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A review of opioid replacement therapy with methadone or buprenorphine on neural development in the newborn

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A REVIEW OF OPIOID REPLACEMENT THERAPY WITH METHADONE OR
BUPRENORPHINE ON NEURAL DEVELOPMENT IN THE NEWBORN

by

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A REVIEW OF OPIOID REPLACEMENT THERAPY WITH METHADONE OR BUPRENORPHINE ON NEURAL DEVELOPMENT IN THE NEWBORN

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ABSTRACT

Opioid replacement therapy with methadone or buprenorphine has been recommended for managing opioid dependence during pregnancy. Although opioid replacement therapy decreases harmful consequences from maternal illicit drug seeking behaviors, the effects of methadone and buprenorphine on neurogenesis and myelination in the developing fetus have not been thoroughly reviewed. Methadone and buprenorphine may alter newborn neurobehavioral functions by impairing neurogenesis and changing the developmental pattern of myelination. This review found that therapeutic doses of methadone and buprenorphine disturb both neurogenesis and myelination in rodents. Methadone and buprenorphine may alter newborn neurobehavioral functions by impairing neurogenesis and changing the developmental pattern of myelination. However, further studies are required to bridge the gap in the understanding between changes in neural development and abnormalities in neurobehavioral function in the newborn.
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LIST OF ABBREVIATIONS

ATP .............................................................. Adenosine triphosphate
BrdU ......................................................................... Bromodeoxyuridine
Ca ........................................................................... Calcium
cAMP .............................................................. Cyclic adenosine monophosphate
DAPI ................................................................. 4’, 6-Diamidino-2-phenylindole
G-protein .................................................. Guanine nucleotide binding protein monophosphate
GABA .......................................................... Gamma amino butyric acid
GDP ................................................................. Guanosine monophosphate
GTP ................................................................. Guanosine triphosphate
K ......................................................................... Potassium
INTRODUCTION

According to the 2013 National Survey on Drug Use and Health, 5.4% of pregnant women reported illicit drug use within the past month, including heroin and prescription opioid analgesics, such as morphine, hydromorphone, codeine, hydrocodone, oxycodone and fentanyl (1). Opioid abuse during pregnancy has been linked to obstetric complications, such as intrauterine growth restriction, neural tube defect, placental dysfunction, premature labor, and spontaneous abortion as well as neonatal abstinence syndrome following birth (2). The percentage of pregnant women abusing opioids specifically has not been determined however, and adverse human pregnancy outcomes from maternal opioid abuse remain poorly understood. Opioid replacement therapy with methadone or buprenorphine, long acting opioid analogues, has been recommended for opioid dependence during pregnancy to minimize symptoms of withdrawal in the fetus, safeguard placental function, and decrease harmful consequences from maternal illicit drug seeking behaviors, such as poor nutrition, infection, and exposure to crime and violence (2). However, both methadone and buprenorphine readily transverse the human placenta (3) which raises the concern for chronically administered exogenous opioids interfering with endogenous opioid systems in the developing fetal central nervous system and ultimately, disrupting neural development.
Mechanisms of Opioid Receptor Signal Transduction

Previous studies have demonstrated that opioids are involved in modulating central nervous system activity, including perception of pain, euphoria, dysphoria, respiration, and function of the hypothalamic-pituitary axis by selectively binding and activating opioid receptors following birth (4). Classically, opioid receptors are a group of G-protein coupled receptors specifically associated with inhibitory heterotrimeric G-proteins and are widely distributed throughout the mammalian central nervous system. Upon activation, opioid receptors undergo a conformational change and induce the inhibitory G-protein alpha subunits to exchange GDP for GTP. The GTP-bound inhibitory G-protein alpha subunit dissociates from the G-protein beta-gamma dimer and inhibits adenylyl cyclase from generating cAMP from ATP. Decreased availability of cAMP leads to decreased activity of cAMP-dependent protein kinase, modifying a variety of downstream intracellular processes. The G-protein beta-gamma dimer binds directly to calcium channels and can decrease voltage dependent channel opening in neurons; inward calcium current decreases which dampens neurotransmitter release. The G-protein beta-gamma dimer can also bind directly to inwardly rectifying potassium channels, leading to an increased inward potassium current and thereby membrane hyperpolarization (4).
Distribution of Opioid Receptors in the Adult Mammalian Central Nervous System

To date, three opioid receptor subtypes have been discriminated – mu (µ), delta (δ) and kappa (κ) – and are widely distributed throughout the adult mammalian central and peripheral nervous systems. Mu opioid receptors demonstrate high affinity for the endogenous opioid peptide beta-endorphin, morphine (full agonist), methadone (full agonist), and buprenorphine (partial agonist), among other exogenous opioids (4). Mu opioid receptors are constitutively expressed and modulate analgesia within the amygdala, substantia nigra, periaqueductal gray, rostroventral medulla, and substantia gelatinosa of Rolando in the dorsal horn. Mu opioid receptors have also been identified within the cerebral cortex, thalamus, striate nucleus, interpeduncular nucleus, and superior and inferior colliculi (5). In contrast to mu opioid receptors, delta opioid receptors bind endogenous enkephalins and are frequently found within the cytoplasm of neurons contributing to the olfactory bulb, substantia gelatinosa of Rolando, amygdala, striate nucleus, and prefrontal cortex (6, 7) as well as specialized mechanosensory end organs and axons surrounding hair follicles (8); cell surface expression of delta opioid receptors has been suggested to occur via regulated secretion (6).

The roles of mu opioid receptors and delta opioid receptors in modulating perception of pain within the spinal cord have been elucidated (9). Activation of mu
opioid receptors, and to a lesser degree delta opioid receptors, expressed on C fiber synaptic terminals in the substantia gelatinosa of Rolando, inhibits their release of excitatory Substance P onto second order sensory neurons, disrupting transmission of thermally and mechanically induced nociceptive input. Activation of mu opioid receptors and delta opioid receptors also inhibits second order sensory neurons directly by increasing potassium conductance post-synaptically, leading to membrane hyperpolarization and reduced excitability and thereby attenuating the perception of pain (9). Delta opioid receptors have also been identified on the presynaptic terminals of GABA (gamma-aminobutyric acid) releasing interneurons synapsing onto glutamatergic principal cells within the hippocampus and are implicated in learning and memory (10).

