Prenatal and postnatal drug exposure: focus on persistent central effects

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Abstract

Clinical studies indicate significant use of prescription, nonprescription and social/recreational drugs by women during pregnancy; however, limited knowledge exists about the detrimental effects that this practice may have on the developing central nervous system of the fetus. Importantly, few experimental and clinical data are available on how gestational exposure could exacerbate the effects of the same or a different drug consumed by the offspring later in life. The present review summarizes recent findings on the central toxicity elicited by several classes of drugs, administered prenatally and postnatally in experimental animals and humans, focusing on prescription and nonprescription analgesics, anti-inflammatory agents, alcohol and nicotine.

Key Words: 3,4-methylenedioxymethamphetamine; acetaminophen; alcohol; chlorpyrifos; dexamethasone; ibuprofen; methadone; neuroinflammation; neurotoxicity; nicotine

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Introduction

As early as the 16th century, the Swiss physician and alchemist Paracelsus affirmed "omnia venenum sunt nec sine veneno quicquam existit. Dosis sola facit ut venenum non fit," stating for the first time that "only the dose makes the poison" (Paracelsus, Responsio ad quasdam accusationes et calumnias suorum aemulorum et obtrectatorum. Defensio III. Descriptionis et designationis nouorum Receptorum). Over the centuries, preclinical and clinical reports have extensively demonstrated that dose is not the only discriminator that confers drug-induced toxicity, as several other factors may participate in this phenomenon. The "developmental origin of health and disease" hypothesis postulates that the exposure to xenobiotics in early life, such as environmental chemicals, prescription, nonprescription or social/recreational drugs, together with other factors that will not be discussed in this review, may be associated with maladies that are manifested at adulthood, including neurological and psychiatric disorders (Arima and Fukuoka, 2020; Gabbianelli et al., 2020; Allegra et al., 2021).

The medical use of prescription and nonprescription ("over-the-counter") medications and the use of social/recreational substances, such as vaped nicotine and alcohol, are major public health concerns for pregnant women. However, investigation into this practice and its effects represents a significant challenge, to our knowledge, in most of the countries. In 1977, the US Food and Drug Administration (FDA) excluded all "women of childbearing potential" from Phase I clinical trials, which usually evaluate the safety of a drug, and from Phase IIa clinical trials, which usually evaluate the efficacy of a drug. A newer draft guidance released by the FDA in 2018 still limits, only under certain circumstances, the inclusion of pregnant women in all phases of clinical trials (US Food and Drug Administration, 2018). Similarly, the inclusion of pregnant women in clinical trials is regulated in the European Union (Petrini, 2014), as well as in Asian countries such as India (Mathur and Swaminathan, 2018). Exceptions are found in China and Japan, where there are guidelines, but no published laws, for clinical trials involving pregnant women. In China, the "Review Methods of Biomedical Research Ethics Involving Human Beings", released by the National Health Commission of China, emphasizes the protection of pregnant women and fetuses (Wang et al., 2011), whereas in Japan, the Clinical Trials Act includes pregnant women together with the elderly as groups that may require special studies due to unique risk/benefit concerns (Nakamura and Shibata, 2020).

Added to these considerations, there is a high rate of non-participation of women in clinical trials during pregnancy. Several studies have explored the reasons why pregnant women chose not to participate, with barriers identified as: low levels of importance/confidence in their contribution to science, uncertainty about effects on them and their unborn children, rushed counseling from clinical trial recruiters, and the negative influence of their partners (Oude Rengerink et al., 2015; Strommer et al., 2018). Taken together, these barriers underscore the importance of education and familiarizing pregnant women, and their partners, to scientific research and methods in order to improve their rates of participation in clinical trials.

Based on these data, pregnant women can be considered "therapeutic orphans" (Wisner et al., 2020), a cross-sectional, web-based, multinational study, involving over nine thousand pregnant women and new mothers with children under 1 year of age from Europe, North and South America and Australia, found that 81.2% of participants reported the use of at least one medication, prescribed or nonprescribed, during pregnancy (Lupattelli et al., 2014). Another multinational report, involving pregnant women from the USA, including American Indians in the northern plains, together with mixed ancestry women from South Africa, found that 56% of pregnant women reported a positive history of vaped nicotine, 61% reported a positive history of drinking alcohol, and 38% reported a positive history of dual exposure (Dukes et al., 2017).

