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# Improving translational relevance: The need for combined exposure models for studying prenatal adversity

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ABSTRACT

Prenatal environmental adversity is a risk factor for neurodevelopmental disorders (NDDs), with the neuroimmune environment proposed to play a role in this risk. Adverse maternal exposures are associated with cognitive consequences in the offspring that are characteristics of NDDs and simultaneous neuroimmune changes that may underlie NDD risk. In both animal models and human studies the association between prenatal environmental exposure and NDD risk has been shown to be complex. Maternal overnutrition/obesity and opioid use are two different examples of complex exposure epidemics, each with their own unique comorbidities. This review will examine maternal obesity and maternal opioid use separately, illustrating the pervasive comorbidities with each exposure to argue a need for animal models of compound prenatal exposures. Many of these comorbidities can impact neuroimmune function, warranting systematic investigation of combined exposures to begin to understand this complexity. While traditional approaches in animal models have focused on modeling a single prenatal exposure or second exposure later in life, a translational approach would begin to incorporate the most prevalent co-occurring prenatal exposures. Long term follow-up in humans is extremely challenging, so animal models can provide timely insight into neurodevelopmental consequences of complex prenatal exposures. Animal models that represent this translational context of comorbid exposures behind maternal obesity or comorbid exposures behind maternal opioid use may reveal potential synergistic neuroimmune interactions that contribute to cognitive consequences and NDD risk. Finally, translational co-exposure models can identify concerning exposure combinations to guide treatment in complex cases, and identify high risk children starting in the prenatal period where early interventions improve prognosis.

## 1. Introduction: the prenatal period as a window of vulnerability and opportunity

The prenatal period is a critical neurodevelopmental window, with maternal environmental experiences shaping offspring brain development and subsequent behaviors (Bale et al., 2010). Maternal experiences affect the developing fetus and can program a risk for neurodevelopmental disorders (NDDs), with perturbations in the maternal-fetal environment playing a well-recognized role in this risk (Allswede et al., 2020). While the prenatal period represents a window of vulnerability, it is also a window of opportunity. Concerted efforts in research and healthcare are continually optimizing prenatal care to promote maternal and infant health. The frequency of routine prenatal healthcare visits gives providers unique opportunities to identify and treat concerns in the United States. Over 75% of women in the U.S. receive appropriate prenatal care (Osterman and Martin, 2018). Prenatal care varies widely based on factors like maternal age, race, education, or geographic location but approximately 50% of U.S. women in groups with low rates of reported care still receive it. (Osterman and Martin, 2018).

While many barriers and challenges exist in prenatal care, pregnant women are often a motivated patient population (Economidoy et al., 2012) and preventative measures taken during pregnancy can directly reduce the number of risk factors a child will face. Once the child is born, follow-up for high risk infants is imperative to identify neurodevelopmental concerns and intervene prior to the manifestation of behavioral health disorders. In contrast to pregnancy, longitudinal childhood assessments are challenging because they require substantial resources and lack standardization (Doyle et al., 2014). This emphasizes the need to identify predictive factors in the prenatal and early postnatal period that categorize a child as high-risk, which can include efforts to reach mothers at risk for delivering without prenatal care and in countries where access to prenatal care is limited.

Based on the literature, maternal environmental risk factors that

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warrant childhood neurobehavioral follow-up include maternal malnutrition, obesity, substance abuse, infection, and stress (Bale et al., 2010; Bölte et al., 2019; Debost et al., 2017; Smith and Reyes, 2017a). Since early interventions are key to improving child developmental outcomes (Tough et al., 2010), understanding maternal risk factors can guide follow-up strategies to ensure high risk children receive appropriate frequency and depth of care. This ideally should include prenatal history. Understanding prenatal environment is vital because maternal risks are associated with an increased likelihood of neonatal complications that significantly exacerbate NDD risk such as preterm birth, low birth weight, and being small for gestational age (Lee and Lin, 2010; Tedesco et al., 2020; Copper et al., 1996; Baer et al., 2019; Forray, 2016; Hofheimer et al., 2020; Indredavik et al., 2010; Arpino et al., 2010). Then as the number of risk factors increase, there is a compounding risk for adverse consequences for the child (Doyle et al., 2014). This makes it imperative to identify, track, and intervene starting in the early prenatal period to understand combined environmental exposures that likely produce a direct increase in the risk for NDDs. While many single prenatal exposures have been identified as increasing risk, combinations of prenatal exposures further escalate risk of NDDs (Kong et al., 2020) and may identify high risk children.

## 2. Neuroimmune consequences of complex prenatal adversity

The immune system is the integrated response unit for addressing the full complexity of environmental challenges (Bennett et al., 2018). The immune system responds to many stimuli that represent potential danger, which can range from infection, trauma, physical stress, deviations in tissue homeostasis, and even psychological stress. In the brain, microglia are the resident immune cells that respond to changes in the environment via multiple pathways, one being via intrinsic activation of toll-like receptors (TLRs) (Bennett et al., 2018; Bachtell et al., 2015). Microglial activation by the environment provides a route for these stimuli to alter brain function and synaptic development, due to the role of microglia in synaptic pruning (Zhan et al., 2014; Squarzoni et al., 2014; Schafer et al., 2012; Frost and Schafer, 2016; VanRyzin et al., 2019; McCarthy et al., 2015). Mounting an immune response is required to effectively adapt, overcome, and protect the organism from environmental challenges. However, compounding environmental risk factors can cause immune activation that exceeds the adaptive range in magnitude and/or time, leading to the development of chronic disease (Bennett et al., 2018).

Environmental adversity in early life is thought to have an embedding effect on immune cells, increasing their responsivity to subsequent stimuli and reducing their ability to resolve inflammation, shifting towards an overall pro-inflammatory state (Miller et al., 2011). Common outcomes of early life adversity, such as elevated stress reactivity and poor lifestyle habits (Miller et al., 2011), can increase the risk of encountering further adversity over the lifespan. This can result in a state of non-resolving inflammation, especially with an increased load of environmental adversity encountered during critical neurodevelopmental periods (Nathan and Ding, 2010). While immune responses are adaptive, this non-resolving inflammation associated with multiple adversities is likely a key contributor to NDDs (Han et al., 2021), however relatively little is understood about combinations of exposures exclusively in the prenatal period.

Prenatally, fetal exposure to a proinflammatory environment via maternal adversity provides a proposed means for altered neuroimmune and behavioral function to increase NDD risk (Smith and Reyes, 2017a). Elevations in markers of maternal inflammation are consistently associated with offspring inflammation, behavioral impairments, and NDDs (Allswede et al., 2020; Rudolph et al., 2018; Lee et al., 2015). This association is evident in the epidemiology of NDDs and has a strong mechanistic premise via the known role of microglia in brain development (Zhan et al., 2014; Squarzoni et al., 2014; Schafer et al., 2012; Frost and Schafer, 2016; VanRyzin et al., 2019; McCarthy et al., 2015).

Maternal inflammation by adverse environmental stimuli has manifold ways to reach the developing fetus. Peripheral maternal cytokine elevations propagate to the placenta and mammary glands, and can result in increases in offspring peripheral and central cytokines (Crew et al., 2016; Reynolds et al., 2015; Frias et al., 2011; Hernandez et al., 2012; Bilbo and Tsang, 2010; Grissom et al., 2016; Sanders et al., 2014; Kang et al., 2014). With numerous routes for inflammation to travel from mother to fetus, a greater burden of prenatal exposures may result in a graded immune response and subsequently amplified impact on offspring cognitive development. A single prenatal exposure may produce a mild immune response that resolves quickly while combined prenatal exposures may produce higher grade immune responses that lead to chronic cytokine elevations (Miller et al., 2011). Single prenatal exposures that may be subthreshold for eliciting an immune response may have the capacity to produce a more severe response when combined with other subthreshold exposures. For example, combined prenatal diesel exhaust particle exposure and prenatal stress elicits an increase in male offspring TLR4 expression in the brain, while either stimulus alone is insufficient to cause such a change (Bolton et al., 2013). Additionally, maternal high fat diet produces minimal changes in basal offspring cytokine or chemokine gene expression (Smith et al., 2020), unless offspring are challenged in adulthood with LPS or a stressor (Grissom et al., 2017). Finally, individuals that may have appropriately graded and timed immune responses to a single exposure could have excessive or chronic responses to combined exposures.

