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Mechanisms underlying brain programming by early-life adversity and nutrition

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CHAPTER 7

Sex-dependence and comorbidities of the early-life adversity induced mental and metabolic disease risks: where are we at?

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Early-life adversity (ELA) is a major risk factor for developing later-life mental and metabolic disorders. However, if and to what extent ELA contributes to the comorbidity and sex-dependent prevalence/presentation of these disorders remains unclear.

We here comprehensively review and integrate human and rodent ELA (pre- and postnatal) studies examining mental or metabolic health in both sexes and discuss the role of the placenta and maternal milk, key in transferring maternal effects to the offspring.

We conclude that ELA impacts mental and metabolic health with sex-specific presentations that depend on timing of exposure, and that human and rodent studies largely converge in their findings. ELA is more often reported to impact cognitive and externalizing domains in males, internalizing behaviors in both sexes and concerning the metabolic dimension, adiposity in females and insulin sensitivity in males.

Thus, ELA seems to be involved in the origin of the comorbidity and sex-specific prevalence/presentation of some of the most common disorders in our society. Therefore, ELA-induced disease states deserve specific preventive and intervention strategies.

Key words
Early-life adversity, mental health, metabolic health, comorbidities, sex-differences, rodent, human, placenta, maternal milk

Abstract

1. Introduction

Exposure to early life adversity (ELA) during critical developmental periods prenatally (PRS)[1–3] or early postnatally (POS)[4,5] increases the risk for numerous health problems later in life, including psychopathology[6,7], cognitive dysfunction[8,9], as well as metabolic and cardiovascular diseases[10,11] (see Hughes and colleagues (2018)[12]). There are several characteristics of these disorders (i.e. specific mental health and metabolic domains, their comorbidity, sex-specific prevalence and presentation), and features of the ELA (i.e. timing and type) exposure that are determinant for understanding how ELA acts upon disease vulnerability across sexes. To gain a better understanding of this, we here comprehensively review and integrate human and rodent ELA (pre- and postnatal) studies examining mental or metabolic health in both sexes. In doing so, we took the following points into consideration.

Firstly, since mental and metabolic health are broad concepts encompassing several major dimensions, we decided to focus on a number of specific mental and metabolic aspects that have been studied in the field of ELA research. Concerning mental health aspects we focused on cognitive, emotional and social dimensions[13], and for metabolic health we considered outcomes related to body composition and insulin/glucose metabolism. Because these various dimensions might be differently impacted by ELA, it is key to consider and assess them separately.

Secondly, mental and metabolic health disorders are often comorbid. For instance, depression and obesity share a bidirectional relationship: obesity increases the risk for depression by 55%, while depression increases the risk for obesity by 58%[14–17]. Depression is also twice as prevalent among those with type 2 diabetes as compared to those without[18]. Another example is the co-occurrence of diabetes and obesity with cognitive decline and Alzheimer's disease[19–21]. The comorbidity of these mental and metabolic disorders suggests that similar pathways might be involved and/or interact, and considering that ELA impacts on vulnerability to both, it is key to understand if and to what extent these pathways may be set in motion by ELA. However, despite the increasing interest in, and acknowledgment of this comorbidity, these disorders have historically often been studied by different experts, only rarely in the context of each other, and even less so in terms of how ELA modulates these disease risks.

Thirdly, these disorders exhibit sex differences in their prevalence and presentation with for example depression being more common in women than in men and depressive women more often suffering from comorbid obesity than do depressive men[22,23]. Considering the increasing evidence that males and females are differentially affected by ELA[24–30] , it is important to understand if and to what extent ELA may contribute to sex differences

in the prevalence and presentation of these (comorbid) mental and metabolic disorders. However, this is hard to study as currently in human studies sex is often a factor that is taken into account by statistical adjustment but rarely specifically tested for with a formal assessment of effect modification, and rodent studies are only very recently being more systematically performed in both sexes.

Fourthly, in order to understand if and to what extent ELA might contribute to vulnerability to mental and metabolic disorders, to their comorbidity and their sex-dependent prevalence/presence, the timing (i.e. prenatal versus postnatal stress) and type (i.e. specifics of the adversity) of ELA exposure could be key determinants. Because currently most research papers only studied one time period (either pre- or postnatal) and focused on a specific ELA exposure, it remains difficult to assess if and how these features of ELA exposure affect outcome characteristics.

Lastly, in order to best evaluate if the current rodent ELA models are suitable to study underlying mechanisms, to identify novel targets and to test proof-of-concept intervention strategies in human patients, it is key to establish if and to what extent the ELA-induced effects are consistent across human and rodent literature.

A comprehensive overview bringing together and integrating the existing literature on ELA effects across these disciplines and from these different subfields is currently lacking. With this review, we aim to overcome these barriers and further our understanding as to whether ELA may contribute to the comorbidity and sex-dependent prevalence/presentation of mental and metabolic disorders. To this end, we set out to assess the consistency of sex differences in effects of ELA on mental and metabolic outcomes across human and rodent studies, and to assess to what extent they depend on the type and timing of ELA. More specifically, we comprehensively review and discuss the prospective human and rodent studies that have (i) included both sexes, (ii) analyzed both sexes separately and (iii) addressed the sex-specific effects of ELA on mental (including cognitive, social and emotional domains) and/or metabolic outcomes. Finally, while addressing the complex mechanisms at the basis of the programming by ELA is out of the scope of this review, we discuss the role of two key biological mediators of the transfer of maternal effects to the offspring during the pre- and postnatal period: the placenta (**Box 1**) and maternal milk (**Box 2**).

Understanding how ELA acts upon disease vulnerability across sexes, diseases and species, is crucial for a better understanding of how ELA programs offspring for life, ultimately providing the basis for a more precise, evidence-based and personalized diagnosis and treatment of disease, and with considerable relevance for study outcome evaluations.

2. Methods

2.1 Literature search

A comprehensive literature search was conducted in PubMed up to March 2020 (**Figure. 1**). For the search terms used for the human and rodent literature, see **Supplementary material 1**. The search was aimed at identifying relevant papers examining the sex-specific effects of ELA on mental and metabolic health outcomes from human and rodent prospective cohort studies. Titles and abstracts of retrieved papers were examined by four authors (LB and JK for the human and SR and KR for the rodent literature). We acknowledge that there is also literature on the effects of ELA in other species (e.g. primates[31], sheep[32] and pigs[33]) but for the purpose of this review, we here focused on rodent and human studies.

For the human literature, the PubMed search identified 1316 research papers, of which 52 research papers covering mental and/or metabolic health outcomes met the inclusion criteria and were included in the current review. The inclusion criteria were; (1) prospective cohort studies examining associations between (2) ELA exposure (either occurring prenatally or postnatally up to 2 years postpartum) including stress, anxiety, depression and other forms of stress (e.g. bereavement, violence, a disaster etc) (See for details **Supplementary table S1.A**). When both prenatal stress (PRS) and postnatal stress (POS) were investigated in the same study and analyzed separately, the specific study was included for both PRS and POS analyses. Studies had to assess either; (3) mental health outcomes (including cognitive functions, externalizing or internalizing behaviors) and/ or (4) metabolic parameters (including insulin/glucose (sensitivity), body weight and body composition). We selected papers that had included; (5) both girls and boys, and (6) had either stratified the analyses for sex, or had tested for an interaction between ELA exposure and offspring sex for any of the outcomes. We included all ages at which the outcomes were measured in the offspring. For the included studies, this ranged from 1 up to 32 years of age (y) with most studies investigating either children or adolescents (47 < 18y, 3 = 18y, 1 = 20y, 1 < 32y). Therefore, we use the term boys/girls when referring to the human study outcomes.

For the rodent literature, our PubMed search led to the identification of 453 research papers of which 182 research papers covering behavioural and/or metabolic health outcomes met the inclusion criteria and were included in the current review. We included; (1) prospective rodent research papers that employed an early life stressor of a physical or psychological nature, and excluded those that were specifically and selectively nutrition-, metabolism- or inflammation-related. The selected ones encompassed either stressors occurring prenatally (PRS) (e.g. prenatal restraint or variable stress) or postnatally (POS)

up to 21 days postpartum (e.g. maternal separation or the limited nesting and bedding material paradigm) (**Supplementary table S1.B**).

The included research papers had to assess either; (2) behavioural outcomes (including cognitive, social, anxiety or depressive-like behaviour) or (3) metabolic outcomes (including bodyweight, adiposity, or insulin/glucose levels/sensitivity) and (4) include both sexes. Finally, (5) considering the importance of the later-life environment ('second-hit') for the ELA-induced phenotypes, for the papers focusing on behavioural effects, studies with secondary stressors (e.g. later-life acute or chronic stress) and for those focusing on metabolic parameters, papers with high-caloric diet exposure later in life, were also included. The behavioural studies with a secondary stressor were not included in the main analyses per domain, but separately discussed in paragraph 4.4.3. Results of mouse and rat experiments were collapsed, unless there was a different effect between species. Another relevant aspect in conceptualizing the findings across many studies is the age at which the outcome was assessed; therefore, we divided rodent studies based on the age of outcome (up to adolescent: <60 days old; adulthood: >= 60 days old). A total of 449 rodent experiments (45 on metabolic parameters; 404 on behavioural parameters) met these inclusion criteria and were included in the current review.
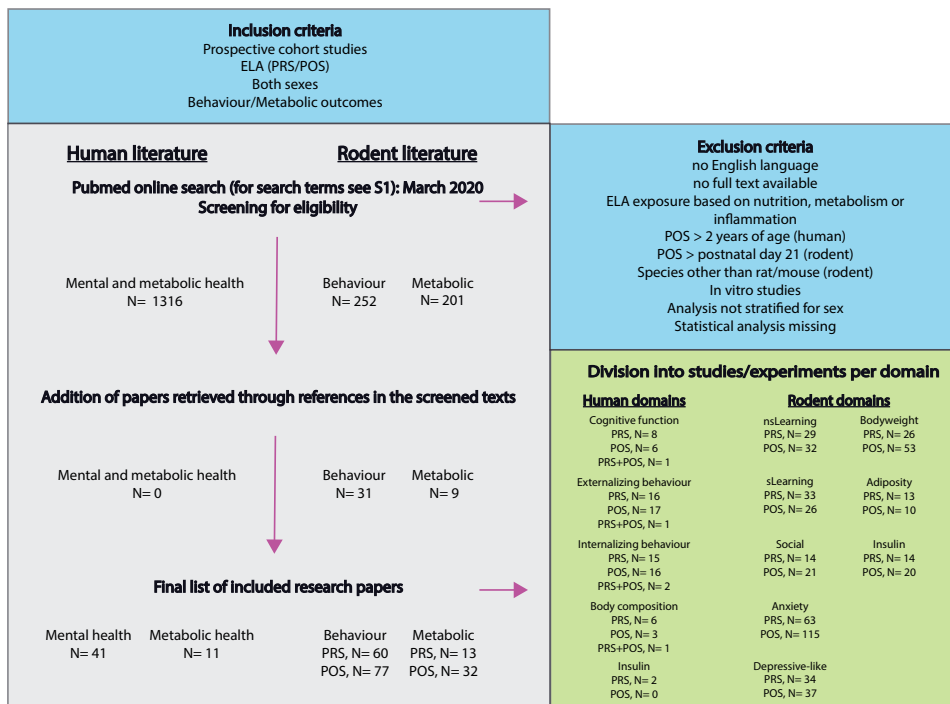

Figure 1. Overview of literature search

2.2 Subdivision into domains

All outcome parameters were categorized into several relevant domains in order to be able to better dissect the effects of ELA on specific aspects of mental health/metabolism for the human literature and behaviour/metabolism for the rodent literature. We therefore divided the various studies/experiments within each research paper by assigning them to the specific domain involved, and scored for each study (human) or experiment (rodent) what the effect of ELA was on that specific domain.

Concerning mental health outcomes, to encompass the cognitive, emotional and social dimensions:

- For the human research papers we included: cognitive function, and externalizing and internalizing behaviours and disorders. Cognitive function included IQ, school performance, attention disengagement, and executive function, as measured with e.g. cognitive tests or attention tasks. Externalizing behaviour and disorders included aggression, hyperactivity, and attention problems, as assessed via questionnaires and/or the presence of the externalizing disorders Attention Deficit Hyperactivity disorder (ADHD) and Conduct Disorder (CD) as readout. Finally, internalizing behaviour and disorders encompassed emotional problems, anxiety and depressive symptoms, as assessed via questionnaires or diagnostic criteria for these disorders (see **Supplementary table S2.A** for all included readouts per domain).

- For rodent research papers, we included and divided the various outcome parameters in the following domains: cognitive (non-stressful and stressful learning), social behaviour and emotional behaviour (anxiety and depressive-like behaviour). Within the cognitive domain, "non-stressful learning (nsLearning)" was assessed with neutral or non-stressful learning tasks (i.e. Object Location Task (OLT) and Object Recognition task (ORT)) and "Stressful learning (sLearning)" was assessed under stressful circumstances, (i.e. Morris Water Maze (MWM), Fear learning/extinction and Active or Passive Avoidance). Social behaviour was tested via social interaction/recognition paradigms that in addition can specifically assess social play and aggressive behaviour. Within emotional behaviour, anxiety-like behaviour was assessed by investigating exploratory activity in the aversive environment (i.e. Open Field Test (OF), Elevated Plus Maze (EPM) and Dark-light box (Da-li-box)). Measures such as coping behaviour (i.e. Forced swimming Test (FST), Tail Suspension Test (TST)) or anhedonia (i.e. Sucrose Preference Test (SPT)), were considered to reflect depressive-like behaviour (**Supplementary table S2.B**).

