Antidepressant use during pregnancy

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Chapter 1

General introduction

The first 1000 days of life

Developmental origins of health and disease

Throughout human history, many supported the notion that events during early childhood are able to influence adult health[1]. In the 1980s this idea gained traction in the scientific world and beyond[1]. Several epidemiological studies were published around this time by Barker and colleagues, showing that low birthweight correlates with an increased risk of developing cardiovascular diseases and metabolic syndrome in adulthood[2]. These results challenged views that emphasize the contribution of genetic predispositions or adult lifestyle on the risk of developing noncommunicable diseases. They facilitated the emergence and popularization of the ever-growing field of Developmental Origins of Health and Disease (DOHaD)[1]. Today, it is well-established that early development harbors exceptional plasticity, and is therefore a time of particular sensitivity to environmental influences. Within constraints determined by genetic makeup, the early pre- and postnatal environment effectively "programs" or conditions the individual, a concept referred to as fetal-, early life-, or developmental programming, conditioning, or plasticity. Belsky and colleagues argue that some individuals are more susceptible than others to early environmental influences, whether they are beneficial or adverse, – in a "for better *and* for worse" manner[3]. Developmental programming has been suggested to affect virtually all physiological systems, ranging from general growth, stem cell lineages, and ageing, to the cardiovascular-, metabolic-, reproductive-, immune-, and nervous system[1]. Highlighting its increasingly recognized public health implications, the World Health Organization has indicated the need to focus on early child development as a means to benefit adult health[4].

Developmental programming of the brain

One particularly fascinating organ whose development and adult functioning is largely shaped within the first 1000 days of life is the brain, as recognized by organizations such as UNICEF[5]. The prenatal and early postnatal period constitutes a period of plasticity for a range of neurological, behavioral and cognitive outcomes. Commonly studied prenatal factors that contribute to long-term brain outcomes are maternal nutrition and stress, which have been shown to affect brain development, learning and memory, emotional behavior and stress responsivity[6] and measures of brain aging[7]. In a human brain imaging study, birth weight was found to correlate to cortical surface area and brain volume at a later age[8]. In addition, it was suggested that early life events may be associated with vulnerability to mental disorders such as major depressive disorder, schizophrenia, autism spectrum disorder, and eating disorders later in life[9]. The brain is in many ways synonymous with our personality, our ability to cope with stressors, our intelligence; for a large part, its health determines our happiness and success in life[10]. Therefore, it is vital that we learn more about the ways in which the early environment can positively or negatively influence these outcomes.

Proposed mechanisms

For a long time, there were few candidate biological mechanisms explaining how early life events could translate into long-term effects on health[1]. If the phenotype of an organism is determined by its genetic makeup, how could environmental factors further modulate this phenotype? In recent years, there has been a growing appreciation for the role of epigenetics – the study of alterations in phenotype that do not stem from alterations in the DNA sequence. Typically, epigenetic modifications refer to chemical modifications of the DNA or its associated proteins. By modulating gene expression, epigenetics is believed to be a mediator between early life environmental influences and later-life health and behavior[11]. For example, there is evidence to suggest that during pregnancy, the placenta can be epigenetically altered as a function of maternal health status, thereby influencing neurodevelopmental programming of the offspring[12].

Maternal depression and SSRI antidepressant use

The fact that maternal health status and lifestyle choices during pregnancy potentially affect the fetus in a profound and long-lasting manner means that we need reliable knowledge on what is beneficial and what is harmful for the developing child. Many cases are intuitive: behaviors that are beneficial to maternal health, such as getting enough sleep, physical activity and a healthy diet, will usually also benefit the child. Similarly, behaviors harmful to maternal health, such as stress, alcohol consumption, or smoking tobacco, are likely to be even more harmful to the child. But what happens if the (future) mother has an illness, and to what extent will it affect her child? And if she needs treatment, will this be in the best interest of her child as well? Unfortunately, many women and their doctors struggle to answer these questions. The answers are not always straightforward, particularly in the case of maternal mental health issues.

Depression during pregnancy

Prevalence

One of the most common mental health problems during pregnancy is depression. Estimates of the prevalence of a major depressive disorder during pregnancy range from 5 to 15%, depending on cohort and research design[13–16]. Perinatal depression is a debilitating disorder which comes at a high cost to the mother as well as her developing child[14].

Developmental outcomes

Maternal depression or depressive symptoms in the perinatal period have been associated with a range of potential health consequences. Complications related to pregnancy, such as preterm delivery, intrauterine growth restriction[17], and preeclampsia[18] have been suggested to increase with maternal depression. Additionally, neurodevelopmental outcomes in children are altered after exposure to maternal depression[19]. Some examples include suboptimal neonatal neurobehavioral functioning[20], lower infant motor development- and language scores[21] and poorer executive functioning capabilities of the child[22]. Brain imaging studies have shown that maternal depressive symptoms during pregnancy correlate positively with connectivity in emotion-regulation networks

in the infant brain[23]. Antenatal maternal depression is also associated with decreased cortical thickness[24,25] and an increased response of the amygdala to negative stimuli in childhood[26]. There is evidence for sex differences in the effects of exposure to maternal depressive symptoms during gestation, with girls showing a larger reduction in connectivity in brain regions relevant to the processing of emotions[27], a larger increase in right amygdala volume[28], and a larger decrease in cortical thickness[29] than boys. Highlighting the potential long-term effects, exposure to maternal depression puts children at a higher risk of developing depression during adolescence[30].

SSRI antidepressant use during pregnancy

Although major depressive disorder is a heterogeneous disease with many potential underlying causes[31], aberrant regulation of the neurotransmitter serotonin has long been considered central to the core symptoms of depression[32]. This is the rationale for employing medication targeting the serotonin system to treat depression. The most popular antidepressants belong to the group of selective serotonin reuptake inhibitors (SSRIs). They target the serotonin transporter (SERT), preventing the reuptake of serotonin, thereby increasing the extracellular levels of serotonin[33]. SSRIs gained popularity in the late 1980s, when fluoxetine (Prozac) was released onto the market[34]. Within a few years, it became the number 1 prescribed drug in North America, and number 2 worldwide[34].

Prevalence

SSRI antidepressants are considered relatively safe to use during pregnancy, as they do not exhibit major teratogenic effects[35]. Therefore, many women continue or start SSRI treatment during pregnancy to combat the debilitating symptoms of depression. In fact, SSRI use during pregnancy has significantly increased over the last few decades[36–39]. Recent surveys of SSRI use in large population studies vary from 2.5-3.3% of pregnancies in Europe[40,41] to 2.7-5.4% in the US[42,43]. This equates to hundreds of thousands of pregnancies every year in these regions alone.

Developmental outcomes

Exposure to SSRIs during gestation has been associated with slight increases in the risk for preterm birth[44], transient withdrawal symptoms, neonatal persistent pulmonary hypertension, and cardiovascular malformations[17]. On the behavioral level, researchers have described a phenomenon termed the "SSRI paradox": while adult SSRI use decreases anxiety and depression, *in utero* SSRI exposure might increase the risk of developing anxiety and depression later in life[45]. For example, exposure to SSRIs during fetal development has been associated with higher levels of anxiety[46], lower motor-, social- emotional- and adaptive behavior skills[47], and a greater likelihood of developing mental and behavioral disorders such as autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), and mental disability[48]. Certain effects seem to be sex-specific; a heightened risk of developing ASD was found only in boys[49]. It should be noted that not all studies find significant effects of in utero exposure to SSRIs and neurobehavioral outcomes[22,50,51]. On the brain level, neuroimaging studies have shown that white and gray matter structure[52] and connectivity[53–55] is altered in offspring after exposure to SSRIs during gestation.

Challenges in human research

Despite the high number of studies investigating the effects of exposure to maternal depression and/or SSRI use during pregnancy in human cohorts, there are important limitations to these types of studies.

Depression vs SSRI

The health outcomes in babies and children who were exposed to maternal depressive symptoms are strikingly similar to those associated with *in utero* exposure to SSRI antidepressants. For scientific advancement of knowledge, but certainly also for better informed treatment decisions, we would like to determine the nature and severity of the effects caused *only* by the depression, *only* by the SSRI, and those potentially mediated by an interaction between the two. This is difficult, because no pregnant women without (a history of) mental health issues are prescribed SSRIs. Therefore, the biggest challenge for researchers studying the role of early SSRI exposure is to control for the confounding factor of maternal psychiatric symptoms[56]. The importance of proper study design and analysis is illustrated by research showing that the increased risk of developing ASD as a result of SSRI exposure during gestation was not significant after controlling for maternal psychiatric diagnosis[57], suggesting it was the underlying maternal illness that caused the effect[57]. One commonly used method to control for maternal illness is to compare SSRI-exposed children to those exposed to unmedicated maternal depression[53–55]. However, these groups might still differ in the intensity or nature of the underlying depressive symptoms[58]. Without properly designed experiments, it remains challenging to demonstrate a causal role for SSRI exposure in mediating any effects[48].

Pathways and mechanisms

Another question difficult to answer with human studies is *how* maternal depressive symptoms and SSRI use during pregnancy contribute to health outcomes in offspring. A likely mechanism is that they affect the fetal brain directly. SSRIs reach the developing fetal brain by crossing the placenta[59] at a time when SERT is widely expressed in the brain[60] and serotonin is a neurotrophic factor[61]. By altering brain serotonin regulation, early SSRI exposure has the potential to modulate brain circuit formation and long-term mental health[62]. How this exactly takes place has yet to be determined. Although neuroimaging studies can yield useful knowledge about brain structure and function at a particular moment[63], human studies do not allow for invasive analyses such as longitudinal tracking of detailed neurobiological and molecular data.