## 3. Translational value of animal models for complex prenatal exposures

Animal models are essential for advancing research on prenatal programming with a single risk factor, and even more so with compounding exposures. To control for and begin to understand these complex exposures, animal models offer control over prenatal variables to an extent not possible with human studies. The short life span of rodents accelerates the pace for studying long term neurobehavioral consequences, which is necessary to gain information fast enough to develop meaningful interventions. Then animal models provide access to fetal and neonatal samples, and realistically the only access to brain samples from this population.

This accentuates the need for continuous evaluation of existing prenatal exposure models, development of new models, and a deep understanding of the translational context or background behind each prenatal exposure. Simplifying a model to a single exposure is required to build a foundational understanding, but to successfully translate findings and improve child health, models need to be relevant to the human population that they are intended to study. This means that at some point, models must directly confront the complexity encountered in humans in a systematic fashion. Furthermore, single exposures may not be sufficient to cause NDDs but combined exposures can act synergistically to confer vulnerability (Debost et al., 2017).

Animal models have demonstrated compounding effects of multiple environmental exposures in altering neuroimmune and behavioral outcomes in the offspring. However, most of these are sequential in nature, and focus on the effects of a single prenatal exposure followed by a second exposure in adolescence or adulthood. These two-hit models demonstrate that a single prenatal exposure alone may not reveal behavioral deficits or could even be advantageous, while a second exposure later in life can result in impairment (Giovanoli et al., 2013, 2016; Bilbo et al., 2005; Makinson et al., 2019; Zhao et al., 2021). With the sequential two-hit approach in mice, maternal immune activation acts synergistically with pubertal stress to reveal behavioral impairments and an increase in hippocampal microglia (Giovanoli et al., 2013, 2016). Additionally, recent evidence shows that maternal immune activation improved performance on a visual discrimination task, although exposed offspring had impaired discrimination and reversal learning if they were challenged with a stressor in adulthood (Zhao et al., 2021). Models less

frequently address combined exposures during the prenatal period but the few that do suggest significant interactions. For example, prenatal stress and diesel exhaust particle exposure in mice acted synergistically to increase neuroinflammatory tone in the offspring brain and impair cognitive function, particularly in male offspring (Bolton et al., 2013). A review on animal models of combined prenatal exposures suggests mild synergistic effects on impairing fetal growth, but they are sparse and subject to bias based on blinding, concealment, and incomplete outcome reporting (Vesterinen et al., 2017). Furthermore, this was only related to fetal growth while cognitive outcomes remain understudied.

Clinical literature is focused on understanding synergistic exposures because humans almost always experience multiple exposures (Debost et al., 2017; Siddika et al., 2019; Clarke et al., 2009; Elliott et al., 2020). Animal models can provide systematic investigation of each exposure alone and in combination, starting with the most prevalent comorbidity and using $2 \times 2$ experimental designs to identify synergistic effects. Then as long-term behavioral follow-up to identify neurodevelopmental issues is challenging in humans, animal models can give an insight on where to focus resources and identify exposure combinations that are the most concerning. This would enable clinicians to leverage the existing prenatal healthcare structure to track exposures prior to birth or shortly after. Understanding common co-occurring exposures may reveal novel interactions, which could relate to a graded neuroimmune response as the number of exposures increase. Then from a clinical perspective, it can help identify and track children who are high risk. Using the translational context behind prevalent prenatal environmental exposures to guide animal models may help identify causative factors or combinations of factors that contribute to NDD risk. As an example, maternal obesity/overnutrition and maternal opioid use are current epidemics that each have a unique constellation of co-occurring exposures. This review highlights these comorbidities, to guide future work on understanding how they may interact.

## 4. Maternal overnutrition/obesity

### 4.1. Behavioral and neuroimmune consequences of maternal obesity/overnutrition

Approximately 20–25% of women are obese at the onset of pregnancy, with 50% being classified as at least overweight (Chen et al., 2018; Branum et al., 2014). Maternal obesity is consistently problematic in the U.S. but has been sharply increasing in other countries, making it a global issue (Chen et al., 2018). Increased maternal body weight is associated with a greater risk of the child developing schizophrenia, autism spectrum disorders (ASD), and attention deficit/hyperactivity disorder (ADHD) (Schaefer et al., 2000; Buss et al., 2012). Maternal overnutrition plays a causal role in maternal overweight status and obesity. The risk for offspring NDDs is associated with maternal nutritional status, even after adjusting for prevalent risk factors like maternal age or nicotine use (Schaefer et al., 2000; Rodriguez et al., 2008). Maternal overnutrition and obesity are associated with impairments in offspring cognitive function, which may explain the risk for NDDs (Smith et al., 2020; Smith and Reyes, 2017b; Grissom et al., 2015; Veena et al., 2016; Iessa and Bérard, 2015).

In animal models, maternal obesogenic HF diets increase cytokine levels at the maternal-fetal interface, with elevated cytokines peripherally in the dam, placenta, and mammary glands (Crew et al., 2016; Reynolds et al., 2015; Frias et al., 2011; Hernandez et al., 2012; Edlow et al., 2019). Consequently, HF offspring display peripheral and central cytokine elevations and reactivity (Bilbo and Tsang, 2010; Grissom et al., 2016; Sanders et al., 2014; Kang et al., 2014), along with evidence of increased microglial activation in the brain (Bilbo and Tsang, 2010; Kang et al., 2014). Increases in inflammatory cytokines and chemokines in HF offspring coincide with cognitive deficits (Graf et al., 2017; McKee et al., 2017) and ablation of microglia during adolescent neurodevelopment can reduce behavioral impulsivity in HF male progeny (Smith et al., 2020) (seeFig. 1).

### 4.2. Translational context of maternal obesity

Since neuroimmune interactions are hypothesized mechanisms for offspring cognitive deficits after maternal overnutrition/obesity, comorbid exposures may lead to non-resolving inflammation and an exacerbated risk for NDDs. Maternal obesity prior to and during gestation is associated with a constellation of health problems and pregnancy complications, many of which are characterized by chronic immune activation. Maternal obesity increases the risk of heightened gestational weight gain, type II diabetes, gestational diabetes, heart disease, hypertension, preeclampsia (Leddy et al., 2008), and even increases the severity of infections (Karlsson et al., 2012; McCartney et al., 2020) (Fig. 2). Children with NDDs are more likely to have a mother that had a

**Fig. 1. Brittany L. Smith:** My research goal is to understand how adverse prenatal environments influence neuroimmune and executive function, and how this may be associated with neurodevelopmental disorders. I have a specific interest in prenatal substance exposure and am actively examining the effects of prenatal opioid exposure. I am working to build a translational research program to understand the molecular basis of prenatal programming after opioid exposure, search for biomarkers to identify children who are high risk, and develop interventions. I completed my dissertation under the mentorship of Dr. James Herman at the University of Cincinnati. While studying chronic stress exposure, I found that microglia and the neuroimmune environment within the prefrontal cortex respond to chronic stress in a manner that depends on stressor modality. Then for my dissertation work, I developed a novel and ethologically relevant stress paradigm based on loss of an enriched environment. As a postdoctoral fellow, I am working under the mentorship of Dr. Teresa Reyes at the University of Cincinnati. I am studying how prenatal experiences affect neuroimmune and executive function. I began my postdoctoral fellowship by studying how maternal high fat diet in mice programs offspring behavioral function, with a focus on the role of microglia in behavioral impulsivity. I went on to obtain K99/R00 funding from NIDA to examine how prenatal opioid exposure affects offspring brain development. I am using rodent models to study offspring behavioral function in domains frequently impaired in opioid exposed children, along with identifying molecular changes in the brain that may contribute to deficits. In humans, I am working to understand the epidemiological context and epigenetic consequences of prenatal opioid exposure.

metabolic condition during pregnancy (Krakowiak et al., 2012), so it is important to understand NDD risk in the context of obesity-related comorbidities. Combinations of these maternal comorbidities increase the risk of offspring NDDs beyond the magnitude seen with a single exposure, such as maternal obesity and diabetes together exacerbating autism risk and more subtly ADHD risk (Kong et al., 2020).