The type and severity of the toxic effects that drugs consumed during pregnancy may elicit on offspring depend on a number of factors including drug dose, gestational time of exposure, drug metabolism and pharmacodynamics, genetics and on the possible existence of an innate resistance of the fetus to the effects of specific drugs (Feghali et al., 2015). Nevertheless, lipophilic molecules, especially those weighing less than 500 Da, can cross placental and blood-brain barriers, thereby passing from the mother to the developing fetus (Al-Enazy et al., 2017). In addition, many molecules can pass into breast milk, which exposes the infant to the effects of these molecules during early postnatal periods, further contributing to persistent, untoward drug effects in newborns.

An important issue that needs further consideration is the central effects on offspring born from mothers who used medications or recreational drugs during pregnancy. Indeed, the possibility exists that exposure to drugs during prenatal life may elicit persistent detrimental effects on the fetal central nervous system that may exacerbate the neurotoxic potential of drugs that the offspring consumes later in life; however, limited information is available in this regard (Stanwood and Levitt, 2004). Most studies of neurodevelopmental toxicology examine the effects of drug exposure within a narrow time window, usually the early postnatal period (Ross et al., 2015). Although the molecular steps responsible for brain development are not fully understood, it is clear that the human brain develops over an extended period of time, beginning in the first trimester of pregnancy (Levitt, 2003); the brain

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continues to develop through adolescence, when myelination and synaptic pruning reach completion (Spear, 2013). This means that particular attention should be paid to the central effects of exposure to drugs between the first trimester of pregnancy and when the offspring reaches adolescence.

In this regard, studies utilizing experimental animals are critical, due to ethical limitations of involving certain categories of people, including adolescents who are minors, in studies of social/recreational substances. Indeed, preclinical studies have shown that several licit and illicit social/recreational substances can induce neurotoxic and neuroinflammatory effects, particularly when administered during adolescence (Pereira et al., 2015; Moratalla et al., 2017; Leung et al., 2019; Jones, 2020; Jayanthi et al., 2021; Shi et al., 2022). This is noteworthy since it is during adolescence that illicit substance use/abuse typically starts, as demonstrated by several long-term human studies. For example, "Monitoring the Future national survey" began in 1975 in order to investigate substance use and related attitudes among USA students across three time periods – lifetime, past year, and past month – until participants reached 50 years of age (Johnston et al., 2022). In early 2022, they reported that the lifetime use of any licit or illicit substance, including inhalants, in 13–18 years old is around 38%; surprisingly, during the coronavirus disease 2019 pandemic they found a trend toward a decrease in the use of many substances, including those most commonly consumed during adolescence such as alcohol, marijuana, and vaped nicotine (Johnston et al., 2022). This downward trend was recently confirmed by Layman and colleagues in their review of 49 studies examining the use of alcohol, cannabis, tobacco, e-cigarettes/vaping, and other drugs by young people during the coronavirus disease 2019 pandemic (Layman et al., 2022). Similarly, the European School Survey Project on Alcohol and Other Drugs, which began in 1995, is a long-term study on licit and illicit social/recreational substance use among 15–16-year-old school students from 35 European Union countries. This report found that 18% of these students had used a substance in their lifetime, with a trend toward decreased use of vaped nicotine and alcohol over the years (Mokinaro et al., 2020). However, these studies only recently concluded that there is no information yet on long-term central effects of substance use in these populations (Mokinaro et al., 2020; Johnston et al., 2022).

In consideration of the importance of the topic, the aim of this review is to summarize the most recent findings of preclinical and clinical studies that investigated the postnatal long-term neurotoxic and neuroinflammatory effects observed in individuals who are drug users and who are born from mothers that used the same or another drug during pregnancy.

Search Strategy

We began with an extensive search strategy to conduct a comprehensive review of the relevant literature, focusing on research studies published in the past 5 years. By using specific search terms ((Prenatal drugs) AND (Postnatal drugs) AND (neurotoxicity)/(neuroinflammation)) in PubMed, we aimed to investigate the postnatal long-term neurotoxic and neuroinflammatory effects observed in humans or experimental animals who used drugs as adolescents/adults and who are born from mothers that used the same or another drug during pregnancy. When reading the titles and abstracts of articles, we used a series of additional keywords (neuronal markers, astrocytes, neurons, microglia, interleukins) with a particular focus on the central biochemical effects of toxicity, rather than on behavioral dysfunctions. If there were few related articles following the strict search criteria, we adjusted the search conditions manually; for example, we relaxed the search period in order to obtain more relevant literature. Through intelligent screening and manual browsing, we analyzed studies related to prenatal plus postnatal drug use and central toxicity.