Concerning metabolic health, we assessed measures of body composition and insulin sensitivity as they were available among the included studies. For the human studies, these consisted of bodyweight, body mass index (BMI) and skinfold thickness, circulating glucose and insulin levels as well as HOMA-IR (a measure of insulin resistance). For rodent experiments, bodyweight, adiposity (DEXA scan or weights of specific adipose

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tissue depots), insulin/glucose levels, HOMA-IR, insulin tolerance tests (ITT) and glucose tolerance tests (GTT) were included.

2.3 Scoring of sex differences in study and experiment outcomes

As study determinants and study outcomes are very heterogeneous it was not feasible to pool results. Thus, in order to get an overview of the consistency of sex differences in the effects of ELA on mental and metabolic health, we scored the reported statistically significant effects ($p<0.05$) in both sexes for all studies/experiments. We realize this is a very crude approach, as it ignores the size of effects and the size of study groups (and number of males and females), factors which potentially influence the chance of finding statistically significant effects, nonetheless it is suitable for our goal of getting such an overview. In addition to take somewhat into account study sizes, we also separately report on outcomes of the larger ($n>500$) studies in humans.

For human metabolic as well as all rodent experiments, we scored the effects in boys/ males and girls/females separately, resulting in one male and one female score per study/ experiment. For example, if in a specific study, bodyweight was increased in girls, but not in boys, girls were scored as 'increased' and boys as 'no effect'. If a rodent investigation included two nsLearning experiments and reported for experiment A impaired nsLearning in both sexes, and for experiment B impaired specifically in males and not females, males were scored as "decreased: 2 studies" and females as "decreased: 1 study". As final outcome, we calculated for both sexes how often ELA decreased, increased, or had no effect on the outcome experiments in each domain.

For human mental health studies, due to the higher complexity of the measured outcomes, we scored separately studies only or predominantly affecting boys or girls or presenting effects in both sexes. For example, in some human studies of mental health, associations were found to be predominantly present in boys/girls compared to the opposite sex. In such cases, studies were classified as 'only or predominantly effect in boys/girls'. Also, in a large number of studies in humans on mental health, a wide variety of behavioural outcomes was reported of which many could not be classified as externalizing or internalizing behavior. In these cases, we only included outcomes that could be clearly classified into these domains.

3. Effect of ELA on mental health outcomes depends on period of exposure, the specific domain as well as sex.

Based on our inclusion and exclusion criteria (see method section and **Fig. 1**), we selected 42 research papers, of which 11 studies addressed cognitive functions, 25 externalizing and 25 internalizing behaviour and disorders.

3.1 Cognition upon ELA exposure, postnatal stress in particular, is more often reported to be affected in boys than girls

When investigating the effects of ELA on cognition, we found that for PRS exposure, only boys were reported to be affected on cognitive aspects in 2 studies and only girls in 1 study, out of a total of 10 studies. Upon POS exposure, only boys were reported to be affected in 3 studies and girls in 1 out of 6 studies. 3 studies in which combined exposure to pre- and postnatal stress was investigated, reported only boys to be affected and 0 in which only girls were affected. For the remaining studies, either boys and girls were equally affected or there were no effects of ELA observed (see **Figure 2A** and **Table 1A** for a detailed overview of all results and references).

Concerning PRS, prenatal anxiety was associated with worse performance in a reaction time task and difficulty with sustained attention in boys, but not in girls in two studies[34,35]. One study reported only girls to show worse inhibitory control after prenatal anxiety exposure[36]. But no sex differences were found in other studies relating prenatal perceived stress and anxiety to worse performance on attention disengagement[37], attention shifting[38], and visuospatial working memory tasks[36,38]. One study did not show associations between prenatal maternal depressive symptoms and IQ in either sex[28].

Regarding POS, boys raised by a mother suffering from postpartum depression exhibited a lower full scale IQ at 3.5 years of age[29]. These effects of POS on IQ in boys persisted beyond early childhood, as also postnatal maternal depression was negatively associated with full scale IQ[28] and with poorer results on academic performance in adolescent boys[40]. Postpartum depression has also been associated with lower verbal intelligence specifically in girls[41].

When only looking at the 'larger' studies (defined as offspring N>500, 2 studies), a study with over 1000 participants, found that while PRS or POS alone did not affect IQ, high levels of depressive symptoms pre- and postnatally decreased IQ scores in children at 5-6 years of age, with boys exhibiting lower full IQ cognitive scores compared to girls, while affecting verbal IQ in both sexes[39]. The other larger study included in this review showed that, while in the complete sample (N=922) prenatal anxiety was related to performance in a reaction time task in both boys and girls, in a subset of highly anxious mothers (n=100), an association between prenatal anxiety and poor performance in a reaction time task was only found in boys[34].

Overall, when looking at all included studies or only the large ones (N>500) for this domain, we find that upon ELA exposure, boys generally are more often reported to develop cognitive problems than girls, which was especially found in the POS and combined PRS/POS studies. Girls, rather than being entirely resilient, repeatedly exhibit a lower verbal IQ.

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These conclusions should be taken with caution, as different ELA exposures and aspects of cognitive functions were assessed here, and a relatively low number of longitudinal prospective human studies investigated the association between ELA and cognitive functions. As such, this highlights the need for additional studies in this direction.

3.2. Externalizing behaviour problems and disorders upon ELA exposure are more often reported in boys than in girls

Concerning the effects of ELA on externalizing behaviours and disorders, we find that boys are more often reported to be affected than girls, both after exposure to PRS (only boys affected on externalizing aspects: 8; only girls affected on externalizing aspects: 3; out of 19 studies), POS (only boys affected on externalizing aspects: 7; only girls affected on externalizing aspects: 1; out of 18 studies) **(Figure 2 A and Table 1A)** and combined PRS and POS (only boys affected on externalizing aspects: 2; only girls affected on externalizing aspects: 0; out of 3 studies).

When reviewing the effects of PRS, prenatal maternal depression and anxiety respectively correlated to externalizing problems in boys at 2.5 years of age[42], and with hyperactivity and attention problems at 5 years of age in boys, but not girls[43]. These effects of PRS extend beyond the early childhood period, as prenatal maternal depression was also associated with externalizing problems at 16-17 years of age, particularly in boys[44]. Moreover, death of a close relative or perceived stress during pregnancy, has been associated with an increased risk for developing ADHD in boys[45,46]. When looking at conduct disorder (CD) risks, also interesting sex differences emerge; maternal distress decreased CD symptoms in girls, while it increased it in boys[47], and similarly, prenatal maternal depression was associated with a higher risk to commit a crime in boys more so than in girls[48].

Some studies also show associations between PRS and externalizing problems in both sexes. For example, prenatal stressful life events and maternal anxiety respectively predicted infant negativity at 6 weeks and 12 months of age[49] as well as childhood conduct problems[50] equally in boys and girls. The few studies concluding that girls are affected and boys are not[51,53] or much less[52] investigated externalizing behaviour at a rather young age (1-4.5 years of age), highlighting the importance of the age at which the various outcomes are studied. More studies that follow children into older age are needed to be able to understand the temporal dynamics of the impact of PRS on behaviour.

Similar to PRS, POS is associated with externalizing problems, specifically in boys. For example, maternal depressive or PTSD symptoms predicted boys externalizing behaviour at 33 months of age measured with ITSEA[51], as well as measured with CBCL at 1.5 and 3 years of age[57-58]. The effects of POS in boys seem to persist into adolescence, as postnatal depressive symptoms were associated with externalizing problems at 16-17 years of age,

only in boys[44]. Yet, postnatal depression was associated with externalizing problems in both boys and girls as measured with CBCL at 4[55] and 12 years of age[56], or with SDQ up until 13 years of age[50], suggesting girls are not completely resilient.

Interestingly, whereas exposure to postnatal depression has been more often reported to be detrimental for the behavioural development of boys, girls raised by a depressed mother postpartum seem to display a more 'mature' phenotype compared to unexposed girls. For example, girls born to women with postnatal depression are better adapted in class, less distractible[57], less likely to use physical aggression[58] and have less externalizing problems[41]. In line with this, combined pre- and postnatal anxiety increased the risk to develop CD symptoms in boys, while it reduced these risks in girls[59]. Interestingly, ELA has been shown to also accelerate sexual maturation in girls[60]. These findings indicate that girls might adapt to adverse maternal emotional states possibly via accelerated maturation of externalizing behavioural regulations. In some cases this adaptation might be beneficial, however on the long term it may lead to other emotional problems, since associations have been reported between social maturity, emotional sensitivity and depressed mood in adolescent girls exposed to maternal prenatal depression[61].

When looking specifically at the large ELA studies (N>500, PRS: 8; POS: 7; PRS/POS: 1), a similar picture emerges for PRS, while for POS, the increased vulnerability in boys is less apparent. For example, in 5 out of 8 large studies, maternal prenatal distress, bereavement, anxiety and depression were associated with increased risk for CD[47], ADHD[45], hyperactivity and attention problems[43] and criminal offenses[48], in boys but not girls. However, two large studies found associations between PRS and externalizing behaviour problems in both sexes[50,62]. For POS and PRS/POS combined, 3 out of 8 large studies reported specifically boys and not girls to be affected; maternal depression and anxiety as well as paternal depression were associated with increased externalizing problems and conduct symptoms in boys[54,63,63]. Three studies found effects of postnatal stress and depression on conduct problems and externalizing behaviour in both sexes[50,55,56].

In conclusion, from the analysis of all included studies, it appears that both PRS and POS are reported to be associated with externalizing behaviour problems mostly in boys. However, when specifically looking at the 16 included large studies (N>500) concerning externalizing behaviour, the increased vulnerability after POS in boys is less apparent, which calls for the need for more large studies in this area. In addition, several studies report that girls exhibit a more mature behaviour and although this adaptation might be beneficial at first, the long-term consequences on emotional functioning should be investigated further[61]. These results highlight the key importance of longitudinal studies that extend into adulthood and further to reach a better understanding of the complex impact of ELA.

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3.3. ELA affects internalizing behaviours and disorders in both boys and girls, depending on the timing of stress exposure

Considering the effects of ELA on later internalizing behaviours and disorders, girls are somewhat more often reported to be affected by PRS (only boys affected on internalizing aspects: 2; only girls affected on internalizing aspects: 5; out of 17 studies), while it is the other way around for POS (only boys affected on internalizing aspects: 5; only girls affected on internalizing aspects: 2; out of 18 studies). Regarding studies investigating combined PRS and POS, no differences could be observed (only boys affected on internalizing aspects: 1; only girls affected on internalizing aspects: 0; out of 4 studies). For a detailed overview of all results and references see **Figure 2A** and **Table 1A**.

For example, prenatal maternal stressful life events have been related to internalizing problems at 1.5 years of age in girls only[49], while prenatal maternal anxiety was positively associated with internalizing behaviour, anxiety and depressive symptoms at 2.5 years of age, predominantly in girls[64]. In addition, maternal prenatal anxiety and depression have been associated with depressive symptoms and emotional disorders in adolescent girls, but not boys[28,65,66]. Although girls have been found to be more vulnerable for the effects of PRS on internalizing problems in many studies, some studies have shown effects in boys as well. For example, two studies found that maternal anxiety and distress during pregnancy predicted emotional symptoms in both sexes at 2 and 3-4 years of age, respectively[43,47]. In addition, depression and high stress during pregnancy predicted internalizing problems at 1-2 years of age[54,67] as well as depression and anxiety symptoms at 20 years of age[68] predominantly in boys. It is unclear what specifically contributes to these differences in study outcome, but the trimester in which PRS occurs may matter in this regard[62,69], possibly due to sex differences during brain development[70].

In contrast to PRS, POS seems to increase vulnerability to develop internalizing behaviour problems more so in boys than in girls. Postnatal depression has been found to predict internalizing problems at 3 and 4 years of age[53,55] as well as at 7 years of age[71] predominantly boys,. Notably, these effects seem to persist until adolescence as postnatal depression predicted offspring depression at 18 years in boys but not girls[66]. Although few studies showed that POS was associated with internalizing behaviour problems in girls, there were some that did point to such an association. For example, one study showed that postnatal depression predicted depressive symptoms in childhood and adolescence in both boys and girls[66,72] and postnatal stress trajectory predicted depression and anxiety symptoms in girls but not boys at 20 years of age[68].

