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Review

Advances in animal models of prenatal opioid exposure

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Neonatal opioid withdrawal syndrome (NOWS) is a growing public health concern. The complexity of *in utero* opioid exposure in clinical studies makes it difficult to investigate underlying mechanisms that could ultimately inform early diagnosis and treatments. Clinical studies are unable to dissociate the influence of maternal polypharmacy or the environment from direct effects of *in utero* opioid exposure, highlighting the need for effective animal models. Early animal models of prenatal opioid exposure primarily used the prototypical opioid, morphine, and opioid exposure that was often limited to a narrow period during gestation. In recent years, the number of preclinical studies has grown rapidly. Newer models utilize both prescription and nonprescription opioids and vary the onset and duration of opioid exposure. In this review, we summarize novel prenatal opioid exposure models developed in recent years and attempt to reconcile results between studies while critically identifying gaps within the current literature.

Highlights

Rates of neonatal opioid withdrawal syndrome (NOWS) have been increasing. Acute withdrawal symptoms are well characterized in NOWS infants, but environmental confounds and difficulties in conducting longitudinal studies make it challenging to elucidate potential long-term clinical effects.

Preclinical animal models have great utility for studying the persisting consequences of perinatal opioid exposure and to address the mechanistic basis of these effects.

Several preclinical perinatal opioid exposure models have been developed. Whereas early animal models primarily used the prototypical opioid, morphine, and a limited opioid exposure period, more recent models have been using various opioid types and different durations of perinatal opioid exposure.

Behavioral phenotypes vary across models, reaffirming the importance of dosage, type of opioid, and duration of exposure for the development of specific phenotypes.

From clinical studies to preclinical models of NOWS

The number of individuals diagnosed with opioid use disorders (OUD) continues to increase due to the ongoing global opioid epidemic[i]. Consequently, there is a growing concern around maternal opioid use during pregnancy and subsequent fetal opioid exposure [1]. NOWS is a disorder characterized by opioid withdrawal symptoms that occurs in neonates at birth upon sudden cessation of **prenatal** (see Glossary) exposure to opioids. Withdrawal symptoms typically arise within 24–72 h after birth, and include gastrointestinal, autonomic, and neurological features [2–4]. The rate of infants diagnosed with NOWS in the USA has grown, with recent estimates suggesting increases of 82% from 2010 to 2017 [5]. Diagnosis rates have plateaued since 2014, potentially due to increased medical intervention and successful maternal OUD treatment [6]. Nevertheless, NOWS diagnoses are at an all-time high, and fetal opioid exposure remains an ongoing public health concern [6].

While acute withdrawal symptoms are well characterized, whether *in utero* opioid exposure produces long-term, deleterious effects remains controversial [7]. Some clinical studies examining outcomes in opioid-exposed infants found no notable differences in neurodevelopment [8–10], whereas other longitudinal studies showed prenatal opioid exposure to be associated with impaired physical development and long-term cognitive and behavioral deficits [11–13]. Given that clinical studies are unable to fully dissociate the influence of confounding environmental factors from the direct effects of *in utero* opioid exposure, animal models are necessary. In addition, because the type of opioid, duration of opioid exposure, maternal polysubstance use, and genetic and epigenetic factors may influence the development and severity of NOWS symptoms, all these factors should be considered when developing preclinical models [2,14–16].

Many of the early preclinical studies of **perinatal** opioid exposure used morphine and fetal exposure that occurred within an extremely narrow **gestational** window (reviewed in [17]). In recent

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years, the number of perinatal opioid exposure models has grown significantly. Newer models vary considerably in key components, including type of opioid, duration of opioid exposure, administration route, and maternal environment. Notably, recent models utilize both prescription and nonprescription opioids and used longer durations of exposure, features that better reflect clinical scenarios. In this review, we summarize phenotypes arising from perinatal opioid exposure across preclinical studies, with a focus on studies published since 2017. Studies are grouped by type of opioid used within the model, with the findings for each study further subdivided into developmental, molecular, and behavioral effects. General phenotypic trends and contradictions between studies are noted and used as the basis for recommendations for future studies.

Variability in preclinical models

Route of administration and maternal environment

In rodent studies, opioids can be administered to the dam to induce prenatal exposure or to the pup during the **postnatal** period. Maternal administration can be achieved orally through drinking water, intravenous self-administration (IVSA), injection, or implantable minipumps, while postnatal offspring exposure can occur through lactation or direct injection. Each administration route has specific considerations associated with it, including temporal and dose control of drug intake, potential stress produced by repeated injections, and the volitional nature of drug exposure. Maternal environment and care may also influence outcomes, dependent on whether offspring remain with their birth mothers or are cross-fostered to opioid-naïve dams [18]. There are conflicting data on how opioids may affect maternal care, because some researchers found no effect [19], while others showed deficits in maternal care [20,21]. Furthermore, if pups remain with the opioid-exposed dam throughout the postnatal period, the amount of opioid transferred through lactation may vary, resulting in offspring potentially experiencing premature opioid withdrawal. Cross-fostering also carries additional considerations, because the practice may produce behavioral and physiological alterations in offspring irrespective of opioid exposure [22].

Duration of exposure

Rodent and human development occurs on substantially different timescales. The gestational period in rodents is equivalent to approximately the first two trimesters of human pregnancy [23–25]. The developmental equivalent of the third trimester in human pregnancy occurs within the first two postnatal weeks in rodents [23–25]. Critical neurodevelopmental events peak during this postnatal period in rodents, including synaptogenesis, microglial maturation, myelination, and synaptic pruning [25–27]. Duration of opioid exposure in models varies, and can include pregestation, partial gestation, all of gestation, all of gestation and postnatal exposure, or postnatal exposure only (Figure 1). Different exposure periods are of utility in both understanding the pharmacological effects of opioids on early development and modeling different clinical scenarios. For example, in humans, a person with OUD may cease drug use upon discovery of pregnancy, or transition to medication-assisted therapy (MAT) during pregnancy. As such, there would be further alterations in gestational opioid exposure due to changes in duration of drug use or opioid type.

Opioid pharmacology

One critical variable to consider when modeling prenatal opioid exposure is opioid type. Early studies primarily used morphine, but newer models use a variety of opioids to better explore the heterogeneity that exists within clinical settings. Each opioid has a unique pharmacological profile that may further influence symptomology. Morphine is a short-acting opioid with high affinity for the mu opioid receptor (MOR) and some affinity for the kappa opioid receptor (KOR) and delta opioid receptor (DOR). Oxycodone, an opioid frequently prescribed for pain management, is a semisynthetic opioid with high MOR affinity and lower KOR and DOR affinity [28]. Heroin and

Glossary

Adolescence: in this review, we define 'adolescence' in rodents as being 4–8 weeks of age.
Adulthood: in this review, we define 'adulthood' in rodents as being >8 weeks of age.
Gestational: period of time between conception and birth; in this review, gestational is used interchangeably with prenatal.
Perinatal: any period of time encompassing some (or all) of the gestational period, birth, and postnatal period.
Postnatal: period of time immediately following birth. In this review, the early postnatal period is defined as postnatal days 1–28 in rodents, or from birth until weaning.
Prenatal: period of time between conception and birth; in this review, prenatal is used interchangeably with gestational.

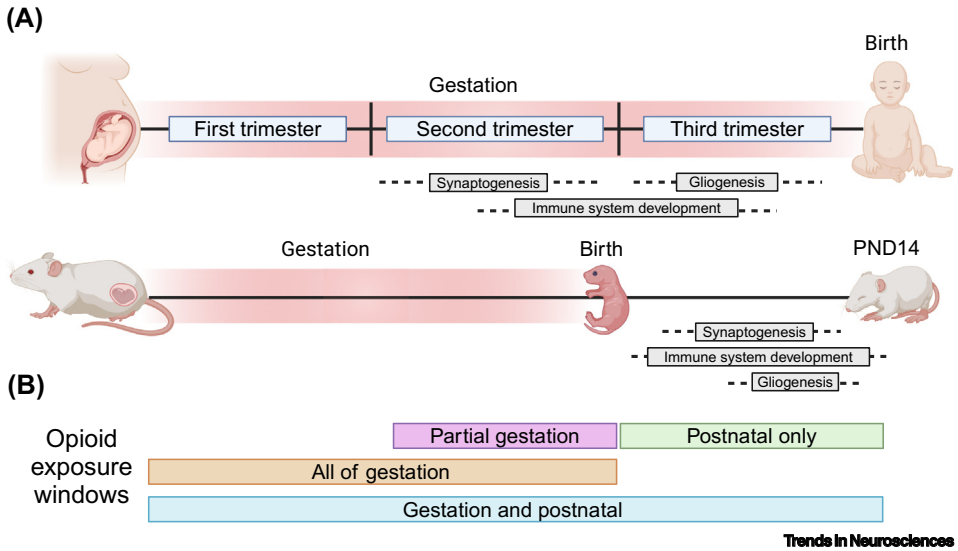

Figure 1. Comparison of human and rodent neurodevelopment and opioid exposure windows. (A) The gestational period of a rodent is approximately equivalent to the first two trimesters of a human pregnancy. Critical neurodevelopmental milestones peak within the second and third trimesters of human pregnancy, while comparable developmental processes in rodents occur throughout the first two postnatal weeks. (B) In preclinical animal models, opioids can be administered throughout different gestational and postnatal timepoints. Figure created with BioRender (biorender.com). Abbreviation: PND, postnatal day.

fentanyl are synthetic opioids commonly used among individuals with OUDs. Heroin is rapidly metabolized into the active metabolite 6-acetylmorphine (6-AM) and then to morphine, both of which have high affinity for MOR [29]. Fentanyl is a short-acting MOR agonist that also binds at KOR and DOR, although with much lower affinity [30]. Buprenorphine and methadone are prescribed as MAT for OUD treatment. Buprenorphine is a partial agonist at MOR and an antagonist at KOR and DOR [31], while methadone is a long-acting MOR agonist [32] (Figure 2).