Use of Prescription and Nonprescription Drugs during Pregnancy/Early Life and Their Relationship with the Onset of Neurotoxic and Neuroinflammatory Effects in Drug Re-Exposed Offspring

Many women take drugs during pregnancy to treat disorders of varying severity. Among these drugs are prescription analgesics, such as opioids, and nonprescription analgesics, such as acetaminophen and ibuprofen, as well as anti-inflammatory drugs, such as glucocorticoids like dexamethasone (DEX).

According to an investigation performed in 2019, 6.6% of women in the U.S. reported the use of prescription opioid analgesics during pregnancy (Ko et al., 2020). Preclinical and clinical studies suggest that use of prescription opioid analgesics during pregnancy can have detrimental neurological consequences on the fetus, which may persist up to postnatal life (van Hoogdalem et al., 2022). Studies in rodents found that prenatal administration of methadone, a synthetic, long-acting, opioid analgesic used for maintenance therapy for individuals dependent on opioids and for the management of chronic pain, can induce a range of central toxic effects on exposed offspring (Merhar et al., 2021; Graeve et al., 2022). For example, a recent study performed in rats examined the neurotoxic and neuroinflammatory effects observed in the offspring of methadone-treated dams (**Figure 1**; Jantzie et al., 2020). In this study, methadone was administered to pregnant rat dams from gestational day 16 until 3 weeks after birth, and therefore the pups received methadone via the maternal milk until they reached preadolescent age (Jantzie et al., 2020). Evaluations performed on pups on postnatal day 10 revealed that methadone administration, compared with gestational exposure to saline, increased cerebral levels of cortical toll-like receptor 4 (TLR4) (a key player in inflammatory signaling in perinatal brain injury) as well as myeloid differentiation primary response protein (MyD88, an initial downstream mediator of TLR4) (Jantzie et al., 2020). Moreover, rat pups subjected to combined prenatal and postnatal exposure to methadone displayed changes in cortical levels of pro-inflammatory mediators, in particular increased levels of interleukin-1β and chemokine CXCL1; these changes were accompanied by altered morphology and reduced arborization of microglia in the somatosensory cortex (Jantzie et al., 2020). Anatomical evaluations performed on pups at postnatal day 21 revealed that combined prenatal and postnatal exposure to methadone was associated with a significant reduction in axonal integrity and myelin expression (Jantzie et al., 2020). In three studies by Grecco et al. (2021, 2022a, 2022b), methadone was administered to oxycodone-dependent female mice from the pre-gestational day 5 until weaning (**Figure 1**). As a consequence, offspring from these treated dams showed delayed development of sensorimotor milestones such as surface righting, forelimb grasp and cliff aversion (Grecco et al., 2021). Moreover, the authors found widespread region- and sex-dependent changes to the proteomic and phosphoproteomic landscape in the dorsal striatum (Grecco et al., 2022b) and in the primary somatosensory cortex (Grecco et al., 2022a). Specifically, in the dorsal striatum, adolescent offspring showed reduced glutamate release and enhanced endocannabinoid signaling, particularly in males (Grecco et al., 2022b). In the cortex, there was reduced neuronal density in the motor cortex (Grecco et al., 2021), while in the primary somatosensory cortex there was reduced GABAergic neurotransmission in layers 2, 3 and 4 and reduced microglia density in layer 1 (Grecco et al., 2022a). Taken together, these preclinical data indicate that exposure to opioids during gestation and early postnatal life may cause detrimental long-term changes in the brains of offspring, which affect both neuronal and microglia cells. Although human studies have investigated opioid-induced neurotoxic and neuroinflammatory effects (Bohnert et al., 2021), in some cases in offspring exposed prenatally (Kim et al., 2021), no human study has yet evaluated the central effects of prenatal opioids administration in drug re-exposed offspring.