When looking at the large-sized studies (PRS: 7; POS: 8), for PRS the female vulnerability disappears and boys and girls seem equally affected, while for POS the increased vulnerability in boys compared to girls remains. For example, one study with over 7000

participants showed that exposure to prenatal maternal depression was associated with depression in girls[66], while another study (n=2868) showed that high stress during pregnancy predicted depression and anxiety, specifically in boys[68]. Both studies assessed depressive symptoms in young adulthood (18-20 years of age), and it is unclear what factors contribute to this discrepancy. Two large studies investigating effects of PRS on internalizing behaviour (emotional symptoms and anxiety) at a younger age (3-5 years), observed similar effects in boys and girls[43,47]. For POS, also within the large studies, boys are more often reported to exhibit a heightened vulnerability. For example, postnatal maternal depression and anxiety were associated with an increased risk for emotional symptoms, internalizing behaviour problems, anxiety and depressive symptoms predominantly in boys [54,55,66,71], and only one study concluded that POS was associated with an increased risk to develop depression and anxiety symptoms, only in girls[68].

In conclusion, when taking into consideration all included studies, the effects of PRS on internalizing behavioural problems seem to be more often reported in girls. However, when looking solely at the larger studies, this trend disappears. For POS, both when all studies are considered, or when focusing only on the larger studies, boys seem more vulnerable to its effects on internalizing behavior with more studies reporting associations only in boys or associations being stronger in boys than in girls. It is important to note that there are discrepancies in the literature for which the reason is not always clear[66,68]. Hence, more research is needed to better understand if and how different factors (e.g. timing of PRS, nature of ELA exposure, age at outcome and the specific assessment method used) contribute to the differential effects of PRS and POS on internalizing behavioural problems in boys and girls. This calls not only for more research in this field, preferably with larger group sizes, but especially for a higher level of standardization in categorizing ELAs and outcome measures.

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Table 1. Overview of human and rodent studies/experiments and the respective behavioural outcomes for the specific domains

A) Human studies (numbers and references) assessing mental health per domain, timing of ELA and sex
B) Rodent experiments (numbers and references) assessing behaviour per domain, timing of ELA, outcome age, species (if relevant) and sex.

A

Domain	Timing ELA	Total studies	Boys more affected	Girls more affected	Both sexes equally affected	No effect in either sex
Cognition	PRS	8	2 [34,35]	1 [36]	3 [37,38,73]	2 [28,39]
	POS	6	3 [28,29,40]	1 [41]	1 [37]	1 [39]
	PRS + POS	1	1 [39]	0	0	0
Externalizing behaviour & disorders	PRS	16	8 [42–48,54]	3 [64,67,69]	3 [49,50,62]	2 [28,63]
	POS	17	9 [42,44,51–54,57,58,63]	2 [41,62]	4 [49,50,55,56]	2 [28,47]
	PRS + POS	1	1 [59]	0	0	0
Internalizing behaviour & disorders	PRS	15	3 [54,67,68]	6 [28,49,62,64–66]	4 [43,47,69,74]	2 [42,63]
	POS	16	6 [52–55,66,71]	2 [61,68]	3 [56,62,72]	5 [28,42,47,49,63]
	PRS + POS	2	1 [59]	0	1 [75]	0

B

Domain	Timing ELA	Outcome age	Species	Total experiments	Increased M	Increased F	Decreased M	Decreased F	No effect in either sex
nsLearning	PRS	Adolescence	rats+mice	15	2 (76,77)	1 (78)	7 (79-83)	5 (76,81,82,84)	5 (78,85-87)
	PRS	Adulthood	rats+mice	14	2 (77,88)	0	1 (89)	2 (82,87)	9 (90-96)
	POS	Adolescence	rats	8	0	0	3 (97,98)	2 (97,99)	4 (97,99-101)
	POS	Adolescence	mice	1	0	0	1 (102)	1 (102)	0
	POS	Adulthood	rats	13	0	0	3 (103-105)	3 (103,104,106)	9 (103,105-110)
	POS	Adulthood	mice	10	0	0	7 (102,111-113)	2 (111)	3 (93,111)
sLearning	PRS	Adolescence	rats+mice	12	0	0	7 (80,82,114-116)	8 (80-82,114-116)	4 (76,116,117)
	PRS	Adulthood	rats+mice	21	2 (89,118)	4 (118-121)	8 (87,88,119,122-126)	8 (82,87,122,125-129)	5 (89,93,96,120,130)
	POS	Adolescence	rats+mice	4	0	0	2 (131)	2 (131)	2 (132,133)
	POS	Adulthood	rats+mice	22	3 (134-136)	1 (137)	5 (112,113,136,138)	5 (113,138-140)	11 (93,110,136,139,141-143)
Social behaviour	PRS	Adolescence	rats (no mice studies)	7	1 (144)	1 (144)	4 (144-147)	2 (145,146)	2 (144)
	PRS	Adulthood	rats+mice	7	0	0	3 (94,96,148)	3 (94,96,149)	3 (93,96,149)
	POS	Adolescence	rats+mice	11	1 (150)	0	6 (101,150-154)	3 (151,154,155)	2 (150)
	POS	Adulthood	rats+mice	10	0	1 (156)	3 (104,135,152)	2 (104,152)	6 (93,111,152,157,158)

Behaviour		Age	Model	N					
Anxiety-like behaviour	PRS	Adolescence	rats+mice	27	7 144,146,147,159–161	12 85,115,144,159–161	2 144,159	0	11 76,85,144,161,162
	PRS	Adulthood	rats+mice	36	14 88,94,121,123,147,163–168	11 88,123,148,164–167,169	1 92	2 77,121	16 79,91,93,94,118,148,165,170–173
	POS	Adolescence	rats	37	14 97,101,133,174–179	9 97,174–177	6 132,179–183	5 132,180–183	17 97–99,105,154,176,178,181,183–187
	POS	Adolescence	mice	10	0	1 188	1 150	1 162	7 102,150,162,188,189
	POS	Adulthood	rats	42	16 103,104,107,135,137,138,141,190–194	9 103,109,137,138,179,192–194	1 195	2 104,135	23 103,104,108,136,138,142,157,158,179,196–202
	POS	Adulthood	mice	26	7 156,188,203–205	10 93,156,188,204–206	2 205	2 203,205	10 93,112,140,188,189,205,207
Depressive-like behaviour	PRS	Adolescence	rats (no mice studies)	11	4 87,146,160,208	2 160,208	4 144	1 144	3 146,147,208
	PRS	Adulthood	rats+mice	23	4 167,169,171	6 84,147,167,209–211	2 120,169	1 120	12 84,87,93,96,120,147,171
	POS	Adolescence	rats+mice	21	15 132,150,174,176,177,189,212,213	10 132,150,174,176,177,185,189,212	0	3 150,213	5 177,186,188
	POS	Adulthood	rats+mice	16	2 190	3 106,188	2 103,106	2 103,157	8 93,106,108,112,113,189,194,200

4. Evidence of sex differences in the effects of ELA on rodent behaviour

To study the effects of ELA exposure on rodents, multiple animal models have been developed which can be divided into PRS and POS exposures (**Supplementary table S1.B**). In total, 60 PRS research papers were selected that comprised a total of 173 experiments, addressing cognitive, emotional or social behaviour, with restraint stress (39%) and variable stress (25%) being the most commonly used PRS models. In total, 77 POS research papers were included which were divided into 231 experiments for which maternal separation (MS; 56%), maternal deprivation (MS; 30%) and the limited bedding and nesting material paradigm (LBN; 31%) were the most often used POS models. For both PRS and POS, anxiety-like behaviour was the most frequently investigated domain (PRS; 39% and POS; 51%).

In this section, we will discuss in detail the findings stratified by behavioural domain, the timing of ELA exposure and the age at which outcomes were measured. The experiments were divided over the domains as follows: PRS: cognitive (non-stressful (29) and stressful (33) learning), social behaviour (14) and emotional behaviour (anxiety (63), depressive-like behaviour (34)) and POS: cognitive (non-stressful (32) and stressful (26) learning), social behaviour (21) and emotional behaviour (anxiety (115), depressive-like behaviour (37)). For a detailed overview of all results and references see Figure 2A and **Table 1B**.

4.1 Impact of ELA on cognitive functions depends on the nature of the learning task, type and timing of ELA exposure and sex

To get more insight into the sex differences in effects of ELA on cognitive functions, we divided the experiments addressing this question into non-stressful and stressful learning, as there is evidence that different processes might be at play when learning under non-stressful or stressful circumstances. When exposed to ELA, one could be best prepared to thrive under later stressful situations, whereas learning can be impaired or suboptimal when the conditions are not stressful. This hypothesis is also known as the 'match-mismatch', or predictive-adaptive-response hypothesis[214–216]. We will first review the effects of PRS and POS on non-stressful learning (nsLearning), followed by the effects on stressful learning (sLearning) (for the included behavioural paradigms see **Supplementary table S2B**). A full overview of the results with references per domain, outcome age, and if relevant species, can be found in **Table 1B**.

4.1.1 Non-stressful learning

PRS does not lead to sex-specific impairments in nsLearning when animals are tested during adolescence (PRS-induced nsLearning impairments in males: 7; females: 5, out of 15 experiments) or in adulthood (males: 1; females: 2, out of 14 experiments). POS leads

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to differential effects in rats and mice and therefore we report these results separately. For POS rat studies, no consistent sex-dependent nsLearning impairments were present across studies either when tested during adolescence (males: 3; females: 2, out of 8 experiments) or in adulthood (males: 3; females: 3, out of 13 experiments). For POS mice studies, effects on learning in adolescent mice has not been studied sufficiently (1 experiment showed learning impairments in both sexes[102]), however when tested in adulthood, POS is reported to more often impair nsLearning in males (males: 7; females: 2, out of 10 experiments).

PRS exposure often led to memory impairments in both sexes when animals were tested during adolescence[81,82,84]. However, some papers report male specific memory impairments[78,80,83] or increased memory[76,77] as well as female specific impaired[76] and increased[78] memory. The specific reason for this discrepancy is not entirely clear, but could be due to for example the specific PRS model used (i.e. early PRS[78] versus late PRS[77]) or the exact age the outcome was measured[76]. When tested in adulthood, PRS-exposure mostly does not affect nsLearning outcome parameters[90–96]. Some papers however report changes in specifically males[77,88,89] or females[82,217]. Based on the information available, there does not seem to be any specific experimental parameter that could be responsible for these differences. This thus calls for a more careful description of experimental details to increase reproducibility of PRS nsLearning studies.

When studying POS effects, as mentioned above, we observed differential effects in rats and mice and we therefore discuss them separately. For rats, when tested during adolescence half [94,97,99,100], and when tested during adulthood a majority[103,105,107–110,218] of POS experiments did not report changes in nsLearning in either sex. Within the experiments showing changes in learning capabilities, there was no sex-or age-dependent effect[97–99,103–105,218]. When POS-exposed mice were tested in adulthood, a male vulnerability to develop learning impairments is clearly present[102,112,207]. These studies all used common ELA models (LBN and maternal separation) and behavioural tests (ORT, OLT, MWM) while the few studies that failed to show this sex effect, either used a less common ELA model (e.g. faecal smell stress[93]) or nsLearning behavioural paradigm (e.g. "Attention-Set-Shifting-Task (ASST)" testing attention or the "T-maze" which rather tests working memory[111]).

In summary, in cases during which PRS and POS affect nsLearning, the stressors mostly impair learning and memory in rodents. When tested during adolescence, no clear sex-differences emerge, while in adulthood, POS exposure is more often reported to lead to males' susceptibility in mice, but not in rats.

4.1.2 Stressful learning
When reviewing the studies addressing the effect of ELA on sLearning, the large majority of both PRS and POS experiments were performed in rats, and behaviour was mostly

tested in adulthood. Based on the studies concerning the adolescent period, neither PRS (less memory in males: 7 females: 8, out of 12 experiments) nor POS (less memory in males: 2; females: 2, out of 4 experiments) lead to sex-specific differences (See Table 1B for all references). When tested in adulthood, both PRS exposed males (more memory: 2; less memory: 8, out of 21 experiments) and females (more memory: 4; less memory: 8, out of 21 experiments) mostly show decreased memory during stressful learning. POS also lead to reduced memory in males (more: 3; less: 5, out of 22 experiments) and females (more: 1; less: 5, out of 22 experiments).

Upon PRS exposure, when tested during adolescence, almost all included papers report reduced memory in both males and females during the stressful learning tasks, with two using a passive avoidance paradigm[114,219] and five using Morris Water Maze[80,82,116,219], and only one paper reported female specific memory impairments[81]. Also when tested in adulthood, no sex-differences and mostly learning impairments were detected even though there is also evidence for both sexes[122,125,126,217] only males[88,119,123,124] or only females[82,127–129] being affected. Additionally, increased memory during stressful learning has been reported as well in adult males[89,118] and females[119,120]. For these experiments, the age of testing (P63 versus P100) and behavioural paradigm (contextual versus cued fear conditioning or Morris Water Maze) could explain the difference in learning outcomes. All above mentioned experiments used common PRS models induced during the late-gestational period, while for example PRS via faecal smell stress during mid gestational period did not affect sLearning in either sex[93].

For POS experiments, only few were performed during adolescence with one paper describing decreased memory in both sexes during contextual and auditory fear conditioning tasks[131] and two papers reporting no change in learning capabilities using the Morris Water Maze paradigm. When tested in adulthood no clear sex-dependent learning differences were detected either. Differences in learning outcome have been reported in either sex, in both rats and mice. For example, two studies showed decreased memory of the stressful event in females and not males[139,140] and others reported male-specific increases in memory during fear conditioning or active avoidance paradigms[134–136]. There is also evidence for impaired learning in both sexes[138,207] or findings in the opposite direction depending on sex, with increased memory specifically in females[137] or decreased memory in males only[112,136]. Lehmann et al. (1999) reported that effectiveness of POS on sLearning highly depends on the timing of the POS model and behaviour paradigm used[136].

