Published in final edited form as:

Front Neuroendocrinol. 2018 October ; 51: 1–13. doi:10.1016/j.yfrne.2017.09.001.

Modeling prenatal opioid exposure in animals: Current findings and future directions

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Abstract

The past decade has seen a drastic rise in the number of infants exposed to opioids in utero. It is unclear what lasting effect this exposure may have on these children. Animal models of prenatal opioid exposure may provide insight into potential areas of vulnerability. The present review summarizes the findings across animal models of prenatal opioid exposure, including exposure to morphine, methadone, buprenorphine, and oxycodone. Details regarding the drug, doses, and duration of treatment, as well as key findings, are summarized in tables with associated references. Finally, significant gaps in the current preclinical literature and future directions are discussed.

Keywords

morphine; methadone; buprenorphine; oxycodone; postnatal; transgenerational; development; mu receptor; HPA

1. Introduction

Over the past decade, recognition of the magnitude of opioid use and misuse in North America has been mounting. Indeed, the dire public health consequences of exposing large numbers of people to highly addictive pharmaceuticals are becoming increasingly evident. To that end, the number of scientific publications with the term “opioid epidemic” in the title have steadily increased from 2 in 2006 to 62 in 2016 (see Figure 1). As the population of opioid users grows, so do the numbers of infants born exposed to opioids *in utero* (Hayes and Brown 2012, McQueen and Murphy-Oikonen 2016). Many of these babies will experience postnatal opioid withdrawal, with symptoms emerging as early as a few hours or as late as several days after birth, depending upon the opioid being used by the mother (Kocherlakota 2014). These withdrawal symptoms, termed neonatal abstinence syndrome (NAS), have been the subject of numerous reports detailing variations in clinical manifestation and treatment protocols (Raffaelli, Cavallaro et al. 2017). The confluence of

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NAS symptoms can include autonomic, gastrointestinal, and neurological features, ranging from excessive crying and irritability, to significant weight loss and seizures (Wiles, Isemann et al. 2014). There are data suggesting that the type of opioid, as well as the dose and timing of exposure can impact the occurrence and severity of NAS (Liu, Jones et al. 2010, Wiles, Isemann et al. 2014, Klamann, Isaacs et al. 2017). Other relevant factors include genetic vulnerabilities (Wachman, Hayes et al. 2013, Wachman, Hayes et al. 2014, Wachman, Hayes et al. 2015, Mactier, McLaughlin et al. 2017), gestational age (Liu, Singh et al. 2015), comorbid disease and polysubstance use (Logan, Brown et al. 2013, McQueen and Murphy-Oikonen 2016). Currently, however, it is not possible to predict which babies will develop NAS following *in utero* opioid exposure. While improving the management of NAS continues to be an area of clinical focus and research, long-term risk following exposure to opioids *in utero*, whether an infant demonstrates NAS or not, remains unclear. A limited number of longitudinal studies are available, although few extend past childhood. Findings to date, however, have reported deficits on a number of cognitive parameters (Nygaard, Moe et al. 2015, Konijnenberg, Sarfi et al. 2016, Nygaard, Slinning et al. 2017) that suggest neurodevelopmental consequences of *in utero* opioid exposure. These data, however, must be interpreted with caution given the potential impact of numerous additional risk factors, including polysubstance use, mother-child interactions, environmental stability, as well as unknown genetic vulnerabilities. Indeed, a recent systematic review and meta-analysis of studies examining opioid exposed infants and preschool children found few significant neurobehavioral deficits, although there was a tendency for overall poorer performance in exposed groups (Baldacchino, Arbuckle et al. 2014). Such findings suggest that, like NAS, the long-term consequences of *in utero* opioid exposure may be highly variable. This variability suggests diverse underlying mechanisms, likely influenced by drug type, dose, and duration of use, coupled with genetic vulnerabilities. Moreover, all of these risk factors may produce effects that can be ameliorated or exacerbated by environmental conditions.

The variability in the development and expression of NAS following *in utero* opioid exposure, coupled with disparities in the potential long-term consequences of such exposure, lends itself to further study using a reductionist approach. Over the past four decades, a variety of animal models have been used to examine the behavioral, physiological, and neural effects of *in utero* exposure to opioids on future offspring. The vast majority of these studies have been carried out in rats, although studies using mice, chicks, and sheep have also been conducted. The opioid most commonly used in these studies is morphine, although methadone, buprenorphine, and occasionally oxycodone, have also been examined. There are a number of key physiological differences that must be considered when attempting to translate preclinical prenatal substance use studies to human populations. As the primary focus of most prenatal opioid studies has been on the nervous system, one must first consider differences in the timing of neurodevelopment across species. For example, mice and rats, with their respective 21–22 day gestation period, are born altricial and on many measures of neurodevelopment are considered at birth (postnatal day 0; PND0) to be comparable to a late second trimester-early third trimester human fetus (Semple, Blomgren et al. 2013). Indeed, postnatal day 7 (PND7) in rodents is often considered similar to a human infant at birth with regard to brain development, although this comparison is based primarily on brain weight relative to body size as a determinant of rapid periods of brain

growth (Dobbing and Sands 1979, Hofman 1983). Thus, even models that expose dams to opioids throughout gestation are only comparable to use in the first and second trimester in humans.

Additional drug administration postpartum, either with direct injections to pups or with drugs passed via mother's milk, have been used to model drug use continuing through the third trimester; however, such studies typically result in lower levels of drug exposure in the pups and have the added confound of impacting offspring via increased postnatal handling/stress (direct injections) or changes in maternal behavior (maternal exposure and direct injections). Again, such findings must be interpreted carefully, as both neonatal stress and alterations in maternal care induce significant offspring effects, many of which overlap with outcomes reported following prenatal opioid exposure (Kaffman and Meaney 2007, Juul, Beyer et al. 2011, Hays, McPherson et al. 2012, Rainekei, Lucion et al. 2014). Given the significant role of endogenous opioids in regulating the neuroendocrine axis (Morley 1981, Bicknell 1985), it is often difficult to dissociate direct effects of fetal opioid exposure, from indirect effects mediated by alterations in maternal endocrine physiology. Indeed, it is perhaps the unique role of endogenous opioids in regulating hypothalamic and pituitary outflow during pregnancy and the postpartum period (Douglas, Johnstone et al. 1998, Wigger, Lorsch et al. 1999, Douglas and Russell 2001, Neumann 2001, Wigger and Neumann 2002, Grattan, Steyn et al. 2008, Gustafsson, Orelan et al. 2008, Russell, Douglas et al. 2008, Brunton, McKay et al. 2009, Brunton and Russell 2011) that results in such diverse offspring outcomes following maternal exposure to high levels of exogenous opioids during this critical period.

In addition to species differences in neurodevelopment, one must also be mindful of the significant differences in the timing, route, type, and dose of opioid being utilized. Many studies initiate opioid exposure during mid-gestation (gestation day; GD). Such studies were designed primarily to expose the fetus to exogenous opioids immediately prior to the development of opioid receptors in brain. Other models initiate opioid exposure prior to conception and then continue drug administration throughout gestation and during the postpartum period. Some studies use a continuous steady-state exposure model with either subcutaneous pellets or minipumps; many of these studies then cross-foster offspring to naïve dams (i.e. place exposed pups with a drug-naïve foster mother) in an attempt to model the effects of sudden withdrawal on neurodevelopmental outcomes. Overall, most studies utilize experimenter-administered drug regimens (subcutaneous, intraperitoneal, or intravenous), although there are some studies that examine exposure via drinking water. Finally, as with all animal studies, species differences in drug metabolism should be considered. Nonetheless, even given the above considerations, the confluence of data from multiple models, across multiple dosing regimens and using various forms of commonly used opioids, have demonstrated significant neurodevelopmental effects of perinatal opioid exposure that can impact behavioral, physiological, and neural functions.

The current review contains a summary of effects observed in offspring as a result of prenatal opioid exposure across a number of animal models as reported in the scientific literature over the last three (or more) decades. The findings have been summarized under specific areas of inquiry. Each summary is accompanied by a table that includes

methodological details with regard to the specifics of the animal model used as well as more detailed information regarding outcomes. Studies in which sex differences were reported are denoted in the tables with an asterisk in the “Age of Testing” column. Prenatal opioid exposure references are included in the tables rather than in the summary paragraph. The following are abbreviations used across all tables: B.I.D. (twice a day); BUP (buprenorphine); BW (body weight); G (gestation day); i.p. (intraperitoneal); MOR (morphine); MET (Methadone); OXY (oxycodone); PN (postnatal day); p.o.(oral); s.c. (subcutaneous); SD (Sprague-Dawley). Abbreviations that are unique to individual tables are found in the table description.

2. Effects on Early Postnatal Development (Table 1.)

Effects of prenatal opioids on gross developmental parameters suggest a limited and drug-dependent effect. Most studies using morphine fail to observe significant effects on litter size, sex distribution, body weight, tooth eruption, or eye opening. In contrast, oxycodone, buprenorphine, and methadone have all been shown to decrease body weight at birth and at least one study suggested craniofacial malformations following high doses of oxycodone. These discrepancies may be due to the differing pharmacological profiles of the various opioids. Effects on measures of postnatal neuronal development have also been reported. In particular, studies suggest decreased dendritic length and spine density during early postnatal development in response to morphine, as well as significant effects on early myelination following both buprenorphine and methadone. A number of studies focused on the effects of prenatal opioids on postnatal striatal development. Significant changes in neurotransmitter content and postsynaptic activity have been reported. Early postnatal modifications in acetylcholine, serotonin, and dopamine systems have been observed within striatal regions. Additional effects on basal levels of enkephalins and nerve growth factor have also been reported. Overall, these findings indicate that prenatal opioid exposure has the potential to modify early neurodevelopment with effects on a broad range of developmental targets including both neurons and glia.

3. Effects on the Endogenous Opioid System (Table 2.)

The endogenous opioid system is a central mediator of pain modulation, reward, and stress responsivity. It consists of three families of opioid peptides: β -endorphin, enkephalins, and dynorphins; as well as three families of receptors: mu opioid receptor (MOR, μ), delta opioid receptor (DOR, δ), and kappa opioid receptor (KOR, κ). There is a widespread yet specific distribution of both peptides and receptors throughout the central and peripheral nervous system that allows for regulation of a wide range of opioid-related behavioral and physiological effects. The opioid analgesics that induce NAS work within this tightly controlled system. Morphine is a principal psychoactive compound found in the opium of the poppy plant and is considered the prototypical opioid analgesic. It has extensive effects in the central nervous system and is thought to act primarily on the MOR, although it has agonist action at all opioid receptors. Oxycodone is a semi-synthetic opioid with high abuse liability that also acts on all three classes of opioid receptors but may have stronger affinity than morphine for the KOR. Methadone is fully synthetic and is unique because it has a much longer half-life than other opioids, making it useful for the management of chronic

pain as well as treatment of opioid withdrawal/addiction. Finally, buprenorphine, another semi-synthetic, is unlike the other drugs discussed, as it is a partial agonist at MOR and KOR and an antagonist at the DOR. The lack of DOR activity may underlie decreased development of tolerance observed with chronic use of buprenorphine.

3.1 Effects on Opioid Receptors

Some of the earliest work using models of prenatal opioid exposure examined effects on the development of the endogenous opioid system. In rodents, opioid receptors are first expressed in peripheral tissues beginning around embryonic day 9.5 (Zhu, Hsu et al. 1998) and in neural tissue around embryonic day 12.5 (Khachaturian, Alessi et al. 1983, Loh, Rius et al. 1991). Indeed, the reason why many studies initiated opioid exposure on gestation day 8 is that they were designed to target the period when opioid receptors are first being expressed. The prevailing hypothesis underlying such studies was that the presence of high levels of exogenous opioids during this sensitive period would induce modifications in the development of endogenous opioids (both receptor and ligand). The majority of these studies examine prenatal morphine exposure and report effects that are region-, age-, and often sex-specific. While there are exceptions, in most brain regions examined, exogenous prenatal opioid exposure results in decreased receptor binding during the early postnatal period, with increased binding observed in adult offspring. These effects have been reported with regard to both the number of binding sites as well as ligand affinity. As the neurodevelopment of opioid receptors follows a caudal to rostral trajectory, the timing of the exposure within this developmental sequence can affect regional expression patterns. Typically, findings suggest downregulation during and/or soon after exposure (i.e. during early postnatal development) with upregulation emerging later in development, likely as a compensatory response to exogenous drug withdrawal. Notably, these effects can also be regulated by the hormonal milieu, which may underlie sex-specific effects on opioid receptor expression and cellular activity observed in prenatally exposed offspring.

