially in females (Schmidt et al., 2012). Further, in European starlings, corticosterone administration in the egg yolk produced nestlings with increased pectoral muscle mass associated to improved flight performance and metabolic capacity (Chin et al., 2009).

In rodents, mice exposed to predator rat urine during the first week of pregnancy produced smaller litters with decreased survival of the offspring (de Catanzaro, 1988). Rats exposed to a live cat predator or predator odour prenatally were lighter at birth while displaying increased blood corticosterone level during the first postnatal week along with stronger induced seizures, linked to decreased hippocampal plasticity (Ahmadzadeh et al., 2011; Korgan et al., 2014; Saboory et al., 2011).

Although not being a prenatal stress, rodent dams exposed to predator odour shortly after parturition (postnatal days 1 to 3) in presence of her offspring displayed an increase in maternal licking-grooming and arched-back nursing behaviours (Coutellier and Würbel, 2009; Mashoodh et al., 2009; McLeod et al., 2007; but see Burton et al., 2006). Further, exposure of lactating mice dams in presence of their pups until weaning (postnatal days 1

to 21) lead to a specific impairment in adult female offspring memory (Coutellier and Würbel, 2009). Rat dams and pups exposed to cat odour on the day of birth lead to increased avoidance to a predator odour in adult animals and decreased stress-related behaviour in adult female offspring in a novel environment (open field) while male offspring showed the opposite effect when compared to unstressed offspring. However, no difference in the glucocorticoid receptor transcript abundance was detected (Mashoodh et al., 2009). Finally, pups exposed to 2,3,5-trimethyl-3-thiazoline during the first 3 postnatal weeks in the dam's presence vocalized more to solicit maternal care on postnatal days 1, 2 and 5 when compared to control pups exposed to a solely aversive odour (butyric acid). In contradiction with the other studies reported here, pup exposed to 2,3,5-trimethyl-3-thiazoline showed decreased immobility when exposed to 2,3,5-trimethyl-3-thiazoline in young adolescence on postnatal day 30 (Ayers et al., 2016).

1.5.5.3 Summary of phenotypic impacts of prenatal exposure to predator or predator cue presence

Although no species has been studied exhaustively at all phenotypic level, the results reported here suggest that prenatal predation stress can affect the offspring integrative phenotype, in accordance with the concept of temperament or behavioural type. Temperament consists of fixed inter-correlated traits over time (or behavioural type if not tested over time) that are determined by the life history of the individual (Réale et al., 2007). Such inter-correlated traits include boldness (opposite to cautiousness or shyness), exploration and activity levels that are reproducible over time and over a range of situations. Epigenetic modifications could likely mediate the developmental trajectories activation although no study has investigated the mechanism associated to this phenomenon yet. Results from this section are summarized in Table 4.

Table 4. Effects of prenatal predator or predator cue presence on the offspring phenotype

Class	Offspring traits											
	Antipredatory behavior	Social behavior	Body size	Body condition	Morphological defense	Metabolic rate	Glucocorticoids production	Transcriptome	HPA axis brain regions IEG*	Chromatin organization processes	Metabolism of steroid processes	Offspring survival
Invertebrate	↑[17]				✔[1]							↑[17]
Fish		↑aff.[9]	↑[11]			↑[9]	↑[9]	✔[11]		↑[11]	↑[11]	
Reptile	↑[4,16,17]		↑[16]		✔[16]							↑[17]
Bird			↓[8]	↓[6]	✔[5]	↑[5,13]						↓[19]
Mammal	↓[3]		↓[2,10,14]	↓[2,10,12]			↑[12,14,15]		↑[10]			↓[7]

*Includes the hippocampus, amygdala and paraventricular nucleus of the hypothalamus
HPA axis: Hypothalamic-Pituitary Adrenal axis
IEG: Immediate Early Gene
Aff.: Affiliation

[1]Agrawal et al. 1999
[2]Ahmadzadeh et al. 2011
[3]Ayers et al. 2016
[4]Bestion et al. 2014
[5]Chin et al. 2009
[6]Clinchy et al. 2004
[7]de Catanzaro 1988
[8]Fontaine and Martins 2006
[9]Giesing et al. 2011
[10]Korgan et al. 2014
[11]Mommer and Bell 2014
[12]Saboory et al. 2011
[13]Schmidt et al. 2012
[14]Sheriff et al. 2009
[15]Sheriff et al. 2010
[16]Shine and Down 1999
[17]Storm and Lima 2010
[18]Uller and Olsson 2006
[19]Zanette et al. 2011

1.6 Rationale

I demonstrated here that mammalian species are responsive and modify their phenotype in response to predator or predator cues presence in wild, semi-natural and laboratory settings. These effects are diverse and spread across the integrative phenotype of the prey: affecting its behaviour, physiology, endocrinology, neural circuit activation and transcript abundance level. However, no study has investigated simultaneously all trait types within a prey species. Although widespread, costly inducible changes in the prey phenotype converge within a trait type between species. This convergence suggests that the mechanisms underlying predator response are either evolutionary conserved or converged over a wide range of species. Additionally, prenatal exposure to predator presence or predator cue triggered several phenotypic modifications generally in accordance with the changes elicited in preys postnatally or in adulthood. Additionally, some chromatin organization genes that are likely involved in epigenetic modifications are up-regulated in response to predation risk although no study investigated direct epigenetic alterations in response to predation or predation cues.

In a lot of instances, studies investigated males phenotypic changes only (e.g. Belzung et al., 2001; Dielenberg et al., 2001; Figueiredo et al., 2003; Roseboom et al., 2007). However, when both sexes were studied, frequent sex differences have been reported (e.g. Clinchy et al., 2011; Coutellier and Würbel, 2009; Mashoodh et al., 2009; Schmidt et al., 2012), suggesting that intensive efforts should be done in the future to investigate predation-induced phenotypic plasticity in males and females.

Therefore, prenatal predator cue exposure should be sufficient to elicit modifications in the long-lasting integrative phenotype of an individual. This phenotype should be consistent with the notion of behavioural type.

Furthermore, epigenetic modifications should be assessed as a mechanism that could maintain the animal phenotype. Finally, sex-specific mechanistic and phenotypic differences were expected.

1.7 Objectives

The objective of this thesis was to understand the long-term impacts of prenatal predator cue exposure. More specifically, I investigated the programming impact of unpredictable and unavoidable predator odour exposure during the prenatal hypothalamic-pituitary adrenal axis development (Charil et al., 2010) on the adult integrative phenotype encompassing behavioural, physiological and endocrine traits as well as the underlying mechanistic transcript abundance and associated epigenetic modifications. Traits assessed will be selected amongst the most widely predation stress-induced traits reported above.

To achieve this objective, I designed a predator odour exposure paradigm (PO) in which pregnant rodent females were exposed to an hour of an unpredictable and inescapable odour at a random time over the second half of pregnancy (gestational day 11 to 18). Controls were exposed to distilled water over the same period and duration at a predictable time. Sex and species-specific variations were evaluated by applying the prenatal predator odour paradigm in both sexes of two different rodent model species: C57BL/6 mice and Long-Evans rats.

1.8 Hypotheses

I hypothesize that the prenatal predator odour exposure paradigm will be sufficient to elicit modifications of the animal's integrative phenotype in a coherent manner, similarly to a behavioural type, that will be advantageous under high predation risk. More specifically:

1) The prenatal predator odour exposure paradigm will elicit adult behavioural modifications including general hypoactivity, decreased foraging in open areas, increased stress-related behaviour and hiding in novel aversive environments and decreased social behaviour when compared to the control phenotype.

2) The prenatal predator odour exposure paradigm will elicit physiological modifications including lower birth and adult weight, increased adult metabolic rate at baseline and in response to predator stress when compared to the control phenotype.

3) The prenatal predator odour exposure paradigm will elicit adult endocrine modifications including increased corticosterone stress reactivity when compared to the control phenotype.

4) The prenatal predator odour exposure paradigm will elicit increased long-term transcript abundance changes of stress-related genes in brain regions implicated in the hypothalamic-pituitary adrenal axis in adults when compared to the control phenotype.

5) The prenatal predator odour exposure paradigm will elicit long-term differential DNA methylation at Cytosine Guanine dinucleotides in key differentially expressed genes in adults when compared to the control phenotype.

6) These differences will display some degree of specific sex- and species-specific modifications.

Chapter 2 General methods

2.1 Animal housing and breeding

C57BL/6 mice and Long-Evans rats were obtained from Charles River Canada (St. Constant, QC). Mice and rats were housed in same-sexed groups (mice: 3-5 per cage, rat: 2 per cage) and maintained on a 12:12 hours light-dark cycle (lights on at 7AM) with *ad libitum* access to food and water. All experimental protocols were approved and conform to the Local Animal Care Committee at the University of Toronto in Scarborough regulatory standards and were in accordance with the guidelines of the Canadian Council on Animal Care.

2.2 Predator odour exposure in pregnant dams

Females were habituated to the exposure rooms, the control room and the predator odour exposure room including a fume hood for the predator odour exposure, for 5 to 7 consecutive days prior to breeding. From gestational days 11 to 18 in mice and from gestational days 12 to 20 in rats, dams were introduced to a novel cage (mice: 15 x 33 cm, rat: 19 x 39 cm) and presented with liquid odours on cotton balls sealed in a petri dish with holes, used to avoid direct contact with the odourants. Dams in the predator odour condition were exposed once per day for one hour during the light cycle (between 7AM and 7PM) for a total of three times to 3 mL of bobcat urine, three times to 5 mL of coyote urine (thepeemart.com), and two (mice) or three (rats) times to 100µl of a 1:5000 solution of 2,3,5-Trimethyl-3-thiazoline dissolved in mineral oil (Contech enterprises #300000368, Mueller and Bale, 2006) in a randomized manner. 2,3,5-Trimethyl-3-thiazoline is a component of fox feces that is very aversive and widely used in predator odour stress exposure studies (Mueller and Bale, 2006; Takahashi et al., 2008; Wallace and Rosen, 2000). Each exposure lasted for one hour, based on previously published protocols (Belzung et al., 2001; Mashoodh et al., 2009; Mueller and Bale, 2007). Control dams were exposed to the same regimen at the same time (Chapters 3-4: 3PM, Chapters 5-6: 4PM) each day, except that distilled water was used instead of the odourants.

2.3 Maternal behaviour in dams and offspring morphological measures

The maternal behaviours of predator odour exposed and control dams were examined on postnatal days 1 through 6. On postnatal day 0, litters were culled to a maximum of 6 pups (litter sizes 3 to 6 pups) in mice and to a maximum of 8 pups in rats. Maternal behaviours were video recorded for 1 hour, 6 times a day throughout the animal's subjective light phase (7AM, 11AM, 3PM) and dark phase (7PM, 11PM, 3AM) (Champagne et al., 2007). Focal maternal behaviours including licking, nursing (high-crouch, low-crouch, supine), hovering, nesting, retrieving and time on the nest were coded using Observer XT 8.5 (Noldus, USA) every 3 minutes for a given observation period (20 observations/period x 6 periods = 120 observations/mother/day). The percentage of maternal behaviours was calculated by dividing the frequency observed by the number of observations made per day.

The morphology of the offspring was examined from birth to adulthood. Sex, body weight and body length from the tip of the snout to the base of the tail were measured on postnatal day 0 using a scale (to the nearest 0.1g) and a Vernier calliper (to the nearest 0.1 mm), respectively. Offspring were left undisturbed except for a weekly weighing and measuring until postnatal day 21, when they were housed in same-sex groups (mice: 3 to 5 per cage, rats: 2 per cage). In mice and rats, measurements of body length continued up to postnatal day 56 during the weekly cage change (**Chapter 3** and **4**) while body weights were measured weekly up to postnatal day 56 and 114 as well as at the time of sacrifice.

2.4 Adult offspring general testing procedures

Animals were handled (3 min/day) for seven consecutive days prior to testing. The order of testing for each behavioural test was pseudo-randomized between prenatal stress groups and sexes. After each behavioural test, the testing apparatus was cleaned with

70% ethanol. Behavioural testing started when offspring were adults (postnatal day 65 or 90). Male and female adult offspring (1 or 2/sex/litter) were tested in each test. The behavioural tests were generally conducted at least one week apart from one another.

2.5 Elevated-plus maze test in adult offspring

The elevated-plus maze comprised two open and two closed arms (mice: 35.5 cm x 5 cm, rat: 45 x 10 cm) connected by a center platform (mice: 5 cm x 5 cm, rat: 10 x 12 cm) elevated 80 cm above the ground. Tests were conducted in a room illuminated with red light. The frequency of visits and time spent in the open arms and closed arms and the distance travelled in both types of arms were measured (5 minutes in rats, 15 minutes in mice), and the total distance travelled was recorded over 15 minutes. The number of boli was counted at the end of the trial. Mice and rats position and activity level was measured continuously using EthoVision XT 10 (Noldus, Toronto, Canada). Rat grooming and risk assessment behaviours (rearing, head dipping, stretch attend posture) were coded using Observer XT 8.5.

2.6 Open field test in adult offspring

In rats, the open field consisted in a square field (Mice: Plexiglas 38.5 cm x 38.5 cm; Rat: opaque, 40.3 cm x 40.3 cm) in a dimly lit room (33.7 lux). Frequency of entry into and duration spent in the centre (Mice: 21 cm x 21 cm; Rat: 10.1 cm x 10.1 cm) and the borders of the arena as well as the total distance traveled during the 15 minutes trial were recorded. The animal's mid-body had to be in the center of the apparatus for the animal to be recorded in that position. Mice and rats position and activity level was measured continuously using EthoVision XT 10.

2.7 Light-Dark transition test in adult offspring

The light-dark transition apparatus consisted of black (dark) and white (light), equal-sized (30 cm x 30 cm) Plexiglas chambers placed in a dimly lit room (33.7 lux). A wall with a small opening (12 cm x 12 cm) allowed passage between the two boxes. The frequency of entries and duration spent in the light box as well as the total distance travelled was recorded over 5 minutes. Mice and rats position and activity level was measured continuously using EthoVision XT 10.

2.8 Predator odour avoidance test and corticosterone response to predator odour exposure in adult offspring

For the predator odour avoidance test, animals were habituated to a procedure room with a fume hood for 3 hours on the day of testing. The predator odour exposure took place in a transparent arena (45 cm x 24 cm) placed in the fume hood. The animals were exposed to 3 mL of bobcat urine at one end of the arena and to a shelter (Mice: 10 cm diameter dome, Rat: 7 cm x 12 cm cylinder) at the other end of the arena.

The corticosterone response to predator odour in offspring was measured after behavioural testing (after 4 to >7 days) accompanied by a 7-day habituation to restraint in a loosely fitted towel. To measure the corticosterone response to predator odour exposure, animals were habituated to a procedure room with a fume hood for 3 hours and then hand-restrained in a loosely fitting towel. Blood was withdrawn from a small nick in the tail for baseline corticosterone measurement (0 minutes) within 3 minutes. Animals were then exposed to 100µl of a 1:5000 2,3,5-Trimethyl-3-thiazoline solution in mineral oil on a cotton ball sealed in a petri dish with holes placed in a transparent cage (Mice: 15 cm x 33 cm, Rat: 19 cm x 30 cm) inside the fume hood for 60 minutes, and a second blood sample was collected to measure levels of corticosterone immediately following this exposure (60 minutes). Animals were then returned to their home cage and left

undisturbed for 60 minutes, after which a third blood sample was collected to measure the recovery corticosterone level (120 minutes). Blood was kept on ice for at least 30 minutes before being centrifuged at 4,000 rotation per minute at 4°C for 20 minutes. Serum was then extracted and stored at -80°C. The amount of corticosterone present in the serum was determined using a radioimmunoassay kit with ^{125}I-labeled anti-corticosterone antibody (MP Biomedicals Inc., CA., USA: sensitivity 7.7 ng/mL, intra-assay coefficient of variation 10.3%).

2.9 Tissue preparation and nucleotide extraction

Mice and rats were sacrificed by CO_2 inhalation followed by decapitation (Mice: postnatal day 130 ± 10; Rats: postnatal day 0 and postnatal day 177 ± 2). Whole brains and liver tissue were flash frozen in isopentane on dry ice and stored at -80°C. The entire hippocampus (Mice: Bregma -1.22 mm to +3.08 mm; Rats: left hemisphere Bregma -2.04 mm to -4.92 mm) and amygdala (Mice: Bregma -0.58 mm to -1.94 mm; Rats: left hemisphere Bregma -1.72 mm to -3.00 mm) were dissected from 50 µm microsections with a Research Cryostat Leica CM3050 S (Leica Biosystems) using stereotaxic coordinates (Mice: Franklin and Paxinos, 1997; Rat: Paxinos and Watson, 1998). RNA and DNA were extracted using a combination of Trizol/chloroform and Allprep kit (Qiagen). RNA was converted to complementary DNA (Applied BioSystems High Capacity cDNA Conversion Kit). Nucleotide quantification and purity were assessed with a spectrophotometer (Nanodrop ND-2000C, Thermo Scientific).

2.10 Transcript abundance analysis by quantitative real-time reverse transcriptase-polymerase chain reaction

In animals, gene transcript abundance in the hippocampus, amygdala and liver were quantified using a StepOne Plus real-time thermocycler and Fast SYBR Green PCR

master mix (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). Primers were designed using sequence information from GeneBank at the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov) and Ensembl (www.useast.ensembl.org). A standard curve was generated with 6 to 11 serial dilutions of a mixture of complementary DNA from all offspring which transcript abundance was measured. Quantification was carried out in triplicate, and the average relative transcript abundance for each sample was used for analysis.

2.11 DNA methylation analysis by bisulfite pyrosequencing

Bisulfite conversion of DNA from each sample (Mice: 2 μg, Rat: 300 ng) was performed (Mice: EpiTect Bisulfite Kit [Qiagen], Rat: EZ DNA Methylation-Gold Kit [Zymo Research, Irvine, CA, USA]). Pyrosequencing was performed with a Pyromark Q96 ID pyrosequencer, and CpG methylation levels were quantified using Pyromark Q-CpG 1.0.9 software. Analysis of two to four replicates was carried out for each sample.

2.12 Statistical analysis

Statistical analyses were carried out using SPSS (IBM).

For the bulk of the statistics (**Chapters 4-6**), data were tested for normality using the Shapiro-Wilk test and were log-transformed, log plus 1-transformed when zeros were present in the distribution, or fraction transformed in that order to achieve normality whenever possible. General linear models or, to correct for litter effects or missing data, linear mixed models were used for analyses. When the data could not be normalized, a Mann-Whitney Test was performed. Data sets with over 30 samples (maternal behaviour, body weight, standard length, overall Cytosine-Guanine dinucleotide methylation levels) per group that could not be normalized were treated as normal distributions as violations of the normality assumption with large samples sizes is not considered problematic

(Ghasemi and Zahediasl, 2012). Effect sizes were calculated using the Cohen's d or d_r (calculated with the residuals in the linear mixed model), with $d \geq 0.5$ indicating moderate and $d \geq 0.8$ indicating large effect sizes, eta squared (η^2) or partial eta-squared ($p\eta^2$) with η^2 or $p\eta^2 \geq 0.06$ indicating moderate and η^2 or $p\eta^2 \geq 0.14$ indicating large effect size (Levine and Hullett, 2002; Rice and Harris, 2005; Rouder et al., 2012). Partial least-squares difference tests or Bonferroni corrected t-tests, general least model, or linear mixed model separated by sex were used for *post hoc* comparisons. Effects were considered statistically significant at $P \leq 0.05$.

Data for maternal behaviours were analyzed using 2(predator odour exposure) x 6(postnatal day) repeated general linear model with litter size as a covariate.

Data for weight, standard length and body weight were analysed using a 2(prenatal treatment) x 10(weighing day), 9(standard length day) or 6(food consumption day) linear mixed model for body weight and food consumption and a repeated measures general linear model for standard length.

A repeated measures general linear model or linear mixed model, with 2(prenatal treatment) x 2(sex) x 3(time point) were used to examine the log-transformed corticosterone levels and adrenocorticotropic hormone levels (Tadross et al., 2010) over time.

Behavioural data for elevated-plus maze, open field and light-dark transition test were analysed using a linear mixed model using prenatal odour exposure as a main effect with litter as a random factor to account for 1-2 pups used per litter. Prenatal predator odour exposure time spent in the different zones (i.e. predator odour zone, neutral zone, shelter zone) was analysed using a linear mixed model using 2(prenatal treatment) x 3(zone relative to odour) main effects, with litter as a random factor. The measures that could not be normalized were compared using a Mann-Whitney Test.

Two(maternal predator odour exposure) x 2(sex) general linear model or linear mixed model followed by post-hoc analyses were used for transcript abundance analysis and litter as a random effect.

A 2(maternal predator odour exposure) x 4-7(Cytosine-Guanine dinucleotide site) general linear model or linear mixed model was used to examine DNA methylation levels between groups. Bonferroni-corrected student's t-test was used to evaluate the prenatal predator odour exposure effect on female transcript abundance analysis and each Cytosine-Guanine dinucleotide methylation level.

Correlational analysis was used to assess the relationship between key variables influenced by prenatal predator odour exposure as functional and mechanistic connections are expected between those variables. Effects were considered statistically significant at $P \leq 0.05$ and some non-significant trends at $P \leq 0.10$ are reported. Pearson correlations, for normal distributions, and Spearman's rho, for non-normal distributions, were used to assess the relationship between variables that were the most different between the controls and prenatally predator odour exposed animals while each sex was analysed separately.

Chapter 3 Predator odour response and underlying sex-specific mechanisms in prenatally predator odour exposed mice

3.1 Publication

Chapter 3 is adapted from the following research article:
St-Cyr, Sophie and McGowan, Patrick O., 2015. Programming of stress-related behavior and epigenetic neural gene regulation in mice offspring through maternal exposure to predator odor. Frontiers in Behavioral Neuroscience. 9:145 doi: 10.3389/fnbeh.2015.00145.

3.2 Approach and hypotheses

This study was designed to examine the behavioural and neural response to prenatal exposure to predator odour in adulthood: an ethologically relevant psychogenic stressor that has been present in rodents' habitat over evolutionary times. Pregnant C57BL/6 mice were exposed daily to unpredictable and inescapable predator odours or distilled water control over the second half of the pregnancy. I hypothesized that prenatally predator odour exposed animals of both sexes would show increased anti-predatory behaviour, endocrine response and transcript abundance of stress-related genes in limbic brain regions underlain by stable epigenetic alterations.

3.3 Material and methods

3.3.1 Mice breeding

For breeding, two normally cycling females were housed with one male between 9AM and 5PM. Female's oestrous phase was not checked prior to breeding to avoid the induction of pseudo-pregnancy. Females were then checked for sperm plugs indicating gestational day 0. Pregnant females were singly housed and weighed every other day throughout the pregnancy. Further details are available in section 2.1 on page 42.

During initial mating, 5 females exposed to 19.4 µl of pure 2,3,5-Trimethyl-3-thiazoline (150 µmol; Wallace and Rosen, 2000) failed to reach parturition. After that, dosing was reduced for the remaining predator odour-exposed females (see below). Of the 23 remaining mated females, two failed to get pregnant and two control litters were excluded due to low pup numbers. A total of 19 mated females gave birth to 11 prenatally predator odour exposed litters and 8 control litters.

3.3.2 Predator odour exposure in pregnant dams

In preliminary testing of the behavioural response to predator odour in adult females, a cage was divided into three zones: far, neutral and near a predator odour source, and the animal's location was measured once per minute over a 15 minute trial. Bobcat and coyote urine elicited anti-predator behaviours, as females exposed to predator odour (sample size = 4 per odour) stayed in the 'far' zone more frequently relatively to females exposed to distilled water control exposure (sample size = 16) (Figure 2A). As well, in additional preliminary testing in a separate group of animals, females showed elevated basal corticosterone levels after one week of exposure to predator odours compared to distilled water control using the same exposure regimen as above (sample size = 4 per group) (Figure 2B). Further procedural details are available in section 2.2 on page 42 and section 2.3 on page 43.

3.3.3 Adult offspring sample sizes

A set of animals was tested in the predator odour avoidance test and for corticosterone radioimmunoassays with predator odour exposure (control male sample size = 13, control female sample size = 8, prenatally predator odour-exposed male sample size = 13, prenatally predator odour-exposed female sample size = 13). For corticosterone measurements, where two animals of the same sex and litter were tested, the samples were pooled in equal amounts. The final sample sizes were: control male sample size = 8, control female sample size = 6, predator odour-exposed male sample size n = 8 and

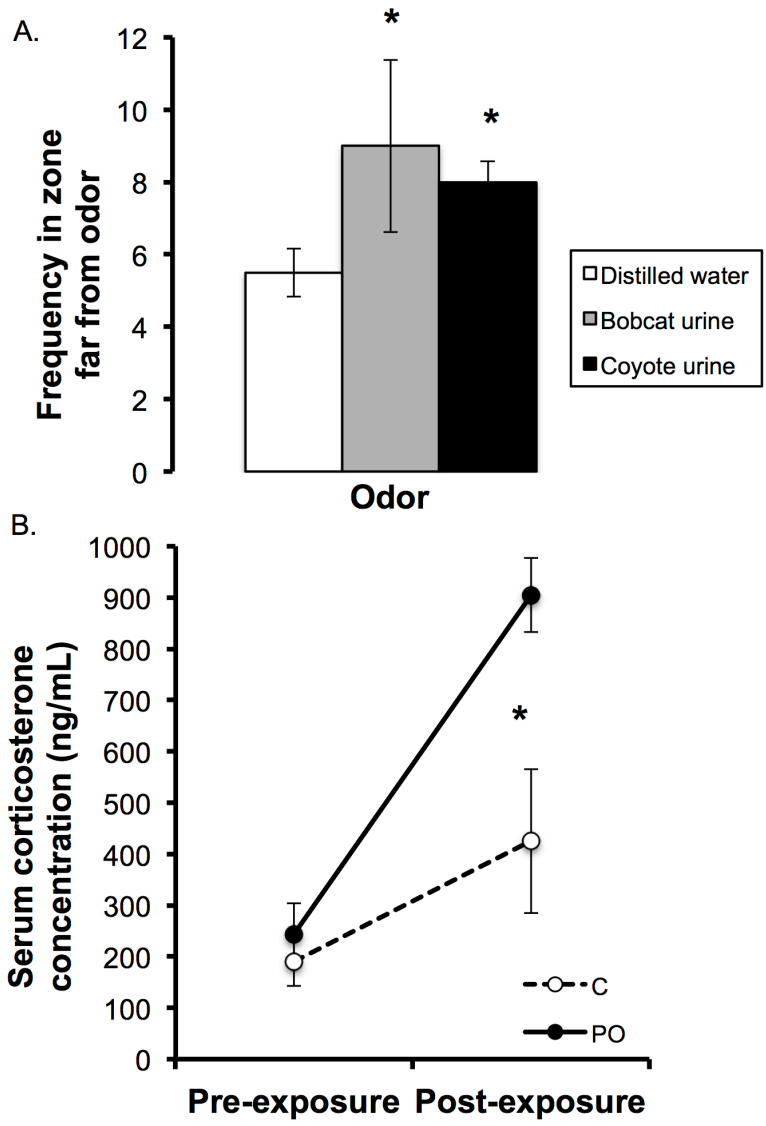


Figure 2. Preliminary exposure to predator odours in adult mice females.

Bobcat (3 mL) and coyote (5 mL) urine elicited avoidance of the predator odour source relative to distilled water control (**A.**) and elevated basal corticosterone levels after a week of unpredictable one hour daily predator odour (2,3,5-Trimethyl-3-thiazoline, bobcat and coyote odour) exposure (**B.**). Data are average ± standard error of the mean. PO: Predator Odour; C: Control. *$P \leq 0.05$, effect of predator odour exposure.

predator odour-exposed female sample size = 8 for each time point. Each sample was assayed in duplicate. A separate set of adult offspring (sample size = 6 per prenatal stress group, 1 mouse/sex/litter) was used for transcript abundance and DNA methylation measurements. Further procedural details are available in sections 2.4 on page 43.

3.3.4 Predator odour avoidance test and corticosterone response to predator odour exposure in adult offspring

In mice, predator odour avoidance, quantified as the average distance from the predator odour over each 10 minutes of the 30 minutes trial, and the total distance travelled during each 10 minutes of the trial were tracked in an automated fashion using EthoVision XT10. Further procedural details are available in section 2.8 on page 45.

3.3.5 Transcript abundance analysis by quantitative real time polymerase chain reaction

The transcript abundance of 7 genes of interest was quantified: Brain-derived neurotrophic factor, glucocorticoid receptor, mineralocorticoid receptor, FK506 binding protein 5, corticosterone-releasing factor, corticosterone-releasing factor receptor 1 and corticosterone-releasing factor receptor 2. The transcript abundance of 4 housekeeping genes (Glyceraldehyde 3-phosphate dehydrogenase, Actin beta, 14-3-3 protein zeta/delta and 18S ribosomal RNA) was also assessed. **Table 5** presents the primers used in this study.

Transcript abundance was quantified relative to 4-3-3 protein zeta/delta, the gene showing the least variance between the prenatal stressed and control offspring according to a previously published algorithm (NormqPCR R script, available at: http://www.bioconductor.org/packages/release/bioc/html/NormqPCR.html). Further procedural details are available in sections 2.9 and 2.10 on page 46.

3.3.6 DNA methylation analysis by bisulfite pyrosequencing

Table 5. Mice primer sequences

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
YWHAZ	TTGAGCAGAAGACGGAAGGT	GAAGCATTGGGGATCAAGAA
NR3C1	AACTGGAATAGGTGCCAAGG	GAGGAGAACTCACATCTGGT
NR3C2	GAAGAGCCCCTCTGTTTGCAG	TCCTTGAGTGATGGGACTGTG
FKBP5	CGGAAAGGCGAGGGATACTC	TTCCCCAACAACGAACACCA
CRF	GCAGCCCTTGAATTTCTTGCA	TCTTCACCCATGCGGATCAG
CRFR1	CGCAAGTGGATGTTCGTCT	GGGGCCCTGGTAGATGTAGT
CRFR2	CCCCTGTGGACACTTTTGGA	AGGTCGTGTTGCAGTAGGTG
BDNF	AGGTTCGAGAGGTCTGACGA	ATGTTTGCGGCATCCAGGTA
*BDNF*mO	AGATGTATTATTTTAAATGCGCGGA	ACTTCCCAACAAACCAAACGA
*BDNF*mN	AGAGTGTTTATTTAGAGGTAGAGGAGGTAT	AACCCATCCCCAAAATTCTAAACTCT
*BDNF*seq	TTTGTTTAGATTAAATGGA	

YWHAZ: 14-3-3 protein zeta/delta, *NR3C1*: Glucocorticoid receptor, *NR3C2*: Mineralocorticoid receptor, *FKBP5*: FK506 binding protein 5, *CRF*: Corticotropin-releasing factor, *CRFR1*: Corticotropin-releasing factor receptor 1, *CRFR2*: Corticotropin-releasing factor receptor 2, *BDNF*: Brain-derived neurotrophic factor, *BDNF*mO: BDNF methylation Outside, *BDNF*mN: BDNF methylation Nested, *BDNF*seq: BDNF sequencing primer.

DNA methylation of the *brain-derived neurotrophic factor* exon *IV* gene in mice was examined by bisulfite pyrosequencing for 6 prenatally predator odour exposed and 6 control female offspring. Biotinylated polymerase chain reaction products were obtained using outside and nested polymerase chain reaction primers targeting four Cytosine-Guanine dinucleotide sites within *brain-derived neurotrophic factor* exon *IV* proximal to the transcription initiation site **Table 5** and Figure 7A (Lubin et al., 2008). Replicates with a coefficient of variation greater than 30% were considered outliers and removed from analysis (sample size = 1 prenatally predator odour exposed and sample size = 1 control offspring). Further procedural details are available in sections 2.11 on page 46.

3.3.7 Statistical analysis

Statistical analysis procedures are described in section 2.12 on page 47.

3.4 Results

3.4.1 Maternal behaviour

Maternal behaviour was examined in predator-odour exposed dams and control dams between postnatal days 1 through 6. There was a significant interaction between postnatal day and predator odour exposure in the percent time that dams spent licking and grooming their offspring [$F_{(5,75)} = 3.04$, $P = 0.02$], with control dams showing increased licking on postnatal day 3 compared to predator odour-exposed dams [$P = 0.02$] (Figure 3). There was no effect of maternal predator odour exposure on the other measures of maternal behaviour examined, the length of pregnancy, litter size, offspring sex ratios, offspring body weight, offspring body length and offspring sex ratio [*P's* > 0.05].

3.4.2 Behavioural and corticosterone response to predator odour exposure in adult offspring

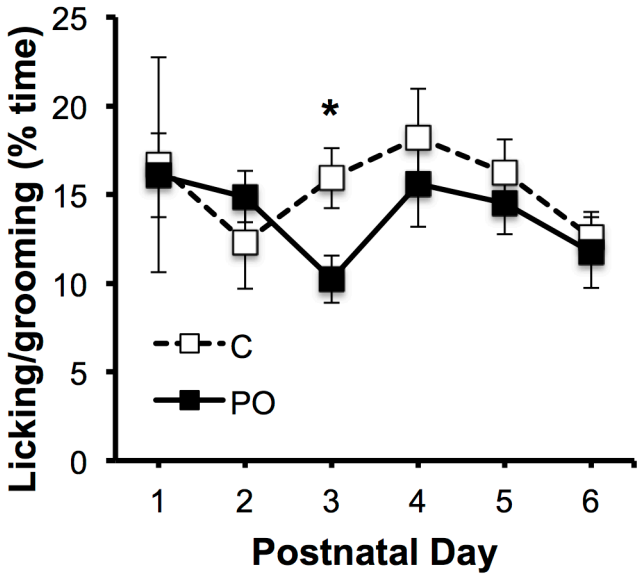


Figure 3. Predator odour-exposed (PO) dams transiently decreased the amount of maternal behaviour provided as measured by the time spent licking the pups compared to control (C) dams.

Data are average ± standard error of the mean. *$P \leq 0.05$, effect of predator odour exposure.

When the offspring reached adulthood, they were tested for their response to predator odour exposure. Overall, the adult offspring of dams exposed to predator odour showed increased avoidance behaviour when exposed to bobcat urine (Figure 4A-C). The offspring of predator odour-exposed dams stayed further away from the predator odour compared to control offspring overall during the 30 minutes trial [$F_{(1, 44)} = 4.29$, $P = 0.04$] (Figure 4D). Male offspring from predator odour-exposed dams stayed farther from the source of the predator odour on average compared to male control offspring [$P < 0.05$ at 0-10 and $P < 0.09$ at 10-20 minutes]. In addition, when exposed to predator odour in adulthood, offspring from predator-odour exposed dams were less active than control offspring, traveling a shorter distance over the course of the trial [$F_{(1, 44)} = 4.38$, $P = 0.04$] (Figure 4E). The effect of dams' predator odour exposure on offspring locomotor activity varied over time [$F_{(2, 88)} = 3.13$, $P = 0.05$, maternal predator odour exposure x time interaction] and as a function of sex [$F_{(1,44)} = 4.45$, $P = 0.04$]. Female offspring from predator odour-exposed dams were also hypoactive compared to control female offspring in the first 10 minutes and last 10 minutes of the trial [P's < 0.05 at 0-10 and 20-30 minutes].

The same subjects were tested for their corticosterone response to 2,3,5-Trimethyl-3-thiazoline predator odour exposures a minimum of 4 days after the completion of behavioural testing. Corticosterone levels were examined at baseline (0 minute), after an hour exposure to predator odour (60 minutes) and an hour after the end of the exposure to assess the return to baseline (120 minutes). Overall, all adult offspring showed a significant rise in corticosterone levels in response to predator odour exposure and a significant decrease in corticosterone levels between the 60 minutes and 120 minutes time points [$F_{(2, 52)} = 24.31$, $P < 0.001$] (Figure 5**)**. The corticosterone response over time differed between offspring from dams exposed to predator odour and control offspring [$F_{(2, 52)} = 4.55$, $P = 0.02$, time x maternal predator odour exposure interaction]. The corticosterone response over time also differed between males and females [$F_{(2, 52)} = 4.24$, $P = 0.02$, time x sex interaction]. There was a significant three-way interaction between all three variables [$F_{(2, 52)} = 6.23$, $P = 0.004$, time x sex x maternal predator odour exposure interaction]. Female offspring from predator odour-exposed dams showed

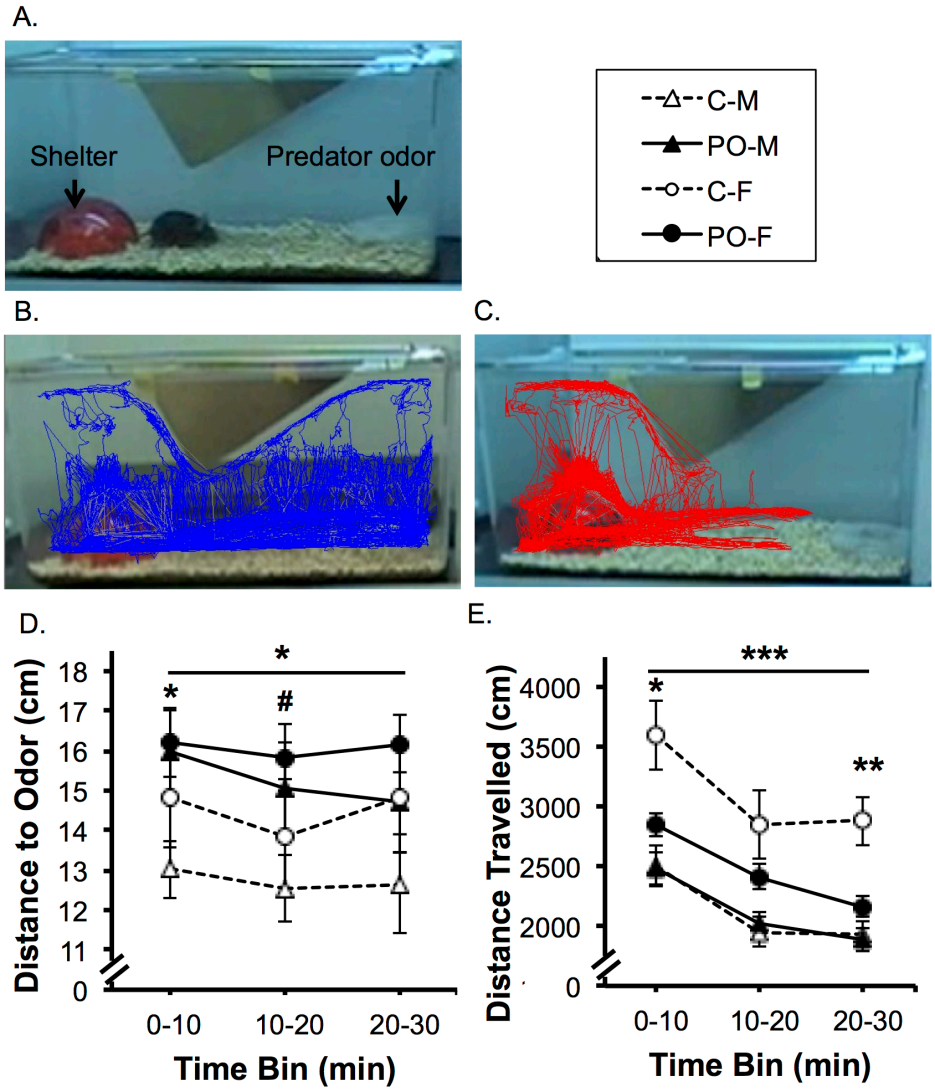


Figure 4. Adult offspring from dams exposed to predator odour during pregnancy (PO) increased avoidance and hypoactivity in predator odour presence compared to control (C) adult offspring.

Predator odour exposure setup (**A.**). Representative 30 minutes track of control (**B**.) and PO (**C.**) adult offspring. Average distance to the predator odour (**D.**) and overall distance travelled (**E.**). Data are average ± standard error of the mean. M: male, F: female. Bars, $*P \leq 0.05$, $***P \leq 0.001$, main effect of maternal predator odour exposure; Bars, $****P \leq 0.0001$, main effect of sex; $^{\#}P \leq 0.10$, $*P \leq 0.05$, $** P \leq 0.01$ post hoc comparison within a sex.

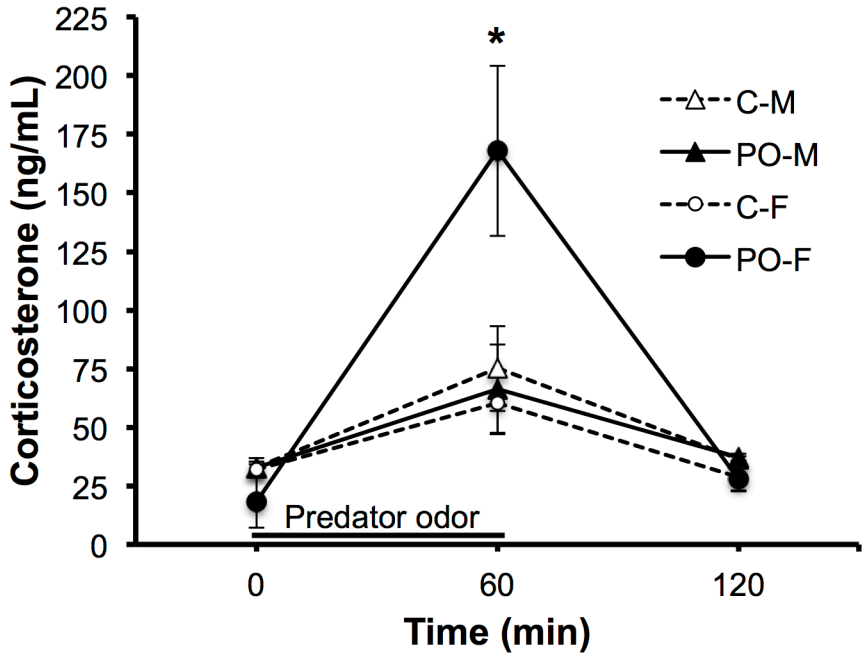


Figure 5. Adult female offspring from dams exposed to predator odour during pregnancy (PO) show an elevated predator odour-challenged level of corticosterone (CORT) to a predator odour compared to control (C) adult offspring.

The black line shows the duration of the predator odour exposure. Data are average ± standard error of the mean. M: Male, F: Female. *$P \leq 0.05$, *post hoc* comparison within female offspring.

significantly higher corticosterone levels immediately after exposure to the predator odour compared to female control offspring [$P = 0.03$].

3.4.3 Transcript abundance analysis in adult offspring

The transcript abundance of stress-related genes in the hippocampus and amygdala was measured in adult offspring from predator odour exposed dams and controls. In the hippocampus, there was a significant interaction between maternal predator odour condition and offspring sex for *brain-derived neurotrophic factor* transcript abundance [$F_{(1,,20)} = 7.70$, $P = 0.01$]. Female offspring showed a higher *brain-derived neurotrophic factor* transcript abundance compared to male offspring [$F_{(1,,20)} = 9.58$, $P < 0.01$], an effect due to significantly higher *brain-derived neurotrophic factor* among control female offspring compared to prenatally predator odour exposed female offspring [$P = 0.03$] (Figure 6A). There was no difference in the transcript abundance of *corticosterone-releasing factor receptor 1* in the hippocampus. In the amygdala, offspring from predator odour–exposed dams showed higher transcript abundance of *corticosterone-releasing factor receptor 1* compared to control offspring [$F_{(1,,20)} = 6.29$, $P = 0.02$], and *corticosterone-releasing factor receptor 1* transcript abundance levels varied as a function of maternal predator odour condition and offspring sex [$F_{(1,20)} = 9.49$, $P < 0.01$, maternal predator odour exposure x sex interaction] (Figure 6B**)**. Prenatally predator odour exposed females showed higher transcript abundance of *corticosterone-releasing factor receptor 1* compared to control females [$P = 0.01$]. Overall, the level of predator-odour induced corticosterone (60 minutes time point) (Figure 5) was positively correlated with the transcript abundance of *corticosterone-releasing factor receptor 1* in the amygdala [$R^2 = 0.25$, $P = 0.01$]. There was no difference in the transcript abundance of *brain-derived neurotrophic factor* in the amygdala. The transcript abundance of the other stress-related genes examined (*glucocorticoid receptor*, *mineralocorticoid receptor, FK506 binding protein 5*, *corticosterone-releasing factor*, and *corticosterone-releasing factor receptor 2*) did not differ in the hippocampus or amygdala of offspring as a function of maternal predator odour exposure or offspring sex.