Nonprescription analgesics utilize different mechanisms of action, such as the inhibition of cyclooxygenase enzymes, in the case of non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, or other mechanisms, yet undefined, in the case of acetaminophen. Nonprescription analgesics consumed by women are generally able to cross placental and blood-brain barriers, thus posing a risk for both the fetus during pregnancy and the infant during breast feeding (Courad et al., 2001; Parepally et al., 2006; Conings et al., 2019). Although several preclinical studies have evaluated the neurotoxic potential of prenatal administration of NSAIDs such as diclofenac sodium (Gokcimen et al., 2007; Ragbetli et al., 2007; Ozyurt et al., 2011) or acetaminophen (Klein et al., 2020), no studies have evaluated the central effects of prenatal NSAID administration in drug re-exposed offspring. In humans, a recent study that involved over a thousand mothers found that 46.1% of them used acetaminophen ten or more times and that 18.4% of them used any ibuprofen-containing medication during pregnancy (Rifas-Shiman et al., 2020). The same study also found that these mothers largely gave acetaminophen and/or ibuprofen to their infants since 65.3% and 39.6% of newborns, respectively, received acetaminophen or ibuprofen six or more times during their first year of life (Rifas-Shiman et al., 2020). Interestingly, the high rates of prenatal and postnatal use of acetaminophen or ibuprofen may have detrimental effects on fetal and child brain development, particularly on executive functions (Liew et al., 2016). Accordingly, these data serve as a warning as to the potential noxious long-term effects on neuronal functions that may arise from exposure of individuals to nonprescription pain relievers during gestation and early postnatal life.

DEX is a corticosteroid, anti-inflammatory drug that can be administered to pregnant women in order to improve fetal lung surfactant production and fetal lung maturity in pregnancies at risk for preterm delivery; DEX has a high capacity to pass through the placenta and into fetal circulation (Kemp et al., 2016; Jobe and Goldenberg, 2018). The effects of prenatal exposure to corticosteroids on the human fetal brain are still ill-defined, although animal studies have reported inhibitory effects on brain growth (Huang et al., 1999) or enhanced noradrenergic synaptic activity in brain areas such as the midbrain and brainstem (Slotkin et al., 1992). Moreover, preclinical studies agree that prenatal exposure to DEX may amplify the detrimental effects that xenobiotics administered later in life may elicit on the brain. This issue was addressed by Slotkin's research group in preclinical experimental animal models (Slotkin et al., 2014). In rats prenatally exposed to DEX, the effects of the organophosphate pesticide chlorpyrifos on the expression of serotonin (5-HT) type 1A and 2 receptors, 5-HT transporter, and on 5-HT turnover were evaluated (**Figure 1**; Slotkin et al., 2014). Treatment with chlorpyrifos alone reduced the expression of 5-HT receptors and 5-HT transporter, as well as 5-HT turnover, and these changes were exacerbated by prenatal exposure to DEX (Slotkin et al., 2014). A study from our research group using mice prenatally

exposed to DEX evaluated the neuroinflammatory and neurotoxic effects in adolescent and adult male mice following administration of the amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA, or "ecstasy") (**Figure 1**; Costa et al., 2021). MDMA is largely consumed by adolescents and young adults for recreational purposes (Gorska et al., 2018; Costa et al., 2019; Costa and Golembiowska, 2022); MDMA not only affects emotional states, but it can also produce neurotoxic effects and glial activation in several animal species (Gudelsky and Yamamoto, 2008; Gorska et al., 2018; Costa et al., 2019). Administration of MDMA to male offspring born to dams treated with DEX enhanced astrogliosis in the substantia nigra pars compacta in both adolescent and adult mice and potentiated dopaminergic neurodegeneration in the substantia nigra pars compacta in adolescent mice compared with matched mice born to dams that were not treated with DEX during pregnancy (Costa et al., 2021). Collectively, these data suggest that fetal exposure to DEX, and possibly other glucocorticoids, during pregnancy may be a risk factor that exacerbates the detrimental effects on the fetal brain induced by xenobiotics and drugs of abuse used by the offspring later in life.

Use of Alcohol and Nicotine during Pregnancy/ Early Life and Their Relationship with the Onset of Neurotoxic and Neuroinflammatory Effects in Substance Re-Exposed Offspring

As mentioned earlier in this review, alcohol and nicotine are social/ recreational substances widely consumed by pregnant women, although their use, either singly or in combination, is far from harmless to the fetus (Shuffrey et al., 2020).