3.2 Effects on analgesia

Opioid-induced analgesia is produced by activating modulatory pain circuits that originate in the midbrain and descend to the dorsal horn of the spinal cord via the rostral ventromedial medulla. There is additional analgesia produced by inhibition of the ascending nociceptive pathways within the dorsal horn of the spinal cord (Basbaum and Fields 1984). The prototypical tests for analgesic efficacy in rodent models are the hot plate (which measures a supraspinal reflex) or tail flick assay (which measures a spinal reflex). In these tests, the latency to respond to a noxious stimulus (heat) is recorded. Analgesics increase the latency. Given the well-known analgesic effects of opioids, a number of studies have examined the effect of prenatal opioid exposure on both pain perception and morphine-induced analgesia. Such studies suggest age-dependent outcomes with hyperalgesia and decreased opioid sensitivity observed during the early postnatal period (i.e. soon after exposure), while increased opioid sensitivity has been reported in adult prenatally exposed offspring. Similar effects have been observed in a number of species and using different dosing regimens. In addition, these effects are observed when offspring are cross-fostered at birth, suggesting they are due to effects incurred during gestational exposure.

3.3 Effects on Reward

Opioids produce their rewarding/reinforcing effects through opioid-dopamine interactions in the ventral tegmental area and the nucleus accumbens (Di Chiara and North 1992, Shippenberg, Bals-Kubik et al. 1993). In animals, reward and addiction-like behaviors are measured via sensitization paradigms (repeated administrations of an opioid induce increased or sensitized locomotor activity), conditioned place preference (an animal will spend more time in an environment paired with a rewarding drug) or self-administration (animal is allowed access to a drug and chooses to ingest the substance). In addition to effects on analgesia, there is also a growing body of literature that indicates altered reward-related behaviors in response to prenatal opioids including increased behavioral sensitization and conditioned place preference. Moreover, these effects are not only observed in response to opioids, as there also appears to be a cross-sensitization to other drugs of abuse, such as methamphetamine, with increased reward-related responses observed in prenatally opioid exposed animals. Additionally, symptoms of opioid withdrawal also appear to be more intense in prenatally exposed offspring as measured using naloxone precipitated withdrawal. Together these findings demonstrate significant effects of prenatal opioid exposure on the development of the endogenous opioid system. Moreover, these developmental modifications appear to coincide with functional effects, including alterations in opioid-mediated analgesia and reward-related behaviors.

4. Effects on the HPA axis and immune system (Table 3.)

Endogenous opioids are well-known regulators of the stress response, with modulatory effects on both the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (Drolet, Dumont et al. 2001). A number of studies have documented significant effects of prenatal opioid exposure on the regulation of the HPA axis. This includes endocrine modifications, such as changes in the release of adrenocorticotropin releasing hormone (ACTH) at baseline and in response to both physiological and psychological stressors. Alterations in adrenal and circulating epinephrine and norepinephrine have also been observed. The effects on corticosterone appear to be dependent on sex and age at the time of testing. In addition, both positive and negative feedback systems can be altered by prenatal opioid exposure. Some of the observed effects are due in part to alterations in monoamines that serve to regulate the HPA axis. Effects due to alterations in glucocorticoid receptor activation have also been demonstrated. Thus, prenatal opioid exposure can significantly impact regulation of the HPA axis. Additionally, the response of the immune system is significantly modified by prenatal opioid exposure. While data in this area are more limited, reported effects include decreased fever response, despite upregulation of fever-inducing cytokines, as well as increased hypoalgesia following an immune challenge using LPS. Both dysregulation of the HPA-axis and immune system are associated with increased risk of disease, and as such these effects of prenatal opioid exposure may have long-term implications for overall health.

5. Effects on social and reproductive-related behaviors (Table 4.)

In addition to regulating the HPA axis, endogenous opioids can also influence the hypothalamic-pituitary-gonadal (HPG) axis, with potential effects at all levels of the this system (Jenkins and Grossman 1993, Bottcher, Seeber et al. 2017). Several studies observe delayed sexual maturation in female prenatally opioid exposed offspring as measured by the timing of vaginal opening. Such effects are likely mediated by changes in the regulation of puberty by endogenous opioids (Bernasconi, Petraglia et al. 1986, Cicero, Schmoeker et al. 1986, Sizonenko 1987). Sexually dimorphic effects on sex behaviors have also been reported following prenatal opioid exposure, with increased sex behaviors reported in males and decreased sex behaviors reported in females. These effects may be due to opioid-mediated alterations in the pre- and postnatal hormonal milieu, which could subsequently alter the development of sexually dimorphic brain regions that regulate reproductive behavior, such as the medial preoptic area or bed nucleus of the stria terminalis (Hammer 1985, Ostrowski, Hill et al. 1987). Effects on sex behavior in offspring may also be mediated by alterations in motivational aspects of sex behaviors, which are regulated by endogenous opioid activity in the ventral tegmental area (van Furth and van Ree 1996). In addition to effects on sex behavior, prenatal opioid exposure can alters play behaviors in both male and female juveniles, as well as maternal behavior in adult females. These findings suggest that one mechanism underlying the effects of prenatal opioid exposure on offspring outcomes is an alteration in the organizational and perhaps activational effects of gonadal hormones.

6. Effects on hippocampal-based learning and associated neural processes (Table 5.)

One brain region that has been investigated extensively following prenatal opioid exposure is the hippocampus. Opioids have a profound influence on the activity of hippocampal neurons (Simmons and Chavkin 1996, Drake, Chavkin et al. 2007) as well as on hippocampal neurogenesis (Sargeant, Miller et al. 2008, Zhang, Loh et al. 2016). The effects of opioid may be particularly relevant to spatial learning in the context of goal-directed behaviors (Retailleau, Etienne et al. 2012). With regard to prenatal opioid exposure, significant disruptions of certain aspects of spatial learning have been noted in a number of studies. The pattern of these effects, however, suggests moderate impairments and delays in learning rather than a severe deficit. Moreover, there are data providing evidence that environmental enrichment can reverse the observed deficiencies. These effects on learning may be related to both the direct effects of opioids on neuronal firing as well as on processes related to neurogenesis. In fact, studies have demonstrated a number of cellular alterations within the hippocampus of exposed offspring. In particular, effects on both long term potentiation (LTP) and long term depression (LTD) have been noted. As LTP and LTD are thought to be critical for learning and memory, such findings may provide a mechanism for the observed effects on spatial learning. In addition, changes in synaptogenesis, including differences in spine density have been noted as well as differences in levels of nerve growth factors and mediators of apoptosis. A number of studies have also implicated changes in the NMDA receptor, a glutamate receptor that is critical for both LTP and LTD; and there is evidence that co-administration of the NMDA receptor agonist dextromethorphan during prenatal

morphine exposure, can attenuate the long-term effects on NMDA receptors. Thus, there is now significant evidence that prenatal opioid exposure can lead to long term changes in neural processes critical for hippocampal-based learning, with additional evidence suggesting that these effects can be modified by postnatal environment.

7. Effects on seizure threshold (Table 6.)

Alterations in neuronal activity in the hippocampus may also play a role in the increased risk of seizures following prenatal opioid exposure. A number of studies administering opioids beginning around mid-gestation have reported significant alterations in epileptiform activity and seizure thresholds in offspring. These effects, however, are complex and often differ based on age and sex. Given that prenatal opioid exposure can impact glutamate receptors and opioid-mediated modulation of GABA in the hippocampus (described in Table 5), effects on seizure activity are perhaps not unexpected. Prenatal opioid exposure has also been demonstrated to modify the effects of estrogen and progesterone on seizure activity. Gonadal hormones are known to modulate seizure thresholds (Scharfman and MacLusky 2006). Thus, the observed effects of prenatal opioid exposure on seizure thresholds once again suggest that significant alterations in endocrine regulation of neuronal activity, particularly in systems normally modulated by endogenous opioids, may mediate some of these effects. As many NAS infants are at high risk for seizures, understanding the mechanism underlying the developmental effects of prenatal opioid exposure on seizure threshold may provide useful insights for better treatment options, particularly for babies demonstrating NAS.

8. Future Directions

Decades of animal research has demonstrated that *in utero* opioid exposure can induce broad neurodevelopmental effects, although largely in the absence of congenital abnormalities associated with other drugs, such as alcohol. While the precise nature of these effects depends upon the drug, dose, and/or timing of exposure, the overall impact appears to be on systems that are normally modulated by endogenous opioids. Given the ubiquitous nature of endogenous opioids, such effects could impact a number of processes, including regulation of enteric, endocrine, and/or immune function. Like other chemicals, during early development endogenous opioids serve as growth factors in both neural and non-neural tissues (Tenconi, Lesma et al. 1996, Zagon, Wu et al. 1999, Sargeant, Miller et al. 2008, Yamamizu, Furuta et al. 2011). Opioids acting at different receptor subtypes have been observed to influence cell proliferation, differentiation and apoptosis in a variety of cell-types (Hu, Sheng et al. 2002, Kim, Clark et al. 2006, Narita, Kuzumaki et al. 2006). Thus, increased exposure of the fetus to circulating opioids has the capacity to modify developmental trajectories. To date, the majority of studies in this area have focused on neurodevelopment with a primary focus on changes to the mu opioid receptor subtype. There remains a paucity of data examining the effects of prenatal opioid exposure on other opioid receptor subtypes or on the interaction between the different opioid receptor subtypes. This lack of knowledge is unfortunate as opioid receptor subtypes are known to form heterotypic dimers (Law, Erickson-Herbrandson et al. 2005). Moreover, changes in the relative expression of different subtypes at both the cellular and regional level may play a

role in some of the effects observed in prenatally exposed offspring. Additionally, we still have a very limited understanding regarding potential effects of prenatal opioid exposure on the development and function of the peripheral and/or enteric nervous systems.

When considering the impact of prenatal opioid exposure, consideration must be given to the fact that endogenous opioids serve as important modulators and, as such, cellular effects stimulated by opioid receptor binding are often activity dependent. Due to this modulatory role, fetal opioid receptor activation induced by exogenous opioids during development, may be modified by both the internal and external milieu of the mother. This activity-dependent modulation likely underlies disparate outcomes observed in offspring exposed to opioids under various prenatal conditions, such as increased environmental stressors or poor nutrition. While not included in the present review, animal models that couple prenatal opioid exposure with additional environmental demands, such as prenatal stress, provide support for the hypothesis that prenatal opioid exposure interacts with environmental conditions to influence outcomes (Ebrahimi, Saboory et al. 2014, Saboory, Ebrahimi et al. 2015). These effects are likely due to opioidergic modulation of hormones and other endocrine-related mediators during gestation (Brunton and Russell 2011). Similarly, opioids also modulate the immune system, with effects observed at classical opioid receptors and at non-classical opioid receptors (e.g. toll-like receptors)(Hutchinson, Shavit et al. 2011). Given the importance of opioids in the regulation of immune function (Liang, Liu et al. 2016), coupled with the increased risk of infectious diseases in opioid-using women (Raketic, Stamatovic Gajic et al. 2013), additional preclinical studies examining opioid-immune interactions during the prenatal period are warranted. Similarly, clinical data has suggested that genetic and epigenetic variants can influence the development of NAS in opioid exposed infants (Wachman, Newby et al. 2011, Bagley, Wachman et al. 2014, Wachman, Hayes et al. 2015). Such findings provide an excellent back translational opportunity to identify mechanisms underlying gene by environment interactions in the context of prenatal opioid exposure. Increasing our understanding of the mechanisms underlying the impact of biological and environmental modifiers may be critical to advancing useful translational animal models of prenatal opioid exposure.