In summary, ELA modulates performance during sLearning paradigms. When tested during adolescence, this does not seem to depend on sex. However, to date, the effects of PRS and POS on sLearning have not been studied sufficiently at a young age. In adulthood, when taking all studies together, no clear sex-differences emerge, but different findings

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exist among research papers. There is evidence that (at least part of) this discrepancy might lie in the specific PRS and POS models and behavioural tests that are used. To clarify this, studies are needed that directly compare the effects of multiple ELA models on sLearning behavioural paradigms.

4.2 ELA is more often reported to lead to altered social behaviour in young male rodents

Within this section we will discuss experiments that addressed the effects of ELA particularly on social behaviour. This is a relatively new research field and only comprised approximately 8% out of the total experiments included in this review.

When effects of ELA on social behaviour were tested during adolescence, changes were most often reported in male rodents exposed to PRS (males: 5; females: 3; out of 7 experiments) or POS (males: 7; females: 3; out of 11 experiments). When animals were tested in adulthood, often no behavioural changes were observed and when altered behaviour was found, this was equally often changed in male and female rodents (3 out of 7 (PRS) or 10 (POS) experiments for both sexes).

When PRS-exposed rats were tested during adolescence (no mice studies met the criteria for this review), males were more often affected. Two experiments showed that specifically males displayed a decrease in social behaviour[144,147], while no experiment showed this for females. However, females are not completely resilient since behavioural changes in both sexes have also been reported[144–146]. The rat strain used and the type and timing of PRS seem to greatly matter for the outcome of social behaviour experiments in young rats. For example, Schroeder and colleagues reported that daily restraint stress during the last week of pregnancy did not alter social behaviour in neither Wistar Kyoto (WKY) nor Wistar rats, while 7 days of restraint stress applied in a random fashion throughout pregnancy led to decreased social behaviour in specifically male WKY rats and increased social behaviour in both male and female Wistar rats[144]. Others have reported that restraint stress during the last trimester decreased social behaviour in male and female Sprague-Dawley rats[145,146], while in Long-Evans, the same PRS paradigm decreased social behaviour only in males[147]. When PRS-exposed rats were tested in adulthood, no sex-dependent behavioural changes were observed. Some report both sexes to be affected[94,96], one specifically males[148] and one specifically females[220]. However Grundwald and colleagues detected the female-specific effect only with a social memory task and not with a social preference task[220]. Additional research is needed to better understand the impact of PRS on adult social behaviour.

In POS-exposed rodents, similar observations were made: adolescent males displayed altered social behaviour more often as compared to females. For rat experiments, the

exact age of testing seems to be a key determinant of the observed effects. In fact, the 3 experiments that showed specifically males displaying decreased social behaviour tested the animals around P40[101,151,152], while the studies reporting only females[151,155] or both males and females[154] to be affected used 25-day-old rats. Mouse strain dependent results have also been reported, Kundakovic and colleagues showed that for mice MS led to increased social behaviour in male C57BL/6 mice while no POS effect was found in Balb/c mice using a social approach task[150]. On the other hand, male MS-exposed Balb/c were more aggressive as compared to control animals, while this was not observed in C57BL/6 mice. Venerosi and colleagues also reported MS-exposed male CD-1 to be more aggressive during a social interaction task as compared to control mice[153]. While most papers did not report any behavioural changes when POS-exposed animals were tested in adulthood[93,111,157,158], some reported behavioural changes in specifically males[135], females[156] or both sexes[104,152]. Kentrop and colleagues reported that the outcome depended on the behavioural paradigm used: no behavioural change was found in either sex using a social interest paradigm, while both sexes underperformed on a social discrimination paradigm, the latter possibly also measuring social memory[152].

Overall, based on the limited papers available that studied ELA effects on social behaviour of rodents, we found that when tested during adolescence, both PRS and POS-exposed males are more often affected than females. In adulthood, often no change in behaviour was observed, and when PRS or POS did affect social behaviour, this was usually similar in both sexes.

4.2 ELA-induced effects on the behavioural problems that are related to anxiety and depressive-like behaviour, are highly dependent on sex, the ELA-model, behavioural paradigm and age tested

4.2.1 Anxiety-like behaviour

When tested during adolescence, PRS females are somewhat more vulnerable to develop increased-anxiety-related behaviour when compared to males (males: 7; females: 12, out of 27 experiments), while when tested during adulthood, no sex-differences are apparent (males: 14; females: 11, out of 36 experiments). Opposed to PRS, POS had differential effects in rats and mice and therefore we will discuss them separately. When POS-exposed mice were tested during adolescence, anxiety-related behaviour was often not affected and when it was, this did not depend on sex (males: 1; females: 2, out of 10 experiments). In adulthood, POS-exposed rodents did develop behavioural changes, however the sex-specificity of these effects is not clear cut for mice (males: 9; females: 12, out of 26 experiments), while in rats, POS-exposed males have been reported to be more often affected, both when tested during adolescence (males: 14; females: 9, out of 37 experiments) and in adulthood (males: 16; females: 9 out of 42 experiments).

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When PRS effects were tested during adolescence, there were several experiments showing that specifically females, and not males, developed increased anxiety-related behaviour[85,115,144]. Others have also reported increased anxiety-related behaviour in both sexes[160,161] or in males specifically[146,147]. Based on these available studies it seems that even though PRS-exposed young female rodents more often develop increased anxiety-related behaviour when compared to males, this likely depends on the rodents' strain and the type and intensity of PRS[144,159]. In adulthood, PRS induced sex-differences were also modest; mostly both PRS-exposed males and females displayed increased anxiety-related behaviour[88,123,164–167]. However, from the experiments showing a sex-effect, increased anxiety-related behaviour was more often reported in males[94,121,147,163,166,168] than in females[148,165,169], possibly indicating increased vulnerability in males.

From POS experiments in rats, both during adolescence and adulthood, approximately half of the experiments did not show altered behaviour in either sex. However, when behaviour was affected, POS was reported to increase anxiety-like behaviour more often in males than in females, both when tested during adolescence and adulthood. The experiments that found the male vulnerability during adolescence mostly used MS as POS model and tested behaviour via OFT and EPM[101,133,178,179]. Similarly, when tested in adulthood, the majority of the experiments showed increased anxiety-related behaviour in POS-exposed males[107,135,141,190,191,193].

In mice, there was no sex-effect of POS when evaluating anxiety-related behaviours. In fact, when tested during adolescence, in the majority of the experiments POS had no effect on behaviour in either sex[102,150,162,188,189]. In adulthood, behavioural changes are observed for both sexes. The specific effect of POS on adult anxiety-related behaviour seems to depend, at least partly, on the behavioural paradigm used to assess anxiety-like behaviour[93,156,188,203–206]. For example, POS-exposed males displayed increased anxious behaviour when assessed with OFT, while females displayed increased anxiety behaviour when assessed with EPM as compared to their respective controls[156]. Another paper reported that, both for the EPM and OFT, POS males were more anxious as compared to their controls, while females were less anxious during the OFT[203]. Interestingly, they only found this decrease in anxiety-related behaviour in females in the diestrous phase of their estrous cycle, indicating that hormonal fluctuations may affect POS-induced anxiety-related behaviour in females. Interestingly, Goodwill et al. (2019) reported females to be more vulnerable to POS in the anxiety domain, but this could only be exposed by continuous home cage video monitoring, while a standard OFT showed both sexes to be more anxious as compared to their respective controls[188]. Lastly, Weiss et al. (2011) reported differential effects depending on the type of POS, i.e. they found a male vulnerability in offspring of MS dams that were additionally stressed by restraint or swim stress during the dam-pup separations while in regular MS offspring, females and not

males were found to display increased anxiety-related behaviours for the same task, a free exploratory paradigm[205].

Taken together, PRS-exposed adolescent female rodents are have been reported to be more affected than males while in adulthood, increased anxiety-like behaviour was observed in both sexes equally. POS-exposed male rats have been reported to more often develop anxiety-related behaviour changes as compared to females, both when tested during adolescence and adulthood, while for mice, the outcome depends on the specific POS model and behavioural paradigm.

4.2.2 Depressive-like behaviour
For ELA effects on depressive-like behaviour, when tested during adolescence, we found altered behaviour to be present more often in males exposed to either PRS (males: more: 4, less: 4; females: more: 2, less: 1; out of 11 experiments) or POS (males: more: 15, less: 0; females: more: 10, less: 3; out of 21 experiments). When tested in adulthood, behaviour was often not affected and when it was, no clear sex-differences became apparent when rodents were exposed to either PRS (males: more: 4, less: 2; females: more: 6, less: 1; out 23 experiments) or POS (males: more: 2, less: 2; females: more: 3, less: 2; out of 16 experiments).

When reviewing effects of PRS on depressive-like behaviour during adolescence, male rats (no studies using mice met the requirements for this review) were more likely to be affected as compared to females[144,146,217]. Similar to anxiety-like behaviour, the outcome depended on the rat strain and timing of PRS model used[144]. In adulthood, firstly, half of the experiments did not show behavioural changes in either males or females[84,93,96,120,147,217,221]. Of note, six of these experiments were part of the same paper in which several PRS time-windows were tested, of which the majority was not effective in modulating depressive-like behaviour in mice[221]. In fact, variable stress applied during the first week of gestation led to increased depressive-like behaviour in males, while the same stressor applied during mid or late gestational period, did not affect depressive-like behaviour in either sex[221]. However, van der Hove et al. (2014) reported increased depressive-like behaviour specifically in male rats exposed to restraint stress during late gestation. This discrepancy could be due to the use of different species (mice versus rats) and a different PRS model (variable stress versus restraint stress)[169,221], as also discussed in this review[222]. Apart from these two papers, most experiments actually report increased anxiety-related behaviour in female rodents exposed to mid- or late-gestational stress[84,147,170,210,211], suggesting a female vulnerability depending on the timing of PRS.

For POS, when tested during adolescence, several experiments showed that in particular males develop depressive-like behaviour[150,174,176,213], even though female offspring is not

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resistant and similar behavioural changes in both sexes have been reported as well[132,15 0,174,176,177,189,212]. All of these studies used MS or MD as POS model, while the exact timing and behavioural paradigm used (see e.g. [176]) differed among experiments. From the details in these papers, it becomes clear that even though young males seem to be more vulnerable to the effects of POS on depressive-like behaviour, the type and timing of POS matters, as well as the behavioural paradigm used to assess behaviour. In adulthood, half of the included experiments reported neither males nor females to be affected by POS[93,108,112,189,200,207,223] and, within the affected studies, there was no sex-difference. Some reported increased depressive-like behaviour, specifically in MS-exposed male rats[190], MS-exposed female rats[218] or LBN-exposed female mice[188]. Decreased depressive-like behaviour has also been reported for MS-exposed male rats[218], MD-exposed female rats[157] or both male and female rats exposed to the LBN paradigm[103]. Thus, where in some cases species or POS model explain the different outcome, in other cases the source of variation in outcomes remains unclear.

In summary, both for PRS and POS, males seem to more often display depressive-like behaviours when animals are tested during adolescence. When tested in adulthood, both PRS and POS affect behaviour in about half of the reported experiments and within the affected experiments no clear sex-difference becomes apparent. For all ELA experiments regarding depressive-like behaviour, the timing and type of ELA and behavioural paradigm used seem to greatly matter for the outcome of an experiment.

4.4 The impact of a 'second-hit' in ELA experiments related to rodent behaviour

The two-hit hypothesis poses that ELA is the 'first hit', that increases the sensitivity to later-life challenges (i.e. the 'second hit'), and as such increases 'allostatic load'[224]. A secondary challenge might then unmask behavioural changes that have been programmed earlier, and are not apparent under basal circumstances. Concerning the behavioural domains, we investigated whether secondary stressors (e.g. acute or chronic stress) affected behavioural outcomes (**Supplementary table S3**). Unfortunately, the effects of secondary stressors on the behaviour of ELA-exposed rodents have not been studied often in a setting where both sexes were included (PRS: 4.0%, POS: 13.9% of all included experiments). Nevertheless, for anxiety- and depressive-like behaviour that was tested in adulthood, sex-specific behavioural changes are observed. For example, postnatally stressed males and not females develop anxiety-like behaviours when exposed to a secondary stressor later in life as compared to control animals exposed to the same secondary stressor only[177,189,202,225]. This is comparable to the POS induced male vulnerability in rats for developing anxiety-like behaviour under standard later-life conditions (paragraph 4.2.1). For depressive-like behaviour on the other hand, no sex-dependent behavioural changes were found when animals where only postnatally stressed early in life and tested

in adulthood (paragraph 4.2.2). However, when POS-exposed animals were exposed to a secondary stressor, specifically the adult females exhibit increased depressive-like behaviour whereas males do not, as compared to controls that were (only) exposed to the same secondary stressor[108,190,226]. These examples highlight the importance of a second-hit in revealing typical aspects of ELA-induced programming. Thus, we encourage the inclusion of a second hit when possible, to study the response of various systems, not only under resting/basal state, but also under conditions of demand, while being activated to respond to the current challenge.