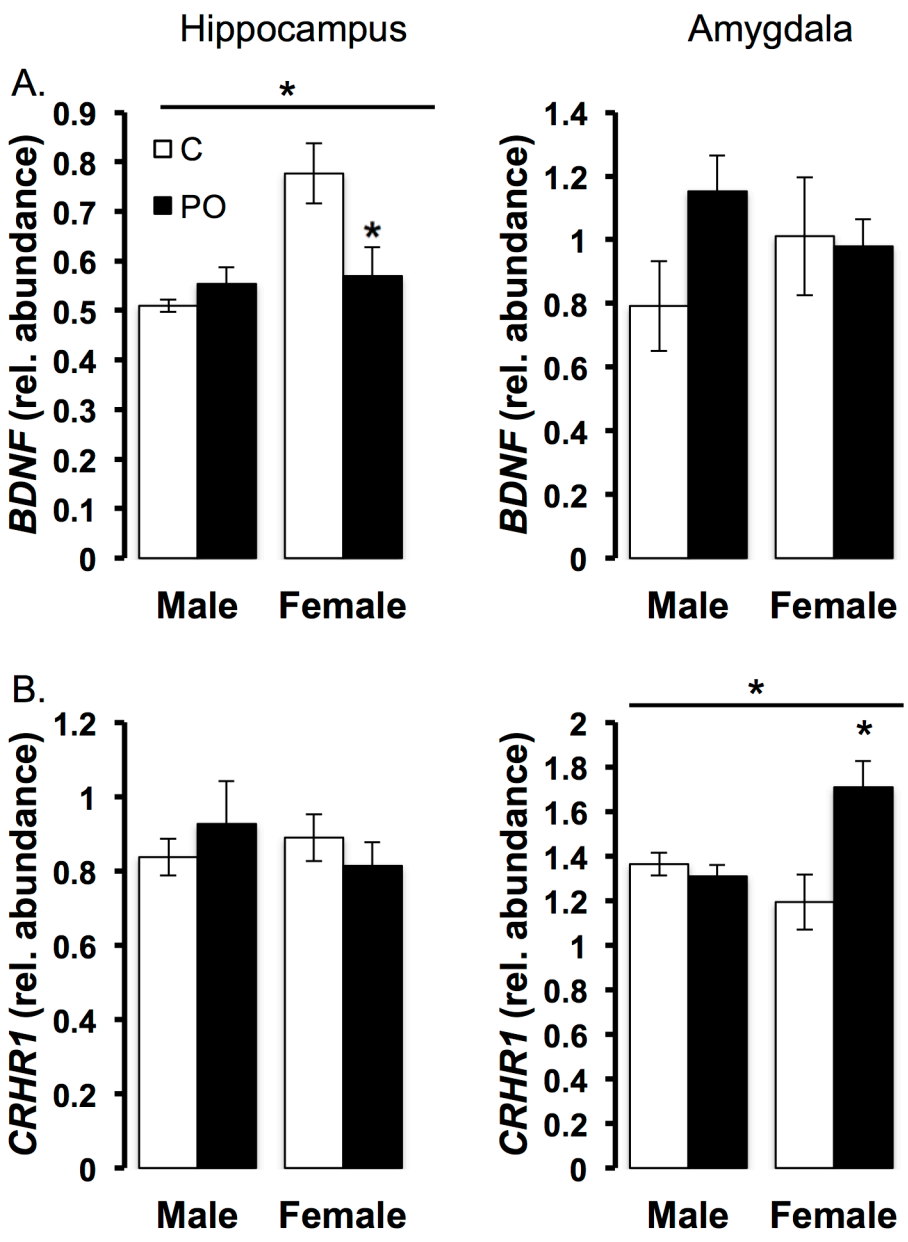


Figure 6. Prenatal exposure to predator odour (PO) leads to differences in transcript abundance in the hippocampus and amygdala of the adult offspring. Respectively, hippocampal and amygdala brain-derived neurotrophic factor (*BDNF*; **A.**) and corticosterone-releasing factor receptor 1 (*CRHR1*; **B.**) transcript relative abundance corrected using the 14-3-3 protein zeta/delta (*YWHAZ*) transcript abundance. Data are average ± standard error of the mean. C: control. Bars, *$P \leq 0.05$, main effect of maternal predator odour exposure; * $P \leq 0.05$, post hoc comparison within sex.

3.4.4 DNA methylation analysis of *brain-derived neurotrophic factor* exon *IV* in adult female offspring

Due to the observed reduction in *brain-derived neurotrophic factor* transcript abundance in the hippocampus of prenatally predator odour exposed female compared to controls and previous reports of altered epigenetic regulation of *brain-derived neurotrophic factor* exon *IV* as a function of early life environmental factors (Lubin et al., 2008; Roth et al., 2009), we examined DNA methylation levels in *brain-derived neurotrophic factor* exon *IV* in the hippocampus of adult female offspring (Figure 7A). Overall, levels of DNA methylation in female offspring differed significantly across Cytosine-Guanine dinuclotides sites within *brain-derived neurotrophic factor* exon *IV* [$F_{(3,21)} = 6.47$, $P = 0.003$]. In particular, Cytosine-Guanine dinucleotide site 3 showed a significantly lower methylation in prenatally predator odour exposed females compared to control females [$t_{(10)} = 4.09$, $P = 0.004$] (Figure 7B). Furthermore, hippocampal *brain-derived neurotrophic factor* transcript abundance was positively correlated with hippocampal *brain-derived neurotrophic factor* exon *IV* Cytosine-Guanine dinucleotide site 3 methylation level in females [$R^2 = 0.46$, $P = 0.03$] (Figure 7C). Methylation levels of other Cytosine-Guanine dinucleotide sites within the exon did not differ significantly as a function of the prenatal treatment or vary in relation to *brain-derived neurotrophic factor* transcript abundance [$P's > 0.05$].

3.5 Discussion

3.5.1 Maternal behaviour in dams exposed to predator odour during pregnancy

Dams exposed to predator odour during pregnancy showed alterations in maternal behaviour consisting of reduced licking of their pups on postnatal day 3. Decreased licking and grooming of pups has been linked to several indices of stress-related behaviour in adult rat and mouse offspring (Champagne et al., 2006; Weaver et al., 2004). Therefore, the modulation of dam maternal behaviour may reflect an adjustment of the

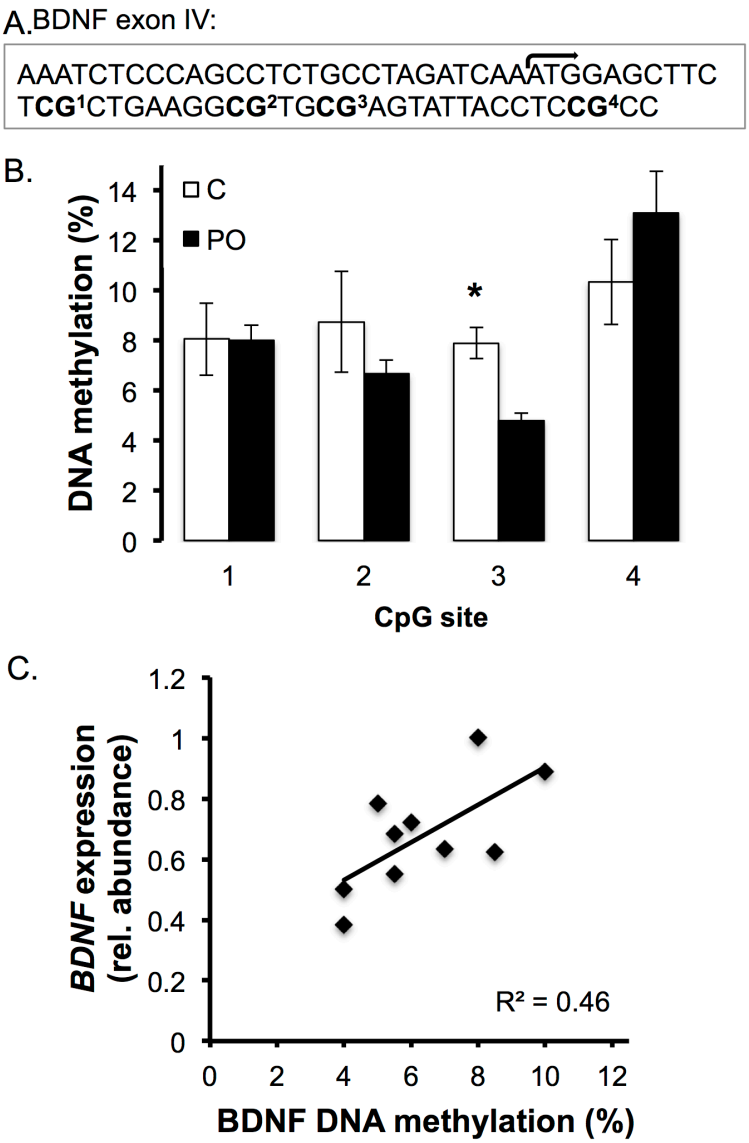


Figure 7. **DNA methylation level of the brain-derived neurotrophic factor (BDNF) exon IV in the hippocampus of female offspring of predator odour exposed (PO) and control (C) dams.**

Region sequenced with transcription initiation site (bent arrow) and Cytosine-Guanine dinucleotide sites (**A.**). DNA methylation level (%) of the 4 sequenced Cytosine-Guanine dinucleotide sites (**B.**). Correlation between the hippocampal *BDNF* transcript abundance and the hippocampal *BDNF* exon *IV* Cytosine-Guanine dinucleotide site 3 methylation level (%) [$P = 0.03$] (**C.**). Data are average ± standard error of the mean. * $P \leq 0.05$, effect of maternal predator odour.

females to a predator odour exposure itself or to cues provided by the pups as a function of the exposure. However, this transient decreased licking in predator odour exposed dams did not correlate with adult offspring phenotypic measures. We also did not detect changes in offspring body weight or growth associated with maternal predator odour exposure or maternal care. Future studies using a cross-fostering design would be important in determining the relative contribution of prenatal and postnatal programming to the adult prenatal predator odour exposed phenotype (e.g. Weaver et al., 2004).

3.5.2 Behavioural and endocrine response to predator odour exposure in adult offspring

The offspring of dams exposed to predator odour during pregnancy displayed an increase in avoidance behaviour to inescapable predator odour in adulthood compared to offspring from control dams. Prenatally predator odour exposed females were also hypoactive in the context of predator odour exposure. Further, prenatally predator odour exposed females displayed a greater increase in circulating corticosterone after exposure to an inescapable predator odour in adulthood compared to control females. Increased corticosterone levels after predator odour exposure in adult rodents have previously been linked to higher hypothalamic-pituitary adrenal axis reactivity and increased stress-related behaviours or previous exposure to predator odour in early postnatal life in both sexes. Similarly, the offspring of gravid crickets exposed to a live predator exhibit increased anti-predator behaviours (McAllister et al., 1999; Zheng and Quirion, 2004). Our data suggest an increase in anti-predator behaviours in adulthood in mice exposed to predator odour prenatally.

3.5.3 Neural transcript abundance in adult offspring

The transcript abundance of stress-related genes was altered in offspring as a function of prenatal predator odour exposure. In the hippocampus, decreased transcript abundance of *brain-derived neurotrophic factor* was observed in female offspring of predator odour-exposed dams. *Brain-derived neurotrophic factor* exon *IV* is the most abundant isoform

of expressed *brain-derived neurotrophic factor* transcripts (Giannotti et al., 2014). During development (Andersen and Sonntag, 2014) and following stress (Giannotti et al., 2014), the transcript abundance of total *brain-derived neurotrophic factor* is closely related to the transcript abundance of *brain-derived neurotrophic factor* exon *IV* mRNA. Notably, we observed changes in *brain-derived neurotrophic factor* in female but not male offspring from predator odour-exposed dams relative to same-sex controls, together with enhanced corticosterone reactivity in female but not male offspring from predator odour-exposed dams. *Brain-derived neurotrophic factor* is a downstream target of an altered glucocorticoid-signalling pathway, and its expression is altered in concert with a dysregulated hypothalamic-pituitary adrenal function (Dwivedi et al., 2007; Lee and Sawa, 2014). There is evidence of decreased transcript abundance of *brain-derived neurotrophic factor* in the hippocampus and amygdala of prenatally stressed animals (Boersma et al., 2014; Lubin et al., 2008). A decrease in hippocampus *brain-derived neurotrophic factor* transcript abundance is also observed following chronic restraint (Bennett and Lagopoulos, 2014) and social defeat stress (Tsankova et al., 2006). Similar to our results, Taliaz et al. (2011) showed that lentivirus-induced reduction in hippocampal *brain-derived neurotrophic factor* transcript abundance leads to prolonged elevation of circulating corticosterone. Early maternal separation, a potent method to potentiate hypothalamic-pituitary adrenal responsiveness in later life, leads to long-term inhibition of *brain-derived neurotrophic factor* transcript abundance in the hippocampus, coupled with impaired hypothalamic-pituitary adrenal response to forced swim (Roceri et al., 2004). Our results are thus consistent with the interpretation that decreased *brain-derived neurotrophic factor* in adult prenatally predator odour exposed females may result from altered stress-related signalling pathways linked to hypothalamic-pituitary adrenal function.

In further support of this interpretation, we found that *corticotropin-releasing factor receptor 1* transcript abundance in the amygdala was increased among prenatally predator odour exposed females but unchanged among males. *Corticotropin-releasing factor receptor 1* transcript abundance was also correlated with the increase in the levels of corticosterone during exposure to a predator odour. As *corticotropin-releasing factor*

receptor 1 mediates the effects of corticotropin-releasing factor, an important activator of the hypothalamic-pituitary adrenal axis (Anisman, 2009), these results implicate *corticotropin-releasing factor receptor 1* level in the increased corticosterone reactivity among female offspring from predator odour-exposed dams that were exposed to predator odour in adulthood. *Corticotropin-releasing factor receptor 1* is highly expressed in limbic areas such as the amygdala, including in the basolateral and central nuclei, where it regulates the activation of stress-related responses (Joëls and Baram, 2009). Our data support those of a recent study in a genetic model of anxiety-like behaviour showing an association between higher levels of stress-induced corticosterone and increased *corticotropin-releasing factor receptor 1* transcript abundance in male mice exposed to chronic mild stress (Zohar and Weinstock, 2011).

3.5.4 *Brain-derived neurotrophic factor* exon *IV* methylation and transcriptional regulation of the *brain-derived neurotrophic factor* in adult female offspring

In adult prenatally predator odour exposed female, Cytosine-Guanine dinucleotide site 3 within *brain-derived neurotrophic factor* exon *IV* showed significantly lower DNA methylation levels compared to control females. Previous evidence has implicated DNA methylation of *brain-derived neurotrophic factor* exon *IV* in transcriptional regulation of the *brain-derived neurotrophic factor* gene as a function of other forms of maternal adversity. For example, Roth et al. (2009) showed that stressed caregivers displaying increased maternal maltreatment showed lower *brain-derived neurotrophic factor* transcript abundance in the medial prefrontal cortex and higher DNA methylation levels of *brain-derived neurotrophic factor* exon *IV* in adult offspring. Several additional studies have also demonstrated the association between the *brain-derived neurotrophic factor* transcript abundance changes and alterations in DNA methylation, including in the context of early-life stress, though lower transcript abundance is not always associated with higher DNA methylation levels, and the specific Cytosine-Guanine dinucleotides implicated in these effects vary in association with experimental parameters (reviewed in Boulle et al., 2012). In the context of maternal predator odour exposure in this study, *brain-derived neurotrophic factor* transcript abundance in adult female offspring was

positively correlated with levels of DNA methylation of *brain-derived neurotrophic factor* exon *IV* Cytosine-Guanine dinucleotide site 3, implicating this epigenetic modification in transcriptional regulation of the *brain-derived neurotrophic factor* gene. The fact that we detect differential methylation at a single site and that this difference is small might be due to the dilution effect of the multiple cell types present in the tissue analysed. However, this change is also strengthened, as it is detected and significant despite the dilution effect. The association between decreased exonic DNA methylation and decreased transcription are supported by a number of other studies, including the relationship between altered DNA methylation within exons and transcriptional regulation of the *glucocorticoid receptor* locus among adult offspring of mothers providing high compared to low levels of maternal care (McGowan et al., 2011). For example, exonic DNA methylation has been shown to associate with the transcriptional repression of retrotransposons and microRNAs within exonic regions as well as the regulation of cryptic alternative transcription start sites (Maor et al., 2015). It is possible that hypermethylation of Cytosine-Guanine dinucleotide site 3 in predator-odour exposed female offspring is associated with as-yet unrecognized regulatory control of *brain-derived neurotrophic factor* exon *IV*. Future studies are required to examine this possibility, for example by targeted site-specific DNA methylation followed by chromatin immunoprecipitation of RNA Polymerase associated with the *brain-derived neurotrophic factor* exon *IV* or in vitro reporter assays (e.g. McGowan et al., 2009). Together, these results suggest an epigenetic mechanism underlying some long-term impacts of the prenatal exposure to predator odour.

3.5.5 Conclusions

In this study, we found evidence for sex-specific effects of prenatal predator odour exposure on behaviour, endocrine response and neural gene transcript abundance. Sex-specific alterations in behaviour and neural gene transcript abundance appear to be a common feature of the impacts of early life adversity (Coutellier and Würbel, 2009; Mashoodh et al., 2009; Zohar and Weinstock, 2011). Responses to prenatal stressors may play both an adaptive role in stressful environments and lead to sex-specific differences

in the risk for psychopathology, including an increased risk for affective disorders among females (Glover, 2011). Viewed from an evolutionary perspective, understanding the impacts of ethologically-relevant stressors such as predator odours is important in elucidating mechanisms of plasticity associated with stress-related phenotypes.

The data reported here indicate that prenatal predator odour exposure alone, a psychological and ethologically-relevant stressor, is sufficient to alter offspring phenotype in a manner that persists to adulthood. We found that prenatal exposure to predator odour changes the animals' phenotype by increasing the stress-related and defensive response. We also observed sex-specific differences in the impacts of prenatal predator odour exposure. To our knowledge, our study provides the first evidence of maternal transmission of behavioural sensitivity to predator odour, an unconditioned olfactory stimulus, and stress-related neural gene regulation through exposure to predator odour alone during pregnancy. Recently, an aversive association between a neutral odour (acetophenone) and mild foot shocks in the father was shown to lead to the retention of the aversive conditioning to this odour over multiple generations of mice offspring (Dias and Ressler, 2014). The potential influence of maternal predator odour exposure over multiple generations remains to be determined, though in several species multigenerational effects of predation exposure have been reported in the ecology literature (Love et al., 2013; Sheriff et al., 2010).

Chapter 4 Maternal programming of sex-specific responses to predator odour stress in adult rats

4.1 Publication

Chapter 4 is adapted from the following research article:
St-Cyr, Sophie, Abuaish, Sameera, Sivanathan, Shathveekan, McGowan, Patrick O., 2017. Maternal programming of sex-specific responses to predator odor stress in adult rats. *Hormones and Behavior.* 94(June 20): 1-12.

4.2 Approach and hypotheses

The present study was designed to (1) study the impact of maternal prenatal predator odour exposure in offspring on anxiety-like behaviour and locomotor responses associated with unconditioned novelty-induced anxiety in commonly used laboratory tests, (2) examine whether prenatal predator odour exposure leads to increased defensive responses and predator odour-induced endocrine stress responses in offspring, (3) examine stress-related gene transcript abundance in limbic regions and associated epigenetic modifications in adult prenatally predator odour exposed offspring and (4) highlight sex differences in the PO offspring adult phenotype. We used Long-Evans rats, an outbred rat strain in which the effects of chronic variable stress in the prenatal period and maternal behaviour on offspring stress-related phenotypes have been extensively investigated (Erickson et al., 2014; Harmon et al., 2009; McGowan et al., 2011; Weaver et al., 2004).

We hypothesized that anxiety-like behaviour, locomotor activity, predator odour-related defensive responses would be increased in prenatally predator odour exposed animals as a result of enhanced hypothalamic-pituitary adrenal stress reactivity. Additionally, we assessed the activity of stress-related genes in the hippocampus and amygdala, limbic structures that have an inhibitory and activational effect, respectively, on the hypothalamic-pituitary adrenal axis reactivity (Steckler, 2005). Finally, we sought to determine the extent to which sex differences in the response to predator odour reflect a general alteration in stress-related behavioural responses.

This study focused on the activity of stress-related genes in the hippocampus and amygdala, limbic structures that have an inhibitory and activational effect, respectively, on the hypothalamic-pituitary adrenal axis reactivity (Steckler, 2005). *FK506 binding protein 5* is a co-chaperone of multiple steroid receptors, including the glucocorticoid receptor and mineralocorticoid receptor, and is essential for mediating the effect of those steroid hormones on gene transcript abundance via transactivation. In the nucleus, the glucorticoid receptor acts as a transcription factor and binds to glucocorticoid receptor elements to alter transcription, including on the FK506 binding protein 5 gene itself to potentiate its transcription, forming an ultra-short regulatory loop. *FK506 binding protein 5* transcript abundance is thus implicated in glucocorticoid sensitivity and stress recovery (Jääskeläinen et al., 2011). *FK506 binding protein 5* is ubiquitously expressed in tissues ranging from brain to liver and heart (Baughman et al., 1997). In the mouse brain, glucocorticoids lead to an up-regulation of *FK506 binding protein 5* transcript abundance in the paraventricular nucleus of the hypothalamus, central amygdala and hippocampus. For example, restraint stress increases the transcript abundance of *FK506 binding protein 5* (Jääskeläinen et al., 2011). Furthermore, *FK506 binding protein 5* polymorphisms have been implicated in mood, affective and anxiety disorders as well as post-traumatic stress disorder development (Binder, 2009; Binder et al., 2008). Interestingly, intronic elements of the *FK506 binding protein 5* gene exhibit demethylation after chronic glucocorticoid exposure through a conserved main glucocorticoid response element enhancer and this difference persists over time (Hubler and Scammell, 2004) making *FK506 binding protein 5* an interesting target for long-term programming effects of glucocorticoid exposure during the hypothalamic-pituitary adrenal axis development.

4.3 Material and methods

4.3.1 Rat breeding

For breeding, two normally cycling females were housed with one male between 9AM and 5PM. Female's oestrous cycle was not checked prior to breeding. Females were then

checked for sperm plugs indicating gestational day 0. Pregnant females were singly housed and weighed every other day throughout the pregnancy. Twenty-one of the 23 rat females that were bred gave birth, resulting in 11 prenatally predator odour exposed litters and 9 control litters. Additional procedural details are available in section 2.1 on page 42.

4.3.2 Predator odour exposure in pregnant dams

Preliminary analysis of serum corticosterone in non-pregnant rat females (sample size = 4 per group) showed a lack of habituation to predator odour exposure over 8 days as an interaction between the time and repeated predator odour exposure effect [$F_{(1, 6)} = 12.331$, $P = 0.01$; $p\,\eta^2 = 0.67$] and an increased stress response to predator odour on the final day of exposure compared to distilled water controls [$t_{(1, 6)} = -5.488$, $P = 0.002$, $d = -3.9$] (Figure 8). These results support the use of successive unpredictable exposures to predator odour to induce an elevated stress response. Additional procedural details are available in sections 2.2 and 2.3 on pages 42 and 43.

4.3.3 Offspring morphological measures

Among rat males, there was 1 prenatally predator odour exposed and 1 control male that were individually housed due to uneven number of animals per group. However they were not considered outliers according to the interquartile range in each statistical measure used.

4.3.4 Adult offspring sample sizes

A subset of offspring (control male sample size = 15, control female sample size = 16 both from 8 litters, prenatally predator-odour exposed male sample size = 12, prenatally predator-odour exposed female sample size = 12 both from 6 litters) was used to examine unconditioned novelty-induced anxiety in commonly-used laboratory tests in the following order: elevated-plus maze, open field and light-dark transition task. An

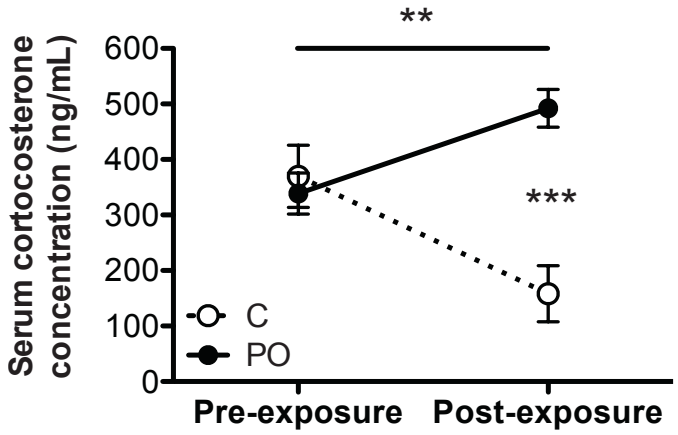


Figure 8. **Preliminary exposure to predator odours in adult female rats.**

Females exposed for 8 days to unpredictable predator odour (PO) 1 hour daily display an elevated circulating corticosterone level post-exposure compared to control (C) females exposed for a week to distilled water. Data are average ± standard error of the mean. ***P* < 0.01, comparison within time point.

independent set of animals (control male sample size = 14, control female sample size = 15, prenatally predator-odour exposed male sample size = 16, prenatally predator-odour exposed female sample size = 12) was used for predator odour exposure and inescapable predator odour exposure for endocrine measurements (control male sample size = 14, control female sample size = 15, prenatally predator-odour exposed male sample size = 16, prenatally predator-odour exposed female sample size = 12). In the stress-tested animals, corticosterone serum levels (control male sample size = 13, control female sample size = 14, prenatally predator-odour exposed male sample size = 16, prenatally predator-odour exposed female sample size = 11) were measured using radioimmunoassay. For subjects with remaining serum, adrenocorticotropic hormone levels were also measured (control male sample size =13, control female sample size = 13, prenatally predator-odour exposed male sample size = 16, prenatally predator-odour exposed female sample size = 10) using radioimmunoassay. When two rats of the same sex and litter adrenocorticotropic hormone level was measured, their serum samples were pooled in equal amounts (pooled samples: control male sample size = 6, control female sample size = 5, prenatally predator-odour exposed male sample size = 7, prenatally predator-odour exposed female sample size = 3). Finally, a subset of postnatal day 0 animals (6 control males, 6 control females, 6 prenatally predator-odour exposed males, 6 prenatally predator-odour exposed females) and postnatal day 177 females (8 control, 6 prenatally predator odour exposed) that were behaviourally tested (1 rat/litter) were used for transcript abundance analysis. Additional procedural details are available in sections 2.4 to 2.7 on pages 43 to 45.

4.3.5 Predator odour avoidance test and corticosterone response to predator odour exposure in adult offspring

The location of the animal within the predator odour avoidance test apparatus, divided into equal thirds (i.e. predator odour zone, neutral zone, shelter zone), was video-recorded over 5 minutes and behaviours were manually coded using Observer XT 8.5.

The amount of adrenocorticotropic hormone present in the serum was determined for the same time points as described for corticosterone previously (see section 2.8, page 45) using the commercially available radioimmunoassay kit with ^{125}I-labeled anti-adrenocorticotropic hormone antibody (MP Biomedicals Inc., CA., USA: adrenocorticotropic hormone sensitivity 5.7 pg/mL, intra-assay coefficient of variation 10.7%).

4.3.6 Transcript abundance analysis by quantitative real time polymerase chain reaction

The transcript abundance patterns of 3 genes *glucocorticoid receptor*, *mineralocorticoid receptor* and *FK506 binding protein 5* were measured in postnatal day 0 (day of birth) offspring. Additionally, the transcript abundance patterns of *glucocorticoid receptor*, *mineralocorticoid receptor*, *FK506 binding protein 5* and *brain-derived neurotrophic factor* were quantified and analysed in adult female offspring. Relative normalization was performed against the average transcript abundance of 5 housekeeping genes (Glyceraldehyde 3-phosphate dehydrogenase, Actin beta, 14-3-3 protein zeta/delta, 18S ribosomal RNA and Ubiquitin C) **(**Vandesompele et al., 2002). Table 6 shows the primers used in this study. Additional procedural details are available in sections 2.9 and 2.10 on pages 46.

4.3.7 DNA methylation analysis by bisulfite pyrosequencing

DNA methylation of the *FK506 binding protein 5* intron *V* gene was examined by bisulfite pyrosequencing for six prenatally predator odour exposed female offspring and six control female offspring. Biotinylated polymerase chain reaction products were obtained using outside and nested polymerase chain reaction primers targeting 7 Cytosine-Guanine dinucleotide sites within *FK506 binding protein 5* intron *V* upstream from a conserved glucocorticoid response element (Kitraki et al. 2015) (Table 6) (Figure 13). Additional procedural details are available in section 2.11 on pages 47.

Table 6. Rat primer sequences

Gene	Forward primer (5'-3')	Reverse primer (5' – 3')
ACTB	5'-TTTGAGACCTTCAACACCCC-3'	5'-ATAGCTCTTCTCCAGGGAGG-3'
18S	5'-ATGGTAGTCGCCGTGCCTA-3'	5'-CTGCTGCCTTCCTTGGATG-3'
GAPDH	5'-ACATCAAATGGGGTGATGCT-3'	5'-GTGGTTCACACCCATCACAA-3'
UBC	5'-CACCAAGAAGGTCAAACAGGAA-3'	5'-AAGACACCTCCCCATCAAACC-3'
YWHAZ	5'-TTGAGCAGAAGACGGAAGGT-3'	5'-GAAGCATTGGGGATCAAGAA-3'
NR3C1	PPR52805B, SAbiosciences, Qiagen, Valencia, CA, USA	
NR3C2	5'-GGCAGCTGCAAAGTCTTCTT-3'	5'-GACAGTTCTTTCGCCGAATC-3'
BDNF	5'-AAAACCATAAGGACGCGGACTT-3'	5'-AAAGAGCAGAGGAGGCTCCAA-3'
FKBP5	5'-GAACCCAATGCTGAGCTTATG-3'	5'-ATGTACTTGCCTCCCTTGAAG-3'
*FKBP5*mO1	5'-TGGAAATTTTTGTTTAGTGTGATTGA-3'	5'-ACCCCAAACTATACAACTTATATTTCA-3'
*FKBP5*mO2	5'-GAAATATAAGTTGTATAGTTTGGGGTTTTT-3'	5'-AACACCCTATTCTAAATATAACTAACAC-3'
*FKBP5*mN1	5'-TGGAAATTTTTGTTTAGTGTGATTGA-3'	5'Biotin-ACCCCAAACTATACAACTTATATTTCA-3'
*FKBP5*mN2	5'-TGGAAATTTTTGTTTAGTGTGATTGA-3'	5'Biotin-AAAACAAAAACTATAAAACTTTTTAATACT-3'
*FKBP5*mN3	5'Biotin-ATAAGTTGTATAGTTTGGGGTTTTTTGTAT-3'	5'-AACACCCTATTCTAAATATAACTAACAC-3'
*FKBP5*seq1	5'-GTTATTGTTTGGGGATAG-3'	
*FKBP5*seq2	5'-ATTAGAGAAGAGAAAGTAGATA-3'	
*FKBP5*seq3		5'-CCTATTCTAAATATAACTAACACAT-3'

ACTB: Beta-actin, *18S*: 18S ribosomal RNA, *GAPDH*: Glyceraldehyde 3-phosphate dehydrogenase, *UBC*: Ubiquitin C, *YWHAZ*: 14-3-3 protein zelta/delta, *NR3C1*: Glucocorticoid receptor, *NR3C2*: Mineralocorticoid receptor, *BDNF*: Brain-derived neurotrophic factor, *FKBP5*: FK506 binding protein 5, *FKBP5*mO1: FKBP5 methylation Outside pair 1 (Cytosine-Guanine dinucleotide 1 to 5), *FKBP5*mO2: FKBP5 methylation Outside pair 2 (Cytosine-Guanine dinucleotide 6 and 7), *FKBP5*mN1: FKBP5 methylation Nested pair 1 (Cytosine-Guanine dinucleotide 1 and 2), *FKBP5*mN2: FKBP5 methylation Nested pair 2 (Cytosine-Guanine dinucleotide 3 to 5), *FKBP5*mN3: FKBP5 methylation Nested pair 3 (Cytosine-Guanine dinucleotide 6 and 7), *FKBP5*seq1: FKBP5 sequencing 1 (Cytosine-Guanine dinucleotide 1 and 2); *FKBP5*seq2: FKBP5 sequencing 2 (Cytosine-Guanine dinucleotide 3 to 5); *FKBP5*seq3: FKBP5 sequencing 3 (Cytosine-Guanine dinucleotide 6 and 7).

4.3.8 Statistical analysis

Statistical analyses procedural details are available in section 2.12 on pages 47.

4.4 Results

4.4.1 Maternal behaviour in dams and offspring morphological measures

There were no differences detected in maternal behaviour between control and predator odour exposed dams. There was no effect of predator odour on total licking or on total nursing [$P > 0.05$] (Figure 9A-B). There was an overall decrease in the dams' total nursing over the first postnatal week [$F_{(5, 13)} = 7.73$, $P = 0.001$, $p\eta^2 = 0.75$]. There were no significant effects of predator odour exposure on hovering, nest-building behaviour, time spent off the nest, self-grooming, eating and drinking by the dam [$P > 0.05$]. There were no significant differences in weight gain during pregnancy, length of pregnancy, litter size, or sex ratio between prenatal predator odour exposure and control offspring [$P > 0.05$].

Offspring weight from postnatal day 0 to 139 and standard length from postnatal day 0 to 56 did not differ between controls and prenatally predator odour exposed offspring [*P's* > 0.05]. However, there was a general increase of weight with time [Male: $F_{(9, 183)} = 2786.835$, $P < 0.0001$, $d_r = 5.2$; Female: $F_{(9, 183)} = 3191.564$, $P < 0.0001$, $d_r = 5.0$] and of standard length with time [Male: $F_{(8, 12)} = 4349.451$, $P < 0.0001$, $p\eta^2 = 1.0$; Female: $F_{(8, 12)} = 4198.548$, $P < 0.0001$, $p\eta^2 = 1.0$].

4.4.2 Stress-related behaviour in adult offspring

On the elevated plus maze, prenatally predator odour exposed adult males laid more boli than adult control male offspring [Mann–Whitney $U = 43.5$, $n_C = 15$, $n_{PO} = 12$, $P = 0.005$ two-tailed, $d = 1.0$] (**Figure 10** A) while female offspring did not differ in the number of

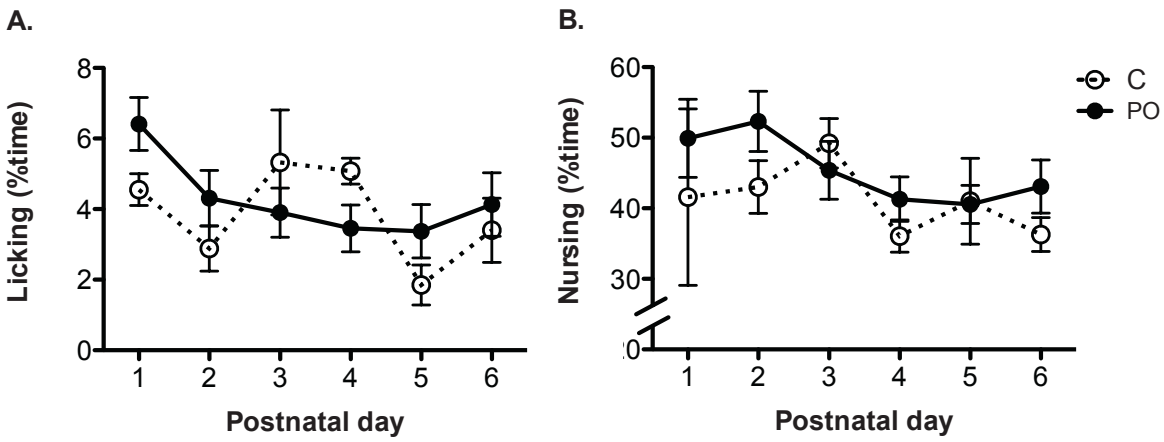


Figure 9. Maternal behaviour is not altered by prenatal predator odour exposure (PO).

Proportion of time spent licking (**A.**) and nursing (**B.**) pups. Data are average ± standard error of the mean. C: control.

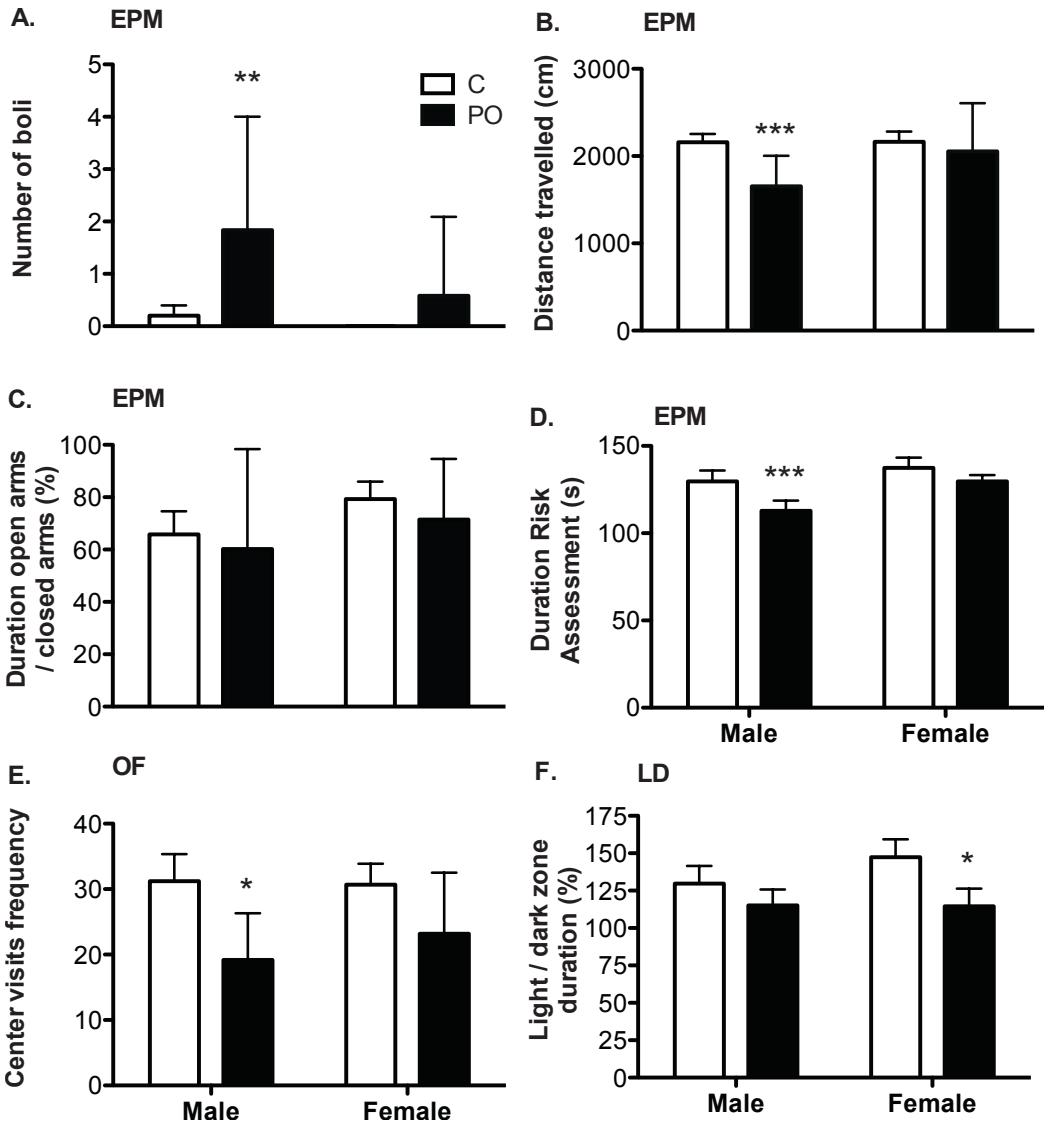


Figure 10. Prenatally predator odour exposed adults (PO) display increased stress-related behaviours compared to control (C) adult rats.

In the elevated-plus maze (EPM), PO males laid more boli (**A.**) and were less active (**B.**) compared to controls. There was no difference in the ratio of time spent on the open arm compared to the closed arms (**C.**) PO males also spent less time performing risk assessment behaviours (**D.**). In the open field (OF), PO male rats entered the center less frequently (**E.**) compared to control rats. In the light-dark transition box (LD), PO female rats spent less time in the light zone (**F.**). Data are average ± standard error of the mean. $*P < 0.05$, $**P < 0.01$, $***P \leq 0.001$ male main effect of prenatal stress; $*P = 0.05$, females main effect of prenatal stress.

boli [$P > 0.05$]. Prenatally predator odour exposed male were less active than control rats in the elevated-plus maze over 15 minutes [Distance travelled: $F_{(1, 25)} = 13.129$, $P = 0.001$, $d_r = -1.8$] (**Figure 10** B) while females showed no prenatal treatment effect [$P > 0.05$]. There were no differences in the ratio of time spent in the open arms relative to the closed arms between prenatally predator odour exposed and control rats [$P > 0.05$] (**Figure 10** C) or the duration or frequency [$P > 0.05$] of open arm visits. Finally, prenatally predator odour exposed males spent less time performing self care (grooming) and risk assessment behaviours (head dipping, rearing, stretch attend posture) [$t_{(1,24)} = 3.862$, $P = 0.001$, $d = 1.5$] (**Figure 10** D), indicative of increased anxiety-like behaviour. Prenatally predator odour exposed and control females did not show difference in risk assessment behaviours [$P > 0.05$].

In the open field, prenatally predator odour exposed male rats showed a decreased center entry frequency [Mann–Whitney $U = 47$, $n_C = 15$, $n_{PO} = 12$, $P = 0.04$ two-tailed, $d = 0.9$] (**Figure 10** E) compared to control offspring, with no differences in females [$P > 0.05$]. Prenatally predator odour exposed rats did not show an activity difference when compared to control rats [$P > 0.05$].

In the light-dark transition task, prenatally predator odour exposed female rats spent less time in the light portion of the apparatus compared to control female rats [$F_{(1, 25)} = 3.986$, $P = 0.05$, $d_r = -0.8$] (**Figure 10** F) while males showed no differences according to their prenatal treatment [$P > 0.05$].

4.4.3 Predator odour exposure in adult offspring

Upon exposure to predator odour in adulthood, the amount of time spent in each of the zones relative to the odour in offspring from both prenatal treatments differed [Male: $F_{(2, 50)} = 25.905$, $P < 0.0001$, $d_r = 2.9$; Female: $F_{(2, 53)} = 54.861$, $P < 0.0001$, $d_r = 2.6$]. Males showed a different use of the zones in each prenatal treatment [$F_{(2, 50)} = 6.256$, $P = 0.002$, $d_r = 3.2$]. More specifically, prenatally predator odour exposed males spent less time in the area closest to the odour [$F_{(1, 16)} = 37.538$, $P = 0.001$, $d_r = -0.6$] (Figure 11A) while

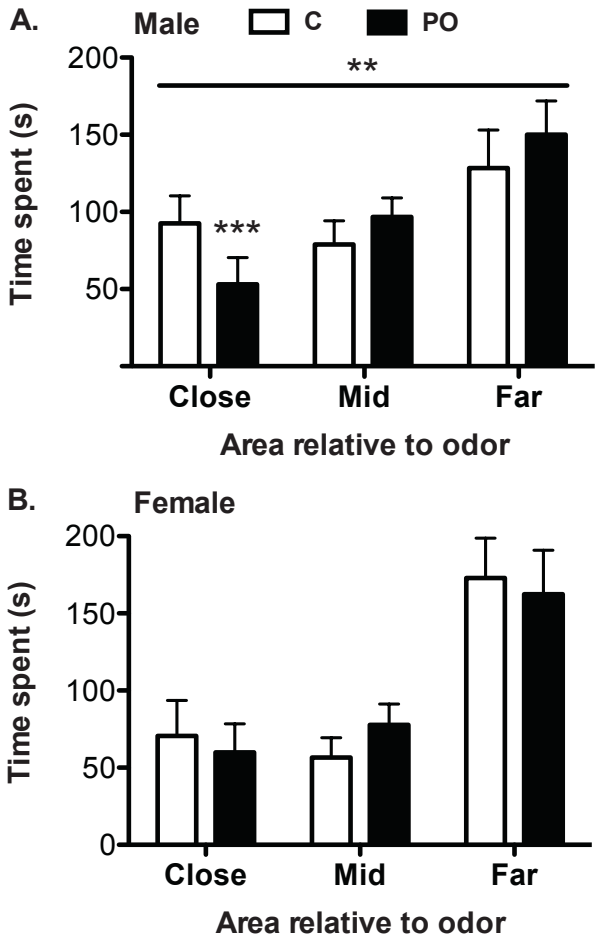


Figure 11. **Prenatally predator odour exposed (PO) males exhibited increased avoidance of the predator odour.**

PO males spend less time in the area close to the odour compared to control (C) males (**A.**) while females show no difference (**B.**). Data are average ± standard error of the mean. Bar: $^{**}P < 0.01$, male interaction effect of prenatal stress and time spent in areas; $^{***}P = 0.001$, male main effect of prenatal stress.

female offspring showed no difference [$P > 0.05$] (Figure 11B). Prenatally predator odour exposed and control rats of both sexes did not show a difference in the time spent in the shelter or the latency to enter the zone closest to the odour [$P > 0.05$].

