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Prenatal Drug Exposures and Neurodevelopmental Programming of Glucocorticoid Signaling

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Abstract

Prenatal neurodevelopment is dependent on precise functioning of multiple signaling pathways in the brain, including those mobilized by glucocorticoids (GC) and endocannabinoids (eCBs). Prenatal exposure to drugs of abuse, including opioids, alcohol, cocaine, and cannabis have been shown to not only impact GC signaling, but also alter functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Such exposures can have long-lasting neurobehavioral consequences, including alterations in stress response in the offspring. Furthermore, cannabis contains cannabinoids that signal via the eCB pathway, which is linked to some components of GC signaling in the adult brain. Given that GCs are frequently used in pregnancy to prevent complications of prematurity, and rates of cannabis use in pregnancy are increasing, the likelihood of fetal co-exposure to these compounds is high, and may have additional implications for long-term neurodevelopment. Here, we present a discussion of GC signaling and the HPA axis, effects of prenatal drug exposures on these pathways and the stress response, and explore the interactions between GC and EC signaling in the developing brain and potential for neurodevelopmental consequences.

Keywords

glucocorticoid; HPA axis; prenatal; cannabinoid; stress

Introduction

While human brain development is not considered complete until the early 3rd decade of life,^{1, 2} early *in utero* neurodevelopmental processes (e.g. neurogenesis and migration, gliogenesis, synaptogenesis, pruning³) are particularly critical for long-term functional neurologic outcomes and represent a period of great vulnerability to exogenous insults. Prenatal exposures to certain drugs, infections, or maternal conditions such as stress, can

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lead to both physical and functional neurodevelopmental alterations via endogenous signaling pathways, affecting downstream regulation of gene transcription and protein expression (Figure 1). Such exposures can target the hypothalamic-pituitary-adrenal (HPA) axis, a system that influences glucocorticoid (GC) signaling pathways and stress responses. *In utero* disruption of this system can lead to persistent changes in molecular mechanisms such as endocannabinoid (eCB) signaling, brain structural formation, and behavioral responses in affected offspring. In this review, we provide an overview of GC signaling and the stress response, present examples of prenatal exposures (substances of abuse and synthetic glucocorticoids or sGCs) that influence GC signaling, the HPA axis and stress response, and explore the molecular basis of interactions between eCB and GC signaling and their potential for influencing neurodevelopmental outcomes.

GCs Mediate HPA Axis Activation and the Stress Response

Physiologic and psychological stressors activate the HPA axis resulting in the coordinated synthesis and secretion of GCs. In brief, stress causes the release of corticotropin releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus, which then stimulates the synthesis and release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary. ACTH subsequently stimulates the synthesis and secretion of GCs, including cortisol in humans and corticosterone in rodents, from the adrenal cortex. GCs, in turn, provide negative feedback by acting on GC receptors (GRs) at each level of the HPA axis, subsequently terminating its activation.^{4, 5} This neuroendocrine regulation is central for reestablishment of physiological homeostasis.⁶ Activation of the HPA axis is directly mediated by extra-hypothalamic regions including the amygdala, as its activation can drive the HPA axis in response to a stressor.⁷ Alternatively, GC-mediated feedback inhibition of HPA axis activity can be mediated by the hippocampus and the prefrontal cortex.⁸

The cellular response to GCs is mediated largely by the GR, a member of the nuclear receptor superfamily of transcriptional regulators.^{9, 10} GR coordinates effects of GCs via two signaling pathways, both of which regulate major cellular processes (Figure 2). The classic genomic actions of GR occur when the receptor, which normally resides in the cytoplasm bound to chaperone proteins such as heat shock protein 90, binds a ligand and undergoes conformational changes. This reconfiguration allows GR to translocate to the nucleus and activate or repress target genes.¹¹ However, in addition to its well-established role as a transcription factor, GR can trigger rapid activation of multiple cytoplasmic signaling cascades through non-classical pathways initiated by ligand binding in various cytoplasmic and membrane compartments. In the central nervous system, non-classical GR signaling cascades converge to influence synaptic plasticity, neurogenesis, and glial plasticity.¹² While GCs bind to another nuclear receptor that controls HPA axis function, the mineralocorticoid receptor (MR),^{13,14} GR is the primary receptor by which GCs mediate the neuroendocrine and genomic response to stress and thus will be the focus of this review.