5. Assessment of ELA effects on later metabolic health in human studies

Relative to the ELA effects on mental health outcomes and behaviour, the metabolic consequences of ELA are understudied. This is reflected by the number of included research papers in this review (human mental health: 42; human metabolic health: 11; rodent behaviour: 131; rodent metabolism: 45). We will discuss in this chapter the current human literature with a focus on the PRS versus POS exposures, sex differences, and the effects on bodyweight, adiposity, and thereafter measures of insulin sensitivity. For a detailed overview of all results and references see **Figure 2B** and **Table 2A**

5.1 Girls are more often reported to be at higher risk for effects of ELA on body composition than boys

Overall, girls seem at a higher risk than boys to develop overweight or adiposity upon ELA. Upon PRS exposure, girls were reported to develop overweight or adiposity somewhat more often than boys (girls: increased: 4, decreased: 0; boys: increased 2, decreased: 2, out of a total of 6 studies). For POS, girls also showed increased risk to develop overweight or adiposity since only "high risk" subgroups of boys were affected (girls: 2; boys: 2 ("high risk" subgroups) out of 3 studies). One study investigating PRS+POS exposure together found increased risk for obesity only in girls.

For example, maternal report of intimate partner violence pre- or postnatally was associated with an increased risk for obesity, specifically in girls[30]. Also, daughters of mothers with a psychiatric illness (and no use of SSRI's) during pregnancy were more likely to become overweight as compared to daughters of healthy mothers, while no such effect was observed in boys[227]. Postpartum maternal distress was associated with an increased waist-to-hip ratio in girls, whereas in boys this was only seen in those with high levels of stress reactivity[228]. Prenatal depression was associated with increased BMI in girls, while for boys this was only true for those whose mothers required hospitalization due to depression[229]. While most studies thus suggest stronger effects of ELA on body composition in girls, there

are also studies reporting ELA-induced metabolic vulnerability in both boys and girls, for example after prenatal maternal stress due to an ice storm[230] and in relation to parental separation during pregnancy[231]. Opposite effects on body composition have only been reported in boys: depression during pregnancy was associated with a lower weight and smaller height among boys, but not girls, while in the same study depression postpartum was associated with higher weight-for-height ratios specifically in girls[229]. While CORT levels are not the same as an actual stressor, they do reflect stress levels. It is interesting to note that also prenatal maternal CORT levels were associated with marginally lower fat mass index (FMI) in boys, but higher FMI in girls at 5 years of age[232].

Concluding, ELA has been reported to more often increase bodyweight in girls. Boys seem less affected and there is some evidence for boys to exhibit a leaner phenotype. Importantly, even though only few studies are available on the effects of ELA on body composition, almost all included studies are considered large (8 out of 9 had more than 500 participants) and thus the observed increased vulnerability for overweight or adiposity in girls seems robust.

5.2 Effects of ELA on insulin sensitivity parameters
There are so far no studies that met inclusion criteria for this review investigating associations between ELA and diabetes. However, two human studies on measures of glucose metabolism and insulin sensitivity after ELA exposure showed no sex differences. In a large study with 1478 participants, prenatal psychosocial stress exposure did not correlate to fasting glucose and insulin resistance (HOMA-IR; measure for insulin resistance based on circulating glucose and insulin levels) in 5 to 6-year-old children[233]. A small study (32 participants) reported a positive association between prenatal stress due to an ice storm with insulin secretion at 13 years of age in both boys and girls[234]. As insulin insensitivity usually develops with age together with unhealthy lifestyle habits[235], it is important to study these insulin parameters in ELA-exposed adults instead of children.

Taken together, there are relatively few studies addressing ELA exposure on metabolic outcomes in humans, however based on the evidence presented above, we tentatively conclude that ELA is more often reported to increase the risk to develop overweight in girls compared to boys, while no clear association was found between ELA and insulin sensitivity parameters in either sex.

Table 2. Overview of human and rodent studies/experiments and the respective metabolic outcomes for the specific domains

A) Human studies (numbers and references) assessing metabolic outcomes: per domain, timing of ELA and sex. B) Rodent experiments (numbers and references) assessing metabolic outcomes: per domain, timing of ELA, outcome age, species (if relevant) and sex.

A

Domain	Timing ELA	Total studies	Increased		Decreased		No effect in either sex
			M	**F**	**M**	**F**	
BW	PRS	6	2 230,231	4 227,230–232	2 229,232	0	1 236
	POS	3	2 228,229	2 228,229	0	0	1 237
	PRS + POS	1	0	1 30	0	0	0
Insulin sensitivity	PRS	2	1 234	1 234	0	0	1 233

B

Domain	Diet	Timing ELA	Outcome age	Total	Increased M	Increased F	Decreased M	Decreased F	No effect in either sex
BW	standard diet	PRS	Adolescence	11	2 [146,238]	3 [146,217,238]	0	0	8 [91,162,239–244]
			Adulthood	10	0	0	2 [243,244]	2 [241,245]	7 [217,238–240,242,246,247]
		POS	Adolescence	26	2 [179,248]	2 [248,249]	12 [103,105,174,180,250–257]	10 [103,105,174,180,251,253–257]	11 [199,201,257–265]
			Adulthood	21	4 [199,248,252,266]	4 [179,199,248,266]	4 [103,140,255,257]	4 [103,140,255,267]	11 [112,201,250,254,257,261,264,265,268–270]
	unhealthy diet	PRS	Adulthood (no adolescent studies)	5	1 [238]	2 [238,239]	2 [239,244]	1 [244]	2 [240,247]
		POS	Adolescence	1	1 [263]	1 [263]	0	0	0
			Adulthood	5	0	2 [265,271]	0	1 [250]	2 [255,270]
Adiposity	standard diet	PRS	Adolescence	3	0	0	1 [217]	0	2 [238,239]
			Adulthood	6	0	0	2 [217,244]	0	4 [238,239,243,247]
		POS	Adolescence	1	0	0	0	0	1 [272]
			Adulthood	5	2 [179,265]	2 [179,265]	1 [269]	1 [269]	2 [250,270]
	unhealthy diet	PRS	Adulthood (no adolescent studies)	4	1 [238]	3 [238,239,247]	2 [239,244]	1 [244]	0
		POS	Adulthood (no adolescent studies)	4	0	2 [265,271]	0	1 [250]	1 [270]

Insulin									
	standard diet	PRS	Adolescence	1	0	0	0	0	1 [238]
			Adulthood	8	1 [246]	4 [238,241,246,247]	2 [239,241]	2 [239,243]	2 [240,244]
		POS	Adolescence	3	1 [255]	1 [255]	2 [256,263]	1 [263]	0
			Adulthood	10	1 [255]	0	5 [248,250,252,266,270]	3 [248,265,266]	3 [179,254,268]
	unhealthy diet	PRS	Adulthood (no adolescent studies)	5	1 [239]	1 [247]	1 [238]	0	2 [240,244]
		POS	Adolescence	2	0	0	0	1 [255]	1 [263]
			Adulthood	5	0	2 [250,271]	2 [255,270]	0	1 [265]

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6. Assessment of sex differences in the effects of ELA on rodent metabolic outcome parameters

It has been proposed that the later life (nutritional) environment eventually determines which ELA-induced phenotype emerges: a healthy lifestyle or positive environment would lead to resilience (lower adiposity, increased insulin sensitivity), while a negative environment, or unhealthy lifestyle, increases vulnerability (increased adiposity, reduced insulin sensitivity)[273]. While in human cohorts, later life dietary conditions are difficult to control for, this is not the case for rodent studies. Therefore, we considered not only the impact of ELA on metabolic health under basal circumstances, but also under different dietary conditions. We will thus next review and discuss the effects of PRS and POS on the various metabolic outcomes (body weight (BW), adiposity and insulin resistance) at two different ages (adolescence and adulthood), and highlight when the outcomes were at basal levels, or in response to a later life dietary challenge (unhealthy diet; UD). For a detailed overview of all results and references see Figure 2B and **Table 2B**.

6.1 Sex-dependent effects of ELA on the bodyweight of rodents depend on the nature of stress exposure and the later life diet

Under standard dietary conditions, PRS does not affect body weight (BW) in most studies, and when it did, there were no strong sex differences in either adolescence (males: increased: 2, decreased: 0; females: increased: 3, decreased: 0 out of 11) or adulthood (males: increased: 0, decreased: 2; females: increased: 0, decreased: 2, out of 10). In adolescence, POS mostly reduced BW (males: decreased: 12, increased: 2; females: decreased: 10, increased: 2, out of 26), while in adulthood, POS either increased or decreased BW (males: increased: 4, decreased: 4; females: increased: 4, decreased: 4, out of 21) similarly in males and females.

When challenging the system with an unhealthy diet (UD) later in life, we found the following: only one study investigated the modulatory effect of UD (high-sucrose only) in adolescence, and an increased BW was observed in both POS exposed males and females. In adulthood, PRS exposure combined with UD, either increased (males: 1; females: 2, out of 5) or decreased (males: 2; females: 1, out of 5) BW compared to control animals on UD without showing strong sex differences. In contrast, for POS a clear sex difference was observed: no studies showed an effect on adult BW in males, while 3 out of 5 studies (2 increased; 1 decreased) reported effects of POS in females.

Under standard dietary conditions, PRS generally did not affect BW in *adolescence*[91,162,239], although some studies reported an increase in adolescent BW in both sexes or in females only[146,217,238]. There are no clear differences in timing of PRS (early versus late in gestation), the age at which BW was measured (early in adolescence versus later in adolescence),

or the species used, that could explain some of the differences between these studies. In adulthood, PRS mostly does not affect BW[238,240,242,246]. For example, chronic mild unpredictable stress during early and mid-gestation, or variable or restraint stress during late gestation did not affect BW in either sex[238,239,242,247]. Few studies showed a reduction in adult BW after PRS[241,243,274], while no studies reported an increase.

In contrast to PRS, POS has stronger effects on BW in both adolescence and adulthood. In *adolescence*, POS often leads to a reduction in BW similarly in both sexes[105,251,253,257]. For example, exposure to the limited bedding and nesting (LBN) paradigm with or without exposure to a substitute mother for one hour per day reduced BW at P27 and 9 weeks of age in males and females[103,253]. Similarly, maternal deprivation (MD) and maternal separation (MS) reduced BW throughout adolescence in male and female offspring[174,180,254–256]. However, some MS studies also showed an increase in adolescent BW[179,248,249]. It is unclear what contributes to these discrepancies. In adulthood, POS alters BW similarly in both sexes. LBN or MD resulted in a reduction[103,140,255], or no effect[112,254,269] on adult BW. In contrast, MS with or without early weaning either increased[179,199,266], or had no effect[250,264,265,270,275] on adult BW. In conclusion, studies show differences in the effect of ELA on BW depending on the timing of stress, and (for POS) the specific model used, but generally with similar effects in both sexes.

When challenging the system with an UD later in life, a different picture emerges. PRS either decreased[244] or increased[238] adult BW when exposed to UD. For example, chronic variable stress early in pregnancy reduced BW in both males and females upon 17 weeks of UD, compared to their respective controls on UD[244]. In contrast, variable stress during late gestation has been shown to increase BW in male and female offspring when exposed to UD post-weaning[238]. Restraint stress during late gestation did not affect BW after 4 weeks of UD[240]. Due to the relatively low number of studies, it remains difficult to understand what underlies the discrepancies between these studies. When looking at the effects of POS combined with UD, females appear more susceptible compared to males. For example, MS combined with either sham injection or early-weaning increased BW when fed a post-weaning UD for either 12 or 17 weeks in females, but not in males[265,271]. In contrast, MD or MS alone followed by postweaning UD for 11 or 16 weeks, did not affect BW in either sex[255,270] suggesting that the type and severity of the postnatal stressor may influence metabolic outcome.

Summarizing, PRS in most studies does not seem to affect BW when animals are on chow diet and there are mixed results when exposed to UD later in life. POS, however, leads to a similar effect on BW in both sexes when fed standard chow, and upon UD later in life POS-exposed females are more affected than males.

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6.2 ELA exposure increases adiposity in female rodents when exposed to an unhealthy diet

Adiposity can be affected by ELA independent of bodyweight[269], and is therefore another important indicator of metabolic health to assess with regard to ELA. Under standard dietary conditions, both in adolescence and in adulthood, in some cases PRS exposure decreased adiposity in males (adolescence: 1 out of 3 studies; adulthood: 2 out of 6 studies) while this was never the case for females. For POS, only one study investigated its effects on adiposity in adolescence and observed no difference in either sex, while in adulthood, POS affected adult adiposity in both sexes in 3 out of 5 studies. When animals are subjected to an unhealthy diet (UD) in adulthood, a clear picture emerges: females specifically gain more adiposity when they are stressed during either pre- or postnatal life (females: 5; males: 1, out of 8). When taking into account timing of ELA; for PRS, 3 out of 4 studies show increased adiposity in adult females, while this was true for only 1 out of 4 studies for males. For POS, adipose tissue accumulation in response to UD was never increased in males, compared to their respective controls (4 out of 4), while females showed increased adiposity in 2 out of 4 studies, and decreased adiposity in 1 out of 4 studies.