Furthermore, mu opioid receptors and kappa opioid receptors modulate the mesolimbic dopamine system, a reward pathway associated with substance dependence and addiction, although not fully understood, in which dopaminergic neurons from the ventral tegmental area of the midbrain project into the nucleus accumbens of the forebrain. GABAergic neurons express mu opioid receptors and continuously inhibit dopaminergic neurons in the ventral tegmental area. Activation of mu opioid receptors prevents GABAergic neurons from inhibiting dopamine release from dopaminergic neurons in the ventral tegmental area and nucleus accumbens. Dopamine released from ventral tegmental area neurons onto the nucleus accumbens contributes to generating positive reinforcement (4).
However, activation of kappa opioid receptors with dynorphins, a family of endogenous opioid peptides, induces dysphoria, a state of emotional distress, by depressing dopamine release from dopaminergic neurons in the ventral tegmental area onto the nucleus accumbens and prefrontal cortex. Kappa opioid receptors are also expressed within the striate nucleus on the presynaptic terminals of dopaminergic neurons, hippocampus, amygdala, dorsal raphe nucleus and substantia nigra (11). Interestingly, chronic opioid and cocaine exposures have been shown to increase the release of dynorphin within the striate nucleus and decrease the number of dopamine receptors within the caudate putamen in mice, potentially serving as a safeguard against dopamine-induced neurotoxicity from drug abuse (12).

**Expression Timeline for Opioid Receptors in the Mammalian Central Nervous System during Prenatal Development**

During prenatal development, opioid receptors and endogenous opioid peptides are expressed within the central nervous system, and growing evidence supports a role for the endogenous opioid system in the developing central nervous system. Rius et al. first detected mu opioid receptors and kappa opioid receptors on embryonic days 12.5 and 14.5, respectively, in rodents, and observed a gradual increase in their expression and anatomic distribution throughout the prenatal period, developing an expression profile comparable to the adult by
embryonic day 17.5; delta opioid receptors were first detected following birth on postnatal day 1 (13). However, beta-endorphin, met-enkephalin, and dynorphin were first detected on embryonic day 11.5, preceding the expression of opioid receptors. Levels of met-enkephalin and dynorphin increased continuously throughout the prenatal period, whereas beta-endorphin peaked on embryonic day 18.5 and subsequently diminished to levels previously observed on embryonic day 14.5 until birth. Rising levels of beta-endorphin, met-enkephalin and dynorphin observed from embryonic day 11.5 through 18.5, in addition to increasing expression of mu opioid receptors and kappa opioid receptors, paralleled an increase in brain mass, possibly implicating the opioid receptor system in central neurodevelopment (13).

**Overview of Methadone and Buprenorphine**

Opioid replacement therapy with methadone or buprenorphine has been recommended for managing opioid dependence during pregnancy. The United States Food and Drug Administration classifies both methadone and buprenorphine as Pregnancy Category C controlled substances, meaning that human controlled studies are insufficient; however, the potential benefits may justify use in pregnant women. To date, a randomized controlled trial comparing opioid replacement therapy with opioid detoxification therapy during pregnancy on newborn outcomes has not been published. In fact, opioid detoxification therapy
during pregnancy presents a risk for relapse and premature labor, and is not regarded as best practice. Methadone maintenance therapy is the current recommended standard of care for treating opioid dependence in pregnant women (2).

Methadone (Figure 1) is a synthetic opioid which is highly selective for and fully agonizes the mu opioid receptor not unlike morphine (Figure 2), and is used to achieve harm reduction from illicit drug use and relieve symptoms of opioid withdrawal. However, overdose on methadone can result in respiratory depression, circulatory collapse and death. In methadone maintenance therapy, opioid dependent individuals report each day as outpatients for a strictly controlled oral dose of methadone and urine toxicology screening for illicit substances. Participation in methadone maintenance therapy on a daily basis offers pregnant women suffering from opioid dependence opportunities for regular and frequent contact with health care providers and education which both have been shown to limit drug seeking behaviors and enhance antenatal care (2).

Buprenorphine (Figure 3) is a semi-synthetic opioid derived from paramorphine and has a long duration of action at mu opioid receptors and kappa opioid receptors. Buprenorphine is a partial agonist at the mu opioid receptor, and an antagonist at the kappa opioid receptor and the delta opioid receptor. Buprenorphine is often prepared as a combination product with naloxone, an opioid receptor antagonist, in a sublingual film or tablet marketed as Suboxone in order to deter intravenous abuse (15). Although naloxone administered
sublingually has a relatively low bioavailability, the National Institute of Drug Abuse recommends that pregnant women with opioid dependence receive buprenorphine monotherapy to avoid exposing the developing fetus to naloxone. As a reasonable alternative to methadone, buprenorphine is provided once a week generally in an outpatient office-based setting by a qualified physician in order to achieve harm reduction from illicit drug use and limit symptoms of opioid withdrawal (14). Buprenorphine demonstrates a lower maximal efficacy upon binding the mu opioid receptor compared to methadone and exhibits a ceiling effect with higher doses which decreases the risk of overdose in opioid dependent individuals compared to methadone (15).

![Figure 1](image.png)

*Figure 1.* The two dimensional structure and three dimensional conformer for methadone. Note the T-shape configuration of the three dimensional conformer common to opioids (16).
Figure 2. The two dimensional structure and three dimensional conformer for morphine. Note the T-shape configuration of the three dimensional conformer common to opioids (17).

Figure 3. The two dimensional structure and three dimensional conformer for buprenorphine. Note the T-shape configuration of the three dimensional conformer common to opioids (18).
AIMS

The purpose of this paper is two-fold: to describe the implications of opioid replacement therapy with methadone or buprenorphine on neurogenesis during prenatal development and to review the current understanding of opioid replacement therapy with methadone or buprenorphine during pregnancy on neonatal and early childhood neurobehavioral outcomes.