As research in this area moves forward, increasing awareness of maternal factors, including effects of opioid exposure prior to conception will be necessary. Studies have documented effects of prenatal opioid exposure on postnatal maternal behaviors in animals (Slamberova, Szilagyi et al. 2001, Fodor, Timar et al. 2014). As noted in the previous tables, some studies attempt to control for this potential confound through the use of cross-fostering, while others include both cross-fostered and non-cross-fostered groups in an effort to parse the effects imbued by pre- versus postnatal maternal influences. However, studies that directly examine the effects of opioid exposure on maternal physiology during pregnancy are limited. This gap in our knowledge based is unfortunate, particularly given the role of endogenous opioids in the endocrine regulation of pregnancy (Brunton, Russell et al. 2008, Grattan, Steyn et al. 2008, Brunton, Russell et al. 2014). Indeed, while studies initiating opioid exposure during gestation have provided insight into effects induced during specific developmental windows; such regimens, make it difficult to dissociate direct fetal effects from those induced by the dam's physiological response to an initial opioid exposure. Moreover, as these drugs are experimenter administered and can initially stimulate the HPA axis, some of the effects

observed in offspring may be mediated in part by maternal stress. Using models that initiate exposure prior to pregnancy and/or utilize voluntary intake can help minimize this potential confound. Certainly, from a translational perspective most women do not initiate opioid use for the first time during pregnancy. Moreover, preclinical findings suggest that even exposure to opioids that occurs prior to conception, in the absence of *in utero* exposure, can alter opioid sensitivity in future offspring (Byrnes, Babb et al. 2011, Johnson, Carini et al. 2011, Vassoler, Wright et al. 2016, Vassoler, Oliver et al. 2017). Such epigenetic effects of prior drug history should be considered, particularly in light of clinical data suggesting that epigenetic modifications within the mu receptor gene (OPRM1) of the mother influences the severity of opioid withdrawal observed in the infant (Wachman, Hayes et al. 2015, Wachman, Hayes et al. 2017).

Finally, as this research advances, it will be important to start moving away from morphine as the prototypical opioid and instead focus on more commonly used opioids, like oxycodone. In addition, data are still lacking on the two opioids currently recommended for use in medical assisted therapy, methadone and buprenorphine. Preclinical studies should also investigate the effects of poly-substance use during gestation. This gap in our knowledge is troubling not simply due to the frequent misuse of opioids with other illicit and licit substances, like cocaine or nicotine; but because many women being maintained on medical assisted therapy are also being prescribed a number of other drugs to treat co-morbid conditions. Careful preclinical assessment may prove useful in determining which drug combinations lead to the best outcome for both mother and offspring. Animal models can provide important insights regarding mechanisms underlying the range of outcomes observed following fetal exposure. The ultimate goal of this work should be to identify both pre- and postnatal treatments, whether pharmacological, nutritional, or behavioral, that can attenuate adverse health outcomes associated with prenatal opioid exposure.

Acknowledgments

This work was supported by the National Institutes of Health (R01DA025674).

References

- Ahmadalipour A, Sadeghzadeh J, Vafaei AA, Bandegi AR, Mohammadkhani R, Rashidy-Pour A. Effects of environmental enrichment on behavioral deficits and alterations in hippocampal BDNF induced by prenatal exposure to morphine in juvenile rats. *Neuroscience*. 2015; 305:372–383. [PubMed: 26272536]
- Bagley SM, Wachman EM, Holland E, Brogly SB. Review of the assessment and management of neonatal abstinence syndrome. *Addict Sci Clin Pract*. 2014; 9(1):19. [PubMed: 25199822]
- Baldacchino A, Arbuckle K, Petrie DJ, McCowan C. Neurobehavioral consequences of chronic intrauterine opioid exposure in infants and preschool children: a systematic review and meta-analysis. *BMC Psychiatry*. 2014; 14:104. [PubMed: 24708875]
- Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annu Rev Neurosci*. 1984; 7:309–338. [PubMed: 6143527]
- Basheer R, Yang J, Tempel A. Chronic prenatal morphine treatment decreases G alpha s mRNA levels in neonatal frontal cortex. *Brain Res Dev Brain Res*. 1992; 70(1):145–148. [PubMed: 1473274]
- Bernasconi S, Petraglia F, Iughetti L, Facchinetti F, Marcellini C, Giovannelli G, Genazzani AR. Endogenous opioid inhibitory tone on LH secretion in normal puberty and in several pubertal disturbances. *Acta Endocrinol Suppl (Copenh)*. 1986; 279:196–201. [PubMed: 3465161]

- Bhat R, Chari G, Rao R. Effects of prenatal cocaine, morphine, or both on postnatal opioid (μ) receptor development. *Life Sci.* 2006; 78(13):1478–1482. [PubMed: 16242731]
- Bhat R, Chari G, Rao R, Wirtshafter D. Prenatal cocaine and morphine alter brain cyclin-dependent kinase 5 (Cdk5) activity in rat pups. *Neurotoxicol Teratol.* 2006; 28(5):625–628. [PubMed: 16962740]
- Bicknell RJ. Endogenous opioid peptides and hypothalamic neuroendocrine neurones. *J Endocrinol.* 1985; 107(3):437–446. [PubMed: 2999285]
- Biglarnia M, Karami M, Hafshejani ZK. Differences in morphine-induced antinociception in male and female offspring born of morphine exposed mothers. *Indian J Pharmacol.* 2013; 45(3):227–231. [PubMed: 23833363]
- Bottcher B, Seeber B, Leyendecker G, Wildt L. Impact of the opioid system on the reproductive axis. *Fertil Steril.* 2017; 108(2):207–213. [PubMed: 28669481]
- Brunton PJ, McKay AJ, Ochedalski T, Piastowska A, Rebas E, Lachowicz A, Russell JA. Central opioid inhibition of neuroendocrine stress responses in pregnancy in the rat is induced by the neurosteroid allopregnanolone. *J Neurosci.* 2009; 29(20):6449–6460. [PubMed: 19458216]
- Brunton PJ, Russell JA. Allopregnanolone and suppressed hypothalamo-pituitary-adrenal axis stress responses in late pregnancy in the rat. *Stress.* 2011; 14(1):6–12. [PubMed: 20666638]
- Brunton PJ, Russell JA. Neuroendocrine control of maternal stress responses and fetal programming by stress in pregnancy. *Prog Neuropsychopharmacol Biol Psychiatry.* 2011; 35(5):1178–1191. [PubMed: 21216265]
- Brunton PJ, Russell JA, Douglas AJ. Adaptive responses of the maternal hypothalamic-pituitary-adrenal axis during pregnancy and lactation. *J Neuroendocrinol.* 2008; 20(6):764–776. [PubMed: 18601699]
- Brunton PJ, Russell JA, Hirst JJ. Allopregnanolone in the brain: protecting pregnancy and birth outcomes. *Prog Neurobiol.* 2014; 113:106–136. [PubMed: 24012715]
- Buisman-Pijlman FT, Gerrits MA, Van Ree JM. Increased opioid release in specific brain areas in animals exposed to prenatal morphine and emotional stress later in life. *Neuroscience.* 2009; 159(1):405–413. [PubMed: 19138727]
- Byrnes JJ, Babb JA, Scanlan VF, Byrnes EM. Adolescent opioid exposure in female rats: transgenerational effects on morphine analgesia and anxiety-like behavior in adult offspring. *Behav Brain Res.* 2011; 218(1):200–205. [PubMed: 21138744]
- Castellano C, Ammassari-Teule M. Prenatal exposure to morphine in mice: enhanced responsiveness to morphine and stress. *Pharmacol Biochem Behav.* 1984; 21(1):103–108.
- Che Y, Sun H, Tan H, Peng Y, Zeng T, Ma Y. The effect of prenatal morphine exposure on memory consolidation in the chick. *Neurosci Lett.* 2005; 380(3):300–304. [PubMed: 15862906]
- Chiang YC, Hung TW, Ho IK. Development of sensitization to methamphetamine in offspring prenatally exposed to morphine, methadone and buprenorphine. *Addict Biol.* 2014; 19(4):676–686. [PubMed: 23551991]
- Chiang YC, Hung TW, Lee CW, Yan JY, Ho IK. Enhancement of tolerance development to morphine in rats prenatally exposed to morphine, methadone, and buprenorphine. *J Biomed Sci.* 2010; 17:46. [PubMed: 20529288]
- Chiang YC, Ye LC, Hsu KY, Liao CW, Hung TW, Lo WJ, Ho IK, Tao PL. Beneficial effects of co-treatment with dextromethorphan on prenatally methadone-exposed offspring. *J Biomed Sci.* 2015; 22:19. [PubMed: 25890152]
- Chiou LC, Yeh GC, Fan SH, How CH, Chuang KC, Tao PL. Prenatal morphine exposure decreases analgesia but not K⁺ channel activation. *Neuroreport.* 2003; 14(2):239–242. [PubMed: 12598737]
- Cicero TJ, Schmoeker PF, Meyer ER, Miller BT, Bell RD, Cytron SM, Brown CC. Ontogeny of the opioid-mediated control of reproductive endocrinology in the male and female rat. *J Pharmacol Exp Ther.* 1986; 236(3):627–633. [PubMed: 3512817]
- Davis CP, Franklin LM, Johnson GS, Schrott LM. Prenatal oxycodone exposure impairs spatial learning and/or memory in rats. *Behav Brain Res.* 2010; 212(1):27–34. [PubMed: 20307587]
- De Vries TJ, Van Vliet BJ, Hogenboom F, Wardeh G, Van der Laan JW, Mulder AH, Schoffeleer AN. Effect of chronic prenatal morphine treatment of μ -opioid receptor-regulated adenylate

- cyclase activity and neurotransmitter release in rat brain slices. *Eur J Pharmacol.* 1991; 208(2):97–104. [PubMed: 1686867]
- Devarapalli M, Leonard M, Briyal S, Stefanov G, Puppala BL, Schweig L, Gulati A. Prenatal Oxycodone Exposure Alters CNS Endothelin Receptor Expression in Neonatal Rats. *Drug Res (Stuttg).* 2016; 66(5):246–250. [PubMed: 26676852]
- Di Chiara G, North RA. Neurobiology of opiate abuse. *Trends Pharmacol Sci.* 1992; 13(5):185–193. [PubMed: 1604711]
- Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev.* 1979; 3(1):79–83. [PubMed: 118862]
- Douglas AJ, Johnstone HA, Wigger A, Landgraf R, Russell JA, Neumann ID. The role of endogenous opioids in neurohypophysial and hypothalamo-pituitary-adrenal axis hormone secretory responses to stress in pregnant rats. *J Endocrinol.* 1998; 158(2):285–293. [PubMed: 9771473]
- Douglas AJ, Russell JA. Endogenous opioid regulation of oxytocin and ACTH secretion during pregnancy and parturition. *Prog Brain Res.* 2001; 133:67–82. [PubMed: 11589146]
- Drake CT, Chavkin C, Milner TA. Opioid systems in the dentate gyrus. *Prog Brain Res.* 2007; 163:245–263. [PubMed: 17765723]
- Drolet G, Dumont EC, Gosselin I, Kinkad R, Laforest S, Trottier JF. Role of endogenous opioid system in the regulation of the stress response. *Prog Neuropsychopharmacol Biol Psychiatry.* 2001; 25(4):729–741. [PubMed: 11383975]
- Dutriez-Casteloot I, Bernet F, Dedieu JF, Croix D, Laborie C, Montel V, Lesage J, Beauvillain JC, Dupouy JP. Hypothalamic-pituitary-adrenocortical and gonadal axes and sympathoadrenal activity of adult male rats prenatally exposed to morphine. *Neurosci Lett.* 1999; 263(1):1–4. [PubMed: 10218896]
- Ebrahimi L, Saboory E, Roshan-Milani S, Hashemi P. Effect of prenatal forced-swim stress and morphine co-administration on pentylenetetrazol-induced epileptic behaviors in infant and prepubertal rats. *Dev Psychobiol.* 2014; 56(6):1179–1186. [PubMed: 24464467]
- Fodor A, Timar J, Zelena D. Behavioral effects of perinatal opioid exposure. *Life Sci.* 2014; 104(1–2):1–8. [PubMed: 24746901]
- Gagin R, Cohen E, Shavit Y. Prenatal exposure to morphine alters analgesic responses and preference for sweet solutions in adult rats. *Pharmacol Biochem Behav.* 1996; 55(4):629–634. [PubMed: 8981594]
- Grattan DR, Steyn FJ, Kokay IC, Anderson GM, Bunn SJ. Pregnancy-induced adaptation in the neuroendocrine control of prolactin secretion. *J Neuroendocrinol.* 2008; 20(4):497–507. [PubMed: 18266946]
- Gustafsson L, Orelund S, Hoffmann P, Nylander I. The impact of postnatal environment on opioid peptides in young and adult male Wistar rats. *Neuropeptides.* 2008; 42(2):177–191. [PubMed: 18082882]
- Hamilton KL, Franklin L, Roy S, Schrott LM. Prenatal opiate exposure attenuates LPS-induced fever in adult rats: role of interleukin-1beta. *Brain Res.* 2007; 1133(1):92–99. [PubMed: 17196563]
- Hamilton KL, Harris AC, Gewirtz JC, Sparber SB, Schrott LM. HPA axis dysregulation following prenatal opiate exposure and postnatal withdrawal. *Neurotoxicol Teratol.* 2005; 27(1):95–103. [PubMed: 15681124]
- Hammer RP Jr. The sex hormone-dependent development of opiate receptors in the rat medial preoptic area. *Brain Res.* 1985; 360(1–2):65–74. [PubMed: 3000537]
- Hayes MJ, Brown MS. Epidemic of prescription opiate abuse and neonatal abstinence. *JAMA.* 2012; 307(18):1974–1975. [PubMed: 22546607]
- Hays SL, McPherson RJ, Juul SE, Wallace G, Schindler AG, Chavkin C, Gleason CA. Long-term effects of neonatal stress on adult conditioned place preference (CPP) and hippocampal neurogenesis. *Behav Brain Res.* 2012; 227(1):7–11. [PubMed: 22061798]
- Hofman MA. Evolution of brain size in neonatal and adult placental mammals: a theoretical approach. *J Theor Biol.* 1983; 105(2):317–332. [PubMed: 6656284]
- Hol T, Niesink M, van Ree JM, Spruijt BM. Prenatal exposure to morphine affects juvenile play behavior and adult social behavior in rats. *Pharmacol Biochem Behav.* 1996; 55(4):615–618. [PubMed: 8981592]