In addition to differential receptor affinities, pharmacokinetic considerations of each opioid should be noted to account for maternal to fetal drug transfer within models. Most opioids readily cross the blood–placental barrier, although each drug type does so at different rates [44–48]. Both *in vitro* studies using human placentas [49,50] and preclinical studies in rats [51] found that buprenorphine had lower placental transfer compared with other opioids, potentially explaining the lower severity and rates of NOWS in buprenorphine-exposed infants. Models that have quantified opioid levels in rodents at the embryonic and neonatal stage found that morphine, oxycodone, fentanyl, 6-AM, buprenorphine, and methadone can accumulate in the fetus following maternal exposure [51–57]. Breastfeeding is not contraindicated in mothers receiving MAT, because transfer of methadone and buprenorphine through lactation is minimal and nursing may benefit NOWS infants [58–60]. In rodents, postnatal transfer through lactation has been shown to be variable. One study, which delivered postnatal oxycodone through lactation, found that drug levels in rat pups were inconsistent and became negligible by postnatal day (P)14 [19]. Thus, studies that rely on lactation alone must take additional care to ensure that opioid levels in offspring remain steady throughout the duration of the intended exposure period.

Impact of withdrawal

Clinical studies have revealed poor outcomes in opioid-exposed infants even in the absence of receiving a NOWS diagnosis [61,62], suggesting that adverse effects arise from prenatal opioid

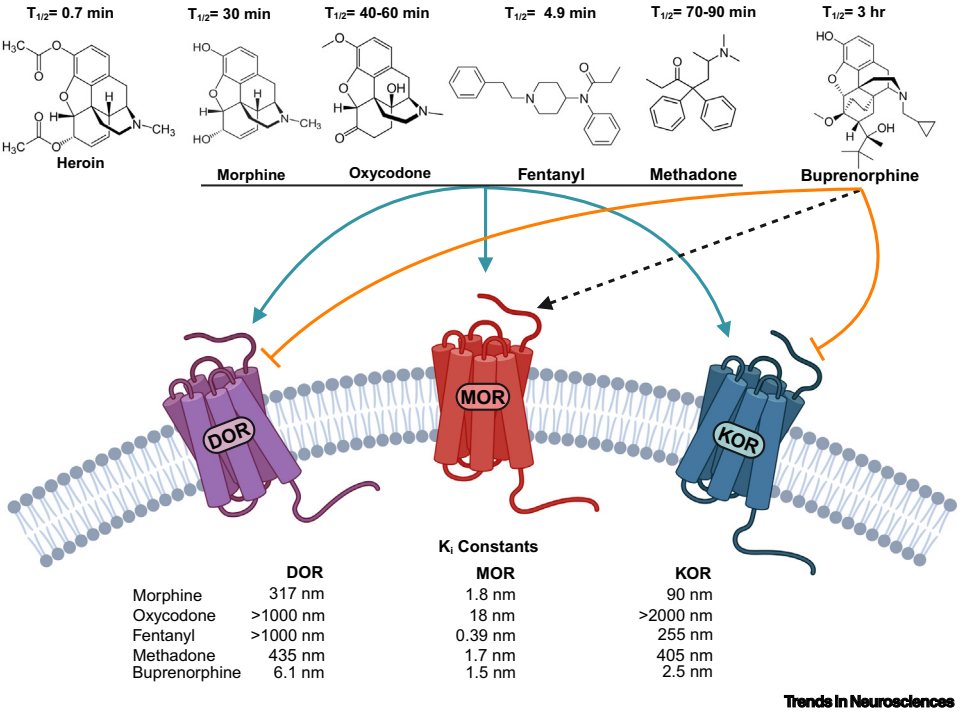

Figure 2. Opioid pharmacology. Schematic of common opioids, half-lives ($T_{1/2}$) in rodents [33–38], and binding affinity at delta opioid receptor (DOR), mu opioid receptor (MOR), and kappa opioid receptor (KOR). Turquoise arrows indicate that the drug is acting as an agonist at the receptor, black broken arrow indicates that the drug is acting as a partial agonist at the receptor, and orange inhibitor arrows indicate that the drug is acting as an antagonist at the receptor. The inhibitory constant (K_i) for each drug is listed in nanomolar (nM) [39–43]. Figure created with BioRender (biorender.com).

exposure alone. However, the potential impact of opioid exposure and withdrawal or the effects of additional postnatal opioid treatment on the infant remain unclear. Thus, there is a need for preclinical studies that model opioid exposure independent of withdrawal and studies that model NOWS directly. Many of the current studies do not indicate whether offspring are experiencing withdrawal. Studies that only administer opioids *in utero* can result in withdrawal around birth, while studies delivering postnatal exposure through injections allow for temporal control of the onset of opioid withdrawal. Other models that continue postnatal opioid exposure through lactation typically do so until weaning. In these studies, pups could experience withdrawal at a later timepoint (typically at P28) or may have tapered off opioids as they begin the transition from feeding through lactation to solid food intake [63]. Future studies can be strengthened by directly addressing these concerns when initially describing a new model. When models are first developed, we recommend that opioid concentrations are measured in offspring throughout the gestational and postnatal periods, and the presence or absence of withdrawal behaviors upon cessation of opioid exposure is clearly documented. In doing so, preclinical studies will be better able to dissociate the effects of opioid exposure from opioid withdrawal.

Morphine models

To broadly characterize how perinatal opioid exposure affects offspring, several models use the prototypical MOR agonist morphine. Morphine is less likely to be misused by humans relative to other opioids. However, its use still has clinical relevance. Morphine is a metabolite of other opioids, including heroin and codeine [64,65]. A recent clinical study found that morphine was among the most abundant opioids detected in a cohort of mothers immediately following

childbirth, and mothers who tested positive for buprenorphine and methadone also frequently tested positive for morphine [66].

Developmental alterations

Models that use morphine show variable effects that may be dependent on duration of exposure or dose. Most models showed that morphine-exposed rodents have lower body weights throughout the first two postnatal weeks [53,67–70], while other models revealed a sex-specific deficit in weight [71] or no deficits in weight [72,73]. Additionally, one study found that exposed rats initially had decreased body length and body weight, but that growth normalized beginning at P28, resulting in weights similar to those of controls in **adulthood** [74]. Multiple studies also found that morphine-exposed mice had increased latencies to reach developmental milestones, including righting reflex and extinguishing of pivoting behavior [53,67,68].

Molecular alterations

Alterations in expression of genes associated with neural development, including synaptic development, myelination, mitochondrial function, and GABAergic and glutamatergic signaling, were prevalent across many brain regions when assayed during both the early postnatal period and **adolescence** in exposed mouse offspring [53,67,70,72,75]. Changes in oxytocin (OT) have been reported, with one study finding alterations in OT levels in the paraventricular nucleus and supraoptic nucleus in rats [73] and another showing upregulation of OT in the medial prefrontal cortex (mPFC) of male mice [72].

Other molecular changes, including increased KOR expression and decreased corticotropin-releasing hormone in the periaqueductal gray in mice [76] and activation of striatal epigenetic histone markers in rats [69], have also been observed. Alterations in neuroimmune signaling have also been noted. Expression of cytokines, microglial markers, and immune genes changed in mice both at birth and in adulthood within the PFC and amygdala [72,77].