Under standard dietary conditions, in adolescence, PRS either had no effect on adiposity in either sex[238,239] or decreased it in males specifically[217]. Similar effects were observed in adulthood: PRS (early or late in gestation) exposed males showed decreased adiposity, while females were unaffected[217,244]. However, other studies reported no effect of PRS (early-mid or late gestation) in either sex[238,239,243,247]. Although it is unclear what contributes to these differences between studies, they suggest males may be somewhat more affected by PRS, and show a leaner phenotype compared to controls.

POS similarly affected adult adiposity in both sexes, but the exact outcome (i.e. more or less adiposity) seems to depend on the POS paradigm. Whereas exposure to LBN led to decreases in adiposity[269], exposure to MS increased adiposity[179,265] in adult males and females. Of note, although POS does not seem to result in clear sex differences in adiposity when fed standard chow, there might be more subtle sex differences. For example, MS increased adipose tissue depot weight in males and females, and while this was the case for only one out of three depots in males, it was true for all three depots in females[179], and LBN led to a higher mesenteric fat percentage, which was particularly the case in females[269]. As the various fat depots have different functional implications[276], this suggest the need for more detailed studies in this area.

When animals are subjected to an unhealthy diet (UD) in adulthood, females gain more adiposity when exposed to PRS or POS[239,247,265,271]. For PRS, in the studies specifically finding increased adiposity in females but not males, UD started in adulthood with a duration of

3-10 weeks, and PRS was applied either early-mid or late during gestation[239,247]. Notably, the one study that observed increased adiposity in both sexes used an UD starting directly after weaning[238]. This suggests that timing and length of exposure of UD are important aspects to take into account when comparing the various studies, and that starting UD exposure at a younger age might overrule the otherwise observed sex differences.

When looking at the effects of POS, similar to PRS, a female-specific increase in adiposity upon UD is observed[265,271], although one study found that while POS did not affect male adiposity, females showed less adiposity after UD[179]. All studies used the MS paradigm and UD started either directly or soon after weaning. The two studies observing more adiposity added either early weaning or sham injections to the MS protocol[265,271], suggesting that the severity of the stressor might influence metabolic outcome.

In conclusion, under standard chow, males may be somewhat more affected by PRS, while POS affects adult males and females similarly. While PRS led to reduced adiposity, POS either increased or decreased adiposity, which is potentially related to the used POS model. When fed an UD, specifically ELA-exposed females accumulated more fat.

6.3 Insulin sensitivity is generally more compromised in male rodents after ELA

High levels of circulating glucose (hyperglycaemia) and insulin (hyperinsulimea) are signs of insulin insensitivity. Insulin sensitivity can be measured with insulin and glucose tolerance tests (ITT; GTT). Under healthy circumstances, blood glucose is expected to quickly decrease in the ITT as well as the GTT. Reduced insulin sensitivity is thus indicated by either high glucose or insulin levels, or a slow glucose clearance. Only one study has investigated the effects of PRS on insulin sensitivity during adolescence, and observed no effects in either sex[238] (Table 1B). In adulthood, PRS increased insulin sensitivity measures more often in females compared to males (males: 1; females: 4, out of 8), while in 2 out of 8 studies for both sexes reduced sensitivity was observed. For POS, adolescent males were affected in 3 (2 decreased; 1 increased) out of 3 studies, and females in 2 (1 decreased; 1 increased) out of 3 studies. In adulthood, POS affected adult insulin sensitivity measures more often in males (males: 6; females: 3, out of 10).

When reviewing the studies including an UD condition later in life, there are no studies addressing the effects of PRS in combination with UD on insulin sensitivity in adolescence. In adulthood, PRS affected insulin sensitivity in 2 out of 5 studies for males and in 1 out of 5 for females. For POS, one study investigated its effects in adolescence and observed detrimental outcomes only in females. In adulthood, POS either decreases (2 out of 5 studies) or does not affect (3 out of 5 studies) insulin sensitivity in males, while in females POS increases (2 out of 5 studies) or did not affect (3 out of 5 studies) insulin sensitivity

measures when fed UD. When combining both adult PRS and adult POS studies, females never show decreased insulin sensitivity when fed UD, while ELA-exposed males on UD showed this in 3 out of 10 studies. See table 1B for references

In adulthood, PRS females more often showed increased insulin sensitivity measurements compared to males[247,250]. For example, exposure to prenatal variable stress during late gestation led to lower insulin levels, lower HOMA-IR and faster glucose clearance in adult females, but not males[247,250]. However, in another study PRS during late gestation did not affect glucose clearance in GTT or ITT[240], and also reduced insulin sensitivity is described upon either early-, mid- or late-PRS exposure in females[243], males[241], and both sexes[239]. These discrepancies between studies do not seem to be explained by the gestational period in which the stress was applied, nor measured outcome (e.g. circulating insulin/ glucose levels versus GTT/ITT) or used species, thus further research is needed to gain a better understanding of the effects of PRS on insulin sensitivity.

When looking at POS, both increased and reduced insulin sensitivity was observed in adolescence[255,256,263]. The studies of Mela and colleagues suggest timing of measurement matters: with the same POS model they found increased insulin levels in males at P36 without affecting females[256], but decreased insulin levels at P45 and P65 in both sexes[255]. In adulthood, males were more often affected, mostly showing reduced insulin sensitivity indicated by e.g. higher circulating glucose[252], and higher circulating insulin levels[250], although some studies also report such effects in both sexes[248,266]. In conclusion, when rodents are fed a standard chow diet, PRS more often increases insulin sensitivity in females compared to males, while POS affects males slightly more often, mainly resulting in decreased insulin sensitivity.

When exposed to an UD later in life, PRS-exposed males are reported to have both increased glucose clearing in GTT and ITT[239], as well as reduced glucose tolerance[238] after PRS during either early-mid or late gestation, respectively. Females were not affected in these studies. However, another study found increased insulin sensitivity measures specifically in females but not males exposed to PRS followed by UD in adulthood[247]. Differences between the studies are timing of stress, as well as the timing and duration of UD.

POS exposure followed by post-weaning UD reduced insulin sensitivity measures in males but not females in two studies[255,270]. Interestingly, in two other studies, POS followed by either post weaning UD or a diet high in fat and carbohydrates led to increased insulin sensitivity measures in females, while males were unaffected[250,271]. These studies indicate sex differences in vulnerability to high caloric diets following POS.
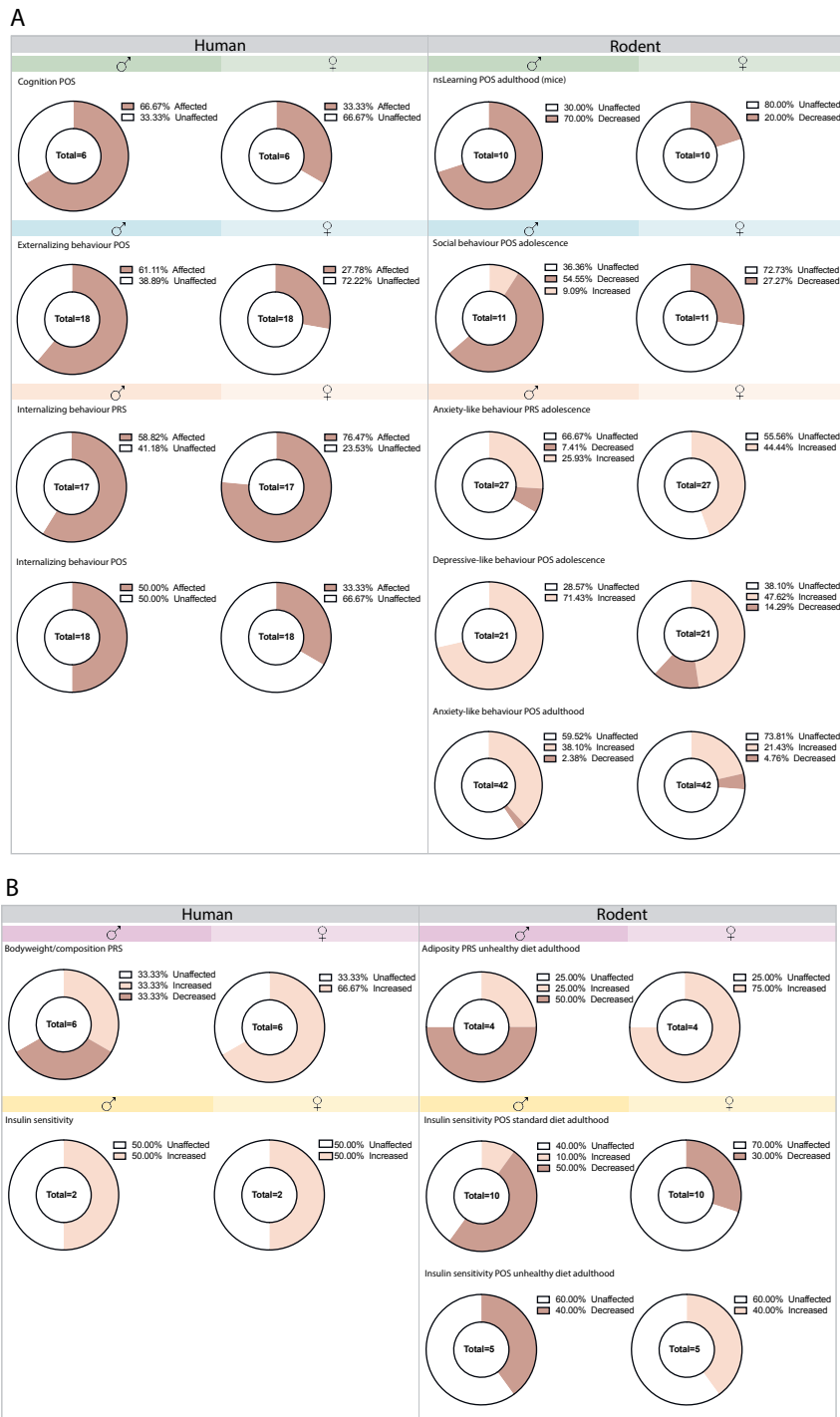
Figure 2. Schematic summary of the sex-dependent effects of early-life adversity on later life mental and metabolic health.

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Taken together, ELA-exposed males are more often reported to exhibit reduced insulin sensitivity, either when fed standard chow or UD, while ELA-exposed females more often show increased insulin sensitivity, depending on the timing of the ELA and diet. However, more studies focusing on sex-dependent effects of ELA on insulin sensitivity are needed, preferably investigating insulin sensitivity in multiple ways (e.g. ITT/GTT as well as basal insulin and glucose levels), at multiple ages, and in response to both standard and UDs.

7. Discussion

In this comprehensive review, we set out to unravel the consistency of sex differences in effects of ELA on later mental and metabolic outcomes across human and rodent studies and assess to what extent they depend on the type and timing of ELA. We did this by reviewing and discussing prospective human and rodent studies that have (i) included both sexes, (ii) analysed both sexes separately and (iii) addressed the sex-specific effects of ELA on core dimensions for mental (cognitive, emotional and social) and/or metabolic (body composition and insulin sensitivity) health. We will here integrate the so far reviewed evidence from the different fields and address these points.

7.1 Sex dependence and convergence of human and rodent literature of ELA-induced effects

Concerning the data from mental health/behavioural domains for human and rodent studies respectively, we can carefully conclude that they mostly go hand in hand. Indeed, ELA seems to lead to specific sex differences and the overall conclusion after integrating human and rodent research and the PRS/POS induced effects across the various mental health/behavioural domains is that males/boys seem to be more often affected by the effects of ELA compared to females/girls. Potential mechanisms for sex-dependent outcomes of ELA are: sex-differences in the development of the fetus in utero[277] and the placenta[278], differential (epigenetic) programming of the HPA-axis[279], differences in hormonal exposures during development[280], differences in breast milk content[281–283] or microbiome[284], or may result from alternate sex-specific evolutionary strategies[285–287]. While addressing the complex mechanisms at the basis of the programming by ELA is out of the scope of this review, we included a brief discussion on the role of the two key biological mediators interfacing the communication between mother and foetus/offspring and essential for the transfer of maternal effects during the critical pre- and post-natal period, i.e. the placenta (**Box 1**) and breast milk (**Box 2**) respectively.

When reviewing the results in more detail, we find that for the considered mental health domains in humans (cognitive, internalizing and externalizing behaviors) and rodents (cognitive, social and emotional) the following picture emerges:

Concerning cognitive functions, ELA exposed boys were more often reported to perform poorer on cognitive tasks as compared to girls. Timing of ELA seems to matter, since the effect was stronger after POS exposure, with evidence for this effect to be lasting up to adolescence (human studies) and into adulthood (rodent studies). Important to note is that girls are not resilient to ELA as their cognitive health is also frequently shown to be affected by PRS and POS and there is initial evidence that ELA might impact girls' verbal intelligence more so than in boys. While we can of course only speculate, this could be due for example to a sex difference in basal level in verbal intelligence to begin with. In fact studies have shown a female advantage in verbal skills[288], which could potentially contribute to this aspect being more sensitive to modulation by ELA in girls. When thinking of the neural basis of this, there is some evidence that male and female intelligence might relay on distinct functional networks[289], which might also explain such sex-dependent impact, but this remains open to future investigations. Similarly there is evidence from rodent studies for females to be impacted at specific ages or settings. Concerning externalizing behaviour in children, such as attention problems, aggression and hyperactivity, especially boys were at risk to develop these type of behavioural changes, both after PRS and POS exposure. While this domain is harder to directly relate to rodent studies, social behaviour (e.g. measuring social interest and sometimes aggression) can be used as a proxy for aspects of externalizing behaviour. Indeed, in line with human literature, especially young male offspring of PRS or POS stressed dams display altered social behaviours. Finally, when considering internalizing behaviours, the picture is a little more complex. We found that both boys and girls exposed to ELA often develop internalizing behaviour problems (i.e. emotional problems, anxiety and depressive-symptoms, and that the specific result (the affected sub-domain and sex-specificity) highly depends on timing of stress exposure and age of outcome. More specifically, the effects of PRS on internalizing behaviour are somewhat stronger in girls, while boys seem more vulnerable for the effects of POS.