However, there is one human organ of interest that is relatively easy to obtain for in-depth study: the placenta. The placenta facilitates exchange of nutrients and waste between the maternal and fetal blood circulations[64]. It also has specialized endocrine functions and produces various neuroactive molecules[64]. Genes expressed in the placenta are known to be epigenetic targets, and are linked to neurodevelopmental outcomes[65]. It is certainly possible that the long-term outcomes of prenatal maternal depressive symptoms and/or SSRI use are mediated by changes in the placenta[66]. A pilot study investigating this hypothesis identified several genes that had altered expression in placentas from depressed women compared to controls and placentas from antidepressant-treated

women compared to controls[67]. In another study, human embryos were treated with fluoxetine and several proteins involved in cell growth, cell proliferation, and inflammation were detected that were not present in control embryos[68]. These types of studies offer unique insights because they allow for in-depth study of human tissue.

Nevertheless, for advancing our understanding of how *in utero* exposure to maternal depression and/or SSRI treatment might alter neurobiological outcomes, we would ideally study the physiology of a pregnant woman and her child and its developing brain, from the circuit-level down to the molecular level. This is unfeasible in humans. Because of the limitations of studies in humans, rodent studies can play a valuable role in developing further knowledge of this subject.

Using rodents to study maternal depressive-like symptoms and SSRI use

Translational value of rodent experiments

Using rodents to model human health and disease

Many controlled experiments that are required to answer questions about human health and disease are not performed in humans. For obvious ethical- but also practical- and financial reasons, model systems like laboratory animals are used instead. Rodents play an important role in this regard, due to the similarities between humans and rodents in their genetic makeup, and by extension their nervous-, cardiovascular-, endocrine-, and immune systems[69]. These similarities, in addition to the ease of husbandry and small size, have made rodents the most commonly used laboratory animals to model aspects of human physiology. Recent advances in genetic engineering technologies have led to staggering new possibilities of studying the effect of particular genes on development and physiology[70]. However, in the search for methods to make precision medicine a reality, laboratory rodents are useful for more than gene variant interpretation[71]. They offer the opportunity for deep phenotyping and integration with "-omic" data in multiple tissues, for investigation of environmental exposures, and for in vivo modeling of phenotypes discovered in humans[71].

Using rodents to study the brain

One of the main benefits of using laboratory rodents in experiments is that they mature much faster than humans. At the same time, key developmental events and the anatomy and functioning of the rodent brain are remarkably similar to that of the human brain[72]. For example, the architecture of neurotransmitter systems such as the serotonin system are fairly conserved across species[56,73,74]. In terms of brain maturation, the first postnatal weeks in rodents approximately correspond to the third trimester of gestation in humans[75]. Overall, rats and mice play an important role in advancing our knowledge of the neurobiology and neuropharmacology of psychiatric disorders[76]. For instance, rodent experiments enable in-depth investigation of molecular alterations at key neurodevelopmental time points that may elicit long-term outcomes.

Rodent models of depressive-like symptoms

Validity of rodent models

Rodent models for human health and disease can be evaluated and compared on several dimensions. The most important and longest-standing criteria used for this purpose are face validity, predictive validity, and construct validity[77]. Face validity refers to the similarity in symptoms between the rodent model and the human condition. Predictive validity requires that manipulations or treatments have similar effects in the rodent model as in humans. Construct validity concerns the pathways or mechanisms of disease that are supposed to be similar in the rodent model and in humans. It is important to acknowledge that many complex human diseases like major depressive disorder do not have a perfectly corresponding rodent model. However, specific aspects of a human disease can often be reproduced quite accurately in rodents, which can be used to further our understanding of biological systems, how they relate to human health and disease, and how they might be manipulated to our advantage[78].

Rodent models of endophenotypes of depression

Generally, rodent models relevant to depression can be divided into three categories: direct genetic manipulations, breeding for a specific behavioral pattern, and environmental manipulations[77]. Purely genetic techniques that cause baseline depressive-like behavior have a relatively low validity, because depression is an episodic disorder that usually manifests itself during or after adolescence[79]. In contrast, rodent models using stress exposure have higher validity, because life adversity or trauma is the most well-established risk factor for depression in humans[78]. Additionally, the dysregulated stress system shows clear parallels between species[78]. There are various methods used to induce stress in laboratory rodents in the context of depression research, such as chronic mild stress, chronic social defeat stress, social instability, loss of enrichment, social transmission of stress, early life stress, and corticosterone supplementation[80]. Rodents with a genetic vulnerability in combination with an environmental stressor might reproduce the human situation best[77,79].

Modeling aspects of maternal depression during pregnancy

Rodent models relevant to perinatal maternal depression also usually use some form of stress to induce the desired phenotype. The stress can either be applied prior to gestation, during gestation, or in the early postnatal period[81]. This postnatal period is still relevant since this includes the rodent equivalent of the human third trimester[75]. Endophenotypes of depression that are observed in dams exposed to stress are behavioral (such as anhedonia or behavioral despair), physiological (such as weight changes), hormonal (such as stress hormones) and neuroanatomical (such as neuronal death)[81]. In addition, reversal of these changes by antidepressant treatment is sometimes observed[81], confirming the predictive validity of these models. Overall, although they are not a perfect representation of the human situation, these rodent models reproduce crucial neurobiological aspects of peripartum depression[81].

Developmental outcomes

In rodents, perinatal exposure to maternal stress affects behavioral outcomes later in life, such as anxiety-like behavior, and learning and memory[82]. Studies have identified neurochemical, molecular, and epigenetic modifications in the brain that are likely candidates for mediating these effects[83,84].

Perinatal SSRI exposure in rodents

Rodent models of perinatal SSRI exposure

To study the effects of perinatal exposure to SSRIs in rodents, researchers administer these drugs during pregnancy, or in the first postnatal weeks, or throughout this entire period. The pups can either be exposed indirectly, through treatment of the mother and then lactation, or by injecting the newborn pups directly. The method of SSRI administration can be subcutaneous, oral, or intraperitoneal. In contrast with most human studies, these animal experiments involve tight control over drug dosing and timing.

Developmental outcomes

Following concerns about the effects of perinatal SSRI exposure, many animal experiments have been performed to examine neurobiological outcomes, as described in numerous literature reviews[56,62,73,74,85–87]. A range of behavioral outcomes have been assessed, such as anxiety-like behavior and stress-coping behavior. Additionally, neurochemical changes in the brain[88–98] and the neuroendocrine response to stress[92,99–106] have been the focus of study. Brain systems and regions that have been studied include the serotonergic system[107], the prefrontal cortex[108,109], the limbic system[99,110,111], the hippocampus[99,111,112], the dorsal raphe nucleus[109,110], and the hypothalamus[99]. Levels of analyses include brain structure and connectivity[110,113–118], neuronal health[92,97,104,108,119–123], protein expression[98,107,113,124–129], gene expression[98,99,103,112,130–136], and epigenetic modifications[100,112,132,137].

Opportunities for rodent research

Separate and combined effects of maternal depressive-like symptoms and SSRI exposure

The studies described in Section 3.2. investigate the effect of maternal depressive-like symptoms, while those in Section 3.3. investigate the effect of SSRI exposure during pregnancy. However, these two might interact; the effect of developmental SSRI exposure on offspring might depend on the presence or absence of a depressive-like phenotype in the mother[62,74]. In order to enhance the translational value of rodent studies on this topic, models have been designed to examine the effects of early SSRI exposure in combination with maternal (pre)gestational stress[62]. It has been shown that (pre)gestational maternal stress and perinatal SSRI exposure interact to affect stress-coping behavior and neurogenesis[122], and serotonin levels in the brain[138,139]. However, the number of studies of this interaction is still limited. Replication studies and studies that investigate the underlying mechanisms are needed.

Novel methods and mechanisms: microbiomics and transcriptomics

Molecular biology techniques offer the opportunity to investigate the "how": which biological pathways mediate the long-term effects of maternal depression and antidepressant use during pregnancy? Technology is rapidly advancing, and the accessibility and affordability of methods that already exist is also growing. This has opened up an era of unprecedented creation and analysis of large, complex datasets on biological systems at various levels. Microbiomics and transcriptomics are two promising "-omics" analyses that may provide new information on the long-term effects of maternal depressive-like symptoms and SSRI exposure.

The gut microbiome is collective genetic material of all the microorganisms residing in the gastrointestinal tract. The gut microbiome and host serotonin regulation influence each other. Some microbes modulate serotonin homeostasis[140], while altered serotonin homeostasis by SSRI treatment is associated with the composition of the gut microbiome in humans[141–144] and rodents[145–150]. In addition, major depressive disorder in humans[141,151–156] and depressive-like symptoms in rodents[157–161] are associated with distinct gut microbiota signatures. During pregnancy, the gut microbiome is remodeled[162], potentially altering its potentially increasing its vulnerability to pharmacological and environmental influences. Importantly, the perinatal period is characterized by a high metabolic demand[163], and the gut microbiota synthesizes a proportion of metabolites that are found in the systemic circulation[164,165]. SSRI use might alter the metabolic output of the maternal gut microbiota, and thereby metabolite supply to the developing fetus. This is a yet unexplored potential pathway to lasting health outcomes.