LITERATURE REVIEW

During prenatal development, opioid receptors and endogenous opioid peptides are expressed within the central nervous system, and growing evidence supports a role for the endogenous opioid system in neurogenesis, myelination and neurotransmitter pathways (20, 22, 25, and 26). Mu opioid receptors have been identified on neural progenitor cells within the developing central nervous system in mice (19). Willner et al. have demonstrated that acute exposure of neural progenitor cells to increasing concentrations of morphine, a full mu opioid receptor agonist, significantly decreased the incorporation of bromodeoxyuridine (BrdU) (Figure 4), a marker for cell proliferation, in neural progenitor cells (20).
Increasing concentrations of morphine reduced the proliferation of neural progenitor cells in vitro. Neural progenitor cells were obtained from the cerebral cortices of mouse embryos at day 14 of gestation. 4', 6-diamidino-2-phenylindole (DAPI) was used to visualize nuclear DNA (adapted from Willner et al., 2015 (20)).

Willner et al. have also demonstrated that acute exposure of neural progenitor cells to increasing concentrations of morphine significantly increased the activity of caspase-3, a biomarker for apoptosis, using in vitro mouse models (Figure 5) (20). Furthermore, they have demonstrated a significant increase in the incorporation of TUJ1, a marker for neuron specific class III beta-tubulin, in the number of bromodeoxyuridine stained cells with increasing concentrations of morphine in vitro (20). Additionally, they reported a significant decrease in the
incorporation of nestin, a marker for neural stem cells, in the number of bromodeoxyuridine stained cells with increasing concentrations of morphine in vitro (Figure 6) (20).

These findings suggest that an acute exposure to morphine, and possibly other full mu opioid receptor agonists such as methadone, may induce proliferating neural progenitor cells to prematurely undergo apoptosis or differentiate into neurons during embryonic development and upset the concerted, transient, and vulnerable sequence of events necessary for normal central nervous system development. Furthermore, the addition of naloxone, an opioid receptor antagonist, to neural progenitor cells exposed to morphine, significantly increased the incorporation of bromodeoxyuridine and the expression of nestin, and decreased caspase-3 activity compared to neural progenitor cells exposed only to morphine, supporting that morphine was producing effects by activating the mu opioid receptor (20). Although these findings support a role for the mu opioid receptor in mediating the proliferation, renewal, and apoptosis of neural progenitor cells, the results from in vitro studies using mice cannot be definitively extrapolated to in vivo studies or to humans.
Figure 5. Increasing concentrations of morphine increased caspase-3 activity of neural progenitor cells in vitro. Neural progenitor cells were obtained from the cerebral cortices of mouse embryos at day 14 of gestation. 4’, 6-diamidino-2-phenylindole (DAPI) was used to visualize nuclear DNA (adapted from Willner et al., 2015 (20)).
Figure 6. Increasing concentrations of morphine decreased the number of neural stem cells and increased the number of developing neurons in vitro. Neural stem cells were obtained from the cerebral cortices of mouse embryos at day 14 of gestation (adapted from Willner et al., 2015 (20)).

Furthermore, the findings from Willner et al. are somewhat limited because prenatal morphine exposure may not be comparable to prenatal methadone exposure. Although morphine (Figure 1) and methadone (Figure 2) are buprenorphine (Figure 3) are mu opioid receptor agonists, they differ in chemical composition, three dimensional conformation, and some interacting residues of the mu opioid receptor in mice (Figure 8) (21). The binding of morphine may induce
the mu opioid receptor to undergo a different conformational change by interacting with a different combination of residues \((Figure 8)\), potentially activating a different cellular signaling pathway and leading to a different effect than the binding of methadone or buprenorphine. However, no significant correlations have been demonstrated between the binding affinity or ligand efficacy and the interaction of ligands with specific residues of the mu opioid receptor \((21)\). For example, naloxone and nalbuphine share 9 residues in the binding pocket of the mu opioid receptor \((Figure 7)\); however, naloxone acts as a mu opioid receptor antagonist and nalbuphine acts as a partial mu opioid receptor agonist \((21)\).
opioid receptor. Naloxone and nalbuphine interact with nine of the same residues in the binding pocket of the mu opioid receptor (21).

Figure 8. Interacting residues within the binding pocket of the murine mu opioid receptor for methadone and morphine. Methadone and morphine have been observed to interact with seven and eight residues, respectively, in the binding pocket of the mu opioid receptor. Methadone and morphine share six residues in the binding pocket of the mu opioid receptor (adapted from Cui et al., 2013 (21)).

The effect of chronic prenatal exposure to methadone on neurogenesis in the developing central nervous system has not been comprehensively studied
using whole animal models. However, Nassogne et al. studied the structural organization of the cerebral cortex in mice chronically exposed to methadone in utero (22). They administered 40 mg/kg of methadone daily to pregnant mice by subcutaneous injection from day 8 of gestation through day 18 of gestation, and reported no significant difference in brain mass between mouse pups with prenatal methadone exposure and controls assessed on embryonic day 17 and postnatal days 0, 11 and 40 (22). They also reported no significant difference in the migration of neurons within the cerebral cortex assessed on postnatal day 11 (Figure 9) and no significant difference in the laminar structure of the cerebral cortex assessed on postnatal day 40 (Figure 10) between mouse pups with chronic prenatal methadone exposure and controls administered an equal volume of saline solution (22).

Figure 9. Cerebral cortex of mouse pups on postnatal day 11 comparing equal volume of saline solution to chronic prenatal methadone exposure. No significant difference detected in the migration of neurons within the cerebral cortex. Cell nuclei
labeled with bromodeoxyuridine (adapted from Nassogne et al., 1998 (22)).

Figure 10. Cerebral cortex of mice on postnatal day 40 comparing chronic prenatal methadone exposure to equal volume of saline solution. No significant difference detected in the horizontal lamination of neurons and the radial alignment of neurons. Sections prepared using cresyl violet stain (adapted from Nassogne et al., 1998 (22)).

Nassogne et al. exposed mouse pups prenatally to supratherapeutic doses of methadone, specifically 40 times greater than the recommended dose for managing opioid dependence in humans (22). Although Nassogne et al. did not identify any abnormalities in the cerebral cortex of mice with repeated prenatal methadone exposure (22), their findings do not necessarily demonstrate that methadone does not interfere with the formation of the cerebral cortex. During prenatal development, cells within the cerebral cortex begin expressing mu opioid
receptors and kappa opioid receptors (13). Methadone binds to mu opioid receptors with a higher affinity than kappa opioid receptors (23); more mu opioid receptors than kappa opioid receptors are occupied when exposed to a therapeutic dose of methadone. However, a supratherapeutic dose of methadone may increase the occupancy and activation of kappa opioid receptors in addition to activating mu opioid receptors. Activated kappa opioid receptors produce several effects which have been observed to counteract and offset the effects of activated mu opioid receptors (24). By activating kappa opioid receptors, Nassogne et al. may have offset the effects of activated mu opioid receptors, producing no overall detectable effect on the structure of the cerebral cortex. In contrast, a therapeutic dose of methadone may have disturbed neurogenesis and the formation of the cerebral cortex by activating mu opioid receptors unopposed.