Behavioral alterations

Morphine-treated mice displayed increases in anxiety-like behavior when tested during the early postnatal period [67], although changes in affective behavior were not observed when tested in adulthood in a different study [53]. Long-term effects on social preference and play behavior were evident in both mice and rats, although results are conflicting and may be further dependent on sex [53,72,73,77]. These social effects may be due, in part, to the alterations in OT levels [72,73]. Deficits have been observed in the five-choice serial reaction time task (5CSRTT) [72,77,78], whereas other cognitive tasks, including Barnes maze and fear conditioning, were not impacted in mice [70]. This indicates that, within cognitive tasks, attention and impulsivity may be preferentially affected over learning and memory. Female mice displayed alterations in sleep patterns in adulthood, whereas this was not found in males [53]. Of interest, in studies that examined opioid reward behavior, no impact of prenatal morphine exposure was observed in mice [53,68,70], while only one study found blunted development of morphine tolerance in adult rats [74]. A summary of preclinical models utilizing morphine can be found in Table 1 (for the full table, see Table S1 in the supplemental information online).

Oxycodone models

The number of drug-related overdose deaths involving oxycodone has plateaued in recent years; however, a significant number of people still use and misuse prescription opioids[ii]. While many novel preclinical models using oxycodone have been developed, clinical statistics indicate that their translational utility may be more limited relative to other drugs, because it is not one of the most abundant opioids detected in pregnant women [66].

Table 1. Models using morphine[a,b]

Exposure	Outcomes			Refs
	Development	Molecular	Behavioral	
P1–14	↓ Body weight ↑ Latencies to developmental milestones	Enrichments of signaling pathways (P15) Downregulation of myelin-associated transcripts (P15)	↑ Anxiety-like behavior in OF (P21)	[67,70]
All gestation–P7	–	↓ Oxytocin-positive cells at P7, ↑ at P14	Altered play behavior (adolescent)	[73]
All gestation–P14	↓ Body weight ↑ Latencies to developmental milestones	Enrichment of synaptic, GABAergic, and myelin systems (P1, P14)	Altered social behavior (adult) Altered sleep patterns (♀) (adult)	[53]
P1–P14	↓ Body weight ↑ Latencies to developmental milestones	–	↓ Marble burying Alterations in morphine locomotor sensitization (adult)	[68]
P1–P14	–	↑ KOR expression ↓ CRH expression (P14)	–	[76]
Preconception–P21	↑ Weight gain (♂) ↓ Weight gain (♀) (adolescent)	Alterations in immune genes (P1, P21) Differential gene expression (P21) Altered levels of microglia, macrophages (adult)	↑ Social behavior (adolescence) Impaired 5CSRTT (adult)	[72,77]
G19–birth	↓ Body weight (♂) ↑ Body temperature	–	–	[71]
Preconception–P30	↓ Body weight	Differential expression of epigenetic histone modifications, proinflammatory factors (P14) ↓ AMPAR and NMDAR expression (P14, P30)	–	[69,75]
E11–E18	–	–	Impaired 5CSRTT (♂) (adult)	[78]
All gestation–birth	↓ Body weight (normalized by P28) ↓ Tibia length (adult) ↓ Fat mass (♂) (adult)	↑ Peripheral cardiovascular risk biomarkers ↓ Adrenergic receptor expression ↓PENK expression in vascular tissues (adult)	↓ Morphine tolerance	[74]

[a]Abbreviations: CRH, corticotropin-releasing hormone; E, embryonic day; G, gestational day; OF, open field; PENK, proenkephalin; –, not determined.
[b]If sex-specific effect, indicated by ♀/♂, timepoint in which assessment was made is indicated in parentheses.

Developmental alterations

There is mixed evidence for how perinatal oxycodone exposure affects development. Similar to findings with morphine, some studies found lower body weights throughout the postnatal period [20,79,80], sex-specific deficits in weight gain [81], or no differences in body weights in exposed rat and mouse offspring [19,82–85]. Other deficits in physiological development, such as reduction in head diameter and alterations in sensorimotor development, have been observed in rats [19,80,83].

Molecular alterations

Changes in opioid receptor expression induced by perinatal oxycodone exposure have been documented. One study found decreased *Oprm1* at P1 in the midbrain in exposed female rat offspring [83]. A study using mice found that expression of genes related to opioid receptors may be differentially affected in a sex-dependent manner [86]. Males had increases in *Oprd1* and females had decreases in *Oprk1* in the hippocampus (HIP) and hypothalamus, whereas there was no effect on *Oprm1* expression in either sex [86]. Another study found that perinatally exposed male rats had reduced microglial phagocytosis of dopamine receptor type 1 (D1R) in the nucleus accumbens (NAc) in adolescence, resulting in higher D1R density in adulthood [20]. Additional molecular alterations, including changes in extracellular vesicle miRNA signatures, differential

expression of hypocretin neuropeptides, increased neuroinflammation, and alterations in brain metabolites, have been found during the early postnatal period and adolescence across multiple brain regions in rats [80,84,87,88].

Behavioral alterations

Nociception and spatial navigation were not altered in offspring following oxycodone exposure when measured during the early postnatal period in rats [19]. Multiple studies found that exposed mouse and rat pups produced higher ultrasonic vocalizations (USVs) [19,72,86], although it is unclear whether this was representative of increased anxiety-like behavior or a symptom of opioid withdrawal. Additional tests did not find increases in other anxiety or depressive-like behaviors [81,82,86]. However, there is evidence of increased marble burying in oxycodone-exposed rat offspring [80,84], which can be interpreted as either increased anxiety or impulsive behavior [89,90]. Thus, it remains unclear how perinatal oxycodone impacts affective behaviors. Unlike models using other opioids, there is evidence that perinatal oxycodone exposure affects opioid reward behavior. In adulthood, oxycodone-exposed male rats displayed impaired extinction of oxycodone conditioned place preference (CPP) [20], and both male and female mice showed increased oxycodone IVSA [85]. Changes within the dopaminergic and/or opioidergic system may be the molecular basis for these effects on reward behavior [20]. No effects on thermal nociception were observed in rats [84], although increased pain sensitivity using the Von Frey assay has been demonstrated in mice [91]. It is possible that the reported molecular changes within the endogenous opioid system may underlie these effects [83,86]. There are conflicting data regarding effects on social behavior. One study found social deficits in mice [86], while others found no change in mice or rats [80,81]. Reports on activity and motor behavior are similarly variable, with one study reporting hyperactivity in rats [82] and others finding reductions in activity in mice and rats [84,86], or no changes in mice [81]. Evidence for how spatial learning and memory may be affected is also mixed, because only one study in mice found deficits in the Barnes maze [86], while other studies in mice and rats report no deficits in memory or cognitive tasks [79,82].

A summary of NOWS models utilizing oxycodone can be found in Table 2 (for the full table, see Table S2 in the supplemental information online).

Synthetic and semisynthetic opioid models: fentanyl and heroin

Patterns of opioid use and drug-related overdoses have shifted in recent years, given that the number of overdose deaths associated with fentanyl has sharply increased in the USA[ii] [93]. Thus, models that utilize fentanyl are particularly timely. A recent study found that fentanyl and norfentanyl (the inactive metabolite of fentanyl) were among the most prevalent substances detected in a cohort of pregnant women during delivery [66]. Although rates of overdose deaths involving heroin have decreased relative to those involving fentanyl, it remains a common illicit opioid[ii] [93]. Case-studies of infants exposed to heroin *in utero* and reports of heroin use during pregnancy affirm the need for preclinical models using this drug [94,95].

Developmental alterations

As with other opioids, perinatal fentanyl exposure resulted in offspring with lower body weights throughout the early postnatal period, although this effect may be sex specific and dose dependent [96,97]. Mouse offspring exposed to heroin *in utero* did not exhibit any developmental deficits; nevertheless, these results may be confounded by the low amounts of drug delivered within the model [55].

Molecular alterations

Transcriptomic analysis of mice exposed to perinatal fentanyl revealed differentially expressed genes (DEGs) in reward and sensory brain regions [98]. Studies in mice found that perinatal

Table 2. Models using oxycodone[a,b]

Exposure	Outcomes			Refs
	Development	Molecular	Behavioral	
Preconception–weaning		Altered gene expression in HIP and HYP (adult) Alterations in gut microbiota (adult)	↑ Immobility in EPM (adult) ↓ In social behavior (P21) Impairment in Barnes maze (adult)	[86,92]
All gestation–weaning	↓ Body weight post weaning ↑ Latency to righting reflex (♀)	–	Altered USVs (P5–P11) Altered thermal nociception (♀) (adult)	[79,81]
G8–G21, P1,3,5	–	–	Hyperactivity (adult)	[82]
Preconception–birth	↓ Body weight ↑ latency to righting reflex ↓ USVs	↓ Microglial engulfment of D1R (♂) (adolescent) ↑ D1R density (♂) (adult)	Impaired extinction in oxycodone CPP (♂) (adult)	[20]
	–	↓ *OPRM1* expression (♀) (P1)	–	[83]
Preconception–P14	–	–	Altered separation-induced USVs (P8)	[19]
Preconception–weaning	↓ Body weight ↓ Head diameter	Altered neuropeptide expression, synaptic vesicle proteosome, miRNA signatures (P14) ↑ Iba1+ cells Altered brain metabolites Impaired synaptic mitochondrial function (adolescent)	↑ Marble burying (adult) Alterations in social behavior (adult) ↓ Pain threshold (adult) ↓ Motor function (adult)	[80,84,87,88,91]
	–	–	↑ Oxycodone self-administration (adult)	[85]

[a]Abbreviations: G, gestational day; EPM, elevated plus maze; HYP, hypothalamus; –, not determined.
[b]If sex-specific effect, indicated by ♀/♂, timepoint in which assessment was made indicated by parentheses.

fentanyl exposure altered circuitry and synaptic transmission in cortical areas, including the primary somatosensory cortex, anterior cingulate cortex, and auditory cortex [99–101]. Additionally, morphological changes of pyramidal neurons in these regions were also observed in mice [100]. Expression of mitochondrial-related genes within the NAc may also be affected by fentanyl exposure in mice [102]. Mice exposed to heroin *in utero* did not show any differences in MOR binding at P1 [55].