Similarly, evidence from rodent studies indicates that both sexes can develop changes related to anxiety and depressive-like behaviour and that this also depends on the type and timing of the ELA model, behavioural paradigm and the age at which the animals were tested. Thus, ELA-exposed boys/males seem to be more vulnerable for the cognitive and externalizing domain, while for the internalizing disorders (e.g. anxiety and depression), both sexes are affected depending on the type and timing of ELA. This suggests that ELA might make men more susceptible to develop internalizing disorders that in the general population are more prevalent among women.

Concerning the metabolic outcomes, before discussing the overall picture, it is important to note that most longitudinal clinical studies included in this review that investigated the effects of ELA on metabolism, report ELA effects in children and adolescents, while rodent studies most often study this aspect in adulthood. This is important because, for

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example, insulin insensitivity often develops with age or after prolonged high-fat diet exposure[235]. Effects of ELA on these parameters may be difficult to observe at a younger age and this is thus a limitation to our study. For our literature search, we did not constrain age, but the oldest age of individuals studied was 32 years. This clearly indicates the need for longitudinal human studies with follow-ups into (late) adulthood. Nonetheless, human studies suggest that girls are more likely to develop overweight or altered waist-hip ratio after ELA exposure.

Similarly, rodent evidence indicates that ELA-exposed females, when exposed to an unhealthy diet later in life, gain more adiposity compared to their respective controls than males. There is also initial evidence for ELA to impact on food choice in both a human[290,291] and rodent setting[180], in a sex-specific manner, which will clearly also have consequences for metabolic health.

Regarding the effects of ELA on glucose metabolism and insulin sensitivity, human studies are extremely sparse and inconsistent: both no association and a non-sex-specific association between ELA and insulin sensitivity parameters are reported. Also, within the relatively few rodent studies that investigated ELA effects on insulin sensitivity and glucose metabolism, there are inconsistencies, but overall males somewhat more often showed a decreased insulin sensitivity, independent of later-life diet, while females rather showed increased insulin sensitivity. While decreased insulin sensitivity has been associated with diabetes[292], the exact implication of increased sensitivity is not well understood. Thus, ELA-exposed females seem to be more vulnerable to develop adiposity, whereas males may be more susceptible for detrimental effects on insulin sensitivity.

7.2 Comorbidity between cognitive and metabolic phenotype

Notable, the comorbidity between metabolic and brain disorders suggest converging or interacting underlying pathways might be at play[14–16,19–21]. Do the comorbidities of these particular conditions indeed share a similar early-life origin?

Human studies suggest a bidirectional relationship between depression and obesity: obesity increases risk for depression and depression is also predictive of obesity[14]. Within this review we found that PRS-exposed girls are more likely to develop depressive behaviours and girls seem more vulnerable to become overweight after ELA exposure. Although the studies that we included in the review did not specifically investigate comorbidity, our results may point to an increased risk for women to suffer from comorbid depression and overweight/obesity after ELA, which could (partly) explain evidence demonstrating that depressive women more often suffer from comorbid obesity than do depressive men[23].

Also for rodent studies, females seem to be more vulnerable for the metabolic and depressive-like phenotype. Effects of ELA on depressive-like behaviours depend on the timing of both stress exposure and behaviour assessment but overall adult females exposed to PRS in late pregnancy are more likely to develop depressive(-like) behaviours in adulthood. This is in line with the metabolic vulnerability at this age since ELA mostly affects adiposity in adult females fed a high fat diet.

Moreover, metabolic diseases (both obesity and diabetes) are linked to the development of, and often co-occur with, cognitive impairments and Alzheimer's disease later in life[19–21,293]. We here report that cognitive deficits following ELA are more pronounced in males/boys, which is supported by a previous meta-analysis[26]. The ELA-induced sex-dependent vulnerability to metabolic alterations depended on the readout. As mentioned above, males have been reported more often to be vulnerable with regard to glucose metabolism/insulin sensitivity in contrast to females being more vulnerable to increased adiposity. In particular the downstream effects of obesity, such as inflammation and insulin resistance are suggested to be involved in mediating its effects on cognition[293,294]. Therefore, it is possible that the observed ELA effects on insulin resistance in males underlie the male vulnerability for ELA induced cognitive impairments.

Taken together, the ELA-induced adiposity and PRS-induced depressive-like phenotype are more pronounced in females/girls, supporting the hypothesis that ELA might be at the origin of this female specific vulnerability and that there might be converging pathways leading to the metabolic and mental disorders. In addition, pathways leading to dementia might differ between men and women, i.e. while for women, the ELA effects on obesity might increase risk, for men, the changes in glucose metabolism/insulin sensitivity may do so.

7.3 Limitations
Throughout this review, it became clear that effects of ELA largely depend on the specific domain, the time of exposure, the specific ELA model, the age of testing and importantly the sex of the offspring. However, in particular for rodent research, even when stratifying the outcomes into these sub-categories, there is considerable discrepancy in the findings and a considerable amount of experiments showed no effect of ELA on the various domains. Some of the aspects contributing to this variation are the breeding method and animal species/strain, early-life environmental factors such as diet composition and cage enrichments, and for behavioural research, the animal facility and experience of the experimenter. While these are clearly important determinants, they might be the hardest to fully standardize and control for. Two specific aspects emerged while reviewing the literature that appear to have an important influence in the context of ELA-induced effects on behaviour and metabolism: a methodological aspect concerning the origin of

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the pregnant dams and a statistical aspect concerning the power of the current studies, which are discussed in the **Supplementary material 2**.

Next, it is important to note that studies addressing mental/behavioural and metabolic health outcomes simultaneously, are currently lacking, and this is the case both for human and rodent literature. These will be needed in the future, in order to be able to more directly relate these two aspects and to further our understanding on their origin(s) and sex-specific co-occurrence. Understanding more about the co-occurrence of various diseases, how they modulate each other and how the treatment of one may impact the other, will give us new insights into possible converging pathways and joint targets.

Another limitation to our study, as shortly referred to above, is the young age of the studied populations in the human studies. Disorders like depression, obesity, type 2 diabetes and dementia tend to develop with increasing age (although depression also affects the young). In order to study sex-differences between effects of ELA on these disorders, studies are needed that include older populations.

Finally, as also stated in the Methods section, in order to gain a better understanding of the consistency of sex differences in effects of ELA on mental and metabolic disorders, we took a fairly crude approach by scoring the statistically significant effects in both sexes for all studies/experiments and looking at the male/female ratio in the total counts of these studies. Also, by taking study/experiment as the unit of observation, we did not take into account the size of the studies. In an attempt to overcome this limitation, we separately reported on outcomes of the larger studies in human (n>500). In our view, this was the best practical way to get an overview of sex differences in effects of ELA, as our aim was to compare studies in humans and rodents with a wide variety of early life stressors and outcomes, making it impossible to perform meta-analysis.

Despite these limitations, our evaluation of the consistency of sex differences in the effects of ELA on mental and metabolic disorders is an important step in better understanding the differences between disorder prevalence and presentation among males and females who experienced ELA, which is important for future research and potentially treatment.
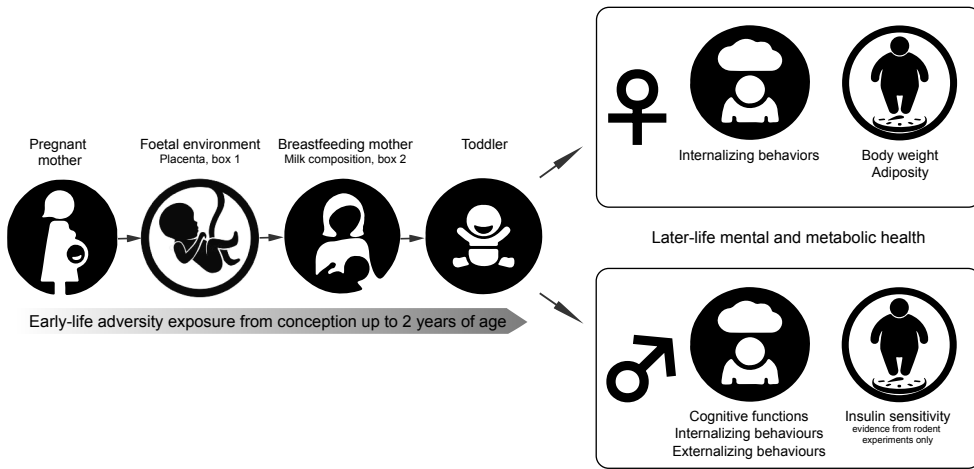
Figure 3. Graphical overview of the sex-dependent effects of early-life adversity on later life mental and metabolic health.

Conclusion

Altogether, our findings show that ELA impacts later life mental and metabolic health differently in males and females, and suggest that ELA plays an important role in the sex-specific prevalence/presentation and comorbidities of these disorders (**Figure 2A,B Figure 3**). As such, ELA-induced disease states deserve specific strategies for the adequate development of preventive or interventional treatments. Researchers are advised to broaden their assessment of the context of population studies, intervention trials, treatments and diagnosis, in order to more regularly include individuals early-life history, sex and comorbidities, laying ultimately the basis for personalized treatment. The high convergence of rodent and human findings across our comprehensive review further reinforces the validity of rodent models to study the biological substrates and exact underlying mechanisms that lead to the 'programming' of disease risk by ELA, and are thus vital in identifying and testing novel targets for future preventive and intervention strategies.

Box 1. Role of the placenta in mediating sex differences during foetal development

All eutherian mammals nourish their offspring via a placenta during gestation. The placenta is responsible for mediating the exchange of nutrients, respiratory gasses, waste products, and hormones between a mother and foetus during pregnancy[295]. Throughout development, maternal blood in the endometrium is closely apposed to foetal blood vessels in the trophoblast allowing for the transfer of materials via diffusion,

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active transport, or selective absorption from mother to child and vice versa[295]. Due to the intimate nature of this maternal-foetal connection, the placenta plays a pivotal role in regulating the growth and development of the embryo and is therefore likely to play an important role in the transference of prenatal stress to the developing offspring.

The placenta is a sexually dimorphic organ. It is unique in that it is the only organ in the animal kingdom that is formed by two genetically different individuals, consisting of both maternally-derived and foetally-derived (and hence gender-specific) tissue. Related in part to sex-specific differences in placental function, males and females differ in terms of growth and development in utero[296,297]. Studies on normal (uncomplicated) pregnancies have reported sex-specific differences in placental weight[298], efficiency[286], gene expression[299–301], hormone production[237,238] and foetal and neonatal morbidity and mortality[285,302]. Moreover, there is compelling evidence that the placenta responds differently to prenatal stress (PRS) depending on the sex of the developing offspring. For example, the male placenta seems to be more susceptible to gestational glucocorticoid exposure (e.g. cortisol induced placental growth[303] and increased vasculature[304]) and prenatal stress (e.g. male placenta exhibit increased expression of PPARα, IGFBP-1, GLUT4, and HIF3α when compared to females[221]), while the female placenta appears to be able to adapt more readily to adverse maternal conditions during pregnancy, such as obesity[305] and asthma[306].

At the heart of all these differences lies a fundamental difference in prenatal growth strategy between the genders, with males investing maximally in foetal growth, while females forego maximal growth to allow for more adaptive flexibility in utero[286,287,307,308]. These sex-specific growth patterns determine the way in which male and female fetuses are capable of coping with the same adverse prenatal intrauterine environment, ultimately driving gender differences in their vulnerability or resilience to prenatal stress. Under favorable conditions, the focus of males on faster growth represents a distinct adaptive advantage that maximizes their fitness. However, this male-specific strategy is also risky, leaving them less adaptable to changes in the intrauterine environment; when confronted with adverse intrauterine conditions they are programmed to prioritize growth even when it is not in their best interest to do so, placing them at a greater risk for morbidities or mortality in utero[286,287,305,307,308]. Females adopt a more measured growth strategy, which allows female placentas to more readily adapt to adverse changes in the intrauterine environment[305]. This more conservative approach enables female fetuses to respond dynamically to maternal disease, enhancing their probability of survival should further maternal insults occur while ultimately lowering their risk of developing negative perinatal outcomes[286,287,305,307,308].

There is thus increasing evidence that stress differentially affects the male and female placenta, potentially contributing to sex differences in adult disease risk after PRS exposure. This likely involves sex-specific programming in the placenta (e.g., [171,279,287,307,309,310]), however, the precise mechanisms are still insufficiently understood and require further research.