Furthermore, the effect of chronic prenatal exposure to methadone on the function of oligodendrocytes, particularly myelination in the developing central nervous system, is not fully understood. Mu opioid receptors and kappa opioid receptors have been identified near the nucleus and widely throughout the cytoplasm, respectively, in oligodendrocyte progenitor cells harvested from mouse pups 0 through 2 days of age, and grown in vitro, whereas delta opioid receptors appear to be absent (Figure 11) (25). Knapp et al. exposed enriched oligodendrocytes to 1 micromolar PL017, a selective mu opioid receptor agonist, and bromodeoxyuridine, a marker for cell proliferation, for 27 hours, and reported a significant increase in the number of cells staining with both O4, a marker for
early oligodendrocyte progenitor cells, and bromodeoxyuridine compared to the control, enriched oligodendrocytes which were not treated with PL017 (25). They also exposed enriched oligodendrocytes to 3 micromolar naloxone in addition to 1 micromolar PL017, and found no significant change in the number of cells staining with both O4 and bromodeoxyuridine, indicating that PL017 increased the proliferation of oligodendrocyte progenitor cells by activating the mu opioid receptor (25). Knapp et al. also exposed enriched oligodendrocytes to 1 micromolar PL017 for 96 hours and bromodeoxyuridine for the last 22 hours, and reported no significant change in the number of oligodendrocyte progenitor cells staining with bromodeoxyuridine compared to the control, enriched oligodendrocytes treated with both PL017 and naloxone (25). Furthermore, Knapp et al. exposed enriched oligodendrocytes to 1 micromolar U50488, a selective kappa opioid receptor agonist, and bromodeoxyuridine for 27 hours, and reported no significant change in the number of oligodendrocyte progenitor cells staining with bromodeoxyuridine compared to the control, enriched oligodendrocytes treated with both U50488 and nor-binaltorphimine, a selective kappa opioid receptor antagonist (25). They also exposed enriched oligodendrocytes to 1 micromolar U50488 and bromodeoxyuridine for 48 hours, and reported no significant change in the number of oligodendrocyte progenitor cells staining with bromodeoxyuridine compared to the control, enriched oligodendrocytes treated with both U50488 and nor-binaltorphimine; however, enriched oligodendrocytes exposed to 1 micromolar U50488 for 48 hours demonstrated a significant increase
in size, from 1660 micrometers$^2$ to 2368 micrometers$^2$ (25). Although these findings support a role for the mu opioid receptor in mediating the proliferation of oligodendrocyte progenitor cells and the kappa opioid receptor in mediating the growth of oligodendrocyte progenitor cells, the results from in vitro studies using mice cannot be definitively extrapolated to in vivo studies or to humans.

**Figure 11.** Oligodendrocytes were immunostained, following six days of enrichment, for delta opioid receptors, kappa opioid receptors, and mu opioid receptors. Polyclonal antibodies and nickel-diaminobenzidine were used. Delta
opioid receptors were not detected. Kappa opioid receptors were detected typically two days following mu opioid receptors (adapted from Knapp et al., 1998 (25)).

Furthermore, Vestal-Laborde et al. administered 9 mg/kg of methadone daily to pregnant rats via subcutaneous osmotic mini-pumps for 28 days starting from day 7 of gestation and reported significant increases in myelin basic protein, myelin oligodendrocyte glycoprotein, and proteolipid proteins in rat pups 11 days of age and 19 days of age with both prenatal and postnatal methadone exposure compared to controls (26). They reported no significant difference in the number of axons with 4 or less loosely wrapped myelin sheaths and the number of axons with more than 4 uncompact myelin sheaths between rat pups 16 days of age with both prenatal and postnatal methadone exposure and controls (26). They found a significant increase in the number of axons with compact myelin sheaths in the corpus callosum of rat pups 16 days of age with both prenatal and postnatal methadone exposure compared to the controls (Figure 12), indicating a premature increase in the number of mature axons; however, the g ratio, calculated as the axon diameter divided by the myelinated fiber diameter, for axons with compact myelin sheaths was not significantly different between groups (26).
Figure 12. Comparing compact myelin sheaths in the corpus callosum of rat pups 16 days of age with both prenatal and postnatal methadone exposure to equal volume saline solution control. A significant increase in the number of axons with compact myelin sheaths was noted with both prenatal and
postnatal methadone exposure. Sections stained using uranyl acetate and lead citrate, and photographed under 5000x magnification with electron microscopy (adapted from Vestal-Laborde et al., 2014 (26)).

Vestal-Laborde et al. have also demonstrated that exposure of oligodendrocyte progenitor cells, harvested from rat pups 3 days of age, to methadone significantly increased the incorporation of thymidine labeled with tritium, and therefore cell proliferation (26). Vestal-Laborde et al. further demonstrated that exposure of preoligodendrocytes, harvested from rat pups 9 days of age, to methadone significantly increased myelin basic protein and myelin oligodendrocyte glycoprotein, markers of highly mature oligodendrocytes, suggesting methadone exposure may induce preoligodendrocytes to prematurely differentiate (26).

Chronic prenatal exposure to methadone may interfere with endogenous opioid systems which are potentially involved in myelin formation in the developing central nervous system. Exposure to exogenous opioids may alter the function of oligodendrocytes and the developmental pattern of myelination in the prenatal brain and spinal cord. The findings of Vestal-Laborde et al. suggest that chronic exposure to methadone may induce oligodendrocyte progenitor cells to undergo proliferation and prematurely myelinate axons during prenatal development and upset the concerted, transient, and vulnerable sequence of events necessary for normal central nervous system development.

In vivo studies also suggest that chronic prenatal exposure to methadone may also interfere with neurotransmitter pathways (27). Guo et al. administered 9
mg/kg of methadone daily to pregnant rats via subcutaneous osmotic mini-pumps from day 8 of gestation through delivery, and found significant decreases in acetylcholine in the striatum of rat pups 4 days of age with prenatal methadone exposure compared to controls (27). However, Guo et al. did not quantify the number of cholinergic neurons in the striatum of rat pups with prenatal methadone exposure for comparison to control values, and did not determine the extent of acetylcholine production in the striatum of rat pups with prenatal methadone exposure for comparison to control values.