A pharmaceutical proxy of the endogenous neurosteroids which mediate cellular consequences of the stress response are sGCs, which include widely used medications such as prednisone, dexamethasone, and betamethasone. sGCs have significant homology to endogenous GCs and activate GR-mediated signaling.¹⁵ Similar to endogenous GCs, sGC

exposure can also regulate the HPA axis, and chronic exposure can lead to iatrogenic Cushing's syndrome. Furthermore, abrupt discontinuation of chronic sGC therapy has the potential to put patients at risk for adrenal insufficiency, where the adrenal cortex does not respond effectively to stressors and endogenous GC secretion is suppressed.¹⁶

Prenatal Exposures to Select Drugs of Abuse Leads to Altered Stress Responses and GC Signaling in Offspring

In addition to sGCs, multiple other compounds and signaling pathways interact with GC signaling and HPA axis function, including during the antenatal period. Pregnancy represents a period of critical human development and exquisite sensitivity to the many teratogens, as well as a time where baseline HPA axis function appears to be downregulated.¹⁷ Despite recommendations to limit exposures to certain medications and drugs,¹⁸ a significant proportion of women continue to use potentially harmful substances during their pregnancies.¹⁹ Such prenatal exposures can lead to neurodevelopmental and behavioral consequences, and the compounds themselves have the potential to interact with endogenous signaling pathways. In particular, prenatal exposures to multiple drugs of abuse have been shown to affect GC signaling in offspring, including modulation of the HPA axis and stress response. For many, these observed effects could persist until adulthood, and thus represent an important point of study to prevent long-term morbidity. While a review of all teratogenic exposures and a comprehensive mechanistic summary is beyond the scope of this paper, we will review four commonly abused substances in pregnancy with specific regard to their relationship with HPA axis regulation and GC signaling: opioids, cocaine, alcohol, and cannabis.

Opioids

In the past decade, opioid use has skyrocketed, and terms like the "opioid epidemic" have become commonplace.^{20–24} The period of pregnancy has not been immune to this exposure, and the number of women seeking treatment for opioid use disorder during pregnancy is also rising.²⁰ Exogenous opioids, such as morphine, oxycodone, methadone, and buprenorphine mediate effects through the endogenous opioid system, which includes the opioid receptors (mu, delta, kappa, and nonclassical nociceptin).^{25–27} In addition to being broadly distributed throughout the central nervous system,^{26, 28} endogenous opioid receptors are expressed in the PVN of the hypothalamus,^{28, 29} the neuroanatomical location also responsible for synthesis of two ACTH secretagogues: CRH and vasopressin.³⁰ It is therefore not surprising that exposure to exogenous opioids alters various outcomes of HPA axis-mediated signaling. As a caveat, however, the mechanism of this influence is likely somewhat dependent on the type of exogenous opioid, receptors involved, whether effects are studied in humans or animals, and duration of exposure.³¹ For example, exposure to high levels of opioid antagonists in healthy human controls produced elevated levels of corticosterone, suggesting opioid signaling may exert baseline inhibitory signaling on the HPA axis.³¹ This observation is supported by clinical observations of adrenal insufficiency in patients with chronic exposure to opioids.^{31–33} Alternatively, in animal and *in vitro* models, opioid exposure can stimulate CRH secretion. In pregnancy, however, opioid signaling generates an inhibitory effect on HPA axis signaling, which may be mediated indirectly by presynaptic inhibitory

actions from increased levels of mu opioid receptor and enkephalin expression that in turn inhibit norepinephrine release that stimulates CRH release.^{29, 34}

In pregnancy, exogenous opioids are able to cross the placenta, and in chronic exposure settings are associated with opiate withdrawal symptoms in neonates, termed neonatal opioid withdrawal syndrome (NOWS).^{20, 35} From a mechanistic standpoint, there is some evidence to suggest that exogenous opioid exposure may also lead to epigenetic changes (e.g. changes in DNA methylation); however, studies comparing such alterations in an inherited model examining parental and progeny DNA are limited.³⁵ In a study by Wachman et al.,³⁶ higher rates of methylation of the mu-opioid receptor gene *OPRM1* were associated with more severe forms of NOWS in opioid-exposed infants, which the authors hypothesized would correlate with lower expression of the receptor.³⁷ It is thus plausible that reducing opioid receptor expression could impact baseline inhibitory actions on the HPA-axis during pregnancy.