4.4.4 Endocrine response to predator odour exposure in adult offspring

Overall, serum corticosterone was significantly higher at 60 minutes after the introduction of the predator odour in all animals [Main effect of time: Female: $F_{(2, 34)} = 6.152$, $P = 0.005$, $d_r = 2.9$; Male: $F_{(2, 12)} = 30.068$, $P < 0.0001$, $d_r = 2.7$] (Figure 12A) with no prenatal treatment effect [$P > 0.05$]. There was no difference in the area under the curve between males or females of both prenatal treatments [$P > 0.05$].

Circulating adrenocorticotropic hormone level changed across time with exposure to the predator odour in both sexes [main effect of time: Female: $F_{(2, 11)} = 5.996$, $P = 0.02$, $d_r = 2.8$; Male: $F_{(2, 36)} = 4.179$, $P = 0.02$, $d_r = 3.0$] (Figure 12B). Serum adrenocorticotropic hormone from adult female offspring was also modulated differently across time in function of the prenatal treatment [Interaction time by prenatal effect: $F_{(2, 11)} = 0.14$, $P = 0.01$, $d_r = 2.6$] **(**Figure 12B) although there was no significant prenatal treatment effect [$P > 0.05$], Furthermore, prenatally predator odour exposed female showed no recovery from stress 120 minutes after the start of predator odour exposure compared to control females [$t_{(1, 12)} = -3.44$, $P = 0.005$, $d = -1.9$]. The time spent in the light portion of the light-dark transition apparatus was negatively correlated with the adrenocorticotropic hormone recovery level in females [*Spearman's rho Correlation Coefficient* = -0.661, n = 10, $P = 0.04$].

4.4.5 Gene transcript abundance analysis in adult offspring

At birth (postnatal day 0), prenatally predator odour exposed females showed an overall increased level of *glucocorticoid receptor* messenger RNA in the amygdala [$t_{(1,7)} = -2.723$, $P = 0.03$, $d = -1.6$] (Figure 13A) (Table 7) when compared to control females, while there was no difference between prenatally predator odour exposed and control

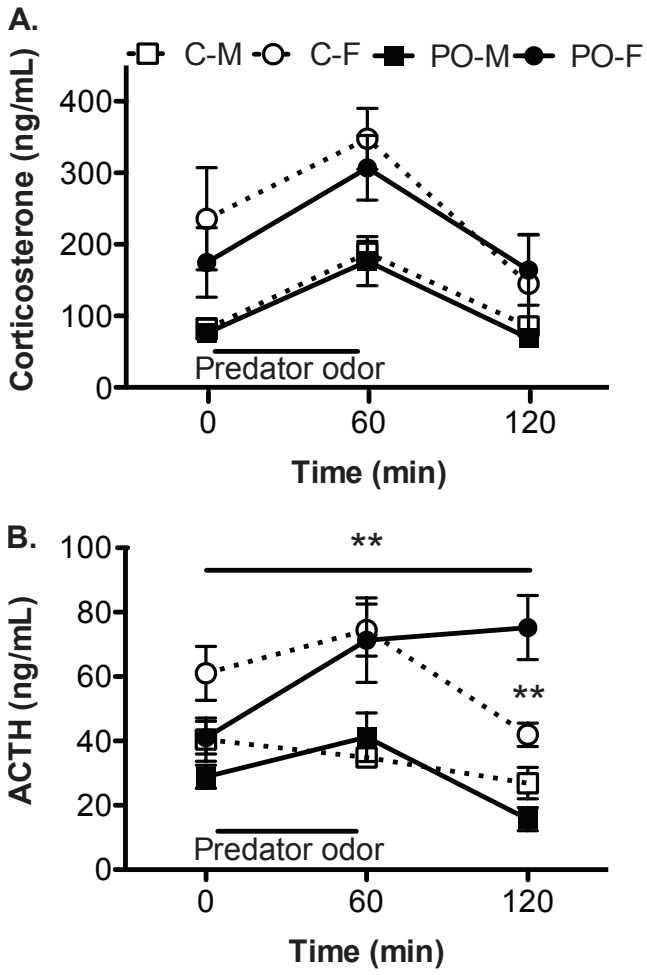
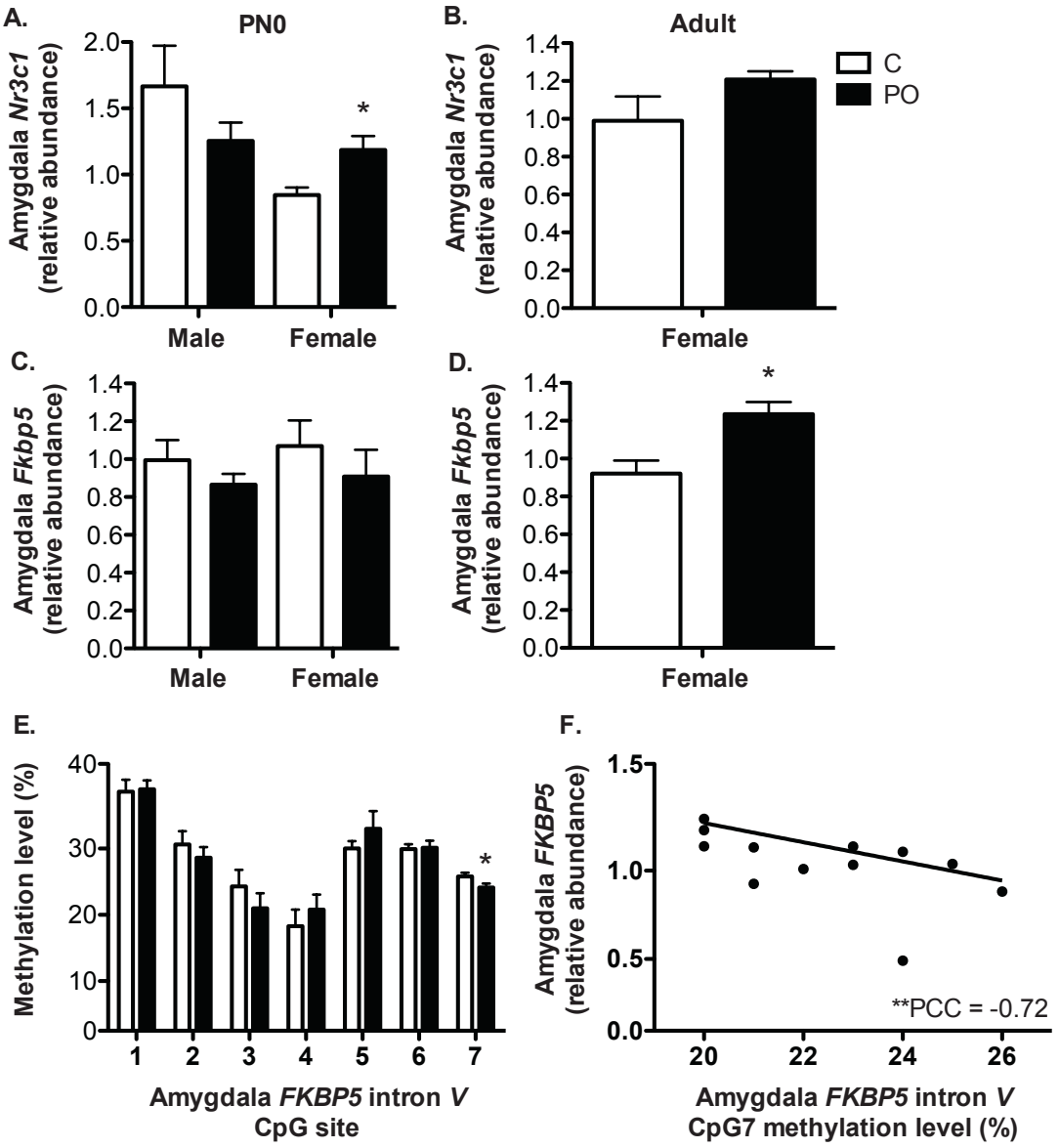


Figure 12. Prenatally predator-odour exposed (PO) adult females affects circulating level of adrenocorticotropic hormone (ACTH), but not corticosterone (CORT), to predator odour compared to control adult offspring.

The CORT level increase after exposure to a predator odour in all groups (**A.**). ACTH levels during the same challenge show a lack of recovery in PO compared to control (C) females (**B.**). M: male; F: female. Data are average ± standard error of the mean. Bars: **P = 0.01, female interaction effect of prenatal stress and time; **P < 0.01, time point female main effect of prenatal stress.



G. *FKBP5* intron *V*

TAG**CG$_1$**TAAAGTTATTAGA**CG$_2$**TTAGTTGTTATAATTAGAGAAGAGAAAGTAGATAT
TTAT**CG$_3$**AGTTAA**CG$_4$**TTTTAGGTTTTGG**CG$_5$**GTTATAGTATTAAAAAGTTTTATAGT
TTTTGTTTTTAGTTTTGTTTTTTGAAATATAAGTTGTATAGTTTGGGGTTTTTTGTAT
TTTAGTTTTTGTTATTGTTGTATTTTTTTTTTTTTTTTATTTTAAT**CG$_6$**GAGAATAAATT
GTTGTTAGTATAT**CG$_7$**AGTTTATGTGTTAGTTATATTTAGAATAGGGTGTTTTGTGT
TTTTTAAAAT

Figure 13. Transcript abundance in postnatal day 0 (PN0) and adult female offspring and FK506 binding protein 5 (FKBP5) intron V methylation in the amygdala is altered by prenatal exposure to predator odour (PO).

In the amygdala at birth, *NR3C1* transcript abundance in PO female offspring is higher than in control (C) female offspring (**A.**) while there is no difference in *FKBP5* transcript

abundance PN0 female offspring (**C.**). Males show no difference in transcript abundance at birth in function of prenatal predator odour exposure (**A., C.**). In adulthood, there is no difference in glucocorticoid receptor (*Nr3c1*) transcript abundance (**B.**) while *FKBP5* transcript abundance is increased in PO females compared to control females (**D.**) in the amygdala. DNA methylation level (%) of the 7 sequenced Cytosine-Guanine dinucleotides sites within the *Fkbp5* intron *V* show alterations by prenatal predator odour exposure in adult females (**E.**). There is a negative correlation between the amygdala *FKBP5* transcript abundance and the amygdala *Fkbp5* exon *V* Cytosine-Guanine dinucleotide site 7 methylation level as shown by the line of best fit (**F.**). The *Fkbp5* intron *V* Cytosine-Guanine dinucleotide (CpG) sites sequenced are in just upstream of a conserved glucocorticoid-response element (GRE; underlined; **G**). Data are average ± standard error od the mean. Bar: $^{**}P = 0.01$ interaction of prenatal predator odour exposure per site effect; $^{*}P < 0.05$, main prenatal predator stress effect; Correlation: $^{**}P < 0.01$.

Table 7. **Transcript relative abundance in the amygdala in control and prenatally predator odour exposed rats on postnatal day 0 (PN0) and in adult offspring.**

Transcript	**Average relative transcript abundance (Mean ± SEM)**		**Mean effect of PO**	
	C	PO	F-value	*P*-value
Male PN0				
NR3C1	1.666 ± 0.751	1.334 ± 0.324	0.983	0.345
NR3C2	1.065 ± 0.354	1.074 ± 0.291	0.002	0.963
FKBP5	0.994 ± 0.239	0.865 ± 0.140	1.257	0.291
Female PN0				
NR3C1	0.845 ± 0.115	1.186 ± 0.232	7.087	**0.032**
NR3C2	1.162 ± 0.594	1.132 ± 0.629	0.005	0.945
FKBP5	1.069 ± 0.271	0.953 ± 0.345	0.279	0.617
Female Adult				
NR3C1	0.989 ± 0.128	1.208 ± 0.043	2.011	0.182
NR3C2	0.925 ± 0.092	0.971 ± 0.054	0.159	0.697
FKBP5	0.921 ± 0.069	1.137 ± 0.060	5.131	**0.040**
BDNF	0.626 ± 0.197	1.039 ± 0.492	0.740	0.407

Transcript abundance was corrected with the average 5 reference genes (Beta-actin, 18S ribosomal RNA, Glyceraldehyde 3-phosphate dehydrogenase, Ubiquitin C and 14-3-3 protein zelta/delta) transcript abundance. Boldface *P*-values are significant. *NR3C1*: Glucocorticoid receptor, *NR3C2*: Mineralocorticoid receptor, *FKBP5*: FK506 binding protein 5, *BDNF*: Brain-derived neurotrophic factor. (PN0: sample size = 6 per group; Adult: Control [C]: sample size = 8, Prenatally predator-odour exposed [PO]: sample size = 6). Boldface: $P < 0.05$.

male offspring in *glucocorticoid receptor* levels in the amygdala on the day of birth [$P > 0.05$] (Figure 13B). The transcript abundance of *FK506 binding protein 5* and *mineralocorticoid receptor* did not differ between prenatally predator odour exposed and control offspring in the amygdala at birth (postnatal day 0) [$P > 0.05$] (Figure 13C) (Table 7).

Since females, but not males, showed stress-related endocrine differences as a function of the maternal predator odour during pregnancy exposure at birth, we examined evidence for alterations in stress-related gene transcript abundance in prenatally predator odour exposed adult females only. Therefore, gene transcript abundance in the amygdala of prenatally predator odour exposed and control females in adulthood were examined. Prenatally predator odour exposed females showed higher transcript abundance of *FK506 binding protein 5* in the amygdala when compared to control females [$t_{(1,12)} = -2.27$, $P = 0.04$, $d = -1.3$] (Figure 13D). The transcript abundance of *glucocorticoid receptor*, *mineralocorticoid receptor* and *brain-derived neurotrophic factor* did not differ between prenatally predator odour exposed and control females in the amygdala [*P's* > 0.05] (Figure 13B) (Table 7). The transcript abundance of *FK506 binding protein 5* in the amygdala of females was positively correlated with their *glucocorticoid receptor* level [*Spearman's rho Correlation Coefficient* = 0.679, n = 14, $P = 0.001$] and *mineralocorticoid receptor* level [*Spearman's rho Correlation Coefficient* = 0.653, n = 14, $P = 0.01$] in the amygdala.

4.4.6 DNA methylation analysis of *FK506 binding protein 5* intron *V* in adult female offspring

Due to the observed difference in *FK506 binding protein 5* transcript abundance in the amygdala of prenatally predator odour exposed females compared to controls and previous reports of altered epigenetic regulation of *Fk506 binding protein 5* intron *V* as a function of early life environmental factors (Kitraki et al., 2015), we examined DNA methylation levels in *Fk506 binding protein 5* intron *V* in the amygdala of adult female offspring (Figure 13E). Overall, levels of DNA methylation in all female offspring

differed significantly across the Cytosine-Guanine dinucleotide sites examined within the *Fk506 binding protein 5* intron *V* [$F_{(6, 51)} = 53.992$, $P < 0.0001$, $d_r = 5.1$] and the effect of prenatal exposure to predator odour on DNA methylation varied across the Cytosine-Guanine dinucleotide sites [Interaction of prenatal stress and Cytosine-Guanine dinucleotide sites: $F_{(6, 51)} = 3.009$, $P = 0.01$, $d_r = 6.0$] **(**Figure 13E). In particular, Cytosine-Guanine dinucleotide site 7 showed significantly lower methylation in prenatally predator odour exposed female rats when compared to controls [$t_{(1,10)} = 2.58$, $P = 0.03$, $d = 1.4$] (Figure 13E). Furthermore, amygdala *FK506 binding protein 5* transcript abundance was negatively correlated with amygdala *FK506 binding protein 5* intron *V* Cytosine-Guanine dinucleotide site 7 methylation levels in females [*Pearson Correlation Coefficient* = -0.72, n = 12, $P = 0.008$] **(**Figure 13F). Methylation levels of other Cytosine-Guanine dinucleotide sites examined within the intron did not differ significantly as a function of the maternal predator odour exposure during pregnancy or vary in relation to *FK506 binding protein 5* transcript abundance [*P*'s > 0.05].

4.5 Discussion

Exposure to cues indicating the threat of predation plays an important role in shaping animal behaviours in natural habitats (Apfelbach et al., 2005; Love et al., 2013). In this study, we found that maternal predator odour exposure in rats programmed stress-related behaviours, in both sexes and a sex-specific endocrine response to predator odour later in life along with gene transcript abundance and DNA methylation. Prenatal predator odour is therefore a potent programming stressor.

Our findings suggest that it is likely that these effects on offspring phenotype resulted from maternal exposure to predator odour during gestation rather than as consequences of changes in maternal physiology or behaviour during the lactational period. Compared to control dams, dams exposed to predator odour during gestation showed similar levels of nursing and licking behaviour, maternal behaviours classically associated with maternal programming of stress-related genes in early postnatal life (Weaver et al., 2004). We

observed an increase in *glucocorticoid receptor* mRNA in the amygdala of female offspring on the day of birth. These results suggest a different effect of maternal predator odour exposure during pregnancy compared to exposure to predator odour during the lactational period, which is associated with increased licking and nursing in dams (Coutellier and Würbel, 2009; Mashoodh et al., 2009; McLeod et al., 2007). Although prenatal stress studies do not always report maternal care provided, the stress normally alters the maternal behaviour (Darnaudéry and Maccari, 2008; Mueller and Bale, 2006; Weinstock, 2008), with changes sometimes being modest (St-Cyr and McGowan, 2015). Predator odour exposure during pregnancy might be particular in this sense but further investigations such as a cross-fostering study could be used to control for effects of alterations in maternal behaviour. However, the long-term morphology of the offspring was not affected by the maternal predator odour exposure during pregnancy, although live prenatal predator exposure and other prenatal stress paradigms such as physical restraint lead to decreased weight at birth and up to weaning (Ahmadzadeh et al., 2011; Korgan et al., 2014; Lesage et al., 2004; Mueller and Bale, 2006; Wilson and Terry, 2013).

Using a variety of standardized tests to assess ethologically relevant anxiety-like behaviours involving approach-avoidance conflict, we found behavioural responses consistent with increased anxiety-like behaviour in prenatally predator odour exposed rats (Bailey and Crawley, 2009). The increase in defecation seen in the prenatally predator odour exposed adult male offspring in the elevated-plus maze is a classic indicator of increased anxiety (Gray and Lalljee, 1974) and is increased over a range of stressful situations and sensitive to pharmacological blockade of corticosterone-releasing hormone receptors (Fukudo, 2012). Our results parallel those observed with prenatal physical restraint stress, typically associated with increased anxiety-like behaviour in these tasks (reviewed in Darnaudéry and Maccari 2008). Further, risk assessment behaviours were decreased in the prenatally predator odour exposed males compared to the control male rats. Self-care (grooming) and exploration (rearing, head dipping and stretch attend posture) are all increased in less anxious animals (Rodgers and Dalvi, 1997). Acute and chronic exposure to a cat predator lead to the suppression of grooming and rearing

behaviours (Blanchard et al., 1998). Cat or cat odour exposure has no effect on open arm exploration in the elevated-plus maze while reducing risk-assessment behaviours such as head dipping, rearing and overall risk assessment behaviour (Adamec et al., 2004b, 1998). Anxiogenic drugs also lead to a reduction in risk assessment behaviours (Adamec et al., 2004a). However, the female's increase in anxiety-like behaviour was weaker as detected in only one of the three tests, suggesting sex-specific novelty-induced anxiety-like behaviour in this study, as discussed below.

Male offspring from predator odour exposed dams showed increased avoidance behaviour in response to acute predator odour exposure in adulthood. On the contrary, prenatally predator odour exposed and control females were already showing strong avoidance to the predator odour including general avoidance, rearing, hiding, defensive burying as well as freezing (see Figure 11). This result is in accordance with other reports of early postnatal and prenatal exposure to predator odour, including our earlier findings in mice, where adult offspring increase their avoidance to predator odour (Mashoodh et al., 2009; St-Cyr and McGowan, 2015). Importantly, in the present study the adult offspring were naïve to direct exposure to the predator odour; they had only been exposed prenatally through their mother. This effect of maternal predator odour exposure during pregnancy on offspring behaviour may have consequences for fitness, as increased avoidance to a predator odour decreases the probability of detection by a predator and therefore impacts survival (Langerhans, 2007), particularly in male rodents that show more extensive dispersal than females in naturalistic conditions (Glover and Hill, 2012).

There is some additional evidence in the literature of sex-specific responses to predator odour exposure. Female rats exhibit increased escape behaviour (wall climbing) during fox urine exposure and a sustained corticosterone response one hour after the end of the odour exposure compared to males (Campbell et al. 2003). In Long-Evans rats exposed to cat odour early postnatally, females in adulthood show a greater decrease in contact and visits near the odour compared to predator odour exposed males (Mashoodh et al., 2009).

Both prenatally predator odour exposed and control rats showed a similar initial rise of corticosterone level after exposure to an inescapable predator odour for the first time. However, the adrenocorticotropic hormone response in prenatally predator odour exposed female offspring was maintained at high levels at the recovery time point, indicating a prolonged adrenocorticotropic hormone response beyond the termination of the stressor. The stress recovery level of adrenocorticotropic hormone was inversely correlated with increased anxiety-like behaviour in the light-dark transition test. Adrenocorticotropic hormone activation has been observed following acute exposure to predator odour in adult animals, and is generally accompanied by a similar corticosterone elevation (Masini et al., 2005; Takahashi et al., 2008). However, Muñoz-Abellán et al. (2011) also reported an increase in adrenocorticotropic hormone without a significant increase in the circulating corticosterone level in response to cat odour exposure. Dissociation between the adrenocorticotropic hormone and corticosterone responses to stress may result from a ceiling effect of elevated corticosterone, adrenocorticotropic hormone variation in bioactivity, or altered adrenal sensitivity to adrenocorticotropic hormone (Poland et al., 1989). The ceiling effect does not apply in this case since we observed a decrease in corticosterone at the recovery time-point (120 minutes). Regarding adrenocorticotropic hormone bioactivity, the immunoreactive adrenocorticotropic hormone measured with the radioimmunoassay is the total adrenocorticotropic hormone from which a subset is bioactive. However, the immunoreactive adrenocorticotropic hormone and the bioactive adrenocorticotropic hormone levels are known to be correlated (Goverde et al., 1989), so adrenocorticotropic hormone variation in bioactivity is unlikely. It is possible that predator odour exposure modifies adrenal sensitivity to adrenocorticotropic hormone, as has been shown in response to chronic stress in adulthood (Bornstein et al., 2008). Further studies are needed to examine adrenal sensitivity in the offspring of dams exposed to gestational predator odour.

On the day of birth (postnatal day 0), we observed a female-specific increase in *glucocorticoid receptor* transcript abundance in the amygdala. Negative feedback inhibition of glucocorticoids mediated by neural transcript abundance of the *glucocorticoid receptor* is functional in the foetus from gestational day 16.5 (Reichardt

and Schütz, 1996). This period coincides with sex-specific transcript abundance of placental 11-beta-hydroxysteroid dehydrogenase 2, when females show reduced 11-beta-hydroxysteroid dehydrogenase 2 and thus reduced maternal glucocorticoid buffering (Pankevich et al., 2009). As effects of prenatal stress on placental 11-beta-hydroxysteroid dehydrogenase 2 transcript abundance have been observed up to the gestational day 20 (Peña et al., 2012), differential glucocorticoid exposure *in utero* may lead to a sex-specific *glucocorticoid receptor* transcript abundance in offspring at birth.

In adulthood, prenatally predator odour exposed females no longer displayed significantly elevated *glucocorticoid receptor* transcript abundance, however the transcript abundance of the *glucocorticoid receptor* moderator *FK506 binding protein 5* was elevated in the amygdala. *Fk506 binding protein 5* is a co-chaperone that modulates the sensitivity of multiple steroid receptors, including the glucocorticoid and mineralocorticoid receptors, and is essential for mediating the effect of those steroid hormones on gene transcript abundance via transactivation. The binding of *FK506 binding protein 5* leads to the translocation of these activated receptors into the nucleus, where the *glucocorticoid receptors* acts as a transcription factor and binds to a glucocorticoid-response element to alter transcription, including on the *FK506 binding protein 5* gene itself to potentiate its transcription, forming an ultra-short regulatory loop. *FK506 binding protein 5* transcript abundance is thus implicated in glucocorticoid sensitivity and stress recovery (Jääskeläinen et al., 2011). *FK506 binding protein 5* is ubiquitously expressed in tissues ranging from brain to liver, skeletal muscles and heart, including in the embryo on gestational days 15 and 16 (Baughman et al., 1997; Freeman et al., 1998). In the mouse brain, glucocorticoids lead to an up-regulation of *FK506 binding protein 5* transcript abundance in the paraventricular nucleus of the hypothalamus, central amygdala and hippocampus. For example, restraint stress increases the transcript abundance of *FK506 binding protein 5* (Jääskeläinen et al., 2011). Furthermore, *FK506 binding protein 5* polymorphisms have been implicated in mood, affective and anxiety disorders as well as post-traumatic stress disorder development (Binder, 2009; Binder et al., 2008). Allele-specific *FK506 binding protein 5* DNA methylation also mediates gene-childhood trauma interactions (Klengel et al., 2013) with different genotype showing differential level of

DNA methylation after early life trauma exposure associated to functional changes. Interestingly, intronic elements of the *FK506 binding protein 5* gene exhibit demethylation after chronic glucocorticoid exposure through a conserved main glucocorticoid-responsive element enhancer and this difference persists over time (Hubler and Scammell, 2004) making *FK506 binding protein 5* an interesting target for long-term programming effects of glucocorticoid exposure during the hypothalamic-pituitary adrenal axis development. As the amygdala has an excitatory effect on the hypothalamic-pituitary adrenal axis response to stress (Steckler, 2005), increased *FK506 binding protein 5* in the amygdala lead to sustained activation of the hypothalamic-pituitary adrenal axis (Gold, 2015). Interestingly, circulating adrenocorticotropic hormone levels in prenatally predator odour exposed females had not recovered two hours after the termination of the predator odour stress, indicating prolonged activation of the stress axis compared to control females.

We further investigated potential mechanisms involved in regulating adult female *FK506 binding protein 5* transcript abundance as a function of maternal predator odour exposure during pregnancy by examining methylation levels at 7 Cytosine-Guanine dinucleotide sites within the *FK506 binding protein 5* intron *V* in the amygdala. The level of DNA methylation of *FK506 binding protein 5* intron *V* is known to show a dose-dependent to administration of the synthetic glucocorticoid dexamethasone in mouse hippocampal neuronal cultures (Yang et al., 2012). Other studies have reported a long-term increase in the *FK506 binding protein 5* transcript abundance correlated with a decrease in the methylation of *FK506 binding protein 5* intron *V* both within the amygdala and hippocampus following a chronic increase in corticosterone or recovery from chronically elevated corticosterone levels (Lee et al., 2010; Sawamura et al., 2015). We found that *FK506 binding protein 5* amygdala transcript abundance correlated negatively with Cytosine-Guanine dinucleotide site 7 methylation levels in the *FK506 binding protein 5* intron *V*. Maternal predator odour exposure during pregnancy had an effect on DNA methylation in *FK506 binding protein 5* intron *V* that varied across Cytosine-Guanine dinucleotide sites overall, and was associated decrease in DNA methylation in Cytosine-Guanine dinucleotide site 7 of large statistical effect size (Cohen's $d = 1.4$). We note that

this difference in methylation emerged despite the use of tissue samples containing mixed cell populations, not limited to a specific neural pathway, which may have diluted the difference in absolute levels of DNA methylation between prenatally predator odour exposed and control rats. We also found that the *FK506 binding protein 5* intron *V* amygdala transcript abundance correlated negatively with Cytosine-Guanine dinucleotide site 7 methylation levels. Together, these data indicate that the observed differential methylation is likely biologically meaningful, though further studies examining the precise mechanism by which maternal predator odour exposure during pregnancy may modify DNA methylation of *FK506 binding protein 5* are needed. This finding suggests a long-term programming role of *FK506 binding protein 5* following developmental stress, while some *FK506 binding protein 5* gene variants increases the probability of developing a mood and anxiety disorders when primed with a life stressors (Zannas and Binder, 2014).

We found a prenatal predator odour exposure by Cytosine-Guanine dinucleotide site interaction over the 7 Cytosine-Guanine dinucleotide sites examined and a specific decrease in the methylation of Cytosine-Guanine dinucleotide site 7 in the prenatally predator odour exposed rats proximal to a glucocorticoid-response element binding motif. Our results suggest that elevated *glucocorticoid receptor* transcript abundance in newborn females as a consequence of maternal predator odour exposure may lead to the long-term programming of *FK506 binding protein 5* through demethylation around the glucocorticoid-response element. Future studies characterizing *glucocorticoid receptor-FK506 binding protein 5* binding and epigenetic modifiers at *FK506 binding protein 5* intron *V* during the developmental period in response to prenatal odour exposure are needed to examine this possibility.

4.5.1 Conclusion

The data reported here indicate that maternal exposure during gestation to predator odours alone, a psychogenic and ethologically-relevant stressor, is sufficient to alter the offspring phenotype in a manner that persists into adulthood. We note that although our

results are consistent with a response to a predator odour, the impacts of the odour's noxious characteristics per se on offspring stress-related phenotype cannot be ruled out. Future studies employing an odour contrast with other noxious stimuli, such as butyric acid (e.g. Ayers et al., 2016), will be useful in definitively probing responses specific to predator odour.

Chapter 5 Prenatal predator odour exposure programs metabolism in adult offspring

5.1 Publication

Chapter 5 is adapted from the following research article manuscript:
St-Cyr, Sophie, Abuaish, Sameera, Welch Jr., Kenneth C., McGowan, Patrick O. Prenatal predator odour exposure programs metabolism in adult offspring. *In preparation.*

5.2 Approach and hypotheses

One of the most important responses to stress is to promote energy mobilization by increasing energy requirements. This energy further mediates the 'fight-or-flight' response, allowing the behavioural response to stress. Key modulators that could be implicated in the mediation of weight loss, reduction of food intake, increase in core body temperature and energy expenditure are the thyrotropin-releasing hormone and thyroid hormones (Lechan and Fekete, 2006). Furthermore, maternal behaviour variations and neonatal administration of thyroxine during the first two postnatal weeks leads to elevated glucocorticoid receptors, basal corticosterone and corticosterone-binding globulin levels (Hellstrom et al., 2012; Meaney et al., 1987). Taken together, increased circulating thyroid hormones levels could be a key mediator of energy requirements in prenatally stressed or prenatally predator odour exposed mice.

The objectives of this study is to evaluate the impact of prenatal predator odour exposure on the adult offspring of both sexes 1) energy consumption over 24 hours, 2) energy consumption under acute stress and 3) thyroxine serum level as a proxy of the hypothalamic-pituitary-thyroid axis activity modulating growth and energy consumption.

We hypothesize that prenatal predator odour exposure will produce adult offspring with decreased body weight and food consumption, increased metabolic rate over 24 hours and under stress as observed in mammals exposed to predators or predator cues prenatally or during adulthood as reported in Table 2 and Table 3. We expect to detect increased thyroxine serum level and incidentally corticosterone-binding globulin liver transcript

abundance if the hypothalamic-pituitary-thyroid axis is in part responsible for an increased overall metabolism in prenatally predator odour exposed animals.

5.3 Material and methods

5.3.1 Mice breeding

For breeding, two normally cycling females were housed with one male overnight. Pregnant females were singly housed on gestational day 10 and weighed every day throughout the pregnancy. Twenty-four of the 41 mated females (58,5%) gave birth to 12 control litters and 12 prenatally predator odour exposed litters. This breeding success rate can be explained by the fact that the female's oestrous phase was not checked prior to breeding. Additional procedural details are available in section 2.1 on page 42.

5.3.2 Fecal corticosterone metabolite levels during pregnancy

Feces were collected from pregnant females for corticosterone metabolites measurements. Females were housed in an empty cage for an hour from 9 to 10 PM for 7 days for habituation prior to mating. Feces were collected during one hour, 6 hours after the stress exposure onset deduced from average mouse metabolism reported by Touma et al. (2003). Stress exposure consistently took place from 4 to 5 PM. A baseline level was collected from undisturbed pregnant females on the day prior to the first odour exposure (gestational day 10) and was compared to the last day of the odour exposure paradigm (gestational day 18). 11 pregnant control females and 13 pregnant predator-odour exposed females were compared.

Fecal corticoid extraction was conducted by drying the frozen feces overnight using a lyophilizer (Labconco), crushed to a dust-like material in liquid nitrogen and weighed (94 grams on average). Ethanol 10 mL (100%) was added to each sample, which was then boiled in a water bath for 20 minutes. Upon removal from the bath, tubes were

centrifuged for 15 minutes at 4,500 rotations per minute and the supernatant was poured off into a glass tube. Ethanol 5 mL was added to the fecal sample tube, and the sample was then vortex for 1 minute, re-centrifuged for 15 minutes at 4,500 rotation per minute, and the supernatant added to the previous 10 mL of extract. Supernatants were evaporated under air using a custom manifold and re-constituted with 1 mL methanol, diluted to 1:40 and stored at -80°C until assay (modified from (Cavigelli et al., 2005; Palme et al., 2013). The amount of corticosterone present in the fecal corticosterone metabolite extracts was determined using a radioimmunoassay kit with ^{125}I-labeled anti-corticosterone antibody (MP Biomedicals Inc., CA., USA: sensitivity 7.7 ng/mL, intra-assay coefficient of variation 10.3%; cross-reactivity: desoxycorticosterone 0.34%, cortisol 0.05%, aldosterone 0.03%, cholesterol/11-desoxycortisol <0.01%).

5.3.3 Maternal behaviour in dams and offspring morphological measures

On top of the classic maternal behaviours measured, the maternal behaviour fragmentation percentage was also evaluated as the number 'on the nest' compared to 'off the nest' behaviour exhibited by the dam (Baram et al., 2012; Rice et al., 2008). Nest quality was also evaluated at the beginning of each observation period (6 times per day) according to a qualitative 1 to 5 scale, with 1 being scattered nesting material and 5 being a completely closed dome-shaped nest (Hess et al., 2008). Additionally, from postnatal day 21 to 65, offspring food consumption was measured weekly. Additional procedural details are available in sections 2.2 and 2.3 on page 42 and 43.

5.3.4 Adult offspring sample sizes

A subset of adult offspring was used to examine metabolic rates in the following order: metabolic rate over 24 hours (control male sample size = 13, control female sample size = 12, prenatally predator-odour exposed male sample size = 13, prenatally predator-odour exposed female sample size = 12) and metabolic rate while exposed for the first time directly to a predator odour (control male sample size = 12, control female sample size = 11, prenatally predator-odour exposed male sample size = 12, prenatally predator-

odour exposed female sample size = 9). The thyroxine level at baseline and after stress was measured in unstressed mice (control male sample size =10, control female sample size = 11, prenatally predator-odour exposed male sample size = 9, prenatally predator-odour exposed female sample size = 10) and restrained mice (control male sample size =10, control female sample size = 11, prenatally predator-odour exposed male sample size = 9, prenatally predator-odour exposed female sample size = 10). Gene transcript abundance was measured in a subset of the thyroxine level at baseline animals (sample size = 6 per prenatal stress group, 1 mouse/sex/litter).

5.3.5 Metabolic rate over 24 hours in adult offspring

The oxygen consumption rate of control and prenatally predator odour exposure adult mice was measured over a 24-hour period using open-flow respirometry. During experiments, mice were individually housed in Sable Systems mouse-sized respirometry cages (Model: CAGE-3721; Las Vegas, NV, USA). Cages consisted of standard mouse-sized polysulfone tubes fitted with filter cover tops and a respirometer manifold (Model: MAN-3721) permitting air intake. Eight respirometer cages were sequentially serially sampled with one of these cages left empty to provide a reference ambient air recording. Cages containing mice were provisioned with food pellets and water. Air was drawn through the cages at 800 mL/minute and flow rate was regulated by a Flowbar-8 flow controller system (Sable Systems). An RM-8 Flow Multiplexer, controlled by Expedata data collection software (V. 1.7.2; Sable Systems) orchestrated the sequential sampling of the reference chamber for 5 minutes, followed by animal chambers 1 to 4 for 5 minutes each, another 5 minutes recording from the reference chamber, and animal chambers 5-7 for 5 minutes each. This sampling regime was repeated continuously over the full 24-hour measurement period over 8 separate days in PN71-127 mice (median PN85).

Ex-current air from the focal respirometry chamber was first drawn through the main pump (and flow meter/controller) of a Turbofox 5 respirometry system (Sable Systems). The airstream was then sub-sampled at a flow rate of approximately 200 mL/minute and this gas passed through the Turbofox's water vapour meter, followed by the CO_2

analyzer, and finally O_2 analyzer. Analog signals from flow meters and gas analyzers were converted to digital signals via the internal A/D converter of the Turbofox. Data were recorded every 5 seconds to computer via Expedata software. The flow-rate from the focal chamber, and O_2 and CO_2 partial pressures were corrected for water vapour dilution using equation 8.6 from Lighton (2008). Drift in the O_2 trace was corrected within the Expedata software suite by fitting a spline function through baseline (reference) chamber recordings. Oxygen consumption rate (in mL/minute) was calculated using equation 11.7 in Lighton (2008). Mice were filmed throughout testing. Afterwards, their activity level was blindly coded continuously throughout the 5-minutes metabolic rate recordings using Observer XT 8.5. The first measurement in the metabolic chamber is elevated due to the initial stress of the novel environment of the metabolic chamber, this measure is considered as a metabolic rate under stress.

5.3.6 Metabolic rate during predator odour exposure in adult offspring

The oxygen consumption rate of adult control and prenatally predator odour exposed mice was measured during a two-hour exposure to an inescapable predator odour (100 µL 2,3,5-trimethyl-3-thiazoline 1:5000). The respirometric setup and analyses were conducted identically to that described above for the 24-hour recording period except for the following details: only 2 cages were used; one cage with a focal animal and one cage left empty (the reference chamber). Cages were placed inside a fume hood in order to contain the predator odour. As a result, the flow-rate through the cages was maintained at ~2000 mL/minute. The baseline (reference chamber) was recorded for 2 minutes followed by the animal metabolic chamber for 8 minutes, and this 10-minute schedule was repeated continuously over the 2 hours recording window. Mice activity level was tracked continuously using EthoVision XT 10. Mice were aged PN98-153 (median 119).

A user error resulted in a needle valve moderating air-flow through the Turbofox being left wide open during a subset of recordings. Because of this error, the flow controller of the Turbofox was unable to maintain a steady flow rate and flow through the chambers fluctuated wildly. The error was not discovered until after data collection. As a result of

uncontrolled flow, calculation of accurate oxygen consumption rate could not be accomplished on this subset of data (11 mice) and these data are excluded from further analyses. The first measurement in the metabolic chamber is elevated due to the initial stress of the metabolic chamber, this measure is perceived as metabolic rate under the stress of the novel environment as well as the initial exposure to the predator odour.

5.3.7 Thyroxine circulating at baseline and in response to restraint stress in adult offspring

The thyroxine level at baseline and after stress was measured in unstressed mice and mice recovering from a restraint stress. On the day of testing, mice were habituated to the procedure room for two hours. Mice were then restrained in a transparent disposable bakery-decorating bag (Wilton) for 20 minutes and returned to their home cage. Restrained mice were sacrificed two hours after the stress onset and trunk blood was collected. Blood was kept on ice at least 30 min before being centrifuged at 4,000 rotation per minute at 4°C for 20 minutes. Serum was then extracted and stored at -80°C. The amount of thyroxine present in duplicate serum samples was determined using a commercially available radioimmunoassay kit with ^{125}I-labeled thyroxine antibody (MP Biomedicals Inc., CA., USA: thyroxine sensitivity 0.76 µg/dL, intra-assay coefficient of variation 11.4%).

5.3.8 Transcript abundance analysis by quantitative real time-polymerase chain reaction

In the mouse liver, the *corticosterone-binding globulin* transcript abundance (Forward primer: (5'- GTCGTCGCTGCACTTAATCG -3'; Reverse primer: 5'- TGGGGATGTACAGGTTCATCTG -3') was normalized against the *Glyceraldehyde 3-phosphate dehydrogenase* (Forward primer: (5'- CCTGCACCACCAACTGCTTA -3'; Reverse primer: 5'- CGTTCAGCTCTGGGATGACC -3') housekeeping gene transcript abundance. Additional statistical analyses procedural details are available in sections 2.9 and 2.10 on page 46.

5.3.9 Statistical analysis

Pregnant fecal corticosterone levels comparisons were analyzed using a linear mixed model with prenatal predator odour exposure and gestational day as main factors and feces weight as a random factor. Within gestational day comparisons were performed using Bonferroni corrected Mann-Whitney Tests.

A linear mixed model with time, light phase or pre- and post- 3AM time as repeated measures and prenatal predator odour exposure and sex as main effects was used to compare the average oxygen consumption rate over 24 hours and activity level while correcting for random factors such as body weight, distance travelled and litter. A linear mixed model with time as a repeated measure and prenatal predator odour exposure and sex as a main effects was used to compare the average oxygen consumption rate during predator odour while correcting random factors (such as body weight, distance travelled, distance to the predator odour and litter). Hour-by-hour comparison were performed using Bonferroni corrected Mann-Whitney Tests.
Thyroxine level comparisons were analyzed using a linear mixed model with prenatal predator odour exposure, sex and presence of restraint stress as main factors and litter as a random factor.

Additional statistical analyses procedural details are available in section 2.12 on page 47.

5.4 Results

5.4.1 Fecal corticosterone metabolite levels during pregnancy

Fecal corticosterone metabolites did not show an overall predator odour exposure effect across the gestational days [$P > 0.05$]. However, the odour exposure treatment influenced the amount of fecal corticosterone metabolites compared to the baseline level [$F_{(1, 12)} = 7.425$, $P = 0.02$, $d_r = 2.46$] (Figure 14). There was no difference between the pregnant

females at baseline [gestational day 10; $P > 0.05$] but predator odour exposed females exhibited higher fecal metabolite corticosterone on the last day of exposure [Mann–Whitney $U = 7.40$, $n_C = 10$, $n_{PO} = 12$, $P = 0.05$ two-tailed, $d = 0.9$] (Figure 14).

5.4.2 Maternal behaviours in dams and offspring morphological measures

Predator odour exposed dams built lower quality nest compared to control dams [$F_{(1, 21)} = 8.9$, $P = 0.007$, $p\eta^2 = 0.30$] over postnatal days 1 to 6 (Figure 15A). Predator odour exposed dams also licked their offspring more frequently than control dams when controlling for the effect of nest quality [$F_{(1, 132)} = 5.481$, $P = 0.02$, $d_r = 1.0$] (Figure 15B**)**, decreasing in all dams over time [$F_{(6, 152)} = 4.736$, $P = 0.001$, $d_r = 5.6$]. There were no differences in the nursing behaviour [$P > 0.05$] (Figure 15C), the maternal behavioural transition, length of pregnancy, pregnancy weight gain, litter size, offspring deaths up to weaning age or offspring sex ratios [$Ps > 0.05$]. Therefore, standard measures (licking) of maternal care and others that are not typically reported (nest quality) were affected by exposure to unpredictable predator odour during the second half of pregnancy.

From birth to postnatal day 21, there was no effect of prenatal predator odour exposure on offspring body weight [$P > 0.05$] while the body weight of the pups increased with time [$F_{(3, 60)} = 602.887$, $P < 0.0001$, $d_r = 7.5$] when correcting for litter size. From birth to adulthood (postnatal day 114), males were heavier than females [$F_{(1, 706)} = 626.669$, $P < 0.0001$, $d_r = -1.8$] and, as expected, body weight increased with time [$F_{(16, 706)} = 999.012$, $P < 0.0001$, $d_r = 5.1$]. There was a significant interaction between prenatal predator odour exposure and sex [$F_{(1, 706)} = 9.515$, $P = 0.002$, $d_r = 6.5$] when correcting for the number of pups in the litter. Males weight increased over time [$F_{(16, 342)} = 660.113$, $P < 0.0001$, $d_r = 5.0$] and prenatally predator odour exposed males weighed less than control males [Prenatal predator odour exposure: $F_{(1, 343)} = 23.03$, $P < 0.0001$, $d_r = -0.8$] (Figure 16A). Females showed no prenatal predator odour exposure effect [$P > 0.05$] while body weight increased with time [$F_{(16, 331)} = 1016.791$, $P < 0.0001$, $d_r = 5.1$] (Figure 16C).

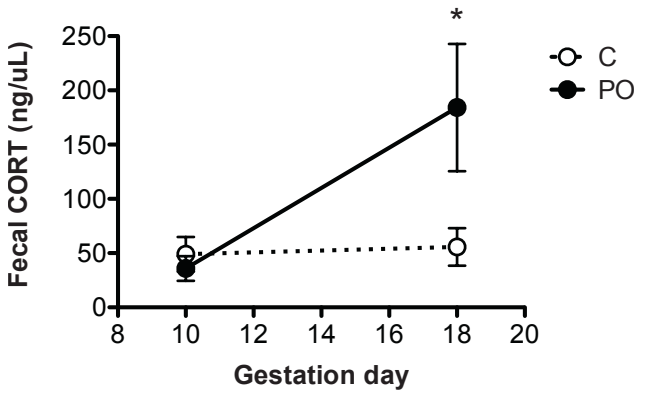


Figure 14. Predator-odour exposed (PO) dams show increased fecal corticosterone (CORT) metabolites after 8 days of predator odour exposure while levels are similar to control (C) pregnant females at baseline.