Furthermore, Robinson administered 9 mg/kg of methadone daily to pregnant rats via subcutaneous osmotic mini-pumps for 28 days starting from day 7 of gestation, and found significant decreases in acetylcholine in the striatum of rat pups 4 days of age with prenatal methadone exposure and rat pups 4 days of age with both prenatal and postnatal methadone exposure compared to controls (28), consistent with findings from Guo et al. However, no significant decreases in acetylcholine in the striatum of rat pups 10 days of age and 21 days of age with either prenatal methadone exposure or prenatal and postnatal methadone exposure were noted in comparison to controls (28).

Robinson measured acetylcholine in the striatum of rat pups with prenatal and postnatal methadone exposure because rats are born at a stage of development comparable to the end of the second trimester in humans (32). Interestingly, cholinergic neurons of the striatum do not express mu opioid receptors and kappa opioid receptors, and only express delta opioid receptors
following birth (28). The pattern of opioid receptor expression on cholinergic neurons of the striatum suggests that decreases in acetylcholine in rat pups with prenatal methadone exposure were not due to methadone directly binding onto cholinergic neurons but rather acting indirectly via neurons communicating with cholinergic neurons.

The effect of chronic prenatal exposure to buprenorphine on neurogenesis in the developing central nervous system has been examined using whole animal models (29). Wu et al. administered 1.0 mg/kg of buprenorphine daily to pregnant mice via intraperitoneal injection from days 7 through 21 of gestation and demonstrated a significant decrease in body mass, brain mass, and brain-to-body mass ratio in mouse pups with chronic prenatal exposure to buprenorphine compared to controls (29). Wu et al. also found a significant increase in the immobility time during both the forced swimming test and the tail suspension test in mouse pups with chronic prenatal exposure to buprenorphine compared to controls (Figure 13A and Figure 13B) (29). The forced swimming test and the tail suspension test are commonly used for assessing depression in rodents (29). Wu et al. demonstrated that mouse pups with chronic prenatal exposure to buprenorphine had a significant increase in depression-like behaviors compared to controls (29). However, these findings are somewhat limited because Wu et al. exposed mouse pups prenatally to a supratherapeutic dose of buprenorphine, greater than the recommended dose of buprenorphine daily for managing opioid dependence in humans.
Figure 13. (A) Comparing immobility times of males and females with prenatal 1.0 mg/kg/day of buprenorphine exposure to 0 mg/kg/day of buprenorphine controls for the forced swimming test (sample size of 10 mouse pups per group). Mouse pups were subjected to the forced swimming test for five minutes. A significant increase in the immobility time for the forced swimming test was detected in mouse pups (males and females) with prenatal exposure to buprenorphine compared to controls. (B) Comparing immobility times of males and females with prenatal 1.0 mg/kg/day of buprenorphine exposure to 0 mg/kg/day of buprenorphine controls for the tail suspension test (sample size of 10 mouse pups per
Mouse pups were subjected to the tail suspension test for six minutes. A significant increase in the immobility time for the tail suspension test was detected in mouse pups (males and females) with prenatal exposure to buprenorphine compared to controls (adapted from Wu et al., 2014 (29)).

Mouse pups with chronic prenatal exposure to buprenorphine also demonstrated a significant reduction in the number of neuronal cells staining positive for NeuN (Figure 14A), a nuclear protein exclusive to neurons, and the quantity of MAP-2, a microtubule associated protein exclusive to neurons, and class 3 beta-tubulin, a microtubule exclusive to neurons, in the prefrontal cortex, compared to controls (Figure 14B) (29). Mouse pups with chronic prenatal exposure to buprenorphine also demonstrated a significant reduction in the quantity of proteins related to neural stem cells, such as nestin, sex determining region Y box 2, and Kruppel-like factor 4, and proteins related to neural precursor cells and immature neurons, such as doublecortin (Figure 15) (29). These findings suggest that chronic exposure to buprenorphine may limit the proliferation and renewal of neural progenitor cells during prenatal development, and potentially interfere with the vulnerable sequence of events necessary for normal central nervous system development. Mu opioid receptors and kappa opioid receptors have been identified on neural progenitor cells within the developing central nervous system in mice (16). Buprenorphine binds to mu opioid receptors with a higher affinity than to kappa opioid receptors in vitro (30), such that more mu opioid receptors than kappa opioid receptors will be occupied when exposed to a
therapeutic dose of buprenorphine. However, a supratherapeutic dose of buprenorphine may increase the occupancy and limit the activation of kappa opioid receptors by endogenous opioid peptides in addition to activating mu opioid receptors. Activated kappa opioid receptors produce several effects which have been observed to counteract and offset the effects of activated mu opioid receptors in mice (24). By exposing mouse pups chronically to supratherapeutic doses of buprenorphine from days 7 through 21 of gestation, Wu et al. may have limited the activation of kappa opioid receptors and consequently, the counteracting effects of kappa opioid receptors on the effects of activated mu opioid receptors. Wu et al. may have observed a more significant decrease in neurogenesis than may be expected from therapeutic doses of buprenorphine as recommended to manage opioid dependence during pregnancy.
Figure 14. (A) Decreased population of neurons in the prefrontal cortex of male and female mouse pups with prenatal exposure to buprenorphine compared to controls. Neurons stained for NeuN. (B) Western blot of protein content
in the prefrontal cortex of mouse pups with prenatal exposure to buprenorphine compared to controls; sample size for each group was 8 mouse pups. A significant decrease in NeuN, MAP-2, and class III beta-tubulin was detected in mouse pups with prenatal exposure to buprenorphine compared to controls. No significant change was detected in GFAP (glial fibrillary acidic protein which is commonly expressed in astrocytes), and GADPH, glyceraldehyde-3-phosphate dehydrogenase, between mouse pups with prenatal exposure to buprenorphine compared to controls (adapted from Wu et al., 2014 (29)).
significant change was detected in GADPH (glyceraldehyde-3-phosphate dehydrogenase) between mouse pups with prenatal exposure to buprenorphine compared to controls (adapted from Wu et al., 2014 (29)).