NDD risk after maternal obesity may be exacerbated by comorbid maternal inflammatory conditions. For example, preeclampsia is described as an immune imbalance that leads to persistently elevated immune activation (Cornelius, 2018). Prenatal preeclampsia exposure impairs offspring cognitive function and increases the risk and severity of NDDs such as ASD, ADHD, and schizophrenia (Gumusoglu et al., 2020). Similarly, maternal diabetes can increase inflammation and lead to an increased risk of NDDs in the offspring (Krakowiak et al., 2012; Van Lieshout and Voruganti, 2008; Zhao et al., 2019; Xu et al., 2014). With maternal obesity, comorbid conditions such as hypertension and diabetes exacerbate the risk of developmental delays or ASD in the child (Kong et al., 2020; Krakowiak et al., 2012).

Furthermore, maternal obesity increases the severity of viral infections like influenza and COVID-19 in pregnant women (Karlsson et al., 2012; McCartney et al., 2020), and increases the incidence of maternal infection-related asthma complications (Murphy et al., 2014). Maternal infection is associated with offspring NDDs, particularly with additional second hit exposures (Debost et al., 2017; Giovanoli et al., 2013). Obesity in pregnancy also increases the risk of offspring infections both in early life and long-term (Gutvirtz et al., 2019; Gutierrez et al., 2021; Griffiths et al., 2016), which could predispose these offspring to a second-hit immune challenge.

In rodents, HF diet exposure to induce maternal obesity can impair glucose tolerance and increase blood pressure (Ge et al., 2013; Yokomizo et al., 2014), although these endpoints are not routinely studied in the context of offspring behavior. Maternal overnutrition, obesity, diabetes, and hypertension each are associated with NDDs in humans, but the impact of combined exposures is lacking (Han et al., 2021). In an attempt to parse out effects in animal models, maternal diabetes can be studied in the absence of maternal obesity. Maternal diabetes impaired offspring neurodevelopment in the early postnatal period and reduced object recognition in females (Sousa et al., 2020). Interestingly, maternal diabetes did not affect offspring microglial morphology but maternal insulin treatment for diabetes did increase microglial number and processes (Sousa et al., 2020). In contrast, maternal dietary intervention reduces offspring microglial activation in response to maternal HF diet (Kang et al., 2014). Treatment strategies for maternal obesity and related comorbidities may impact offspring neuroimmune outcomes.

Collectively, maternal adversity such as obesity increases inflammation and is associated with an increased risk of offspring NDDs, but that association is variable (Meyer, 2019). Obesity-related comorbidities may confer NDD vulnerability by increasing the severity of inflammation at the maternal-fetal interface. While many factors during the prenatal and postnatal periods can increase or decrease susceptibility (Meyer, 2019), the challenge of postnatal long term follow-up emphasizes the need to understand factors during the prenatal period. Studying the impact of maternal obesity together with obesity-related conditions that increase disease severity will enable clinicians who treat pregnant women with complex experiences to take into account the long term cognitive health of the child, especially if maternal treatments can influence offspring outcomes.

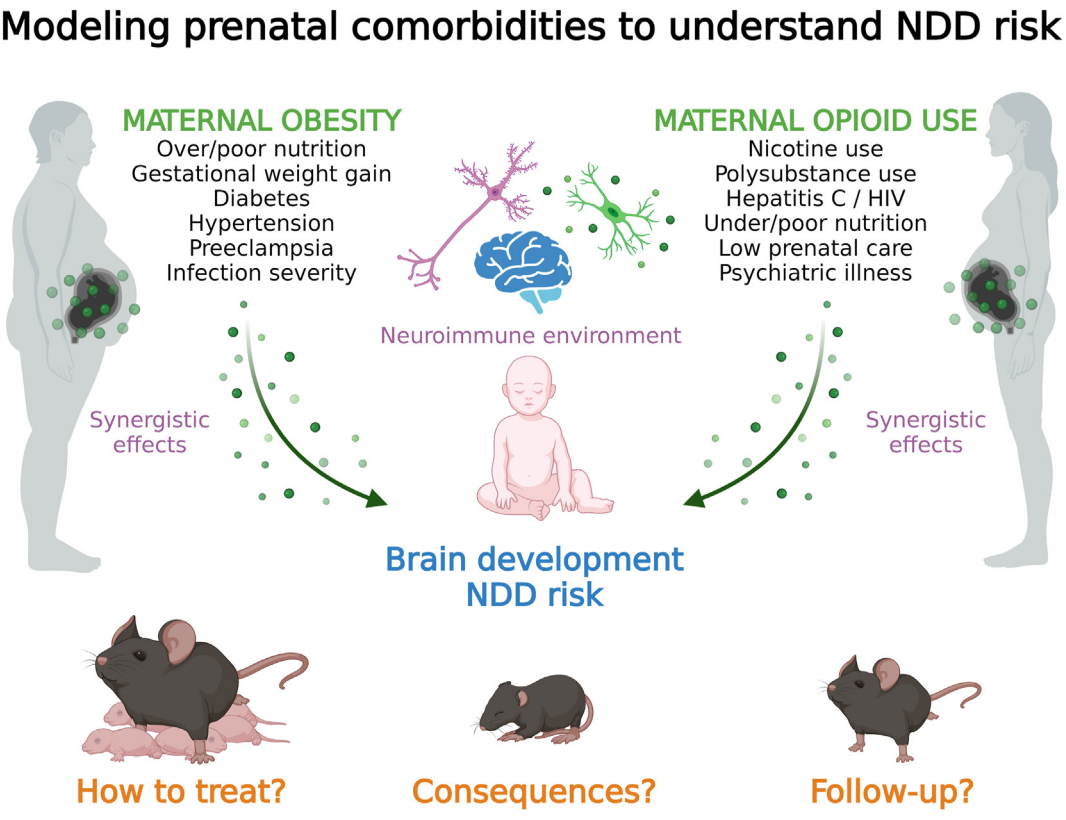


**Fig. 2. Modeling prenatal comorbidities to understand NDD risk:** Maternal obesity and maternal opioid use have distinct comorbidities that have the capacity to increase neuroinflammatory signaling in the offspring and result in behavioral impairments to increase neurodevelopmental disorder (NDD) risk. Multiple prenatal comorbidities are present in the human population, but exposure combinations are seldom assessed in animal models. Due to the ability of the immune system to have a graded response to increasing stimuli severity and the multiple routes for inflammatory cytokines to reach the developing fetus, combined prenatal exposures may act synergistically. Understanding these possible synergistic interactions in rodent models will guide how to treat mothers with complex comorbidities, identify offspring consequences to facilitate interventions, and improve long term follow-up by identifying high risk children. (Created with BioRender.com).