Data are average ± standard error of the mean. Bars: Predator odour exposure during pregnancy effect *$P \leq 0.05$.

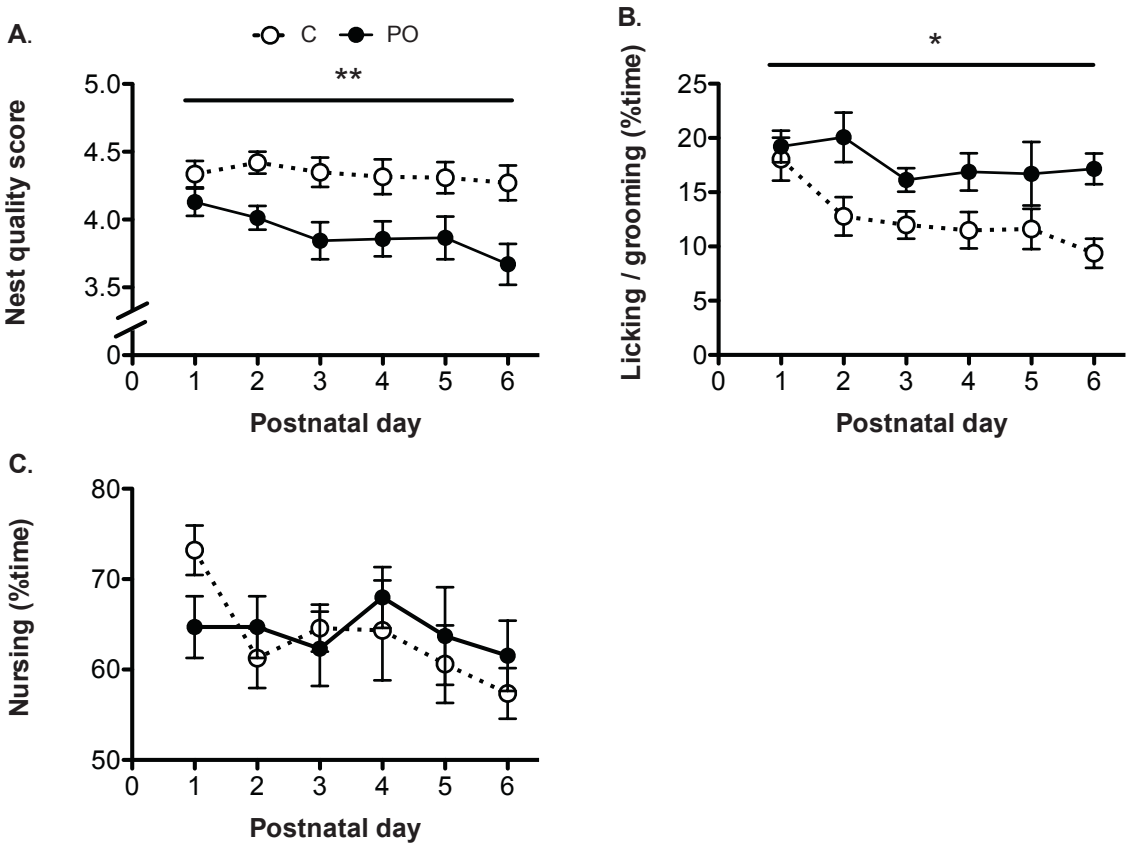


Figure 15. Dams exposed to predator odour during pregnancy (PO) show maternal behaviour alterations.

PO dams show decreased nest quality (**A.**) and increased licking-grooming when correcting for the nest quality (**B.**) compared to control (C) dams. PO dams do not show alterations in nursing behaviour (**C.**). Data are average ± standard error of the mean. Bars: Predator odour exposure during pregnancy effect *$P \leq 0.05$, **$P \leq 0.01$.

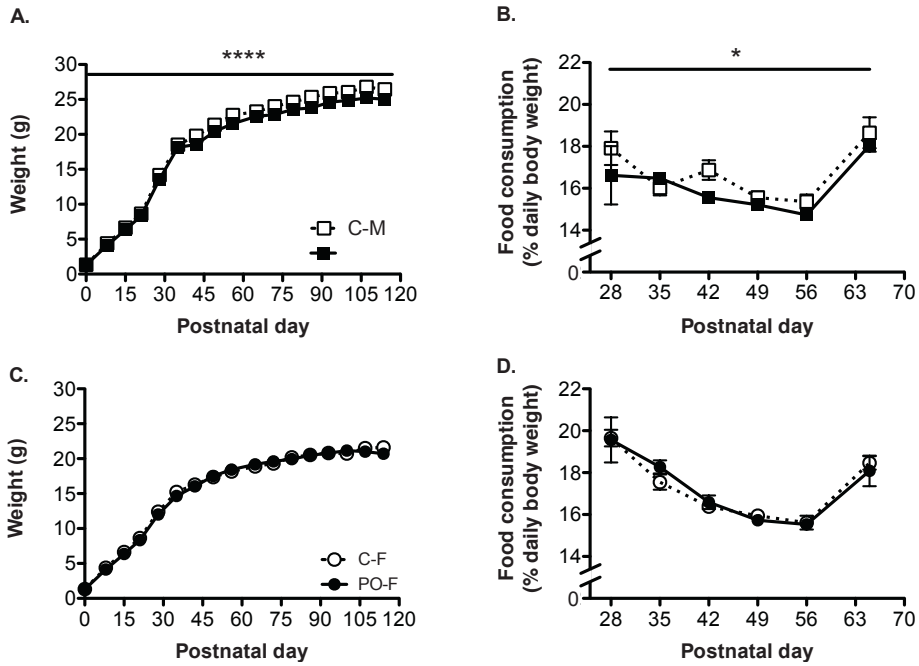


Figure 16. Prenatal predator-odour exposure affects male weight and food consumption.

Prenatally predator-odour exposed (PO) male (M) offspring show decreased body weight (**A**) compared to control (C) male offspring. There was no difference between PO and C female (F) offspring weight (**B**). Food consumption was decreased overall in PO males when compared to control males (**C**) while PO females did not differ from controls (**D**). Data are average ± standard error of the mean. Bars: Predator odour exposure during pregnancy effect *$P \leq 0.05$, ****$P \leq 0.0001$.

Prenatal predator odour exposure did not affect the age of female sexual maturation [$P > 0.05$].

Offspring presented no prenatal predator odour exposure effect on food consumption [$P > 0.05$] with females generally consuming a greater percentage of their body weight per day than males [$F_{(1, 38)} = 11.87$, $P = 0.001$, $d_r = 0.5$; Figure 16C]. Food consumption as a proportion of body weight decreased up to postnatal day 56 with a subsequent increase on postnatal day 65, forming an inverted U-shape curve. Male PO offspring showed a reduction in their food consumption as juveniles up to early adulthood compared to control males [Prenatal predator odor exposure: $F_{(1, 112)} = 5.472$, $P = 0.02$, $d_r = -0.5$] (Figure 16B) with males generally showing the described inverted U-shape curve of food consumption over time [$F_{(5, 112)} = 7.429$, $P < 0.0001$, $d_r = 7.7$]. Similarly, females offspring showed a similar inverted U-shape curve of food consumption over time [$F_{(5, 116)} = 24.604$, $P < 0.0001$, $d_r = 4.7$] (Figure 16D), but no PO exposure effect [$P > 0.05$].

5.4.3 Metabolic rate over 24 hours in adult offspring

Average oxygen consumption rate over 24 hours showed no prenatal treatment influence [$P > 0.05$], while varying with time of day when correcting for body weight and activity level [Time effect: $F_{(23, 1004)} = 25.916$, $P < 0.0001$, $d_r = 5.8$]. Prenatal treatment however influenced the oxygen consumption rate throughout the circadian cycle [Prenatal treatment x time interaction: $F_{(23, 1004)} = 2.132$, $P = 0.001$, $d_r = 4.5$] (**Figure 17** A). Prenatally predator odour exposed mice showed a different average oxygen consumption rate at 1PM, 3PM, 10PM, 3AM and 10AM [$P \leq 0.002$]. Right at the start of the trial (1PM) and from 3AM to 1PM on the following day, prenatally predator odour exposed mice showed a higher average oxygen consumption rate while between 3PM and 1AM,

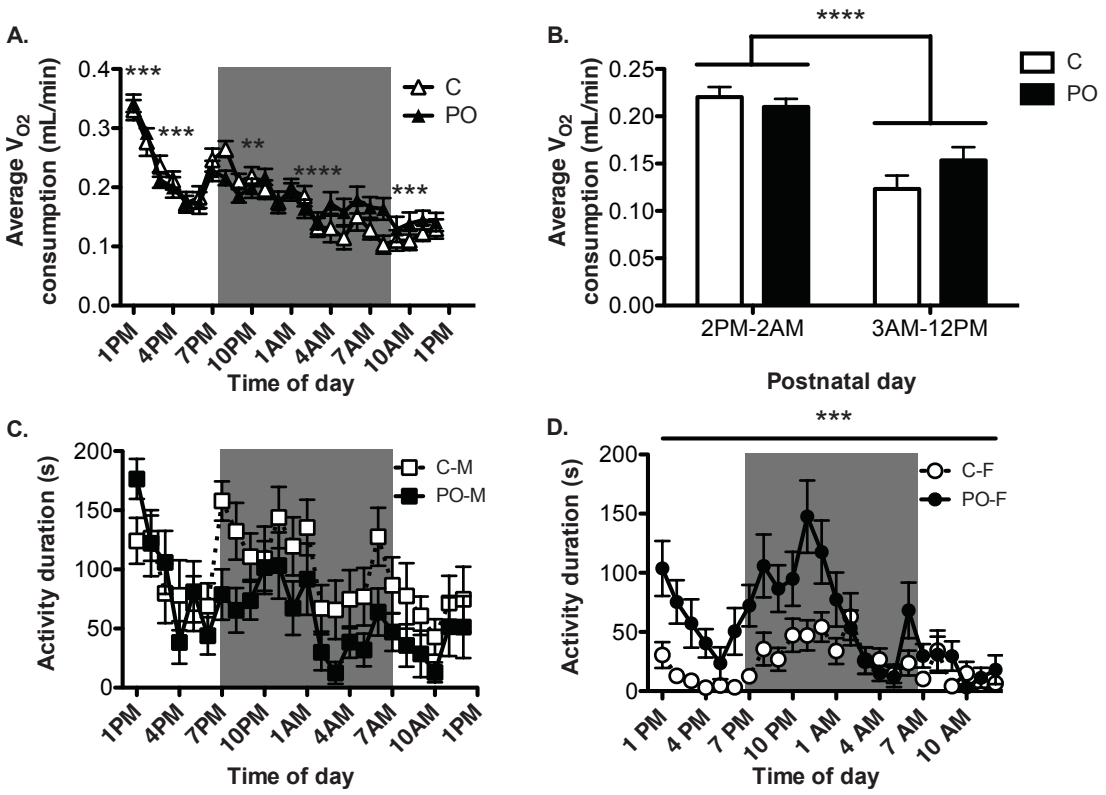


Figure 17. **Prenatally predator-odour exposed (PO) adult offspring show altered average oxygen consumption rate ($\dot{V}_{O_2}$) over 24 hours.**

PO adult offspring show differential average $\dot{V}_{O_2}$ during 24 hours compared to control (C) mice (**A.**). There is also a general decrease in average $\dot{V}_{O_2}$ after 3 AM in all animals (**B.**). Males (M) show activity level variation over 24 hours (**C.**) while PO females (F) display increased overall activity (**D.**) when compared to control females. Data are average ± standard error of the mean. Shade: dark (active) phase of the light-dark cycle. Connected bar: time effect $^{****}P \leq 0.0001$; Straight bar: prenatal predator odour exposure main effect $^{***}P \leq 0.001$; prenatal predator odour exposure effect $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$.

prenatally predator odour exposed mice show a decreased average oxygen consumption rate.

The lowest energetic needs in rats and mice, as indicated by the minimal levels of corticosterone, activity and metabolic markers, is observed daily approximately between 3AM and 1PM of the dark cycle (Kohsaka et al., 2007; Malisch et al., 2008; Spiga et al., 2014). Accordingly, there was an effect of the time, when comparing the oxygen consumption rate between 2PM and 2AM to the oxygen consumption rate between 3AM to 12AM [Half day effect: $F_{(2, 1051)} = 204.494$, $P < 0.0001$, $dr = -0.8$] along with a prenatal predator odour exposure on the oxygen consumption rate before or after 3AM [Half day x prenatal predator odour exposure effect: $F_{(2, 1051)} = 14.87$, $P < 0.0001$, $d_r = 6.8$] (**Figure 17** B). This result indicated that prenatal predator odour exposure affected the daily oxygen consumption rate. The oxygen consumption rate of prenatally predator odour exposed mice was exaggerated during the circadian period when the energetic necessity was the lowest (3AM-1PM) while being punctually reduced during the normally most energetically costly period of the day (2PM-2AM) when compared to control mice. This effect could be summarized as a flattening of the oxygen consumption rate circadian rhythm in the prenatally predator odour exposed animals.

The activity level, when corrected for body weight, varied across the circadian cycle [Time effect: $F_{(23,1008)} = 8.902$, $P < 0.0001$, $d_r = 5.7$], and was higher overall in males when compared to females [Sex effect: $F_{(1, 42)} = 30.838$, $P < 0.0001$, $d_r = -0.6$]. Activity was also influenced differently by prenatal predator odour exposure and sex [Prenatal predator odour exposure x sex effect: $F_{(1, 42)} = 13.988$, $P = 0.001$, $d_r = 2.8$]. Activity in mice of both sexes varied with the time of day [Day effect: Male $F_{(23, 518)} = 5.127$, $P < 0.0001$, $dr = 1.7$; Female $F_{(23, 444)} = 6.402$, $P > 0.0001$, $dr = 5.9$] (**Figure 17** C-D).

Additionally, prenatally predator odour exposed females showed increased activity compared to control females [Prenatal predator odour exposure effect: $F_{(1,20)} = 15.759$, $P = 0.001$, $dr = 0.7$] and prenatal predator odour exposure influenced time of day

differences [Prenatal predator odour exposure effect x time effect: $F_{(23, 444)}$ = 2.147, P = 0.002, d_r = 13.7] (**Figure 17** D).

The quality of the nest built was negatively correlated with the average activity level over the same period [*Pearson Correlation Coefficient* = -0.36, n = 36, P = 0.03]. Similarly, and in females only, the overall activity was significantly negatively correlated with the average nest quality over the first six postnatal days [*Pearson Correlation Coefficient* = -0.741, n = 16, P = 0.001]. However, there was no difference in the quality of the nest built over the 24 hours of the oxygen consumption rate measurements [$P > 0.05$].

5.4.4 Metabolic rate during predator odour exposure in adult offspring

Average oxygen consumption rate measured over a two-hour predator odour exposure varied with time [$F_{(16, 363)}$ = 16.633, $P < 0.0001$, d_r = 6.6] and according to the prenatal treatment [Prenatal predator odour exposure effect: $F_{(1, 366)}$ = 7.308, P = 0.007, d_r = 2.0] while correcting for body weight, activity and distance to predator odour (Figure 18A-B). When we excluded the first average oxygen consumption rate measure, elevated due to the initial stress of being in the metabolic chamber (Careau et al., 2011), the prenatal predator odour exposure influence was still present [$F_{(1, 334)}$ = 6.959, P = 0.009, d_r = 2.0] while varying over time [$F_{(15, 347)}$ = 8.54, $P < 0.0001$, d_r = 5.7].

The distance travelled in the apparatus varied over time [$F_{(16, 621)}$ = 9.501, $P < 0.0001$, d_r = 5.8] when correcting for the body weight of the mice (Figure 18C). The distance to predator odour varied with time [$F_{(16, 621)}$ = 15.426, $P < 0.0001$, d_r = 6.0] and between sexes [$F_{(1, 37)}$ = 5,541, P = 0.02, d_r = 0.5] **(**Figure 18D-E) with females staying further away from the predator odour.

There was a significant negative correlation between the average oxygen consumption rate during predator odour exposure and the nest quality over the first postnatal week [*Pearson Correlation Coefficient* = -0.42, n = 25, P = 0.04].

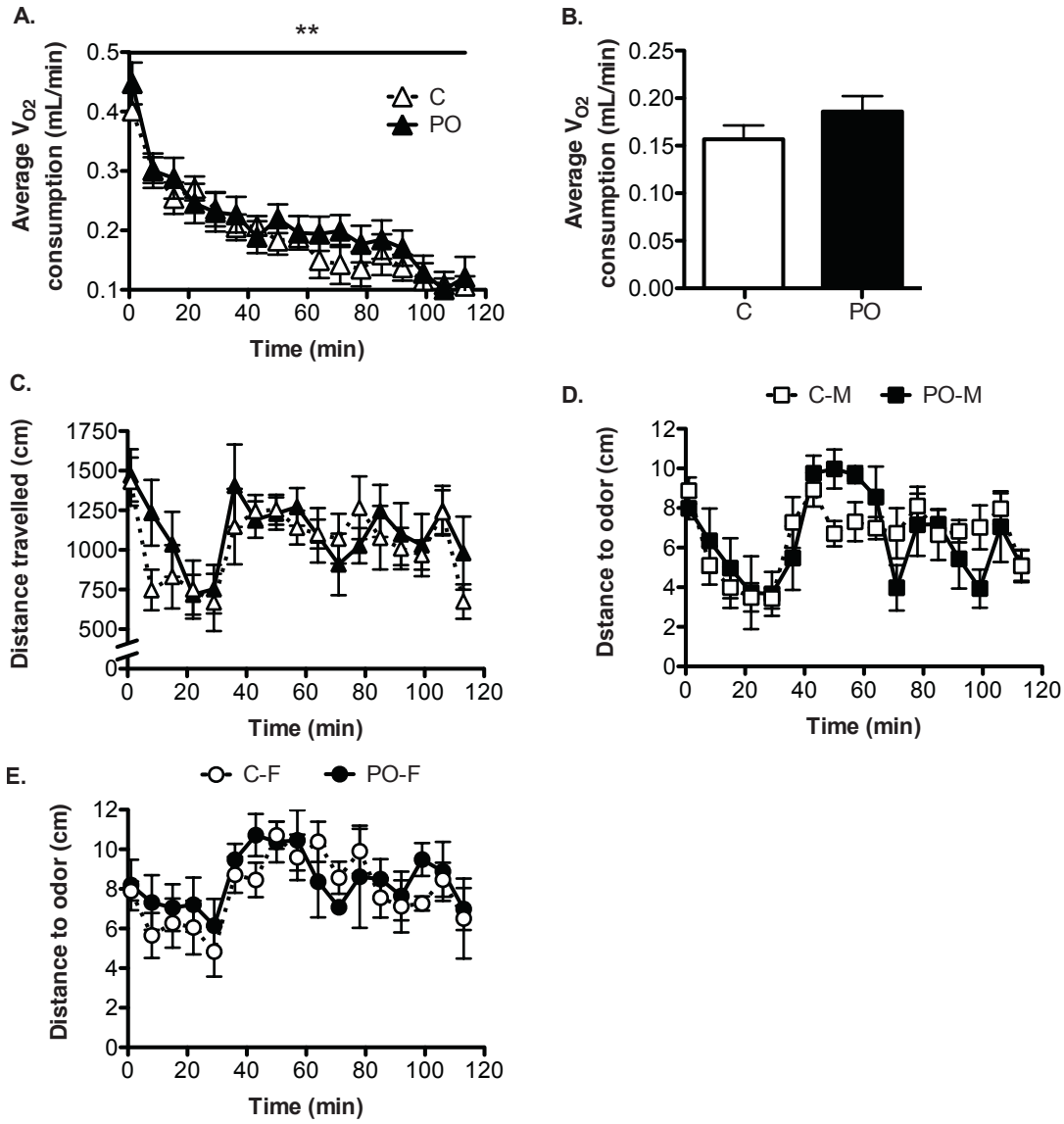


Figure 18. **Prenatal predator odour exposure (PO) increases the average oxygen consumption rate ($\dot{V}_{O_2}$) during exposure to predator odour.**

PO mice display a greater average $\dot{V}_{O_2}$ consumption compared to control (C) mice and decreases with time in both groups (**A.-B.**). Distance travelled varied over time in control and PO offspring (**C.**). The distance of animal to the predator odour varied with time in male (M; **D.**) and female (F; **E.**) offspring. Data are average ± standard error of the mean. Straight line: Prenatal predator odour effect $^{**}P < 0.01$

5.4.5 Circulating levels of thyroxine in adult offspring at baseline and in response to restraint stress

Prenatally predator odour exposed mice exhibit an increased thyroxine level compared to control animals [$F_{(1, 11)}$ = 6.313, P = 0.02, d_r = 0.5] (**Figure 19** A), with an impact of the restraint stress versus the baseline level [Restraint effect: $F_{(1, 28)}$ = 16.088, P < 0.0001, d_r = 0.5]. Females also had higher level than males [$F_{(1, 15)}$ = 8.740, P = 0.004, d_r = 1.1] (**Figure 19** B). At baseline, the thyroxine circulating level was not influenced by prenatal predator odour exposure [Ps > 0.05] (**Figure 19** A), while females had higher level than males [$F_{(1, 37)}$ = 7.882, P = 0.008, $p\eta^2$ = 0.19] (**Figure 19** B). After a restraint stress, however, prenatally predator odour exposed mice exhibited a higher thyroxine circulating level [$F_{(1, 35)}$ = 6.182, P = 0.02, $p\eta^2$ = 0.16] (**Figure 19** A). The basal serum thyroxine level was positively correlated with the amount of licking-grooming provided during the first postnatal week [*Pearson Correlation Coefficient* = -0.49, n = 32, P = 0.004]. The basal serum thyroxine level was also negatively correlated with the 24 hour activity level [*Pearson Correlation Coefficient* = -0.39, n = 31, P = 0.03].

5.4.6 Liver *corticosterone-binding globulin* transcript abundance in adult offspring

There was no difference in the *corticosterone-binding globulin* transcript abundance between the prenatal treatments [P > 0.05]. However, females showed a higher transcript abundance of liver *corticosterone-binding globulin* compared to males [$F_{(1, 19)}$ = 6.049, P = 0.02, d_r = 1.1] (Figure 20A).

There was also a positive correlation between the *corticosterone-binding globulin* liver transcript abundance level and the thyroxine level under restraint [*Pearson Correlation Coefficient* = 0.54, n = 19, P = 0.01] (Figure 20B).

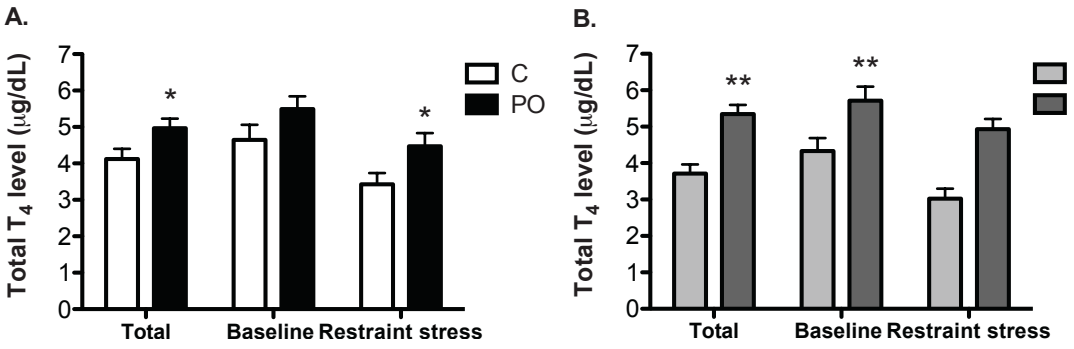


Figure 19. Prenatal predator odour exposure (PO) animals exhibit elevated thyroxine (T_4) levels, especially following a restraint stress.

PO mice exhibited higher T_4 level overall and after restraint compared to control (C) mice (**A.**). Females (F) had higher circulating T_4 level overall and at baseline compared to males (M) (**B.**). Data are average ± standard error of the mean. Main prenatal predator odour exposure effect $*P \leq 0.05$; within restraint prenatal predator odour exposure effect $*P \leq 0.05$. Sex effect $**P \leq 0.01$.

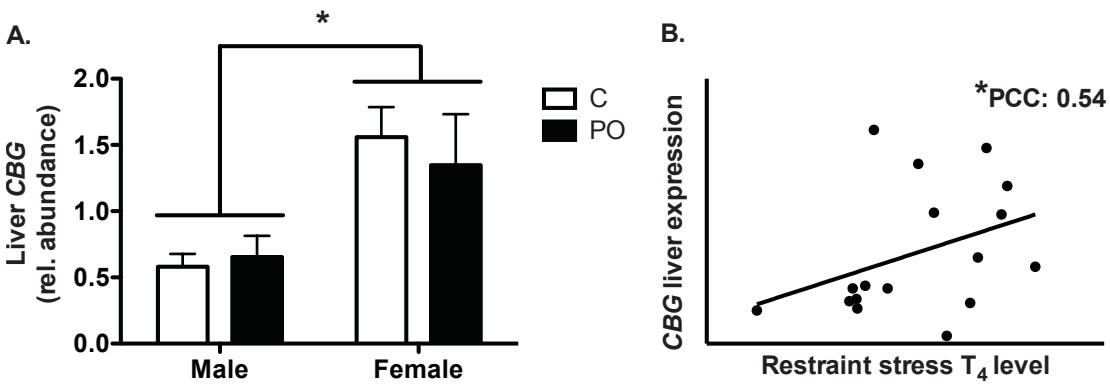


Figure 20. **Baseline *corticosterone-binding globulin* (*CBG*) transcript abundance is not affected by prenatal predator odour exposure (PO).**

Females showed higher levels of *CBG* than males (**A.**). *CBG* liver transcript abundance was positively correlated with thyroxine (T_4) levels after a restraint stress (**B.**). C: Control. Data are average ± standard error of the mean. Connected bar: Sex effect, $*P < 0.05$; Pearson's correlation coefficient: $*P < 0.05$.

5.5 Discussion

5.5.1 Maternal behaviour and offspring body weight

Predator odour exposure did not impact the amount of dam's maternal nursing, total time spent on the nest or the amount of behavioural transition, a measure of behavioural fragmentation during the first postnatal week. However, the classical maternal licking and grooming behaviour was increased in predator odour exposed dams during the first postnatal week, similarly to dams exposed to predator or predator odour during the first postnatal week (Coutellier and Würbel, 2009; Mashoodh et al., 2009; McLeod et al., 2007). Interestingly, pup licking and grooming as well as handling produce a modest but significant decrease in pup body temperature throughout the pre-weaning period (Sullivan et al., 1988). Further, pregnant dams exposed to predator odour built less intricate nests than control dams over the first postnatal week. A higher quality nest increases insulation and decreases radiated heat and the litter oxygen consumption by as much as 25%, as nesting is a thermoregulatory behaviour (Bryant and Hails, 1975; Gaskill et al., 2013). Pups are poikilothermic until the third postnatal week, and are therefore susceptible to temperature variations during that time (Harshaw and Alberts, 2012) and males are more susceptible to die from low temperatures during that interval (Berry and Bronson, 1992). Mice provided with nesting material under cold stress for four weeks show a reduction in food consumption and higher end weight compared to cold stressed mice in the absence of nesting material. These results indicate a reduction in energy used for thermogenesis in mice provided with nesting material (Gaskill et al., 2013). Furthermore, mothers building higher quality nests produce bigger litters with offspring surviving better and weighing more at 40 days of age (Bult and Lynch, 1997). Finally, in adulthood, a mouse line which was selected for high nest building abilities under low temperature showed greater basal metabolic rates, lowered food consumption and higher body temperature, indicating higher energetic efficiency when compared to control lines not selected for their nest building abilities (Berry and Bronson, 1992; Lacy et al., 1978).

Overall, these results suggest that pups exposed to predator odour prenatally are raised in a cooler environment with greater temperature variations during the first postnatal week, via the lower quality nest and increased licking and grooming received. These maternal behaviour changes could be sufficient to modify growth and increase thermoregulatory demands and their long-term metabolism, as seen in mice raised at lower temperatures (Bult and Lynch, 1997; Hart and Heroux, 1953; Héroux, 1970; Lacy et al., 1978). This difference could also explain the changes in growth as well as the increase in the hypothalamic-pituitary thyroid axis activity (through the thyroxine level), a regulator of the metabolic rate as observed in adults in response to a four week cold stress challenge (Gaskill et al., 2013). A positive correlation between licking-grooming behaviour and basal thyroxine level also suggests a potential role for maternal behaviour in the programming of the thyroxine level, though this remains to be examined.

Prenatally predator odour exposed males show a modest but significant decrease in weight from weaning to adulthood accompanied by a decrease in food consumption at least from weaning to early adulthood. Decreased body weight has been commonly observed following prenatal stress such as chronic variable stress (Mueller and Bale, 2006).

5.5.2 Offspring metabolic rate in adult offspring

Prenatal stress can affect the circadian rhythm of locomotor activity, the sleep-wake cycle and sleep fragmentation of pups (Maccari et al., 2003). The circadian rhythm can be permanently programmed through differential circadian corticosterone secretion in stressed pregnant females acting on foetal glucocorticoid receptor present in the suprachiasmatic nucleus during early development (Koehl et al., 1999, 1997; Reppert, 1983). Circadian changes following prenatal restraint and bright light consisted of an increase in corticosterone released throughout the day in females with an additional corticosterone secretion peek two hours before the dark period in rat offspring of both sexes (Koehl et al., 1999). On the contrary, chronic stress in adulthood leads to short-term modifications in sleep patterns only (Maccari et al., 2003).

Prenatally predator-odour exposed adult offspring showed a difference in their circadian oxygen consumption rate independently of their body weight. This difference was dependent on the time of the day, with prenatally predator odour exposed mice showing a differential metabolic rate, decreased between 1PM and 3AM and increased between 3AM and 12PM relative to controls. Interestingly, serum corticosterone, activity and metabolic markers exhibit their lower circadian level between 3AM and 1PM in mice and rats, following predictable lower energetic needs during that time (Kohsaka et al., 2007; Malisch et al., 2008; Spiga et al., 2014). Furthermore, thyrotropin-releasing hormone and thyroxine serum levels show a circadian cycle that is similar to the corticosterone cycle (Campos-Barros et al., 1997).

Regarding activity, while males showed no activity difference throughout the day, females from predator-exposed dams showed hyperactivity overall compared to control females. Furthermore, overall female activity during 24 hours was correlated with the dam's nest quality over the first postnatal week so that this activity level might be programmed. Increased activity is a common behavioural feature observed in response to prenatal stress (Burton et al., 2006; Koenig et al., 2005).

These differences show that the prenatal predator odour exposure treatment lead to alterations in the long-term metabolic rate of the offspring with an increase in energy being spent during the normally low energetic need period. As this difference was still apparent after controlling for locomotor activity, this difference in oxygen consumption rate was due to changes in heat production through the metabolic activity (Refinetti, 2003). These results are in line with deer mice and wild rats acclimated for several weeks to cold temperature (4 to 6 °C), enhancing their heat production capabilities (Hart and Heroux, 1953; Héroux, 1970). High energy consumption is also related to a negative trade-off with growth, as observed in prenatally predator odour exposed males. Furthermore, the standard metabolic rate is an indicator of the daily energy expenditure (Biro and Stamps, 2010) and environmental factors in the juvenile period such as temperature and diet can determine metabolic rate in adulthood. Finally, the resting metabolic rate has been correlated with behavioural traits (boldness), activity,

productivity (growth, foraging activity) and ultimately fitness in a wide range of animal species (Biro and Stamps, 2010; Kasumovic, 2013).

The difference in the metabolic rate observed here might be part of the programming to predator presence in the environment, allowing the animal to be in a constant ready state. In accordance with this observation, stickleback fish gravid females exposed to simulated predator attacks produced eggs that contained higher concentrations of corticosterone and oxygen consumption rate compared to control group (Giesing et al., 2011). Song sparrows exposed to corticosterone supplementation mimicking an acute stressor (such as frequent encounters with predators) during development and growth exhibit an increase in standard metabolic rate and diurnal metabolism, especially in females (Schmidt et al., 2012). In turn, increased metabolic rate leads to increased energy expenditure in the overnight low activity period (Spencer and Verhulst, 2008). Finally, increased foetal glucocorticoid exposure also leads to changes in liver metabolism (McMillen and Robinson, 2005), the organ responsible for energy mobilization for example through an increase in glucocorticoid receptor expression and in enzymes responsible for glucose metabolism. Interestingly, modifications in thyroid function is generally positively correlated with the rhythmicity of behavioural and biological circadian rhythms such as temperature, corticosterone and activity level (Campos-Barros et al., 1997).

However, contrary to their mother, there is no evidence for a difference on the nest quality that the mice built over the 24 hours of the metabolic rate measurement. Therefore, the nest does not appear to be responsible for the change in metabolic rate although it is correlated with the activity level of the mice. A future study could evaluate the nest quality built by mice from predator-exposed dams over a longer period, which was avoided here to prevent isolation of mice for a prolonged period.

We detected an overall increase in oxygen consumption rate when mice are exposed to predator odour. This result was expected as females from predator-odour exposed dams displayed a higher circulating corticosterone to predator odour while mice from predator-odour exposed dams of both sexes showed increased avoidance to predator odour when a

shelter was provided (St-Cyr and McGowan, 2015). This difference is not explained by the activity level or distance to the predator odour. Stress increases energy consumption and the mobilization of energy through the 'fight or flight' response. These results are also in accordance with the stress-induced hyperthermia detected in reaction to a wide range of stressors in parallel to energy mobilization and heart rate increase (Bouwknecht et al., 2007; Campeau et al., 2008; Chabot et al., 1996; Rorick-Kehn et al., 2005). Finally, females also show a correlation between their average circadian oxygen consumption rate and the average oxygen consumption rate under stress.

5.5.3 Circulating levels of thyroxine in adult offspring

The hypothalamic-pituitary thyroid axis consists of the hypothalamus releasing thyrotropin-releasing hormone that reaches the pituitary through portal circulation. The pituitary in turn releases thyroid-stimulating hormone into general circulation that eventually reaches the thyroid gland and induces the release of thyroid hormones (thyroxine and triiodothyronine). The hypothalamic-pituitary adrenal axis is under negative feedback at several levels including through the thyroid-stimulating hormone and, to a lesser extent, thyroid hormones (Zoeller et al., 2007). Circulating thyroxine is carried in the blood bound at 75% to the thyroxine-binding globulin synthesized in the liver and to transthyretin and albumin (15%) (Zoeller et al., 2007).

As rodent pups are poikilotherms until the third postnatal week, thermoregulation is under maternal influence and plays a role in the development of the thyroid gland (Harshaw and Alberts, 2012; Samel, 1968). Overall, nest quality differences over the first postnatal week are an important factor responsible for the phenotypic difference observed in prenatally predator odour exposed offspring.

Thyrotropin-releasing hormone and thyroid hormones are master regulators of feeding behaviour, thermogenesis and locomotor activation (Lechan and Fekete, 2006) through weight loss and increase in core body temperature, activity and energy expenditure (Lechan and Fekete, 2006). A thyroid hormone-mediated energy expenditure increase

occurs through an increase in oxygen consumption (up to 35% when compared to baseline), as early as during the first postnatal week (Haidmayer and Hagmüller, 1981; Tirri et al., 1968). In accordance with this, adult prenatally predator odour exposed males were lighter, and both sexes altered their oxygen consumption rate throughout the day as well as during predator odour exposure.

There is also a cross-talk between the hypothalamic-pituitary thyroid and the hypothalamic-pituitary adrenal axis. Increases in the thyroxine level induce an increase in corticosterone-releasing factor, corticosterone and corticosterone-binding globulin levels in early postnatal life as well as in adulthood (D'Agostino and Henning, 1981; Dakine et al., 2000; Helmreich et al., 2005; Sanchez-Franco et al., 1989). Basal level of circulating thyroid hormone is correlated with the spontaneous exploratory behaviour in the open field (Helmreich and Tylee, 2011). On the contrary, stress decrease the hypothalamic-pituitary thyroid activity and the thyrotropin-releasing hormone, thyroid-stimulating hormone, transthyretin and thyroid hormone levels, as observed for example during the recovery from immobilization stress (Helmreich and Tylee, 2011). In accordance with those results, mice from predator odour-exposed dams showed an overall increase in total thyroxine level, especially following stress, compared to control mice.

Thyroid hormones are necessary for normal development during the late embryogenic period (embryonic day 17-18; Williams, 2008) while the thyroid-stimulating hormone increases immediately after birth (Yun et al., 1982). Thyroid hormones levels are negatively correlated with the intracellular glucocorticoid receptor concentration in the hippocampus during development (Meaney et al., 1987). Maternal behaviour such as increase licking-grooming behaviour and neonatal administration of thyroxine during the first two postnatal weeks leads to elevated glucocorticoid receptor, basal corticosterone and corticosterone-binding globulin levels (Hellstrom et al., 2012; Meaney et al., 1987). Thyroxine supplementation leads to an increase in the *corticosterone-binding globulin* transcript abundance and binding capacity in the general circulation (D'Agostino and Henning, 1981; Dakine et al., 2000; Helmreich et al., 2005; Koehl et al., 1999; Sanchez-Franco et al., 1989). This likely happens through the action of thyroid hormones on the

liver during the first two postnatal weeks (D'Agostino and Henning, 1981). Although we do not find differences in baseline thyroxine serum levels, there is a positive correlation between the thyroxine level under restraint and the *corticosterone-binding globulin* transcript abundance at baseline. As *corticosterone-binding globulin* is the delivery molecule of corticosterone and maintains a reservoir of readily releasable glucocorticoids (Perogamvros et al., 2012), it would be interesting in a follow-up study to measure the corticosterone-binding globulin transcript abundance and serum level under restraint stress to evaluate its possible implication in the stress reactivity of mice from predator-odour exposed dams.

Transcription levels of key players of the hypothalamic-pituitary thyroid axis should be assessed to evaluate the effect of prenatal predator odour exposure on the hypothalamic-pituitary thyroid axis. As the thyrotropin-releasing hormone, thyroxine-binding globulin and transthyretin determine the plasmatic amount of thyroxine and its availability, future steps will include measuring the thyrotropin-releasing hormone transcript abundance level within the paraventricular nucleus of the hypothalamus and the liver thyroxine-binding globulin and transthyretin transcript abundance. Measuring these transcript abundance levels will help to model the hypothalamic-pituitary thyroid axis functioning following prenatal predator odour. Transthyretin is a good target for early life programming since its transcript abundance is modulated with stress (Kohda et al., 2005; Wakasugi et al., 1986) and has been linked to differences in predation stress (Lavergne et al., 2014).

5.5.4 Conclusion

In conclusion, both prenatal predator odour exposure and likely the associated modifications triggered in maternal behaviour affect the offspring. Consequently, the adult offspring show fundamental differences in energy consumption throughout the circadian cycle. Males showed stronger physiological modifications through a decrease in body weight. Finally, thyroid hormone levels are increased in prenatally predator odour exposed mice of both sexes. Thyroid hormones might therefore be part of the

programming of prenatal predator odour exposure leading to the increase in the female predator odour stress response and both sexes stress-related behaviours. Therefore, a solely psychogenic stressor that is ecologically relevant is sufficient to modify the integrative adult offspring phenotype.

Chapter 6 Stress-related behaviour to anxiogenic naturalistic, laboratory commonly-used and social situations in prenatally predator odour exposed mice

6.1 Publication

Chapter 6 is adapted from the following research article manuscript:
St-Cyr, Sophie, Abuaish, Sameera, McGowan, Patrick O. Prenatal predator odour exposure programs an increase in stress-related behaviour to naturalistic, laboratory commonly-used and social tests in adult offspring. *In preparation*.

6.2 Approach and hypotheses

Animals exposed to predator or predator cues decreased exploratory behaviour, activity and social interactions in the animals. Boldness (opposite to cautiousness or shyness), exploration and activity are inter-correlated behaviours that are reproducible over time over a range of situations, consistent with the notion of temperament or behavioural type. Temperament consists of fixed inter-correlated traits over time (or behavioural type if not tested over time) and determined by the life history of the individual (Réale et al., 2007). One of those life history traits is predation pressure.

The objective of this study is to evaluate whether prenatal predator odour exposure stress could program the offspring behavioural type encompassing less exploratory, active and social behaviour. Therefore, the first aim is to evaluate the impact of prenatal predator odour exposure on the adult offspring stress-related behaviour in 1) stressful exploratory-based commonly-used laboratory tests, 2) stressful naturalistic situations as well as 3) social investigation, recognition and interaction.

We hypothesize that prenatal predator odour exposure will lead to adult offspring showing a behavioural type with decreased boldness, exploration and activity in a wide range of stressful commonly-used laboratory, naturalistic and social situations. More specifically, we hypothesize that prenatal predator odour exposure will lead to an increase

in stress-related behaviour in a range of exploration-based laboratory commonly-used and naturalistic stressors while decreasing social investigation, recognition and interaction.

6.3 Material and methods

6.3.1 Mice breeding

For breeding, two normally cycling females were housed with one male overnight. Pregnant females were singly housed on gestational day 10 and weighed every day throughout the pregnancy. Twenty-four of the 41 mated females (58,5%) gave birth to 12 control litters and 12 prenatally predator odour exposed litters. This breeding success rate can be explained by the fact that the female's oestrous phase was not checked prior to breeding. Additional procedural details are available in sections 2.1 and 2.2 on page 42.

6.3.2 Adult offspring sample sizes

An subset of male and female adult offspring (1-2/sex/litter) was used to examine social and stress-related behaviour in the following order: social recognition test (control male sample size = 7, control female sample size = 7, prenatally predator-odour exposed male sample size = 13, prenatally predator-odour exposed female sample size = 14), restraint stress followed by the elevated-plus maze (control male sample size = 14, control female sample size = 11, prenatally predator-odour exposed male sample size = 13, prenatally predator-odour exposed female sample size = 10), mouse defense test battery (control male sample size = 13, control female sample size = 11, prenatally predator-odour exposed male sample size = 11, prenatally predator-odour exposed female sample size = 10) and foraging task (control male sample size = 13, control female sample size = 11, prenatally predator-odour exposed male sample size = 12, prenatally predator-odour exposed female sample size = 10). An independent cohort of mice was submitted to a set of social and anxiety-like behaviours tests in the following order: elevated-plus maze (control male sample size = 16, control female sample size = 14, prenatally predator-

odour exposed male sample size = 20, prenatally predator-odour exposed female sample size = 20), social choice test (control male sample size = 7, control female sample size = 7, prenatally predator-odour exposed male sample size = 13, prenatally predator-odour exposed female sample size = 14) and social interaction test (pairs for control male sample size = 7, control female sample size = 5, prenatally predator-odour exposed male sample size = 8, prenatally predator-odour exposed female sample size = 10). Additional procedural details are available in section 2.4 to 2.7 on page 43 to 45.

6.3.3 Elevated-plus maze and elevated-plus maze plus restraint

The elevated-plus maze apparatus and general procedure is described in section 2.5 on page 43. Mice were then restrained in a transparent disposable bakery decorating bag (Wilton) for 15 minutes and left to recover and groom for 5 minutes in a clean cage before being submitted to the elevated-plus maze test.

6.3.4 Mouse defense test battery

The mouse defense test battery is based on Griebel and Beeské (2011). Briefly, the tested mouse was exposed to a rat puppet (Folkmanis, 30 cm long x 13 cm wide x 10 cm high) that was kept in a soiled male rat cage to smell like a rat. Mice were exposed to the rat puppet in an oval runway (40 cm wide x 30 cm high x 2 times 200 cm straight segments joined by two 40 cm curved segments separated by a median wall) **(**Figure 21). Activity was first recorded over a 3-minute pre-test familiarization period followed by a predator avoidance test where the rat was introduced at an end of the runway and brought up to the mouse at a 50 cm/seconds speed until the subject runs away or is brought into contact with the rat. The chase/flight test consisted of a 200 cm/seconds chase by the rat over 15 m in which the rat stayed at a distance of 20 cm. For the straight alley test, the subject was constrained between partitions placed in the runway 60 cm apart. The rat was introduced at one end of the alley for 30 seconds. The forced contact test consisted of three, one-second approach of the rat within the straight alley. Finally, a three-minute

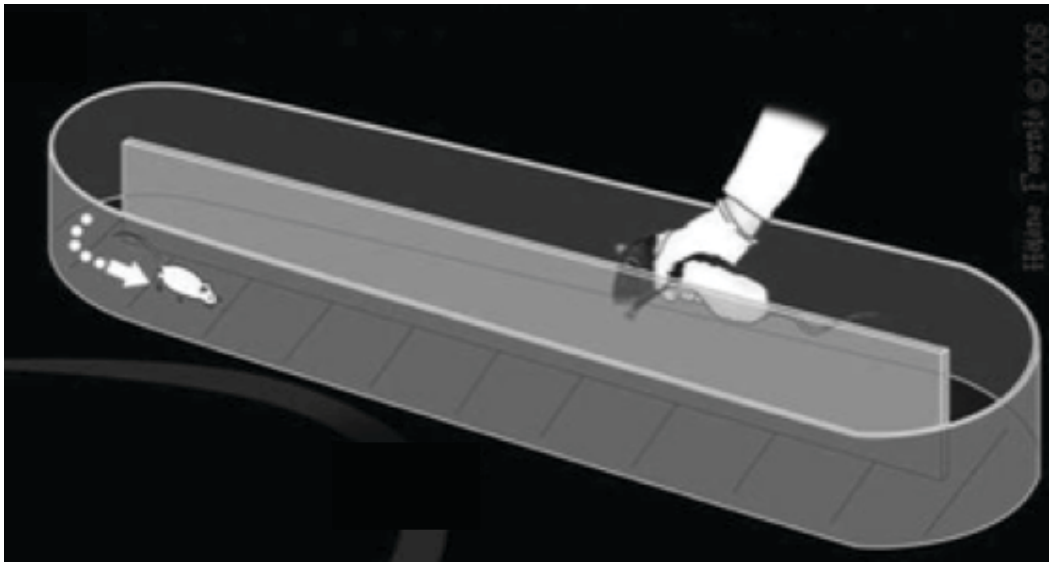


Figure 21. Mouse defense test battery apparatus.