Long-term behavioral effects of exposure to maternal depressive-like symptoms and SSRI use during pregnancy are thought to be the result of changes in brain development. Despite evidence that the corticolimbic- and somatosensory system in the brain play a role[45], the developmental pathways are largely unknown. Rodents offer the opportunity to study the brain down to the molecular level at the developmental stage of choice. RNA sequencing (RNAseq) and subsequent bioinformatics analyses yield a complete picture of the gene expression in a particular brain region at a particular time. Although targeted gene expression assays have been used in this field to examine serotonin-[131,133] and neurotrophin-related[100,103] genes, unbiased approaches such as RNAseq can generate new insights.

Sex differences

Both human and animal research has traditionally been conducted mainly in male subjects. However, it is known that many physiological systems, including the brain, exhibit sex-specific characteristics[166]. For example, developmental exposure to stress affects males and females differently and might be the reason for differential susceptibility to certain mental disorders between the two sexes[167]. Additionally, evidence suggests that the brain serotonin system matures at a different rate in males than females[62]. A growing awareness of the importance of studying both sexes, compounded by requirements from funding bodies and ethical approval boards, is now leading to an increase in studies focusing on females[168]. Importantly, when both sexes are investigated, sex-specific effects are often observed in the neurodevelopmental effects of early exposure to SSRIs[118,139,169] and prenatal stress[139,170]. However, many researchers still use exclusively males in their

studies, as is the case for all transcriptome-wide analyses of the brain after early SSRI exposure published so far[99,111,112]. More studies are therefore needed to determine if and how males and females might be differentially affected at the molecular level.

Improving translatability of animal studies

In line with the "3Rs" of animal research, Replacement, Reduction, and Refinement, continuous efforts are needed to maximize the utility of the animal studies[171]. One approach to this is to make better use of all the animal experiments that have already been performed in the past, by combining studies in a systematic review. Systematic reviews have long been considered to provide the highest form of medical scientific evidence, but are surprisingly rarely performed on animal studies[172]. This despite the fact that human clinical trials are usually based on the results of preclinical evidence[172]. Although systematic reviews of animal studies gained some popularity over the past decade, there is still a lot of untapped potential in using them to summarize, analyze, evaluate and communicate the results of animal studies[173]. For example, meta-analyses are powerful tools for combining the results of animal studies, especially considering that individual studies are often underpowered[174].

Experimental approach

The work using laboratory rats described in this thesis consists of a two-part animal model with the aim of mimicking key aspects of maternal depressive-like symptoms and SSRI use in humans. The first part is a model of maternal vulnerability, and the second part is treatment with SSRIs in the peripartum period.

Model for maternal vulnerability

SERT genotype X stress interaction

The animal model is based on findings from a human population study on a Gene X Environment interaction published in the early 2000s[175]. With rising interest in behavioral genetics, and fueled by developments in molecular biology techniques, several influential studies by the same research group were published in *Science* showing evidence that particular genotypes related to serotonin homeostasis modulate the effect of adverse experiences on the risk for developing psychopathology[176]. For example, they showed that a polymorphism in monoamine oxidase A (*MAOA*), coding for the enzyme that breaks down monoaminergic neurotransmitters such as serotonin, interacted with childhood maltreatment to influence antisocial behavior in men[177].

Their next study focused on a polymorphism in the promoter region of the *SERT* gene; the 5-HTT gene-linked polymorphic region (5-HTTLPR)[178]. This polymorphism is naturally present in the human population and modulates the transcription of the SERT[178]. The short (s) allele is associated with lower SERT gene expression than the long (l) allele[178,179]. About 50% of the population has an "s/l" genotype, and about 20% an "s/s" genotype[178]. The research group hypothesized that the 5-HTTLPR polymorphism acts as a moderator of the risk of developing depression, based on three lines of evidence[175]. First, SERT knockout mice were shown to display more fearful behaviors[180] and a heightened adrenocorticotropic hormone (ACTH) response to acute

injection stress[181] compared to their wild type counterparts, suggesting that genetically-determined lowered SERT expression leads to a more stress-reactive phenotype. Second, in rhesus macaques the "s" allele is associated with decreased serotonergic function only in individuals exposed to stress early in life[182]. Third, in humans the "s" allele is associated with enhanced reactivity of the amygdala to fearful stimuli, suggesting that the stress response is modulated by the 5-HTTLPR[183].

Indeed, the results of their study on 847 men and women showed that the association between the number of stressful life events, such as employment-, housing- or relationship stressors, and the probability of a major depressive episode was weakest among "l/l" allele carriers, stronger in "s/l" carriers, and strongest in those with an "s/s" genotype[175]. In addition, childhood maltreatment only predicted adult depression in those carrying at least one "s" allele[175]. These results inspired many follow-up studies in various human cohorts[184], as well as animal experiments[185].

Heterozygous serotonin transporter knockout (SERT$^{+/-}$) rat

Although the SERT polymorphism occurs naturally in humans and other primates, it does not in rodents. Therefore, researchers have turned to genetic knockout models in laboratory rodents to study the effects of these gene variants in a controlled environment. Since genetic manipulation tools in mice are more advanced than in rats, SERT knockout mice already existed before the year 2000[186]. A rat SERT knockout model was long awaited, because rats offer advantages over mice in research areas covering complex behaviors, physiology and pharmacology[76,187]. In the early 2000s, N-ethyl-N-nitrosurea (ENU)-driven target-selected mutagenesis was used to introduce a premature stop codon in the rat SERT gene, resulting in a non-functional protein product and the first SERT knockout Wistar rats (Slc6a41^{Hubr})[188,189]. While SERT protein and gene expression are completely absent in homozygous knockout (SERT$^{-/-}$) animals, heterozygous knockout animals (SERT$^{+/-}$) show only a 40-50% decreased expression of SERT compared to wildtype animals[186,190]. This is similar to the expression in human "s" allele carriers. Combined with neurochemical evidence, this suggests that SERT$^{+/-}$ animals are translationally relevant[186,190,191].

Early life stress in SERT$^{+/-}$ female rats

To study the effects of maternal depressive-like symptoms, the experimental work in this thesis involves a rat model of maternal vulnerability (MV), based on the human SERT X stress interaction studies described above. First, SERT$^{+/-}$ rats were mated, leading to litters with SERT$^{+/+}$, SERT$^{+/-}$, and SERT$^{-/-}$ offspring (F0). After birth at postnatal day 0 (PND0), litters were allocated to either a stressful- or a control rearing condition.

The stressful rearing condition involved separating the pups from the dam for 6 hours daily from PND2 until PND15 (Figure 1). This procedure, known as maternal separation, is a commonly used method of inducing early life stress in laboratory rodents[192]. The maternal separation procedure was initiated every day at the same time. Pups were taken from their home cage, and placed per litter in a smaller cage with sawdust bedding. The cage was moved to another room to prevent communication with the dam, and kept warm on a heating mat to aid the pups in maintaining body temperature. The control rearing condition involved handling the pups for 15 minutes daily from PND2 until PND15 (Figure 1). This method was chosen to control for the effect

of enhanced maternal care that is usually observed when pups are reunited with the dam[193]. At PND21, pups were ear punched for identification, and the punched tissue was used for SERT genotyping. They were then weaned, and socially housed with same-sex animals.

The SERT$^{+/-}$ female offspring from these nests (F1) possess a genetic predisposition to stress vulnerability. In adulthood, previously stressed SERT$^{+/-}$ females show anhedonia, an endophenotype associated with depressive symptoms[194]. Compared to their control counterparts with the same vulnerable genotype, stressed females have a lower preference for sucrose, and lower gene expression of nerve growth factor in the basolateral amygdala and the paraventricular nucleus in the brain[194]. Because these SERT$^{+/-}$ females become mothers themselves (to generate F2), they are referred to as maternal vulnerability (MV) females, who have either been stressed in early life (sMV) or control handled (cMV) (Figure 1).

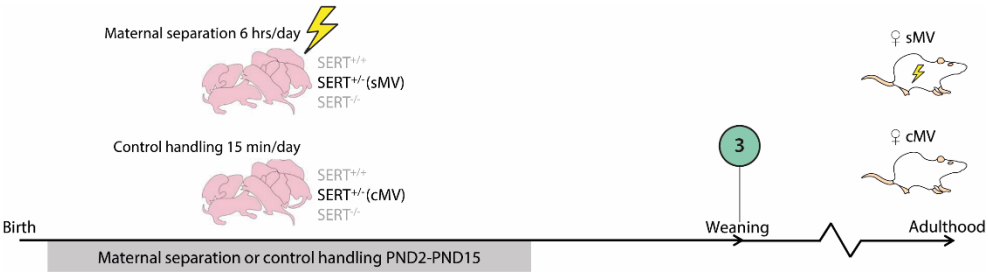

Figure 1: Early life stress in SERT$^{+/-}$ females. *The encircled number (3) refers to its respective chapter number. Fecal samples were collected at weaning age (3).*

Perinatal fluoxetine treatment

sMV and cMV female rats were paired with wildtype males for 24 hours. This was gestational day 0 (GD0). Dams were treated with the SSRI fluoxetine (FLX) or vehicle (Veh) for 6 weeks: from the start of pregnancy (GD1) until the end of lactation (PND21) (Figure 2). Every day, the dams were weighed and then received an oral dose of 10 mg/kg FLX or Veh (1% methylcellulose).