Behavioral alterations

In mice, perinatal fentanyl exposure has been shown to alter locomotion, affective behavior, and produce sensory maladaptation in adolescence [97,100,101]. Fentanyl-exposed mice also exhibited impaired auditory discrimination and lower levels of engagement in auditory tasks in adulthood [97]. These behavioral deficits are hypothesized to result from alterations within the somatosensory cortex and auditory cortex, which were observed using the same model [97,100]. In late adolescence, fentanyl-exposed mice displayed altered morphine-induced antinociception, but did not show differences in opioid reward [96]. Heroin-exposed female mice displayed hyperactivity and heroin-induced locomotor sensitization [55].

A summary of NOWS models utilizing fentanyl and heroin can be found in Table 3 (for the full table, see Table S3 in the supplemental information online).

MAT models: buprenorphine and methadone

Pregnant individuals diagnosed with OUDs may receive buprenorphine or methadone as treatment [95,103,104]. A recent clinical study found that buprenorphine and the active metabolite

Table 3. Models using synthetic and semisynthetic opioids[a,b]

Exposure	Outcomes			Refs
	Development	Molecular	Behavioral	
Fentanyl: P4–P9	↓ Body weight	–	↓ Baseline analgesic response ↑ Morphine-induced hyperalgesia (adult)	[96]
Fentanyl: all gestation–P21	Sex- and age-dependent alterations in body weight	Transcriptomic changes in reward and sensory brain regions (adolescent) Alterations in mitochondrial-related gene expression (adolescent) Altered pyramidal neuron morphology (adolescent) Deficits in S1 function (adolescent) Alterations in A1 function (adult)	↑ Anxiety-like behaviors (adolescent) Impairment in auditory discrimination task (adult) Hyperactivity Sensory maladaptation (adolescent)	[97–102]
Heroin: E12, E15, E18	–	–	Hyperactivity ↑ Heroin-induced locomotion (♀) (adolescent)	[55]

[a]Abbreviations: A1, primary auditory cortex; E, embryonic; S1, primary somatosensory cortex; –, not determined.
[b]If sex-specific effect, indicated by ♀/♂, timepoint in which assessment was made indicated in parentheses.

norbuprenorphine were among the most prevalent substances detected in a cohort of pregnant women during childbirth [66]. Furthermore, there is emerging evidence that maternal treatment with buprenorphine is associated with more favorable outcomes and fewer NOWS diagnoses compared with infants born to mothers receiving methadone treatment [105,106]. Given these statistics, it may be more clinically relevant for models to use buprenorphine over methadone. However, pharmacokinetic considerations may favor the use of methadone. Methadone concentrations were found to be much higher than those of buprenorphine in rodent fetal tissue following maternal administration [51]. Although we believe achieving sufficient fetal opioid concentrations should be prioritized when developing models of perinatal exposure, studies using both buprenorphine and methadone are critical. There is widespread use of both drugs during pregnancy, and preclinical studies using MAT may serve to guide clinical recommendations. Some models also include a prepregnancy exposure period using a different opioid, which closely reflects a clinical scenario in which a patient with OUD transitions to MAT upon discovery of pregnancy [57,107–112]. Although this practice is not standard in developing models that use methadone or buprenorphine, it should be considered and perhaps more widely adopted because it produces a more translationally relevant model.

Developmental alterations

Developmental deficits have been documented in both methadone and buprenorphine-exposed mice, including lower weights throughout development, changes in body temperature, and increased latencies to reach developmental milestones [21,51,56,57,113]. Other developmental deficits in methadone-exposed offspring have been characterized, including lower brain weight, decreased bone volume, hyperactivity, and increases in USVs [57,114]. Buprenorphine-exposed mice showed molecular and cellular differences in the developing brain during gestation, including decreases in neural progenitor cell proliferation, reductions in cortical thickness, and decreases in corticogenesis [112].

Molecular alterations

Widespread molecular changes have been observed following methadone exposure in rats, including reduction of brain-derived neurotrophic factor (BDNF) and GABAergic proteins in the cortex and HIP, resulting in decreased excitability in dentate gyrus (DG) cells [115,116]. Other processes that impact neuronal excitability, such as myelination, were also affected by prenatal

methadone exposure. Reports of global attenuation of myelin revealed decreased levels of myelin-specific proteins and accelerated apoptosis of oligodendrocytes during the early postnatal period in rats [117,118]. Studies in mice showed alterations in synaptic function and circuitry in cortical regions, primarily within the primary motor cortex and somatosensory cortex, and in the dorsal striatum [57,107–109]. Furthermore, methadone exposure was found to reduce functional brain-wide connectivity in mice [119]. A growing area of research focuses on alterations in immune activation induced by perinatal opioid exposure. Studies in rats using methadone found that offspring had increased cytokines and immune markers in the brain and periphery, as well as altered reactivity to immune challenges [56,120,121]. Decreased MOR binding in the early postnatal period was shown after both perinatal buprenorphine and methadone exposure in rats [122]. There is less research investigating the molecular alterations induced by buprenorphine exposure, although one study found that mice had an increase in tyrosine hydroxylase-positive neurons in the ventral tegmental area in adulthood [112].

Behavioral alterations

Cognitive deficits involving learning and memory have been well documented in models of methadone exposure, although they have not been demonstrated in buprenorphine models [77,113,115,116,123]. These cognitive impairments may arise from the decreased excitability in HIP DG cells following prenatal methadone exposure [115,116]. There is also evidence that perinatal methadone exposure alters response to alcohol reward in mice; however, it is unclear how reward circuitry may be affected or whether this phenotype extends to other psychoactive drugs [110]. Fewer behaviors are impacted following buprenorphine. When tested in adolescence, there were no effects of exposure on anxiety-like behaviors or stress response in rats [21], but male mice exposed to buprenorphine *in utero* displayed hyperactivity at P60 [112]. Buprenorphine-exposed mice displayed enhanced baseline nociceptive sensitivity during the hotplate test, but did not differ from controls when given an injection of morphine [21].

A summary of NOWS models utilizing buprenorphine and methadone can be found in Table 4 (for the full table, see Table S4 in the supplemental information online).

Phenotypic commonalities across models

Models discussed in this review use a wide range of exposure paradigms and exhibit varying phenotypes. Nevertheless, some phenotypic trends persist across multiple studies. Developmental delays and lower body weights are generally observed, regardless of the opioid used or duration of exposure. Recurring themes in molecular alterations include changes in synaptic development, as well as GABAergic and glutamatergic signaling. Long-term alterations in the dopaminergic system were found in studies using both oxycodone [20] and buprenorphine [112]. There is also converging evidence that systemic immune and neuroimmune function may be altered by prenatal opioid exposure, specifically arising from alterations in microglia and cytokines [56,72,120,121]. Opioids are known immunomodulators [124]. This may underlie clinical evidence showing that children diagnosed with NOWS may have immune dysfunction [125–127]. While additional preclinical studies should be conducted to better understand the scope of these effects, investigations on opioid-induced immunomodulation are an emerging and clinically relevant direction for research.