Given the recent rise in rates of prenatal opioid use, clinical studies have attempted to specifically characterize consequences of exogenous opioid exposures and whether effects are persistent; however, observed clinical effects are variable and subjective to confounding factors.^{23, 38–43} In a review by Byrnes and Vassoler⁴⁴ of animal studies of prenatal chronic opioid exposure, various consequences on HPA axis function have been reported, including: no change in basal or stress-induced ACTH or corticosterone, decreased ACTH in response to restraint stress, and variable sensitivity to dexamethasone negative feedback.⁴⁴ Behaviorally, studies frequently identify increased rates of stress-like anxiogenic or depressed behavior in exposed progeny.⁴⁵ One study by Nakhjiri, *et al.*⁴⁶ even evaluated the potential consequences of combined opioid- and stress-related effects in adult rat offspring after a 3-day prenatal exposure to morphine, restraint stress, or the combination thereof.⁴⁶ Elevated levels of circulating vasopressin were observed in male offspring, and escalated anxiety behaviors of increased locomotor activity in the elevated plus maze were seen in both sexes after morphine and stress exposure. While results did not reach statistical significance for both male and female groups in all conditions, the authors also reported that prenatal morphine exposure alone reduced anxiety-like behaviors. These findings suggest that combined prenatal exposure to opioids and stress may differentially regulate long-term outcomes in offspring from isolated exposures to either.

Cocaine

The neurostimulants represent another class of drugs abused during pregnancy, and includes cocaine, methamphetamines, and prescription stimulants such as those used for treatment of attention deficit hyperactivity disorder. Of these, cocaine is one of the most commonly abused neurostimulants during pregnancy, although both methamphetamine and prescription stimulant use is increasing.^{47–49} Cocaine is a neurostimulant that inhibits monoamine reuptake in the brain and indirectly activates the HPA axis.³⁴ In adult humans and animals, cocaine exposure increases rates of GC secretion, i.e. cortisol in humans and corticosterone in rats.^{50–52} Furthermore, in patients with chronic cocaine use, lower levels of GR mRNA expression is detected in whole blood, which authors postulate is a likely consequence of

receptor down-regulation due to increased systemic circulation of cortisol associated with cocaine use.⁵²

Prenatal cocaine exposure has also been associated with altered HPA axis regulation even remote to *in utero* exposure. In juvenile rats prenatally exposed to cocaine, serum basal cortisol levels were increased as compared to controls and resulted in a more robust increase to a CRH stimulation test.⁵³ In human studies, adolescents with prenatal cocaine exposure have a higher basal cortisol level, but demonstrate a diminished cortisol activation response to stressors compared to unexposed controls.⁵⁴ Such longitudinal human studies are subject to confounders, however, since prenatal cocaine exposure, as with many other illicit drugs, frequently does not occur in isolation from other factors that can affect long-term psychopathological outcomes, e.g. low-income socioeconomic status, maternal stress, additional drug and alcohol exposures.⁵⁵ Such co-exposures may exacerbate or dampen physiological and/or behavioral responses to stress; for example, in a longitudinal study by Min *et al.*,⁵⁶ adolescents exposed to cocaine *in utero* in conjunction with childhood maltreatment demonstrated poorer coping skills compared to those unexposed to cocaine, or those exposed to cocaine or childhood maltreatment alone.⁵⁶

Cocaine exposure influences epigenetic modifications such as DNA methylation,^{57, 58} including in the prenatal setting,⁵⁷ and also via alterations in histone acetylation that can lead to lasting effects on gene and protein expression remote to prenatal exposures.⁵⁹ While longitudinal studies evaluating these specific effects in progeny have yet to be completed to investigate observed effects on the HPA axis, these modifications may represent potential mechanisms through which prenatal cocaine exposure alters HPA axis programming.