Box 2. Sex-specificity in maternal milk composition

Newborn mammals are initially fed with maternal milk[311], a rich bioactive liquid responsible for the transfer of nutrients, immune factors, hormones and microbiota from mother to offspring. Maternal milk composition is highly dynamic; within a single mother, large compositional differences exist within a single feeding (foremilk/hindmilk), across the day and over longer time periods/lactation stages. Maternal milk composition also varies greatly between different mothers, dependent on various environmental and maternal factors such as maternal diet and maternal BMI[312]. Interestingly, several experimental and clinical observations suggest that infant sex might be another driver of variability in maternal milk composition.

Evidence for sex-specific maternal milk composition has been collected in various animal species. The primate work of Hinde *et al*. in rhesus macaques (*Macaca mulatta*) has shown that milk produced for male singleton offspring is higher in energy and fat content, but lower in volume and calcium compared to milk produced for female offspring[313–315]. A cross-fostering study in bank voles (*Myodes glareolus*) demonstrated that all-female litters receive more milk than all-male litters, independent of maternal size or condition[316]. Similarly, a study analyzing numerous lactation records of >1.4 million Holstein dairy cows (*Bos taurus*) reports higher volume and energy content of milk produced for daughters compared to milk for sons[317]. Since calves are removed from their mother on the day of birth and as milking occurs in a mechanical and standardized fashion, the sex-specific milk production is suggested to be the result of prenatal programming of the mammary gland by foetal sex. Under different conditions, maternal nursing behaviour could possibly regulate milk production in a different direction. Indeed, among captive red deers (*cervus elaphus hispanicus*), another ruminant species, milk volume and protein, fat and lactose content has been shown to be greater for sons than for daughters[318]. Also in marsupials, kangaroos (*Macropus giganteus*) and wallabies (*Macropus eugenii*), milk for sons is found to be higher in protein content but similar in volume and total energy[319].

Clinical observations that preterm boys respond differently to early nutritional interventions than girls[320] point towards sex-specific early-life nutritional requirements, in humans too. Interestingly, breastfed same-sex twins are found to be taller and heavier than breastfed opposite-sex twins[321], possibly because milk composition cannot be

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tailored to both sexes at the same time. This suggests that adaptation of milk composition to infant sex could be a potential mechanism via which optimal growth and development is achieved in both sexes.

So far, only a few studies have addressed the relationship between infant sex and human milk composition and the results are somewhat conflicting. Some studies report no effect of infant sex on human milk macronutrient and energy content[322], or microbiota profiles[323]. Three studies, all with a modest sample size (25-61) report higher energy and fat content in milk for sons compared to daughters[281–283]. On the contrary, Hanh et al. report higher carbohydrate and energy content in milk for daughters[324]. In addition a study found that Kenyan mothers with a high social economic status produced milk with a higher fat content for sons, while poor mothers produced milk with a higher fat content for daughters[325]. Another study by Yahya et al. reports higher phosphor content and higher volume for sons, but higher calcium content in milk for daughters[326].

Altogether, the current evidence collected in various mammalian species, hints toward sex-specificity in maternal milk composition, and that a potentially differential adaptation of maternal milk composition to maternal stress depending on the sex of the infant might contribute to the sex-dependent effects of POS on offspring/children, but further studies are required to establish this.

Author Contributions

AK and SdR, conceptualized and supervised this study and reviewed and edited the manuscript. Literature search was performed and screened by KR and SR for the rodent aspect and by LB and JK for the human aspect. KR and SR analysed the included papers, prepared figures and tables and wrote the manuscript. EN contributed to writing of the box concerning the maternal milk and TE and BP to the box on the placenta. All authors contributed to editing the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary material

Supplementary material 1. Pubmed Search terms.

Available upon request

Supplementary material 2. Additional factors impacting the outcome of ELA experiments

Origin of pregnant dams impacts on the offspring's later life outcome
Purchasing pregnant dams versus breeding within the own facility has been shown to be a modulating factor in preclinical ELA studies[26]. Indeed, transportation during pregnancy is stressful and thus acts as a stressor (first "hit"), both in controls as well as in the experimental animals prior to the actual ELA paradigm they will be exposed to, thereby altering the original design. This can affect the CTL dam and offspring as well as the response of the dam and offspring to ELA exposure, and thereby on the assessed outcome in the offspring possibly leading to incorrect conclusions. Nevertheless, several laboratories report ordering their dams pregnant as this saves time and can facilitate cross-fostering to assure large litters.

Also within our literature search, we found some papers reporting ordering of pregnant dams. Within our review, the majority of the included research papers have reported in house breeding (breeding in house: 70.3%, ordering pregnant dams: 20.3%, not reported: 9.3%) and in particular, this aspect is mostly relevant for studies performed in rats, as ordering of pregnant mice happens only rarely (rats: 17.5%, mice: 1.5%).

Nevertheless, we saw that this was a modulating factor in the case of e.g. PRS effects on nsLearning and POS effects on anxiety-like behaviour, both when tested in adulthood. Indeed, when stratifying the experiments based on the origin of the dam, we found that PRS-exposure lead to increased memory impairment in females among studies where rodents were bred in house while there was no sex-difference in PRS-exposed offspring from dams that were ordered pregnant. Similarly, the increased male POS-induced vulnerability to anxiety-like behaviour was most pronounced in POS males that were born to dams that were ordered pregnant.

As this was impacting only a minority of the papers included in this review our overall conclusions concerning the effects of ELA remain reliable. Nevertheless, this is certainly an element to pay attention to and to avoid such cofounder, we would recommend to standardise this aspect and choose to only breed in house when interested in studying the effects of ELA.

Limitation of studies related to their experimental power

Low statistical power is an aspect that has been identified as a key problem across all neurosciences, and thus concerning ELA studies as well, as it reduces the chance of detecting a true effect and the probability that statistical significant results reflect true effects[327,328]. Concerning the human studies, a limitation in drawing overall conclusions when comparing studies based on different cohorts is that the size of the various human cohorts varies largely, therefore, within this review we separately reported on outcomes of the larger studies in human.

Concerning the rodent studies, in the current review, despite the lack of a systematic check of the statistical power for each study (group sizes were mostly between 5-15 rodents, with often multiple experimental groups) and considering the well acknowledged individual variation when testing rodent behaviour, is plausible to assume that, at least part of the reviewed research papers might be underpowered. In fact it has been highlighted that a large proportion of the currently existing studies are underpowered, challenging the reliability and reproducibility of animal research across labs and experiments[26,327,329].

With the increasing complexity of experimental designs, often including second hits and possible interventions, it remains difficult to increase power and perform studies with sufficient animals per experimental group. Therefore, Bonapersona and colleagues have recently suggested an alternative solution for the problem of statistical power in preclinical research, called RePAIR, which includes previously obtained information in order to reduce the required number of animals and increase statistical power[329]. While this solution is applicable to tests or behavioural paradigms that are frequently used, other solutions concerning experimental design and statistical analysis have been suggested as well in order to maximize power[328] and improving the reliability of experiments.

Chapter 7

Supplementary table S1. Included ELA's for human and rodent studies.

ELA= early-life adversity

A. ELA exposures (human)

PRS	Anxiety symptoms
	Depressive symptoms
	Stressful life events
	Perceived stress
	Job stress
	Parental cohabitation
	Exposure to flood
	Exposure to bereavement
	Exposure to ice storm
POS	Anxiety symptoms
	Depressive symptoms
	Stressful life events
	Post-traumatic stress disorder
PRS+POS	Anxiety symptoms
	Depressive symptoms
	Intimate partner violence

B. ELA models (rodent)

PRS	Restraint stress
	Unpredictable restraint stress
	Restraint and forced swim stress
	Chronic mild unpredictable/variable stress
	Noise and/or swim stress
	Predator exposure
	Elevated platform stress
	Social stress
	Social defeat with aggressive lactating rat
	Psychological stress
	Footshock stress
POS	Maternal separation (MS)
	MS + early weaning
	MS + social isolation
	MS + sham injection
	Unpredictable MS
	Maternal deprivation (MD)
	Limited bedding and nesting (LBN)
	LBN + substitute mother
	Neonatal isolation
	Feacal smell stress
	Forced swim stress
	Maternal immobilization stress

Supplementary table S2. Included readouts per domain for human and rodent studies

A.   Questionnaires and tasks used per domain for human studies

Domain	Outcome	Questionnaire or task
Cognition	Attention	Eye tracking attention disengagement paradigm
		Continuous performance task
		Reaction time task
	Executive functions / inhibitory control	The Hearts and Flowers task
		Flanker task
	IQ	Wechsler Abbreviated Scale of Intelligence
		Wechsler Preschool Primary Scale of Intelligence
	Verbal IQ	Peabody Picture Vocabulary Test-Revised
	School performance	General Certificate of Secondary Education
	Visuospatial memory	Sequential memory test
	Cognitive functions	Cambridge Neuropsychological Test Automated Battery
Externalizing behaviour & disorders	Total externalizing behaviour	Strength and difficulties questionnaire (SDQ)
		Child behaviour checklist (CBCL)
		Infant-Toddler Social Emotional Assessment
		Brief Infant-Toddler Social Emotional Assessment
		Caregiver-Teacher Report Form
		Youth Self Report
		Rutter Revised Preschool Scales
		The Preschool Behaviour Checklist
		The Temperament Assessment Battery
	ADHD & CD symptoms	First time ADHD medication/hospitalization
		Index of psychiatric problems
		Diagnostic Interview Schedule according to DSM-IV
		Child and Adolescent Psychiatric Assessment
		Preschool Age Psychiatric Assessment
		The Development and Well-Being Assessment (DAWBA)
	Aggresive behaviour	Peer play
	Criminality	Number of criminal offences

Internalizing behaviour & disorders	Total internalizing behaviour	Strength and difficulties questionnaire (SDQ)
		Child behaviour checklist (CBCL)
		Brief Infant-Toddler Social and Emotional Assessment
		Infant-Toddler Social Emotional Assessment
		Rutter Revised Preschool Scales
	Depression and anxiety symptoms	Dimensions of Depression Profile for Children and Adolescents, CES-D
		Depression Anxiety Stress Scale
		Kiddie Schedule for Affective Disorders and Schizophrenia
		Index of psychiatric symptoms in children
		Revised Clinical Interview Schedule
		Diagnostic Interview Schedule according to DSM-IV
		Spence Preschool Anxiety Scale
		Children's Depression Symptoms Inventory
		Mood and Feelings Questionnaire-Short Version (MFQ)
		Preschool Age Psychiatric Assessment
		Child and Adolescent Psychiatric Assessment (CAPA)
		The Development and Well-Being Assessment (DAWBA)

B.   Behavioural paradigms per domain for rodent studies

Domain	Behaviour paradigm
nsLearning	Object Location Task
	Object Recognition Task
	Barnes-maze
	Y-maze
	T-maze
	Water T-maze
	Attention-Set-Shifting-Task
sLearning	Fear conditioning
	Active avoidance
	Passive avoidance
	Morris Water Maze
	Emotional learning

Social behaviour	Social interaction
	Social investigation/approach
	Social preference
	Social play/fighting
	Social recognition/memory
	Competitive behaviour
	Aggression
Anxiety-like behaviour	Open Field Test
	Elevated Plus Maze
	Plus Maze
	Elevated Zero Maze
	Dark-light box
	Novelty-suppressed feeding/drinking
	Thigmotaxis
	Novelty seeking
	Continuous video monitoring
	Acoustic startle response test
	Hole-board
	Emergence test
	Free exploratory paradigm
	Defensive burying
Depressive-like behaviour	Forced Swimming Test
	Swim Escape Test
	Open Space Swimming Test
	Tail Suspension Test
	Learned Helplessness
	Sucrose Preference Test
	Sucrose consumption
	Sucrose Negative Contrast Test

Chapter 7

Supplementary table S3. Rodent experiments assessing behaviour of ELA exposed animals in response to a secondary stressor, per domain, timing of ELA, outcome age and sex. ELA= early-life adversity

Domain	Timing ELA	Outcome age	Species	Total experiments	Increased, compared to control + secondary stress		Decreased, compared to control + secondary stress		No effect in either sex, compared to control + secondary stress
					M	F	M	F	
nsLearning	PRS	Adolescence	mice	3	0	0	0	0	3 [78]
	POS	Adulthood	rats	4	0	1 [105]	1 [108]	0	2 [105,108]
sLearning	POS	Adulthood	rats	3	0	0	0	0	3 [141,226]
Anxiety-like behaviour	PRS	Adulthood	rats+mice	2	1 [209]	1 [209]	0	0	1 [169]
	POS	Adolescence	rats+mice	3	0	1 [189]	0	0	2 [105,189]
	POS	Adulthood		13	5 [177,189,190,202,225]	1 [190]	0	2 [177,225]	8 [108,141,189,198,226]
Depressive-like behaviour	PRS	Adulthood	rats	2	0	0	0	0	2 [169]
	POS	Adolescence	mice	1	0	0	0	0	1 [189]
	POS	Adulthood	rats+mice	8	0	4 [108,190,226]	2 [190]	0	4 [177,189,226]