There are few reported behavioral phenotypes consistently reproduced across multiple models. Perhaps most surprising is the paucity of data supporting altered drug taking behavior, given concerns that early-life drug exposure predisposes an individual to substance use later in life. Of all models discussed in this review, only two studies using oxycodone found opioid reward response and drug taking to be affected. One potential explanation is that environment, rather

Table 4. Models using MAT[a,b]

Exposure	Outcomes			Refs
	Development	Molecular	Behavioral	
MET: preconception–P28	↓ Body weight ↓ Body length Altered behavioral development	↓ Cell density, altered neuronal properties in M1 and S1 (P22) Altered glutamatergic and endocannabinoid signaling (adolescent) Altered protein expression in M1 (P21–P36) Altered anatomical and functional connectivity (adult)	↑ Separation-induced USVs (♀) (P7) Altered sensitivity to alcohol reward (adult)	[57,107–111]
MET: G3–G20	–	Altered hippocampal DG cell function (♀) (adult)	Impaired NOR Impaired spontaneous alteration (♀) (adult) Impaired FC (♀) (adult)	[115,116]
MET: preconception–P14	↓ Body weight ↓ Brain weight (P1)	↓ BDNF and GABAergic proteins in PFC and HIP (adolescent)	↑ Center time in OF Impairment in NOR Impairment in T-maze (♀) (adolescent)	[113]
MET: all gestation–P21	–	↓ MOR binding (P1–P21)	Impairment in discrimination task, NOR, MWM (adult)	[122,123]
MET: G7–P19	–	Attenuation of myelin development ↑ Apoptosis of oligodendrocytes, microglial activation (P7) ↓ Expression of myelin proteins (P7)	–	[117,118]
MET: E16–P21	↓ Body weight Altered brain structure (P21)	↑ Serum and brain cytokines (P10, P21) Altered microglia morphology (P10) Hypersecretion of inflammatory cytokines in PBMCs at baseline and post LPS	Impairments in discrimination and learning task (adult)	[56,120,121]
MET: G50–birth	–	↑ Plasma cortisol levels (P2)	–	[114]
BUP: E0.5–birth	↓ Embryonic body width	↓ Neural progenitor cell proliferation Altered corticogenesis (E18.5) ↑ Th+ cells in VTA (adult)	Hyperactivity (adult)	[112]
BUP: preconception–P21	↓ Body weight ↑ Latencies to developmental milestones ↓ Body temperature	–	↓ Pain sensitivity (adolescent)	[21]
BUP: preconception	↑ Body weight (P19) ↓ Weight gain (♀) (Adolescent)	–	–	[77]

[a]Abbreviations: BUP, buprenorphine; E, embryonic day; FC, fear conditioning; G, gestational day; LPS, lipopolysaccharide; M1, primary motor cortex; MET, methadone; MWM, Morris water maze; NOR, novel object recognition; OF, open field; PBMC, peripheral blood mononuclear cell; S1, primary somatosensory cortex; TH, tyrosine hydroxylase; VTA, ventral tegmental area.
[b]If sex-specific effect, indicated by ♀/♂, timepoint in which assessment was made indicated in parentheses.

than perinatal opioid exposure, may have a more critical role in the development of substance use disorders.

Notable contradictory results arise across models. Multiple studies using methadone and morphine produced robust cognitive deficits, but most studies using oxycodone failed to show any effects in a battery of cognitive tasks. Similarly, models using oxycodone and fentanyl showed some increases in anxiety-like behavior, but studies using morphine, methadone, and buprenorphine generally did not observe any effects on affective behavior. The basis for these differences remains unclear. In all cases, the studies utilized different doses, types of opioid, and exposure paradigms. Discrepancies in results are probably not due to a single factor, but rather the convergence of multiple variables. These differences across models reaffirm the significance of dosage, type of opioid, and duration of exposure for development of phenotypes.

It remains difficult to reconcile molecular and behavioral data across models of perinatal opioid exposure. Many studies have investigated the effects of perinatal opioid exposure on molecular *or* behavior alterations exclusively, while only a few have explored the molecular underpinnings of behavior within the same model. Furthermore, due to substantial differences between models, it is almost impossible to attribute molecular changes uncovered in one study to behavioral phenotypes produced in other, even across studies using the same type of opioid.

Concluding remarks and future directions

Although many advances have been made in studying the effects of prenatal opioid exposure, it is essential to recognize the limitations of current studies (see Outstanding questions). For instance, the effects of maternal polysubstance use represent a notable gap in the current literature and remain to be studied. Other prescription and nonprescription psychoactive drugs, including alcohol, benzodiazepines, selective serotonin reuptake inhibitors (SSRIs), cannabis, and nicotine, are known to exacerbate NOWS [128–130] Clinical studies have found a large proportion of pregnant women who test positive for opioids also test positive for other substances [130–132], but the potential effects on offspring remain unclear. In addition to opioid exposure, there are also many potential variables that may affect infant development after a NOWS diagnosis, including genetic factors, socioeconomic status, familial history of other disorders, and environment. While it is not possible to model all variables in laboratory animals, the introduction of additional variables, such as addiction-associated genetic variants or maternal and/or offspring stress, could provide an interesting and unique lens from which to study prenatal opioid exposure. One recent study delivered a 'secondary stressor' to rats following opioid exposure by inducing minor traumatic brain injury and found that it further exacerbated NOWS outcomes [84]. Another study in rats found that adolescent nicotine exposure following perinatal oxycodone exposure did not significantly alter offspring outcomes [133]. The full scope of the effects of future stressors on opioid exposed offspring have yet to be explored.

In addition to effects in the central nervous system (CNS), recent studies have begun to investigate the influence of perinatal opioid exposure on the periphery. Peripheral immune dysfunction [121], changes in gut microbiota [92,134], and cardiovascular and metabolic alterations [74] have been noted across different models. Future studies should be conducted to explore additional systemic changes arising beyond the CNS.

Numerous clinical studies have been conducted to assess treatment options in NOWS infants, although there remains no standard of care across hospitals. First-line pharmacological treatment is often opioid replacement therapy with oral morphine or methadone [14,15], although emerging evidence supports buprenorphine as a more efficacious treatment [135–139]. Unlike most disorders, in which preclinical treatment studies pre-date and far outnumber controlled clinical trials, few studies analyze the efficacy of treatments in animal models of prenatal opioid exposure. A small number of preclinical animal studies have utilized opioid-based pharmacological treatments to reduce withdrawal symptoms and early-life deficits, although efficacy in treating potential long-term phenotypes remains unclear [55,76,114]. In animal models, nonopioidergic treatments, including clonidine (an α_2-adrenergic agonist [140]) and environmental enrichment, have also been shown to ameliorate some NOWS-related deficits [101,141].

When considering the complexity of prenatal opioid exposure, no one standard animal model will be sufficient to account for all variables. While this may provide some challenges in reconciling results and data interpretation, each model provides a unique vantage point in modeling different aspects and conditions of *in utero* opioid exposure. Human NOWS is heterogeneous, and individual diagnoses may present under different circumstances or with varying symptoms. Thus,

Outstanding questions

General phenotypic patterns can be drawn from preclinical studies of neonatal opioid exposure, although it remains difficult to reconcile results due to variability between animal models. Given that each opioid has different pharmacokinetic and pharmacodynamic properties, altering opioid type or dose may affect experimental outcomes. How can consideration of dose and opioid type be used to better compare preclinical data?

It remains unclear what impact, if any, opioid withdrawal may have on producing persisting phenotypes beyond the perinatal period. Do long-term consequences of prenatal opioid exposure arise from exposure alone, or from the combination of opioid exposure and withdrawal?

Some pregnant individuals who test positive for opioids also test positive for additional psychoactive substances. Preclinical NOWS models have yet to examine the possible outcomes of polysubstance exposure. How does prenatal polysubstance exposure affect NOWS phenotypes?

How do genetic factors influence the development and severity of NOWS?

How do additional environmental factors, such as maternal stress, infection, or injury, affect NOWS phenotypes, and how can this be incorporated into preclinical models?

Many clinical studies have been conducted to assess both pharmacological and nonpharmacological treatments for NOWS symptoms, although preclinical studies assessing treatments are largely lacking. How can preclinical NOWS models be used to study potential therapies?

the diversity in preclinical models provides an advantage when attempting to elucidate the many potential clinical outcomes arising from prenatal opioid exposure.

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Declaration of interests

The authors declare no competing interests in relation this work.