Alcohol

Alcohol use during pregnancy is relatively common, particularly early in pregnancy. A study of pregnant women in Australia found that only 41% of women did not have any exposure to alcohol during pregnancy, and while a general trend of decreasing use as pregnancy progressed was observed (87% of these women stopped drinking once they found out they were pregnant), 27% of women surveyed continued to use alcohol at some level throughout pregnancy.⁶⁰ Exposure to alcohol *in utero* is associated with a well-described syndrome and collection of physical and psychological complications defined as fetal alcohol syndrome (FAS). Severity of effects is variable without a clear dose-response pattern or known “safe” level of exposure during pregnancy,⁶¹ and thus collectively the group of features that can occur after *in utero* exposure to alcohol has more appropriately been termed “Fetal Alcohol Spectrum Disorders” (FASDs).⁶²

Common neurobehavioral consequences among FASDs include developmental delay, intellectual disability, hyperactivity, and hypersensitivity to stress.⁶³ Animal, molecular, and cellular studies have corroborated the clinical observation of abnormal stress response through characterization of altered HPA function, GC signaling, and stress-related behavioral studies after prenatal alcohol exposure. That is, prenatal alcohol exposure can both variably affect basal corticosterone levels in rodents,^{63–65} and differentially regulate other HPA axis regulated hormones and associated stress responses.

A study by Lu, *et al.*⁶⁵ evaluating adult male rats that were exposed chronically to alcohol *in utero* demonstrated differential regulation of HPA axis hormones compared to controls and after exposure to stressors. Alcohol-exposed rats demonstrated decreased basal levels of ACTH compared to unexposed controls, and while basal corticosterone levels were not significantly different between the 2 groups, corticosterone levels in the alcohol-exposed group were significantly increased relative to unexposed animals after exposure to a stressor. A similar pattern was observed for CRH (i.e. significantly more induced by stress in alcohol-exposed group). Baseline arginine vasopressin levels were lower in the alcohol-exposed group, but after stress were significantly elevated compared to controls.⁶⁵ Therefore, prenatal alcohol exposure may generate a hypersensitive programming of the HPA axis to stressful stimuli. In fetal rats exposed to prenatal alcohol, GR mRNA expression is increased in the hippocampus, along with a concurrent increase in GAD67 mRNA and protein expression in GABAergic neurons.⁶⁵ The increase in GABAergic neurons may represent a compensatory response to the environment with excessive GC signaling, leading to alterations in hypothalamic function in adulthood via an imbalance of excitatory/inhibitory input.⁶⁵ Of particular note, even periconceptional exposure to alcohol has been shown to trigger a sexually dimorphic alteration in HPA function in rats. Specifically, in a study by Burgess, *et al.*,⁶⁶ female rat progeny exposed to periconceptional alcohol have significant decreases in basal corticosterone levels, as well as significant increases in levels of hippocampal GR gene expression.⁶⁶ A separate study by Lucia *et al.*⁶⁷ investigating a similar paradigm found subtle increases in anxiety-like behavior and short-term spatial memory impairment in female rat progeny exposed to periconceptional alcohol.⁶⁷ These observations are of particular relevance given that a majority of women who drink alcohol during pregnancy stop once they are pregnant, but still potentially expose the fetus to alcohol during a critical neurodevelopmental period and could inadvertently increase susceptibility of offspring to aberrant HPA axis function and stress responses. Mechanistically, long-lasting effects on the stress response in offspring triggered by fetal alcohol exposure have been linked to increased methylation within the proopiomelanocortin (POMC) gene promoter in cells from the arcuate nucleus of the hypothalamus leading to a reduction in POMC expression.⁶⁸ This effect was furthermore shown to be due to increased expression of the methyl CpG binding protein MeCP2.⁶⁸ Changes in specific histone modifications also contribute to fetal alcohol effects on POMC expression in the hypothalamus.^{69–71}

Cannabis

In recent years in the United States, movements to legalize marijuana, or cannabis, for medicinal and recreational purposes have broadened. Concurrently, popular acceptance of the use of marijuana, or its cannabinoid (CB) derivatives such as ⁹-tetrahydrocannabinol (THC), has increased, including during pregnancy. Recent studies have found that rates of marijuana use during pregnancy are rising and approaching nearly 4% in all groups,⁷² though when prevalence measures do not rely on self-report and are based on testing of urine or umbilical cord blood, rates frequently exceed 20%.^{73, 74} Given the ability of lipophilic CBs to readily cross the placenta,^{75–77} concerns from the medical and research communities have arisen about the potential detrimental effects of *in utero* exposure.