Prenatal alcohol administration has been shown to cause fetal alcohol spectrum disorders, which consist of various behavioral and cognitive problems, and increased addiction risk. Brain imaging studies in experimental animals and children prenatally exposed to ethanol have identified structural changes in several brain regions, including basal ganglia, corpus callosum, cerebellum and hippocampus, that may account for the cognitive deficits (Lebel et al., 2011; Milbocker et al., 2022). Importantly, prenatal exposure to alcohol affects not only neuronal but also glial cell viability (Aronne et al., 2011; Guizzetti et al., 2014). In particular, the study by Doremus-Fitzwater et al. (2020) evaluated the expression of inflammatory-related genes in the hippocampus, amygdala and paraventricular nucleus of the hypothalamus of male and female rats born from alcohol-treated dams and challenged with a binge-like dose of alcohol as adolescents or adults (**Figure 2**). They reported increased expression of interleukin-6 and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-α, with decreased expression of interleukin-1β and tumor necrosis factor-α, in all the three brain areas evaluated (Doremus-Fitzwater et al., 2020). The study by Pascual et al. (2017) evaluated the immune system activation in wildtype and TLR4-knockout mice born from dams exposed to alcohol during pregnancy and lactation (**Figure 2**). In cortical areas of postnatal day 20 (adolescent) and postnatal day 66 (adult) wildtype offspring there were increased levels of specific interleukins/ chemokines (interleukin-1β, interleukin-17, macrophage inflammatory proteins-1α, and fractalkine), increased activation of microglial markers, and reduction in several synaptic (synaptotagmin, synapsin IIa) and myelin proteins (proteolipid protein and myelin basic protein) (Pascual et al., 2017). In contrast, TLR4-knockout mice were protected against alcohol-induced cytokine/chemokine production. Taken together, these studies suggest that prenatal alcohol stimulates the immune system of offspring, and, in this regard, TLR4 activation may play an important role.

While in utero, the fetus can be exposed to nicotine in several ways, ranging from second-hand smoke to the use of nicotine cessation aids. Preclinical and clinical studies generally agree that nicotine affects the developing fetal brain, particularly at cortical (El Marroun et al., 2014; Bryden et al., 2016) and striatal (Dwyer et al., 2019) levels. Interestingly, the central noxious effects of nicotine can be exacerbated by other substances, such as ethanol, that are consumed by mothers during pregnancy; indeed, an increased toxic effect has been demonstrated in the cerebellum of children that were prenatally exposed to both nicotine and alcohol, compared to children prenatally exposed to either substance alone (de Zeeuw et al., 2012). Moreover, both preclinical and clinical studies have reported the presence of cognitive impairments in the offspring of mothers who used nicotine during pregnancy (Schneider et al., 2011; Ramsay et al., 2016). Nevertheless, these impairments appeared to be milder than those observed in children born to mothers who used alcohol or were exposed to other xenobiotics during pregnancy (Huizink and Mulder, 2006). Furthermore, another detrimental effect that may arise from exposure to nicotine during pregnancy may be an increased risk for the offspring to develop nicotine dependence (Kandel et al., 2007; Okoli et al., 2016; De Genna et al., 2017; Rissanen et al., 2021). Preclinical studies have suggested that the neurotoxic effects of nicotine may be potentiated when prenatal exposure to nicotine is followed by re-exposure to this substance in later stages of life. In a similar manner to their studies examining prenatal exposure to DEX (Slotkin et al., 2014), the pre- and postnatal effects of nicotine were masterfully explored by studies performed by Slotkin and coworkers (Abreu-Villaca et al., 2004; Slotkin et al., 2007). In two studies, nicotine was administered to pregnant rat dams from gestational day 4 until the birth of pups; then from postnatal day 30 to postnatal day 47 adolescent rats that were prenatally exposed to nicotine were treated with nicotine (**Figure 3**; Abreu-Villaca et al., 2004; Slotkin et al., 2007). The neurotoxic effects of nicotine were evaluated in offspring 1 (Abreu-Villaca et al., 2004) or 6 months (Slotkin et al., 2007) after the completion of the postnatal nicotine treatment. At 1 month, rats that were exposed to nicotine both prenatally and during adolescence displayed higher levels of neurotoxicity in the midbrain and cerebral cortex, with corresponding elevations in the membrane/total protein ratio (likely reflecting neuronal loss and enhanced gliosis) compared to rats that were exposed to nicotine either prenatally or during adolescence alone (**Figure 3**; Abreu-Villaca et al., 2004). Moreover, when evaluated at six months after postnatal adolescent treatment with nicotine, female offspring showed persistent deficits in cerebrocortical choline acetyltransferase activity and hemicholinium-3 binding to the presynaptic choline transporter, a pattern consistent with cholinergic hypoactivity, along with reduction of 5HT1A receptors and upregulation of 5HT2 receptors, compared to offspring exposed to prenatal nicotine alone (**Figure 3**; Slotkin et al., 2007). In a separate study, Mychasiuk et al. (2013) treated female rats with nicotine following mating and for the duration of their pregnancies (**Figure 3**). On postnatal day 60, adolescent rats born from nicotine-treated dams received daily subcutaneous injections of nicotine for 14 consecutive days (Mychasiuk et al., 2013). Combined prenatal and adolescent exposure to nicotine differentially affected female and male offspring compared to same sex offspring that received nicotine either prenatally or during adolescence alone (**Figure 3**; Mychasiuk et al., 2013). For example, the combined nicotine exposure in female offspring led to spine density changes in the parietal and prefrontal cortex as well as dendritic morphology differences in the nucleus accumbens; in contrast, male offspring had dendritic morphology changes in the dorsal agranular insular cortex (**Figure 3**; Mychasiuk et al., 2013). Taken together, these studies suggest that adolescents whose mothers used nicotine during pregnancy may have an increased sex-dependent vulnerability to the neurotoxic effects of nicotine. An interesting investigation performed by Buck et al. (2019) explored the neurotoxic effects of prenatal and perinatal nicotine exposure over two generations of mice (**Figure 3**). In this study, female mice were exposed to nicotine starting on pre-gestational day 30 for the duration of their pregnancy, and for another 3 weeks after delivery (generation F1); these female F1 mice were subsequently mated with drug-naïve male mice in order to produce the F2 generation of mice (Buck et al., 2019). Therefore, the F2 mice were not directly exposed to nicotine. Their results showed molecular changes in both F1 and F2 offspring, which included altered nicotinic acetylcholine receptor binding, dopamine transporter dysfunction and DNA hypomethylation in the striatum and prefrontal cortex, as well as behavioral changes such as hyperactivity and increased risk-taking (**Figure 3**; Buck et al., 2019). Accordingly, these findings suggest the existence, in experimental models, of multigenerational neurotoxic effects of nicotine at both molecular and behavioral levels.