## 5. Maternal opioid use

### 5.1. Behavioral and neuroimmune consequences of maternal opioid use

Opioid use during pregnancy has increased by over 130% in the past decade, contributing to an average 80% increase in neonatal opioid withdrawal syndrome (NOWS) across the U.S. (Tolia et al., 2015; Desai et al., 2014; Pan and Yi, 2013; Hirai et al., 2021). Opioid drugs readily cross the placenta and the blood brain barrier to reach the fetal brain (Gabrielsson and Paalzow, 1983; Farid et al., 2008), where they have been shown to affect neurodevelopment. Children born to opioid-using mothers have cognitive problems related to NDDs, including hyperactivity, inattention, impulsivity, poor planning, lowered adaptability, and impaired working memory (Hickey et al., 1995; Nygaard et al., 2016; Ornoy et al., 1996; Slinning, 2004; Levine and Woodward, 2018; Wahlsten and Sarman, 2013). These problems persist into adolescence and early adulthood and are present even if the mother was on methadone or buprenorphine treatments for opioid use disorder (Levine and Woodward, 2018; Wahlsten and Sarman, 2013; Nygaard et al., 2017). Recently, maternal opioid use has also been associated with an elevated risk of ADHD and ASD symptoms and diagnoses in the child (Azuine et al., 2019; Rubenstein et al., 2019; Haugland et al., 2018).

In rodent models, offspring exposed prenatally to opioids displayed learning and memory deficits in basic cognitive tasks (Šlamberová et al., 2001; Wang and Han, 2009; Lu et al., 2012; Zagon et al., 1979). Recently, Jantzie et al, 2020 found that prenatal methadone exposure impaired executive function and cognitive flexibility in the visual discrimination reversal task (Jantzie et al., 2020). In conjunction with behavioral deficits, methadone increased peripheral inflammation maternally and in the offspring (Jantzie et al., 2020; Newville et al., 2020). Notably, methadone exposed offspring had increased morphological activation of microglia, increased cortical expression of TLR4 and TLR4 adapter protein Myd88, along with increased protein levels of cytokine IL-1b and chemokine Cxcl1 (Jantzie et al., 2020). Furthermore, peripheral blood mononuclear cells of offspring exposed prenatally to methadone had increased proinflammatory activity, both at baseline and after stimulation (Newville et al., 2020).

The proinflammatory effect of prenatal opioid exposure on the offspring brain may seem paradoxical given that opioids are considered immunosuppressive (Eisenstein, 2019; Roy et al., 2011). However, this evidence has focused largely on peripheral immune cells while in microglia, morphine has been shown to induce proinflammatory signaling (Merighi et al., 2013; Wang et al., 2012). Morphine (the immediate metabolite of heroin), along with a wide range of opioids including methadone, buprenorphine, oxycodone, and fentanyl can activate TLR4 in the CNS environment (Wang et al., 2012; Hutchinson et al., 2010). Given the role of microglia in brain development, microglial activation may provide a direct route for maternal opioid use to interfere with offspring neurodevelopment and subsequent behavioral function.

### 5.2. Translational context of maternal opioid use

Based on rodent models, maternal opioid use may induce a neuroinflammatory state in the offspring that is associated with impaired cognitive function. In addition to the neuroinflammatory potential of opioids, it is critical to note that in humans, the maternal opioid using population has high rates of other exposures that could potentially act synergistically to increase neuroinflammation, impair cognitive function, and increase NDD risk (Fig. 2).

Pregnant women who use opioids are more likely to use other substances and combinations of multiple substances (Smith et al.). Polysubstance use of nicotine, cocaine, methamphetamine, and benzodiazepines are disproportionately higher in pregnant women who test positive for opioids (Smith et al.). Cocaine and other drugs can activate TLR4 (Northcutt et al., 2015), so it is possible that polysubstance use could heighten TLR4 activation of microglia and further increase NDD risk. Polysubstance use in the context of opioid use is particularly concerning due to the synergistic effects on addictive properties, overdose risk, and increased severity of NOWS (Compton et al., 2021; Choo et al., 2004; Desai et al., 2015; Jansson et al., 2012, 2017; Sanlorenzo et al., 2019; Kaltenbach et al., 2012). NOWS treatment is notoriously challenging for infants exposed prenatally to multiple substances, who frequently require longer hospital lengths of stay (Choo et al., 2004; Jansson et al., 2012; Sanlorenzo et al., 2019; Kaltenbach et al., 2012). However, long-term neuroimmune status and cognitive function in polydrug exposed infants remains unknown.

Opioid use during pregnancy is highly comorbid with nicotine use to the extent that co-treatment for dual use of these two substances has been recommended as standard of care (Morris and Garver-Apgar, 2020). Up to 95% of opioid-using mothers also use nicotine, especially women taking methadone or buprenorphine, medications that are used to treat opioid use disorder (Smith et al.; Chisolm et al., 2013). In humans, prenatal nicotine exposure was associated with offspring cognitive and behavioral problems (Daseking et al., 2015; Tiesler and Heinrich, 2014), along with an increased risk for ADHD (Wang, 2018; Ross et al., 2015) and schizophrenia (Niemelä et al., 2016). In rodents, offspring exposed prenatally to nicotine had executive function deficits (Bryden et al., 2016; Schneider et al., 2011), increased peripheral cytokines as newborns (Mohsenzadeh et al., 2014), and increased brain cytokine levels as adults (Chan et al., 2016). Furthermore, prenatal nicotine exposure has been shown to be an important mediating variable for the cognitive deficits reported in children exposed prenatally to opioids (Nelson et al., 2020). This evidence supports the idea that inclusion of prenatal nicotine exposure in preclinical rodent models of maternal opioid use would greatly enhance the translational validity of these studies.

Beyond polysubstance use, pregnant women who use opioids are at a greater risk for a range of infections. Intravenous drug use is a primary causal route because it increases the chance of exposure, particularly to HIV or hepatitis C. Additionally, opioid use is associated with immunosuppression that impairs host defense, leading to increased incidence and severity of opportunistic infections such as pneumonia and influenza, in addition to HIV and hepatitis C (Roy et al., 2011). Hepatitis C exposure is particularly high in the context of opioid use, present in over 40% of pregnant women who use opioids (Fajemirokun-Odudeyi et al., 2006). The neurodevelopmental consequences of prenatal or perinatal hepatitis C exposure are unknown, let alone in combination with prenatal opioid exposure. While prenatal exposure to opioids, hepatitis C, or nicotine have not been shown to cause overt structural damage to the developing brain, white matter lesions have been recently reported in human infants exposed prenatally to all three of these stimuli (Merhar et al., 2019). In rodents, Iba-1 expression was increased after exposure to a combination of nicotine, morphine, and HIV but not with any of these stimuli alone (Cornwell et al., 2020). This emphasizes the importance of leveraging animal models to understand how these exposures interact in the prenatal period.

HIV has been surging in the context of opioid use disorder where treatment strategies are working to combine medication distribution for opioid use disorder with antiretroviral therapy (Bach et al., 2015; Tofighi et al., 2019). The consequences of prenatal exposure to opioids and HIV are also unknown, but children exposed perinatally to HIV experienced developmental delays and had impaired neurobehavioral development as toddlers (Wu et al., 2018; Wedderburn et al., 2019). At the neuroimmune level, morphine acts synergistically in the context of HIV and pneumonia co-infection to enhance cytokine synthesis and TLR activation in microglia (Dutta et al., 2012). However, this interaction is unknown after prenatal co-exposure. There is a need to understand the neurodevelopmental consequences of prenatal opioid exposure combined with comorbid maternal infections, because the little that is known suggests these exposures may act synergistically.