Furthermore, the effect of chronic prenatal exposure to buprenorphine on the function of oligodendrocytes, particularly myelination, is not fully understood. Sanchez et al. administered 0.3 mg/kg or 1.0 mg/kg of buprenorphine daily to pregnant rats via subcutaneous osmotic mini-pumps for 28 days starting from day 7 of gestation (33). They reported significant increases in all four major isoforms of myelin basic protein, consisting of 14.0, 17.0, 18.5, and 21.5 kDa isoforms, in rat pups 12 days of age (beginning of brain myelination), 19 days of age (peak of brain myelination), and 26 days of age (end of brain myelination) with both prenatal and postnatal 0.3 mg/kg of buprenorphine exposure compared to the controls (Figure 16A, Figure 16B, Figure 16C) (33). Myelin basic protein is a marker of highly mature oligodendrocytes (31). Sanchez et al. exposed rat pups to buprenorphine both prenatally and postnatally because rats are born at a stage of development comparable to the end of the second trimester in humans; the early postnatal period in rats is comparable to the third trimester in humans (32). A dose of 0.3 mg/kg of buprenorphine once a day in rodents is comparable to the dose recommended for managing opioid dependence in humans (33).

They also reported significant decreases in all four major isoforms of myelin basic protein in rat pups 12 days of age with both prenatal and postnatal 1.0 mg/kg of buprenorphine exposure compared to the controls (Figure 16A) (33). However,
they reported no significant difference in any of the four major isoforms of myelin basic protein in rat pups 19 days of age and 26 days of age with both prenatal and postnatal 1.0 mg/kg of buprenorphine exposure compared to the controls (Figure 16B and Figure 16C) (33). A dose of 1.0 mg/kg of buprenorphine once a day in rodents is comparable to an overexposure of buprenorphine in humans (33).

Furthermore, Sanchez et al. reported no significant difference in the number of myelinated axons in the corpus callosum of rat pups 26 days of age with both prenatal and postnatal 0.3 mg/kg of buprenorphine exposure compared to controls (33). They reported a significant decrease in the number of myelinated axons in the corpus callosum of rat pups 26 days of age with both prenatal and postnatal 1.0 mg/kg of buprenorphine exposure compared to controls (Figure 17) (33). They also reported a significant increase in the number of large diameter myelinated fibers and a significant decrease in the thickness of the myelin sheath of large diameter myelinated fibers in rats 26 days of age with prenatal and postnatal exposure to either 0.3 mg/kg of buprenorphine or 1.0 mg/kg of buprenorphine daily (Figure 18) (33). However, they reported no significant change in the number of unmyelinated axons in the corpus callosum of rat pups 26 days of age with prenatal and postnatal exposure to 0.3 mg/kg of buprenorphine or 1.0 mg/kg of buprenorphine compared to the controls (33).
Figure 16. (A) Western blot of the four major isoforms of myelin basic protein, consisting of 14.0, 17.0, 18.5, and 21.5 kDa isoforms, in the whole brain of rat pups 12 days of age. A significant increase in all four major isoforms of myelin basic protein was detected in rat pups with prenatal and postnatal exposure to 0.3 mg/kg of buprenorphine compared to the controls (c). A significant decrease in all four major isoforms of myelin basic protein was detected in rat pups with prenatal and postnatal exposure to 1.0 mg/kg of buprenorphine compared to the controls (c). (B) Western blot of the four major isoforms of myelin basic protein, consisting of 14.0, 17.0, 18.5, and 21.5 kDa isoforms, in the whole brain of rat pups 19 days of
A significant increase in all four major isoforms of myelin basic protein was detected in rat pups with prenatal and postnatal exposure to 0.3 mg/kg of buprenorphine compared to the controls (c). No significant difference in any of the four major isoforms of myelin basic protein was detected in rat pups with prenatal and postnatal exposure to 1.0 mg/kg of buprenorphine compared to the controls (C). Western blot of the four major isoforms of myelin basic protein, consisting of 14.0, 17.0, 18.5, and 21.5 kDa isoforms, in the whole brain of rat pups 26 days of age. A significant increase in all four major isoforms of myelin basic protein was detected in rat pups with prenatal and postnatal exposure to 0.3 mg/kg of buprenorphine compared to the controls (c). No significant difference in any of the four major isoforms of myelin basic protein was detected in rat pups with prenatal and postnatal exposure to 1.0 mg/kg of buprenorphine compared to the controls (c). Beta-actin was used as a loading control (adapted from Sanchez et al., 2008 (33)).

Figure 17. Myelinated axons in the corpus callosum of rat pups 26 days of age. Stained using toluidine blue and photographed at 1000 times magnification. A significant decrease myelinated axons was detected in rat pups with prenatal and postnatal exposure to 1.0 mg/kg of buprenorphine compared to the controls and 0.3 mg/kg of buprenorphine (adapted from Sanchez et al., 2008 (33)).
Sanchez et al. also reported a significant decrease in myelin associated glycoprotein in the corpus callosum of rat pups 12 days of age with prenatal and postnatal 1.0 mg/kg buprenorphine exposure compared to controls and 0.3 mg/kg of buprenorphine exposure (33). They also reported a significant increase in myelin
associated glycoprotein in the corpus callosum of rat pups 26 days of age with prenatal and postnatal 0.3 mg/kg or 1.0 mg/kg of buprenorphine exposure compared to controls; myelin associated glycoprotein was significantly greater in rat pups with 0.3 mg/kg of buprenorphine exposure compared to 1.0 mg/kg of buprenorphine exposure (33).

Chronic prenatal exposure to therapeutic doses of buprenorphine may interfere with endogenous opioid systems which are potentially involved in myelin formation in the developing central nervous system. Myelin basic protein, a marker of highly mature oligodendrocytes, was significantly increased with prenatal and postnatal exposure to 0.3 mg/kg of buprenorphine (33). These findings suggest that chronic exposure to therapeutic levels of buprenorphine may induce cells within the oligodendrocyte lineage to prematurely differentiate and change axon growth and the developmental pattern of myelination, potentially upsetting the vulnerable sequence of events necessary for normal central nervous system development.