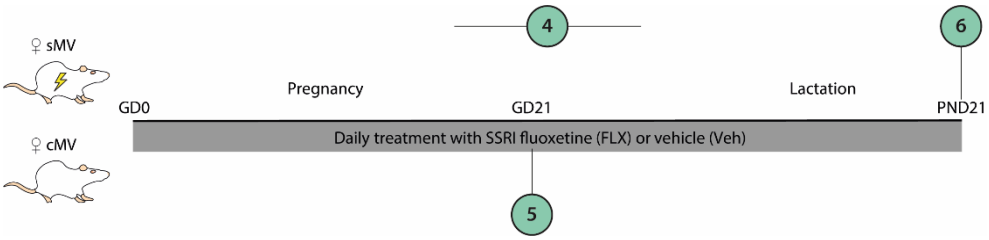

Figure 2: Perinatal fluoxetine treatment. *The encircled numbers (4, 5, and 6) refer to their respective chapter numbers. Fecal samples were collected throughout pregnancy and lactation (4), placentas were collected at GD21 (5), and offspring brains were collected at weaning age (6).*

Outcome measures

Behavioral consequences for the offspring using this experimental approach have been described elsewhere[195]. In short, developmental fluoxetine exposure is associated with reduced ultrasonic vocalizations in pups, reduced social play behavior in male and female juveniles, and reduced social behavior in male adults[196]. In addition, it increases anxiety-like behavior and anhedonia in females, but not in males[197]. In adult males, perinatal FLX exposure reduces aggression[198]. Overall sexual performance is not affected, but a reduced mounting frequency is observed[198]. Lastly, perinatal FLX exposure alters aspects of female circadian behavior, but only after a non-photic challenge[199]. Maternal sMV phenotype is associated with increased social behavior in adult female offspring. Overall, developmental FLX exposure has sex-specific long-term effects on behavior that are stronger than those of the maternal sMV phenotype.

This thesis describes the potential molecular underpinnings of these alterations in behavior, using the same animal model. The microbiome and metabolic output were studied in fecal samples as described in **Chapters 3 and 4** (Figure 1 and 2), gene expression was examined in the placenta in **Chapter 5** (Figure 2) and transcriptomics and epigenetics were investigated in offspring brain regions in **Chapter 6** (Figure 2).

Aim and outline of this thesis

Aim of thesis

The overall aim of this PhD thesis is to examine potential mechanisms linking perinatal SSRI antidepressant exposure to long-term health outcomes, using a rat model of maternal depressive-like symptoms. To this end, the following studies were conducted and described in this thesis:

Chapter 2: meta-analyses

The aim of **Chapter 2** was to determine whether there is an overall effect of perinatal SSRI exposure in animals on behavioral outcomes in all previously published studies. Using a systematic search, we identified all available studies of this topic. Then, we performed meta-analyses corresponding to 9 behavioral domains. We also investigated the potential modulating roles of animal sex, the presence or absence of stress exposure, and timing of SSRI exposure.

Chapter 3: the juvenile microbiome

Chapter 3 was designed to characterize the influence of SERT genotype on the gut microbiota composition in juvenile animals (Figure 1). In addition, we investigated the effect of early life stress exposure and its potential interactions with SERT genotype on the gut microbiota. To this end, we collected fecal samples at weaning age and used 16S ribosomal RNA marker gene sequencing and bioinformatics techniques to characterize features of the microbiome.

Chapter 4: the maternal microbiome

In **Chapter 4**, we hypothesized that a depressive-like phenotype, SSRI treatment, and their combination affect the microbial community composition and function during pregnancy and the postpartum period. Therefore, we collected weekly fecal samples during pregnancy and lactation for 16S rRNA gene sequencing (Figure 2). Moreover, to investigate whether changes in the gut microbiome relate to changes in metabolic output, we performed targeted metabolomic analyses.

Chapter 5: placental gene expression

Chapter 5 describes a study aimed at validating changes in placental gene expression in pregnancies characterized by maternal depressive symptoms and SSRI use as described in the human literature. We collected rat placentas for analysis just before the moment of natural birth (Figure 2). Then, we measured expression of genes related to the stress- and serotonin system, neurogenesis, signal transduction, and angiogenesis.

Chapter 6: brain transcriptomics and epigenetics

The aim of **Chapter 6** was to assess sex-specific molecular alterations in the prefrontal cortex and basolateral amygdala of juvenile rats. After exposure to a maternal depressive-like phenotype and/or SSRIs, brains of male and female offspring were collected at weaning age (Figure 2). RNA sequencing was used to identify differences in transcriptomic state, and subsequent DNA methylation of targeted genes was used to correlate gene expression to epigenetic regulation.

Chapter 7: general discussion

In **Chapter 7**, all previous chapters are summarized and potential areas of synergy discussed. The strengths, limitations and implications of the results presented in this thesis are outlined. Finally, suggestions for future studies are presented.

References

1. Hanson, M. A. & Gluckman, P. D. Early Developmental Conditioning of Later Health and Disease: Physiology or Pathophysiology? *Physiol. Rev.* **94**, 1027–1076 (2014).
2. Barker, D. J. The fetal and infant origins of adult disease. *BMJ* **301**, 1111 (1990).
3. Belsky, J., Bakermans-Kranenburg, M. J. & van IJzendoorn, M. H. For Better and For Worse. *Curr. Dir. Psychol. Sci.* **16**, 300–304 (2007).
4. World Health Organization. Meeting report: Nurturing human capital along the life course: Investing in early child development. 28 (2013).
5. Unicef. The first 1,000 days of life: The brain's window of opportunity. https://www.unicef-irc.org/article/958-the-first-1000-days-of-life-the-brains-window-of-opportunity.html.
6. Lindsay, K. L., Buss, C., Wadhwa, P. D. & Entringer, S. The Interplay Between Nutrition and Stress in Pregnancy: Implications for Fetal Programming of Brain Development. *Biol. Psychiatry* **85**, 135–149 (2019).
7. Franke, K., Gaser, C., Roseboom, T. J., Schwab, M. & de Rooij, S. R. Premature brain aging in humans exposed to maternal nutrient restriction during early gestation. *Neuroimage* **173**, 460–471 (2018).
8. Walhovd, K. B. *et al.* Long-term influence of normal variation in neonatal characteristics on human brain development. *Proc. Natl. Acad. Sci.* **109**, 20089–20094 (2012).
9. Bale, T. L. *et al.* Early life programming and neurodevelopmental disorders. *Biol. Psychiatry* **68**, 314–319 (2010).

10. Blair, C. & Raver, C. C. Poverty, Stress, and Brain Development: New Directions for Prevention and Intervention. *Acad. Pediatr.* **16**, S30–S36 (2016).
11. Szyf, M. & Bick, J. DNA Methylation: A Mechanism for Embedding Early Life Experiences in the Genome. *Child Dev.* **84**, 49–57 (2013).
12. Nugent, B. M. & Bale, T. L. The omniscient placenta: Metabolic and epigenetic regulation of fetal programming. *Front. Neuroendocrinol.* **39**, 28–37 (2015).
13. Becker, M., Weinberger, T., Chandy, A. & Schmukler, S. Depression During Pregnancy and Postpartum. *Curr. Psychiatry Rep.* **18**, 32 (2016).
14. Dadi, A. F., Miller, E. R., Bisetegn, T. A. & Mwanri, L. Global burden of antenatal depression and its association with adverse birth outcomes: an umbrella review. *BMC Public Health* **20**, 173 (2020).
15. Underwood, L., Waldie, K., D'Souza, S., Peterson, E. R. & Morton, S. A review of longitudinal studies on antenatal and postnatal depression. *Arch. Womens. Ment. Health* **19**, 711–720 (2016).
16. Gavin, N. I. *et al.* Perinatal depression: A systematic review of prevalence and incidence. *Obstet. Gynecol.* **106**, 1071–1083 (2005).
17. Ornoy, A. & Koren, G. SSRIs and SNRIs (SRI) in Pregnancy: Effects on the Course of Pregnancy and the Offspring: How Far Are We from Having All the Answers? *Int. J. Mol. Sci.* **20**, 2370 (2019).
18. Palmsten, K., Setoguchi, S., Margulis, A. V., Patrick, A. R. & Hernández-Díaz, S. Elevated Risk of Preeclampsia in Pregnant Women With Depression: Depression or Antidepressants? *Am. J. Epidemiol.* **175**, 988–997 (2012).
19. Gentile, S. Untreated depression during pregnancy: Short- and long-term effects in offspring. A systematic review. *Neuroscience* **342**, 154–166 (2017).
20. Osborne, S. *et al.* Antenatal depression programs cortisol stress reactivity in offspring through increased maternal inflammation and cortisol in pregnancy: The Psychiatry Research and Motherhood – Depression (PRAM-D) Study. *Psychoneuroendocrinology* **98**, 211–221 (2018).
21. O'Leary, N. *et al.* Antenatal depression and the impact on infant cognitive, language and motor development at six and twelve months postpartum. *Early Hum. Dev.* **134**, 41–46 (2019).
22. Hutchison, S. M., Mâsse, L. C., Brain, U. & Oberlander, T. F. A 6-year longitudinal study: Are maternal depressive symptoms and Selective Serotonin Reuptake Inhibitor (SSRI) antidepressant treatment during pregnancy associated with everyday measures of executive function in young children? *Early Hum. Dev.* **128**, 21–26 (2019).
23. Qiu, A. *et al.* Prenatal maternal depression alters amygdala functional connectivity in 6-month-old infants. *Transl. Psychiatry* **5**, e508–e508 (2015).
24. Sandman, C. A., Buss, C., Head, K. & Davis, E. P. Fetal Exposure to Maternal Depressive Symptoms Is Associated With Cortical Thickness in Late Childhood. *Biol. Psychiatry* **77**, 324–334 (2015).
25. El Marroun, H. *et al.* Prenatal Exposure To Maternal and Paternal Depressive Symptoms and Brain Morphology: a Population-Based Prospective Neuroimaging Study in Young Children. *Depress. Anxiety* **33**, 658–666 (2016).
26. van der Knaap, N. J. F. *et al.* Maternal depressive symptoms during pregnancy are associated with amygdala hyperresponsivity in children. *Eur. Child Adolesc. Psychiatry* **27**, 57–64 (2018).
27. Soe, N. N. *et al.* Perinatal maternal depressive symptoms alter amygdala functional connectivity in girls. *Hum. Brain Mapp.* **39**, 680–690 (2018).
28. Wen, D. J. *et al.* Influences of prenatal and postnatal maternal depression on amygdala volume and microstructure in young children. *Transl. Psychiatry* **7**, e1103–e1103 (2017).
29. Lebel, C. *et al.* Prepartum and Postpartum Maternal Depressive Symptoms Are Related to Children's Brain Structure in Preschool. *Biol. Psychiatry* **80**, 859–868 (2016).
30. Pawlby, S., Hay, D. F., Sharp, D., Waters, C. S. & O'Keane, V. Antenatal depression predicts depression in adolescent offspring: Prospective longitudinal community-based study. *J. Affect. Disord.* **113**, 236–243 (2009).
31. Beijers, L., Wardenaar, K. J., van Loo, H. M. & Schoevers, R. A. Data-driven biological subtypes of depression: systematic review of biological approaches to depression subtyping. *Mol. Psychiatry* **24**, 888–900 (2019).
32. Coppen, A. The biochemistry of affective disorders. *Br. J. Psychiatry* **113**, 1237–1264 (1967).
33. Claassen, V., Davies, J. E., Hertting, G. & Placheta, P. Fluvoxamine, a Specific 5-Hydroxytryptamine Uptake Inhibitor. *Br. J. Pharmacol.* **60**, 505–516 (1977).
34. Pereira, V. S. & Hiroaki-Sato, V. A. A brief history of antidepressant drug development: from tricyclics to beyond