Modified from Griebel and Beeské (2011).

post-test activity recording was made of the entire runway. Mouse position and activity level were measured continuously using EthoVision XT 10.

6.3.5 Mouse foraging test

Measuring the giving-up density in a depletable food patch is a measure of the quitting harvest rate, which is a way to titrate food to safety (Brown and Kotler, 2004). In natural animal populations, predator presence, cues of predator presence and foraging patch cover density determine the giving-up density. Here, we are testing the effect of rat odour, a predator of the mouse (Galef, 1970; Karli, 1956) which odour is aversive and increases the latency to consume a treat (Merali et al., 2003), on the giving-up density in a laboratory setting. This protocol is a modification from Troxell-Smith et al. (2016) and represents a high-throughput way to evaluate foraging behaviour in a laboratory setting. The rationale is that prenatally predator odour exposed mice especially should leave a higher giving-up density in risky open patches compared to concealed patches.

The foraging task consisted of a 45 cm long x 24 cm wide x 20.5 cm high transparent cages. The cages contained bedding, 4 foraging patches, a dish with water and two 10 cm high platforms with an accessing angled ramp. Two patches were exposed on the top of the platforms (patch 1 and 2) and two were covered by the platforms (patch 3 and 4) (Figure 22). The foraging patches (ramequins) contained a mix of 8 grams of sand and 3.5 grams of hulled sunflower seeds. Prior to testing, mice were exposed to hulled sunflower seeds for 4 consecutive days (habituation day 1-4). On the fifth day of habituation, mice were exposed to a small novel cage (15 cm x 33 cm) with a shallow foraging patch (1.5 cm deep) containing a high concentration of sunflower seeds and seeds on top from 9AM to 12PM. On the sixth day of habituation, the same protocol as day 5 was applied with the petri dish replaced by a ramequin. The habituation phases were used to ensure that the mice would consume the bait and learn to dig to access the food in the foraging patches. Mice were then housed overnight in the testing room for habituation and food-deprived for 17 hours prior to the test. Eight mice, blind to each other with white partitions, were tested at a time in 4 rows of 2 cages in between which

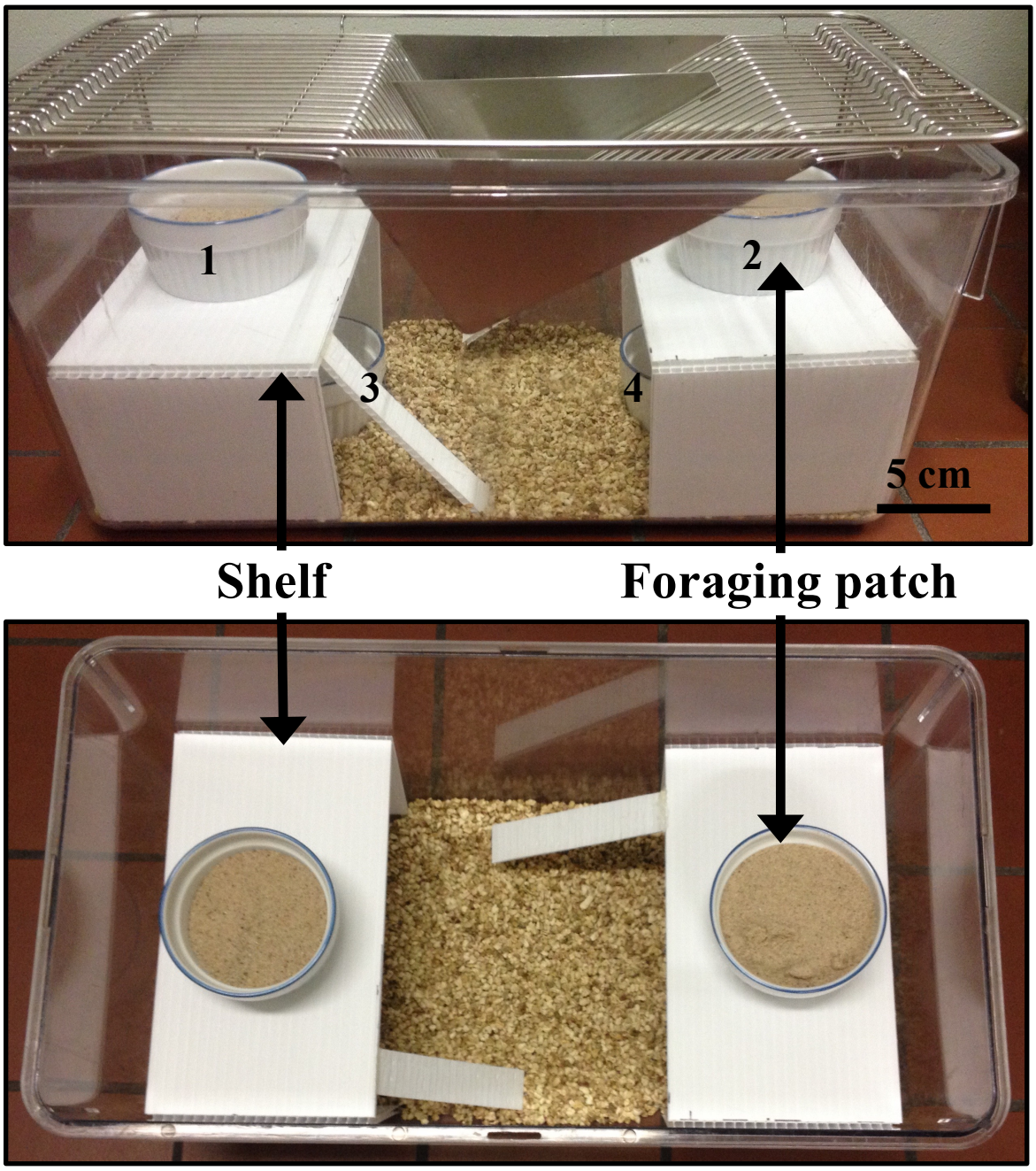


Figure 22. Mouse foraging test apparatus.

Modified from the protocol from Troxell-Smith et al. (2016). The ratio of giving-up densities (GUD; food left in a patch) from the top exposed patches to the bottom concealed patches is calculated as: (GUD patch 1 + GUD patch 2 / total initial food) / (GUD patch 3 + GUD patch 4 / total initial food).

soiled rat cages were placed. The test lasted 6 hours, from 9AM to 3PM. The giving-up density was measured for each patch following the test. The ratio of the giving-up density from the top exposed patches to the bottom concealed patches was calculated: (giving-up density patch 1 + giving-up density patch 2 / total initial food)/(giving-up density patch 3 + giving-up density patch 4 / total initial food).

6.3.6 Mouse social choice and social recognition test

The social choice test consisted of an arena divided into a center neutral zone between two choice zones (15 x 24 cm each), placed in a dimly lit room (33.7 lux). The choice zones contained a wire cup (diam. 8.5 cm) enclosing a familiar mouse (from the same home cage as the focal mouse) and an unfamiliar mouse (from a different litter and home cage than the focal mouse). The focal mouse was placed in the center of the arena and the frequency of entering and time spent in each choice zone were measured over 10 minutes.

The social recognition test consisted of four consecutive five-minute exposures to an unknown juvenile #1 followed by a five-minute exposure to an unknown juvenile #2, with inter-trial times of 10 minute on the first day. On day 2, juvenile #1 was presented once and, after a 10 minutes inter-trial interval, juvenile #3 was exposed for the first time. Each trial lasted five minutes. Three juvenile CD-1 mice with a maximum of 20% weight difference and aged between postnatal day 25 and 40 were presented as unknown conspecific models. An initial six-minutes baseline activity trial was performed on day 1 prior any unknown animal presentation. Measurements included time spent in the juvenile zone, difference in time spent in the juvenile zone across encounters and distance travelled during the activity trial and the sociality trials. In both tests, mice position and activity level were measured continuously using EthoVision XT 10.

6.3.7 Mouse social interaction test

The social interaction test took place in a Plexiglas square arena (38.5 cm x 38.5 cm). Two unfamiliar mice (different litter and home cage) from the same prenatal stress group,

sex and presenting minimal weight difference (≤ 5%) were put simultaneously in the arena for 15 minutes. The focal interactions were coded continuously using Observer XT 8.5 for 5 minutes based on Grant and Mackintosh (1963).

6.3.8 Statistical analysis

The elevated-plus maze plus restraint and mouse defense test battery tests were analyzed using a linear mixed model with prenatal treatment and sex as main effects and litter as a random factor. For the foraging test, the giving-up density ratios were compared using a Mann-Whitney Test using prenatal treatment as the main factor while the giving-up densities were compared using a linear mixed model with prenatal treatment, sex and patch as main effects and litter and body weight as random factors while the percentage of food eaten and replicate exposed and concealed patches giving-up densities were compared using Mann-Whitney Test using prenatal treatment as the main factor.

The social choice test ratio of time spent in the unknown compared to the known conspecific zone and distance travelled were compared using a linear mixed model with prenatal treatment and sex as main effects and random effects (e.g. litter and distance travelled). In the social recognition test, the time spent in the juvenile zone was corrected for the latency to enter the juvenile zone. The duration of time spent in the juvenile zone during the encounters was analyzed using a linear mixed model encounter as a repeated measure, prenatal treatment and sex as main effects and litter and distance travelled as random effects. The ratio of time spent in the juvenile zone on the first compared to the fifth encounter was analyzed using a Mann-Whitney Test with prenatal treatment as the main effect. In the social interaction test, behaviours were compared using Mann-Whitney Tests with prenatal treatment as the main factor except for the duration of total interactions, which was analyzed using a linear mixed model with prenatal treatment and sex as main effects and litter as a random effect.

Additional statistical analyses procedural details are available in section 2.12 on page 47.

6.4 Results

6.4.1 Elevated-plus maze and elevated-plus maze following 15 minutes of restraint

In the elevated-plus maze, prenatally predator odour exposed mice spent less time in the open arm of the elevated-plus maze than the control mice [$F_{(1, 64)}$ = 5.094, P = 0.03, d_r = -0.6] (**Figure 23**A) and males spent more time in the open arm than females [$F_{(1, 63)}$ = 5.055, P = 0.03, d_r = -1.0] while controlling for the distance travelled. Prenatally predator odour exposed mice also spent less time in the open arm relative to the closed arm compared to control mice [$F_{(1, 62)}$ = 6.458, P = 0.01, d_r = -0.7] (**Figure 23**B) while this ratio was greater in males than females [$F_{(1, 62)}$ = 18.241, P < 0.0001, d_r = -1.1]. The distance travelled was greater in prenatally predator odour exposed mice than in control mice [$F_{(1, 64)}$ = 5.094, P = 0.03, d_r = 0.7] (**Figure 23**C), with females travelling greater distance than males [$F_{(1, 63)}$ = 5.055, P = 0.03, d_r = 0.5] over the 15 minutes of the test.

In the elevated-plus maze plus restraint test, prenatally predator odour exposed mice showed decreased the number of visits to the open arm compared to control mice [$F_{(1, 46)}$ = 5.531, P = 0.01, d = -2,3] (**Figure 23**D) when controlling for the distance travelled. Prenatally predator odour exposed mice also exhibit decreased distance travelled over the 15 minutes of the test [$F_{(1, 42)}$ = 7.262, P = 0.01, d_r = -0.6]. Additionally, the prenatal treatment influenced the distance travelled in a sex-specific manner [Prenatal stress x sex interaction: $F_{(1, 42)}$ = 6.009, P = 0.02, d_r = 3.7] (**Figure 23**E). Within females, prenatally predator odour exposed mice suppressed their activity as measured by the distance travelled compared to control mice [$F_{(1, 19)}$ = 13.119, P = 0.002, d_r = -1.5] (**Figure 23**E) while males showed no difference [P > 0.05].

6.4.2 Open field test and light-dark transition test

In the open field, prenatally predator odour exposed mice spent more time in the center of the arena than control offspring [$F_{(1, 54)}$ = 9.084, P = 0.004] (Figure 24A) when controlled

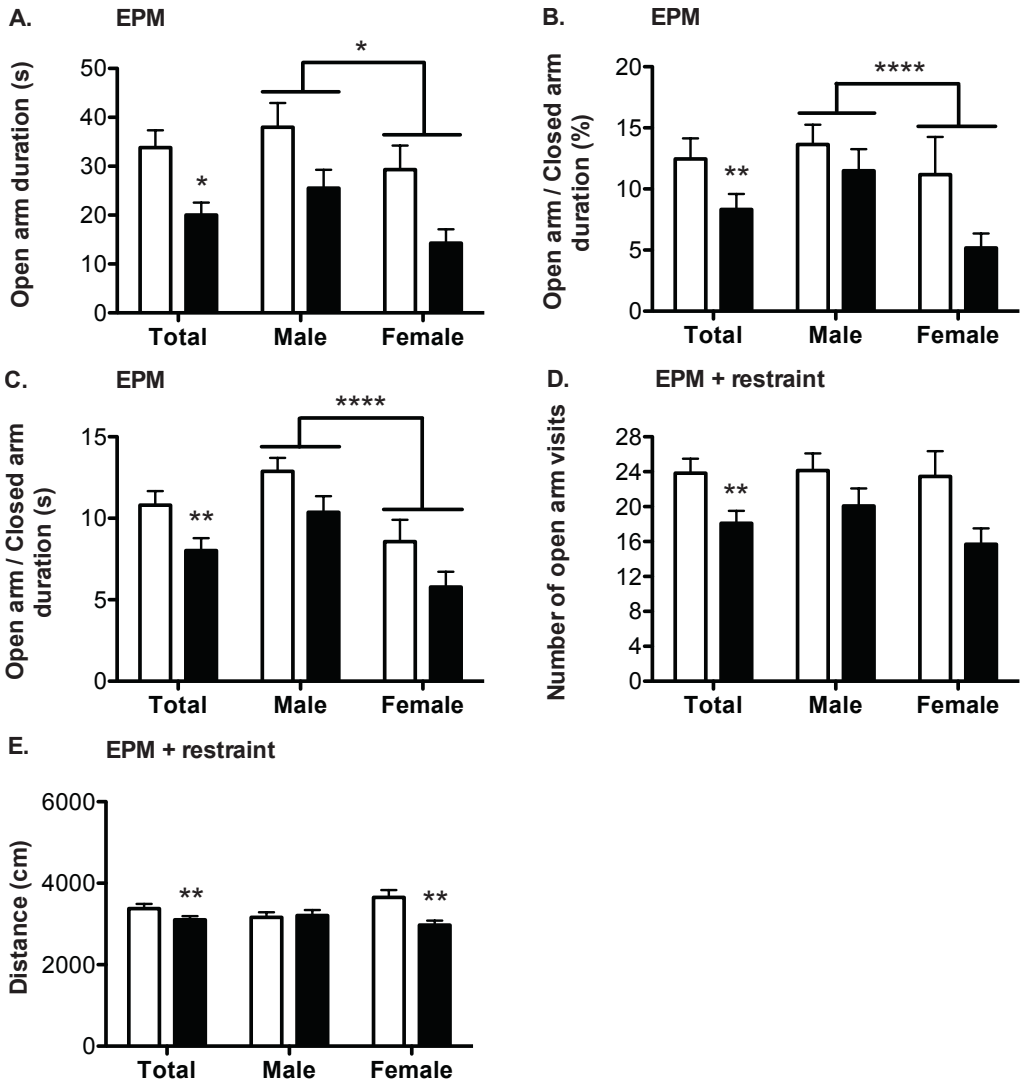


Figure 23. Prenatal predator odour exposure (PO) increases stress-related behaviour in the elevated-plus maze (EPM) and the EPM plus restraint tests in adulthood.

In the EPM, prenatally stressed offspring showed decreased time spent in the open arm (**A.**) and decreased their ratio of time spent in the open arm on the closed arm (**B.**) and increased their activity level (**C.**) compared to control (C) offspring. In the EPM plus restraint test, PO mice showed decreased visits in the open arms (**D.**) as well as general hypoactivity, especially PO females (**E.**). Data are average ± standard error of the mean. Connected bars: main sex effect $*P \leq 0.05$, $****P \leq 0.0001$; main prenatal predator odour effect and within sex prenatal predator odour effect $*P \leq 0.05$, $**P \leq 0.01$.

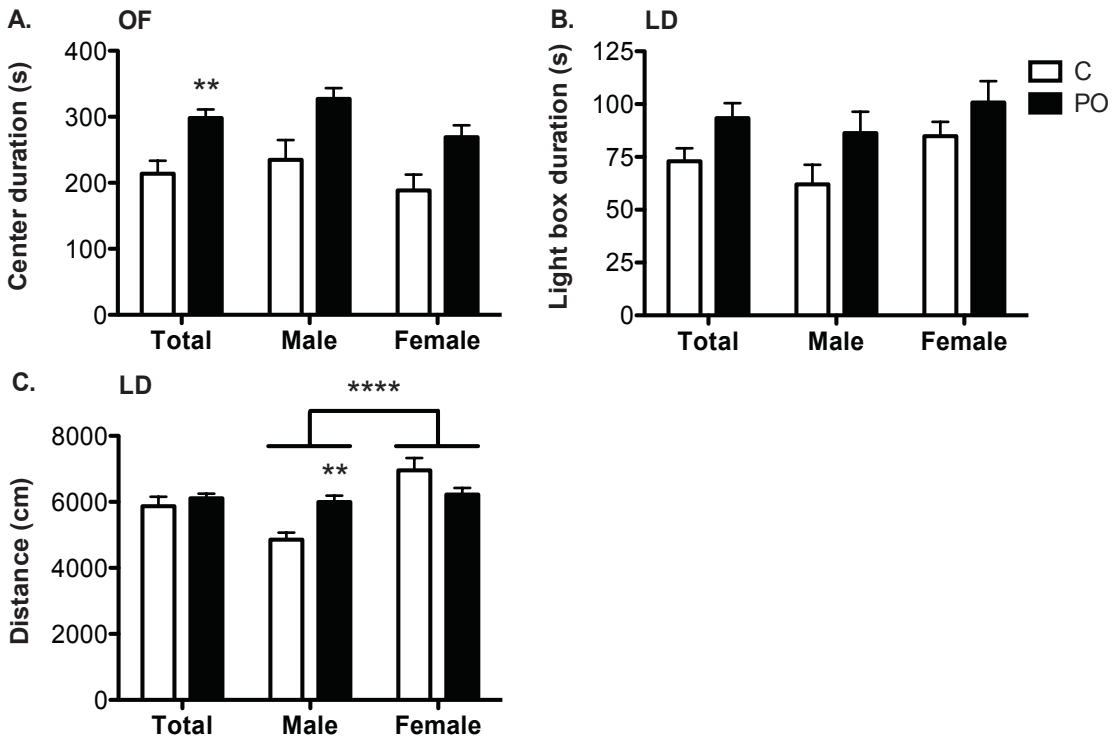


Figure 24. Prenatal predator odour exposure (PO) affects the stress-related behaviour and activity in the open field (OF) and light-dark transition task (LD) in adulthood.

In the OF, prenatally stressed offspring showed an increase in time spent in the center (**A.**) compared to control (C) offspring. In the LD, PO animals spent the same amount of time in the light aversive box as control offspring (**B.**) while males were hyperactive compared to control males (**C.**). Data are average ± standard error of the mean. Connected bars: main sex effect ****$P \leq 0.0001$; main prenatal predator odour effect and within male prenatal predator odour effect **$P \leq 0.01$.

for the distance travelled. Distance travelled did not differ between sex or prenatal treatment [$P > 0.05$].

In the light-dark transition test, prenatally predator odour exposed offspring spent the same amount of time in the aversive light portion of the arena than control offspring [$P > 0.05$] (Figure 24B). However, females generally travelled a greater distance then males [sex effect: $F_{(1, 69)} = 15.378$, $P < 0.0001$] (Figure 24C) while the prenatal treatment influencing the distance travelled in a sex-specific manner [Prenatal predator odour exposure x sex interaction: $F_{(1, 69)} = 7.701$, $P = 0.007$]. Further, prenatal predator odour exposed males were hyperactive compared to control males [$F_{(1, 35)} = 7.515$, $P = 0.01$] (Figure 24C). Females showed no difference in distance travelled [$P > 0.05$].

6.4.3 Mouse defense test battery

In the mouse defense test battery, the overall flight time from the rat puppet (time taken to run 15 meters) was shorter in prenatally predator odour exposed compared to control mice [$F_{(1,29)} = 4.552$, $P = 0.04$, $d_r = -0.9$] (**Figure 25**A). The associated frequency of stops to orient towards the rat showed a prenatally predator odour exposed impact at the sex level [Prenatal stress x sex interaction: $F_{(1,33)} = 4.48$, $P = 0.04$, $d_r = 3.6$]. Prenatally predator odour exposed males showed a decrease in the number of orientation stops [$F_{(1,19)} = 18.754$, $P = 0.02$, $d_r = -1.6$] (**Figure 25**B) when compared to control males while females showed no such difference [$P > 0.05$]. There were no other differences in the predator avoidance test, straight alley test or forced contact test [$Ps > 0.05$].

Between the initial habituation and final activity trial, mice exhibited an overall increase in the frequency of rearing [$F_{(1,43)} = 218.709$, $P < 0.0001$, $d_r = 2.9$] (**Figure 25**C), a risk assessment behaviour, and wall climbing [$F_{(1,43)} = 9.008$, $P = 0.004$, $d_r = 0.7$], an escaping behaviour. These increases took place in both prenatal treatment offspring groups. The distance travelled did not vary before and after the predator exposure trial [$P > 0.05$], but distance was influenced by the prenatal exposure differently in each sex [Prenatal exposure x sex interaction: $F_{(1,41)} = 3.801$, $P = 0.05$, $d_r = 3.6$] with prenatally

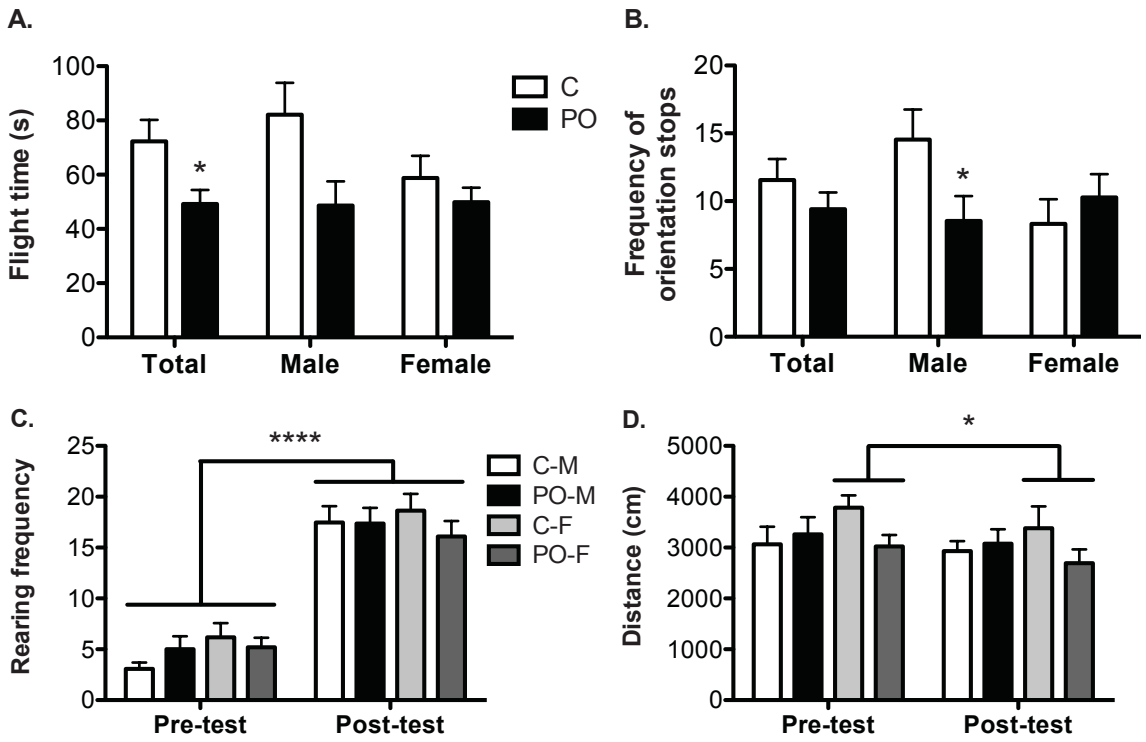


Figure 25. Adult offspring of prenatally exposed to predator-odour (PO) show increased anti-predatory behaviour in the Mouse Defense Test Battery when compared to control (C) offspring.

In the chase/flight test, PO offspring showed a decrease in flight time (time taken to run 15 meters; **A.**) with a decrease in the number of stops with orientation towards the rat in PO compared to control male mice (**B.**). All tested groups showed an increase in post-test rearing (**C.**) and PO females (F) showed a decrease in overall activity compared to control females (**D.**). Data are average ± standard error of the mean. Large connected bars: main time (pre- and post-test) effect ****$P \leq 0.0001$. Connected bars: main female prenatal predator odour exposure effect *$P \leq 0.05$. Main and within-male maternal predator odour exposure effect and *$P \leq 0.05$.

predator-odour exposed females showing an overall decrease in distance travelled compared to control female mice [$F_{(1,19)}$ = 4.407, P = 0.05, d_r = 0.7] (**Figure 25**D).

6.4.4 Foraging test

The ratio of the top on bottom giving-up densities showed a significant prenatal predator odour exposure effect [Mann–Whitney U = -1.935, n_C = 24, n_{PO} = 22, P = 0.05 two-tailed, d = 3.4] (Figure 26A). This result indicated that prenatally predator odour exposed offspring had a higher giving-up density on the top exposed patches versus the bottom-concealed patches. In other words, prenatally predator odour exposed mice decreased feeding on the exposed patches versus to the concealed patches, when compared to control offspring. When looking at the overall top and bottom giving-up densities, the concealed patches were consumed more thoroughly than exposed patches in all groups [$F_{(1,44)}$ = 14.564, P < 0.0001, d_r = -0.7]. Further, prenatal predator odour exposure impacted the consumption in differentially covered patches [Prenatal treatment x patch interaction: $F_{(1,44)}$ = 4.27, P = 0.05, d_r = 3.6] while correcting for body weight (Figure 26B). Overall, the adult prenatally predator odour exposed mice consumed the same amount of food as control mice when correcting for their body weight [P > 0.05]. Finally, there was no difference within duplicate concealed patches and duplicate exposed patches giving-up densities [P > 0.05].

6.4.5 Social choice and social recognition test

In the social choice test, prenatally predator odour exposed mice spent relatively more time in the unknown conspecific zone than in the known conspecific zone [$F_{(1, 37)}$ = 7.302, P = 0.01, d_r = 0.9] (Figure 27A) when compared to control mice. There was no difference in the distance travelled due to the prenatal treatment [P > 0.05].

In the social recognition test, there was no difference in the initial activity level (distance travelled during the activity trial) between prenatal predator odour exposure and control offspring [P > 0.05] (Figure 27B).

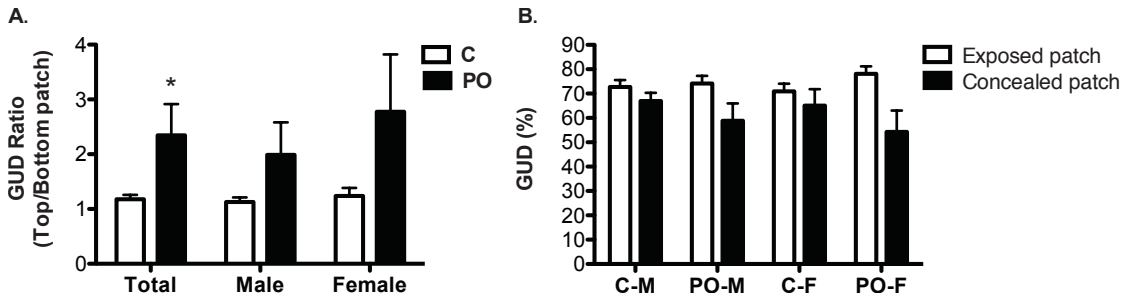


Figure 26. Adult offspring of predator-odour exposed dams during pregnancy (PO) forage more in concealed than in exposed patches when compared to control (C) adult offspring.

PO offspring showed an increase in the ratio of giving up density (GUD) between exposed and concealed patches (**A.**). Percentage of GUD in the top and bottom foraging patches (**B.**). Data are average ± standard error of the mean. M: Male, F: Female. Main maternal predator odour exposure effect, $*P \leq 0.05$.

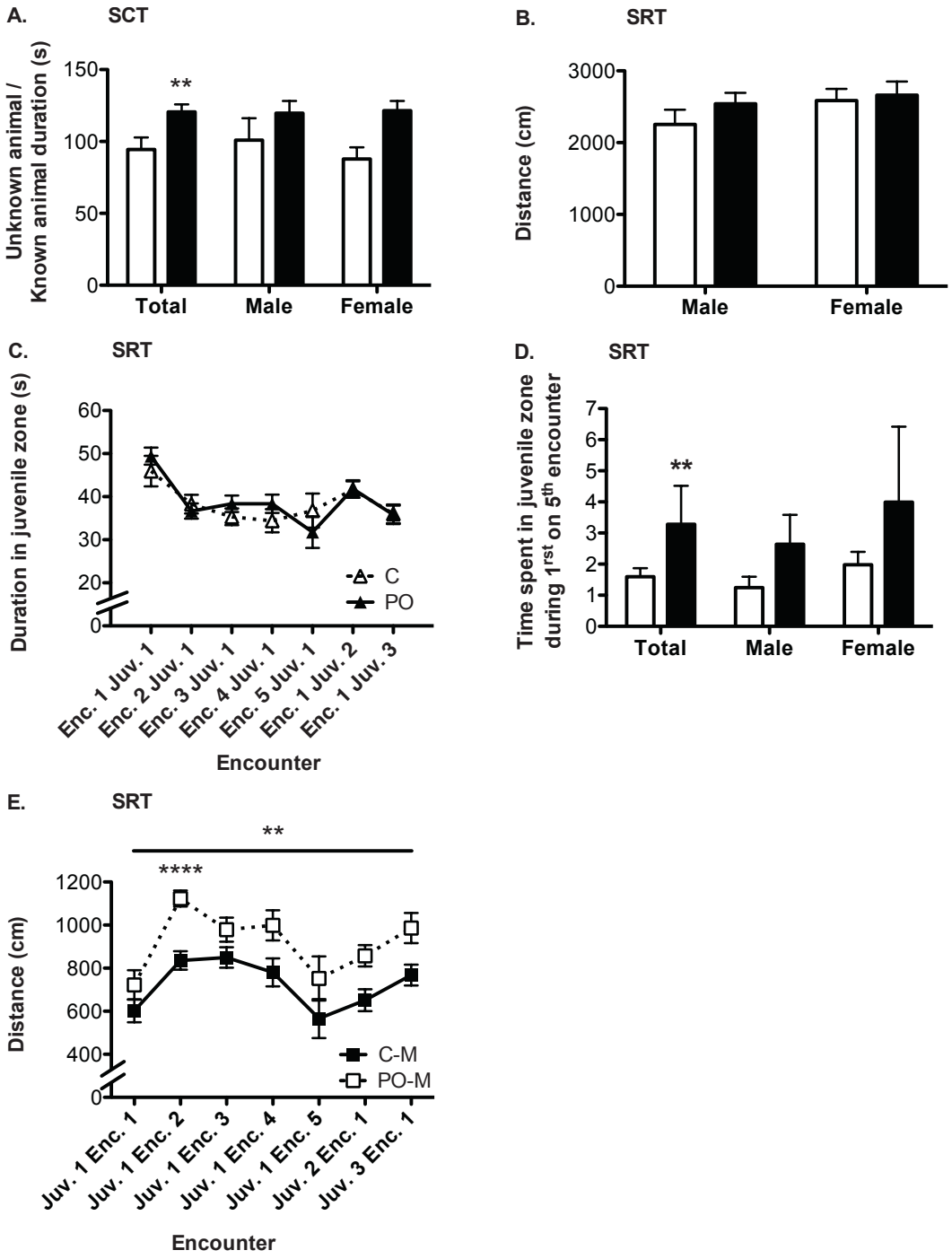


Figure 27. Prenatally predator odour exposed (PO) offspring increase social investigation during an initial encounter with an unkoen conspecific with no difference in social recognition when compared to control (C) offspring.

In the social choice test (SCT), PO mice of both sexes showed increased investigation of an unknown compared to a known conspecific (**A.**). In the social recognition test (SRT),

The duration of time spent in the juvenile zone for encounters 1 through 5 with the same juvenile showed no difference between the prenatal exposure treatments [$P > 0.05$], after correction for the latency to enter the juvenile zone and distance moved. Time spent in proximity to the juvenile decreased with time in all prenatal groups [Encounter effect: $F_{(4,2307)} = 40.229$, $P < 0.0001$, $d_r = 3.0$] (Figure 27C), indicating a normal social memory in prenatally predator-odour exposed mice. However, prenatal predator odour exposure distance travelled in the baseline activity trial showed no difference with prenatal treatment exposure (**B.**). The time spent near the juvenile varied over successive encounters (**C.**). All mice showed a decrease in the time spent in the juvenile zone between the first and fifth encounter with an unknown juvenile. This ratio difference was most pronounced in PO mice of both sexes, indicating an increase in social investigation during a first encounter with an unknown animal (**D.**). PO males (M) showed an overall decrease in their activity (distance travelled) compared to control males (**E.**) in the SRT. Distance travelled changed across the encounters in both males (**E.**) and females (F; **F.**). Data are average ± standard error of the mean. Bar: main prenatal predator odour exposure effect $^{**}P \leq 0.01$. Angled bar: Encounter effect $^{****}P \leq 0.0001$. Within sex prenatal predator odour exposure effect $^{****}P \leq 0.0001$.

influenced the duration spent in the juvenile zone over successive encounters [Prenatal treatment x encounter interaction: $F_{(4,2207)} = 3.553$, $P = 0.007$, $d_r = 5.7$].

When comparing the ratio of time spent in the juvenile zone on a first versus a fifth encounter, prenatally predator odour exposed mice showed a greater decrease in the time spent in the juvenile zone over time compared to control mice [Mann–Whitney $U = 340$, $n_C = 22$, $n_{PO} = 21$, $P = 0.003$ two-tailed, $d = 0.9$] (Figure 27D). When comparing the first encounter with an unknown juvenile individual on a first, second and third occasion (Juvenile 1 first encounter, juvenile 2 first encounter and juvenile 3 first encounter), all mice spent decreasing time with the juvenile conspecific on successive encounters [$F_{(2,2143)} = 84.069$, $P < 0.0001$, $d_r = 3.0$]. Mice from predator odour exposed dams therefore investigated a conspecific longer specifically during the first encounter with an unknown conspecific.

Therefore, prenatally predator odour exposed mice had a similar social recognition and memory over 24 hours when compared to controls when controlling for the distance travelled by individuals. However, the first time prenatally predator odour exposed offspring encountered an unknown conspecific, there was an increase in the time spent investigating that conspecific as shown in both the social choice test and social recognition test.

Contrary to the initial basal activity test that showed no difference between the prenatal treatment groups (Figure 27B), the distance moved during the social encounter varied according to the encounter [Distance x encounter interaction: $F_{(6, 264)} = 2.484$, $P < 0.0001$, $d_r = 4.4$] while the prenatal treatment influenced the sexes differently [Prenatal predator odour exposure x sex interaction: $F_{(1, 40)} = 8.289$, $P = 0.006$, $d_r = 3.6$]. More specifically, prenatally predator odour exposed males showed a generalized hypoactivity during social encounters when compared to control males [$F_{(1, 22)} = 10.375$, $P = 0.004$, $d_r = 0.9$] (Figure 27E) along with an encounter effect [$F_{(6, 138)} = 16.681$, $P < 0.0001$, $d_r = 4.3$]. Additionally, prenatally predator odour exposed males specifically showed a decrease in the distance they travelled on the 2nd encounter with an unknown juvenile

conspecific when compared to control males [$P < 0.0001$]. Females activity varied according to the encounters only [$F_{(6, 114)} = 7.144$, $P < 0.0001$, $d_r = 4.4$] (Figure 27F). Therefore, although the exploration of the novel apparatus did not alter the distance travelled, the presence of a juvenile individual lead to a modification of the activity level in prenatally predator odour exposed mice, particularly a reduction in the activity of prenatally predator odour exposed males.

6.4.6 Social interaction test

Prenatal predator odour exposure leads to an overall decrease in the number of social interactions performed (pooled displacement, offensive, defensive, sniffing, following, jumping on, crawling, escaping and approaching behaviours) when freely interacting with an unknown conspecific [Mann–Whitney $U = 330.5$, $n_C = 27$, $n_{PO} = 36$, $P = 0.03$ two-tailed, $d = 0.6$] (**Figure 28**A). This difference was particularly pronounced among negative (aversive or aggressive) interactions (including displacement, offensive, defensive and escaping behaviour) [Frequency: Mann–Whitney $U = 280$, $n_C = 27$, $n_{PO} = 36$, $P = 0.004$ two-tailed, $d = 0.8$; Duration: Mann–Whitney $U = 258$, $n_C = 27$, $n_{PO} = 36$, $P = 0.002$ two-tailed, $d = 0.9$] (**Figure 28**B). Prenatally predator odour exposed mice approached the unknown conspecific less frequently than control mice [Mann–Whitney $U = 351$, $n_C = 27$, $n_{PO} = 36$, $P = 0.05$ two-tailed, $d = 0.5$] (**Figure 28**C). Finally, prenatally predator odour exposed mice displaced the unknown conspecific less frequently than control mice [Duration: Mann–Whitney $U = 273.5$, $n_C = 27$, $n_{PO} = 36$, $P = 0.003$ two-tailed, $d = 0.8$; Frequency: Mann–Whitney $U = 293$, $n_C = 27$, $n_{PO} = 36$, $P = 0.007$ two-tailed, $d = 0.7$] (**Figure 28**D).

6.5 Discussion

In this study, I investigated whether prenatal predator odour exposure stress could program the long-term offspring shy behavioural type encompassing less exploratory, active and social behaviour. These behaviours should be consistent in a variety of situa-

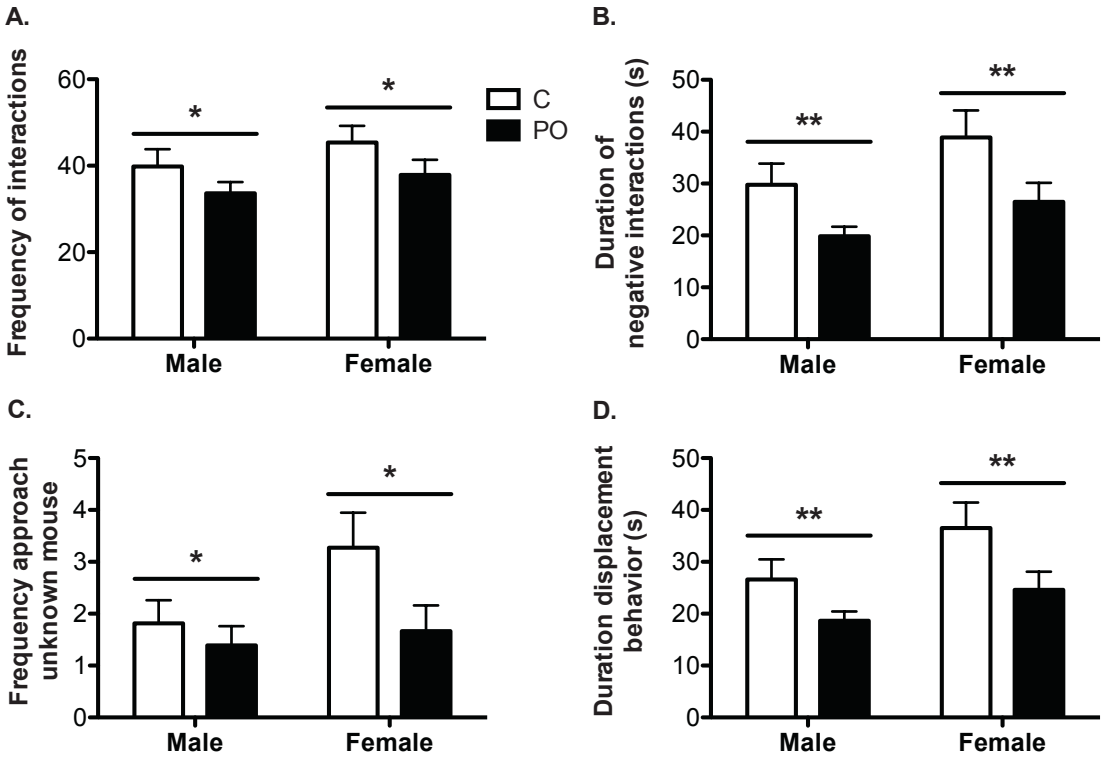


Figure 28. Prenatally predator odour exposed adult mice (PO) decrease their social interactions with an unknown individual when compared to control (C) adult offspring.

The total number of interaction was decreased between unknown PO mice compared to unknown control conspecifics meeting for the first time (**A.**). PO mice also interacted negatively for shorter amount of time (**B.**) than control mice. PO mice also approached the unknown mouse less frequently than the control mice (**C.**). PO mice spent less time displacing an unknown individual than control mice (**D.**). Data are average ± standard error of the mean. Bars: Prenatal predator odour exposure main effect, $*P \leq 0.05$, $**P < 0.01$.

tions including behaviours relevant for the fitness of the individual such as foraging and anti-predatory behaviours. Overall, we discovered that prenatal predator odour exposed offspring of both sexes showed a behavioural type consistent in a variety of stressful situations including: robust commonly-used exploration-based stressful situations, anti-predatory behaviour, foraging or social encounters. Generally, boldness, exploration and activity in stressful situation were decreased in mice from predator-odour exposed dams of both sexes.

6.5.1 Stress-related behaviour in exploratory-based commonly-used stressful situations and naturalistic tests

Offspring of both sexes exposed prenatally to predator odour showed increased anxiety-like behaviour to a variety of exploratory-based stressors both commonly-used in the laboratory and naturalistic. Commonly-used laboratory stressors included the elevated-plus-maze (Carobrez and Bertoglio, 2005), open field, light-dark transition test (Gould et al., 2009) and elevated-plus maze following a 15 minutes restraint stress (modified from Albonetti and Farabollini, 1992; Zimprich et al., 2014) while naturalistic stressors included the standardized mouse defense test battery (Griebel and Beeské, 2011) and a foraging test (modified from Troxell-Smith et al., 2016).

The elevated-plus maze test is the hallmark commonly used test to evaluate anxiety-like behaviour. Prenatally predator odour exposed offspring of both sexes show increased anxiety-like behaviour through decreased time spent in open arms, the aversive portion of the maze (Carobrez and Bertoglio, 2005). Increased activity was also observed in the prenatally predator odour exposed offspring. This increase in spontaneous locomotor activity could be due to the sudden darkness as the test took place under red light (Nasello et al., 1998), a stressor to which the prenatally predator odour exposed animals would be programmed to respond to. Elevated-plus maze measurements of anxiety-like behaviour were further used to assess the effect of a standardized restraint stress applied before the test. Prenatally predator odour exposed mice showed higher anxiety-like behaviour in the elevated-plus maze after being acutely pre-stressed using restraint stress.

Further, we observed a global reduction in activity following restraint, a hallmark effect of restraint stress (Buynitsky and Mostofsky, 2009; Padovan and Guimarães, 2000) and a drastic difference compared to the hyperactivity observed in the initial elevated-plus maze test. This hypoactivity was more important in prenatally predator odour exposed females compared to control females, suggesting higher sensitivity to the restraint stress in prenatally predator odour exposed females or differential perception by both sexes.