Conclusions

In summary, preclinical studies indicate that certain drugs used during pregnancy can induce detrimental central effects in exposed offspring. Moreover, these prenatal effects may be potentiated, in some cases in a sex-specific manner, following postnatal use of the same or different drugs by the offspring. So far, no systematic characterization has been performed to uncover the mechanism(s) underlying the neurotoxic/neuroinflammatory effects that may be elicited by prenatal drug exposure. Similarly, little is known about the mechanism(s) that may lead to the exacerbation of neurotoxic/neuroinflammatory effects elicited by postnatal use of specific drugs in animals that were prenatally exposed to the same or different drugs. In humans, there is a lack of studies that have considered the crucial role of genetics, aging and the postnatal environment on these noxious drug effects. Notably, the central changes observed in offspring described by the few studies reported in the literature are regardless of whether the drugs used during pregnancy are prescription, nonprescription or social/recreational; it is in fact arguable that licit social/recreational substances such as nicotine or anti-inflammatory drugs like DEX produce less dramatic deficits than opioid drugs such as methadone. Given the considerable number of women that use drugs during pregnancy, and considering the worldwide limitations in the involvement of pregnant women in clinical trials, more preclinical models are warranted to investigate how and to what extent prenatal and postnatal drug exposure may elicit detrimental effects on the brains of offspring. In addition, considering the high rate of non-participation by pregnant women in clinical trials, it is essential to increase their and their partners' knowledge and trust in scientific research and methods in order to overcome the barriers to their participation in clinical trials.