In vivo studies suggest that chronic prenatal exposure to buprenorphine may also interfere with neurotransmitter pathways (28). Robinson administered 0.3 mg/kg buprenorphine daily to pregnant rats via subcutaneous osmotic mini-pumps for 28 days starting from day 7 of gestation, and found significant decreases in acetylcholine in the striatum of rat pups 4 days of age with prenatal buprenorphine exposure (28). Rat pups 4 days of age with prenatal and postnatal 0.3 mg/kg/day
buprenorphine exposure also demonstrated decreases in striatal acetylcholine although non-significant (28).

Robinson measured acetylcholine in the striatum of rat pups with prenatal and postnatal methadone or buprenorphine exposure because rats are born at a stage of development comparable to the end of the second trimester in humans (32). Cholinergic neurons of the striatum do not express mu and kappa opioid receptors, and only express delta opioid receptors following birth (28). The pattern of opioid receptor expression on cholinergic neurons of the striatum suggests that decreases in acetylcholine in rat pups with prenatal buprenorphine exposure were not due to buprenorphine directly binding onto cholinergic neurons but rather acting indirectly via neurons communicating with cholinergic neurons. Interestingly, rat pups 4 days of age with prenatal and postnatal 0.3 mg/kg/day buprenorphine exposure did not demonstrate a significant decrease in striatal acetylcholine which requires further study of the mechanisms involved.

Methadone maintenance therapy and buprenorphine monotherapy are effective options for managing opioid dependence during pregnancy, minimizing the harmful consequences from maternal drug seeking behaviors, and improving antenatal care (2). However, both methadone and buprenorphine readily transverse the human placenta (3) which raises the concern for chronically administered exogenous opioids interfering with endogenous opioid systems in the vulnerable fetal central nervous system and translating into poor central nervous system outcomes later. In rodent models, methadone has been demonstrated to
bind at least twice up to 14 times as many neurons in the developing central
nervous system of the fetus than in the adult central nervous system of the mother;
however, this study’s investigators did not determine whether methadone was
binding to pharmacologically active opioid receptors in the fetus (34). Children with
a history of repeated exposure to therapeutic levels of methadone or
buprenorphine in utero may however require routine follow-up and monitoring
throughout infancy and/ or early childhood.

McGlone et al. reported that infants six months of age born to women with
opioid dependence and maintained on methadone (with polysubstance
exposures), scored lower than healthy drug-free controls in all developmental
areas assessed by Griffiths Mental Development Scales, including locomotor
skills, personal and social skills, hearing and language skills, eye and hand
coordination, and performance, after adjusting for maternal cigarette smoking and
alcohol consumption (35). Furthermore, newborn infants exposed to methadone in
utero have a decreased occipital frontal head circumference at birth compared with
healthy drug-free controls matched for age, demographic, and maternal cigarette
smoking, and by six months of age, differences in occipital frontal head
circumference between infants exposed to methadone in utero and healthy drug-
free controls were no longer significant (35). In addition to a decreased occipital
frontal head circumference at birth, several newborn infants exposed to
methadone in utero also demonstrated strabismus and nystagmus (35). In
contrast, Baldacchino et al. performed a meta-analysis using five case-control
studies and found no significant difference in neurobehavioral outcomes for both infants and preschool aged children repeatedly exposed to opioids only (mothers excluded for polysubstance use) in utero compared to healthy drug-free controls by assessing general cognition, executive function, memory, language, psychomotor skills, and personal and social skills (36). Interestingly, both infants and preschool aged children repeatedly exposed to opioids in utero performed poorer than healthy drug-free controls on all neurobehavioral testing (36). However, these findings are somewhat limited and may be unreliable because Baldacchino et al. only analyzed a small number of studies with a small sample size which reduces the chance of detecting a true effect on neurobehavioral outcomes for both infants and preschool aged children from repeated exposure to opioids in utero. Furthermore, Baldacchino et al. did not specifically examine neurobehavioral outcomes for both infants and preschool aged children with respect to repeated methadone versus buprenorphine exposure in utero.

Previous studies have rarely examined a history of repeated exposure to methadone or buprenorphine in utero on neonatal and/ or early childhood neurodevelopmental and behavioral outcomes, and the findings, which have been published, are inconsistent. However, neonatal withdrawal syndrome has been well documented and described (37). Neonatal withdrawal syndrome is a complex multi-system disorder and refers to a group of signs and symptoms of withdrawal from gestational opioid exposure in newborn infants, including, but not limited to, high-pitched crying, hyperactivity, tremors, seizures, hypersensitivity to stimuli,
excessive sucking, poor feeding and rapid breathing (37). Newborn infants with a history of chronic in utero exposure to buprenorphine had lower incidence of neonatal withdrawal syndrome, required considerably less morphine to manage withdrawal symptoms, and had shorter length of hospital stay compared to newborn infants with a history of chronic in utero exposure to methadone (37).

Neurobehavioral outcomes for the newborn infant with a history of chronic in utero exposure to either methadone or buprenorphine are not well understood. Coyle et al. examined neurobehavioral outcomes in a double blind study for newborn infants, specifically birth through postnatal day 30, with regard to a history of repeated in utero exposure to methadone versus buprenorphine, and found that newborn infants exposed to buprenorphine scored significantly lower on average than newborn infants exposed to methadone in excitability, arousal, and hypertonia, using the Neonatal Intensive Care Unit Network Neurobehavioral Scale; high scores for excitability, arousal, and hypertonia indicate dysregulated neurobehavioral functioning (38). The Neonatal Intensive Care Unit Network Neurobehavioral Scale provides a comprehensive assessment of central nervous system development and integrity in vulnerable infants with prenatal substance exposure (38). They also reported that newborn infants exposed to buprenorphine demonstrated fewer signs of stress-abstinence and required less handling to achieve a quiet-alert state (better self-regulation) compared to newborn infants exposed to methadone; however, neurobehavioral outcomes improved in newborn infants exposed to either methadone or buprenorphine by postnatal day 30 (38).
In addition, Coyle et al. corroborated that newborn infants chronically exposed to buprenorphine in utero had lower incidence of neonatal withdrawal syndrome, had shorter length of hospital stay to manage neonatal withdrawal syndrome, and required less morphine to treat withdrawal symptoms compared to newborn infants chronically exposed to methadone in utero (38). Newborn infants with a history of repeated in utero exposure to buprenorphine demonstrated less adverse neurobehavioral outcomes and severity of withdrawal than their methadone counterparts (38) which suggests that buprenorphine may be the better option for managing opioid dependence during pregnancy with regard to minimizing harm to the developing central nervous system in the embryo or fetus.