CBs signal primarily through established eCB signaling pathways within the brain, and in humans, marijuana use typically has been shown to have activating effects on the HPA axis, including increased levels of cortisol after administration, and a reduced response in cortisol elevation after a stressor in chronic compared to non-users.⁷⁸ Conversely, many other studies have demonstrated the significant role of eCBs as effectors of GC-mediated negative feedback on the HPA axis, thus highlighting the complexity of the relationship of these two signaling systems.⁷⁹ Despite the establishment of a scientific premise for eCB pathway interactions at multiple points with GC signaling, (see section below: *Glucocorticoid and Endocannabinoid Signaling Pathways Reciprocally Modulate One Another*) few studies have evaluated lasting effects of prenatal CB exposure on the HPA axis. Sexually dimorphic responses within HPA axis effectors have been noted in rats exposed to THC *in utero*. Specifically, female rats demonstrate increased expression of CRH in the medial basal hypothalamus and increased plasma corticosterone levels as adults, whereas male rats demonstrated decreased levels.⁸⁰ Another study evaluated levels of ACTH and corticosterone in adult rat serum after prenatal THC exposure, both at basal levels and after morphine-induced place preference testing. Following low dose THC exposure, females showed elevated basal levels of both ACTH and corticosterone compared to controls, while males demonstrated no significant change or lower levels, respectively. ACTH increased in both groups after place preference testing, more significantly so in males. Corticosterone levels were also increased in females while decreased in males, and place preference testing led to a significant increase in males but a blunted response in females. The authors hypothesize that because a similar hormonal and response pattern was seen in rats exposed to prenatal stress, THC exposure may have led to maternal HPA axis elevation.⁸¹ While a precise mechanism has not been described, fetal exposure to cannabis, as with other drugs of abuse, can impact gene expression in select brain regions triggered most likely by select alterations in select histone modifications leading to long lasting changes in gene expression,⁸² which thus could represent a potential mechanism for these persistent effects of exposure.

Despite these early findings, however, longitudinal clinical studies evaluating long-term consequences of prenatal CB exposure in humans have been inconsistent.⁸³ Considering the scientific literature supporting the role endogenous GC/eCB signaling in regulating the HPA axis and stress response, as well as associations of prenatal drug exposure and adverse neurological outcomes previously mentioned, the mechanisms underlying drug use during pregnancy and alterations in GC and/or eCB signaling *in utero* that contribute to enduring consequences in the exposed offspring ought to be further explored.

Prenatal Exposure to Exogenous GCs Leads to Alterations in Cerebral Architecture, HPA Axis Function, and Programming of the Stress Response

Premature birth affects nearly 10% of all pregnancies in the United States⁸⁴ and complications of prematurity are the second leading cause of infant mortality.⁸⁵ In 1972, Liggins and Howie published their seminal work demonstrating that prenatal administration of sGCs (e.g. betamethasone or dexamethasone) ameliorates multiple infant complications of premature delivery, including neonatal respiratory distress syndrome, necrotizing enterocolitis, cerebral intraventricular hemorrhage, and overall mortality.⁸⁶ Their findings

continue to influence medical practice today, and a course of antenatal steroids is indicated for pregnant women who are at imminent risk within 7 days for preterm delivery between the age of viability and gestational week 34.⁸⁷ Neurodevelopmental studies over the ensuing decades, however, have identified potential consequences of antenatal steroid exposure. Postmortem neonatal brain tissue from both animals and humans analyzed after antenatal sGC exposure have demonstrated decreased brain volume in mice,⁸⁸ decreased hippocampal cell density in humans and animals,^{89–91} and a delayed pattern of cerebral myelination in sheep.⁹² Human studies have also demonstrated long-term neuropsychiatric consequences, in particular increased rates of attention problems, lower cognitive scores, and executive dysfunction.⁹³ Though premature birth itself can have significant consequences for neurodevelopment,^{94, 95} certain studies have evaluated children who were exposed to steroids *in utero* for concern for premature delivery, but then were carried to term (occurring in increasing frequency at approximately 25–30% of sGC-exposed pregnancies).^{96, 97} This population of patients was not immune to neurologic consequences of sGC exposure. In a study by Modi, *et al.*⁹⁸ evaluating structural brain development, decreased cortical surface area and decreased complexity of cortical folding were noted in this population.⁹⁸ Furthermore, in a study by Davis, *et al.*,⁹⁹ GC-exposed term-born children were noted to have cortical thinning on brain MRI, particularly affecting the anterior cingulate gyrus.⁹⁹