ketamine. *Acta Neuropsychiatr.* **30**, 307–322 (2018).
35. Gentile, S. The Safety of Newer Antidepressants in Pregnancy and Breastfeeding. *Drug Saf.* **28**, 137–152 (2005).
36. Bakker, M. K., Kölling, P., van den Berg, P. B., de Walle, H. E. K. & de Jong van den Berg, L. T. W. Increase in use of selective serotonin reuptake inhibitors in pregnancy during the last decade, a population-based cohort study from the Netherlands. *Br. J. Clin. Pharmacol.* **65**, 600–606 (2008).
37. Jimenez-Solem, E. *et al.* Prevalence of Antidepressant Use during Pregnancy in Denmark, a Nation-Wide Cohort Study. *PLoS One* **8**, e63034 (2013).
38. Cooper, W. O., Willy, M. E., Pont, S. J. & Ray, W. A. Increasing use of antidepressants in pregnancy. *Am. J. Obstet. Gynecol.* **196**, 544.e1-544.e5 (2007).
39. Andrade, S. E. *et al.* Use of antidepressant medications during pregnancy: a multisite study. *Am. J. Obstet. Gynecol.* **198**, 194.e1–5 (2008).
40. Zoega, H. *et al.* Use of SSRI and SNRI Antidepressants during Pregnancy: A Population-Based Study from Denmark, Iceland, Norway and Sweden. *PLoS One* **10**, e0144474 (2015).
41. Jordan, S. *et al.* Selective Serotonin Reuptake Inhibitor (SSRI) Antidepressants in Pregnancy and Congenital Anomalies: Analysis of Linked Databases in Wales, Norway and Funen, Denmark. *PLoS One* **11**, e0165122 (2016).
42. Hayes, R. M. *et al.* Maternal antidepressant use and adverse outcomes: a cohort study of 228,876 pregnancies. *Am. J. Obstet. Gynecol.* **207**, 49.e1-49.e9 (2012).
43. Huybrechts, K. F. *et al.* Antidepressant Use Late in Pregnancy and Risk of Persistent Pulmonary Hypertension of the Newborn. *JAMA* **313**, 2142 (2015).
44. Alwan, S., Friedman, J. M. & Chambers, C. Safety of Selective Serotonin Reuptake Inhibitors in Pregnancy: A Review of Current Evidence. *CNS Drugs* **30**, 499–515 (2016).
45. Homberg, J. R., Schubert, D. & Gaspar, P. New perspectives on the neurodevelopmental effects of SSRIs. *Trends Pharmacol. Sci.* **31**, 60–65 (2010).
46. Hanley, G. E., Brain, U. & Oberlander, T. F. Prenatal exposure to serotonin reuptake inhibitor antidepressants and childhood behavior. *Pediatr. Res.* **78**, 174–180 (2015).
47. Hanley, G. E., Brain, U. & Oberlander, T. F. Infant developmental outcomes following prenatal exposure to antidepressants, and maternal depressed mood and positive affect. *Early Hum. Dev.* **89**, 519–524 (2013).
48. Halvorsen, A., Hesel, B., Østergaard, S. D. & Danielsen, A. A. In utero exposure to selective serotonin reuptake inhibitors and development of mental disorders: a systematic review and meta-analysis. *Acta Psychiatr. Scand.* **139**, 493–507 (2019).
49. Harrington, R. A., Lee, L.-C., Crum, R. M., Zimmerman, A. W. & Hertz-Picciotto, I. Prenatal SSRI Use and Offspring With Autism Spectrum Disorder or Developmental Delay. *Pediatrics* **133**, e1241–e1248 (2014).
50. Viktorin, A. *et al.* Association of Antidepressant Medication Use During Pregnancy With Intellectual Disability in Offspring. *JAMA Psychiatry* **74**, 1031 (2017).
51. Lupattelli, A. *et al.* Effect of Time-Dependent Selective Serotonin Reuptake Inhibitor Antidepressants During Pregnancy on Behavioral, Emotional, and Social Development in Preschool-Aged Children. *J. Am. Acad. Child Adolesc. Psychiatry* **57**, 200–208 (2018).
52. Podrebarac, S. K. *et al.* Antenatal exposure to antidepressants is associated with altered brain development in very preterm-born neonates. *Neuroscience* **342**, 252–262 (2017).
53. Lugo-Candelas, C. *et al.* Associations between brain structure and connectivity in infants and exposure to selective serotonin reuptake inhibitors during pregnancy. *JAMA Pediatr.* **172**, 525–533 (2018).
54. Rotem-Kohavi, N. *et al.* Alterations in Resting-State Networks Following In Utero Selective Serotonin Reuptake Inhibitor Exposure in the Neonatal Brain. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* **4**, 39–49 (2019).
55. Videman, M. *et al.* Newborn Brain Function Is Affected by Fetal Exposure to Maternal Serotonin Reuptake Inhibitors. *Cereb. Cortex* **27**, 3208–3216 (2017).
56. Millard, S. J., Weston-Green, K. & Newell, K. A. The effects of maternal antidepressant use on offspring behaviour and brain development: Implications for risk of neurodevelopmental disorders. *Neurosci. Biobehav. Rev.* **80**, 743–765 (2017).
57. Sorensen, M. J. *et al.* Antidepressant exposure in pregnancy and risk of autism spectrum disorders. *Clin. Epidemiol.* **5**, 449 (2013).