- Hou Y, Tan Y, Belcheva MM, Clark AL, Zahm DS, Coscia CJ. Differential effects of gestational buprenorphine, naloxone, and methadone on mesolimbic mu opioid and ORL1 receptor G protein coupling. *Brain Res Dev Brain Res*. 2004; 151(1–2):149–157. [PubMed: 15246701]
- Hu S, Sheng WS, Lokensgard JR, Peterson PK. Morphine induces apoptosis of human microglia and neurons. *Neuropharmacology*. 2002; 42(6):829–836. [PubMed: 12015209]
- Hung CJ, Wu CC, Chen WY, Chang CY, Kuan YH, Pan HC, Liao SL, Chen CJ. Depression-like effect of prenatal buprenorphine exposure in rats. *PLoS One*. 2013; 8(12):e82262. [PubMed: 24367510]
- Hutchings DE, Hamowy AS, Williams EM, Zmitrovich AC. Prenatal administration of buprenorphine in the rat: effects on the rest-activity cycle at 22 and 30 days of age. *Pharmacol Biochem Behav*. 1996; 55(4):607–613. [PubMed: 8981591]
- Hutchings DE, Zmitrovich AC, Brake SC, Church SH, Malowany D. Prenatal administration of methadone in the rat increases offspring acoustic startle amplitude at age 3 weeks. *Neurotoxicol Teratol*. 1993; 15(3):157–164. [PubMed: 8336677]
- Hutchinson MR, Shavit Y, Grace PM, Rice KC, Maier SF, Watkins LR. Exploring the neuroimmunopharmacology of opioids: an integrative review of mechanisms of central immune signaling and their implications for opioid analgesia. *Pharmacol Rev*. 2011; 63(3):772–810. [PubMed: 21752874]
- Jenkins PJ, Grossman A. The control of the gonadotrophin releasing hormone pulse generator in relation to opioid and nutritional cues. *Hum Reprod*. 1993; 8(Suppl 2):154–161. [PubMed: 8276951]
- Jiang J, He X, Wang MY, Sui N. Early prenatal morphine exposure impairs performance of learning tasks and attenuates in vitro heterosynaptic long-term potentiation of intermediate medial mesopallium in day-old chicks. *Behav Brain Res*. 2011; 219(2):363–366. [PubMed: 21219936]
- Johnson NL, Carini L, Schenk ME, Stewart M, Byrnes EM. Adolescent opiate exposure in the female rat induces subtle alterations in maternal care and transgenerational effects on play behavior. *Front Psychiatry*. 2011; 2:29. [PubMed: 21713113]
- Juul SE, Beyer RP, Bammler TK, Farin FM, Gleason CA. Effects of neonatal stress and morphine on murine hippocampal gene expression. *Pediatr Res*. 2011; 69(4):285–292. [PubMed: 21178816]
- Kaffman A, Meaney MJ. Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights. *J Child Psychol Psychiatry*. 2007; 48(3–4):224–244. [PubMed: 17355397]
- Khachaturian H, Alessi NE, Munfakh N, Watson SJ. Ontogeny of opioid and related peptides in the rat CNS and pituitary: an immunocytochemical study. *Life Sci*. 1983; 33(Suppl 1):61–64. [PubMed: 6141503]
- Kim E, Clark AL, Kiss A, Hahn JW, Wesselschmidt R, Coscia CJ, Belcheva MM. Mu- and kappa-opioids induce the differentiation of embryonic stem cells to neural progenitors. *J Biol Chem*. 2006; 281(44):33749–33760. [PubMed: 16954126]
- Kirby ML. Recovery of spinal cord volume in postnatal rats following prenatal exposure to morphine. *Brain Res*. 1983; 282(3):211–217. [PubMed: 6831244]
- Klaman SL, Isaacs K, Leopold A, Perpich J, Hayashi S, Vender J, Campopiano M, Jones HE. Treating Women Who Are Pregnant and Parenting for Opioid Use Disorder and the Concurrent Care of Their Infants and Children: Literature Review to Support National Guidance. *J Addict Med*. 2017; 11(3):178–190. [PubMed: 28406856]
- Kocherlakota P. Neonatal abstinence syndrome. *Pediatrics*. 2014; 134(2):e547–561. [PubMed: 25070299]
- Konijnenberg C, Sarfi M, Melinder A. Mother-child interaction and cognitive development in children prenatally exposed to methadone or buprenorphine. *Early Hum Dev*. 2016; 101:91–97. [PubMed: 27614330]
- Kunko PM, Smith JA, Wallace MJ, Maher JR, Saady JJ, Robinson SE. Perinatal methadone exposure produces physical dependence and altered behavioral development in the rat. *J Pharmacol Exp Ther*. 1996; 277(3):1344–1351. [PubMed: 8667196]
- Laborie C, Dutriez-Casteloot I, Montel V, Dickes-Coopman A, Lesage J, Vieau D. Prenatal morphine exposure affects sympathoadrenal axis activity and serotonin metabolism in adult male rats both

under basal conditions and after an ether inhalation stress. *Neurosci Lett*. 2005; 381(3):211–216. [PubMed: 15896472]

- Law PY, Erickson-Herbrandson LJ, Zha QQ, Solberg J, Chu J, Sarre A, Loh HH. Heterodimerization of mu- and delta-opioid receptors occurs at the cell surface only and requires receptor-G protein interactions. *J Biol Chem*. 2005; 280(12):11152–11164. [PubMed: 15657030]
- Liang X, Liu R, Chen C, Ji F, Li T. Opioid System Modulates the Immune Function: A Review. *Transl Perioper Pain Med*. 2016; 1(1):5–13.
- Lin CS, Tao PL, Jong YJ, Chen WF, Yang CH, Huang LT, Chao CF, Yang SN. Prenatal morphine alters the synaptic complex of postsynaptic density 95 with N-methyl-D-aspartate receptor subunit in hippocampal CA1 subregion of rat offspring leading to long-term cognitive deficits. *Neuroscience*. 2009; 158(4):1326–1337. [PubMed: 19041927]
- Litto WJ, Griffin JP, Rabii J. Influence of morphine during pregnancy on neuroendocrine regulation of pituitary hormone secretion. *J Endocrinol*. 1983; 98(2):289–295. [PubMed: 6875436]
- Liu AJ, Jones MP, Murray H, Cook CM, Nanan R. Perinatal risk factors for the neonatal abstinence syndrome in infants born to women on methadone maintenance therapy. *Aust N Z J Obstet Gynaecol*. 2010; 50(3):253–258. [PubMed: 20618243]
- Liu WF, Singh K, Faisal M, Li S. Maternal methadone treatment and neonatal abstinence syndrome. *Am J Perinatol*. 2015; 32(11):1078–1086. [PubMed: 25915141]
- Logan BA, Brown MS, Hayes MJ. Neonatal abstinence syndrome: treatment and pediatric outcomes. *Clin Obstet Gynecol*. 2013; 56(1):186–192. [PubMed: 23314720]
- Loh YP, Rius RA, Elkabes S, Bem W, Coscia CJ. Prenatal expression of pro-opiomelanocortin mRNA, POMC-derived peptides, and mu-opiate receptors in the mouse embryo. *NIDA Res Monogr*. 1991; 111:96–112. [PubMed: 1663583]
- Mactier H, McLaughlin P, Gillis C, Osselton MD. Variations in Infant CYP2B6 Genotype Associated with the Need for Pharmacological Treatment for Neonatal Abstinence Syndrome in Infants of Methadone-Maintained Opioid-Dependent Mothers. *Am J Perinatol*. 2017; 34(9):918–921. [PubMed: 28320034]
- McQueen K, Murphy-Oikonen J. Neonatal Abstinence Syndrome. *N Engl J Med*. 2016; 375(25):2468–2479. [PubMed: 28002715]
- Mei B, Niu L, Cao B, Huang D, Zhou Y. Prenatal morphine exposure alters the layer II/III pyramidal neurons morphology in lateral secondary visual cortex of juvenile rats. *Synapse*. 2009; 63(12):1154–1161. [PubMed: 19670310]
- Mithbaokar P, Fiorito F, Della Morte R, Maharajan V, Costagliola A. Chronic maternal morphine alters calbindin D-28k expression pattern in postnatal mouse brain. *Synapse*. 2016; 70(1):15–23. [PubMed: 26418221]
- Morley JE. The endocrinology of the opiates and opioid peptides. *Metabolism*. 1981; 30(2):195–209. [PubMed: 6258010]
- Narita M, Kuzumaki N, Miyatake M, Sato F, Wachi H, Seyama Y, Suzuki T. Role of delta-opioid receptor function in neurogenesis and neuroprotection. *J Neurochem*. 2006; 97(5):1494–1505. [PubMed: 16696856]
- Nasiraei-Moghadam S, Sherafat MA, Safari MS, Moradi F, Ahmadiani A, Dargahi L. Reversal of prenatal morphine exposure-induced memory deficit in male but not female rats. *J Mol Neurosci*. 2013; 50(1):58–69. [PubMed: 22864979]
- Neumann ID. Alterations in behavioral and neuroendocrine stress coping strategies in pregnant, parturient and lactating rats. *Prog Brain Res*. 2001; 133:143–152. [PubMed: 11589127]
- Niu L, Cao B, Zhu H, Mei B, Wang M, Yang Y, Zhou Y. Impaired in vivo synaptic plasticity in dentate gyrus and spatial memory in juvenile rats induced by prenatal morphine exposure. *Hippocampus*. 2009; 19(7):649–657. [PubMed: 19115391]
- Nygaard E, Moe V, Slinning K, Walhovd KB. Longitudinal cognitive development of children born to mothers with opioid and polysubstance use. *Pediatr Res*. 2015; 78(3):330–335. [PubMed: 25978800]
- Nygaard E, Slinning K, Moe V, Walhovd KB. Cognitive function of youths born to mothers with opioid and poly-substance abuse problems during pregnancy. *Child Neuropsychol*. 2017; 23(2):159–187. [PubMed: 26471942]