Although obesity has been associated with increased prescription opioid use in the general population (Stokes et al., 2020), this association is not evident in pregnant women (Whiteman et al., 2014). Conversely,

pregnant women with opioid use disorders are more likely to be underweight (Nagarajan and Goodman, 2020). Poor nutrition is pervasive in drug using populations and some adverse effects of prenatal drug exposure are possibly attributed to malnutrition (Tonkiss et al., 1996; Wright and Walker, 2007). Undernutrition and nutritional deficiencies are just beginning to be recognized and addressed in pregnant women with opioid use disorders (Nagarajan and Goodman, 2020). Nutritional deficiencies are common across undernutrition and overnutrition, as both result in a lack of key nutrients either via low food access or excess consumption of nutritionally poor quality foods. Consequently, maternal undernutrition is also associated with offspring NDDs with a likely contribution of the neuroimmune environment (Smith and Reyes, 2017b).

While prenatal care is largely accessible across the United States, pregnant substance using women are more likely to deliver without prenatal care (Maupin et al., 2004). Substance using women frequently have significant social, psychological, economic, and legal barriers that prevent them from getting adequate care during pregnancy (Stone, 2015). Understanding common co-exposures in this population may lead to improved evidence-based treatment strategies that help dissolve barriers and stigma, along with efforts to increase global access to prenatal care. Finally, the prevalence of psychiatric illness is extremely high in the maternal opioid using population, an important comorbidity that warrants its own review (Noose Glovak et al., 2020; Arnaudo et al., 2017).

## 6. Conclusion: understanding prenatal complexity to predict risk versus resiliency

Clinically, comorbid maternal exposures make it difficult to definitively test associations between a single exposure and the risk for offspring NDDs. These comorbidities are especially prevalent in the context of maternal obesity and maternal opioid use, and a number of them are associated with neuroimmune and behavioral consequences. Animal models can eliminate confounds to help disentangle whether a single exposure alone contributes to NDD risk.

Comorbid exposures may act synergistically to exacerbate the risk for complications, possibly via a graded neuroimmune response to an increased number of stimuli. However, these synergistic outcomes have yet to be systematically assessed in relation to cognitive function and NDD risk. Complex co-occurring exposures are associated with an increased risk of neonatal complications, making mothers and babies difficult to treat from early on. Clinical practice lacks a preclinical understanding of co-exposure contributions to long term neurodevelopment, which is particularly problematic due to the challenges of long-term follow up.

Animal models have the capacity to address these complex problems and ideally should capture some of the complexity seen in the human populations that they are intended to study. Studying the most concerning exposures and exposure combinations can establish a means for predicting risk versus resilience starting in the prenatal period. In complex cases, this would help allocate long term resources to the most high risk children. Simultaneously, it would use the existing U.S. prenatal healthcare system or encourage increased access to prenatal care worldwide to adjust prenatal and early postnatal interventions to encourage resiliency.

Interestingly, there are some children exposed to extremely complex prenatal adversities that thrive against all odds. Neuroimmune and behavioral responses to adverse prenatal environments alternatively could serve a protective role as necessary adaptations to these early life stimuli. It is important to assess the context and duration of these responses to help determine their valence, in conjunction with continued work on the role of microglia in shaping the development of higher level cognitive processes. As the field progresses, the translational context of prenatal exposures will promote the clinical relevance of preclinical models.

## Declaration of competing interest

None.

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## References

Allswede, D.M., Yolken, R.H., Buka, S.L., Cannon, T.D., 2020. Cytokine concentrations throughout pregnancy and risk for psychosis in adult offspring: a longitudinal case-control study. The Lancet Psychiatry 7, 254–261.
Arnaudo, C.L., Andraka-Christou, B., Allgood, K., 2017. Psychiatric Co-morbidities in pregnant women with opioid use disorders: prevalence, impact, and implications for treatment. Curr. Addict. Reports 4, 1–13.
Arpino, C., et al., 2010. Preterm birth and neurodevelopmental outcome: a review. Child's Nerv. Syst. 26, 1139–1149.
Azuine, R.E., et al., 2019. Prenatal risk factors and perinatal and postnatal outcomes associated with maternal opioid exposure in an urban, low-income, multiethnic US population. JAMA Netw. Open 2, 1–14.
Bach, P., et al., 2015. Association of patterns of methadone use with antiretroviral therapy discontinuation: a prospective cohort study. BMC Infect. Dis. 15, 1–7.
Bachtell, R., et al., 2015. Targeting the toll of drug abuse: the translational potential of toll-like receptor 4. CNS Neurol. Disord. - Drug Targets 14, 692–699.
Baer, R.J., et al., 2019. Risk of preterm and early term birth by maternal drug use. J. Perinatol. 39, 286–294.
Bale, T.L., et al., 2010. Early life programming and neurodevelopmental disorders. Biol. Psychiatr. 68, 314–319.
Bennett, J.M., Reeves, G., Billman, G.E., Sturmberg, J.P., 2018. Inflammation-nature's way to efficiently respond to all types of challenges: implications for understanding and managing 'the epidemic' of chronic diseases. Front. Med. 5, 1–30.
Bilbo, S., Tsang, V., 2010. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. Faseb. J. 24, 2104–2115.
Bilbo, S.D., et al., 2005. Neonatal infection induces memory impairments following an immune challenge in adulthood. Behav. Neurosci. 119, 293–301.
Bölte, S., Girdler, S., Marschik, P.B., 2019. The contribution of environmental exposure to the etiology of autism spectrum disorder. Cell. Mol. Life Sci. 76, 1275–1297.
Bolton, J.L., et al., 2013. Maternal stress and effects of prenatal air pollution on offspring mental health outcomes in mice. Environ. Health Perspect. 121, 1075–1082.
Branum, A.M., Kirmeyer, S.E., Gregory, E.C.W., 2014. Prepregnancy body mass index by maternal characteristics and state: data from the birth certificate. Natl. Vital Stat. Rep. 65, 1–11, 2016.
Bryden, D.W., et al., 2016. Prenatal nicotine exposure impairs executive control signals in medial prefrontal cortex. Neuropsychopharmacology 41, 716–725.
Buss, C., et al., 2012. Impaired executive function mediates the association between maternal pre-pregnancy body mass index and child ADHD symptoms. PloS One 7.
Chan, Y.L., et al., 2016. Impact of maternal cigarette smoke exposure on brain inflammation and oxidative stress in male mice offspring. Sci. Rep. 6, 1–12.
Chen, C., Xu, X., Yan, Y., 2018. Estimated global overweight and obesity burden in pregnant women based on panel data model. PloS One 13, 1–14.
Chisolm, M.S., et al., 2013. A comparison of cigarette smoking profiles in opioid-dependent pregnant patients receiving methadone or buprenorphine. Nicotine Tob. Res. 15, 1297–1304.
Choo, R.E., Huestis, M.A., Schroeder, J.R., Shin, A.S., Jones, H.E., 2004. Neonatal abstinence syndrome in methadone-exposed infants is altered by level of prenatal tobacco exposure. Drug Alcohol Depend. 75, 253–260.
Clarke, M.C., Tanskanen, A., Huttunen, M., Whittaker, J.C., Cannon, M., 2009. Evidence for an interaction between familial liability and prenatal exposure to infection in the causation of schizophrenia. Am. J. Psychiatr. 166, 1025–1030.
Compton, W.M., Valentino, R.J., DuPont, R.L., 2021. Polysubstance use in the U.S. opioid crisis. Mol. Psychiatr. 26, 41–50.
Copper, R.L., et al., 1996. The preterm prediction study: maternal stress is associated with spontaneous preterm birth at less than thirty-five weeks' gestation. Am. J. Obstet. Gynecol. 175, 1286–1292.
Cornelius, D.C., 2018. Preeclampsia: from inflammation to immunoregulation. Clin. Med. Insights Blood Disord. 11.
Cornwell, W.D., et al., 2020. Tobacco smoke and morphine alter peripheral and CNS inflammation following HIV infection in a humanized mouse model. Sci. Rep. 10, 1–12.
Crew, R.C., Waddell, B.J., Mark, P.J., 2016. Maternal obesity induced by a 'cafeteria' diet in the rat does not increase inflammation in maternal, placental or fetal tissues in late gestation. Placenta 39, 33–40.
Daseking, M., Petermann, F., Tischler, T., Waldmann, H.C., 2015. Smoking during pregnancy is a risk factor for executive function deficits in preschool-aged children. Geburtshilfe Frauenheilkd 75, 64–71.
Debost, J.C.P.G., et al., 2017. Joint effects of exposure to prenatal infection and peripubertal psychological trauma in schizophrenia. Schizophr. Bull. 43, 171–179.