The open field and light-dark transition tests were less consistent and informative of the prenatally predator odour exposed stress-related phenotype. We find increased exploration in prenatally predator odour exposed animals in the open field with no difference in activity. Further, we detect no exploration difference in the light-dark transition test in prenatally predator odour exposed animals but hypoactivity in prenatally predator odour exposed males compared to control males. Those results are contradictory to the elevated-plus maze and elevated-plus maze plus restraint tests were unexpected and should be interpreted with caution. Ramos (2008) found that behaviours in the elevated-plus maze and open field are not clearly overlapping and even correlate negatively with the light-dark transition test light exploration, which suggests that these tests measure inconsistent and different aspects of anxiety. Further, C57BL/6 mice showed a decrease in their stress-related phenotype in these tests compared to other mouse strains (Kulesskaya and Voikar, 2014), possibly decreasing this test discrimination potential between the control and prenatally predator odour exposed animals. In addition, several studies observe opposite effects in the elevated-plus maze and open field (Ennaceur, 2014; Ramos, 2008). Further, it has been shown that the open field and light-dark transition test do not consistently elicit a corticosterone increase (Ader et al., 1967; Walsh and Cummins, 1976) and corticosterone injections does not increase the stress-related behaviours in those tests (Cai et al., 2006; Ennaceur, 2014; Gregus et al., 2005; Kalynchuk et al., 2004; Murray et al., 2008). In future studies, further assessment of the risk assessment behaviours (rearing, head dipping and stretch-attend posture, grooming) could be helpful to interpret the open field and light-dark transition results as they are strongly correlated with the circulating corticosterone level of the animal (Rodgers et al., 1999).

The mouse defense test battery is a standardized test to evaluate defensive behaviour in response to threatening stimuli including predators. For mice, laboratory rats elicit such a reaction, especially flight and risk assessment, as risk assessment behaviours are meant to gather information about the threat source (Griebel and Beeské, 2011). Rats are predators of mice, as up to 77% of wild and domestic laboratory rats predate on mice (Galef, 1970; Karli, 1956) and mice show aversion to brightly lit compartments following rat odour exposure (Hebb et al., 2003). In the mouse defense test battery, all mice display an increase in rearing and wall climbing, risk assessment and escape behaviours respectively, at the end of the rat exposure, confirming the aversive nature of rats to mice of both prenatal treatments. Prenatally predator odour exposed females are additionally hypoactive compared to control females in the empty apparatus, a well documented anti-predatory behaviour (Apfelbach et al., 2005; Blanchard and Blanchard, 1989). When the predator chased the mice, prenatally predator odour exposed mice took less time to flee over 15 meters than control mice. In addition, prenatally predator odour exposed males also stopped less frequently to orient themselves toward the rat, a risk-assessment behaviour decreasing the efficiency of the flight response in this case. These observations indicate that the prenatally predator odour exposed mice are more efficient in their anti-predator flight response than control mice.

The foraging test was developed to assess foraging, a natural behaviour exhibited by mice in the wild to find and gather food. The foraging rate of prey species is determined by the interaction of several costs of foraging including the predation risk (Brown and Kotler, 2004). The giving-up density is measured as the amount of food left in a depletable food patch after a given foraging time, and indicates a trade-off of food for safety, a well-documented effect of predation (Brabrand and Faafeng, 1993; Christensen and Persson, 1993; Dickman, 1992). The amount of food left in a patch is determined by several factors including the perceived 'riskiness' of the patch, that can be altered by its degree of coverage. It is riskier or perceived riskier to forage in exposed patches when compared to concealed ones (Troxell-Smith et al., 2016), especially in a risky environment that exhibit cues of predator presence (Shrader et al., 2008; Thorson et al., 1992). In this study, I used uniform rat odour to create a risky environment (see Hebb et al., 2003) over exposed and

concealed foraging patches. The results indicated that, although consuming the same total amount of food, the control and prenatally predator odour exposed adult offspring distributed their foraging effort across the patches differently. Prenatally predator odour exposed mice left more food in the exposed patches compared to the concealed ones while correcting for their body weight when compared to control offspring. Prenatally predator odour exposed offspring therefore favoured food consumption in patches perceived safer under uniform predator odour. This foraging task is a high-throughput way to test the foraging behaviour in a laboratory setting and should be included in behavioural test batteries aiming at uncovering stress-associated phenotypes.

Overall, these laboratory exploratory-based commonly-used and naturalistic stressors indicated that prenatally predator odour exposed mice are more sensitive to stress, as shown through their increased stress-related behaviours across a variety of situations, with some minor sex differences. Prenatally predator odour exposed mice therefore seem to be programmed up to adulthood for increased reactivity to a variety of stressful situations. Finally, these results bridge naturalistic and the most robust laboratory commonly-used stressors by indicating a general decrease in exploration across a variety of situations including an elevated novel environment, forced restraint, flight anti-predatory behaviour and foraging in a risky environment (except in the open field). These results indicate that the elevated-plus maze, the most commonly-used exploration-based laboratory test is informative of naturalistic behaviour while having an incidence on fitness. These results are also in accordance with the increase in stress reactivity observed at the endocrine, metabolic and transcript abundance level in limbic regions accompanied by stable DNA methylation marks observed previously in prenatally predator odour exposed animals (St-cyr et al., 2017; St-Cyr and McGowan, 2015; see Chapter 3 to 5).

6.5.2 Social behaviour

As predicted, prenatally predator odour exposed adults showed a decrease in reciprocal social interactions when exposed with an unknown conspecific in the social interaction test. We also observed an unexpected normal social recognition memory in the prenatally

predator odour exposed mice. However, and most surprisingly, prenatally predator odour exposed mice showed an increase in the initial social investigation of an unknown conspecific, a robust observation made twice in different cohorts of animals. This difference is not present in subsequent encounters or first encounters with other unknown conspecifics. This lack of social recognition memory deficit and specific increase in social investigation during the first encounter with an unknown conspecific are in contradiction with other prenatal stressors that lead to a decrease in social recognition memory and no change or a decrease in social investigation (Grundwald et al., 2016; Jones et al., 2010; Lee et al., 2007; reviewed in Kundakovic and Jaric, 2017). Further, social motivation can be disrupted through neonatal exposure to stressors as well as general stress and shifted towards aggressive or defensive behaviour (Sandi and Haller, 2015).

However, in line with observations in prenatally predator odour exposed mice, male mice overexpressing *corticosterone-releasing factor* starting in the early postnatal period (postnatal day 4-8) show enhanced social investigation during the first encounter with an unknown mouse along with normal short-term social recognition memory in adulthood (Kasahara et al., 2011). This sustained *corticosterone-releasing factor* overexpression is present in the paraventricular nucleus of the hypothalamus, in the central nucleus of the amygdala, in the hippocampus and in the cerebral cortex. Although prenatally predator odour exposed mice show no difference in *corticosterone-releasing factor* transcript abundance in the hippocampus and amygdala, females showed a specific increase in *corticosterone-releasing hormone receptor 1* transcript abundance in the amygdala, potentiating corticosterone-releasing hormone detection and response in this brain structure (St-Cyr and McGowan, 2015). Overexpression of corticosterone-releasing hormone in the paraventricular nucleus of the hypothalamus could explain the prenatally predator odour exposed mice differences in both sexes and should be assessed.

Another possibility to explain these results is that the increase in initial social investigation would be triggered by stressed unknown conspecifics. This is likely since the unknown conspecifics presented are loosely restrained in a small wire cup (Buynitsky

and Mostofsky, 2009). Since prenatally predator odour exposed mice exhibit increased stress-related behaviour and behavioural modifications in stressful situations, it is likely that prenatally predator odour exposed mice would be more sensitive to bystander stress. We know that a prenatal stress can carry this effect as rat pups prenatally exposed to a bystander stress, where pregnant mothers were housed with a pregnant female stressed by an elevated platform exposure, showed decreased exploration in a novel environment (Mychasiuk et al., 2011). In the future, the amount of circulating corticosterone in the loosely restrained mouse and the observer mouse should be measured.

6.5.3 Behavioural type

We demonstrate here that an ethologically mild and ecologically-relevant prenatal stress is sufficient to elicit modifications in a range of significant stress-induced and social behaviours. We observed only minor sex differences within the prenatally predator odour exposed mice behavioural responses. Both sexes displayed an increase in stress-related behaviour as detected through the exploration and foraging in novel risky environments, anti-predatory behaviour and social behaviour alterations. This result is particularly interesting as we previously observed differential endocrine responses, transcript abundance in limbic regions and stable DNA methylation modifications in prenatally predator odour exposed females compared to prenatally predator odour exposed male mice. These results suggest that sex distinct mechanisms are leading both sexes to a convergent behavioural phenotype of prenatal predator odour exposed mice.

Overall, prenatally predator odour exposed adult mice of both sexes are consistently more timid and less exploratory, active and sociable than control mice. However, the detected decrease in sociability was specific to freely interacting conspecifics while their social memory was not affected. We found that these traits were reproducible over a range of situations, consistent with the notion of a behavioural type (Réale et al., 2007) in which fixed inter-correlated traits are determined by the life history of the individual. Taken together, we suggest that the behavioural phenotype of prenatally predator odour exposed mice is consistent with the concept of temperament as predator presence cues trigger the

programming of a coherent offspring phenotype to react to frequent environmental stressors.

Chapter 7 Discussion

7.1 Potential underlying causes of sex differences in prenatal stress phenotype

One of the recurrent findings of the studies of prenatal predator odour exposure described herein is the sex-specific phenotype found. More precisely, the phenotype was similar at the behavioural and somewhat at the physiological level but differed at the endocrine, transcript abundance and methylation modifications level. The anti-predatory behaviour can be elicited acutely (within seconds to minutes), suggesting it may be advantageous for both sexes to express behaviours responsible for immediate survival.

Prenatal stress affects both sexes, but for decades the bulk of the research has been done on males only (Ahmadzadeh et al., 2011; Campos-Barros et al., 1997; Kraszpulski et al., 2006; Lemaire et al., 2000; Mueller and Bale, 2008; Saboory et al., 2011; Salm et al., 2004; reviewed in Charil et al. 2010). Furthermore, some research suggest that male offspring are more susceptible to prenatal stress (Mueller and Bale, 2008) while others suggest the opposite (Glover et al., 2010; Weinstock, 1997).

When investigated, sex differences are widely reported in response to perinatal stressors including predator or predator cues presence. For example, Mashoodh et al. (2009) found differential impacts of early postnatal exposure to predator odour in male and female offspring. Adult female offspring showed a less-anxious phenotype while males showed a more anxious phenotype in the open field. Coutellier and Würbel (2009) also found that the female offspring of predator odour exposed mice dams showed a reduced object recognition memory. The opposite was observed in male offspring, indicating that the observed increased maternal care might be able to rescue the adverse effects of early predator odour exposure in this model. Chronic variable stress in the third week of pregnancy in rats leads to increased anxiety-like behaviour in the elevated-plus-maze in young adult offspring and is associated with increased corticotropin-releasing factor and corticotropin-releasing factor receptor 1 transcript abundance in the amygdala of female offspring and increased corticotropin-releasing factor receptor 2 transcript abundance in

offspring of both sexes (Zohar and Weinstock, 2011). Another study found that pregnant laboratory rats exposed to social defeat over 5 days in their last week of pregnancy produced adult offspring displaying a greater hypothalamic-pituitary adrenal axis response to a restraint stress compared to control offspring (Brunton and Russell, 2010). Associated with this response, they found differential transcript abundance of the hippocampal mineralocorticoid receptor and in the amygdala levels of glucocorticoid receptor and corticotropin-releasing factor in both sexes but decreased in hippocampal glucocorticoid receptor transcript abundance in females only. Thus, in these studies, prenatal stress in female offspring leads to broader impacts on neural gene transcript abundance in limbic structures than in male offspring. Responses to prenatal stressors may play both an adaptive role in stressful environments and lead to sex-specific differences in the incident risk for psychopathology, including an increased risk for affective disorders among females (Glover, 2011). Viewed from an evolutionary perspective, understanding the impacts of ethologically-relevant stressors such as predator odours may be important in elucidating mechanisms of sex plasticity associated with stress-related phenotypes.

As described in section 1.3.4 on page 12, sex differences in the hypothalamic-pituitary adrenal axis are widespread and start perinatally through sex-specific exposure to glucocorticoids *in utero* and access to maternal care. Prenatally, placental 11β-hydroxysteroid dehydrogenase is less active from gestational day 16 onward in males than females, suggesting that male foetuses are exposed to higher glucocorticoid levels (Burton and Waddell, 1994). Moreover, from gestational day 16.5 onward, female foetuses receive lower maternal glucocorticoid buffering through decreased placental 11β-hydroxysteroid dehydrogenase level in response to stress (Glover and Hill, 2012; Pankevich et al., 2009). These sex-specific mechanisms both lead to an increase in foetal glucocorticoids exposure in late pregnancy. Postnatally in rodents, males also receive increased maternal behaviour in the form of ano-genital licking and handling (Moore and Morelli, 1979). Ano-genital licking is an essential maternal care stimulating urine production and release in the pup while regulating the development of endocrine, emotional and cognitive response to stress (Champagne et al., 2003). Therefore, maternal

behaviour may have a greater potential to modulate the offspring phenotype or rescue prenatal stress in male than female offspring.

Within the brain, prenatal stress leads to sex-specific smaller hypothalamic nuclei in females (Anderson et al., 1985) and feminization of the cerebral cortex structure and size in males (Fleming et al., 1986). These sex-specific changes are not surprising per se as the brain transcriptome diverges between sexes by 14% and by 70% in the liver (Vigé et al., 2008). It is likely that these differences are associated to sex- and tissue-specific epigenetic modifications present near regulatory elements, though this is mostly unexplored.

Further, sex steroids interact with the hypothalamic-pituitary adrenal axis and modulate the stress response and vice-versa. In rodents, gonadectomised males typically exhibit increased adrenocorticotropic hormone and corticosterone response to stress (Gaskin and Kitay, 1971; Seale et al., 2004). On the contrary, androgens supplementation to gonadectomised animals lead to an increase in exploration in the elevated-plus maze and the open field and decreased the glucocorticoid response to restraint stress (Edinger and Frye, 2005; Gaskin and Kitay, 1971). Circulating testosterone is also typically reduced in prenatally stressed animals (e.g. Richardson et al., 2006) which express increased stress-related behaviours. Furthermore, testosterone supplementation in prenatally stressed males can reduce their increased stress response (Kapoor and Matthews, 2011). Similarly, oestrogen, luteinizing hormones and follicle-stimulating hormones are inhibited by glucocorticoid secretion. On the contrary, oestrogens potentiate the corticosterone-releasing hormone and arginine-vasopressin secretion and lead to hyper-responsiveness of the hypothalamic-pituitary adrenal axis by decreasing the glucocorticoid receptor binding in multiple brain regions including the hypothalamus and hippocampus (Chrousos, 1998). Further, ovariectomy of prenatal restraint stress female rescues their increased stress-related phenotype while also being more responsive to the effect of 17β-estradiol supplementation (Ordyan et al., 2014). Therefore, androgens and oestrogens are both repressed by the hypothalamic-pituitary adrenal axis. However, androgens decrease

the hypothalamic-pituitary adrenal axis activation while oestrogen activates the hypothalamic-pituitary adrenal axis and prolongs its action.

Taken together, these sex-specific differences in prenatal glucocorticoid exposure, maternal care received and circulating sex steroids can underlie the general increased susceptibility of female offspring to psychopathology linked to increased anxiety, depression and stress responses of the hypothalamic-pituitary adrenal axis while males are more likely to develop learning and memory deficits (Glover and Hill, 2012).

7.2 Caveats and limitations

One of the confounds present in animal manipulation done in this thesis studies is the sex of the experimenter or handler. Sorge et al. (2014) demonstrated that male odours and male experimenters specifically induce pain inhibition in mice and rats while female experimenters do not and a male experimenter was used in this thesis. The male experimenter conducted some of the behavioural testing in the rat cohort (see Chapter 4, pages 69-95). However, he tested all the rats in the commonly-used exploratory-based tests, and not a subset of them, so that all the animals were submitted to the same experimenter within a behavioural test. All the mice (Chapters 3, 5 and 6, pages 49-68 and 96-150) were handled and tested by myself or Sameera Abuaish. However, I was pregnant during the entire animal handling and testing period, a factor that has not been studied so far.

The behavioural tests were conducted during least indicated period for testing, the light phase of the light-dark cycle. The light phase is the period of low activity in rodents. The circadian period of testing has been assumed to lead to changes in the behaviours recorded. However, behavioural testing of rodents during the light phase has been associated with contradictory results such as behavioural inhibition in stress-eliciting commonly-used tests and cognition impairments (Roedel et al., 2006) or no modifications in those same tests (Beeler et al., 2006; Bertoglio and Carobrez, 2002; Chaudhury and

Colwell, 2002) although the general activity level can be affected. However, social behaviour testing is not affected by the time of day (Yang et al., 2008). Further, reversal of the daily activity periods is a normal adaptation to the presence of predation stress as reported in a natural rat population (Fenn and MacDonald, 1995). Therefore, night behavioural testing is preferable although there is no absolute consensus in the literature on that subject.

Another limitation of the thesis is the interpretation of the stress-related and social behaviour as a consistent shy behavioural type. This interpretation is debatable as the associated literature is somewhat diverse and contradictory (MacKay and Haskell, 2015). An alternative to this interpretation of the behavioural phenotype of mice from predator odour-exposed dams is the relationship with the life history of *Mus musculus. M. musculus* a social species that is territorial and dispersing, two key factors that can vary with environmental factors and influence the adult phenotype (Berry, 1970; Berry and Bronson, 1992; Brust et al., 2015).

Finally, one could question the validity of the phenotype described in this thesis as semi-domesticated strains of animals could present artifacts rather than a relevant adaptive phenotype. The strains we used were semi-domesticated in order to minimize the inter-individual variability in order to detect as much as possible the effect of the prenatal stress. Some differences between laboratory strains and natural populations of rodents can be found (Calisi and Bentley, 2009) although natural populations and laboratory studies complement results from each other (Clinchy et al., 2011a). It is also very difficult to evaluate how the predator odour exposure paradigm relates to the natural predatory stress imposed on natural populations of *Mus musculus*. The methodologies to evaluate or manipulate predation pressure are very diverse and should be unified in the future (Apfelbach et al., 2005; Moll et al., 2017). However, to my knowledge, *Mus musculus* in a natural environment avoid traps when predator odours are present (Dickman, 1992) and reduce foraging in open habitats under predation stress (Nolte et al., 1994). However, other types of manipulated predator cues such as fox scats placed in a semi-natural enclosure did not affect the survival, offspring body weight, fecundity or population size

of *Mus domesticus* (Powell and Banks, 2004) indicating that the type of predator cues can elicit different effects on preys. To evaluate the relative intensity of the predator stress imposed in the prenatal predator odour exposure paradigm, further investigation of the intensity of predation pressure in untouched rodent populations is required.

7.3 Prenatal predator odour exposure programs an integrative phenotype in mice and rat offspring

This section offers an overview and comparison of the male and female from predator-exposed dams phenotypes as well as the mice and rat offspring from predator-exposed dams phenotypes. Additionally, comparisons of the phenotype induced by predator or predator cue exposure, prenatal restraint stress or prenatal chronic variable stress are provided. These comparisons are summarized in **Error! Reference source not found.**-11.

7.3.1 Pregnancy and maternal behaviour

7.3.1.1 Pregnancy and maternal behaviours phenotype of predator odour-exposed dams

Overall, and in both species and cohorts of animals, the pregnancy and litter size of the mice was not altered by the prenatal predator-odour exposure paradigm. However, in the first mouse cohort, several abortions happened following the exposure to a relatively high concentration of 2,3,5-trimethyl-3-thiazoline.

We detected a decrease in the classic maternal programming behaviour licking-grooming in predator odour exposed dams, but only after accounting for the decrease in the nest quality built by the dam, a non-traditionally-examined maternal behaviour. The decrease in nest quality is associated with a decrease in the nest temperature and increase in the energetic needs of pups (Bryant and Hails, 1975; Gaskill et al., 2013). This modification

had not been detected initially as nest quality was not assessed in the first mice and rat cohorts of offspring from predator odour-exposed dams. The decreased nest quality change could be unique feature of this type of prenatal stress. However, this particular trait has not been classically assessed in other prenatal stress paradigms, making a direct comparison difficult.

7.3.1.2 Pregnancy and maternal behaviours phenotype in response to typical predation stress

Studies involving direct observations of maternal behaviour under predator or predator cue exposure during the perinatal period are scarce. Smaller broods and litters with decreased survival at birth were observed in song sparrows and mice exposed to predators or predation cues (de Catanzaro, 1988; Zanette et al., 2011). Additionally, some passerine species increase their maternal investment by increasing the nestling feeding rate in a wild population under high nest predation pressure (Fontaine and Martin, 2006). The only occurrence where prenatal predator or predator cue exposure was associated with a modification of parental nest quality was found in black goby fish males that decreased their nest-building behaviour in presence of predatory cods (Magnhagen, 1990). These findings remain sparse and more effort should be directed to detect the general effects of predator stress on prey's parental behaviour.

7.3.1.3 Pregnancy and maternal behaviours phenotype in response to a traditional prenatal restraint stress and prenatal chronic variable stress

Maternal behaviour associated to chronic variable stress during pregnancy has not been extensively studied, but no obvious differences have been reported (Mueller and Bale, 2006). Although restraint stress during pregnancy is not associated with specific alterations in maternal behaviour, some adoption studies indirectly demonstrated that the offspring phenotype is influenced by the mother both prenatally and postnatally (Barlow et al., 1978; Barros et al., 2006). A systematic assessment of maternal behaviours in

chronically variably stressed and chronically restrained pregnant dams would be important to facilitate comparisons between prenatal stressor types in the future.

7.3.1.4 Comparison highlights between the prenatal stress paradigms effects on dams pregnancy and maternal behaviour

The prenatal predator odour exposure paradigm reported in this thesis consisted of acute predator odour exposure that did not last over the full circadian period unlike most of the previous predator or predator cues experiments reported here. This heterogeneity complicated the direct comparison to predator or predator cues exposure-associated maternal behaviours, although maternal behaviours are modified to some extent in both cases. Further, the lack of systematic maternal behaviour investigation in previous prenatal restraint stress and prenatal chronic variable stress paradigms made direct comparisons difficult to achieve. However, although not specifically described, we know that postnatal maternal effects influence the prenatal restraint stress rodent phenotype. Overall, it is likely the postnatal maternal effects have an impact on the prenatally predator odour exposed adult animal phenotype although direct comparisons with other prenatal stressors were difficult. This finding highlights the value of systematically assessing the maternal behaviours to assess perinatal stress impacts on the offspring phenotype.

7.3.2 Behaviour

7.3.2.1 Behavioural phenotype of rodent offspring from predator odour-exposed dams

Overall, stress-related behaviours in offspring of both sexes from predator odour exposed dams were consistently potentiated by prenatal predator odour exposure in a variety of circumstances. This increase in stress-related behaviours was detected in circumstances ranging from commonly used exploration-based laboratory tests to naturalistic foraging and predator or predator odour exposure as well as direct social interactions.

Within traditional exploration-based laboratory tests paradigms, we observed a variety of altered stress-related behaviours. Defecation, risk assessment and time spent in the open arm indicated increased stress-related behaviours in the elevated-plus maze, with mice showing hyperactivity overall and male rats showing hypoactivity. In the open field, the species showed opposite responses, with mice increasing and male rat decreasing time spent in the center of the apparatus, suggesting a decrease and an increase in stress-related behaviour in this test, respectively. Rat females were not different from control females in this test. Finally, within the light-dark transition apparatus, rat females were the only ones to decrease the time spent in the light zone, a stress-related behaviour, while rat males and mice of both sexes were similar to control animals. These tests were not consistent within and between the species. Several variables around the execution of these tests, especially the size of the apparatus, can influence the behaviour of animals. Interestingly, the elevated-plus maze was adapted to each species and has been the most consistent test to find potentiation of the stress-related behaviours. The open field was of a different size in both species, with rats having a white box with textured surface while mice were placed in a transparent smooth arena. The light-dark transition apparatus was the same in both species. However, this particular light-dark transition apparatus did not respect the traditional one third light two thirds dark proportions common in the literature (e.g. Gould et al., 2009). These considerations could be adding to the variability observed here. Interestingly, these same apparatus similarly yielded some variability and sex differences when discriminating between control rats and rats presenting increased baseline corticosterone and hypothalamic-pituitary adrenal axis reactivity (Sasaki et al., 2014, 2013). Therefore, the results we obtained were generally expected and tend toward an increase in stress-related behaviours in offspring from predator odour-exposed dams. However, measuring other aspects of stress-induced behaviours is needed to put these results in perspective. Alternatively, it is worth considering that these traditional tests present situations that are not directly related to the specific prenatal stress applied in this thesis.

Live predator models and predator odour exposure are known to lead to increase stress-related and anti-predatory behaviours in both prenatally predator-odour exposed species

and sexes. In the above studies, mice from predator-exposed dams presented for the first time directly to a predator odour showed an expected hypoactivity and increased avoidance by staying further away from the odour. Similarly, male rats visited the area closest to predator odour less frequently. Female rats did not show a behavioural impact in relation to predator odour presentation. Although the overall effect of avoidance converged among the species, the fact that we did not find strong behavioural impacts in rats might be due to the smaller presentation apparatus in proportion to the animal size (the apparatus was the same for mice and rats). In rats, this particular apparatus might have been too small to express a full array of anti-predatory behaviour. Specifically, Campeau et al. (2008) reviewed the literature about predator odour-elicited behaviours and detected a shift in the behavioural response to an escapable in comparison to an inescapable predator odour.

Control and mice from predator odour-exposed dams exposed to a 'live' predator model (mouse defense test battery) expressed anti-predatory behaviours: hypoactivity and increased escape behaviours. This result indicated that all animals reacted to the predator stress. Nonetheless, the mice from predator odour-exposed dams exhibited a faster, sustained and more efficient fleeing response to a 'live' predator. Additionally, mice from predator-exposed dams displayed a stronger tendency to forage in a protected habitat under a uniform predation threat compared to control animals.

Finally, social behaviour was assessed in mice and indicated an expected reduction in free social interactions. However, there was no impairment of the prenatal predator odour exposure on social recognition and memory. Furthermore, there was an increase in social investigation during the first in a lifetime encounter with an unknown mouse that was loosely constrained under a wire cup (diameter 8.5 cm). This stranger mouse could not freely interact with the focal mouse. Although there is no confirmed explanation for this particular behaviour, it might be due to greater *corticosterone-releasing hormone* transcript abundance in the paraventricular nucleus of the hypothalamus or susceptibility to bystander stress. These hypotheses should be investigated in follow-up studies.

7.3.2.2 Behavioural phenotype elicited by typical predation stress

Rats exposed to 2,3,5-trimethyl-3-thiazoline prenatally exhibit decreased freezing (an anti-predatory behaviour) during the juvenile period (Ayers et al., 2016). On the contrary, gravid crickets and lizards exposed to predatory stress produced juveniles and adults expressing stronger anti-predatory behaviours and consequently survival to predatory attacks (Bestion et al., 2014; Shine and Downes, 1999; Storm and Lima, 2010; Uller and Olsson, 2006). In adulthood, prey species exposed to predator or predator cue generally decreased their foraging in open habitats and general activity (Belzung et al., 2001; Dickman, 1992). Simultaneously, an increase in the avoidance and refuging behaviours and a decrease in the amount of social interactions performed is detected (Blanchard and Blanchard, 1989; Blundell et al., 2005; Zangrossi and File, 1992). Additionally, predator exposure before the elevated-plus maze testing lead to an increase in risk assessment behaviours and time spent in the open arm in female mice and male rats (Adamec et al., 2006; Zangrossi and File, 1992).

7.3.2.3 Behavioural phenotype elicited by the traditional prenatal restraint stress and prenatal chronic variable stress

Prenatal restraint stress and prenatal chronic variable stress elicit behavioural phenotypes generally potentiating stress-related behaviours in commonly-used exploratory-based laboratory tests while simultaneously increasing the activity (distance travelled) in these tests (De Souza et al., 2013; Dong et al., 2015; Mueller and Bale, 2008; Wilson and Terry, 2013). Immobility was increased in other inescapable stress circumstances (forced swim, tail suspension test; Morley-Fletcher et al., 2003; Mueller and Bale, 2008). Prenatal restraint stress and prenatal chronic variable stress also lead to a general decrease in social interactions performed (De Souza et al., 2013; Lee et al., 2007). However, in these experiments no foraging or anti-predatory assessment has been conducted on prenatally restrained or prenatally chronically variably stressed rodents. However, these prenatal stressors would be expected to decrease the foraging in open habitats and modify anti-predatory tactics.

7.3.2.4 Comparison highlights between the behavioural phenotype elicited by the various prenatal stress paradigms

The behavioural phenotype of animals from predator odour-exposed dams agrees with the behavioural modifications observed in animals exposed to predator stress. The results from 5 tests (predator odour exposure, elevated-plus maze, elevated-plus maze following restraint, mouse defense test battery and foraging test) showed a converging increase in stress-related behaviours. The open field and light-dark transition tests yielded contradictory results, especially in mice, and no direct comparison were possible for these tests with animals exposed to predator stress.

The behavioural phenotype of rats from predator-exposed dams generally agrees with the results found in prenatally restrained stressed and prenatally chronically variably stressed animals although there were some variations with the mice phenotype regarding the general activity level of the rodents. The comparisons are limited by the lack of information in the literature about the impact of prenatal restraint stress and prenatal chronic variable stress on the foraging and predator or predator cue exposure associated anti-predatory behaviours (Error! Reference source not found.).

7.3.3 Physiology

7.3.3.1 Physiological phenotype elicited by rodents from predator odour-exposed dams

Prenatal predator odour exposure leads to a modest decrease in male mice weight, appearing after birth, especially in the post-weaning period. This difference was not detected in female mice and rats of both sexes. Mice from predator odour-exposed dams also exhibited an increase in their average oxygen consumption rate when presented for the first time with an inescapable predator odour. Further, over a normal circadian cycle, the average oxygen consumption rate in mice from predator odour-exposed dams was

Table 8. Comparative effects of stress on behaviour

Treatment	Behaviour									
	Foraging	Refuging/ Avoidance	Activity	Vigilance/ Immobility	Habitat shift	Circadian activity shift	Social behaviour	Startle response	Analgesia	Learning
Predation consensual effect	↓	↑	↓	↑	✔	✔	↓aff. ↑agg.	↑	↑	↑↓
PO male mice	OH ↓	↑	POEspecif.↓	N.A.	N.A.	Partially ↓	↓aff. ↑Initial inv.=memory	N.A	N.A	N.A
PO female mice	OH ↓	↑	POEspecif.↓	N.A.	N.A.	↑	↓aff. ↑Initial inv.=memory	N.A	N.A	N.A
PO male rat	N.A.	↑	↓	N.A.	N.A.	N.A.	N.A	N.A	N.A	N.A
PO female rat	N.A.	↑	=	N.A.	N.A.	N.A.	N.A.	N.A	N.A	N.A
PRS animal	↓Baseline M[6]	↑[1,2,4,5,7]	↑[2,5]	↑[4]	N.A	N.A.	↓aff.[1,2,3,4]	↑[4]	N.A	↓[5]

PO: Prenatal predator Odor exposure
PRS: Prenatal Restraint Stress
POE specif.: Predator Odor Exposure specific
OH: Open Habitat
N.A.: Not Available

M: Male
Aff: Affiliation
Agg: Aggression
✔: Induced trait
Initial inv.: Initial investigation

[1]De Souza et al. 2013
[2]Dong et al. 2015
[3]Matrisciano et al. 2013
[4]Morley-Fletcher et al. 2003
[5]Vallée et al. 1997
[6]Vallée et al. 1996
[7]Xu et al. 2014

increased in normally low energetic demand periods in both sexes when compared to control mice.

7.3.3.2 Physiological phenotype elicited by typical predation stress

Predation stress generally leads to lower weight or weight loss. For example, prenatal exposure to acute predator presence lead to a general decrease in weight in mice and rat at birth and during the first postnatal week (Ahmadzadeh et al., 2011; Korgan et al., 2014). Postnatally, weight loss is detected in adult female mice after a week of acute predator exposure (e.g. Wang et al., 2011). Predation stress also acutely increases the metabolic rate of prey species (Campeau et al., 2008; Schmidt et al., 2012).

7.3.3.3 Physiological phenotype elicited by the traditional prenatal restraint stress and prenatal chronic variable stress

Prenatal restraint stress lead to a male-specific decrease in weight at birth which can normalize in adulthood (Lesage et al., 2004; Vallee et al., 1996). On the contrary, prenatal chronic variable stress leads to an increase in the mice weight at birth which can last up to adulthood (Mueller and Bale, 2006).

7.3.3.4 Comparison highlights between the physiological phenotype elicited by the various prenatal stress paradigms

The detected weight difference in males from predator odour-exposed dams was not specifically converging with any of the other models, as we did not detect changes in birth weight. This change might be explained by the fact that the prenatal predator odour exposure paradigm was milder than the prenatal live predator exposure responsible for decrease weight at birth. Animals from predator-exposed dams showed an increase in acute oxygen consumption rate to predator odour similar to the predator-induced increase in metabolic rate. To my knowledge, metabolic modifications to stress have not been

investigated in the prenatal restraint stress and prenatal chronic variable stress models (Table 9).

7.3.4 Endocrinology

7.3.4.1 Endocrine phenotype elicited by the prenatal predator odour exposure

When exposed to a potent predator odour (2,3,5-trimethyl-3-thiazoline), rats and mice females showed increased stress sensitivity in a species-specific manner while presenting unaltered baseline levels of stress hormones. Female mice showed an increased corticosterone release right after the cessation of the stress accompanied by a normal stress recovery while female rats showed a prolonged adrenocorticotropic hormone release following predator odour exposure. Mice from predator odour-exposed dams also presented an elevated thyroid hormone (thyroxine) circulating level at baseline and following a restraint stress.

7.3.4.2 Endocrine phenotype elicited by typical predation stress

Predator and predator odour presence lead to increased or prolonged circulating glucocorticoid levels and adrenocorticotropic hormone response (Campbell et al., 2003; Campeau et al., 2008; File et al., 1993; Masini et al., 2005, 2009; Thomas et al., 2006). An increase in baseline glucocorticoid level is also observed in response to prenatal live predator exposure in rat pups (Saboory et al., 2011). In adulthood however, baseline circulating corticosterone was globally unaltered (Sotnikov et al., 2011; Wang et al., 2011). Thyroxine circulating level in response to predation stress was not investigated.

7.3.4.3 Endocrine phenotype elicited by the traditional prenatal restraint stress and prenatal chronic variable stress

Similarly, prenatal restraint stress and prenatal chronic variable stress lead to an increase in the glucocorticoid response (Koenig et al., 2005; Morley-Fletcher et al., 2003)

Table 9. Comparative effects of stressors on physiology

Treatment	Trait	
	Growth / Weight	Metabolic rate
Predation consensual effect	↓	↑
PO male mice	Birth =, Ados. Adult ↓	↑
PO female mice	=	↑
PO male rat	=	N.A.
PO female rat	=	N.A.
Laboratory PRS/CVS animals	Birth ↓↑,Adult M ↓	N.A.

PO: Prenatal predator Odor exposure

PRS: Prenatal Restraint Stress

CVS: Chronic Variable Stress

Ados.: Adolescent

M: Male

N.A.: Not Available

accompanied with a lower baseline corticosterone in response to restraint stress (Lesage et al., 2004; Tamashiro et al., 2009), a stressor they experience for the first time directly. Adrenocorticotropic hormone and thyroxine levels were not investigated in these prenatal stress paradigms.

7.3.4.4 Comparison highlights between endocrine phenotypes elicited by the various prenatal stress paradigms

The rodents from predator odour-exposed dams show an endocrine response to predator odour that is similar to the impact of prenatal and postnatal exposure to predator or predator cues (Table 10). The baseline alteration in corticosterone level in prenatal restraint stress and prenatal chronic variable stress exposed animals and their increase corticosterone response to restraint stress however differ from the adult phenotype of rodents from predator-exposed dams (*Sameera Abuaish, personal communication*).

7.3.5 Gene transcript abundance and epigenetic modifications

7.3.5.1 Gene transcript abundance and epigenetic phenotype elicited by the prenatal exposure to predator odours

Female rodents from predator odour-exposed dams showed an increase in stress-related genes transcript abundance in the hippocampus (mouse) and amygdala (mouse and rat). The corticotropin-releasing hormone receptor 1 transcript abundance was increased in the female mouse amygdala. Further, the brain-derived neurotrophic factor transcript abundance was decreased in female mice hippocampus, a gene responsive to stress and associated to neuronal survival. Female rat presented an increase in the transcript abundance of glucocorticoid receptor (at birth) and FK506 binding protein 5 (in adulthood) in the amygdala. No differences were detected in the limbic brain regions (hippocampus and amygdala) investigated in mouse and rat males. Further, the changes in brain-derived neurotrophic factor and FK506 binding protein 5 transcript abundance were accompanied by correlated changes in DNA methylation level within their exon and

Table 10. Comparative effects of stressors on hormone levels

Treatment	Trait			
	Baseline GC	Stress response	ACTH	Thyroxine
Predation consensual effect	↑	↑	↑	N.A.
PO male mice	=	=	N.A.	↑
PO female mice	=	↑	N.A.	↑
PO male rat	=	=	=	N.A.
PO female rat	=	=	Recov.↑	N.A.
Laboratory PRS/CVS animals	Birth ↓	↑	N.A.	N.A.

PO: Prenatal predator Odor exposure
PRS: Prenatal Restraint Stress
CVS: Chronic Variable Stress
GC: Glucocorticoids
ACTH: Adrenocorticotropic hormone
N.A.: Not Available
Recov.: Recovery level

promoter, respectively.

7.3.5.2 Gene transcript abundance and epigenetic modifications phenotype elicited by typical predation stress

Predator or predator odour exposure lead to an increase in the transcript abundance of stress-related genes, especially the corticosterone-releasing factor which is the most consistently reported (Figueiredo et al., 2003; Roseboom et al., 2007; Thomson et al., 2012). Transthyretin, a thyroid hormone transporter down-regulated during stress, presented a strong decrease in hippocampal transcript abundance in snowshoe hares under high predation stress (Lavergne et al., 2014). This reduction is most likely a downstream effect of the elevated stress experienced by these animals. Further, a general brain region activation through the transcription of immediate-early genes was also detected in brain regions such as the hippocampus and amygdala (Asok et al., 2013; Masini et al., 2005). Epigenetic modifications have not been investigated in the context of predator stress exposure.

7.3.5.3 Gene transcript abundance and epigenetic phenotype elicited by the traditional prenatal restraint stress and prenatal chronic variable stress

The prenatal restraint stress and prenatal chronic variable stress lead rodents to exhibit a decreased glucocorticoid receptor transcript abundance (Koehl et al., 1999; Mueller and Bale, 2008) accompanied by modified DNA methylation in males prenatally exposed to chronic variable stress (Mueller and Bale, 2008). Further, the brain-derived neurotrophic factor was expressed at a lower level in both prenatal exposure paradigms along with a general increase in brain-derived neurotrophic factor methylation (Boersma et al., 2014; Dong et al., 2015; Monteleone et al., 2014). Finally, rodents prenatally exposed to chronic variable stress presented an increased corticosterone-releasing hormone transcript abundance (Mueller and Bale, 2008).

7.3.5.4 Comparison highlights between the gene transcript abundance and epigenetic modifications phenotype elicited by the various prenatal stress paradigms

The phenotype of rodents from predator odour-exposed dams reported here regarding gene transcript abundance and epigenetic modifications was detected in female limbic structures only. It is not excluded, and most probable, that males from predator odour-exposed dams present differences in transcript abundance or epigenetic modifications in other brain regions or in unexplored to date stress-related genes. The paraventricular nucleus of the hypothalamus is an especially promising brain region in this regard as it is the master regulator of the hypothalamic-pituitary adrenal and hypothalamic-pituitary thyroid axis (Tsigos and Chrousos, 2002; Zoeller et al., 2007). In the future, a transcriptome analysis using RNA sequencing (RNA-Seq) of the paraventricular nucleus of the hypothalamus would be especially useful to elucidate the male underlying modifications in transcript abundance.

Although the impact of predator stress on gene transcript abundance has only been explored for candidate genes associated with the hypothalamic-pituitary adrenal axis, the reported effects are in accordance with the modifications found in females from predator odour-exposed dams. The prenatal restraint stress and prenatal chronic variable stress phenotype generally produces effects (decreased brain-derived neurotrophic factor and increased corticosterone-releasing factor transcript abundance) in accordance with those observed in the females from predator odour-exposed dams (decreased brain-derived neurotrophic factor and increased corticosterone-releasing factor receptor 1 transcript abundance). However, not only is the sustained decrease in glucocorticoid receptor transcript abundance found in both traditional prenatally stressed phenotypes not found in females from predator odour-exposed dams, but we see the opposite in rat females at birth (Table 11).

Table 11. Comparative effects of stressors on transcript abundance and epigenetic modifications

Treatment	Transcript abundance				Epigenetic modification	
	NR3C1	CRF family	BDNF	Other stress-related genes	BDNF DNA methylation	Stress-related genes DNA methylation
Predation consensual effect	N.A.	↑	N.A.	↑	N.A.	N.A.
PO male mice	=	=	=	=	N.A.	N.A.
PO female mice	=	amy.↑	hipp.↓	=	↓	N.A.
PO male rat	=	=	=	=	N.A.	N.A.
PO female rat	Birth amy.↑	=	=	FKBP5 amy.↑	N.A.	↓
Laboratory PRS/CVS animals	↓	M↑	↓	✔	↑	✔

PO: Prenatal predator Odour exposure

PRS: Prenatal Restraint Stress

CVS: Chronic Variable Stress

NR3C1: Glucocorticoid receptor

N.A.: Not Available

Amy.: Amygdala

CRF: Corticotropin-Releasing Factor

M: Male

BDNF: Brain-Derived Neurotrophic Factor

Hipp.: Hippocampus

AVP: Ariginine VasoPressin

FKBP5: FK506 Binding Protein 5

✔: Induced trait

7.3.6 Comparison of the integrative phenotype elicited by the predator stress, the traditional prenatal stress paradigms and the prenatal predator odour exposure

The phenotype of rodents from predator odour-exposed dams is generally consistent and convergent through all trait types with increased stress-reactivity at the behavioural, physiological, endocrine, gene transcript abundance and epigenetic level. In both sexes mice and rats show increased stress-related behaviour although some sex and species specificities were detected that could in part be explained through the test protocols and apparatus differences. Interestingly, in both mice and rats, we found a strong and consistent sex difference with females from predator odour-exposed dams showing alterations at the endocrine, gene transcript abundance and epigenetic modifications indicating increased stress reactivity. Therefore, although the endocrine, gene transcript abundance and regulation mechanisms are sex-specific, the general behavioural and physiological (oxygen consumption) phenotype converges between the sexes. The male endocrine, gene transcript abundance and epigenetic modifications are yet to be detected and could be present within the paraventricular nucleus of the hypothalamus. On the other hand, species differences could be due in part to the strain used here as Long-Evans outbred rats and C57BL/6 inbred mouse exhibit very different genetic variability.

It is relevant to note that the phenotype of mouse and rat from predator odour-exposed dams converge toward a common increase in stress-related behaviours and female stress-related genes transcript abundance and epigenetic pathways. This convergence highlights the efficiency and redundancy inherent to the hypothalamic-pituitary adrenal axis, making it especially efficient to detect stressful environmental information. The hypothalamic-pituitary adrenal axis input can be detected and then potentiated at the olfactory receptor level (receptor abundance, wiring intensity), initial production of the signal at the hypothalamus and pituitary level (corticosterone-releasing factor, arginine-vasopressin and adrenocorticotropic hormone transcription and release), downstream at the adrenal level (corticosterone and corticosterone-binding globulin transcription and

release) and finally at the feedback level within several limbic brain regions (e.g. amygdala and hippocampal glucocorticoid receptor and co-chaperone transcription). Programming at any of these levels of regulation may be sufficient to mediate this increased stress reactivity phenotype and influence the other regulatory levels of the hypothalamic-pituitary adrenal axis regulation. This complexity and redundancy explains how the hypothalamic-pituitary adrenal axis is especially well suited for programming during the perinatal life. Ultimately, all of these levels should be investigated in the rodents from predator odour-exposed dams to provide a more complete mechanistic understanding of the underlying prenatal predator odour exposure programming.

The prenatal predator odour exposure phenotype is overall in agreement with the general phenotypic adaptations elicited by predator or predator cue presence in mammalian preys (Table 3) regarding the increase in stress-related behaviours in several contexts, increase metabolism, endocrine response and available information about the gene transcript abundance modifications detected in juvenile and adult animals. However, the prenatal predator or predator cue exposure has been less studied and mostly confined to early life (newborns and juveniles) (Table 4), which makes direct comparison difficult and underscores the value of the approach and analysis of the integrative prenatal predator odour exposure phenotype presented in this thesis. Therefore, the prenatal predator odour exposure phenotype can be interpreted as the programming of adaptations useful in a situation where predators are present. Notably, these adaptations (increase live predator and predator odour avoidance) are present in adult animals before any direct exposure to predator or predator cues.