- O'Callaghan JP, Holtzman SG. Prenatal administration of morphine to the rat: tolerance to the analgesic effect of morphine in the offspring. *J Pharmacol Exp Ther.* 1976; 197(3):533–544. [PubMed: 932990]
- O'Callaghan JP, Holtzman SG. Prenatal administration of levorphanol or dextrophan to the rat: analgesic effect of morphine in the offspring. *J Pharmacol Exp Ther.* 1977; 200(2):255–262. [PubMed: 14248]
- Ostrowski NL, Hill JM, Pert CB, Pert A. Autoradiographic visualization of sex differences in the pattern and density of opiate receptors in hamster hypothalamus. *Brain Res.* 1987; 421(1–2):1–13. [PubMed: 2825908]
- Raffaelli G, Cavallaro G, Allegaert K, Wildschut ED, Fumagalli M, Agosti M, Tibboel D, Mosca F. Neonatal Abstinence Syndrome: Update on Diagnostic and Therapeutic Strategies. *Pharmacotherapy.* 2017; 37(7):814–823. [PubMed: 28519244]
- Raineki C, Lucion AB, Weinberg J. Neonatal handling: an overview of the positive and negative effects. *Dev Psychobiol.* 2014; 56(8):1613–1625. [PubMed: 25132525]
- Raketic D, Stamatovic Gajic B, Gajic T, Jovanovic M. Women and addiction (alcohol and opiates): comparative analysis of psychosocial aspects. *Srp Arh Celok Lek.* 2013; 141(9–10):648–652. [PubMed: 24364228]
- Retailleau A, Etienne S, Guthrie M, Boraud T. Where is my reward and how do I get it? Interaction between the hippocampus and the basal ganglia during spatial learning. *J Physiol Paris.* 2012; 106(3–4):72–80. [PubMed: 22033208]
- Ricalde AA, Hammer RP Jr. Perinatal opiate treatment delays growth of cortical dendrites. *Neurosci Lett.* 1990; 115(2–3):137–143. [PubMed: 2172870]
- Rimanoczy A, Slamberova R, Bar N, Vathy I. Morphine exposure prevents up-regulation of MR and GR binding sites in the brain of adult male and female rats due to prenatal stress. *Int J Dev Neurosci.* 2006; 24(4):241–248. [PubMed: 16698219]
- Rimanoczy A, Slamberova R, Riley MA, Vathy I. Adrenocorticotropin stress response but not glucocorticoid-negative feedback is altered by prenatal morphine exposure in adult male rats. *Neuroendocrinology.* 2003; 78(6):312–320. [PubMed: 14688444]
- Rimanoczy A, Slamberova R, Vathy I. Prenatal morphine exposure alters estrogen regulation of kappa receptors in the cortex and POA of adult female rats but has no effects on these receptors in adult male rats. *Brain Res.* 2001; 894(1):154–156. [PubMed: 11245827]
- Robinson SE. Effect of prenatal opioid exposure on cholinergic development. *J Biomed Sci.* 2000; 7(3):253–257. [PubMed: 10810245]
- Robinson SE. Effects of perinatal buprenorphine and methadone exposures on striatal cholinergic ontogeny. *Neurotoxicol Teratol.* 2002; 24(2):137–142. [PubMed: 11943501]
- Robinson SE, Guo H, Maher JR, McDowell KP, Kunko PM. Postnatal methadone exposure does not prevent prenatal methadone-induced changes in striatal cholinergic neurons. *Brain Res Dev Brain Res.* 1996; 95(1):118–121. [PubMed: 8873982]
- Robinson SE, Maher JR, Wallace MJ, Kunko PM. Perinatal methadone exposure affects dopamine, norepinephrine, and serotonin in the weanling rat. *Neurotoxicol Teratol.* 1997; 19(4):295–303. [PubMed: 9253008]
- Robinson SE, Mo Q, Maher JR, Wallace MJ, Kunko PM. Perinatal exposure to methadone affects central cholinergic activity in the weanling rat. *Drug Alcohol Depend.* 1996; 41(2):119–126. [PubMed: 8809500]
- Russell JA, Douglas AJ, Brunton PJ. Reduced hypothalamo-pituitary-adrenal axis stress responses in late pregnancy: central opioid inhibition and noradrenergic mechanisms. *Ann N Y Acad Sci.* 2008; 1148:428–438. [PubMed: 19120138]
- Saboory E, Ebrahimi L, Roshan-Milani S, Hashemi P. Interaction of prenatal stress and morphine alters prolactin and seizure in rat pups. *Physiol Behav.* 2015; 149:181–186. [PubMed: 26056076]
- Sanchez ES, Bigbee JW, Fobbs W, Robinson SE, Sato-Bigbee C. Opioid addiction and pregnancy: perinatal exposure to buprenorphine affects myelination in the developing brain. *Glia.* 2008; 56(9):1017–1027. [PubMed: 18381654]
- Sargeant TJ, Miller JH, Day DJ. Opioidergic regulation of astroglial/neuronal proliferation: where are we now? *J Neurochem.* 2008; 107(4):883–897. [PubMed: 18786168]

- Scharfman HE, MacLusky NJ. The influence of gonadal hormones on neuronal excitability, seizures, and epilepsy in the female. *Epilepsia*. 2006; 47(9):1423–1440. [PubMed: 16981857]
- Schindler CJ, Slamberova R, Vathy I. Prenatal morphine exposure decreases susceptibility of adult male rat offspring to bicuculline seizures. *Brain Res*. 2001; 922(2):305–309. [PubMed: 11743964]
- Schindler CJ, Slamberova R, Vathy I. Cholera toxin B decreases bicuculline seizures in prenatally morphine- and saline-exposed male rats. *Pharmacol Biochem Behav*. 2004; 77(3):509–515. [PubMed: 15006461]
- Schindler CJ, Veliskova J, Slamberova R, Vathy I. Prenatal morphine exposure alters susceptibility to bicuculline seizures in a sex- and age-specific manner. *Brain Res Dev Brain Res*. 2000; 121(1): 119–122. [PubMed: 10837900]
- Schrott LM, Franklin L, Serrano PA. Prenatal opiate exposure impairs radial arm maze performance and reduces levels of BDNF precursor following training. *Brain Res*. 2008; 1198:132–140. [PubMed: 18262500]
- Schrott LM, Sparber SB. Suppressed fever and hypersensitivity responses in chicks prenatally exposed to opiates. *Brain Behav Immun*. 2004; 18(6):515–525. [PubMed: 15331122]
- Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol*. 2013; 106–107:1–16.
- Shavit Y, Cohen E, Gagin R, Avitsur R, Pollak Y, Chaikin G, Wolf G, Yirmiya R. Effects of prenatal morphine exposure on NK cytotoxicity and responsiveness to LPS in rats. *Pharmacol Biochem Behav*. 1998; 59(4):835–841. [PubMed: 9586839]
- Shen YL, Chen ST, Chan TY, Hung TW, Tao PL, Liao RM, Chan MH, Chen HH. Delayed extinction and stronger drug-primed reinstatement of methamphetamine seeking in rats prenatally exposed to morphine. *Neurobiol Learn Mem*. 2016; 128:56–64. [PubMed: 26743042]
- Simmons ML, Chavkin C. Endogenous opioid regulation of hippocampal function. *Int Rev Neurobiol*. 1996; 39:145–196. [PubMed: 8894847]
- Shippenberg TS, Bals-Kubik R, Herz A. Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. *J Pharmacol Exp Ther*. 1993; 265(1):53–59. [PubMed: 8386244]
- Sithisarn T, Bada HS, Dai H, Reinhardt CR, Randall DC, Legan SJ. Effects of perinatal oxycodone exposure on the response to CRH in late adolescent rats. *Neurotoxicol Teratol*. 2008; 30(2):118–124. [PubMed: 18255259]
- Sizonenko PC. Normal sexual maturation. *Pediatrician*. 1987; 14(4):191–201.
- Slamberova R, Bar N, Vathy I. Long-term effects of prenatal morphine exposure on maternal behaviors differ from the effects of direct chronic morphine treatment. *Dev Psychobiol*. 2003; 43(4):281–289. [PubMed: 15027411]
- Slamberova R, Riley MA, Vathy I. Cross-generational effect of prenatal morphine exposure on neurobehavioral development of rat pups. *Physiol Res*. 2005; 54(6):655–660. [PubMed: 15717852]
- Slamberova R, Rimanoczy A, Bar N, Schindler CJ, Vathy I. Density of mu-opioid receptors in the hippocampus of adult male and female rats is altered by prenatal morphine exposure and gonadal hormone treatment. *Hippocampus*. 2003; 13(4):461–471. [PubMed: 12836915]
- Slamberova R, Rimanoczy A, Cao D, Schindler CJ, Vathy I. Alterations of prenatal morphine exposure in mu-opioid receptor density in hypothalamic nuclei associated with sexual behavior. *Brain Res Bull*. 2005; 65(6):479–485. [PubMed: 15862919]
- Slamberova R, Rimanoczy A, Riley MA, Vathy I. Hypothalamo-pituitary-adrenal axis-regulated stress response and negative feedback sensitivity is altered by prenatal morphine exposure in adult female rats. *Neuroendocrinology*. 2004; 80(3):192–200. [PubMed: 15583475]
- Slamberova R, Rimanoczy A, Schindler CJ, Vathy I. Cortical and striatal mu-opioid receptors are altered by gonadal hormone treatment but not by prenatal morphine exposure in adult male and female rats. *Brain Res Bull*. 2003; 62(1):47–53. [PubMed: 14596891]
- Slamberova R, Szilagyi B, Vathy I. Repeated morphine administration during pregnancy attenuates maternal behavior. *Psychoneuroendocrinology*. 2001; 26(6):565–576. [PubMed: 11403978]

- Slamberova R, Velisek L, Vathy I. Prenatal morphine exposure alters N-methyl-D-aspartate- and kainate-induced seizures in adult male rats. *Pharmacol Biochem Behav.* 2000; 65(1):39–42. [PubMed: 10638634]
- Szeto HH, Zhu YS, Amione J, Clare S. Prenatal morphine exposure and sleep-wake disturbances in the fetus. *Sleep.* 1988; 11(2):121–130. [PubMed: 3381053]
- Tan JW, Duan TT, Zhou QX, Ding ZY, Jing L, Cao J, Wang LP, Mao RR, Xu L. Impaired contextual fear extinction and hippocampal synaptic plasticity in adult rats induced by prenatal morphine exposure. *Addict Biol.* 2015; 20(4):652–662. [PubMed: 24903743]
- Tao PL, Chen CF, Huang EY. Dextromethorphan attenuated the higher vulnerability to inflammatory thermal hyperalgesia caused by prenatal morphine exposure in rat offspring. *J Biomed Sci.* 2011; 18:64. [PubMed: 21861871]
- Tempel A, Yang J, Basheer R. Prenatal morphine exposure differentially alters expression of opioid peptides in striatum of newborns. *Brain Res Mol Brain Res.* 1995; 33(2):227–232. [PubMed: 8750881]
- Tenconi B, Lesma E, DiGiulio AM, Gorio A. High opioid doses inhibit whereas low doses enhance neuritogenesis in PC12 cells. *Brain Res Dev Brain Res.* 1996; 94(2):175–181. [PubMed: 8836576]
- van Furth WR, van Ree JM. Sexual motivation: involvement of endogenous opioids in the ventral tegmental area. *Brain Res.* 1996; 729(1):20–28. [PubMed: 8874873]
- Vassoler FM, Oliver DJ, Wyse C, Blau A, Shtutman M, Turner JR, Byrnes EM. Transgenerational attenuation of opioid self-administration as a consequence of adolescent morphine exposure. *Neuropharmacology.* 2017; 113(Pt A):271–280. [PubMed: 27729240]
- Vassoler FM, Wright SJ, Byrnes EM. Exposure to opiates in female adolescents alters mu opiate receptor expression and increases the rewarding effects of morphine in future offspring. *Neuropharmacology.* 2016; 103:112–121. [PubMed: 26700246]
- Vathy I. Effects of prenatal morphine exposure on rat heterotypical sexual behavior. *Physiol Behav.* 1999; 66(4):667–671. [PubMed: 10386912]
- Vathy I, He HJ, Iodice M, Hnatzuk OC, Rimanoczy A. Prenatal morphine exposure differentially alters TH-immunoreactivity in the stress-sensitive brain circuitry of adult male and female rats. *Brain Res Bull.* 2000; 51(3):267–273. [PubMed: 10718519]
- Vathy I, Katay L. Effects of prenatal morphine on adult sexual behavior and brain catecholamines in rats. *Brain Res Dev Brain Res.* 1992; 68(1):125–131. [PubMed: 1521318]
- Vathy I, Komisaruk BR. Differential effects of prenatal morphine exposure on analgesia produced by vaginocervical stimulation or systemic morphine administration in adult rats. *Pharmacol Biochem Behav.* 2002; 72(1–2):165–170. [PubMed: 11900784]
- Vathy I, Rimanoczy A, Slamberova R. Prenatal exposure to morphine differentially alters gonadal hormone regulation of delta-opioid receptor binding in male and female rats. *Brain Res Bull.* 2000; 53(6):793–800. [PubMed: 11179845]
- Vathy I, Slamberova R, Rimanoczy A, Riley MA, Bar N. Autoradiographic evidence that prenatal morphine exposure sex-dependently alters mu-opioid receptor densities in brain regions that are involved in the control of drug abuse and other motivated behaviors. *Prog Neuropsychopharmacol Biol Psychiatry.* 2003; 27(3):381–393. [PubMed: 12691773]
- Vathy I, Veliskova J, Moshe SL. Prenatal morphine exposure induces age-related changes in seizure susceptibility in male rats. *Pharmacol Biochem Behav.* 1998; 60(3):635–638. [PubMed: 9678646]
- Vathy IU, Etgen AM, Barfield RJ. Effects of prenatal exposure to morphine on the development of sexual behavior in rats. *Pharmacol Biochem Behav.* 1985; 22(2):227–232. [PubMed: 3983215]
- Vathy IU, Etgen AM, Rabii J, Barfield RJ. Effects of prenatal exposure to morphine sulfate on reproductive function of female rats. *Pharmacol Biochem Behav.* 1983; 19(5):777–780. [PubMed: 6647511]
- Velisek L, Slamberova R, Vathy I. Prenatal morphine exposure suppresses mineralocorticoid receptor-dependent basal synaptic transmission and synaptic plasticity in the lateral perforant path in adult male rats. *Brain Res Bull.* 2003; 61(6):571–576. [PubMed: 14519453]