Resources

[i]www.unodc.org/unodc/en/data-and-analysis/world-drug-report-2023.html

[ii]www.cdc.gov/drugoverdose/deaths/opioid-overdose.html

Supplemental information

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References

1. Elmore, A.L. *et al.* (2021) Prescription opioid use among women of reproductive age in the United States: NHANES, 2003-2018. *Prev. Med.* 153, 106846
2. Sutter, M.B. *et al.* (2014) Neonatal opioid withdrawal syndrome. *Obstet. Gynecol. Clin. N. Am.* 41, 317–334
3. Kraft, W.K. *et al.* (2016) Neonatal abstinence syndrome: pharmacologic strategies for the mother and infant. *Semin. Perinatol.* 40, 203–212
4. Weller, A.E. *et al.* (2021) Neonatal Opioid Withdrawal Syndrome (NOWS): a transgenerational echo of the opioid crisis. *Cold Spring Harb. Perspect. Med.* 11, a039669
5. Hirai, A.H. *et al.* (2021) Neonatal abstinence syndrome and maternal opioid-related diagnoses in the US, 2010-2017. *JAMA* 325, 146–155
6. Leech, A.A. *et al.* (2020) Neonatal abstinence syndrome in the United States, 2004-16. *Health Aff. (Millwood)* 39, 764–767
7. Maguire, D.J. *et al.* (2016) Long-term outcomes of infants with neonatal abstinence syndrome. *Neonatal Netw.* 35, 277–286
8. Kaltenbach, K. and Finnegan, L.P. (1984) Developmental outcome of children born to methadone maintained women: a review of longitudinal studies. *Neurobehav. Toxicol. Teratol.* 6, 271–275
9. Kaltenbach, K. and Finnegan, L.P. (1987) Perinatal and developmental outcome of infants exposed to methadone in-utero. *Neurotoxicol. Teratol.* 9, 311–313
10. Kaltenbach, K. *et al.* (2018) Prenatal exposure to methadone or buprenorphine: early childhood developmental outcomes. *Drug Alcohol Depend.* 185, 40–49
11. Wilson, G.S. *et al.* (1979) The development of preschool children of heroin-addicted mothers: a controlled study. *Pediatrics* 63, 135–141
12. Nygaard, E. *et al.* (2016) Behavior and attention problems in eight-year-old children with prenatal opiate and polysubstance exposure: a longitudinal study. *PLoS One* 11, e0158054
13. Kilbey, M.M. and Khurshed, A., eds (1991) *Methodological Issues in Controlled Studies on Effects of Prenatal Exposure to Drug Abuse*, National Institute on Drug Abuse
14. Logan, B.A. *et al.* (2013) Neonatal abstinence syndrome: treatment and pediatric outcomes. *Clin. Obstet. Gynecol.* 56, 186–192
15. Reddy, U.M. *et al.* (2017) Opioid use in pregnancy, neonatal abstinence syndrome, and childhood outcomes: executive summary of a joint workshop by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, American College of Obstetricians and Gynecologists, American Academy of Pediatrics, Society for Maternal-Fetal Medicine, Centers for Disease Control and Prevention, and the March of Dimes Foundation. *Obstet. Gynecol.* 130, 10–28
16. Wachman, E.M. and Farrer, L.A. (2019) The genetics and epigenetics of neonatal abstinence syndrome. *Semin. Fetal Neonatal Med.* 24, 105–110
17. Byrnes, E.M. and Vassoler, F.M. (2018) Modeling prenatal opioid exposure in animals: current findings and future directions. *Front. Neuroendocrinol.* 51, 1–13
18. Myers, A.M. *et al.* (2023) Maternal care behavior and physiology moderate offspring outcomes following gestational exposure to opioids. *Dev. Psychobiol.* 65, e22433
19. Zanni, G. *et al.* (2021) Maternal continuous oral oxycodone self-administration alters pup affective/social communication but not spatial learning or sensory-motor function. *Drug Alcohol Depend.* 221, 108628
20. Smith, C.J. *et al.* (2022) Prenatal opioid exposure inhibits microglial sculpting of the dopamine system selectively in adolescent male offspring. *Neuropsychopharmacology* 47, 1755–1763
21. Wallin, C.M. *et al.* (2019) Gestational buprenorphine exposure: effects on pregnancy, development, neonatal opioid withdrawal syndrome, and behavior in a translational rodent model. *Drug Alcohol Depend.* 205, 107625
22. Bartolomucci, A. *et al.* (2004) Cross fostering in mice: behavioral and physiological carry-over effects in adulthood. *Genes Brain Behav.* 3, 115–122
23. Dobbing, J. and Sands, J. (1979) Comparative aspects of the brain growth spurt. *Early Hum. Dev.* 3, 79–83
24. Barr, G.A. *et al.* (2011) Changing mechanisms of opiate tolerance and withdrawal during early development: animal models of the human experience. *ILAR J.* 52, 329–341
25. Semple, B.D. *et al.* (2013) Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog. Neurobiol.* 106-107, 1–16
26. Zeiss, C.J. (2021) Comparative milestones in rodent and human postnatal central nervous system development. *Toxicol. Pathol.* 49, 1368–1373
27. Clancy, B. *et al.* (2007) Extrapolating brain development from experimental species to humans. *Neurotoxicology* 28, 931–937
28. Ordonez Gallego, A. *et al.* (2007) Oxycodone: a pharmacological and clinical review. *Clin. Transl. Oncol.* 9, 298–307
29. Sawynok, J. (1986) The therapeutic use of heroin: a review of the pharmacological literature. *Can. J. Physiol. Pharmacol.* 64, 1–6
30. Suzuki, J. and El-Haddad, S. (2017) A review: fentanyl and non-pharmaceutical fentanyls. *Drug Alcohol Depend.* 171, 107–116
31. Gudin, J. and Fudin, J. (2020) A narrative pharmacological review of buprenorphine: a unique opioid for the treatment of chronic pain. *Pain Ther.* 9, 41–54
32. Walsh, S.L. and Strain, E.C. (2006) Pharmacology of methadone. In *The Treatment of Opioid Dependence* (Strain, E.C. and Stitzer, M.L., eds), pp. 59–76, John Hopkins University Press