In our lab, we have confirmed certain detrimental effects using a mouse model of prenatal dexamethasone (DEX) administration. A single dose of DEX at embryonic day 14.5 leads to decreased brain-to-body weight ratio, cortical thickness, and cortical surface area of the brain at E17.5.⁸⁸ However, despite the smaller cortical surface area and thickness, the cellular structure of the cortex was found to be more densely packed, particularly in cortical layer V, and had associated decreased dendritic complexity.⁸⁸

Antenatal sGC exposure has also been linked to long-term altered HPA axis function and stress responses. In studies performed in our laboratory, adult male mice exposed *in utero* to a single dose of antenatal DEX demonstrated significantly increased presence in the center and open arms of the open field in an elevated plus maze, and decreased time in the closed arms, and less time floating in the forced swim test compared to controls. These behavioral tests suggest that the stress response was lessened compared to controls.⁸⁸ Human infants exposed to sGC and delivered prematurely were found to have dampened cortisol responses compared to unexposed, while those exposed to sGCs *in utero* but delivered at term had a greater response than controls.¹⁰⁰ sGC-exposed children were also noted to have blunted circadian regulation of cortisol.⁹⁷ Furthermore, female school-aged children exposed to prenatal sGC were found to have significant elevations in salivary cortisol levels after being introduced to a stressor as compared to unexposed female controls, and also compared to males with antenatal sGC exposure.¹⁰¹

eCB Signaling Influences Neurodevelopment

eCB signaling in the central nervous system occurs through activation of cannabinoid receptor 1 (CBR1, encoded by the *CNR1* gene), the most abundantly expressed G-protein coupled receptor in the brain,¹⁰² as well as through the cannabinoid receptor 2 (CBR2).^{103, 104} Cannabinoid receptor (CBR) activity is regulated by binding of the endogenous eCB

ligands, 2-arachidonylglycerol (2-AG) and arachidonylethanolamine (AEA). Synthesis of 2-AG and AEA is catalyzed by diacylglycerol lipase and phospholipase D enzymes, respectively, in response to increases in intracellular postsynaptic cell activation.^{105–107} In the mature brain, eCBs diffuse across the synaptic cleft in a retrograde manner to bind presynaptic CBRs and influence a variety of intracellular signaling cascades responsible for mediating synaptic plasticity and homeostatic metabolic processes.^{108–111} CBR also affects other neurogenic areas, such as the hippocampal subgranular and subventricular zones.^{102, 112, 113} In neural progenitor stem cells (NPSC), CB1R and CB2R activity is coupled to the activation of the ERK and PI3K pathways controlling neural progenitor proliferation and cell fate decisions (Figure 2),^{114–116} as well as neurotransmitter release from newborn neurons.^{112, 117} Localized primarily on axonal growth cones of differentiating neurons,^{118–120} CB1R expression increases with neuronal differentiation^{102, 121} and influences neurite outgrowth,¹²² long-range subcortical projections,^{120, 123} and developmental expression of the Ctip2/Satb2 transcriptional axis that differentially guides NPSC fate and cortical layer expansion in the developing forebrain.¹²⁴

In the developing brain, eCB accumulation is established by the balance between its biosynthetic and metabolic enzymes. The fine tuning of eCB availability within distinct brain regions, coupled with, differences in CB1R expression and downstream coupling contribute to the complex effects of eCBs on neuronal specification and cell fate in the developing brain.¹⁰² In recent years, it has become evident that prenatal exposure to THC, the main psychoactive component of marijuana, can alter eCB signaling and evoke multiple long-lasting functional alterations. This alteration results from THC targeting of CB1Rs on developing cortical neurons,¹²⁵ as well as THC-mediated alterations in feed forward and feedback cellular communication in the hippocampus triggered by downregulation of hippocampal cholecystokinin-expressing interneurons, a prominent CB1R-expressing neuronal population throughout development.¹²⁶ Additionally, prenatal THC has been reported to compromise microtubule stability and cytoskeletal dynamics via CB1R activation and phosphorylation of the Superior Cervical Ganglion 10 (SCG10)/stathmin-2 microtubule binding protein, leading to altered neuronal circuitry in adulthood.¹²⁷