- Velisek L, Veliskova J, Moshe SL, Vathy I. Prenatal morphine exposure alters ovarian steroid hormonal regulation of seizure susceptibility. *Brain Res.* 1998; 796(1–2):247–256. [PubMed: 9689475]
- Veliskova J, Moshe SL, Vathy I. Prenatal morphine exposure differentially alters seizure susceptibility in developing female rats. *Brain Res Dev Brain Res.* 1999; 116(1):119–121. [PubMed: 10446353]
- Vestal-Laborde AA, Eschenroeder AC, Bigbee JW, Robinson SE, Sato-Bigbee C. The opioid system and brain development: effects of methadone on the oligodendrocyte lineage and the early stages of myelination. *Dev Neurosci.* 2014; 36(5):409–421. [PubMed: 25138998]
- Villarreal DM, Derrick B, Vathy I. Prenatal morphine exposure attenuates the maintenance of late LTP in lateral perforant path projections to the dentate gyrus and the CA3 region in vivo. *J Neurophysiol.* 2008; 99(3):1235–1242. [PubMed: 18199817]
- Wachman EM, Hayes MJ, Brown MS, Paul J, Harvey-Wilkes K, Terrin N, Huggins GS, Aranda JV, Davis JM. Association of OPRM1 and COMT single-nucleotide polymorphisms with hospital length of stay and treatment of neonatal abstinence syndrome. *JAMA.* 2013; 309(17):1821–1827. [PubMed: 23632726]
- Wachman EM, Hayes MJ, Lester BM, Terrin N, Brown MS, Nielsen DA, Davis JM. Epigenetic variation in the mu-opioid receptor gene in infants with neonatal abstinence syndrome. *J Pediatr.* 2014; 165(3):472–478. [PubMed: 24996986]
- Wachman EM, Hayes MJ, Sherva R, Brown MS, Davis JM, Farrer LA, Nielsen DA. Variations in opioid receptor genes in neonatal abstinence syndrome. *Drug Alcohol Depend.* 2015; 155:253–259. [PubMed: 26233486]
- Wachman EM, Hayes MJ, Sherva R, Brown MS, Shrestha H, Logan BA, Heller NA, Nielsen DA, Farrer LA. Association of maternal and infant variants in PNOC and COMT genes with neonatal abstinence syndrome severity. *Am J Addict.* 2017; 26(1):42–49. [PubMed: 27983768]
- Wachman EM, Newby PK, Vreeland J, Byun J, Bonzagni A, Bauchner H, Philipp BL. The relationship between maternal opioid agonists and psychiatric medications on length of hospitalization for neonatal abstinence syndrome. *J Addict Med.* 2011; 5(4):293–299. [PubMed: 21857233]
- Wang Y, Yao Y, Li Y, Nie H, He X. Prenatal morphine exposure during late embryonic stage enhances the rewarding effects of morphine and induces the loss of membrane-bound protein kinase C- α in intermediate medial mesopallium in the chick. *Neurosci Lett.* 2017; 639:25–30. [PubMed: 27989573]
- Wang Y, Yao Y, Nie H, He X. Implication of protein kinase C of the left intermediate medial mesopallium in memory impairments induced by early prenatal morphine exposure in one-day old chicks. *Eur J Pharmacol.* 2017; 795:94–100. [PubMed: 27940175]
- Wigger A, Lorsch P, Oehler I, Keck ME, Naruo T, Neumann ID. Nonresponsiveness of the rat hypothalamo-pituitary-adrenocortical axis to parturition-related events: inhibitory action of endogenous opioids. *Endocrinology.* 1999; 140(6):2843–2849. [PubMed: 10342876]
- Wigger A, Neumann ID. Endogenous opioid regulation of stress-induced oxytocin release within the hypothalamic paraventricular nucleus is reversed in late pregnancy: a microdialysis study. *Neuroscience.* 2002; 112(1):121–129. [PubMed: 12044477]
- Wiles JR, Isemann B, Ward LP, Vinks AA, Akinbi H. Current management of neonatal abstinence syndrome secondary to intrauterine opioid exposure. *J Pediatr.* 2014; 165(3):440–446. [PubMed: 24948346]
- Wong CS, Lee YJ, Chiang YC, Fan LW, Ho IK, Tien LT. Effect of prenatal methadone on reinstated behavioral sensitization induced by methamphetamine in adolescent rats. *Behav Brain Res.* 2014; 258:160–165. [PubMed: 24157336]
- Wu CC, Hung CJ, Shen CH, Chen WY, Chang CY, Pan HC, Liao SL, Chen CJ. Prenatal buprenorphine exposure decreases neurogenesis in rats. *Toxicol Lett.* 2014; 225(1):92–101. [PubMed: 24321744]
- Wu VW, Mo Q, Yabe T, Schwartz JP, Robinson SE. Perinatal opioids reduce striatal nerve growth factor content in rat striatum. *Eur J Pharmacol.* 2001; 414(2–3):211–214. [PubMed: 11239921]
- Yamamizu K, Furuta S, Katayama S, Narita M, Kuzumaki N, Imai S, Nagase H, Suzuki T, Narita M, Yamashita JK. The kappa opioid system regulates endothelial cell differentiation and pathfinding in vascular development. *Blood.* 2011; 118(3):775–785. [PubMed: 21460241]

- Yang SN, Huang LT, Wang CL, Chen WF, Yang CH, Lin SZ, Lai MC, Chen SJ, Tao PL. Prenatal administration of morphine decreases CREBSerine-133 phosphorylation and synaptic plasticity range mediated by glutamatergic transmission in the hippocampal CA1 area of cognitive-deficient rat offspring. *Hippocampus*. 2003; 13(8):915–921. [PubMed: 14750654]
- Yang SN, Yang JM, Wu JN, Kao YH, Hsieh WY, Chao CC, Tao PL. Prenatal exposure to morphine alters kinetic properties of NMDA receptor-mediated synaptic currents in the hippocampus of rat offspring. *Hippocampus*. 2000; 10(6):654–662. [PubMed: 11153711]
- Zagon IS, Wu Y, McLaughlin PJ. Opioid growth factor and organ development in rat and human embryos. *Brain Res*. 1999; 839(2):313–322. [PubMed: 10519055]
- Zhang Y, Loh HH, Law PY. Effect of Opioid on Adult Hippocampal Neurogenesis. *ScientificWorldJournal*. 2016; 2016:2601264. [PubMed: 27127799]
- Zhu Y, Hsu MS, Pintar JE. Developmental expression of the mu, kappa, and delta opioid receptor mRNAs in mouse. *J Neurosci*. 1998; 18(7):2538–2549. [PubMed: 9502813]
- Zmitrovich AC, Brake SC, Liu PY, Hamowy AS, Hutchings DE. Prenatal administration of methadone in the rat: acoustic startle amplitude and the rest-activity cycle at 30 days of age. *Neurotoxicol Teratol*. 1994; 16(3):251–255. [PubMed: 7935258]

Highlights

- Animal models of prenatal opioid exposure demonstrate significant developmental effects in offspring.
- Outcomes of prenatal opioid exposure vary by drug, dose, duration, and timing of exposure.
- Improving the translational value of preclinical models is crucial given the significant increase in the number of exposed infants.

Scientific Publications with Titles Referring to the Opioid Epidemic

Figure 1.

The number of scientific publications from 2006–2016 as referenced in PubMed that use the phrase “opioid epidemic” in the title.

Table 1

Prenatal opioids affect development.

Age at Exposure	Drug	Age at Testing	Offspring Effects	Reference
Physical				
G11–18 SD rats	MOR: 10 mg/kg b.i.d., s.c.	PN1–21 ♂ and ♀ F1 and F2	↑ righting latency both generations ↓ anogenital distance in second generation ♂	(Slamberova, Riley et al. 2005)
G7–PN4 SD rats	MET: 9 mg/kg/day, pumps	PN4–22 ♂ and ♀ (cross-fostered)	↑ acquisition of righting reflex and negative geotaxic response ↓ somatic growth thru PND21 ↓ spontaneous activity in pups exposed to MET both pre- and postnatally ↑ spontaneous activity in postnatal only MET exposure	(Kunko, Smith et al. 1996)
G7–21 SD rats	OXY 0.5–15 mg/kg escalating, p.o.	PN 1–28 ♂	↓ Birth weight ↑ craniofacial malformations ↑ Endothelin A (ET _A) and ↓ Endothelin B (ET _B) expression in whole brain on PND1 and 7 ↑ ETB expression on PND28	(Devarapalli, Leonard et al. 2016)
G7–21 SD rats	BUP: 0.3 or 1.0 mg/kg/day, i.p.	PN21 ♂ and ♀	↓ BW and ↓ brain mass ↑ depressive-like behavior ↓ plasma BDNF and 5HT ↑ oxidative stress in cortical tissue ↓ BDNF/TrkB-mediated signaling in cortical tissue	(Hung, Wu et al. 2013)
G7–21 SD rats	BUP: 1.0 mg/kg/day, i.p.	PN21 ♂ and ♀	↓ BW ↓ brain mass ↑ depressive-like behavior ↓ expression of a number of neuronal cell markers, neural progenitor markers, growth factors (BDNF) in PFC	(Wu, Hung et al. 2014)
G8-parturition SD rats	MET: 9 mg/kg/day pump	PN21–23 ♂ and ♀ (cross-fostered)	↓ BW in pups ↑ acoustic startle Effects resolve by PND30	(Hutchings, Zmitrovich et al. 1993, Zmitrovich, Brake et al. 1994)
G8–21 SD rats	BUP: 0.3, 1.0, 3.0 mg/kg/day, pump	PN1–30 ♂ and ♀ (cross-fostered)	↑ pup mortality with 2 higher doses.	(Hutchings, Hamowy et al. 1996)
Neuronal				
G12–PN5 SD rats	MOR: 10 mg/kg/day, pump	PN5 ♂	↓ total dendritic length in higher order dendritic branches with terminal dendrites having the greatest reduction	(Ricalde and Hammer 1990)

Age at Exposure	Drug	Age at Testing	Offspring Effects	Reference
G11–18 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	PN25	↓ length, branch number and spine density of layer II/III pyramidal neurons in lateral secondary visual area	(Mei, Niu et al. 2009)
GD7-PN14 SD rats	MET: 9 mg/kg/day, pump	PN10, 16, 19	↑ myelin basic proteins, myelin proteolipid protein, and myelin-oligodendrocyte glycoprotein ↑ number of myelinated axons with highly compacted myelin sheaths	(Vestal-Laborde, Eschenroeder et al. 2014)
G7-PN21 SD rats	BUP: 0.0, 0.3, 1.0 mg/kg/day, pump	PN12, 19, and 26	Dose dependent changes in myelin basic proteins ↓ number of myelinated axons at PN26 in 1.0 mg/kg group Differences in ratio of small/large diameter axons ↑ percentage of large diameter axons with thinner sheaths	(Sanchez, Bigbee et al. 2008)
G121–129 sheep	MOR: 400 ug/h/day, pump	fetal lambs	↑ arousal and loss of quiet sleep and REM sleep on Day 1; Similar effects following drug withdrawal	(Szeto, Zhu et al. 1988)
<i>Striatal</i>				
G7-PN21 SD rats	BUP: 0.3, 3.0 mg/kg/day; MET: 9 mg/kg/day, pump	PN4–21 ♂ and ♀* (cross-fostered)	↓ STR ACh content PN4 both BUP and MET ↑ STR ACh content PN21 in 0.3 BUP ♂ exposed both pre- and postnatally ↓ STR ACh content PN21 in 3.0 BUP exposure both pre- and postnatally	(Robinson 2002)
G7-PN21 SD rats	BUP: 1.5 mg/kg/day, pump; MET: 9 mg/kg/day, pump	PN4 or 10 ♂ and ♀ (cross-fostered)	↓ STR ACh content and expression of ChAT PND4 Effects dissipate with development	(Robinson 2000)
G7-PN4 SD rats	MET: 9 mg/kg/day, pump	PN4 ♂ and ♀* (cross-fostered)	↓ STR ACh content in both pre- and postnatal MET ♂ and ♀ ↓ 5-HIAA content in STR following prenatal exposure ↓ DA turnover in STR in prenatal only ↑ DA and ACh turnover in STR	(Robinson, Guo et al. 1996, Robinson, Mo et al. 1996)
G7-PN21 SD rats	MET: 9 mg/kg/day, pump	PN21 ♂ and ♀* (cross-fostered)	↓ DA turnover in frontal cortex but not STR of postnatal MET ♂ ↑ NE turnover in HIPPO of prenatal MET ♂ ↑ 5-HIAA in STR and parietal cortex in postnatal MET ♂&♀.	(Robinson, Maher et al. 1997)
	MOR: 0.5–1 mg/kg/day	E21 brains	↑ D1-stimulated cAMP in striatal slices (No effect of DAMGO)	(De Vries, Van Vliet et al. 1991)