One way to represent the difference in the integrative stress response detected in rodents from predator odour-exposed dams is represented in Error! Reference source not found.. The baseline or control animal stress response is graded as an inverted U-shape (based on the balancing of pressure and performance also known as the Yerkes-Dodson law from Broadhurst (1956) in response to intermediate levels of stress. In rodents from predator odour-exposed dams, the stress response appears to be elicited by a broadening of the window of stress

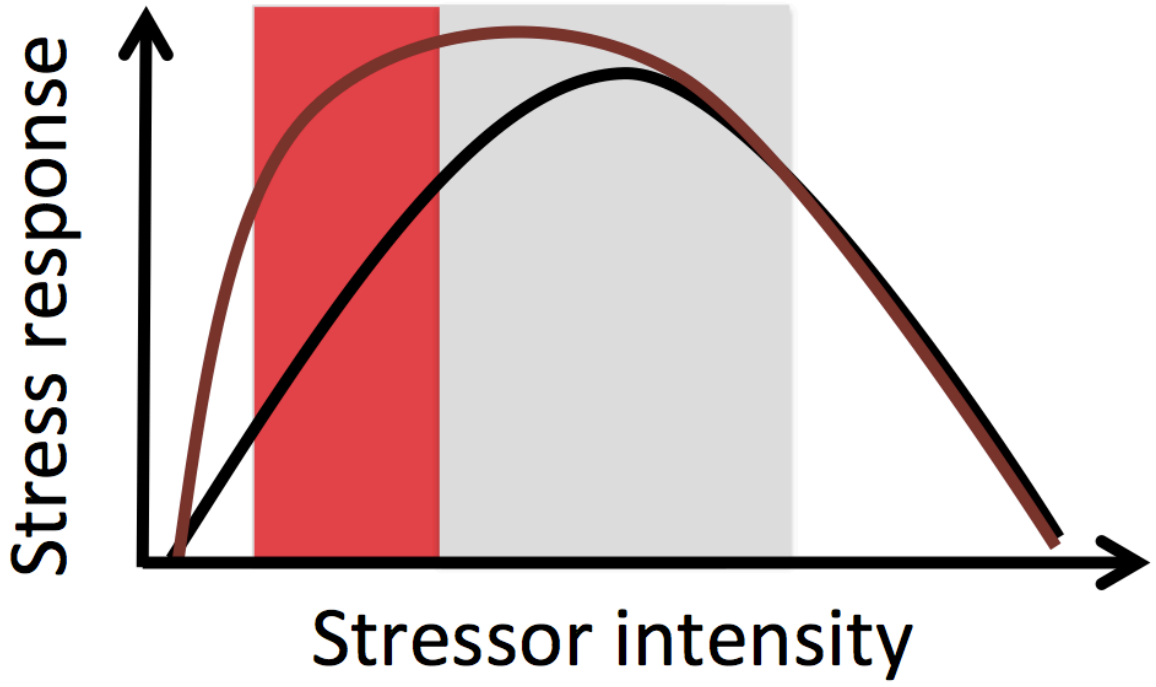


Figure 29. Stress response window in control and prenatally predator odour exposed rodents.

Control rodents (black curve) show a stress response to an intermediate window of stressor intensity (grey rectangle). However, prenatally predator odour exposed rodents, especially females, seem to respond to a wider window (red rectangle extension) of stressors through a response to lower intensity stressors (dark red curve). According to the prenatal predator odour exposure phenotype, this detection of lower intensity stressors might be especially oriented toward predator and predator cue-oriented stressors.

intensity spanning to milder stressor intensities, most probably especially when these stressors are associated to predator presence or predator cues.

The prenatal chronic variable stress and prenatal restraint stress phenotypes are generally converging behaviourally, although anti-predatory behaviour and foraging behaviour have not been assessed. However, there is divergence from the prenatal predator odour exposure phenotype in several aspects including the increased weight at birth observed in rodents prenatally exposed to chronic variable stress and the decreased baseline glucocorticoids and glucocorticoid receptor transcript abundance in both prenatal restraint stress and prenatal chronic variable stress exposed rodents. There was also no possible comparison of the metabolic rate with the rodents from predator odour-exposed dams as these measures have not been investigated in relation to prenatal restraint stress or prenatal chronic variable stress. The decreased birth weight and increased baseline corticosterone detected in rodents prenatally restrained or exposed to chronic variable stress is also of interest as a translational phenotype as it can trigger the 'metabolic syndrome' described earlier (see section 1.2.1 on page 2) along with chronic stress impacts described in Table 1. Therefore, one of the main differences between the prenatal predator odour exposed and prenatal restraint stress/prenatal chronic variable stress phenotypes is the way their impacts can be interpreted.

7.4 Prenatal predator odour exposure phenotype and the prenatal stress hypotheses

The prenatal predator odour exposure integrative phenotype does not match the classically described low-birth weight and incidental 'thrifty phenotype' or metabolic syndrome (Gluckman and Hanson, 2006; McMillen and Robinson, 2005). For example, rodents from predator odour-exposed dams are not developing hyperphagia or obesity typical to the metabolic syndrome (McMillen and Robinson, 2005). However, in the future, an insulin-resistance test should be conducted on the rodents from predator odour-exposed dams to rule out the classical increase risk of developing diabetes. The prenatal

predator odour exposure phenotype is therefore distinct from traditionally studied prenatal restraint stress and prenatal chronic variable stress paradigms.

The prenatal predator odour exposure phenotype better agrees with the mismatch and predicted adaptive response hypotheses (Bateson and Gluckman 2011) stating that developmental forecasting determines the individual developmental trajectory. The increase in rodents from predator odour-exposed dams responsiveness to predator and predator cue presence can be interpreted as a phenotype appropriate to the prenatal predicted environment. The prenatal predator odour exposure phenotype can be interpreted as an adaptive response to a naturally occurring range of cues and signals as predicted by the predicted adaptive response hypothesis (Bateson et al., 2014). The prenatal predator odour exposure phenotype detected may maximize the offspring fitness through increasing survival as a result of an increase in the expression of several increased anti-predatory behaviours. However, direct fitness consequences should be evaluated in the future.

Finally, prenatal predator odour exposure elicits long-term phenotypic modifications in rodents that appear to be mediated at least in part, though long-term epigenetic modifications, especially in females. As this paradigm leads to adaptive outcomes, it should be used to study the persistence of the impacts of early life stress over several downstream generations in absence of the original stressor to observe its persistence in time.

References

Abramsky, Z., Strauss, E., Subach, A., Kotler, B.P., Riechman, A., 1996. The effect of barn owls (Tyto alba) on the activity and microhabitat selection of Gerbillus allenbyi and G. pyramoidum. Oecologia 105, 313–319. doi:10.1007/BF00328733

Adamec, R., Bartoszyk, G.D., Burton, P., 2004a. Effects of systemic injections of Vilazodone, a selective serotonin reuptake inhibitor and serotonin 1 A receptor agonist, on anxiety induced by predator stress in rats. European Journal of Pharmacology 504, 65–77. doi:10.1016/j.ejphar.2004.09.009

Adamec, R., Burton, P., Blundell, J., Murphy, D.L., Holmes, A., 2006. Vulnerability to mild predator stress in serotonin transporter knockout mice. Behavioural Brain Research 170, 126–140. doi:10.1016/j.bbr.2006.02.012

Adamec, R., Kent, P., Anisman, H., Shallow, T., Merali, Z., 1998. Neural plasticity, neuropeptides and anxiety in animals—implications for understanding and treating affective disorder following traumatic stress in humans. Neuroscience & Biobehavioral Reviews 23, 301–318.

Adamec, R., Walling, S., Burton, P., 2004b. Long-lasting, selective, anxiogenic effects of feline predator stress in mice. Physiology & Behavior 83, 401–410. doi:10.1016/j.physbeh.2004.08.029

Adamec, R.E., Blundell, J., Burton, P., 2003. Phosphorylated cyclic AMP response element binding protein expression induced in the periaqueductal gray by predator stress: Its relationship to the stress experience, behavior and limbic neural plasticity. Progress in Neuro-Psychopharmacology and Biological Psychiatry 27, 1243–1267. doi:10.1016/j.pnpbp.2003.09.017

Adamec, R.E., Blundell, J., Burton, P., 2005. Neural circuit changes mediating lasting brain and behavioral response to predator stress. Neuroscience and Biobehavioral Reviews 29, 1225–1241. doi:10.1016/j.neubiorev.2005.05.007

Ader, R., Friedman, S.B., Grota, L.J., 1967. “Emotionality” and adrenal cortical function: Effects of strain, tests, ad the 24-hour corticosterone rhythm. Animal Behaviour 15,

37–44.

Agrawal, A.A., Laforsch, C., Tollrian, R., 1999. Transgenerational induction of defences in animals and plants. Nature 401, 60–63. doi:10.1038/43425

Ahmadzadeh, R., Saboory, E., Roshan-Milani, S., Pilehvarian, A.A., 2011. Predator and restraint stress during gestation facilitates pilocarpine-induced seizures in prepubertal rats. Developmental Psychobiology 53, 806–812. doi:10.1002/dev.20555

Albonetti, M.E., Farabollini, F., 1992. Behavioural responses to single and repeated in male and female rats restraint. Behavioural processes 28, 97–110.

Allis, C., Jenuwein, T., Reinberg, D., Caparros, M., 2007. Epigenetics. Cold Spring Harbor Laboratory Press, New York.

Andersen, S.L., Sonntag, K.C., 2014. Juvenile methylphenidate reduces prefrontal cortex plasticity via D3 receptor and BDNF in adulthood. Frontiers in Synaptic Neuroscience 6, 1–8. doi:10.3389/fnsyn.2014.00001

Anderson, D.K., Rhees, R.W., Fleming, D.E., 1985. Effects of prenatal stress on differentiation of the sexually dimorphic nucleus of the preoptic area (SDN-POA) of the rat brain. Brain Research 332, 113–118. doi:10.1016/0006-8993(85)90394-4

Anisman, H., 2009. Cascading effects of stressors and inflammatory immune system activation : implications for major depressive disorder. Journal of Psychiatry & Neuroscience 34, 4–20.

Apfelbach, R., Blanchard, C.D., Blanchard, R.J., Hayes, R.A., McGregor, I.S., 2005. The effects of predator odors in mammalian prey species: a review of field and laboratory studies. Neuroscience and Biobehavioral Reviews 29, 1123–1144. doi:10.1016/j.neubiorev.2005.05.005

Armario, A., Escorihuela, R.M., Nadal, R., 2008. Long-term neuroendocrine and behavioural effects of a single exposure to stress in adult animals. Neuroscience and Biobehavioral Reviews 32, 1121–1135. doi:10.1016/j.neubiorev.2008.04.003

Asok, A., Ayers, L.W., Awoyemi, B., Schulkin, J., Rosen, J.B., 2013. Immediate early gene and neuropeptide expression following exposure to the predator odor 2 , 5-dihydro-2 , 4 , 5-trimethylthiazoline. Behavioural Brain Research 248, 85–93. doi:10.1016/j.bbr.2013.03.047

Ayers, L.W., Asok, A., Blaze, J., Roth, T.L., Rosen, J.B., 2016. Changes in dam and pup behavior following repeated postnatal exposure to a predator odor (TMT): A preliminary investigation in long-evans rats. Developmental Psychobiology 58, 176–184. doi:10.1002/dev.21362

Bailey, K.R., Crawley, J.N., 2009. Anxiety-Related Behaviors in Mice, in: Buccafusco, J.J., Boca, R. (Eds.), Methods of Behavior Analysis in Neuroscience. Taylor & Francis Group, LLC.

Banik, A., Anand, A., 2011. Loss of learning in mice when exposed to rat odor: a water maze study. Behavioural Brain Research 216, 466–471. doi:10.1016/j.bbr.2010.07.035

Baram, T.Z., Davis, E.P., Obenaus, A., Sandman, C.A., Small, S.L., Solodkin, A., Stern, H., 2012. Fragmentation and unpredictability of early-life experience in mental disorders. American Journal of Psychiatry 169, 907–915. doi:10.1176/appi.ajp.2012.11091347

Barlow, S.M., Knight, A.F., Sullivan, F.M., 1978. Delay in postnatal growth and development of offspring produced by maternal restraint stress during pregnancy in the rat. Teratology 18, 211–218. doi:10.1002/tera.1420180206

Barros, V.G., Rodriguez, P., Martijena, I.D., Pérez, A., Molina, V.A., Antonelli, M.C., 2006. Prenatal stress and early adoption effects on benzodiazepine receptors and anxiogenic behavior in the adult rat brain. Synapse 60, 609–618. doi:10.1002/syn.20336

Bateson, P., Gluckman, P., 2011. Plasticity, Robustness, Development and Evolution. Campbridge University Press, Campbridge.

Bateson, P., Gluckman, P., Hanson, M., 2014. The biology of developmental plasticity and the Predictive Adaptive Response hypothesis. The Journal of Physiology 592, 2357–68. doi:10.1113/jphysiol.2014.271460

Baughman, G., Wiederrecht, G.J., Chang, F., Martin, M.M., Bourgeois, S., 1997. Tissue distribution and abundance of human FKBP51, and FK506-binding protein that can mediate calcineurin inhibition. Biochemical and Biophysical Research Communications 232, 437–443. doi:10.1006/bbrc.1997.6307

Beeler, J.A., Prendergast, B., Zhuang, X., 2006. Low amplitude entrainment of mice and

the impact of circadian phase on behavior tests. Physiology & Behavior 87, 870–880. doi:10.1016/j.physbeh.2006.01.037

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. doi:10.1016/j.psyneuen.2015.06.012

Belzung, C., El, W., Moindrot, N., Griebel, G., 2001. Behavioral and neurochemical changes following predatory stress in mice. Neuropharmacology 41, 400–408.

Bennett, M.R., Lagopoulos, J., 2014. Stress and trauma: BDNF control of dendritic-spine formation and regression. Progress in Neurobiology 112, 80–99. doi:10.1016/j.pneurobio.2013.10.005

Berry, R.J., 1970. The Natural History of the House Mouse. Field Study 3, 219–262.

Berry, R.J., Bronson, F.H., 1992. Life-History and Bioeconomy of the House Mouse. Biological Reviews 67, 519–550.

Bertoglio, L.J., Carobrez, A.P., 2002. Behavioral profile of rats submitted to session 1-session 2 in the elevated plus-maze during diurnal/nocturnal phases and under different illumination conditions. Behavioural Brain Research 132, 135–143.

Bestion, E., Teyssier, A., Aubret, F., Clobert, J., Cote, J., 2014. Maternal exposure to predator scents : offspring phenotypic adjustment and dispersal. Proceedings of the Royal Society B-Biological Sciences 281, 20140701. doi:10.1098/rspb.2014.0701.

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 34, 186–195. doi:10.1016/j.psyneuen.2009.05.021

Binder, E.B., Bradley, R.G., Epstein, M.P., Deveau, T.C., Mercer, K.B., Heim, C.M., Nemeroff, C.B., Schwartz, A.C., Cubells, J.F., 2008. Association of FKBP5 Polymorphisms and Childhood Abuse With Risk of Posttraumatic Stress Disorder Symptoms in Adults. JAMA: The Journal of the American Medical Association 299, 1291–1305. doi:10.1016/j.jsbmb.2011.07.002.Identification

Biro, P.A., Stamps, J.A., 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends in Ecology and

Evolution 25, 653–659. doi:10.1016/j.tree.2010.08.003

Blanchard, R.J., Blanchard, D.C., 1989. Antipredator defensive behaviors in a visible burrow system. Journal of Comparative Psychology 103, 70–82. doi:10.1037/0735-7036.103.1.70

Blanchard, R.J., Nikulina, J.N., Sakai, R.R., McKittrick, C., McEwen, B., Blanchard, D.C., 1998. Behavioral and endocrine change following chronic predatory stress. Physiology and Behavior 63, 561–569. doi:10.1016/S0031-9384(97)00508-8

Blundell, J., Adamec, R., Burton, P., 2005. Role of NMDA receptors in the syndrome of behavioral changes produced by predator stress. Physiology & Behavior 86, 233–243. doi:10.1016/j.physbeh.2005.07.012

Boersma, G.J., Lee, R.S., Cordner, Z. a, Ewald, E.R., Purcell, R.H., Moghadam, A. a, Tamashiro, K.L., 2014. Prenatal stress decreases Bdnf expression and increases methylation of Bdnf exon IV in rats. Epigenetics 9, 1–11. doi:10.4161/epi.27558

Boonstra, R., Dantzer, B., Delehanty, B., Fletcher, Q., Sheriff, M., 2014. Equipped for Life in the Boreal Forest: The Role of the Stress Axis in Mammals. Arctic 67, 82–97. doi:http://dx.doi.org/10.14430/arctic4357

Boonstra, R., Hik, D., Singleton, G.R., Tinnikov, A., 1998. the Impact of Predator-Induced Stress on the Snowshoe Hare Cycle. Ecology Monographs 79, 371–394. doi:10.1890/0012-9615(1998)068[0371:TIOPIS]2.0.CO;2

Bornstein, S.R., Engeland, W.C., Ehrhart-Bornstein, M., Herman, J.P., 2008. Dissociation of ACTH and glucocorticoids. Trends in Endocrinology and Metabolism 19, 175–180. doi:10.1016/j.tem.2008.01.009

Boulle, F., van den Hove, D.L.A., Jakob, S.B., Rutten, B.P., Hamon, M., van Os, J., Lesch, K.-P., Lanfumey, L., Steinbusch, H.W., Kenis, G., 2012. Epigenetic regulation of the BDNF gene: implications for psychiatric disorders. Molecular Psychiatry 17, 584–596. doi:10.1038/mp.2011.107

Bouwknecht, A.J., Olivier, B., Paylor, R.E., 2007. The stress-induced hyperthermia paradigm as a physiological animal model for anxiety: A review of pharmacological and genetic studies in the mouse. Neuroscience and Biobehavioral Reviews 31, 41–59. doi:10.1016/j.neubiorev.2006.02.002

Bowers, M. a, Dooley, J.L.J., Url, S., 1993. Predation Hazard and Seed Removal by

Small Mammals: Mircohabitat versus Patch Sacle Effects. Oecologia 94, 247–254. doi:10.1007/BF00341324

Bowman, R.E., Maclusky, N.J., Sarmiento, Y., Frankfurt, M., Gordon, M., Luine, V.N., 2004. Sexually dimorphic effects of prenatal stress on cognition, hormonal responses, and central neurotransmitters. Endocrinology 145, 3778–3787. doi:10.1210/en.2003-1759

Brabrand, Å., Faafeng, B., 1993. Habitat shift in roach (Rutilus rutilus) induced by pikeperch (Stizostedion lucioperca) introduction: predation risk versus pelagic behaviour. Oecologia 95, 38–46. doi:10.1007/BF00649504

Broadhurst, P., 1956. Emotionality and the Yerkes-Dodson Law. Journal of Experimental Psychology 54, 345–352.

Brown, J.S., Kotler, B.P., 2004. Hazardous duty pay and the foraging cost of predation. Ecology Letters 7, 999–1014. doi:10.1111/j.1461-0248.2004.00661.x

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. Journal of Neuroendocrinology 22, 258–271. doi:10.1111/j.1365-2826.2010.01969.x

Brust, V., Schindler, P.M., Lewejohann, L., 2015. Lifetime development of behavioural phenotype in the house mouse (Mus musculus). Frontiers in Zoology 12, S17. doi:10.1186/1742-9994-12-S1-S17

Bryant, D.M., Hails, C.J., 1975. Mechanisms of heat conservation in the litters of mice (Mus musculus L.). Comparative Biochemistry and Physiology - Part A: Physiology 50, 99–104. doi:10.1016/S0010-406X(75)80208-8

Bult, A., Lynch, C.B., 1997. Nesting and fitness: Lifetime reproductive success in house mice bidirectionally selected for thermoregulatory nest-building behavior. Behavior Genetics 27, 231–240. doi:10.1023/A:1025610130282

Burton, C., Lovic, V., Fleming, A.S., 2006. Early adversity alters attention and locomotion in adult Sprague-Dawley rats. Behavioral Neuroscience 120, 665–675. doi:10.1037/0735-7044.120.3.665

Burton, P.J., Waddell, B.J., 1994. 11 beta-hydroxysteroid dehydrogenase in the rat placenta: developmental changes and the effects of altered glucocorticoid exposure.

Journal of Endocrinology 143, 505–513.

Buynitsky, T., Mostofsky, D.I., 2009. Restraint stress in biobehavioral research: Recent developments. Neuroscience and Biobehavioral Reviews 33, 1089–1098. doi:10.1016/j.neubiorev.2009.05.004

Cai, W.H., Blundell, J., Han, J., Greene, R.W., Powell, C.M., 2006. Postreactivation glucocorticoids impair recall of established fear memory. Journal of Neuroscience 26, 9560–9566.

Calisi, R.M., Bentley, G.E., 2009. Lab and field experiments: Are they the same animal? Hormones and Behavior 56, 1–10. doi:10.1016/j.yhbeh.2009.02.010

Calogero A, GaIIucci WT, Gold PW, C.G., 1988. Multiple feedback regulatory loops upon rat hypothalamic corticotropin releasing hormone secretion. The Journal of Clinical Investigation 82, 767– 74. doi:10.1172/JCI113677

Campbell, T., Lin, S., DeVries, C., Lambert, K., 2003. Coping strategies in male and female rats exposed to multiple stressors. Physiology & behavior 78, 495–504. doi:10.1016/S0031-9384(03)00033-7

Campeau, S., Nyhuis, T.J., Sasse, S.K., Day, H.E.W., Masini, C.V., 2008. Acute and chronic effects of ferret odor exposure in Sprague-Dawley rats. Neuroscience and Biobehavioral Reviews 32, 1277–1286. doi:10.1016/j.neubiorev.2008.05.014.Acute

Campos-Barros, A., Musa, A., Flechner, A., Hessenius, C., Gaio, U., Meinhold, H., Baumgartner, A., 1997. Evidence for circadian variations of thyroid hormone concentrations and type II 5'-iodothyronine deiodinase activity in the rat central nervous system. Journal of Neurochemistry 68, 795–803. doi:10.1046/j.1471-4159.1997.68020795.x

Careau, V., Thomas, D., Pelletier, F., Turki, L., Landry, F., Garant, D., Réale, D., 2011. Genetic correlation between resting metabolic rate and exploratory behaviour in deer mice (Peromyscus maniculatus). Journal of Evolutionary Biology 24, 2153–63. doi:10.1111/j.1420-9101.2011.02344.x

Carobrez, A.P., Bertoglio, L.J., 2005. Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. Neuroscience and Biobehavioral Reviews 29, 1193–1205. doi:10.1016/j.neubiorev.2005.04.017

Cavigelli, S.A., Monfort, S.L., Whitney, T.K., Mechref, Y.S., Novotny, M., McClintock,

M.K., 2005. Frequent serial fecal corticoid measures from rats reflect circadian and ovarian corticosterone rhythms. Journal of Endocrinology 184, 153–163. doi:10.1677/joe.1.05935

Chabot, D., Gagnon, P., Dixon, E.A., 1996. Effect of Predator Odors on Heart Rate and Metabolic Rate of Wapiti (Cervus elaphus canadensis). Journal of Chemical Ecology 22, 839–868.

Chali, D., Enquselassie, F., Gesese, M., 1998. A case-control study on determinants of rickets. Ethiopian Medical Journal 36, 227–234.

Champagne, F.A., Francis, D.D., Mar, A., Meaney, M.J., 2003. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiology & Behavior 79, 359–371. doi:10.1016/S0031-9384(03)00149-5

Champagne, F.A., Weaver, I.C.G., Diorio, J., Dymov, S., Szyf, M., Meaney, M.J., 2006. Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. Endocrinology 147, 2909–2915. doi:10.1210/en.2005-1119

Charil, A., Laplante, D.P., Vaillancourt, C., King, S., 2010. Prenatal stress and brain development. Brain Research Reviews 65, 56–79. doi:10.1016/j.brainresrev.2010.06.002

Chaudhury, D., Colwell, C.S., 2002. Circadian modulation of learning and memory in fear-conditioned mice. Behavioural Brain Research 133, 95–108.

Chin, E.H., Love, O.P., Verspoor, J.J., Williams, T.D., Rowley, K., Burness, G., 2009. Juveniles exposed to embryonic corticosterone have enhanced flight performance. Proceedings of the Royal Society B: Biological Sciences 276, 499–505. doi:10.1098/rspb.2008.1294

Choi, J.-S., Kim, J.J., 2010. Amygdala regulates risk of predation in rats foraging in a dynamic fear environment. Proceedings of the National Academy of Sciences 107, 21773–21777. doi:10.1073/pnas.1010079108

Christensen, B., Persson, L., 1993. Species-specific antipredatory behaviours: effects on prey choice in different habitats. Behavioral Ecology and Sociobiology 32, 1–9. doi:10.1007/BF00172217

Christianson, D., Creel, S., 2010. A nutritionally mediated risk effect of wolves on elk. Ecology 91, 1184–1191. doi:10.1890/09-0221.1

Chrousos, G.P., 1998. Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. Annals of the New York Academy of Sciences 851, 311–335. doi:10.1111/j.1749-6632.1998.tb09006.x

Chrousos, G.P., Gold, P.W., 1992. The Concepts of Stress and Stress System Disorders Overview of. JAMA: The Journal of the American Medical Association 267, 1244–52. doi:10.1001/jama.267.9.1244

Clancy, B., Darlington, B., Finlay, B.L., 2001. Translating developmental time across species. Neuroscience 105, 7–17.

Clinchy, M., Schulkin, J., Zanette, L.Y., Sheriff, M.J., McGowan, P.O., Boonstra, R., 2011a. The neurological ecology of fear: Insights neuroscientists and ecologists have to offer one another. Front. Behav. Neurosci. 4, 21. doi:10.3389/fnbeh.2011.00021

Clinchy, M., Zanette, L., Charlier, T.D., Newman, A.E.M., Schmidt, K.L., Boonstra, R., Soma, K.K., 2011b. Multiple measures elucidate glucocorticoid responses to environmental variation in predation threat. Oecologia 166, 607–614. doi:10.1007/s00442-011-1915-2

Clinchy, M., Zanette, L.Y., Boonstra, R., Wingfield, J.C., Smith, J.N.M., 2004. Balancing food and predator pressure induces chronic stress in songbirds. Proceedings of the Royal Society B-Biological Sciences 271, 2473–2479. doi:10.1098/rspb.2004.2913

Coutellier, L., Würbel, H., 2009. Early environmental cues affect object recognition memory in adult female but not male C57BL/6 mice. Behavioural Brain Research 203, 312–315. doi:10.1016/j.bbr.2009.05.001

Creel, S., Christianson, D., Liley, S., Winnie, J.A., 2007. Predation Risk Affects Reproductive Physiology and Demography of Elk. Science 315, 960–960. doi:10.1126/science.1135918

Creel, S., Winnie, J.A., Christianson, D., Liley, S., 2008. Time and space in general models of antipredator response: tests with wolves and elk. Animal Behaviour 76, 1139–1146. doi:10.1016/j.anbehav.2008.07.006

Creel, S., Winnie, J., Maxwell, B., Hamlin, K., Creel., M., 2005. Elk Alter Habitat Selection as an Antipredator Response to Wolves. Ecology 86, 3387–3397.

doi:10.1890-05-0032

Crews, D., Gore, A.C., Hsu, T.S., Dangleben, N.L., Spinetta, M., Schallert, T., Anway, M.D., Skinner, M.K., 2007. Transgenerational epigenetic imprints on mate preference. Proceedings of the National Academy of Sciences 104, 5942–5946. doi:10.1073/pnas.0610410104

Cruz-Topete, D., Cidlowski, J.A., 2014. One hormone, two actions: Anti- And pro-inflammatory effects of glucocorticoids. NeuroImmunoModulation 22, 20–32. doi:10.1159/000362724

D'Agostino, J., Henning, S.J., 1981. Hormonal control of postnatal development of corticosteroid-binding globulin. The American Journal of Physiology 240, E402-6.

Dakine, N., Oliver, C., Grino, M., 2000. Thyroxine modulates corticotropin-releasing factor but not arginine vasopressin gene expression in the hypothalamic paraventricular nucleus of the developing rat. Journal of Neuroendocrinology 12, 774–783. doi:10.1046/j.1365-2826.2000.00520.x

Dallman, M.F., Levin, N., Cascio, C.S., Akana, S.F., Jacobson, L., Kuhn, R.W., 1989. Pharmacological evidence that the inhibition of diurnal adrenocorticotropin secretion by corticosteroids is mediated via type I corticosterone-preferring receptors. Endocrinology 124, 2844–2850.

Darnaudéry, M., Maccari, S., 2008. Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. Brain Research Reviews 57, 571–585. doi:10.1016/j.brainresrev.2007.11.004

Darnaudéry, M., Perez-Martin, M., Bélizaire, G., MacCari, S., Garcia-Segura, L.M., 2006. Insulin-like growth factor 1 reduces age-related disorders induced by prenatal stress in female rats. Neurobiology of Aging 27, 119–127. doi:10.1016/j.neurobiolaging.2005.01.008

Davis, M., 1992. The role of the amygdala in fear and anxiety. Annual Review in Neuroscience 15, 353–375.

de Catanzaro, D., 1988. Effect of predator exposure upon early pregnancy in mice. Physiology & Behavior 43, 691–696. doi:10.1016/0031-9384(88)90365-4

De Souza, M.A., Centenaro, L.A., Menegotto, P.R., Henriques, T.P., Bonini, J., Achaval, M., Lucion, A.B., 2013. Prenatal stress produces social behavior deficits and alters

the number of oxytocin and vasopressin neurons in adult rats. Neurochemical Research 38, 1479–1489. doi:10.1007/s11064-013-1049-5

Dias, B.G., Ressler, K.J., 2014. Parental olfactory experience influences behavior and neural structure in subsequent generations. Nature Neuroscience 17, 89–96. doi:10.1038/nn.3594

Diaz, R., Brown, R.W., Seckl, J.R., 1998. Distinct ontogeny of glucocorticoid and mineralocorticoid receptor and 11beta-hydroxysteroid dehydrogenase types I and II mRNAs in the fetal rat brain suggest a complex control of glucocorticoid actions. The Journal of Neuroscience 18, 2570–2580.

Dickman, C.R., 1992. Predation and Habitat Shift in the House Mouse, Mus Domesticus. Ecology 73, 313–322.

Dielenberg, R.A., Hunt, G.E., McGregor, I.S., 2001. "When a rat smells a cat": The distribution of Fos immunoreactivity in rat brain following exposure to a predatory odor. Neuroscience 104, 1085–1097. doi:10.1016/S0306-4522(01)00150-6

Dong, E., Dzitoyeva, S.G., Matrisciano, F., Tueting, P., Grayson, D.R., Guidotti, A., 2015. BDNF epigenetic modifications associated with schizophrenia-like phenotype induced by prenatal stress in mice. Biological Psychiatry 77, 589–596. doi:10.1016/j.biopsych.2014.08.012.BDNF

Dwivedi, Y., Razavi, H., Pandey, G., 2007. Antidepressants reverse corticosterone-mediated decrease in BDNF expression: Differential regulation of specific exons by antidepressants and corticosterone. Neuroscience 139, 1017–1029. doi:10.1016/j.neuroscience.2005.12.058

Edinger, K., Frye, C., 2005. Testosterone's anti-anxiety and analgesic effects may be due in part to actions of its 5α-reduced metabolites in the hippocampus. Psychoneuroendocrinology 30, 418–430. doi:http:11dx.doi.org/10.1016/j.psyneuen.2004.11.001

Eghbal-Ahmadi, M., Hatalski, C., Lovenberg, T., Avishai-Eline, S., Chalmers, D., Baram, T., 1998. The developmental profile of the corticotropin releasing factor receptor (CRF2) in rat brain predicts distinct age-specific functions. Brain Research. Developmental Brain Research 107, 81–90. doi:10.1016/j.immuni.2010.12.017.Two-stage

Ennaceur, A., 2014. Tests of unconditioned anxiety - Pitfalls and disappointments. Physiology and Behavior 135, 55–71. doi:10.1016/j.physbeh.2014.05.032

Erickson, Z.T., Falkenberg, E. a, Metz, G.A.S., 2014. Lifespan psychomotor behaviour profiles of multigenerational prenatal stress and artificial food dye effects in rats. PloS One 9, e92132. doi:10.1371/journal.pone.0092132

Fendt, M., Endres, T., Lowry, C.A., Apfelbach, R., McGregor, I.S., 2005. TMT-induced autonomic and behavioral changes and the neural basis of its processing. Neuroscience and Biobehavioral Reviews 29, 1145–1156. doi:10.1016/j.neubiorev.2005.04.018

Fenn, M.G.P., MacDonald, D.W., 1995. Use of Middens by Red Foxes: Risk Reverses Rhythms of Rats. Journal of Mammalogy 76, 130–136.

Figueiredo, H.F., Bodie, B.L., Tauchi, M., Dolgas, C.M., Herman, J.P., 2003. Stress Integration after Acute and Chronic Predator Stress: Differential Activation of Central Stress Circuitry and Sensitization of the Hypothalamo-Pituitary-Adrenocortical Axis. Endocrinology 144, 5249–5258. doi:10.1210/en.2003-0713

File, S., Zangrossi Jr, H., Sanders, F., Mabbutt, P., 1993. Dissociation Between Behavioral and Corticosterone Responses on Repeated Exposures to Cat Odor 54, 1109–1111.

Fleming, D.E., Anderson, R.H., Rhees, R.W., Kinghorn, E., Bakaitis, J., 1986. Effects of prenatal stress on sexually dimorphic asymmetries in the cerebral cortex of the male rat. Brain Research Bulletin 16, 395–398.

Fontaine, J.J., Martin, T.E., 2006. Parent birds assess nest predation risk and adjust their reproductive strategies. Ecology Letters 9, 428–434. doi:10.1111/j.1461-0248.2006.00892.x

Freeman, T.C., Dixon, A.K., Campbell, E.A., Tait, T.M., Richardson, P.J., Rice, K.M., Maslen, G.L., Metcalfe, A.D., Streuli, C.H., Bentley, D.R., 1998. FKBP5. MGI Direct Data Submission.

Fukudo, S., 2012. Hypothalamic-Pituitary-Adrenal Axis in Gastrointestinal Physiology, in: Johnson, L.R. (Ed.), Physiology of the Gastrointestinal Tract. Academic Press, pp. 791–816.

Galef, B.G.J., 1970. Aggression and timidity: Responses to novelty in feral Norway rats.

Journal of Comparative and Physiological Psychology 70, 370–381.

Galliot, E., Levaillant, M., Beard, E., Millot, J.-L., Pourié, G., 2010. Enhancement of spatial learning by predator odor in mice: involvement of amygdala and hippocampus. Neurobiology of Learning and Memory 93, 196–202. doi:10.1016/j.nlm.2009.09.011

Gaskill, B.N., Gordon, C.J., Pajor, E.A., Lucas, J.R., Davis, J.K., Garner, J.P., 2013. Impact of nesting material on mouse body temperature and physiology. Physiology & Behavior 110–111, 87–95. doi:10.1016/j.physbeh.2012.12.018

Gaskin, J., Kitay, J., 1971. Hypothalamic and pituitary regulation of adrenocortical function in the hamster: effects of gonadectomy and gonadal hormone replacement. Endocrinology 89, 1047–1053. doi:10.1210/endo-89-4-1047

Gewolb, I.H., Warshaw, J.B., 1986. Fetal and maternal corticosterone and corticosteroid binding globulin in the diabetic rat gestation. Pediatric Research 20, 155–160.

Ghasemi, A., Zahediasl, S., 2012. Normality Tests for Statistical Analysis: A Guide for Non-Statisticians. International Journal of Endocrinology and Metabolism 10, 486–489. doi:10.5812/ijem.3505

Giannotti, G., Caffino, L., Calabrese, F., Racagni, G., Riva, M. a, Fumagalli, F., 2014. Prolonged abstinence from developmental cocaine exposure dysregulates BDNF and its signaling network in the medial prefrontal cortex of adult rats. International Journal of Neuropharmacology 17, 625–634. doi:10.1017/S1461145713001454

Giesing, E.R., Suski, C.D., Warner, R.E., Bell, A.M., 2011. Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. Proceedings of the Royal Society B: Biological Sciences 278, 1753–1759. doi:10.1098/rspb.2010.1819

Glover, V., 2011. Annual research review: Prenatal stress and the origins of psychopathology: An evolutionary perspective. Journal of Child Psychology and Psychiatry and Allied Disciplines 52, 356–367. doi:10.1111/j.1469-7610.2011.02371.x

Glover, V., Hill, J., 2012. Sex differences in the programming effects of prenatal stress on psychopathology and stress responses: An evolutionary perspective. Physiology & Behavior 106, 736–740. doi:10.1016/j.physbeh.2012.02.011

Glover, V., O'Connor, T.G., O'Donnell, K., 2010. Prenatal stress and the programming of the HPA axis. Neuroscience and Biobehavioral Reviews 35, 17–22. doi:10.1016/j.neubiorev.2009.11.008

Gluckman, P.D., Hanson, M.A., 2006. Developmental origins of health and disease (DOHaD): Mechanisms and implications, in: Neill, J.D., Plant, T.M., Pfaff, D.W., Chalis, J.R.G., de Fretser, D.M., Richards, J.A.S., Wassarman, P.M. (Eds.), Knobil and Neill's Physiology of Reproduction. Elsevier Inc., San Diego, pp. 2975–2990. doi:10.1016/B978-0-12-515400-0.50061-0

Gold, P.W., 2015. The organization of the stress system and its dysregulation in depressive illness. Molecular Psychiatry 20, 1–16. doi:10.1038/mp.2014.163

Gould, T.D., Dao, D.T., Kovacsics, C.E., 2009. Mood and Anxiety Related Phenotypes in Mice. Neuromethods 42, 1–20. doi:10.1007/978-1-60761-303-9

Goverde, H.J.M., Pesman, G.J., Smals, A.G.H., 1989. The bioactivity of immunoreactive Adrenocorticotrophin in human blood is dependent on the secretery state of the pituitary gland. Clinical Endocrinology 31, 255–265.

Grant, E.C., Mackintosh, J.H., 1963. A comparison of the social postures of some common laboratory rodents. Behaviour 21, 246–259. doi:10.1163/156853963X00185

Gray, J.A., Lalljee, B., 1974. Sex differences in emotional behaviour in the rat: correlation between open-field defecation and active avoidance. Animal Behaviour 22, 856–861.

Gregus, A., Wintink, A.J., Davis, A.C., Kalynchuk, L.E., 2005. Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. Behavioural Brain Research 156, 105–114. doi:10.1016/j.bbr.2004.05.013

Griebel, G., Beeské, S., 2011. The Mouse Defense Test Battery : A Model Measuring Different Facets of Anxiety- Related Behaviors 1 Background and Historical Overview. Neuromethods 63, 97–106. doi:10.1007/978-1-61779-313-4_6

Grundwald, N.J., Benítez, D.P., Brunton, P.J., 2016. Sex-Dependent Effects of Prenatal Stress on Social Memory in Rats: A Role for Differential Expression of Central Vasopressin-1a Receptors. Journal of Neuroendocrinology 28.

doi:10.1111/jne.12343

Haidmayer, R., Hagmüller, K., 1981. Influence of Ambient Temperature on Calorigenic Action of Thyroid Hormones in Young Mice. European Journal of Physiology 391, 125–128.

Halasz, I., Rittenhouse, P.A., Zorilla, E.P., Redei, E., 1997. Sexually dimorphic effects of maternal adrenalectomy on hypothalamic corticotrophin-releasing factor, glucocorticoid receptor and anterior pituitary POMC mRNA levels of rat neonates. Developmental Brain Research 100, 198–204.

Hales, C.N., Barker, D.J.P., 1992. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetalogia 35, 595–601. doi:10.1093/ije/dyt133

Hales, C.N., Barker, D.J.P., 2001. The thrifty phenotype hypothesis: Type 2 diabetes. British Medical Bulletin 60, 5–20. doi:10.1093/bmb/60.1.5

Harmon, K.M., Greenwald, M.L., McFarland, A., Beckwith, T., Cromwell, H.C., 2009. The effects of prenatal stress on motivation in the rat pup. Stress 12, 250–258. doi:10.1038/cdd.2010.172.MicroRNAs

Harshaw, C., Alberts, J.R., 2012. Group and Individual Regulation of Physiology and Bahevior: A Behavioral, Thermographic, and Acoustic Study of Mouse Development. Physiology & Behavior 106, 670–682. doi:10.1016/j.physbeh.2012.05.002

Hart, J.S., Heroux, O., 1953. A comparison of some seasonal and temperature-induced changes in Peromyscus: cold restistance, metabolism, and pelage insulation. Canadian Journal Zoology 31, 528–534. doi:10.1139/z53-037

Hebb, A.L.O., Zacharko, R.M., Dominguez, H., Laforest, S., Gauthier, M., Levac, C., Drolet, G., 2003. Changes in brain cholecystokinin and anxiety-like behavior following exposure of mice to predator odor. Neuroscience 116, 539–551. doi:10.1016/S0306-4522(02)00710-8

Hellstrom, I.C., Dhir, S.K., Diorio, J.C., Meaney, M.J., 2012. Maternal licking regulates hippocampal glucocorticoid receptor transcription through a thyroid hormone-serotonin-NGFI-A signalling cascade. Philosophical Transactions of the Royal Society B: Biological Sciences 367, 2495–2510. doi:10.1098/rstb.2012.0223

Helmreich, D.L., Parfitt, D.B., Lu, X.Y., Akil, H., Watson, S.J., 2005. Relation between

the Hypothalamic-Pituitary-Thyroid (HPT) axis and the Hypothalamic-Pituitary-Adrenal (HPA) axis during repeated stress. Neuroendocrinology 81, 183–192. doi:10.1159/000087001

Helmreich, D.L., Tylee, D., 2011. Thyroid hormone regulation by stress and behavioral differences in adult male rats. Hormones and Behavior 60, 284–291. doi:10.1016/j.yhbeh.2011.06.003

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. Journal of Neuroendocrinology 6, 341–5.

Herman, J.P., 2010. Regulation of the HPA axis by acute and chronic stress, in: Koob, G.F., Le Moal, M., Thompson, R.F. (Eds.), Encyclopedia of Behavioral Neuroscience. Academic Press, London, pp. 149–153.

Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y., Ostrander, M.M., Choi, D.C., Cullinan, W.E., 2003. Central mechanisms of stress integration: Hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Frontiers in Neuroendocrinology 24, 151–180. doi:10.1016/j.yfrne.2003.07.001

Herman, J.P., Ostrander, M.M., Mueller, N.K., Figueiredo, H., 2005. Limbic system mechanisms of stress regulation: Hypothalamo-pituitary- adrenocortical axis. Progress in Neuro-Psychopharmacology and Biological Psychiatry 29, 1201–1213. doi:10.1016/j.pnpbp.2005.08.006

Héroux, O., 1970. Pathological Consequences of Artificial Cold Acclimatization. Nature 227, 88–89.

Hess, S.E., Rohr, S., Dufour, B.D., Gaskill, B.N., Pajor, E. a, Garner, J.P., 2008. Home improvement: C57BL/6J mice given more naturalistic nesting materials build better nests. Journal of the American Association for Laboratory Animal Science : JAALAS 47, 25–31.

Hillerer, K.M., Reber, S.O., Neumann, I.D., Slattery, D.A., 2011. Exposure to Chronic Pregnancy Stress Reverses Peripartum-Associated Adaptations: Implications for Postpartum Anxiety and Mood Disorders. Endocrinology 152, 3930–3940. doi:10.1210/en.2011-1091

Homberg, J.R., 2012. The stress-coping (mis)match hypothesis for nature ?? nurture

interactions. Brain Research 1432, 114–121. doi:10.1016/j.brainres.2011.11.037

Hubler, T.R., Scammell, J.G., 2004. Intronic hormone response elements mediate regulation of FKBP5 by progestins and glucocorticoids. Cell Stress and Chaperones 9, 243–52. doi:10.1379/CSC-32R.1

Jääskeläinen, T., Makkonen, H., Palvimo, J.J., 2011. Steroid up-regulation of FKBP51 and its role in hormone signaling. Current Opinion in Pharmacology 11, 326–331. doi:10.1016/j.coph.2011.04.006

Jedrzejewski, W., Rychlik, L., Jedrzejewska, B., 1993. Responses of Bank Voles to Odours of Seven Species of Predators: Experimental Data and Their Relevance to Natural Predator-Vole Relationships. Oikos 68, 251–257.

Joëls, M., Baram, T.Z., 2009. The neuro-symphony of stress. Nature Reviews Neuroscience 10, 459–466. doi:10.1038/nrn2632.