58. Jha, S. C. *et al.* Antenatal depression, treatment with selective serotonin reuptake inhibitors, and neonatal brain structure: A propensity-matched cohort study. *Psychiatry Res. Neuroimaging* **253**, 43–53 (2016).
59. Rampono, J. *et al.* Placental Transfer of SSRI and SNRI Antidepressants and Effects on the Neonate. *Pharmacopsychiatry* **42**, 95–100 (2009).
60. Gaspar, P., Cases, O. & Maroteaux, L. The developmental role of serotonin: news from mouse molecular genetics. *Nat. Rev. Neurosci.* **4**, 1002–1012 (2003).
61. Teissier, A., Soiza-Reilly, M. & Gaspar, P. Refining the Role of 5-HT in Postnatal Development of Brain Circuits. *Front. Cell. Neurosci.* **11**, 139 (2017).
62. Brummelte, S., Mc Glanaghy, E., Bonnin, A. & Oberlander, T. F. Developmental changes in serotonin signaling: Implications for early brain function, behavior and adaptation. *Neuroscience* **342**, 212–231 (2017).
63. Glasser, M. F. *et al.* The Human Connectome Project's neuroimaging approach. *Nat. Neurosci.* **19**, 1175–1187 (2016).
64. Maltepe, E. & Fisher, S. J. Placenta: The Forgotten Organ. *Annu. Rev. Cell Dev. Biol.* **31**, 523–552 (2015).
65. Lester, B. M. & Marsit, C. J. Epigenetic mechanisms in the placenta related to infant neurodevelopment. *Epigenomics* **10**, 321–333 (2018).
66. Velasquez, J. C., Goeden, N. & Bonnin, A. Placental serotonin: implications for the developmental effects of SSRIs and maternal depression. *Front. Cell. Neurosci.* **7**, 1–7 (2013).
67. Olivier, J. D. A. *et al.* The effects of antenatal depression and antidepressant treatment on placental gene expression. *Front. Cell. Neurosci.* **8**, 1–17 (2015).
68. Kaihola, H. *et al.* Effects of Fluoxetine on Human Embryo Development. *Front. Cell. Neurosci.* **10**, (2016).
69. Rosenthal, N. & Brown, S. The mouse ascending: perspectives for human-disease models. *Nat. Cell Biol.* **9**, 993–9 (2007).
70. Gurumurthy, C. B. & Lloyd, K. C. K. Generating mouse models for biomedical research: technological advances. *Dis. Model. Mech.* **12**, dmm029462 (2019).
71. Kent Lloyd, K. C., Robinson, P. N. & MacRae, C. A. Animal-based studies will be essential for precision medicine. *Sci. Transl. Med.* **8**, 352ed12-352ed12 (2016).
72. Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M. & Noble-Haeusslein, L. J. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog. Neurobiol.* **106–107**, 1–16 (2013).
73. Gingrich, J. A. *et al.* New Insights into How Serotonin Selective Reuptake Inhibitors Shape the Developing Brain. *Birth Defects Res.* **109**, 924–932 (2017).
74. Bourke, C. H., Stowe, Z. N. & Owens, M. J. Prenatal Antidepressant Exposure: Clinical and Preclinical Findings. *Pharmacol. Rev.* **66**, 435–465 (2014).
75. Workman, A. D., Charvet, C. J., Clancy, B., Darlington, R. B. & Finlay, B. L. Modeling Transformations of Neurodevelopmental Sequences across Mammalian Species. *J. Neurosci.* **33**, 7368–7383 (2013).
76. Ellenbroek, B. & Youn, J. Rodent models in neuroscience research: is it a rat race? *Dis. Model. Mech.* **9**, 1079–1087 (2016).
77. Neumann, I. D. *et al.* Animal models of depression and anxiety: What do they tell us about human condition? *Prog. Neuro-Psychopharmacology Biol. Psychiatry* **35**, 1357–1375 (2011).
78. Bale, T. L. *et al.* The critical importance of basic animal research for neuropsychiatric disorders. *Neuropsychopharmacology* **44**, 1349–1353 (2019).
79. Czéh, B., Fuchs, E., Wiborg, O. & Simon, M. Animal models of major depression and their clinical implications. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* **64**, 293–310 (2016).
80. Gururajan, A., Reif, A., Cryan, J. F. & Slattery, D. A. The future of rodent models in depression research. *Nat. Rev. Neurosci.* **20**, 686–701 (2019).
81. Bogi, E., Belovicova, K., Csatlosova, K. & Dubovicky, M. Animal models of maternal depression for monitoring neurodevelopmental changes occurring in dams and offspring. *Interdiscip. Toxicol.* **10**, 35–39 (2017).
82. Weinstock, M. Prenatal stressors in rodents: Effects on behavior. *Neurobiol. Stress* **6**, 3–13 (2017).
83. Babenko, O., Kovalchuk, I. & Metz, G. A. S. Stress-induced perinatal and transgenerational epigenetic programming of brain development and mental health. *Neurosci. Biobehav. Rev.* **48**, 70–91 (2015).

84. Bale, T. L. Epigenetic and transgenerational reprogramming of brain development. *Nat. Rev. Neurosci.* **16**, 332–344 (2015).
85. Glover, M. E. & Clinton, S. M. Of rodents and humans: A comparative review of the neurobehavioral effects of early life SSRI exposure in preclinical and clinical research. *Int. J. Dev. Neurosci.* **51**, 50–72 (2016).
86. Grieb, Z. A. & Ragan, C. M. The effects of perinatal SSRI exposure on anxious behavior and neurobiology in rodent and human offspring. *Eur. Neuropsychopharmacol.* 1–16 (2019) doi:10.1016/j.euroneuro.2019.07.239.
87. Ornoy, A. Neurobehavioral risks of SSRIs in pregnancy: Comparing human and animal data. *Reprod. Toxicol.* **72**, 191–200 (2017).
88. Zohar, I., Shoham, S. & Weinstock, M. Perinatal citalopram does not prevent the effect of prenatal stress on anxiety, depressive-like behaviour and serotonergic transmission in adult rat offspring. *Eur. J. Neurosci.* **43**, 590–600 (2016).
89. Yu, Q. *et al.* Dopamine and serotonin signaling during two sensitive developmental periods differentially impact adult aggressive and affective behaviors in mice. *Mol. Psychiatry* **19**, 688–698 (2014).
90. Nagano, R., Nagano, M., Nakai, A., Takeshita, T. & Suzuki, H. Differential effects of neonatal SSRI treatments on hypoxia-induced behavioral changes in male and female offspring. *Neuroscience* **360**, 95–105 (2017).
91. Altieri, S. C. *et al.* Perinatal vs genetic programming of serotonin states associated with anxiety. *Neuropsychopharmacology* **40**, 1456–70 (2015).
92. Gemmel, M. *et al.* Perinatal fluoxetine effects on social play, the HPA system, and hippocampal plasticity in pre-adolescent male and female rats: Interactions with pre-gestational maternal stress. *Psychoneuroendocrinology* **84**, 159–171 (2017).
93. Grimm, V. E. & Frieder, B. Prenatal and early postnatal exposure to zimelidine: behavioral, neurochemical and histological findings in rats. *Int. J. Neurosci.* **33**, 225–235 (1987).
94. Glazova, N. Y. *et al.* Effects of neonatal fluvoxamine administration on the physical development and activity of the serotoninergic system in white rats. *Acta Naturae* **6**, 98–105 (2014).
95. Hilakivi, L. A., Hilakivi, I. & Kiianmaa, K. Neonatal antidepressant administration suppresses concurrent active (REM) sleep and increases adult alcohol consumption in rats. *Alcohol Alcohol Suppl.* **1**, 339–43 (1987).
96. Hilakivi, L. A., Stenberg, D., Sinclair, J. D. & Kiianmaa, K. Neonatal desipramine or zimeldine treatment causes long-lasting changes in brain monoaminergic systems and alcohol related behavior in rats. *Psychopharmacology (Berl).* **91**, 403–409 (1987).
97. Ishiwata, H., Shiga, T. & Okado, N. Selective serotonin reuptake inhibitor treatment of early postnatal mice reverses their prenatal stress-induced brain dysfunction. *Neuroscience* **133**, 893–901 (2005).
98. Pinheiro, I. L. *et al.* Neonatal fluoxetine exposure modulates serotonergic neurotransmission and disturb inhibitory action of serotonin on food intake. *Behav. Brain Res.* **357–358**, 65–70 (2019).
99. Bourke, C. H., Stowe, Z. N., Neigh, G. N., Olson, D. E. & Owens, M. J. Prenatal exposure to escitalopram and/or stress in rats produces limited effects on endocrine, behavioral, or gene expression measures in adult male rats. *Neurotoxicol. Teratol.* **39**, 100–109 (2013).
100. Boulle, F. *et al.* Developmental fluoxetine exposure increases behavioral despair and alters epigenetic regulation of the hippocampal BDNF gene in adult female offspring. *Horm. Behav.* **80**, 47–57 (2016).
101. Knaepen, L. *et al.* Developmental Fluoxetine Exposure Normalizes the Long-Term Effects of Maternal Stress on Post-Operative Pain in Sprague-Dawley Rat Offspring. *PLoS One* **8**, e57608 (2013).
102. Popa, D., Léna, C., Alexandre, C. & Adrien, J. Lasting syndrome of depression produced by reduction in serotonin uptake during postnatal development: evidence from sleep, stress, and behavior. *J. Neurosci.* **28**, 3546–54 (2008).
103. Boulle, F. *et al.* Prenatal stress and early-life exposure to fluoxetine have enduring effects on anxiety and hippocampal BDNF gene expression in adult male offspring. *Dev. Psychobiol.* **58**, 427–438 (2016).
104. Gobinath, A. R., Workman, J. L., Chow, C., Lieblich, S. E. & Galea, L. A. M. M. Maternal postpartum corticosterone and fluoxetine differentially affect adult male and female offspring on anxiety-like behavior, stress reactivity, and hippocampal neurogenesis. *Neuropharmacology* **101**, 165–178 (2016).
105. Pivina, S. G., Fedotova, Y. O., Akulova, V. K. & Ordyan, N. E. Effects of selective inhibitors of serotonin reuptake on the anxiety behavior and activity of the pituitary-adrenal system in prenatally stressed male rats. *Neurochem. J.* **5**, 47–51 (2011).