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Drug	Reference	Protocol applied	Effects
Methadone	Grecco et al., 2021	From PG 14, female C57BL/6J mice were treated with increasing doses of **control or oxycodon** (PG 14: 10 mg/kg; PG 13: 20 mg/kg; from PG 12 to PG 6: 30 mg/kg, all administered s.c.). From PG 5, oxycodone-treated mice were transitioned to **control or methadone** (10 mg/kg s.c.) until weaning of offspring.	Prenatal methadone administration: • produced substantial differences in offspring physical growth, motor activity in an open field, sensorimotor milestone acquisition and sex-dependent differences in USVs emission; • reduced neuronal density in the motor cortex of female mice; • disrupted motor neuron intrinsic properties and local circuit connectivity of female mice.
	Grecco et al., 2022a	Same protocol applied in Grecco et al., 2021	Prenatal methadone administration: • diminished GABAergic synapses in primary somatosensory cortex layer 2/3 and 4, particularly in adolescent males; • reduced microglia density in the upper layer of the primary somatosensory cortex of adolescent females; • produced wide-ranging differences between adolescent male and female mice in cortical protein abundance and phosphorylation patterns.
	Grecco et al., 2022b	Same protocol applied in Grecco et al., 2021	Prenatal methadone administration: • disrupted the endocannabinoid-mediated long-term synaptic depression in the dorsolateral striatum of adolescent males; • produced wide-ranging differences between adolescent male and female mice in striatal protein abundance and phosphorylation patterns.
	Jantzie et al., 2020	From GD 16, pregnant Sprague Dawley rat dams were implanted with osmotic mini pumps for 28 days of continuous **control or methadone** (8–16 mg/kg s.c., 0.25 µL/h flow rate) infusion.	Perinatal methadone administration: • increased pro-inflammatory ILs in the peripheral circulation; • reprogramed and primed lymphocyte; • induced a sustained peripheral immune hyper-reactivity; • increased cerebral TLR4, Myd88, and microglial activation; • induced diffusion tensor imaging abnormalities; • induced functional brain injury and cognitive deficits in adult animals.
DEX	Costa et al., 2021	From GD 14, pregnant C57BL/6J mice dams were treated with **control or DEX** (0.05 mg/kg s.c. per day) until delivery. On PND 28 (adolescents) or on PND 84 (adults), male offspring received 4 administrations of **vehicle or MDMA** in one day (20 mg/kg i.p., 2 h apart).	Prenatal DEX: • worsened MDMA-induced nigral neurodegeneration in adolescent mice; • elevated MDMA-induced nigral astrogliosis in adolescent and adult mice.
	Slotkin et al., 2014	On GD 17, 18 and 19, Sprague-Dawley rat dams received injections of either **control or DEX** (0.2 mg/kg s.c.). On PND 1–4, offspring mice pups were daily injected with **control or chlorpyrifos** (1 mg/kg in a volume of 1 ml/kg s.c.).	Prenatal DEX administration in brain regions containing 5-HT neuronal cell bodies and synaptic projections of chlorpyrifos-treated mice: • reduced the 5-HT_{1A} receptor binding in both sexes, particularly in females; • increased the 5-HT_2 receptor binding in male, but the effect declined with ageing; • decreased the SERT binding in males on PND 60 (adolescents), but increased SERT binding on PND 100 (adults); • decreased the 5-HT_2 receptor and SERT binding in females.

Figure 1 | Dose, protocol and effects observed in offspring in preclinical studies focused on methadone and dexamethasome (DEX) administration during pregnancy/ early life.
5-HT: Serotonin; GD: gestational day; i.p.: intraperitoneal; IL: interleukin; MDMA: 3,4-methylenedioxymethamphetamine; Myd88: myeloid differentiation primary response protein; PG: pregestational day; PND: postnatal day; s.c.: subcutaneous; SERT: 5-HT transporter; TLR4: cortical toll-like receptor 4; USVs: ultrasonic vocalizations.

Drug	Reference	Protocol applied	Effects
Alcohol	Doremus-Fitzwater et al., 2020	From GD 6 to GD 10, pregnant Long-Evans rat dams were first weaned onto the liquid control diet. From GD 11 to GD 20, pregnant dams had access to **control** liquid diet or to a liquid diet containing 35% of daily calories from **alcohol** (6.7% ethanol vol/vol; total calories = 1.02 kcal/g). At birth, offspring from these dams were cross-fostered to untreated dams and remained with their foster moms until the time of weaning at PND 21. On PND 35 (adolescents) and on PND 90 (adults) rats were acutely challenged with a binge-like dose of **alcohol** (4 g/kg, i.g.) or **tap water**.	Acute alcohol challenge in adolescent male and female rats: • increased the gene expression of Il-6, Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-α in the hippocampus, amygdala and paraventricular nucleus of the hypothalamus; • suppressed the gene expression of Tumor necrosis factor-α in the paraventricular nucleus of the hypothalamus. Acute alcohol challenge in adult male and female rats: • increases the gene expression of Il-6, Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-α in the hippocampus, amygdala and paraventricular nucleus of the hypothalamus; • suppressed the gene expression of Tumor necrosis factor-α in the hippocampus.
	Pascual et al., 2017	For 2 months before mating, female C57BL/6 wild-type and TLR4 knockout mice (C57BL/6 background) received solid diet and either **drinking water** or **10% alcohol** solution (v/v) in their drinking water *ad libitum*. The alcohol concentration in drinking water was progressively increased for the first 2 weeks to finally reach 12.8 ± 1.2 g/kg of body weight. At the conclusion of these 2 months, control and alcohol-treated females were exposed overnight to males, and the appearance of a copulation plug was considered to be GD 0. After mating, pregnant mice were individually housed and dams were maintained with solid diet and either water or 10% alcohol solution during gestation and lactation.	Developmental alcohol in wild-type mice, compared with the respective control group: • upregulated cortical IL-1β, fractalkine and macrophage inflammatory proteins-1α in 15-day-old fetuses; • upregulated cortical IL-1β, fractalkine, monocyte chemoattractant protein-1, macrophage inflammatory proteins-1α, IL-17, increased cerebral microglial markers and downregulated cortical myelin-related proteins on PND 0 (newborns); • upregulated cortical IL-1β, increased cerebral microglial markers and downregulated cortical myelin-related proteins on PND 20; • upregulated cortical IL-1β, increased a cerebral microglial marker and downregulated cortical myelin-related proteins on PND 66. Developmental alcohol in TLR4 knockout mice compared with the corresponding control wild-type group: • upregulated cortical IL-1β, fractalkine, macrophage inflammatory proteins-1α and IL-17 on PND 0 (newborns); • upregulated cortical fractaline on PND 20.