Desai, R.J., Hernandez-Diaz, S., Bateman, B.T., Huybrechts, K.F., 2014. Increase in prescription opioid use during pregnancy among medicaid-enrolled women. Obstet. Gynecol. 123, 997–1002.
Desai, R.J., et al., 2015. Exposure to prescription opioid analgesics in utero and risk of neonatal abstinence syndrome: population based cohort study. BMJ 350, h2102.
Doyle, L.W., et al., 2014. Long term follow up of high risk children: who, why and how? BMC Pediatr. 14, 1471–2431.
Dutta, R., et al., 2012. Morphine modulation of Toll-like receptors in microglial cells potentiates neuropathogenesis in a HIV-1 model of coinfection with pneumococcal pneumoniae. J. Neurosci. 32, 9917–9930.
Economidoy, E., Klimi, A., Vivilaki, V.G., 2012. Caring for substance abuse pregnant women: the role of the midwife. Health Sci. J. 6, 161–169.
Edlow, A.G., et al., 2019. Placental macrophages: a window into fetal microglial function in maternal obesity. Int. J. Dev. Neurosci. 77, 60–68.
Eisenstein, T.K., 2019. The role of opioid receptors in immune system function. Front. Immunol. 10, 1–20.
Elliott, A.J., et al., 2020. Concurrent prenatal drinking and smoking increases risk for SIDS: safe Passage Study report. EClinicalMedicine 19, 100247.
Fajemirokun-Odudeyi, O., et al., 2006. Pregnancy outcome in women who use opiates. Eur. J. Obstet. Gynecol. Reprod. Biol. 126, 170–175.
Farid, W., Dunlop, S., Tait, R., Hulse, G., 2008. The effects of maternally administered methadone, buprenorphine and naltrexone on offspring: review of human and animal data. Curr. Neuropharmacol. 6, 125–150.
Forray, A., 2016. Substance use during pregnancy. F1000Research 5, 1–9.
Frias, A.E., et al., 2011. Maternal high-fat diet disturbs uteroplacental hemodynamics and increases the frequency of stillbirth in a nonhuman primate model of excess nutrition. Endocrinology 152, 2456–2464.
Frost, J.L., Schafer, D.P., 2016. Microglia: architects of the developing nervous system. Trends Cell Biol. 26, 587–597.
Gabrielsson, J.L., Paalzow, L.K., 1983. A physiological pharmacokinetic model for morphine disposition in the pregnant rat. J. Pharmacokinet. Biopharm. 11, 147–163.
Ge, J., et al., 2013. Why does a high-fat diet induce preeclampsia-like symptoms in pregnant rats? Neural Regen. Res. 8, 1872–1880.
Giovanoli, S., et al., 2013. Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice, 339, 1095–1100.
Giovanoli, S., et al., 2016. Preventive effects of minocycline in a neurodevelopmental two-hit model with relevance to schizophrenia. Transl. Psychiatry 6, e772–e779.
Graf, A.E., et al., 2017. Maternal high fat diet exposure is associated with increased hepcidin levels, decreased myelination, and neurobehavioral changes in male offspring. Brain Behav. Immun. 58, 369–378.
Griffiths, P.S., Walton, C., Samsell, L., Perez, M.K., Piedimonte, G., 2016. Maternal high-fat hypercaloric diet during pregnancy results in persistent metabolic and respiratory abnormalities in offspring. Pediatr. Res. 79, 278–286.
Grissom, N.M., Herdt, C.T., Desilets, J., Lidsky-Everson, J., Reyes, T.M., 2015. Dissociable deficits of executive function caused by gestational adversity are linked to specific transcriptional changes in the prefrontal cortex. Neuropsychopharmacology 40, 1353–1363.
Grissom, N.M., George, R., Reyes, T.M., 2016. Suboptimal nutrition in early life affects the inflammatory gene expression profile and behavioral responses to stressors. Brain Behav. Immun. https://doi.org/10.1016/j.bbi.2016.10.013.
Grissom, N.M., George, R., Reyes, T.M., 2017. Suboptimal nutrition in early life affects the inflammatory gene expression profile and behavioral responses to stressors. Brain Behav. Immun. 63, 115–126.
Gumusoglu, S.B., Chilukuri, A.S.S., Santillan, D.A., Santillan, M.K., Stevens, H.E., 2020. Neurodevelopmental outcomes of prenatal preeclampsia exposure. Trends Neurosci. 43, 253–268.
Gutierrez, M.J., Nino, G., Hong, X., Wang, X., 2021. Maternal pre-pregnancy weight and early life lower respiratory tract infections in a low-income urban minority birth cohort. Sci. Rep. 11, 1–8.
Gutvirtz, G., Wainstock, T., Landau, D., Sheiner, E., 2019. Maternal obesity and offspring long-term infectious morbidity. J. Clin. Med. 8, 1466.
Han, V.X., et al., 2021. Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. Transl. Psychiatry 11.
Haugland, S., et al., 2018. Symptoms associated with attention deficit/hyperactivity disorder and autism spectrum disorders in school-aged children prenatally exposed to substances. Subst. Abuse Res. Treat. 12, 117822181876577.
Hernandez, L.L., Grayson, B.E., Yadav, E., Seeley, R.J., Horseman, N.D., 2012. High fat diet alters lactation outcomes: possible involvement of inflammatory and serotonergic pathways. PloS One 7, 3–10.
Hickey, J.E., Suess, P.E., Newlin, D.B., Spurgeon, L., Porges, S.W., 1995. Vagal tone regulation during sustained attention in boys exposed to opiates in utero. Addict. Behav. 20, 43–59.
Hirai, A.H., Ko, J.Y., Owens, P.L., Stocks, C., Patrick, S.W., 2021. Neonatal abstinence syndrome and maternal opioid-related diagnoses in the US, 2010-2017. Jama 325, 146.
Hofheimer, J.A., et al., 2020. Psychosocial and medical adversity associated with neonatal neurobehavior in infants born before 30 Weeks' gestation, 87, 721–729.
Hutchinson, M.R., et al., 2010. Evidence that opioids may have toll-like receptor 4 and MD-2 effects. Brain Behav. Immun. 24, 83–95.
Iessa, N., Bérard, A., 2015. Update on prepregnancy maternal obesity: birth defects and childhood outcomes. J. Pediatr. Genet. 4, 71–83.
Indredavik, M.S., et al., 2010. Perinatal risk and psychiatric outcome in adolescents born preterm with very low birth weight or term small for gestational age. J. Dev. Behav. Pediatr. 31, 286–294.