The importance of the role of eCB signaling in neurodevelopment is of particular importance at this time, given rising rates of marijuana use during pregnancy,^{72, 128, 129} a drug which contains multiple compounds which activate the CB system¹³⁰ and has the potential to affect neurodevelopmental outcomes.^{83, 131}

GC and eCB Signaling Pathways Reciprocally Modulate One Another

The interaction between GC and eCB signaling pathways has been observed in a variety of physiological processes including the stress response, emotional memory, reward sensitivity, energy homeostasis, inflammation, pain, and bone health.⁷⁹ In the mature brain, CB1R activation is necessary for rapid GC-mediated negative feedback of HPA axis activation, which is generated primarily by GR mobilization of eCBs 2-AG and AEA.^{132–135} Specifically, eCBs sustain GC-induced inhibition of spontaneous neuronal activity,¹³⁶ non-genomic glucocorticoid-induced inhibition of glutamate release in the PVN of the hypothalamus,¹³⁷ as well as glucocorticoid-induced enhancement of emotional memory

processing in the amygdala.^{138, 139} In contrast, GCs acting via genomic mechanisms can reduce eCB signaling via downregulation of CB1R expression.^{140, 141} For a more detailed background review focused on the interactions between eCB and GC signaling, we refer the reader to the recent review by Balsevich *et al.*⁷⁹ In contrast to multiple molecular mechanisms identified for GC regulation of eCB signaling in a variety of physiological systems⁷⁹ the precise impact of eCBs on GC signaling in the developing brain remain largely unknown.

sGCs Modulate eCB Signaling

Exposure to DEX may also promote and increase eCB signaling. For example, anti-inflammatory effects of synthetic GCs such as DEX are mediated in part through inhibition of COX-2 synthesis.^{142, 143} COX-2 cleaves AEA and 2-AG to form arachidonic acid, thus terminating eCB action.^{144, 145} In the brain, COX-2 decreases eCB signaling *in vivo*, as revealed in a study demonstrating decreased anxiety-like behaviors and increased eCB signaling in mice treated with selective COX-2 inhibitors.¹⁴⁴ These findings suggest that sGC-mediated suppression of COX-2 may promote increased eCB signaling.^{79, 146} The GC mediated change in eCB content occurs through GR, as opposed to MR, as reported in studies investigating the role of corticosteroid receptors in eCB-induced decrease of freezing behavior in rats.¹⁴⁷ While the interplay of endogenous GC and eCB signaling in health and disease have been established in the mature brain,⁷⁹ prenatal exposure to teratogens or other exogenous substances that may influence GC/eCB pathway interactions in the developing brain is less known. Most of the insight into this largely unexplored field has been gained using animal models of prenatal stress to define the associations between exposure to excess GCs (i.e. corticosterone) *in-utero* and altered GC signaling responses later in life.

Specific mechanisms for GC regulation of eCB signaling have been revealed in adult brain with many studies demonstrating effects of GCs on eCB synthesis and release.^{79, 148} However, mechanisms responsible for the reciprocal regulation of GC signaling by eCBs are largely unknown. There is limited evidence supporting an effect of CB signaling on GR expression¹⁴⁹ and the kinases mobilized by eCB signaling in the developing brain (e.g. Akt)^{102, 112} are unlikely to directly impact GR phosphorylation.^{150, 151} However, many transcription factors whose activity can be regulated upon phosphorylation by CBR activated kinases (e.g. AP-1 family members¹⁵² CREB¹⁰²) are well known to co-occupy GC regulated target genes with GR and influence ultimate transcriptional response.^{153–156} Furthermore, many transcriptional coregulators that participate in GR transcriptional responses are themselves regulated via phosphorylation¹⁵⁷ in particular by some kinases that are downstream of CBR activation.¹⁰² Ultimately, the implementation of genomic studies in model *in vitro* and *in vivo* systems will be essential to identify the mechanisms of cross-talk between the eCB system and GR transcriptional responses.