106. Salari, A.-A., Fatehi-Gharehlar, L., Motayagheni, N. & Homberg, J. R. Fluoxetine normalizes the effects of prenatal maternal stress on depression- and anxiety-like behaviors in mouse dams and male offspring. *Behav. Brain Res.* **311**, 354–367 (2016).
107. Maciag, D. *et al.* Neonatal Antidepressant Exposure has Lasting Effects on Behavior and Serotonin Circuitry. *Neuropsychopharmacology* **31**, 47–57 (2006).
108. Rebello, T. J. *et al.* Postnatal Day 2 to 11 Constitutes a 5-HT-Sensitive Period Impacting Adult mPFC Function. *J. Neurosci.* **34**, 12379–12393 (2014).
109. Soiza-Reilly, M. *et al.* SSRIs target prefrontal to raphe circuits during development modulating synaptic connectivity and emotional behavior. *Mol. Psychiatry* **24**, 726–745 (2019).
110. Simpson, K. L. *et al.* Perinatal antidepressant exposure alters cortical network function in rodents. *Proc. Natl. Acad. Sci.* **108**, 18465–18470 (2011).
111. Glover, M. E. *et al.* Early-life exposure to the SSRI paroxetine exacerbates depression-like behavior in anxiety/depression-prone rats. *Neuroscience* **284**, 775–797 (2015).
112. Sarkar, A. *et al.* Hippocampal HDAC4 Contributes to Postnatal Fluoxetine-Evoked Depression-Like Behavior. *Neuropsychopharmacology* **39**, 2221–2232 (2014).
113. Forcelli, P. A. & Heinrichs, S. C. Teratogenic effects of maternal antidepressant exposure on neural substrates of drug-seeking behavior in offspring. *Addict. Biol.* **13**, 52–62 (2008).
114. Lee, L.-J. Neonatal fluoxetine exposure affects the neuronal structure in the somatosensory cortex and somatosensory-related behaviors in adolescent rats. *Neurotox. Res.* **15**, 212–23 (2009).
115. Rayen, I., Steinbusch, H. W. M., Charlier, T. D. & Pawluski, J. L. Developmental fluoxetine exposure facilitates sexual behavior in female offspring. *Psychopharmacology (Berl).* **231**, 123–133 (2014).
116. Rayen, I., Steinbusch, H. W. M., Charlier, T. D. & Pawluski, J. L. Developmental fluoxetine exposure and prenatal stress alter sexual differentiation of the brain and reproductive behavior in male rat offspring. *Psychoneuroendocrinology* **38**, 1618–29 (2013).
117. Smit-Rigter, L. A. *et al.* Prenatal fluoxetine exposure induces life-long serotonin 5-HT$_3$ receptor-dependent cortical abnormalities and anxiety-like behaviour. *Neuropharmacology* **62**, 865–70 (2012).
118. Zhou, X. *et al.* Behavioral training reverses global cortical network dysfunction induced by perinatal antidepressant exposure. *Proc. Natl. Acad. Sci.* **112**, 2233–2238 (2015).
119. da Silva, A. I. *et al.* Fluoxetine treatment of rat neonates significantly reduces oxidative stress in the hippocampus and in behavioral indicators of anxiety later in postnatal life. *Can. J. Physiol. Pharmacol.* **92**, 330–7 (2014).
120. Ko, M.-C., Lee, L. J.-H., Li, Y. & Lee, L.-J. Long-term consequences of neonatal fluoxetine exposure in adult rats. *Dev. Neurobiol.* **74**, 1038–1051 (2014).
121. Lee, L.-J. & Lee, L. J.-H. Neonatal fluoxetine exposure alters motor performances of adolescent rats. *Dev. Neurobiol.* **72**, 1122–32 (2012).
122. Rayen, I., van den Hove, D. L., Prickaerts, J., Steinbusch, H. W. & Pawluski, J. L. Fluoxetine during Development Reverses the Effects of Prenatal Stress on Depressive-Like Behavior and Hippocampal Neurogenesis in Adolescence. *PLoS One* **6**, e24003 (2011).
123. Zheng, J. *et al.* Neonatal exposure to fluoxetine and fluvoxamine alteres spine density in mouse hippocampal CA1 pyramidal neurons. *Int. J. Clin. Exp. Pathol.* **4**, 162–8 (2011).
124. Capello, C. F. *et al.* Serotonin transporter occupancy in rats exposed to serotonin reuptake inhibitors in utero or via breast milk. *J. Pharmacol. Exp. Ther.* **339**, 275–85 (2011).
125. Francis-Oliveira, J. *et al.* Fluoxetine exposure during pregnancy and lactation: Effects on acute stress response and behavior in the novelty-suppressed feeding are age and gender-dependent in rats. *Behav. Brain Res.* **252**, 195–203 (2013).
126. Kummet, G. J. *et al.* Neonatal SSRI Exposure Programs a Hypermetabolic State in Adult Mice. *J. Nutr. Metab.* **2012**, 1–8 (2012).
127. Nagano, M., Liu, M., Inagaki, H., Kawada, T. & Suzuki, H. Early intervention with fluoxetine reverses abnormalities in the serotonergic system and behavior of rats exposed prenatally to dexamethasone. *Neuropharmacology* **63**, 292–300 (2012).
128. Kiryanova, V., Meunier, S. J., Vecchiarelli, H. A., Hill, M. N. & Dyck, R. H. Effects of maternal stress and perinatal

fluoxetine exposure on behavioral outcomes of adult male offspring. *Neuroscience* **320**, 281–96 (2016).

129. Matsumoto, A. K. *et al.* Co-exposure to fish oil or folic acid does not reverse effects in the progeny induced by maternal exposure to fluoxetine. *Neurotoxicol. Teratol.* **56**, 1–8 (2016).
130. Soga, T., Wong, D. W., Putteeraj, M., Song, K. P. & Parhar, I. S. Early-life citalopram-induced impairments in sexual behavior and the role of androgen receptor. *Neuroscience* **225**, 172–184 (2012).
131. Ehrlich, D. E. *et al.* Prenatal stress, regardless of concurrent escitalopram treatment, alters behavior and amygdala gene expression of adolescent female rats. *Neuropharmacology* **97**, 251–258 (2015).
132. Karpova, N. N., Lindholm, J., Pruunsild, P., Timmusk, T. & Castren, E. Long-lasting behavioural and molecular alterations induced by early postnatal fluoxetine exposure are restored by chronic fluoxetine treatment in adult mice. *Eur. Neuropsychopharmacol.* **19**, 97–108 (2009).
133. Meyer, L. R. *et al.* Perinatal SSRI exposure permanently alters cerebral serotonin receptor mRNA in mice but does not impact adult behaviors. *J. Matern. Neonatal Med.* **31**, 1393–1401 (2018).
134. Sarkar, A., Chachra, P. & Vaidya, V. A. Postnatal fluoxetine-evoked anxiety is prevented by concomitant 5-HT2A/C receptor blockade and mimicked by postnatal 5-HT2A/C receptor stimulation. *Biol. Psychiatry* **76**, 858–68 (2014).
135. Galindo, L. C. M. *et al.* Neonatal serotonin reuptake inhibition reduces hypercaloric diet effects on fat mass and hypothalamic gene expression in adult rats. *Int. J. Dev. Neurosci.* **46**, 76–81 (2015).
136. Ishikawa, C. & Shiga, T. The postnatal 5-HT1A receptor regulates adult anxiety and depression differently via multiple molecules. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* **78**, 66–74 (2017).
137. Toffoli, L. V *et al.* Maternal exposure to fluoxetine during gestation and lactation affects the DNA methylation programming of rat's offspring: modulation by folic acid supplementation. *Behav. Brain Res.* **265**, 142–147 (2014).
138. Velasquez, J. C. *et al.* In Utero Exposure to Citalopram Mitigates Maternal Stress Effects on Fetal Brain Development. *ACS Chem. Neurosci.* (2019) doi:10.1021/acschemneuro.9b00180.
139. Gemmel, M., Kokras, N., Dalla, C. & Pawluski, J. L. Perinatal fluoxetine prevents the effect of pre-gestational maternal stress on 5-HT in the PFC, but maternal stress has enduring effects on mPFC synaptic structure in offspring. *Neuropharmacology* **128**, 168–180 (2018).
140. Spohn, S. N. & Mawe, G. M. Non-conventional features of peripheral serotonin signalling-the gut and beyond. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 412–420 (2017).
141. Valles-Colomer, M. *et al.* The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat. Microbiol.* **4**, 623–632 (2019).
142. Jackson, M. A. *et al.* Gut microbiota associations with common diseases and prescription medications in a population-based cohort. *Nat. Commun.* **9**, 1–8 (2018).
143. Zhernakova, A. *et al.* Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science (80-.).* **352**, 565–569 (2016).
144. Vich Vila, A. *et al.* Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nat. Commun.* **11**, 362 (2020).
145. Cussotto, S. *et al.* Differential effects of psychotropic drugs on microbiome composition and gastrointestinal function. *Psychopharmacology (Berl).* **236**, 1671–1685 (2019).
146. Zhu, H.-Z. *et al.* Xiaoyaosan improves depressive-like behavior in rats with chronic immobilization stress through modulation of the gut microbiota. *Biomed. Pharmacother.* **112**, 108621 (2019).
147. Lukić, I. *et al.* Antidepressants affect gut microbiota and Ruminococcus flavefaciens is able to abolish their effects on depressive-like behavior. *Transl. Psychiatry* **9**, 133 (2019).
148. Lyte, M., Daniels, K. M. & Schmitz-Esser, S. Fluoxetine-induced alteration of murine gut microbial community structure: evidence for a microbial endocrinology-based mechanism of action responsible for fluoxetine-induced side effects. *PeerJ* **7**, e6199 (2019).
149. Fung, T. C. *et al.* Intestinal serotonin and fluoxetine exposure modulate bacterial colonization in the gut. *Nat. Microbiol.* **4**, 2064–2073 (2019).
150. Sun, L. *et al.* Fluoxetine ameliorates dysbiosis in a depression model induced by chronic unpredicted mild stress in mice. *Int. J. Med. Sci.* **16**, 1260–1270 (2019).
151. Naseribafrouei, A. *et al.* Correlation between the human fecal microbiota and depression. *Neurogastroenterol.*