33. Lee, J.C. *et al.* (2022) Development of an effective monoclonal antibody against heroin and its metabolites reveals therapies have mistargeted 6-monoacetylmorphine and morphine over heroin. *ACS Cent. Sci.* 8, 1464–1470
34. Handal, M. *et al.* (2002) Pharmacokinetic differences of morphine and morphine-glucuronides are reflected in locomotor activity. *Pharmacol. Biochem. Behav.* 73, 883–892
35. Chan, S. *et al.* (2008) Sex differences in the pharmacokinetics, oxidative metabolism and oral bioavailability of oxycodone in the Sprague-Dawley rat. *Clin. Exp. Pharmacol. Physiol.* 35, 295–302
36. Kalvass, J.C. *et al.* (2007) Pharmacokinetics and pharmacodynamics of seven opioids in P-glycoprotein-competent mice: assessment of unbound brain EC50,u and correlation of in vitro, preclinical, and clinical data. *J. Pharmacol. Exp. Ther.* 323, 346–355
37. Ling, G.S. *et al.* (1981) Methadone: radioimmunoassay and pharmacokinetics in the rat. *J. Pharmacol. Exp. Ther.* 217, 147–151
38. Yu, S. *et al.* (2006) Pharmacokinetics of buprenorphine after intravenous administration in the mouse. *J. Am. Assoc. Lab. Anim. Sci.* 45, 12–16
39. Corbett, A.D. *et al.* (1993) Selectivity of ligands for opioid receptors. In *Opioids* (Herz, A. *et al.*, eds), pp. 645–679, Springer
40. Ben Haddou, T. *et al.* (2014) Exploring pharmacological activities and signaling of morphinans substituted in position 6 as potent agonists interacting with the mu opioid receptor. *Mol. Pain* 10, 48
41. Raynor, K. *et al.* (1994) Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. *Mol. Pharmacol.* 45, 330–334
42. Codd, E.E. *et al.* (1995) Serotonin and norepinephrine uptake inhibiting activity of centrally acting analgesics: structural determinants and role in antinociception. *J. Pharmacol. Exp. Ther.* 274, 1263–1270
43. Toll, L. *et al.* (1998) Standard binding and functional assays related to medications development division testing for potential cocaine and opiate narcotic treatment medications. *NIDA Res. Monogr.* 178, 440–466
44. Kokki, M. *et al.* (2012) Intravenous oxycodone for pain relief in the first stage of labour–maternal pharmacokinetics and neonatal exposure. *Basic Clin. Pharmacol. Toxicol.* 111, 182–188
45. Lam, J. *et al.* (2012) Central nervous system depression of neonates breastfed by mothers receiving oxycodone for postpartum analgesia. *J. Pediatr.* 160, 33–37
46. Shum, S. *et al.* (2021) Predicting maternal-fetal disposition of fentanyl following intravenous and epidural administration using physiologically based pharmacokinetic modeling. *Drug Metab. Dispos.* 49, 1003–1015
47. Iwersen, S. *et al.* (1998) Evidence of gestational heroin exposure by comparative analysis of fetal and maternal body fluids, tissues, and hair in a heroin-related death. *J. Anal. Toxicol.* 22, 296–298
48. Concheiro, M. *et al.* (2010) Maternal buprenorphine dose, placenta buprenorphine, and metabolite concentrations and neonatal outcomes. *Ther. Drug Monit.* 32, 206–215
49. Nanovskaya, T. *et al.* (2002) Transplacental transfer and metabolism of buprenorphine. *J. Pharmacol. Exp. Ther.* 300, 26–33
50. Gordon, A.L. *et al.* (2010) (R)- and (S)-methadone and buprenorphine concentration ratios in maternal and umbilical cord plasma following chronic maintenance dosing in pregnancy. *Br. J. Clin. Pharmacol.* 70, 895–902
51. Kongstorp, M. *et al.* (2019) High accumulation of methadone compared with buprenorphine in fetal rat brain after maternal exposure. *J. Pharmacol. Exp. Ther.* 371, 130–137
52. Griffin, B.A. *et al.* (2019) In utero exposure to norbuprenorphine, a major metabolite of buprenorphine, induces fetal opioid dependence and leads to neonatal opioid withdrawal syndrome. *J. Pharmacol. Exp. Ther.* 370, 9–17
53. Dunn, A.D. *et al.* (2023) Molecular and long-term behavioral consequences of neonatal opioid exposure and withdrawal in mice. *Front. Behav. Neurosci.* 17, 1202099
54. Craft Jr., J.B. *et al.* (1983) Placental passage and uterine effects of fentanyl. *Anesth. Analg.* 62, 894–898
55. Kvello, A.M.S. *et al.* (2019) A monoclonal antibody against 6-acetylmorphine protects female mice offspring from adverse behavioral effects induced by prenatal heroin exposure. *J. Pharmacol. Exp. Ther.* 368, 106–115
56. Jantzie, L.L. *et al.* (2020) Prenatal opioid exposure: the next neonatal neuroinflammatory disease. *Brain Behav. Immun.* 84, 45–58
57. Grecco, G.G. *et al.* (2021) Prenatal methadone exposure disrupts behavioral development and alters motor neuron intrinsic properties and local circuitry. *Elife* 10, e66230
58. Pritham, U.A. (2013) Breastfeeding promotion for management of neonatal abstinence syndrome. *J. Obstet. Gynecol. Neonatal. Nurs.* 42, 517–526
59. Welle-Strand, G.K. *et al.* (2013) Breastfeeding reduces the need for withdrawal treatment in opioid-exposed infants. *Acta Paediatr.* 102, 1060–1066
60. Wojnar-Horton, R.E. *et al.* (1997) Methadone distribution and excretion into breast milk of clients in a methadone maintenance programme. *Br. J. Clin. Pharmacol.* 44, 543–547
61. Hall, E.S. *et al.* (2019) Developmental disorders and medical complications among infants with subclinical intrauterine opioid exposures. *Popul. Health Manag.* 22, 19–24
62. Leyenaar, J.K. *et al.* (2021) Infant mortality associated with prenatal opioid exposure. *JAMA Pediatr.* 175, 706–714
63. König, B. and Markl, H. (1987) Maternal care in house mice. *Behav. Ecol. Sociobiol.* 20, 1–9
64. Jakobsson, G. *et al.* (2021) Heroin-related compounds and metabolic ratios in postmortem samples using LC-MS-MS. *J. Anal. Toxicol.* 45, 215–225
65. Milone, M.C. (2012) Laboratory testing for prescription opioids. *J. Med. Toxicol.* 8, 408–416
66. Smith, B.L. *et al.* (2022) Rates of substance and polysubstance use through universal maternal testing at the time of delivery. *J. Perinatol.* 42, 1026–1031
67. Borrelli, K.N. *et al.* (2021) Sex differences in behavioral and brainstem transcriptomic neuroadaptations following neonatal opioid exposure in outbred mice. *eNeuro* 8, ENEURO.0143-21.2021
68. Robinson, S.A. *et al.* (2020) Neurobehavioral effects of neonatal opioid exposure in mice: influence of the OPRM1 SNP. *Addict. Biol.* 25, e12806
69. Wu, P.L. *et al.* (2021) Enhanced H3K4 trimethylation in TNF-alpha promoter gene locus with cell apoptosis in the ventral-medial striatum following opioid withdrawal of neonatal rat offspring from morphine-addicted mothers. *Mediat. Inflamm.* 2021, 9828995
70. Borrelli, K.N. *et al.* (2023) Decreased myelin-related gene expression in the nucleus accumbens during spontaneous neonatal opioid withdrawal in the absence of long-term behavioral effects in adult outbred CFW mice. *Neuropharmacology* 240, 109732
71. Stevens, S. and Mohan, S. (2021) Opioid withdrawal behavior in spiny mice: a novel preclinical model of neonatal opioid withdrawal syndrome (NOWS). *Heliyon* 7, e06694
72. Smith, B.L. *et al.* (2022) Perinatal morphine exposure leads to sex-dependent executive function deficits and microglial changes in mice. *eNeuro* 9, ENEURO.0238-22.2022
73. Harder, H.J. *et al.* (2023) Perinatal opioid exposure leads to decreased social play in adolescent male and female rats: potential role of oxytocin signaling in brain regions associated with social reward. *Horm. Behav.* 153, 105384
74. Ahmed, N. *et al.* (2023) Prenatal morphine exposure increases cardiovascular disease risk and programs neurogenic hypertension in the adult offspring. *Hypertension* 80, 1283–1296
75. Wu, P.L. *et al.* (2018) Long-lasting alterations in gene expression of postsynaptic density 95 and inotropic glutamatergic receptor subunit in the mesocorticolimbic system of rat offspring born to morphine-addicted mothers. *Biomed. Res. Int.* 2018, 5437092
76. Lepore, G. *et al.* (2023) Buprenorphine reduces somatic withdrawal in a mouse model of early-life morphine exposure. *Drug Alcohol Depend.* 248, 109938
77. Smith, B.L. *et al.* (2023) Perinatal morphine but not buprenorphine affects gestational and offspring neurobehavioral outcomes in mice. *Neurotoxicology* 99, 292–304
78. Alaee, E. *et al.* (2021) Prenatal exposure to morphine impairs attention and impulsivity in adult rats. *Psychopharmacology* 238, 2729–2741