Although Coyle et al.'s findings suggest that opioid replacement therapy with buprenorphine during pregnancy minimizes withdrawal symptoms and adverse neurobehavioral outcomes, such as excitability, arousal and hypertonia, in the newborn infant compared with methadone, they are limited by confounding factors, such as possibly unreported maternal use of alcohol, tobacco, and illegal substances during pregnancy, and a small sample size. Furthermore, Jones et al. previously performed a double blind study comparing neurobehavioral outcomes for newborn infants, birth through postnatal day 14, with respect to a history of repeated in utero exposure to methadone versus buprenorphine (39). Jones et al. reported that newborn infants with a history of chronic in utero exposure to methadone scored lower than their buprenorphine counterparts in excitability and arousal, using the Neonatal Intensive Care Unit Network Neurobehavioral Scales
(39), which contradicts Coyle et al.’s recent findings. The inconsistencies between these two independent studies may be due to different maternal histories of substance use, unreported maternal use of alcohol, tobacco, and illegal substances during pregnancy, and timing of exposure to methadone or buprenorphine during prenatal development.
CONCLUSION

Increasing evidence supports a crucial role for endogenous opioid systems in prenatal brain and spinal cord development; repeated exposure to methadone or buprenorphine may interfere with endogenous opioid systems, potentially compromising neurogenesis, myelination, and neurotransmitter pathways in the developing central nervous system, and impairing neurobehavioral functions. This literature review found that the effect of chronic prenatal exposure to therapeutic doses of methadone on the proliferation, differentiation, and apoptosis of neural progenitor cells has not been recently studied although methadone maintenance therapy is the current recommended standard of care for treating opioid dependence in pregnant women. Interestingly, the effect of chronic prenatal exposure to buprenorphine on neurogenesis has not been studied using comparable therapeutic doses of buprenorphine in mice. Furthermore, buprenorphine has not been administered to mouse pups during the early postnatal period which is comparable to the third trimester in humans, in addition to the prenatal period, in order to model human gestation in its entirety. The findings from chronic prenatal only exposure to supratherapeutic doses of buprenorphine in mice may not be translatable to chronic prenatal exposure to therapeutic doses of buprenorphine in humans. Additionally, chronic exposure to therapeutic doses of methadone has been shown to increase the proliferation of oligodendrocyte progenitors, induce preoligodendrocytes to prematurely differentiate, and change the pattern of myelination in the developing central
nervous system whereas chronic exposure to therapeutic doses of buprenorphine on oligodendrocytes and myelination of the central nervous system has not been as thoroughly studied. It would be interesting to examine the conduction velocity of large diameter myelinated fibers with decreased myelin sheath thickness following chronic prenatal and postnatal exposure to therapeutic doses of buprenorphine and the conduction velocity of axons with compact myelin following chronic prenatal and postnatal exposure to therapeutic doses of methadone which have actually not been studied to date. Other future studies should be geared towards bridging the gap between changes in the developing central nervous system following chronic methadone or buprenorphine exposure and changes in neurobehavioral outcomes. Newborn and early childhood neurobehavioral outcomes following repeated exposure to methadone or buprenorphine in utero are not well understood, are complicated by polysubstance exposure, a deprived rearing environment, and dysfunctional parents, and clearly warrant further research and longitudinal studies.
REFERENCES


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EDUCATION

• Master of Science – Medical Science, Boston University School of Medicine, Boston, MA
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PROFESSIONAL/RESEARCH EXPERIENCE

Study Coordinator, Albert Einstein College of Medicine – Bronx, NY
Conducted research visits to determine the efficacy of an abstinence reinforcing contingency management intervention compared to a performance feedback control condition on improving HIV viral load suppression in HIV-infected opioid-dependent individuals with suboptimal opioid agonist and HIV treatment.

Operations Intern, Emerging Med – New York, NY
May 2010 - Aug. 2010
Managed clinical trial databases for Florida Cancer Clinical Trials online matching service.

Research Assistant, The Methodist Hospital Research Institute – Houston, TX
Studied cardiac muscle excised after death from a heart failure patient having undergone trial stem cell treatment.

Debakey Summer Student Scholar, The Methodist Hospital – Houston, TX
Observed multiple surgeries - aortic valve replacement, robotic mitral valve repair, coronary bypass using internal mammary artery, heart transplant, lung transplant, and double kidney transplant with ipsilateral placement - performed live in the operating room and followed up with patients in recovery.
Clinical Research Assistant, Boston Medical Center – Boston, MA  
Assisted researchers in testing the effectiveness of Seroquel in reducing alcohol dependence in heavy drinkers.

Patient Navigator, American Cancer Society – Kingston, NY  
Served as a medical advocate at Ulster Radiation and Oncology Center.

Office Associate, American Cancer Society – Kingston, NY  
Coordinated ACS subsidized cancer screenings for local low-income populations.

Office Assistant, Alzheimer’s Association – Poughkeepsie, NY  
Connected Alzheimer’s patients and caregivers to local support groups and offered emotional comfort.

PROFESSIONAL CERTIFICATIONS
  • Professional Rescuer CPR – Certified January 25, 2009  
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VOLUNTEER WORK/ COMMUNITY SERVICE
Playroom Assistant, Latifa Hospital – Dubai, UAE  
Supervised and played with pediatric inpatients suffering from sickle cell anemia, cystic fibrosis, and thalassemia to raise their spirits during hospitalization.

CPR Instructor, Hatta Hospital - Hatta, UAE  
Organized CPR public awareness campaigns and trained local villagers in basic adult CPR and abdominal thrusts.

Teaching Assistant, Dubai Autism Center – Dubai, UAE  
Helped children diagnosed with autism spectrum disorders practice social, cognitive and motor skills hoping to achieve functional independence.

  • Weekend Visitor, DOROT – New York, NY 2012  
  • Surgical Liaison, Beth Israel Deaconess Medical Center – Boston, MA 2007 - 2008  
  • Volunteer, Breast Cancer Walk – Kingston, NY and Boston, MA 2005 - 2008
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- Bank of America: Joe Martin Scholarship, 2008 - 2011
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- Ultimate Frisbee, 2009 - 2011
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