Motil. **26**, 1155–1162 (2014).
152. Jiang, H. *et al.* Altered fecal microbiota composition in patients with major depressive disorder. *Brain. Behav. Immun.* **48**, 186–194 (2015).
153. Zheng, P. *et al.* Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol. Psychiatry* 1–11 (2016) doi:10.1038/mp.2016.44.
154. Kelly, J. R. *et al.* Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *J. Psychiatr. Res.* **82**, 109–118 (2016).
155. Aizawa, E. *et al.* Possible association of Bifidobacterium and Lactobacillus in the gut microbiota of patients with major depressive disorder. *J. Affect. Disord.* **202**, 254–257 (2016).
156. Chen, Z. *et al.* Comparative metaproteomics analysis shows altered fecal microbiota signatures in patients with major depressive disorder. *Neuroreport* **29**, 417–425 (2018).
157. Park, A. J. *et al.* Altered colonic function and microbiota profile in a mouse model of chronic depression. *Neurogastroenterol. Motil.* **25**, (2013).
158. Marin, I. A. *et al.* Microbiota alteration is associated with the development of stress- induced despair behavior. *Nat. Publ. Gr.* 1–10 (2017) doi:10.1038/srep43859.
159. Golubeva, A. V *et al.* Prenatal stress-induced alterations in major physiological systems correlate with gut microbiota composition in adulthood. *Psychoneuroendocrinology* **60**, 58–74 (2015).
160. Wong, M.-L. *et al.* Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition. *Mol. Psychiatry* **21**, 1–9 (2016).
161. O'Mahony, S. M. *et al.* Early Life Stress Alters Behavior, Immunity, and Microbiota in Rats: Implications for Irritable Bowel Syndrome and Psychiatric Illnesses. *Biol. Psychiatry* **65**, 263–267 (2009).
162. Nuriel-Ohayon, M., Neuman, H. & Koren, O. Microbial changes during pregnancy, birth, and infancy. *Front. Microbiol.* **7**, 1–13 (2016).
163. Herrera, E. Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. *Eur. J. Clin. Nutr.* **54**, S47–S51 (2000).
164. Wikoff, W. R. *et al.* Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 3698–703 (2009).
165. Marcobal, A. *et al.* A metabolomic view of how the human gut microbiota impacts the host metabolome using humanized and gnotobiotic mice. *ISME J.* **7**, 1933–1943 (2013).
166. Joel, D. *et al.* Sex beyond the genitalia: The human brain mosaic. *Proc. Natl. Acad. Sci.* **112**, 15468–15473 (2015).
167. Bale, T. L. & Epperson, C. N. Sex differences and stress across the lifespan. *Nat. Neurosci.* **18**, 1413–1420 (2015).
168. Prendergast, B. J., Onishi, K. G. & Zucker, I. Female mice liberated for inclusion in neuroscience and biomedical research. *Neurosci. Biobehav. Rev.* **40**, 1–5 (2014).
169. Zhang, J. *et al.* Neonatal citalopram exposure decreases serotonergic fiber density in the olfactory bulb of male but not female adult rats. *Front. Cell. Neurosci.* **7**, 67 (2013).
170. Mueller, B. R. & Bale, T. L. Sex-Specific Programming of Offspring Emotionality after Stress Early in Pregnancy. *J. Neurosci.* **28**, 9055–9065 (2008).
171. MacArthur Clark, J. The 3Rs in research: a contemporary approach to replacement, reduction and refinement. *Br. J. Nutr.* **120**, S1–S7 (2018).
172. Sandercock, P. & Roberts, I. Systematic reviews of animal experiments. *Lancet (London, England)* **360**, 586 (2002).
173. Hooijmans, C. R. & Ritskes-Hoitinga, M. Progress in Using Systematic Reviews of Animal Studies to Improve Translational Research. *PLoS Med.* **10**, e1001482 (2013).
174. Hooijmans, C. R., IntHout, J., Ritskes-Hoitinga, M. & Rovers, M. M. Meta-Analyses of Animal Studies: An Introduction of a Valuable Instrument to Further Improve Healthcare. *ILAR J.* **55**, 418–426 (2014).
175. Caspi, A. *et al.* Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301**, 386–9 (2003).
176. Jaffee, S. R., Price, T. S. & Reyes, T. M. Behavior genetics: Past, present, future. *Dev. Psychopathol.* **25**, 1225–1242 (2013).
177. Caspi, A. *et al.* Role of genotype in the cycle of violence in maltreated children. *Science* **297**, 851–4 (2002).
178. Lesch, K. P. *et al.* Association of anxiety-related traits with a polymorphism in the serotonin transporter gene

regulatory region. *Science* **274**, 1527–31 (1996).
179. Little, K. Y. *et al.* Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. *Am. J. Psychiatry* **155**, 207–13 (1998).
180. Murphy, D. L. *et al.* Genetic perspectives on the serotonin transporter. *Brain Res. Bull.* **56**, 487–94 (2001).
181. Li, Q. *et al.* Reduction of 5-hydroxytryptamine (5-HT)(1A)-mediated temperature and neuroendocrine responses and 5-HT(1A) binding sites in 5-HT transporter knockout mice. *J. Pharmacol. Exp. Ther.* **291**, 999–1007 (1999).
182. Bennett, A. J. *et al.* Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Mol. Psychiatry* **7**, 118–122 (2002).
183. Hariri, A. R. *et al.* Serotonin transporter genetic variation and the response of the human amygdala. *Science* **297**, 400–3 (2002).
184. Culverhouse, R. C. *et al.* Collaborative meta-analysis finds no evidence of a strong interaction between stress and 5-HTTLPR genotype contributing to the development of depression. *Mol. Psychiatry* 1–10 (2017) doi:10.1038/mp.2017.44.
185. Carola, V. & Gross, C. Mouse models of the 5-HTTLPR × stress risk factor for depression. *Curr. Top. Behav. Neurosci.* **12**, 59–72 (2012).
186. Bengel, D. *et al.* Altered brain serotonin homeostasis and locomotor insensitivity to 3, 4-methylenedioxymethamphetamine ('Ecstasy') in serotonin transporter-deficient mice. *Mol. Pharmacol.* **53**, 649–55 (1998).
187. Homberg, J. R., Wöhr, M. & Alenina, N. Comeback of the Rat in Biomedical Research. *ACS Chem. Neurosci.* **8**, 900–903 (2017).
188. Smits, B. M. G., Mudde, J., Plasterk, R. H. A. & Cuppen, E. Target-selected mutagenesis of the rat. *Genomics* **83**, 332–334 (2004).
189. Smits, B. M. G. *et al.* Generation of gene knockouts and mutant models in the laboratory rat by ENU-driven target-selected mutagenesis. *Pharmacogenet. Genomics* **16**, 159–169 (2006).
190. Homberg, J. R. *et al.* Characterization of the serotonin transporter knockout rat: a selective change in the functioning of the serotonergic system. *Neuroscience* **146**, 1662–76 (2007).
191. Houwing, D. J., Buwalda, B., van der Zee, E. A., de Boer, S. F. & Olivier, J. D. A. The Serotonin Transporter and Early Life Stress: Translational Perspectives. *Front. Cell. Neurosci.* **11**, 1–16 (2017).
192. Vetulani, J. Early maternal separation: a rodent model of depression and a prevailing human condition. *Pharmacol. Reports* **65**, 1451–1461 (2013).
193. Orso, R. *et al.* How Early Life Stress Impact Maternal Care: A Systematic Review of Rodent Studies. *Front. Behav. Neurosci.* **13**, 1–17 (2019).
194. Houwing, D. J., Ramsteijn, A. S., Riemersma, I. W. & Olivier, J. D. A. Maternal separation induces anhedonia in female heterozygous serotonin transporter knockout rats. *Behav. Brain Res.* **356**, 204–207 (2019).
195. Houwing, D. J. Antidepressant treatment: for better or worse? Neurodevelopmental outcomes in rat offspring. (University of Groningen, 2020).
196. Houwing, D. J. *et al.* Subjecting Dams to Early Life Stress and Perinatal Fluoxetine Treatment Differentially Alters Social Behavior in Young and Adult Rat Offspring. *Front. Neurosci.* **13**, 1–15 (2019).
197. Houwing, D. J. *et al.* Perinatal fluoxetine treatment and dams' early life stress history alter affective behavior in rat offspring depending on serotonin transporter genotype and sex. *Submitt. Manuscr.* (2019).
198. Houwing, D. J. *et al.* Perinatal fluoxetine treatment and dams' early life stress history have opposite effects on aggressive behavior while having little impact on sexual behavior of male rat offspring. *Submitt. Manuscr.* (2019).
199. Houwing, D. J. *et al.* Perinatal fluoxetine treatment disrupts the circadian response to a phase-shifting challenge in female rats. *Submitt. Manuscr.* (2019).