Age at Exposure	Drug	Age at Testing	Offspring Effects	Reference
G7–21 SD rats			No difference in forskolin-stimulated cAMP ↑ electrically evoke NE release from neocortical slices	
G13–20 SD rats	MOR: 2 mg/kg/day i.p.	PN1, 7,14, 28	↓ Cyclin-dependent kinase 5 in brain	(Bhat, Chari et al. 2006)
G15–21 SD rats	MOR: 75 mg pellet s.c.	PN0	↑ proenkephalin mRNA, ↓ metenkephalin mRNA, ↓ enkephalins in STR ↓ G alpha s mRNA in frontal cortex	(Basheer, Yang et al. 1992, Tempel, Yang et al. 1995)
G7-PN21 SD rats	BUP: 1.5 mg/kg/day; MET: 9 mg/kg/day, pump	PN4 or 10 ♂ and ♀ (cross-fostered)	↓ NGF content in STR with no change in NGF mRNA	(Wu, Mo et al. 2001)
Premating: 7d–PN18 CD1 mice	MOR 30 or 60 mg/kg/day i.p.	PN18	Region dependent changes in Calbindin-D28K positive neurons	(Mithbaokar, Fiorito et al. 2016)

Abbreviations: **ACh** (acetylcholine); **ChAT** (choline acetyl transferase); **DA** (dopamine); **D1** (dopamine D1 receptor); **5-HIAA** (5-hydroxyindoleacetic acid); **HIPP** (hippocampus); **NE** (norepinephrine); **NGF** (nerve growth factor); **REM** (rapid eye movement); **STR** (striatum);

* sex-specific effects

Table 2

Prenatal opioid exposure affects development of endogenous opioid system

Age at Exposure	Drug	Age at Testing	Offspring Effects	Reference
Effects on Receptor Binding				
G12–21 Wistar rats	MOR: 5 mg/kg q.i.d. or 10 mg/kg b.i.d.	E21, PND3–60	↓ mu receptor binding in spinal cord up to PND30; ↑ mu receptor binding in spinal cord at PND60 differences in # of binding sites and affinity	(Kirby 1983)
G11–18 SD rats	MOR: 5–10 mg/kg/day s.c.	Adult ♂ and ♀ *	↑ mu receptor density in CA1 and CA3 of ♀ but not ♂ Effects regulated by gonadal hormones	(Slamberova, Rimanoczy et al. 2003)
Premating: 7d Gestation 21d Postpartum SD rats	MOR: 2 mg/kg/day i.p. ↑ by 1 mg/kg every 14 days	PN14	↓ mu opioid receptor density in STR, thalamus and amygdala No effect observed in NAc, HIPP or cortex Tolerance to morphine-induced tail-flick response	(Chiou, Yeh et al. 2003)
G13–20 SD rats	MOR: 2 mg/kg/day i.p.	PN1, 7, 14, 28	↑ mu receptor mRNA and binding in whole brain observed at PN1 and 7 but not PN14 or 28	(Bhat, Chari et al. 2006)
G11–18 SD rats	MOR: 5–10 mg/kg/day s.c.	PN70–90, ♂ and ♀ *	↓ mu receptor binding in MPOA of ♂ ↑ mu receptor binding in VMH of OVX ♀, which significantly decreases with EB (opposite effect observed in controls)	(Slamberova, Rimanoczy et al. 2005)
G11–18 SD rats	MOR: 5–10 mg/kg/day s.c.	Adult ♂	↓ mu receptor binding in BLA ↑ mu receptor binding in CeA, PMCoA and NAc	(Vathy, Slamberova et al. 2003)
G11–18 SD rats	MOR: 5–10 mg/kg/day s.c.	Adult ♂ and ♀	No effect on mu receptor binding in cerebral cortex, cingulate cortex or STR	(Slamberova, Rimanoczy et al. 2003)
G11–18 SD rats	MOR: 5–10 mg/kg/ b.i.d., s.c.	Adult ♂ and ♀	No difference in delta-opioid receptors in intact ♂ Effects on hormonal regulation of delta-opioid receptors in frontal cortex and STR in ♂ and ♀	(Vathy, Rimanoczy et al. 2000)
G11–18 SD rats	MOR: 5–10 mg/kg/ b.i.d., s.c.	Adult ♂ and ♀ *	↑ kappa receptor binding in frontal cortex and POA of OVX ♀ No effect on kappa receptor binding in ♂	(Rimanoczy, Slamberova et al. 2001)
G10-PN7	BUP: 0.5, 1.0 mg/kg/day MET: 5 mg/kg/day, pumps	PN2 and PN7 ♂ and ♀ *	↓ DAMGO-induced GTPγS binding in ♂ PN2 (primarily VTA and NAc) both BUP and MET	(Hou, Tan et al. 2004)

Age at Exposure	Drug	Age at Testing	Offspring Effects	Reference
SD rats			↑ nociceptin/orphanin FQ-induced GTPγS binding in NAc and LS of PN2 ♂ BUP	
Effects on Analgesic Response				
Premating: 7d Gestation SD rats	MOR: 2mg/kg/day s.c. ↑ daily by 1 mg/kg/day	PN14 and 18	↑ hyperalgesia (plantar); blocked by co-administration of dextromethorphan ↑ NR1 and NR2B mRNA and protein in spinal cord: attenuated by dextromethorphan	(Tao, Chen et al. 2011)
G11–18 SD rats	MOR: 5–10 mg/kg/day s.c.	Adult ♀	Tolerance to morphine-induced tail-flick ↑ analgesic response to vaginal stimulation	(Vathy and Komisaruk 2002)
G5–12 SD rats	MOR: 5–10 mg/kg/day s.c.	PN21–55 ♂ and ♀	↓ morphine-induced analgesia (hotplate)	(O'Callaghan and Holtzman 1976)
G5–12 SD rats	Levorphanol or dextrophan 1.0–1.75 mg/kg/b.i.d., s.c.	5 or 9 weeks ♂ and ♀	Levorphanol produced same tolerance to morphine-induced analgesia as gestational morphine while dextrophan had no effects	(O'Callaghan and Holtzman 1977)
Premating G1-14 CD1 mice	MOR: 10–40 mg/kg/day increasing	Adult ♂ and ♀	↑ analgesic response to both morphine and stress ↑ activity in response to morphine	(Castellano and Ammassari-Teule 1984)
G12–18 Fischer rats	MOR: 0.75–12.0 mg/day slow-release emulsion	PN70–84 ♂ and ♀ (cross-fostered)	↑ morphine analgesia ↑ preference for saccharin solution	(Gagin, Cohen et al. 1996)
G14–16 Wistar rats	MOR: 0.5 or 5 mg/kg/day s.c.	Adult ♂ and ♀*	↑ sensitivity to the antinociceptive effects of morphine in ♂ (5 mg/kg dose only)	(Biglarnia, Karami et al. 2013)
G3–20 SD rats	MOR 2–4 mg/kg/day s.c. MET: 7 mg/kg/day BUP: 3 mg/kg/day	Adult ♂ and ♀*	↑ mortality in BUP group ↑ MOR-induced analgesic tolerance in all groups largest effect in BUP group	(Chiang, Hung et al. 2010)
Effects on Reward				
G3–20 SD rats	MOR: 2–4 mg/kg b.i.d. s.c., increasing	Adult ♂	↑ time to extinguish and drug-primed reinstatement of methamphetamine CPP and self-admin	(Shen, Chen et al. 2016)
G3–20 SD rats	MET: 3 mg/kg, b.i.d. s.c.	PN1, PN30 and adulthood	↑ MET-induced CPP ↓ Oprm1 and Oprl1 in spinal cord ↑ paw movement during postnatal withdrawal ↑ pain sensitivity; many effects reversed by co-administration of dextromethorphan	(Chiang, Ye et al. 2015)

Age at Exposure	Drug	Age at Testing	Offspring Effects	Reference
G5–12 SD rats	MOR: 5–10 mg/kg/day s.c.	Adult ♂ and ♀	Tolerance to the disruptive effects of morphine but not amphetamine on fixed responding	(Wagner, Jarvis et al. 1986)
E5-E8 chick embryos	MOR: 1 mg/kg every other day into the allantoic sac	Post hatch day 1 and 4	↓ reward learning/MOR CPP	(Jiang, He et al. 2011)
G17–21 SD rats	MOR: 10 mg/kg, t.i.d.	Young adult ♂ (130– 150 g)	↑ naloxone precipitated withdrawal following 3 days of morphine (75 mg pellet sc)	(Koyuncuoglu and Aricioglu 1993)

Summary of effects related to the development of the endogenous opioid system and effects on offspring responses to exogenously administered opioids across multiple animals models of prenatal opioid exposure. Abbreviations: **BLA** (basolateral amygdala); **CeA** (central amygdala); **CPP** (conditioned place preferences); **EB** (estradiol benzoate); **HIPP** (hippocampus); **LS** (lateral septum); **MPOA** (medial preoptic area); **Nac** (nucleus accumbens); **PMCoA** (posteromedial cortical amygdala); **POA** (preoptic area); **STR** (striatum); **VMH** (ventromedial hypothalamus);

*
sex-specific effects

Table 3

Prenatal opioid exposure affects endocrine and immune responses

Age at Exposure	Drug	Age at Testing	Offspring Effects	References
G11–18 Wistar rats	MOR: 10 mg/kg s.c., b.i.d.	PN90 ♂	No effect on basal or ether stress-induced plasma ACTH or CORT ↓ Adrenal NE at baseline and post-recovery ↓ Adrenal PNMT 60 and 90 min post ether stress ↑ 5HT content in HIPP and HYPO post ether stress No effect on BW or adrenal weight	(Laborie, Dutriez-Casteloot et al. 2005)
G11–18 SD rats	MOR: 10 mg/kg, b.i.d.	Adult ♀	No effects on basal or stress-induced CORT ↓ Restraint-stress induced ACTH ↓ sensitivity to DEX negative feedback Effects modulated by estrous cycle	(Slamberova, Rimanoczy et al. 2004)
G11–18 SD rats	MOR: 5–10 mg/kg, b.i.d.	Adult ♂ (cross-fostered)	No effects on basal or stress-induced CORT ↓ Restraint stress-induced ACTH No effect on sensitivity to DEX negative feedback	(Rimanoczy, Slamberova et al. 2003)
G11–18 SD rats	MOR: 10 mg/kg, b.i.d.	Adult ♂ and ♀ *	↑ MR and GR binding in HIPP of saline injected ♂ (prenat a l stressor) prevented by MOR ↓ MR in HIPP and GR in HYP in saline injected ♀, prevented by MOR and cycle dependent.	(Rimanoczy, Slamberova et al. 2006)
G11–18 SD rats	MOR: 10 mg/kg, b.i.d.	Adult ♂	↓ adrenal NE and Epi ↑ circulating Epi at rest	(Dutriez-Casteloot, Bernet et al. 1999)
G8-PN1,3,5 Dark agoutirats	OXY: 0.8 mg/kg/day i.v.	PN45 ♂ and ♀ *	↑ CRH-stimulated ACTH in ♂ No difference in CRH-stimulated CORT in ♂ No effect on CRH-stimulated ACTH or CORT in ♀	(Sithisarn, Bada et al. 2008)
Premating and gestation, 28d SD rats	l-alpha-acetylmethadol 0.2 or 1.0 mg/kg/day, p.o.	PN18 and Adult ♂ and ♀ * (cross-fostered)	↓ CORT 2h following immune stressor and 15 min following social stressor on PN18 ↑ CORT 3h after immune stressor in adults ↑ startle response in 1 mg-treated ♂ No effects on open field	(Hamilton, Harris et al. 2005)
G12–18	MOR 0.75–12 mg/kg/day s.c. increasing doses	Adult ♂	↓ cytotoxic activity of NK cells ↓ LPS-induced fever	(Shavit, Cohen et al. 1998)