Jansson, L.M., et al., 2012. Pregnancies exposed to methadone, methadone and other illicit substances, and poly-drugs without methadone: a comparison of fetal neurobehaviors and infant outcomes. Drug Alcohol Depend. 122, 213–219.
Jansson, L.M., et al., 2017. Maternal buprenorphine treatment and infant outcome. Drug Alcohol Depend. 180, 56–61.
Jantzie, L.L., et al., 2020. Prenatal opioid exposure: the next neonatal neuroinflammatory disease. Brain Behav. Immun. 84, 45–58.
Kaltenbach, K., et al., 2012. Predicting treatment for neonatal abstinence syndrome in infants born to women maintained on opioid agonist medication. Addiction 107, 45–52.
Kang, S.S., Kurti, A., Fair, D.A., Fryer, J.D., 2014. Dietary intervention rescues maternal obesity induced behavior deficits and neuroinflammation in offspring. J. Neuroinflammation 11, 156.
Karlsson, E.A., Marcelin, G., Webby, R.J., Schultz-Cherry, S., 2012. Review on the impact of pregnancy and obesity on influenza virus infection. Influenza Other Respi. Viruses 6, 449–460.
Kong, L., Chen, X., Gissler, M., Lavebratt, C., 2020. Relationship of prenatal maternal obesity and diabetes to offspring neurodevelopmental and psychiatric disorders: a narrative review. Int. J. Obes. 44, 1981–2000.
Krakowiak, P., et al., 2012. Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. Pediatrics 129, e1121–e1128.
Leddy, M.A., Power, M.L., Schulkin, J., 2008. The impact of maternal obesity on maternal and fetal health. Rev. Obstet. Gynecol. 1, 170–178.
Lee, H.C., Lin, H.C., 2010. Maternal bipolar disorder increased low birthweight and preterm births: a nationwide population-based study. J. Affect. Disord. 121, 100–105.
Lee, B.K., et al., 2015. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. Brain Behav. Immun. 44, 100–105.
Levine, T.A., Woodward, L.J., 2018. Early inhibitory control and working memory abilities of children prenatally exposed to methadone. Early Hum. Dev. 116, 68–75.
Lu, R., Liu, X., Long, H., Ma, L., 2012. Effects of prenatal cocaine and heroin exposure on neuronal dendrite morphogenesis and spatial recognition memory in mice. Neurosci. Lett. 522, 128–133.
Makinson, R., Lloyd, K., Grissom, N., Reyes, T.M., 2019. Exposure to in utero inflammation increases locomotor activity, alters cognitive performance and drives vulnerability to cognitive performance deficits after acute immune activation. Brain Behav. Immun. 80, 56–65.
Maupin, R., et al., 2004. Characteristics of women who deliver with no prenatal care. J. Matern. Neonatal Med. 16, 45–50.
McCarthy, M.M., Pickett, L.A., VanRyzin, J.W., Kight, K.E., 2015. Surprising origins of sex differences in the brain. Horm. Behav. 76, 3–10.
McCartney, S.A., et al., 2020. Obesity as a contributor to immunopathology in pregnant and non-pregnant adults with COVID-19. Am. J. Reprod. Immunol. 84, 1–9.
McKee, S.E., Grissom, N.M., Herdt, C.T., Reyes, T.M., 2017. Methyl donor supplementation alters cognitive performance and motivation in female offspring from high-fat diet–fed dams. *FASEB J.* fj. 201601172R. https://doi.org/10.1096/fj.201601172R.
Merhar, S.L., et al., 2019. White matter injury and structural anomalies in infants with prenatal opioid exposure. Am. J. Neuroradiol. 40, 2161–2165.
Merighi, S., et al., 2013. Morphine mediates a proinflammatory phenotype via m-opioid receptor-PKCε-Akt-ERK1/2 signaling pathway in activated microglial cells. Biochem. Pharmacol. 86, 487–496.
Meyer, U., 2019. Neurodevelopmental resilience and susceptibility to maternal immune activation. Trends Neurosci. 42, 793–806.
Miller, Gregory E., Chen, Edith, Parker, K.J., 2011. Psychological stress in childhood and susceptibility to the chronic diseases of aging. Psychol. Bull. 137, 959–997.
Mohsenzadeh, Y., Rahmani, A., Cheraghi, J., Pyrani, M., Asadollahi, K., 2014. Prenatal exposure to nicotine in pregnant rat increased inflammatory marker in Newborn Rat. Mediat. Inflamm. 2014, 274048 https://doi.org/10.1155/2014/274048.
Morris, C.D., Garver-Apgar, C.E., 2020. Nicotine and opioids: a call for Co-treatment as the standard of care. J. Behav. Health Serv. Res. 47, 601–613.
Murphy, V., Powell, H., Gibson, P., 2014. The role of maternal obesity in susceptibility to respiratory viral infection and exacerbation rate in pregnant women with asthma. Eur. Respir. J. 44, 224.
Nagarajan, M.K., Goodman, D., 2020. Not just substance use: the critical gap in nutritional interventions for pregnant women with opioid use disorders. Publ. Health 180, 114–116.
Nathan, C., Ding, A., 2010. Nonresolving Inflammation. *Cell* 140, 871–882.
Nelson, L.F., et al., 2020. Cognitive outcomes of young children after prenatal exposure to medications for opioid use disorder: a systematic review and meta-analysis. JAMA Netw. open 3, e201195.
Newville, J., Maxwell, J.R., Kitase, Y., Robinson, S., Jantzie, L.L., 2020. Perinatal opioid exposure primes the peripheral immune system toward hyperreactivity. Front. Pediatr. 8, 1–12.
Niemelä, S., et al., 2016. Prenatal nicotine exposure and risk of schizophrenia among offspring in a national birth cohort. Am. J. Psychiatr. 173, 799–806.
Noose Glovak, S., et al., 2020. Pregnant women misusing opioids: depression, anxiety, stress, and neonatal opioid withdrawal syndrome. J. Affect. Disord. Reports 2, 100025.
Northcutt, A.L., et al., 2015. DAT isn't all that: cocaine reward and reinforcement require Toll-like receptor 4 signaling. Mol. Psychiatr. 20, 1525–1537.
Nygaard, E., Slinning, K., Moe, V., Walhovd, K.B., 2016. Behavior and attention problems in eight- year-old children with prenatal opiate and poly-substance exposure: a longitudinal study. PloS One 11, 1–21.
Nygaard, E., Slinning, K., Moe, V., Walhovd, K.B., 2017. Cognitive function of youths born to mothers with opioid and poly-substance abuse problems during pregnancy. Child Neuropsychol. 23, 159–187.