79. Minakova, E. *et al.* (2021) Ontogenetic oxycodone exposure affects early life communicative behaviors, sensorimotor reflexes, and weight trajectory in mice. *Front. Behav. Neurosci.* 15, 615798
80. Odegaard, K.E. *et al.* (2020) Characterization of the intergenerational impact of in utero and postnatal oxycodone exposure. *Transl. Psychiatry* 10, 329
81. Minakova, E. *et al.* (2022) Perinatal oxycodone exposure causes long-term sex-dependent changes in weight trajectory and sensory processing in adult mice. *Psychopharmacology (Berl)* 239, 3859–3873
82. Sithisarn, T. *et al.* (2017) The effects of perinatal oxycodone exposure on behavioral outcome in a rodent model. *Front. Pediatr.* 5, 180
83. Vassoler, F.M. *et al.* (2018) Oxycodone self-administration during pregnancy disrupts the maternal-infant dyad and decreases midbrain OPRM1 expression during early postnatal development in rats. *Pharmacol. Biochem. Behav.* 173, 74–83
84. Gowen, A.M. *et al.* (2023) In utero and post-natal opioid exposure followed by mild traumatic brain injury contributes to cortical neuroinflammation, mitochondrial dysfunction, and behavioral deficits in juvenile rats. *Brain Behav. Immun. Health* 32, 100669
85. Zhang, Y. *et al.* (2024) Effect of prenatal and early post-natal oxycodone exposure on the reinforcing and antinociceptive effects of oxycodone in adult C57BL/6 J mice. *Psychopharmacology (Berl)* 241, 359–377
86. Martin, R.E. *et al.* (2021) Maternal oxycodone treatment results in neurobehavioral disruptions in mice offspring. *eNeuro* 8, ENEURO.0150-21.2021
87. Odegaard, K.E. *et al.* (2022) Distinct synaptic vesicle proteomic signatures associated with pre- and post-natal oxycodone-exposure. *Cells* 11, 1740
88. Shahjin, F. *et al.* (2019) Brain-derived extracellular vesicle microRNA signatures associated with in utero and postnatal oxycodone exposure. *Cells* 9, 21
89. Balemans, M.C. *et al.* (2010) Reduced exploration, increased anxiety, and altered social behavior: Autistic-like features of euchromatin histone methyltransferase 1 heterozygous knockout mice. *Behav. Brain Res.* 208, 47–55
90. Nicolas, L.B. *et al.* (2006) A combined marble burying-locomotor activity test in mice: a practical screening test with sensitivity to different classes of anxiolytics and antidepressants. *Eur. J. Pharmacol.* 547, 106–115
91. Odegaard, K.E. *et al.* (2020) A holistic systems approach to characterize the impact of pre- and post-natal oxycodone exposure on neurodevelopment and behavior. *Front. Cell Dev. Biol.* 8, 619199
92. Lyu, Z. *et al.* (2022) Long-term effects of developmental exposure to oxycodone on gut microbiota and relationship to adult behaviors and metabolism. *mSystems* 7, e0033622
93. Jones, C.M. *et al.* (2018) Changes in synthetic opioid involvement in drug overdose deaths in the United States, 2010-2016. *JAMA* 319, 1819–1821
94. Kale-Cekinmez, E. *et al.* (2012) Two newborns of heroin-addicted mothers suffering neonatal withdrawal syndrome. *Turk. J. Pediatr.* 54, 421–424
95. Brogly, S.B. *et al.* (2018) Prenatal treatment and outcomes of women with opioid use disorder. *Obstet. Gynecol.* 132, 916–922
96. Crawford, C.A. *et al.* (2023) Effects of neonatal fentanyl on late adolescent opioid-mediated behavior. *Front. Neurosci.* 17, 1094241
97. Alipio, J.B. *et al.* (2021) Enduring consequences of perinatal fentanyl exposure in mice. *Addict. Biol.* 26, e12895
98. Olusakin, J. *et al.* (2023) Transcriptomic profiling of reward and sensory brain areas in perinatal fentanyl exposed juvenile mice. *Neuropsychopharmacology* 48, 1724–1734
99. Xue, B. *et al.* (2022) Perinatal opioid exposure results in persistent hypoconnectivity of excitatory circuits and reduced activity correlations in mouse primary auditory cortex. *J. Neurosci.* 42, 3676–3687
100. Alipio, J.B. *et al.* (2021) Perinatal fentanyl exposure leads to long-lasting impairments in somatosensory circuit function and behavior. *J. Neurosci.* 41, 3400–3417
101. Alipio, J.B. *et al.* (2022) Environmental enrichment mitigates the long-lasting sequelae of perinatal fentanyl exposure in mice. *J. Neurosci.* 42, 3557–3569
102. Calarco, C.A. *et al.* (2021) Mitochondria-related nuclear gene expression in the nucleus accumbens and blood mitochondrial copy number after developmental fentanyl exposure in adolescent male and female C57BL/6 mice. *Front. Psychiatry* 12, 737389
103. Zankl, A. *et al.* (2021) Opioid treatment for opioid withdrawal in newborn infants. *Cochrane Database Syst. Rev.* 7, CD002059
104. Anon. (2017) Committee Opinion No. 711: opioid use and opioid use disorder in pregnancy. *Obstet. Gynecol.* 130, e81–e94
105. Jones, H.E. *et al.* (2010) Neonatal abstinence syndrome after methadone or buprenorphine exposure. *N. Engl. J. Med.* 363, 2320–2331
106. Suarez, E.A. *et al.* (2022) Buprenorphine versus methadone for opioid use disorder in pregnancy. *N. Engl. J. Med.* 387, 2033–2044
107. Grecco, G.G. *et al.* (2022) Alterations of brain microstructures in a mouse model of prenatal opioid exposure detected by diffusion MRI. *Sci. Rep.* 12, 17085
108. Haggerty, D.L. *et al.* (2023) Prenatal methadone exposure selectively alters protein expression in primary motor cortex: Implications for synaptic function. *Front. Pharmacol.* 14, 1124108
109. Grecco, G.G. *et al.* (2022) Prenatal opioid exposure impairs endocannabinoid and glutamate transmission in the dorsal striatum. *eNeuro* 9, ENEURO.0119-22.2022
110. Grecco, G.G. *et al.* (2022) Prenatal opioid exposure reprograms the behavioural response to future alcohol reward. *Addict. Biol.* 27, e13136
111. Grecco, G.G. *et al.* (2022) Sex-dependent synaptic remodeling of the somatosensory cortex in mice with prenatal methadone exposure. *Adv. Drug Alcohol Res.* 2, 10400
112. Schlagal, C.R. *et al.* (2021) Maternal opioid exposure culminates in perturbed murine neurodevelopment and hyperactive phenotype in adolescence. *Neuroscience* 463, 272–287
113. Lum, J.S. *et al.* (2021) Prenatal methadone exposure impairs adolescent cognition and GABAergic neurodevelopment in a novel rat model of maternal methadone treatment. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 110, 110281
114. Safa, A. *et al.* (2020) Pharmacological prevention of neonatal opioid withdrawal in a pregnant guinea pig model. *Front. Pharmacol.* 11, 613328
115. Gamble, M.E. *et al.* (2022) Prenatal methadone exposure leads to long-term memory impairments and disruptions of dentate granule cell function in a sex-dependent manner. *Addict. Biol.* 27, e13215
116. Gamble, M.E. and Diaz, M.R. (2023) Prenatal methadone exposure leads to disruptions in adult-born dentate granule cell survival and female persistent fear responding. *Addict. Neurosci.* 8, 100120
117. Gibson, J.M. *et al.* (2022) Perinatal methadone exposure attenuates myelination and induces oligodendrocyte apoptosis in neonatal rat brain. *Exp. Biol. Med. (Maywood)* 247, 1067–1079
118. Oberoi, R. *et al.* (2019) Diverse changes in myelin protein expression in rat brain after perinatal methadone exposure. *Acta Neurobiol. Exp. (Wars)* 79, 367–373
119. Chin, E.M. *et al.* (2023) In utero methadone exposure permanently alters anatomical and functional connectivity: a preclinical evaluation. *Front. Pediatr.* 11, 1139378
120. Newville, J. *et al.* (2020) Perinatal opioid exposure primes the peripheral immune system toward hyperreactivity. *Front. Pediatr.* 8, 272
121. Madurai, N.K. *et al.* (2022) Methadone alters the peripheral inflammatory and central immune landscape following prenatal exposure in rats. *Adv. Drug Alcohol Res.* 2, 10792
122. Kongstorp, M. *et al.* (2020) Prenatal exposure to methadone or buprenorphine alters micro-opioid receptor binding and downstream signaling in the rat brain. *Int. J. Dev. Neurosci.* 80, 443–453
123. Kongstorp, M. *et al.* (2020) Prenatal exposure to methadone or buprenorphine impairs cognitive performance in young adult rats. *Drug Alcohol Depend.* 212, 108008
124. Roy, S. and Loh, H.H. (1996) Effects of opioids on the immune system. *Neurochem. Res.* 21, 1375–1386

125. Arter, S. *et al.* (2021) Diagnoses during the first three years of life for children with prenatal opioid exposure and neonatal abstinence syndrome using a large maternal infant data hub. *J. Pediatr. Nurs.* 61, 34–39
126. Milliren, C.E. *et al.* (2021) Pediatric hospital readmissions for infants with neonatal opioid withdrawal syndrome, 2016-2019. *Hosp. Pediatr.* 11, 979–988
127. Ko, J.Y. *et al.* (2021) Maternal opioid exposure, neonatal abstinence syndrome, and infant healthcare utilization: a retrospective cohort analysis. *Drug Alcohol Depend.* 223, 108704
128. Bakhireva, L.N. *et al.* (2022) Severity of neonatal opioid withdrawal syndrome with prenatal exposure to serotonin reuptake inhibitors. *Pediatr. Res.* 91, 867–873
129. Lowe, J.R. *et al.* (2022) Early developmental trajectory of children with prenatal alcohol and opioid exposure. *Pediatr. Res.*, Published online August 10, 2022. https://doi.org/10.1038/s41390-022-02252-z
130. 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
131. Wachman, E.M. *et al.* (2011) The relationship between maternal opioid agonists and psychiatric medications on length of hospitalization for neonatal abstinence syndrome. *J. Addict. Med.* 5, 293–299
132. Page, K. *et al.* (2022) Prevalence of marijuana use in pregnant women with concurrent opioid use disorder or alcohol use in pregnancy. *Addict. Sci. Clin. Pract.* 17, 3
133. Flores, A. *et al.* (2023) Impact of adolescent nicotine exposure in pre- and post-natal oxycodone exposed offspring. *J. NeuroImmune Pharmacol.* 18, 413–426
134. Grecco, G.G. *et al.* (2021) Prenatal opioid administration induces shared alterations to the maternal and offspring gut microbiome: a preliminary analysis. *Drug Alcohol Depend.* 227, 108914
135. Hudak, M.L. *et al.* (2012) Neonatal drug withdrawal. *Pediatrics* 129, e540–e560
136. Kraft, W.K. *et al.* (2017) Buprenorphine for the treatment of the neonatal abstinence syndrome. *N. Engl. J. Med.* 376, 2341–2348
137. Jones, H.E. *et al.* (2008) Treatment of opioid-dependent pregnant women: clinical and research issues. *J. Subst. Abus. Treat.* 35, 245–259
138. Hall, E.S. *et al.* (2016) A cohort comparison of buprenorphine versus methadone treatment for neonatal abstinence syndrome. *J. Pediatr.* 170, 39–44
139. McPhail, B.T. *et al.* (2021) Opioid treatment for neonatal opioid withdrawal syndrome: current challenges and future approaches. *J. Clin. Pharmacol.* 61, 857–870
140. Merhar, S.L. *et al.* (2021) Phenobarbital and clonidine as secondary medications for neonatal opioid withdrawal syndrome. *Pediatrics* 147, e2020017830
141. Sithisarn, T. *et al.* (2022) Understanding the effects of opioids vs non-opioids in the treatment of neonatal abstinence syndrome, an in vitro model. *Front. Pediatr.* 10, 1068330