Figure 2 | Dose, protocol and effects observed in offspring in preclinical studies focused on alcohol administration during pregnancy/early life.
GD: Gestational day; i.g.: intragastric; IL: interleukin; PND: postnatal day.

Drug	Reference	Protocol applied	Effects
Nicotine	Abreu-Villaça et al., 2004	From GD 4, pregnant Sprague Dawley rat dams were implanted with osmotic mini pumps for 14 days of continuous **control or nicotine bitartrate** (6 mg/kg s.c.) infusion. From PND 30, male and female offsping were implanted with osmotic mini pumps for 17 days of continuous **control or nicotine bitartrate** (6 mg/kg s.c.) infusion.	Prenatal nicotine administration: • produced cell losses in the midbrain and cerebral cortex, with corresponding elevations in the membrane/total protein ratio. Adolescent nicotine treatment: • produced sex-dependent effects in females, particularly on protein biomarkers. Adolescent nicotine exposure worsened the effects induced by prenatal nicotine exposure.
	Buck et al., 2019	From PG 30, female C57BL/6J mice received **control or nicotine** (200 µg/mL) in place of water for all the duration of their pregnancy, and for other three weeks after delivery (generation F1); these female F1 mice were subsequently mated with drug-naïve male mice in order to produce the F2 generation of mice.	Prenatal nicotine administration: • down-regulated nicotinic acetylcholine receptors in the striatum of F1 mice; • up-regulated nicotinic acetylcholine receptors in the striatum and down-regulated the same receptors in the PFC of F2 mice; • impaired the uptake ability of the dopamine transporter in the striatum and PFC of F2 mice.
	Mychasiuk et al., 2013	On GD 0, Long–Evans rat dams received daily injections of **control or nicotine** (0.3 mg/kg s.c.) until delivery. On PND 60, male and female offsping were treated for 14 days with **control or nicotine** (0.3 mg/kg s.c.).	Prenatal nicotine administration increased: • dendritic complexity of neurons in the AID and NAc of males; • dendritic complexity in Par1 but decreased dendritic complexity of neurons in NAc of females. Adolescent nicotine treatment decreased: • dendritic complexity of neurons in Par1 and PFC but increased dendritic complexity in AID of males; • dendritic complexity in PFC and NAc but increased complexity in AID of females. Prenatal and adolescent nicotine exposure produced additive effects : • in AID of males; • in Par1, PFC and NAc of females.
	Slotkin et al., 2007	On GD 4, pregnant Sprague Dawley rat dams were implanted with osmotic mini pumps for 14 days of continuous **control or nicotine bitartrate** (6 mg/kg s.c.) infusion. On PND 30, male and female offsping were implanted with osmotic mini pumps for 17 days of continuous **control or nicotine bitartrate** (6 mg/kg s.c.) infusion.	Prenatal nicotine administration produced male-specific: • persistent deficits in cerebrocortical choline acetyltransferase activity and hemicholinium-3 binding to the presynaptic choline transporter; • reduction of 5HT_{1A} receptors and upregulation of 5HT_2 receptors. Adolescent nicotine treatment alone produced: • effects that were similar to prenatal nicotine. Prenatal and adolescent nicotine exposure produced effects in female that were similar to those observed after single administration in male.

Figure 3 | Dose, protocol and effects observed in offspring in preclinical studies focused on nicotine administration during pregnancy/early life.
5-HT: Serotonin; AID: dorsal agranular insular cortex; GD: gestational day; NAc: nucleus accumbens; Par1: parietal cortex; PFC: prefrontal cortex; PG: pregestational day; PND: postnatal day; s.c.: subcutaneous.

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