Ornoy, A., Michailevskaya, V., Lukashov, I., Bar-Hamburger, R., Harel, S., 1996. The developmental outcome of children born to heroin-dependent mothers, raised at home or adopted. Child Abuse Negl. 20, 385–396.
Osterman, M.J.K., Martin, J.A., 2018. Timing and adequacy of prenatal care in the United States, 2016. Natl. Vital Stat. Rep. 67.
Pan, I.J., Yi, H.Y., 2013. Prevalence of hospitalized live births affected by alcohol and drugs and parturient women diagnosed with substance abuse at liveborn delivery: United States, 1999-2008. *Matern.* Child Health J 17, 667–676.
Reynolds, C.M., Vickers, M.H., Harrison, C.J., Segovia, S.A., Gray, C., 2015. Maternal high fat and/or salt consumption induces sex-specific inflammatory and nutrient transport in the rat placenta. Phys. Rep. 3 e12399–e12399.
Rodriguez, A., et al., 2008. Maternal adiposity prior to pregnancy is associated with ADHD symptoms in offspring: evidence from three prospective pregnancy cohorts. Int. J. Obes. 32, 550–557.
Ross, E.J., Graham, D.L., Money, K.M., Stanwood, G.D., 2015. Developmental consequences of fetal exposure to drugs: what we know and what we still must learn. Neuropsychopharmacology 40, 61–87.
Roy, S., et al., 2011. Opioid drug abuse and modulation of immune function: consequences in the susceptibility to opportunistic infections. J. Neuroimmune Pharmacol. 6, 442–465.
Rubenstein, E., et al., 2019. Brief report: maternal opioid prescription from preconception through pregnancy and the odds of autism spectrum disorder and autism features in children. J. Autism Dev. Disord. 49, 376–382.
Rudolph, M.D., et al., 2018. Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring. Nat. Neurosci. 21, 765–772.
Sanders, T.R., Kim, D.W., Glendining, K.A., Jasoni, C.L., 2014. Maternal obesity and IL-6 lead to aberrant developmental gene expression and deregulated neurite growth in the fetal arcuate nucleus. Endocrinology 155, 2566–2577.
Sanlorenzo, L.A., et al., 2019. Increased severity of neonatal abstinence syndrome associated with concomitant antenatal opioid and benzodiazepine exposure. Hosp. Pediatr. 9, 569–575.
Schaefer, C.A., et al., 2000. Maternal prepregnant body mass and risk of schizophrenia in adult offspring. Schizophr. Bull. 26, 275–286.
Schafer, D.P., et al., 2012. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74, 691–705.
Schneider, T., et al., 2011. Prenatal exposure to nicotine impairs performance of the 5-choice serial reaction time task in adult rats. Neuropsychopharmacology 36, 1114–1125.
Siddika, N., et al., 2019. Synergistic effects of prenatal exposure to fine particulate matter (PM2.5) and ozone (O3) on the risk of preterm birth: a population-based cohort study. Environ. Res. 176, 108549.
Šlamberová, R., et al., 2001. Prenatal morphine exposure differentially alters learning and memory in male and female rats. Physiol. Behav. 73, 93–103.
Slinning, K., 2004. Foster placed children prenatally exposed to poly-substances: attention-related problems at ages 2 and 4 1/2. Eur. Child Adolesc. Psychiatr. 13, 19–27.
Smith, B.L., Reyes, T.M., 2017a. Offspring neuroimmune consequences of maternal malnutrition: potential mechanism for behavioral impairments that underlie metabolic and neurodevelopmental disorders. Front. Neuroendocrinol. 47.
Smith, B.L., Reyes, T.M., 2017b. Offspring neuroimmune consequences of maternal malnutrition: potential mechanism for behavioral impairments that underlie metabolic and neurodevelopmental disorders. Front. Neuroendocrinol. 47, 109–122.
Smith, B. L. et al. Rates of Substance and Polysubstance Use through Universal Maternal Testing at the Time of Delivery. submitted for publication.
Smith, B.L., Laaker, C.J., Lloyd, K.R., Hiltz, A.R., Reyes, T.M., 2020. Adolescent microglia play a role in executive function in male mice exposed to perinatal high fat diet. Brain Behav. Immun. 84, 80–89.
Sousa, F.J., et al., 2020. Sex differences in offspring neurodevelopment, cognitive performance and microglia morphology associated with maternal diabetes: putative targets for insulin therapy. Brain, Behav. Immun. - Heal. 5, 100075.
Squarzoni, P., et al., 2014. Microglia modulate wiring of the embryonic forebrain. Cell Rep. 8, 1271–1279.
Stokes, A., et al., 2020. Obesity and incident prescription opioid use in the U.S., 2000–2015. Am. J. Prev. Med. 58, 766–775.
Stone, R., 2015. Pregnant women and substance use: fear, stigma, and barriers to care. Heal. Justice 3.
Tedesco, R.P., et al., 2020. The role of maternal infection in preterm birth: evidence from the brazilian multicentre study on preterm birth (EMIP). Clinics 75, 1–6.
Tiesler, C.M.T., Heinrich, J., 2014. Prenatal nicotine exposure and child behavioural problems. Eur. Child Adolesc. Psychiatr. 23, 913–929.
Tofighi, B., et al., 2019. Perspectives on the HIV continuum of care among adult opioid users in New York City: a qualitative study. Harm Reduct. J. 16, 1–9.
Tolia, V.N., et al., 2015. Increasing incidence of the neonatal abstinence syndrome in U.S. neonatal ICUs. Obstet. Gynecol. Surv. 70, 551–552.
Tonkiss, J., Harrison, R.H., Galler, J.R., 1996. Differential effects of prenatal protein malnutrition and prenatal cocaine on a test of homing behavior in rat pups. Physiol. Behav. 60, 1013–1018.
Tough, S.C., Siever, J.E., Benzies, K., Leew, S., Johnston, D.W., 2010. Maternal well-being and its association to risk of developmental problems in children at school entry. BMC Pediatr. 10.
Van Lieshout, R.J., Voruganti, L.P., 2008. Diabetes mellitus during pregnancy and increased risk of schizophrenia in offspring: a review of the evidence and putative mechanisms. J. Psychiatry Neurosci. 33, 395–404.
VanRyzin, J.W., et al., 2019. Microglial phagocytosis of newborn cells is induced by endocannabinoids and sculpts sex differences in juvenile rat social play. Neuron 102, 435–449 e6.
Veena, S.R., et al., 2016. Association between maternal nutritional status in pregnancy and offspring cognitive function during childhood and adolescence ; a systematic review. BMC Pregnancy Childbirth. https://doi.org/10.1186/s12884-016-1011-z.
Vesterinen, H.M., Morello-Frosch, R., Sen, S., Zeise, L., Woodruff, T.J., 2017. Cumulative effects of prenatal-exposure to exogenous chemicals and psychosocial stress on fetal growth: systematic-review of the human and animal evidence. PloS One 12.
Wahlsten, V.S., Sarman, I., 2013. Neurobehavioural development of preschool-age children born to addicted mothers given opiate maintenance treatment with buprenorphine during pregnancy. Acta Paediatr. Int. J. Paediatr. 102, 544–549.
Wang, Y., 2018. Maternal smoking and attention- deficit/hyperactivity disorder in Offspring : a meta-analysis. Pediatrics 141.
Wang, Y., Han, T.Z., 2009. Prenatal exposure to heroin in mice elicits memory deficits that can be attributed to neuronal apoptosis. Neuroscience 160, 330–338.
Wang, X., et al., 2012. Morphine activates neuroinflammation in a manner parallel to endotoxin. Proc. Natl. Acad. Sci. Unit. States Am. 109, 6325–6330.
Wedderburn, C.J., et al., 2019. Neurodevelopment of HIV-exposed uninfected children in South Africa: outcomes from an observational birth cohort study. Lancet Child Adolesc. Heal. 3, 803–813.
Whiteman, V.E., et al., 2014. Maternal opioid drug use during pregnancy and its impact on perinatal morbidity, mortality, and the costs of medical care in the United States. J. Pregnancy 2014.
Wright, A., Walker, J., 2007. Management of women who use drugs during pregnancy. Semin. Fetal Neonatal Med. 12, 114–118.
Wu, J., et al., 2018. Neurodevelopmental outcomes in young children born to HIV-positive mothers in rural Yunnan, China. Pediatr. Int. 60, 618–625.
Xu, G., Jing, J., Bowers, K., Liu, B., Bao, W., 2014. Maternal diabetes and the risk of autism spectrum disorders in the offspring: a systematic review and meta-analysis. J. Autism Dev. Disord. 44, 766–775.
Yokomizo, H., et al., 2014. Maternal high-fat diet induces insulin resistance and deterioration of pancreatic β-cell function in adult offspring with sex differences in mice. Am. J. Physiol. Endocrinol. Metab. 306, 1163–1175.
Zagon, I.S., McLaughlin, P.J., Thompson, C.I., 1979. Learning ability in adult female rats perinatally exposed to methadone. Pharmacol. Biochem. Behav. 10, 889–894.
Zhan, Y., et al., 2014. Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. Nat. Neurosci. 17, 400–406.
Zhao, L., et al., 2019. p>The association of maternal diabetes with attention deficit and hyperactivity disorder in offspring: a meta-analysis</p>. Neuropsychiatric Dis. Treat. 15, 675–684.
Zhao, X., Tran, H., DeRosa, H., Roderick, R.C., Kentner, A.C., 2021. Hidden Talents: poly (I:C)-induced maternal immune activation improves mouse visual discrimination performance and reversal learning in a sex-dependent manner. Genes, Brain Behav 1. https://doi.org/10.1111/gbb.12755.