Jones, K.L., Smith, R.M., Edwards, K.S., Givens, B., Tilley, M.R., Beversdorf, D.Q., 2010. Combined effect of maternal serotonin transporter genotype and prenatal stress in modulating offspring social interaction in mice. Int. J. Dev. Neurosci. 28, 529–536. doi:10.1016/j.ijdevneu.2010.05.002

Kalynchuk, L.E., Gregus, A., Boudreau, D., Perrot-Sinal, T.S., 2004. Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. Behavioral Neuroscience 118, 1365–1377. doi:10.1037/0735-7044.118.6.1365

Kapoor, A., Matthews, S.G., 2011. Testosterone is involved in mediating the effects of prenatal stress in male guinea pig offspring. The Journal of Physiology 589, 755–766. doi:10.1113/jphysiol.2010.200543

Karli, P., 1956. The Norway Rat ' s Killing Response to the White Mouse : An Experimental Analysis. Behaviour 10, 81–103.

Kasahara, M., Groenink, L., Kas, M.J.H., Bijlsma, E.Y., Olivier, B., Sarnyai, Z., 2011. Influence of transgenic corticotropin-releasing factor (CRF) over-expression on social recognition memory in mice. Behavioural Brain Research 218, 357–362. doi:10.1016/j.bbr.2010.12.029

Kasumovic, M.M., 2013. The multidimensional consequences of the juvenile environment: Towards an integrative view of the adult phenotype. Animal

Behaviour 85, 1049–1059. doi:10.1016/j.anbehav.2013.02.009

Kitraki, E., Nalvarte, I., Alavian-Ghavanini, A., Rüegg, J., 2015. Developmental exposure to bisphenol A alters expression and DNA methylation of Fkbp5, an important regulator of the stress response. Molecular and Cellular Endocrinology 417, 191–199. doi:10.1016/j.mce.2015.09.028

Klengel, T., Mehta, D., Anacker, C., Rex-haffner, M., Pruessner, J.C., Pariante, C.M., Pace, T.W.W., Mercer, K.B., Mayberg, H.S., Bradley, B., Nemeroff, C.B., Holsboer, F., Heim, C.M., Ressler, K.J., Rein, T., Binder, E.B., 2013. Allele-specific FKBP5 DNA demethylation mediates gene – childhood trauma interactions. Nature Neuroscience 16, 33–41. doi:10.1038/nn.3275

Koehl, M., Barbazanges, A., Le Moal, M., Maccari, S., 1997. Prenatal stress induces a phase advance of circadian corticosterone rhythm in adult rats which is prevented by postnatal stress. Brain Research 759, 317–320. doi:10.1016/S0006-8993(97)00394-6

Koehl, M., Darnaudéry, M., Dulluc, J., Van Reeth, O., 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. Journal of Neurobiology 40, 302–15. doi:10.1002/(SICI)1097-4695(19990905)40

Koenig, J.I., Elmer, G.I., Shepard, P.D., Lee, P.R., Mayo, C., Joy, B., Hercher, E., Brady, D.L., 2005. Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. Behavioral Brain Research 156, 251–261. doi:10.1016/j.bbr.2004.05.030

Kohda, K., Jinde, S., Iwamoto, K., Bundo, M., Kato, N., Kato, T., 2005. Maternal separation stress drastically decreases expression of transthyretin in the brains of adult rat offspring. The International Journal of Neuropsychopharmacology 9, 201. doi:10.1017/S1461145705005857

Kohsaka, A., Laposky, A.D., Ramsey, K.M., Estrada, C., Joshu, C., Kobayashi, Y., Turek, F.W., Bass, J., 2007. High-Fat Diet Disrupts Behavioral and Molecular Circadian Rhythms in Mice. Cell Metabolism 6, 414–421. doi:10.1016/j.cmet.2007.09.006

Korgan, A.C., Green, A.D., Perrot, T.S., Esser, M.J., 2014. Limbic system activation is

affected by prenatal predator exposure and postnatal environmental enrichment and further moderated by dam and sex. Behavioral Brain Research 259, 106–118. doi:10.1016/j.bbr.2013.10.037

Korpimaki, E., Koivunen, V., Hakkarainen, H., 1996. Microhabitat use and behavior of voles under weasel and raptor predation risk: Predator facilitation? Behavioral Ecology 7, 30–34. doi:10.1093/beheco/7.1.30

Kraszpulski, M., Dickerson, P. a, Salm, a K., 2006. Prenatal stress affects the developmental trajectory of the rat amygdala. Stress 9, 85–95. doi:10.1080/10253890600798109

Kulesskaya, N., Voikar, V., 2014. Assessment of mouse anxiety-like behavior in the light-dark box and open-field arena: Role of equipment and procedure. Physiology & Behavior 133, 30–38. doi:10.1016/j.physbeh.2014.05.006

Kundakovic, M., Jaric, I., 2017. The epigenetic link between prenatal adverse environments and neurodevelopmental disorders. Genes 8, 104. doi:10.3390/genes8030104

Lacy, R.C., Lynch, C.B., Lynch, G.R., 1978. Developmental and adult acclimation effects of ambient temperature regulatio of mice selected for high and low levels of nest-building. Journal of Comparative Physiology 123, 185–192.

Langerhans, R.B., 2007. Evolutionary consequences of predation: avoidance, escape, reproduction, and diversification, in: Elewa, A.M.T. (Ed.), Predation in Organisms: A Distinct Phenomenon. Springer-Verlag, Heidelberg, pp. 177–220. doi:10.1007/978-3-540-46046-6

Lavergne, S.G., McGowan, P.O., Krebs, C.J., Boonstra, R., 2014. Impact of high predation risk on genome-wide hippocampal gene expression in snowshoe hares. Oecologia 176, 613–624. doi:10.1007/s00442-014-3053-0

Lechan, R.M., Fekete, C., 2006. Chapter 12: The TRH neuron: a hypothalamic integrator of energy metabolism. Progress in Brain Research 153, 209–235. doi:10.1016/S0079-6123(06)53012-2

Lee, P.R., Brady, D.L., Shapiro, R.A., Dorsa, D.M., Koenig, J.I., 2007. Prenatal stress generates deficits in rat social behavior: Reversal by oxytocin. Brain Research 1156, 152–167. doi:10.1016/j.brainres.2007.04.042

Lee, R.S., Sawa, A., 2014. Environmental stressors and epigenetic control of the hypothalamic-pituitary-adrenal-axis (HPA-axis). Neuroendocrinology 100, 278–287. doi:10.1159/000369585

Lee, R.S., Tamashiro, K.L.K., Yang, X., Purcell, R.H., Harvey, A., Willour, V.L., Huo, Y., Rongione, M., Wand, G.S., Potash, J.B., 2010. Chronic corticosterone exposure increases expression and decreases deoxyribonucleic acid methylation of Fkbp5 in mice. Endocrinology 151, 4332–4343. doi:10.1210/en.2010-0225

Lemaire, V., Koehl, M., Le Moal, M., Abrous, D.N., 2000. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proceedings of the National Academy of Sciences of the United States of America 97, 11032–11037. doi:10.1073/pnas.97.20.11032

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, 291–296. doi:10.1677/joe.0.1810291

Levine, S., 1957. Infantile experience and resistance to physiological stress. Science 126, 405.

Levine, T.R., Hullett, C.R., 2002. Eta-squared, partial eta-squared, and misreporting of effect size in communication research. Human Communication Research 28, 612–625. doi:10.1111/j.1468-2958.2002.tb00828.x

Lighton, J.R.B., 2008. Measuring Metabolic Rates: A Manual for Scientists. Oxford University Press, New York.

Lima, S.L., 1998. Nonlethal effects in the ecology of predator-prey interactions. BioScience 48, 25–34. doi:10.2307/1313225

Longland, W.S., Price, M. V, 1991. Direct Observations of Owls and Heteromyid Rodents : Can Predation Risk Explain Microhabitat Use? Ecology 72, 2261–2273.

Love, O.P., McGowan, P.O., Sheriff, M.J., 2013. Maternal adversity and ecological stressors in natural populations: the role of stress axis programming in individuals, with implications for populations and communities. Functional Ecology 27, 81–92. doi:10.1111/j.1365-2435.2012.02040.x

Lubin, F.D., Roth, T.L., Sweatt, J.D., 2008. Epigenetic regulation of bdnf gene

transcription in the consolidation of fear memory. Journal of Neuroscience 28, 10576–10586. doi:10.1523/JNEUROSCI.1786-08.2008

Lugo, D.I., Pintar, J.E., 1996. Ontogeny of basal and regulated secretion from POMC cells of the developing anterior lobe of the rat pituitary gland. Developmental Biology 173, 95–109. doi:10.1006/dbio.1996.0009

Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature Reviews Neuroscience 10, 434–445. doi:10.1038/nrn2639

Maccari, S., Darnaudery, M., Morley-Fletcher, S., Zuena, A.R., Cinque, C., Van Reeth, O., 2003. Prenatal stress and long-term consequences: Implications of glucocorticoid hormones. Neuroscience and Biobehavioral Reviews 27, 119–127. doi:10.1016/S0149-7634(03)00014-9

MacKay, J.R.., Haskell, M.., 2015. Consistent Individual Behavioral Variation: The Difference between Temperament, Personality and Behavioral Syndromes. Animals (Basel) 5, 455–478.

Magnhagen, C., 1990. Reproduction under Predation Risk in the Sand Goby, Pomatoschistus-Minutus, and the Black Goby, Gobius-Niger - the Effect of Age and Longevity. Behavioral Ecology and Sociobiology 26, 331–335.

Malisch, J.L., Breuner, C.W., Gomes, F.R., Chappell, M.A., Garland, T., 2008. Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior. General and Comparative Endocrinology 156, 210–217. doi:10.1016/j.ygcen.2008.01.020

Maor, G., Yearim, A., Ast, G., 2015. The alternative role of DNA methylation in splicing regulation. Trends in Genetics 31, 274–280. doi:10.1016/j.tig.2015.03.002

Marieb, E., Hoehn, K., 2012. Human anatomy & physiology, 9th ed. ERPI, New York.

Mashoodh, R., Sinal, C.J., Perrot-Sinal, T.S., 2009. Predation threat exerts specific effects on rat maternal behaviour and anxiety-related behaviour of male and female offspring. Physiology & Behavior 96, 693–702. doi:10.1016/j.physbeh.2009.01.001

Masini, C.V., Sauer, S., Campeau, S., 2005. Ferret odor as a processive stress model in rats: Neurocheminal, behavioral and endocrine evidence. Behavioral Neuroscience

119, 280–292. doi:10.1037/0735-7044.119.1.280

Masini, C. V., Sasse, S.K., Garcia, R.J., Nyhuis, T.J., Day, H.E.W., Campeau, S., 2009. Disruption of neuroendocrine stress responses to acute ferret odor by medial, but not central amygdala lesions in rats. Brain Research 1288, 79–87. doi:10.1016/j.brainres.2009.07.011

Matrisciano, F., Tueting, P., Dalal, I., Kadriu, B., Grayson, D.R., Davis, J.M., Nicoletti, F., Guidotti, A., 2013. Epigenetic modifications of GABAergic interneurons are associated with the schizophrenia-like phenotype induced by prenatal stress in mice. Neuropharmacology 68, 184–194. doi:10.1016/j.neuropharm.2012.04.013.EPIGENETIC

McAllister, A.K., Katz, L.C., Lo, D.C., 1999. Neurotrophins and synaptic plasticity. Annual Review in Neuroscience 22, 295–318. doi:10.1146/annurev.neuro.22.1.295

McGowan, O.P., Sasaki, A., D'Alessio, A., Cymov, S., Labonte, B., Szyf, M., Turecki, G., Meaney, J.M., 2009. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nature Neuroscience 12, 342–348. doi:10.1038/nn.2270

McGowan, P.O., Suderman, M., Sasaki, A., Huang, T.C.T., Hallett, M., Meaney, M.J., Szyf, M., 2011. Broad epigenetic signature of maternal care in the brain of adult rats. PloS One 6, e14739. doi:10.1371/journal.pone.0014739

McHugh, S.B., Deacon, R.M.J., Rawlins, J.N.P., Bannerman, D.M., 2004. Amygdala and ventral hippocampus contribute differentially to mechanisms of fear and anxiety. Behavioral Neuroscience 118, 63–78. doi:10.1037/0735-7044.118.1.63

McLeod, J., Sinal, C.J., Perrot-Sinal, T.S., 2007. Evidence for non-genomic transmission of ecological information via maternal behavior in female rats. Genes, Brain and Behavior 6, 19–29. doi:10.1111/j.1601-183X.2006.00214.x

McMillen, I.C., Robinson, J.S., 2005. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiological Reviews 85, 571–633. doi:10.1152/physrev.00053.2003

Meaney, M.J., Aitken, D.H., Sapolsky, R.M., 1987. Thyroid Hormones Influence the Development of Hippocampal Glucocorticoid Receptors in the Rat: A Mechanism for the Effects of Postnatal Handling on the Development of the Adrenocortical

Stress Response. Neuroendocrinology 45, 278–283.

Meaney, M.J., Sapolsky, R.M., Aitken, D.H., McEwen, B.S., 1985. [3H]dexamethasone binding in the limbic brain of the fetal rat. Brain Res 355, 297–300.

Merali, Z., Levac, C., Anisman, H., 2003. Validation of a simple, ethologically relevant paradigm for assessing anxiety in mice. Biological Psychiatry 54, 552–565. doi:10.1016/S0006-3223(02)01827-9

Moll, R.J., Redilla, K.M., Mudumba, T., Muneza, A.B., Gray, S.M., Abade, L., Hayward, M.W., Millspaugh, J.J., Montgomery, R.A., 2017. The many faces of fear: a synthesis of the methodological variation in characterizing predation risk. Journal of Animal Ecology 86, 749–765. doi:10.1111/1365-2656.12680

Mommer, B.C., Bell, A.M., 2014. Maternal experience with predation risk influences genome-wide embryonic gene expression in threespined sticklebacks (Gasterosteus aculeatus). PLoS ONE 9. doi:10.1371/journal.pone.0098564

Monteleone, M.C., Adrover, E., Pallarés, M.E., Antonelli, M.C., Frasch, A.C., Brocco, M.A., 2014. Prenatal stress changes the glycoprotein gpm6a gene expression and induces epigenetic changes in rat offspring brain. Epigenetics 9, 152–160. doi:10.4161/epi.25925

Moore, C.L., Morelli, G.A., 1979. Mother rats interact differently with male and female offspring. Journal of Comparative and Physiological Psychology 93, 677–84. doi:10.1037/h0077599

Moore, R.Y., Eichler, V.B., 1972. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Research 42, 201–206. doi:10.1016/0006-8993(72)90054-6

Morgan, C.P., Bale, T.L., 2011. Early Prenatal Stress Epigenetically Programs Dysmasculinization in Second-Generation Offspring via the Paternal Lineage. Journal of Neuroscience 31, 11748–11755. doi:10.1523/JNEUROSCI.1887-11.2011

Mori, T., Yanagisawa, Y., Kitani, Y., Sugiyama, M., Kishida, O., Nishimura, K., 2015. Gene expression profiles in Rana pirica tadpoles following exposure to a predation threat. BMC Genomics 16, 258. doi:10.1186/s12864-015-1389-4

Morley-Fletcher, S., Rea, M., Maccari, S., Laviola, G., 2003. Environmental enrichment during adoslescence reverses the effects of prenatal stress on play behaviour and

HPA axis reactivity in rats. European Journal of Neuroscience 18, 3367–3374.

Mueller, B.R., Bale, T.L., 2006. Impact of prenatal stress on long term body weight is dependent on timing and maternal sensitivity. Physiology & Behavior 88, 605–614. doi:10.1016/j.physbeh.2006.05.019

Mueller, B.R., Bale, T.L., 2007. Early prenatal stress impact on coping strategies and learning performance is sex dependent. Physiology & Behavior 91, 55–65. doi:10.1016/j.physbeh.2007.01.017

Mueller, B.R., Bale, T.L., 2008. Sex-specific programming of offspring emotionality following stress early in pregnancy. Journal of Neuroscience 28, 9055–9065. doi:10.1523/JNEUROSCI.1424-08.2008.

Muneoka, K., Mikuni, M., Ogawa, T., Kitera, K., Kamei, K., Takigawa, M., Takahashi, K., 1997. Prenatal dexamethasone exposure alters brain monoamine metabolism and adrenocortical response in rat offspring. The American Journal of Physiology 273, R1669-75. doi:10.1111/j.1471-4159.1992.tb08348.x

Munhoz, C.D., Sorrells, S.F., Caso, J.R., Scavone, C., Sapolsky, R.M., 2010. Glucocorticoids exacerbate lipopolysaccharide-induced signaling in the frontal cortex and hippocampus in a dose-dependent manner. Journal of Neuroscience 30, 13690–8. doi:10.1523/JNEUROSCI.0303-09.2010

Muñoz-Abellán, C., Rabasa, C., Daviu, N., Nadal, R., Armario, A., 2011. Behavioral and endocrine consequences of simultaneous exposure to two different stressors in rats: interaction or independence? PloS One 6, e21426. doi:10.1371/journal.pone.0021426

Murray, F., Smith, D.W., Hutson, P.H., 2008. Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. European Journal of Pharmacology 583, 115–127. doi:10.1016/j.ejphar.2008.01.014

Mychasiuk, R., Schmold, N., Ilnytskyy, S., Kovalchuk, O., Kolb, B., Gibb, R., 2011. Prenatal bystander stress alters brain, behavior, and the epigenome of developing rat offspring. Developmental Neuroscience 33, 159–169. doi:10.1159/000330034

Nanda, S., Qi, C., Roseboom, P.H., Kalin, N.H., 2008. Predator stress induces behavioral inhibition and amygdala somatostatin receptor 2 gene expression. Genes, Brain and

Behavior 7, 639–648. doi:10.1111/j.1601-183X.2008.00401.x

Nasello, A.G., MacHado, C., Bastos, J.F., Felicio, L.F., 1998. Sudden darkness induces a high activity-low anxiety state in male and female rats. Physiology & Behavior 63, 451–454. doi:10.1016/S0031-9384(97)00462-9

Nelson, R.J., 2000. An Introduction to Behvioral Endocrinology, 2nd ed. Sinauer, Sunderland, U.S.A.

Ng, P.C., 2000. The fetal and neonatal hypothalamic-pituitary-adrenal axis. Archives of Disease in Childhood. Fetal and Neonatal Edition 82, F250-4. doi:10.1136/fn.82.3.F250

Nolte, D., Mason, J., Epple, G., Aronov, E., Campbell, D., 1994. Why are predator urines aversive to prey? Journal of Chemical Ecology 20, 1505–1516.

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 : monthly journal of the Association of Physicians 93, 323–333. doi:10.1093/qjmed/93.6.323

Ordyan, N.E., Pivina, S.G., Akulova, V.K., Rakitskaya, V. V., 2014. Effects of Prenatal Stress and 17??-Estradiol on Anxiety and Depressive Behavior in Ovariectomized Female Rats. Neuroscience and Behavioral Physiology 44, 871–878. doi:10.1007/s11055-014-9995-1

Ozanne, S.E., Hales, C.N., 2004. Catch-up growth and obesity in male mice. Nature 427, 411–412. doi:10.1038/427411a

Padovan, C.M., Guimarães, F.S., 2000. Restraint-induced hypoactivity in an elevated plus-maze. Brazilian Journal of Medical and Biological Research 33, 79–83. doi:10.1590/S0100-879X2000000100011

Palme, R., Touma, C., Arias, N., Dominchin, M.F., Lepschy, M., 2013. Steroid extraction: Get the best out of faecal samples. Wiener Tierarztliche Monatsschrift 100, 238–246.

Pankevich, D.E., Mueller, B.R., Brockel, B., Bale, T.L., 2009. Prenatal stress programming of offspring feeding behavior and energy balance begins early in pregnancy. Physiology & Behavior 98, 94–102. doi:10.1016/j.physbeh.2009.04.015

Paxinos, G., Watson, C., 1998. The rat brain in stereodaxic coordinates, Academic P. ed. San Diego.

Peña, C.J., Monk, C., Champagne, F.A., 2012. Epigenetic effects of Prenatal stress on 11β-Hydroxysteroid Dehydrogenase-2 in the Placenta and fetal brain. PLoS ONE 7, 1–9. doi:10.1371/journal.pone.0039791

Perogamvros, I., Ray, D.W., Trainer, P.J., 2012. Regulation of cortisol bioavailability—effects on hormone measurement and action. Nature Reviews Endocrinology 8, 717–727. doi:10.1038/nrendo.2012.134

Phillips, D.I., Walker, B.R., Reynolds, R.M., Flanagan, D.E., Wood, P.J., Osmond, C., Barker, D.J., Whorwood, C.B., 2000. Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. Hypertension 35, 1301–1306. doi:10.1161/01.HYP.35.6.1301

Plata-Salaman, C.R., Ilyin, S.E., Turrin, N.P., Gayle, D., Flynn, M.C., Bedard, T., Merali, Z., Anisman, H., 2000. Neither acute nor chronic exposure to a naturalistic (predator) stressor influences the interleukin-1beta system, tumor necrosis factor-alpha, transforming growth factor-beta1, and neuropeptide mRNAs in specific brain regions. Brain Research Bulletin 51, 187–193. doi:10.1016/S0361-9230(99)00204-X

Poland, R.E., Hanada, K., Rubin, R.T., 1989. Relationship of nocturnal plasma bioactive and immunoreactive ACTH concentrations to cortisol secretion in normal men. Acta Endocrinol. 121, 857–865.

Powell, F., Banks, P.B., 2004. Do house mice modify their foraging behaviour in response to predator odours and habitat? Animal Behaviour 67, 753–759. doi:10.1016/j.anbehav.2003.08.016

Qian, X., Droste, S.K., Gutièrrez-Mecinas, M., Collins, A., Kersanté, F., Reul, J.M.H.M., Linthorst, A.C.E., 2011. A rapid release of corticosteroid-binding globulin from the liver restrains the glucocorticoid hormone response to acute stress. Endocrinology 152, 3738–3748. doi:10.1210/en.2011-1008

Qian, X., Droste, S.K., Lightman, S.L., Reul, J.M.H.M., Linthorst, A.C.E., 2012. Circadian and ultradian rhythms of free glucocorticoid hormone are highly synchronized between the blood, the subcutaneous tissue, and the brain. Endocrinology 153, 4346–4353. doi:10.1210/en.2012-1484

Ramos, A., 2008. Animal models of anxiety: do I need multiple tests? Trends in Pharmacological Sciences 29, 493–498. doi:10.1016/j.tips.2008.07.005

Réale, D., Reader, S.M., Sol, D., Mcdougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. Biological Reviews 82, 291–318. doi:10.1111/j.1469-185X.2007.00010.x

Refinetti, R., 2003. Metabolic heat production, heat loss and the circadian rhythm of body temperature in the rat. Experimental Physiology 88, 423–429. doi:10.1113/eph8802521

Reichardt, H.M., Schütz, G., 1996. Feedback control of glucocorticoid production is established during fetal development. Molecular Medicine 2, 735–44.

Reppert, S.M., 1983. Maternal Coordination of the Fetal Biological Clock in Utero Author (s): Steven M . Reppert and William J . Schwartz. Science 220, 969–971.

Reynolds, R.M., 2001. Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. Journal of Clinical Endocrinology and Metabolism 86, 245–250. doi:10.1210/jcem.86.1.7145

Rice, C.J., Sandman, C.A., Lenjavi, M.R., Baram, T.Z., 2008. A novel mouse model for acute and long-lasting consequences of early life stress. Endocrinology 149, 4892–4900. doi:10.1210/en.2008-0633

Rice, M.E., Harris, G.T., 2005. Comparing Effect Sizes in Follow-Up Studies : ROC Area, Cohen's d , and r. Law and Human Behavior 29, 615–620. doi:10.1007/s10979-005-6832-7

Richardson, H.N., Zorrilla, E.P., Mandyam, C.D., Rivier, C.L., 2006. Exposure to repetitive versus varied stress during prenatal development generates two distinct anxiogenic and neuroendocrine profiles in adulthood. Endocrinology 147, 2506–2517. doi:10.1210/en.2005-1054

Roceri, M., Cirulli, F., Pessina, C., Peretto, P., Racagni, G., Riva, M. a., 2004. Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. Biological Psychiatry 55, 708–714. doi:10.1016/j.biopsych.2003.12.011

Rodgers, R., Haller, J., Holmes, A., Halasz, J., Walton, T., Brain, P., 1999. Corticosterone response to the plus-mazeHigh correlation with risk assessment in rats and mice. Physiology & Behavior 68, 47–53. doi:10.1016/S0031-9384(99)00140-7

Rodgers, R.J., Dalvi, A., 1997. Anxiety, defence and the elevated plus maze. Neuroscience and Biobehavioral Reviews 21, 801–810.

Roedel, A., Storch, C., Holsboer, F., Ohl, F., 2006. Effects of light or dark phase testing on behavioural and cognitive performance in DBA mice. Laboratory Animals 40, 371–381.

Rorick-Kehn, L.M., Hart, J.C., McKinzie, D.L., 2005. Pharmacological characterization of stress-induced hyperthermia in DBA/2 mice using metabotropic and ionotropic glutamate receptor ligands. Psychopharmacology 183, 226–240. doi:10.1007/s00213-005-0169-2

Roseboom, P.H., Nanda, S.A., Bakshi, V.P., Trentani, A., Newman, S.M., Kalin, N.H., 2007. Predator threat induces behavioral inhibition, pituitary-adrenal activation and changes in amygdala CRF-binding protein gene expression. Psychoneuroendocrinology 32, 44–55. doi:10.1016/j.psyneuen.2006.10.002

Roth, T.L., Lubin, F.D., Funk, A.J., Sweatt, J.D., 2009. Lasting epigenetic influence of early-life adversity on the BDNF gene. Biological Psychiatry 65, 760–769. doi:10.1016/j.biopsych.2008.11.028

Rouder, J.N., Morey, R.D., Speckman, P.L., Province, J.M., 2012. Default Bayes factors for ANOVA designs. Journal of Mathematical Psychology 56, 356–374. doi:10.1016/j.jmp.2012.08.001

Rozenberg, A., Parida, M., Leese, F., Weiss, L.C., Tollrian, R., Manak, J.R., 2015. Transcriptional profiling of predator-induced phenotypic plasticity in Daphnia pulex. Frontiers in Zoology 12, 18. doi:10.1186/s12983-015-0109-x

Rundle, S.E., Funder, J.W., 1988. Ontogeny of Corticotropin-Releasing Factor and Arginine Vasopressein in the Rat. Neuroendocrinology 47, 374–378.

Saboory, E., Ahmadzadeh, R., Roshan-Milani, S., 2011. Prenatal exposure to restraint or predator stresses attenuates field excitatory postsynaptic potentials in infant rats. International Journal of Developmental Neuroscience 29, 827–831. doi:10.1016/j.ijdevneu.2011.09.001

Sainio, E.L., Lehtola, T., Roininen, P., 1988. Radioimmunoassay of total and free corticosterone in rat plasma: measurement of the effect of different doses of corticosterone. Steroids 51, 609–622.

Salm, A.K., Pavelko, M., Krouse, E.M., Webster, W., Kraszpulski, M., Birkle, D.L., 2004. Lateral amygdaloid nucleus expansion in adult rats is associated with exposure to prenatal stress. Developmental Brain Research 148, 159–167. doi:10.1016/j.devbrainres.2003.11.005

Samel, M., 1968. Thyroid Function during Postnatal Development in the Rat. General and Comparative Endocrinology 10, 229–234.

Sanchez-Franco, F., Fernandez, L., Fernandez, G., Cacicedo, L., 1989. Thyroid Hormone Action on ACTH Secretion. Hormone and Metabolic Research 21, 550–552.

Sandi, C., Haller, J., 2015. Stress and the social brain : behavioural effects and neurobiological mechanisms. Nature Reviews 16, 290–304. doi:10.1038/nrn3918

Sasaki, A., de Vega, W., Sivanathan, S., St-Cyr, S., McGowan, P., 2014. Maternal high-fat diet alters anxiety behavior and glucocorticoid signaling in adolescent offspring. Neuroscience 11, 92–101. doi:10.1016/j.neuroscience.2014.04.012

Sasaki, A., de Vega, W.C., St-Cyr, S., Pan, P., McGowan, P.O., 2013. Perinatal high fat diet alters glucocorticoid signaling and anxiety behavior in adulthood. Neuroscience 240, 1–12. doi:10.1016/j.neuroscience.2013.02.044

Sawamura, T., Klengel, T., Armario, A., Jovanovic, T., Norrholm, S.D., Ressler, K.J., Andero, R., 2015. Dexamethasone Treatment Leads to Enhanced Fear Extinction and Dynamic Fkbp5 Regulation in Amygdala. Neuropsychopharmacology 1–15. doi:10.1038/npp.2015.210

Schmidt, K.L., Macdougall-Shackleton, E. a, Macdougall-Shackleton, S. a, 2012. Developmental stress has sex-specific effects on nestling growth and adult metabolic rates but no effect on adult body size or body composition in song sparrows. The Journal of Experimental Biology 215, 3207–17. doi:10.1242/jeb.068965

Schneider, J.S., Anderson, D.W., Kidd, S.K., Sobolewski, M., Cory-Slechta, D.A., 2016. Sex-dependent effects of lead and prenatal stress on post-translational histone modifications in frontal cortex and hippocampus in the early postnatal brain. NeuroToxicology 54, 65–71. doi:10.1016/j.neuro.2016.03.016

Schraft, H.A., Clark, R.W., 2017. Kangaroo rats change temperature when investigating rattlesnake predators. Physiology & Behavior In press. doi:10.1016/j.physbeh.2017.02.004

Schulkin, J., Morgan, M.A., Rosen, J.B., 2005. A neuroendocrine mechanism for sustaining fear. Trends in Neurosciences 28, 629–635. doi:10.1016/j.tins.2005.09.009

Seale, J., Wood, S., Atkinson, H., Harbuz, M., Lightman, S., 2004. Gonadal steroid replacement reverses gonadectomy-induced changes in the corticosterone pulse profile and stress-induced hypothalamic-pituitary-adrenal axis activity of male and female rats. Journal of Neuroendocrinology 16, 989–998. doi:10.1111/j.1365-2826.2004.01258.x

Sheriff, M.J., Krebs, C.J., Boonstra, R., 2009. The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. Journal of Animal Ecology 78, 1249–1258. doi:10.1111/j.1365-2656.2009.01552.x

Sheriff, M.J., Krebs, C.J., Boonstra, R., 2010. The ghosts of predators past : population cycles and the role of maternal programming under fluctuating predation risk. Ecology 91, 2983–2994.

Shine, R., Downes, S.J., 1999. Can pregnant lizards adjust their offspring phenotypes to environmental conditions? Oecologia 119, 1–8.

Shrader, A.M., Brown, J.S., Kerley, G.I.H., Kotler, B.P., 2008. Do free-ranging domestic goats show " landscapes of fear "? Patch use in response to habitat features and predator cues. Journal of Arid Environments 72, 1811–1819. doi:10.1016/j.jaridenv.2008.05.004

Snyder, J.S., Soumier, A., Brewer, M., Pickel, J., Cameron, H.A., 2011. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature 476, 458–61. doi:10.1038/nature10287

Sorge, R.E., Martin, L.J., Isbester, K.A., Sotocinal, S.G., Rosen, S., Tuttle, A.H., Wieskopf, J.S., Acland, E.L., Dokova, A., Kadoura, B., Leger, P., Mapplebeck, J.C.S., Mcphail, M., Delaney, A., Wigerblad, G., Schumann, A.P., Quinn, T., Frasnelli, J., Svensson, C.I., Sternberg, W.F., Mogil, J.S., 2014. Olfactory exposure to males, including men, causes stress and related analgesia in rodents. Nature Methods 11, 629–632. doi:10.1038/nmeth.2935

Sotnikov, S. V, Markt, P.O., Umriukhin, a E., Landgraf, R., 2011. Genetic predisposition to anxiety-related behavior predicts predator odor response. Behavioral Brain

Research 225, 230–234. doi:10.1016/j.bbr.2011.07.022

Spencer, K.A., Verhulst, S., 2008. Post-natal exposure to corticosterone affects standard metabolic rate in the zebra finch (Taeniopygia guttata). General and Comparative Endocrinology 159, 250–256. doi:10.1016/j.ygcen.2008.09.007

Spiga, F., Walker, J.J., Terry, J.R., Lightman, S.L., 2014. HPA axis-rhythms. Comprehensive Physiology 4, 1273–1298. doi:10.1002/cphy.c140003

St-Cyr, S., Abuaish, S., Sivanathan, S., Mcgowan, P.O., 2017. Maternal programming of sex-specific responses to predator odor stress in adult rats. Hormones and Behavior 94, 1–12. doi:10.1016/j.yhbeh.2017.06.005

St-Cyr, S., McGowan, P.O., 2015. Programming of stress-related behavior and epigenetic neural gene regulation in mice offspring through maternal exposure to predator odor. Frontiers in Behavioral Neuroscience 9, 145. doi:10.3389/fnbeh.2015.00145

Staples, L.G., McGregor, I.S., Hunt, G.E., 2009. Long-lasting FosB/??FosB immunoreactivity in the rat brain after repeated cat odor exposure. Neuroscience Letters 462, 157–161. doi:10.1016/j.neulet.2009.06.069

Steckler, T., 2005. Handbook of Stress and the Brain. Elsevier Science, San Diego.

Storm, J.J., Lima, S.L., 2010. Mothers forewarn offspring about predators: a transgenerational maternal effect on behavior. The American Naturalist 175, 382–390. doi:10.1086/650443

Sullivan, R.M., Wilson, D. a, Leon, M., 1988. Physical stimulation reduces the brain temperature of infant rats. Developmental Psychobiology 21, 237–250. doi:10.1002/dev.420210304

Tadross, J.A., Patterson, M., Suzuki, K., Beale, K.E., Boughton, C.K., Smith, K.L., Moore, S., Ghatei, M.A., Bloom, S.R., 2010. Augurin stimulates the hypothalamo-pituitary-adrenal axis via the release of corticotrophin-releasing factor in rats. British Journal of Pharmacology 159, 1663–1671. doi:10.1111/j.1476-5381.2010.00655.x

Takahashi, A., Nishi, A., Ishii, A., Shiroishi, T., Koide, T., 2008. Systematic analysis of emotionality in consomic mouse strains established from C57BL/6J and wild-derived MSM/Ms. Genes, Brain and Behavior 7, 849–858. doi:10.1111/j.1601-183X.2008.00419.x

Takahashi, L.K., Chan, M.M., Pilar, M.L., 2008. Predator odor fear conditioning: Current

perspectives and new directions. Neuroscience and Biobehavioral Reviews 32, 1218–1227. doi:10.1016/j.neubiorev.2008.06.001.

Taliaz, D., Loya, A., Gersner, R., Haramati, S., Chen, A., Zangen, A., 2011. Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. Journal of Neuroscience 31, 4475–4483. doi:10.1523/JNEUROSCI.5725-10.2011

Tamashiro, K., Terrillion, C., Hyun, J., Koenig, J., Moran, T., 2009. Prenatal Stress or High-Fat Diet Increases Susceptibility to Diet-Induced Obesity in Rat Offspring. Diabetes 58, 1116–1125. doi:10.2337/db08-1129.

Thomas, R.M., Urban, J.H., Peterson, D.A., 2006. Acute exposure to predator odor elicits a robust increase in corticosterone and a decrease in activity without altering proliferation in the adult rat hippocampus. Experimental Neurology 201, 308–315. doi:10.1016/j.expneurol.2006.04.010

Thomson, J.S., Watts, P.C., Pottinger, T.G., Sneddon, L.U., 2012. Hormones and Behavior Plasticity of boldness in rainbow trout, Oncorhynchus mykiss: do hunger and predation influence risk-taking behaviour? Hormones and Behavior 61, 750–757. doi:10.1016/j.yhbeh.2012.03.014

Thorson, J.M., Morgan, R.A., Brown, J.S., Norman, J.E., 1992. Direct and indirect cues of predatory risk and patch use by fox squirrels and thirteen-lined ground squirrels. Behavioral Ecology 9, 151–157.

Tirri, R., Pantio, M., Tarkkonen, H., 1968. Metabolic Effects of Thyroxine in Infant Rats. Experientia 24, 365–367.

Touma, C., Sachser, N., Möstl, E., Palme, R., 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. General and Comparative Endocrinology 130, 267–278. doi:10.1016/S0016-6480(02)00620-2

Tribollet, E., Goumaz, M., Raggenbass, M., Dreifuss, J.J., 1991. Appearance and transient expression of vasopressin and oxytocin receptors in the rat brain. Journal of Receptor Research 11, 333–346.

Troxell-Smith, S.M., Tutka, M.J., Albergo, J.M., Balu, D., Brown, J.S., Leonard, J.P., 2016. Foraging decisions in wild versus domestic Mus musculus: What does life in the lab select for? Behavioural Processes 122, 43–50. doi:10.1016/j.beproc.2015.10.020

Tsankova, N.M., Berton, O., Renthal, W., Kumar, A., Neve, R.L., Nestler, E.J., 2006. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nature Neuroscience 9, 519–525. doi:10.1038/nn1659

Tsigos, C., Chrousos, G.P., 2002. Hypothalamic – pituitary – adrenal axis, neuroendocrine factors and stress. Journal of Psychosomatic Research 53, 865–871.

Uller, T., Olsson, M., 2006. Direct Exposure to Corticosterone During Embryonic Development Influences Behaviour in an Ovoviviparous Lizard. Ethology 112, 390–397. doi:10.1111/j.1439-0310.2006.01164.x

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. European Journal of Neuroscience 11, 2906–16. doi:10.1046/j.1460-9568.1999.00705.x

Vallee, M., Mayo, W., Maccari, S., Le Moal, M., Simon, H., 1996. Long-term effects of prenatal stress and handling on metabolic parameters: relationship to corticosterone secretion response. Brain Research 712, 287–292. doi:0006-8993(95)01459-4 [pii]

Vallée, M., Mayo, W., Moal, M., Maccari, S., Vallée, M., Mayo, W., Dellu, F., Le Moal, M., 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. The Journal of Neuroscience 17, 2626–2636.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology 3, RESEARCH0034. doi:10.1186/gb-2002-3-7-research0034

Vigé, A., Gallou-Kabani, C., Junien, C., 2008. Sexual dimorphism in non-mandelian inheritance. Pediatric Research 63, 340–347.

Wakasugi, S., Maeda, S., Shimada, K., 1986. Structure and Expression of the Mouse Prealbumin Gene. Journal of Biochemistry 100, 49–58.

Wallace, K.J., Rosen, J.B., 2000. Predator odor as an unconditioned fear stimulus in rats: Elicitation of freezing by trimethylthiazoline, a component of fox feces. Behavioral Neuroscience 114, 912–922. doi:10.1037//0735-7044.114.5.912

Walsh, R.N., Cummins, R.A., 1976. The open-field test: a critical review. Psychological

Bulletin 83, 482–504.

Wang, Z., Wang, B., Lu, J., 2011. Behavioral and physiological responses of striped field mice (Apodemus agrarius) to predator odor. Integrative Zoology 6, 334–340. doi:10.1111/j.1749-4877.2011.00262.x

Weaver, I.C.G., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., Meaney, M.J., 2004. Epigenetic programming by maternal behavior. Nature Neuroscience 7, 847–854. doi:10.1038/nn1276

Weinstock, M., 1997. Does Prenatal Stress Impair Coping and Regulation of Hypothalamic–Pituitary-Adrenal Axis? Neuroscience and Biobehavioral Reviews 21, 1–10.

Weinstock, M., 2008. The long-term behavioural consequences of prenatal stress. Neuroscience and Biobehavioral Reviews 32, 1073–1086. doi:10.1016/j.neubiorev.2008.03.002

Welberg, L.A.M., Seckl, J.R., Holmes, M.C., 2000. Inhibition of 11[beta]-hydroxysteroid dehydrogenase, the feto-placental barrier to maternal glucocorticoids, permanently programs amygdala glucocorticoid receptor mRNA expression and anxiety-like behavior in the offspring. Eur J Neurosci 12, 1047–1054.

Wiedenmayer, C.P., Barr, G.A., 2001. Developmental changes in responsivity to threat are stimulus-specific in rats. Developmental Psychobiology 39, 1–7. doi:10.1002/dev.1022

Williams, G.R., 2008. Neurodevelopmental and neurophysiological actions of thyroid hormone. Journal of Neuroendocrinology 20, 784–794. doi:10.1111/j.1365-2826.2008.01733.x

Wilson, C.A., Terry, A. V., 2013. Variable maternal stress in rats alters locomotor activity, social behavior, and recognition memory in the adult offspring. Pharmacology Biochemistry and Behavior 104, 47–61. doi:10.1016/j.pbb.2012.12.015

Windle, R.J., Wood, S.A., Kershaw, Y.M., Lightman, S.L., Ingram, C.D., 2013. Adaptive changes in basal and stress-induced HPA activity in lactating and post-lactating female rats. Endocrinology 154, 749–761. doi:10.1210/en.2012-1779

Wingfield, J.C., Ramenofsky, M., 1999. Hormones and the behavioral ecology of stress,

in: Baum, P.H.M. (Ed.), Stress Physiology of Animals. Sheffield Academic, Sheffield, pp. 1–51.

Xu, L., Sun, Y., Gao, L., Cai, Y.Y., Shi, S.X., 2014. Prenatal restraint stress is associated with demethylation of corticotrophin releasing hormone (CRH) promoter and enhances CRH transcriptional responses to stress in adolescent rats. Neurochemical Research 39, 1193–1198. doi:10.1007/s11064-014-1296-0

Yang, M., Weber, M.D., Crawley, J.N., 2008. Light phase testing of social behaviors: not a problem. Frontiers in Neuroscience 2, 186–191. doi:10.3389/neuro.01.029.2008

Yang, X., Ewald, E.R., Huo, Y., Tamashiro, K.L., Salvatori, R., Sawa, A., Wand, G.S., Lee, R.S., 2012. Glucocorticoid-induced loss of DNA methylation in non-neuronal cells and potential involvement of DNMT1 in epigenetic regulation of Fkbp5. Biochemical and Biophysical Research Communications 420, 570–575. doi:10.1016/j.bbrc.2012.03.035

Youngson, N.A., Whitelaw, E., 2008. Transgenerational Epigenetic Effects. Annual Review of Genomics and Human Genetics 9, 233–257. doi:10.1146/annurev.genom.9.081307.164445

Yuan, M., Liu, L.J., Xu, L.Z., Guo, T.Y., Yue, X.D., Li, S.X., 2016. Effects of environmental stress on the depression-like behaviors and the diurnal rhythm of corticosterone and melatonin in male rats. Sheng Li Xue Bao 68, 215–223. doi:10.13294/j.aps.2016.0035

Yun, K., Sugihara, H., Izumi, M., Hirayu, H., Ohta, K., Yonemitsu, N., 1982. Relationship between colloid droplets of thyroid follicular cells and serum thyroglobulin concentration in neonatal rats after thyrotropin releasing hormone (TRH) injection. Endokrinologie 79, 362–366.

Zanette, L.Y., White, A.F., Allen, M.C., Clinchy, M., 2011. Perceived predation risk reduces the number of offspring songbirds produce per year. Science 334, 1398–1401. doi:10.1126/science.1210908

Zangrossi, H., File, S.E., 1992. Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. Brain Research Bulletin 29, 381–388. doi:10.1016/0361-9230(92)90072-6

Zannas, A.S., Binder, E.B., 2014. Gene-environment interactions at the FKBP5 locus:

Sensitive periods, mechanisms and pleiotropism. Genes, Brain and Behavior 13, 25–37. doi:10.1111/gbb.12104

Zheng, W.-H., Quirion, R., 2004. Comparative signaling pathways of insulin-like growth factor-1 and brain-derived neurotrophic factor in hippocampal neurons and the role of the PI3 kinase pathway in cell survival. Journal of Neurochemistry 89, 844–852. doi:10.1111/j.1471-4159.2004.02350.x

Zimprich, A., Garrett, L., Deussing, J.M., Wotjak, C.T., Fuchs, H., Gailus-Durner, V., de Angelis, M.H., Wurst, W., Hölter, S.M., 2014. A robust and reliable non-invasive test for stress responsivity in mice. Frontiers in Behavioral Neuroscience 8, 125. doi:10.3389/fnbeh.2014.00125

Zoeller, R.T., Tan, S.W., Tyl, R.W., 2007. General background on the hypothalamic-pituitary-thyroid (HPT) axis. Critical Reviews in Toxicology 37, 11–53. doi:10.1080/10408440601123446

Zohar, I., Weinstock, M., 2011. Differential Effect of Prenatal Stress on the Expression of Cortiocotrophin-Releasing Hormone and its Receptors in the Hypothalamus and Amygdala in Male and Female Rats. Journal of Neuroendocrinology 23, 320–328. doi:10.1111/j.1365-2826.2011.02117.x

Zuena, A.R., Mairesse, J., Casolini, P., Cinque, C., Alemà, G.S., Morley-Fletcher, S., Chiodi, V., Spagnoli, L.G., Gradini, R., Catalani, A., Nicoletti, F., Maccari, S., 2008. Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. PLoS ONE 3. doi:10.1371/journal.pone.0002170