Prenatal Stress and eCB/GC Signaling Interactions

Although the sex-specific effects of excess CBs and/or GCs are well characterized,^{158–160} the eCB-dependent response to stress or its interactions with GC signaling following early life stress is an understudied area of research that may address a significant population of

individuals. Some animal studies provide evidence for exposure to excess CBs during adulthood as a mechanism for reversing the effects of stress.¹⁶¹ For example, chronic administration of the synthetic CB1/2R agonist WIN55,212-2 prevented early life stress-induced impairments in short-term and working memory tasks in mice. This was accompanied by a normalization of CB1R and GR levels that are normally increased or decreased, respectively, following early life stress.¹⁶² The anxiolytic¹⁶³ and neurocognitive¹⁶⁴ benefits of CBs during adulthood are proposed to be due to an impact on hippocampal neurogenesis.

Studies have shown that prenatal stress regulates CB1R expression in a sexually dimorphic manner, reducing CB1R expression in the cortex, striatum, hippocampus, and amygdala of male rats, while increasing CB1R expression in the hippocampus of female rats.¹⁶⁵ This response is not observed in adult female rats, who exhibit downregulation of CB1R receptors in the hippocampus, amygdala, and prefrontal cortex following stress.¹⁶⁶ The eCB-dependent response to stress is dependent not only on sex, but also on age. Studies suggest that the adult brain is more resilient to THC-induced changes in the transcriptome and lipidome landscape of the hippocampus when compared to the developing brain¹⁶⁷ and to development of drug tolerance following chronic THC administration.¹⁶⁸

Conclusion

While prenatal exposure to multiple substances of abuse have the potential to create downstream consequences for GC signaling and the stress response, particular attention should be given to cannabis exposure given its propensity for signaling within the GC system. Furthermore, given that marijuana use in pregnancy is rising, and approximately 1/10 pregnancies may be at risk for premature delivery and thus have the potential to receive sGCs, the likelihood of *in utero* co-exposure to synthetic CBs and sGCs in a subset of pregnant women is high. Additionally, prenatal stress may influence use of CBs in pregnancy. Exposure to such multiple agents could produce an altered GC/eCB signaling response leading to unique developmental consequences in the offspring. While the scientific literature on co-exposure to sGC, stress, and marijuana during pregnancy is currently lacking, the insights gained from future studies on this topic will further the understanding of how prenatal co-exposures can potentially program brain development, HPA axis function and GC signaling, and the stress response, and additionally how such consequences can contribute to the psychopathological onset of neurodevelopmental disorders associated with early life adversity.

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Figure 1: Prenatal exposure to stress and substances of abuse impact neurodevelopmental outcome.

Following prenatal exposure to stress and/or teratogenic agents, multiple points of interaction converge to influence endogenous glucocorticoid (GC) signaling, hypothalamic/pituitary/adrenal (HPA) axis regulation, and the stress response. Ultimately, these may result in developmental abnormalities that persist into adulthood.

Figure 2: Interactions between glucocorticoid and endocannabinoid signaling pathways that impact glucocorticoid receptor control of transcription.

The cellular response to glucocorticoids (GC) is mediated largely by the glucocorticoid receptor (GR), located in the cytoplasm bound to chaperones (e.g. heat shock protein 90 [HSP90]), along with other chaperone (Ch) and co-chaperone (coCh) proteins. A fraction of GR can also be localized at the plasma membrane. The classical response to GCs occurs following ligand binding to and nuclear translocation of GR, leading to its direct or indirect association with glucocorticoid responsive elements (GRE) or other DNA sequences resulting in transcriptional activation or repression. For simplicity, direct GR/GRE binding is shown. Non-classical, rapid GC signaling occurs through a membrane-associated GR, which upon ligand binding can activate multiple downstream cytoplasmic signaling pathways including but not exclusively through the modulation of G-protein coupled receptor (GPCR) activity. Cannabinoid receptors (CBR) are GPCRs that respond to endocannabinoids (eCB) via an autocrine or paracrine mechanism or exogenous cannabinoids that can be plant-derived or synthetic. eCBs also signal via intracellular CBRs (not shown). These receptors can ultimately impact cAMP or MAPK (e.g. Ras/Ras/MEK/ERK) signaling. The direct or

indirect phosphorylation of transcription factors (TF), such as CREB, or coregulators that co-occupy GC-regulated target genes following CBR activation provides a potential mechanism of GR/CBR crosstalk that influences GC regulated transcription. Direct modulation of CBR activity by GR has not been documented although is plausible given established effects of GR on other GPCRs.

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