Age at Exposure	Drug	Age at Testing	Offspring Effects	References
Fischer rats			↓ LPS-induced hyperalgesia ↑ LPS-induced hypoalgesia	
Premating:7d Gestation:21d SD rats	l-alpha-acetylmethadol 0.2 or 1.0 mg/kg/day p.o.	Adult ♂ (cross-fostered)	↓ BW one week postpartum ↓ LPS-induced fever ↑ IL-1B protein in hypothalamus following LPS	(Hamilton, Franklin et al. 2007)
E4–20 chick embryos	NLAAM: 2.5, 5, or 10mg/kg/egg	Post hatch Day 1–18	↓ LPS-induced fever ↑ injection-induced CORT ↓ hypersensitivity response to phytohemagglutinin	(Schrott and Sparber 2004)

Summary of effects related to the hypothalamic pituitary adrenal axis and immune system across multiple animals models of prenatal opioid exposure. Abbreviations: **ACTH** (adrenocorticotropin hormone); **CORT** (corticosterone); **CRH** (corticotropin releasing hormone); **DEX** (dexamethasone); **Epi** (epinephrine); **GR** (glucocorticoid receptor); **HIPP** (hippocampus); **HYPO** (hypothalamus); **IL-1B** (interleukin 1 beta); **LPS** (lipopolysaccharide); **MR** (mineralocorticoid receptor); **NE** (norepinephrine); **PNMT** (phenyl ethanolamine N-methyltransferase)

* sex-specific effects

Table 4

Prenatal opioid exposure affects social and reproductive-related behaviors.

Age at Exposure	Drug	Age at Testing	Offspring Effects	Reference
G5–12 SD rats	MOR: 5–10 mg/kg/day s.c.	PN21–90 ♂ and ♀ *	Delayed VO in ♀ ↓ sexual behavior ♀ ↑ BW at PND90 ♀ modest effects on ♂ sexual behavior	(Vathy, Etgen et al. 1983, Vathy, Etgen et al. 1985)
G5–12 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	PN1–60	Delayed VO in ♀ ↓suckling-induced PRL in mothers ↓sensitivity of HPA to gonadal steroids at PN15	(Litto, Griffin et al. 1983)
G11–18 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	Adult ♂ and ♀ *	↓ ♀ sexual behavior ↑♂ sexual behavior ↓ NE in hypothalamus of ♀ ↓ NE in hypothalamus of ♂	(Vathy and Katay 1992)
G11–18 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	Adult ♂ and ♀ *	↑ expression of ♂ sexual behaviors in ♀ rats in response to testosterone; No effects of E + P in ♂	(Vathy 1999)
G11–18 SD rats	MOR: 5–10 mg/kg/day s.c.	Adult ♀ offspring mated to naïve ♂	↑ active nursing and ↓passive nursing ↑rapid pup retrieval	(Slamberova, Bar et al. 2003)
G8–21 SD rats	MOR: 10 mg/kg/day s.c.	PN21, 28, 90	↑ pinning PN21 ↑social grooming PN21 ↑ social approach ↓social avoidance	(Hol, Niesink et al. 1996)
G8–G21 Wistar rats	MOR:10 mg/kg/day s.c.	PN21	↑endogenous opioid release following mild stress ↑ pinning during social play ♂ and ♀ No effect on BW or sensorimotor reflexes	(Buisman-Pijlman, Gerrits et al. 2009)
G11–18 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	Adult ♂ and ♀ *	↑ TH-ir in PVN and LC of ♂ ↓ TH-ir in LC of OVX ♀ No difference in E-treated	(Vathy, He et al. 2000)

Summary of effects related to social and other reproductive-related behaviors across multiple animal models of prenatal opioid exposure.

Abbreviations: **E** (estrogen); **LC** (locus coeruleus); **NE** (norepinephrine); **P** (progesterone); **PRL** (prolactin); **PVN** (paraventricular nucleus); **TH** (tyrosine hydroxylase);

* sex-specific effects

Table 5

Prenatal opioid exposure affects learning and memory processes.

Age at Exposure	Drug	Age at Testing	Behavioral Effects	Neural Effects	Reference
G11–18 SD Rat	MOR: b.i.d.	PN22–31	Deficit in spatial memory (Y maze)	↓Population spike LTP but not EPSP LTP ↓GABA-containing neurons in DG	(Niu, Cao et al. 2009)
G11–18 SD rats	MOR 5–10 mg/kg b.i.d. s.c.	Adult ♂		↓baseline EPSP and synaptic plasticity in lateral perforant path	(Velisek, Slamberova et al. 2003)
G11–18 SD rats	MOR 5–10 mg/kg b.i.d. s.c.	PN109–120 ♂		↓LTP in lateral perforant path No effect in LTP in medial perforant path	(Villareal, Derrick et al. 2008)
G11–18 Wistar rats	MOR 5–10 mg/kg b.i.d. s.c.	PN51–57 ♂ and ♀	↑ anxiety-like behavior ↑deficits in passive avoidance memory Some deficits improved with environmental enrichment	↓BDNF in hippocampus Reversed by environmental enrichment	(Ahmadipour, Sadeghzadeh et al. 2015)
G1–13 G1–21 Wistar rats	MOR 0.2–0.8mg/day, increasing, p.o.	PN28–70 ♂ and ♀ *	Sex, age, and dose-dependent impairments on passive avoidance memory with ♀ more vulnerable than ♂	↑ Bax/Bcl-2 ratio in DG of ♂ and ♀ ↓caspase-3 activation in HIPP ♂ and ♂. ↓BDNF in ♀ HIPP ↓ activation of CaMKII in HIPP ♂ and ♀	(Nasiraei-Moghadam, Sherafat et al. 2013)
G9–18 SD rats	MOR 5–10 mg/kg b.i.d. s.c.	PN56–70 ♂	Impaired acquisition but increased maintenance of contextual fear memory with impaired extinction ↓anxiety-like behavior	Impaired HIPP CA1 LTP and LTD in response to fear conditioning	(Tan, Duan et al. 2015)
G3-G20 SD rats	MET: 3 mg/kg, b.i.d. s.c.	PN1, PN30 and Adults	No effect on spatial learning and memory	No effect on hippocampal LTP or LTD in PN14–21 slices	(Chiang, Ye et al. 2015)
G1–13 G1–21	MOR: 0.01–0.08 mg/ml, p.o.	PN28 and Adults ♀ and ♂ *	↓ passive avoidance learning and retention (24h) in ♀ and ♂	↑ Bax/Bcl-2 ratio and cleaved caspase-3 in ♀ and adolescent ♂ in the HIPP ↓ BDNF in ♀	(Nasiraei-Moghadam, Sherafat et al. 2013)

Age at Exposure	Drug	Age at Testing	Behavioral Effects	Neural Effects	Reference
Wistar rats					
Prenating: 7d				↓LTD in CA1 pyramidal cells	
Gestation-PN30	MOR: 2–7 mg/kg b.i.d., increasing	PN28–31	↑escape latency on Day 1 of MWM task but no differences on subsequent days.	No effect on LTP in CA1 ↓pCREB in CA1 ↓expression range of glutamatergic synaptic plasticity in CA1 neurons	(Yang, Huang et al. 2003)
SD rats					
Prenating and			↑reference memory errors		
Gestation-PN30	OXY: 15 mg/kg/day p.o.	Adult ♂	Deficits on MW M following long but not short intertrial interval		(Davis, Franklin et al. 2010)
SD rats			↓use of spatial strategies		
Prenating: 7d					
Gestation-PN7	MOR: 2–7 mg/kg b.i.d., increasing	PN14 hippocampal slices		Altered kinetic properties of synaptic NMDA receptors in CA1 pyramidal neurons	(Yang, Yang et al. 2000)
SD rats					
Prenating and			↑reference and working memory errors on radial arm maze		
Gestation-PN7	l-alpha-acetylmethadol 1.0 mg/kg/day p.o.	Adult ♂ (cross-fostered)		↓ BDNF precursor protein in HIPP ♂	(Schrott, Franklin et al. 2008)
SD rats			↓percent correct deficits apparent during training	No effect on TrkB	
Prenating: 7d					
Gestation	MOR: 2mg/kg/day s.c. ↑1 mg/kg/day throughout	PN14 and PN45–59	↓BW P1–P14 but not P21–P45 ↑errors in working and reference memory on spatial task	↓PSD-95, NR1, NR2A and NR2B mRNA and protein in CA1 at PN14 and 45	(Lin, Tao et al. 2009)
SD rats					
E12–16 chick embryos	MOR: 20 mg/kg/day into airspace	Post hatch day 1	↓long term memory using a passive avoidance task (deficits at 120 min but not 30 min)		(Che, Sun et al. 2005)
E5-E8 chick embryos	MOR: 1 mg/kg every other day into allantoic sac	Post hatch day 2		↓PKCα activity in the left IMM ↑ translocation of PKC δ from cytosol to membrane in left IMM	(Wang, Yao et al. 2017)

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Age at Exposure	Drug	Age at Testing	Behavioral Effects	Neural Effects	Reference
E5-E8 chick embryos	MOR: 1 mg/kg every other day into allantoic sac	Post hatch day 2	↓passive avoidance learning		(Wang, Yao et al. 2017)
E5-E8 chick embryos	MOR: 1 mg/kg every other day into allantoic sac	Post hatch day 1 and 4	↓passive avoidance learning ↓reward learning/MOR CPP	↓paired-pulse facilitation and heterosynaptic LTP in IMM <i>in vitro</i> (IMM is comparable to mammalian HIPP)	(Jiang, He et al. 2011)

Summary of effects related to learning and memory and related neural processes across multiple animals models of prenatal opioid exposure: **BDNF** (brain derived neurotrophic factor); **CPP** (conditioned place preference); **DG** (dentate gyrus); **EPSP** (excitatory postsynaptic potential); **HIPP** (hippocampus); **IMM** (intermediate medial mesopallium); **LTD** (long-term depression); **LTP** (long-term potentiation); **MWM** (Morris water maze);

* sex-specific effects

Table 6

Prenatal opioid exposure affects epileptiform activity

Age at Exposure	Drug	Age at Testing	Offspring Effects	Reference
G11–18 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	Adult ♂	↓ susceptibility to tonic-clonic bicuculline seizures in intact ♂	(Schindler, Slamberova et al. 2001)
G11–18 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	PN25, 38 ♂ and ♀ *	↑ bicuculline-induced seizure susceptibility at PN25 in ♂ ↓ bicuculline-induced seizure susceptibility at PN38 in ♂ No effects in ♀	(Schindler, Veliskova et al. 2000)
G11–18 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	Adult ♂	↓ proenkephalin and ↑ prodynorphin mRNA in DG. ↓ met-enkephalin and ↑ dynorphin B levels in DG ↓ dynorphin-derived peptides in CA3 ↑ mu receptor but not delta receptor in HIPP	(Schindler, Slamberova et al. 2004)
G11–18 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	PN15, 25, 38 and Adult ♂	↑ threshold to clonic flurothyl-induced seizures in adults No effect at PN15, emerging effect at PN25, adult-like effect at PN38	(Vathy, Veliskova et al. 1998)
G11–18 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	Adult ♀	↓ tonic-clonic seizure threshold in the presence E and P ↓ development of epileptiform activity <i>in vitro</i> in the presence E	(Velisek, Veliskova et al. 1998)
G11–18 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	PN15, 25, 38 and Adult ♀	↓ clonic and tonic-clonic seizure threshold at PN15 No effects at PN25 or 38	(Veliskova, Moshe et al. 1999)
G11–18 SD rats	MOR: 5–10 mg/kg b.i.d. s.c.	Adult ♂	Dose dependent changes in NMDA and kainic acid-induced seizure activity	(Slamberova, Velisek et al. 2000)

Summary of effects related to seizure activity across multiple animals models of prenatal opioid exposure.

Abbreviations: **DG** (dentate gyrus); **E** (estrogen); **HIPP** (hippocampus); **P** (progesterone);*
sex-specific effects