IMPORTANT DEVELOPMENTS: CANNABINOID EFFECTS AT DIFFERENT STAGES OF LIFE
Learning Objectives

• Understand how the endocannabinoid system relates to neurotransmitters and brain circuits

• Become familiar with the significant *in utero* to adult epidemiology studies of cannabis use and their effect on brain development

• Be able to discuss the relationship between cannabinoids and neurogenesis
The Brain as Chicken Soup

Diffuse Neurotransmitter Systems

- **Glycine**
- **Glutamate**
- **Gama-aminobutyric acid (GABA)**

The Brain and Circuits

Defined Neurotransmitter Systems

- Dopamine
- Serotonin
- Norepinephrine
- Acetylcholine
- Histamine

The Endocannabinoid System (ECS)

- **ENDOCANNABINOIDS**
  - Phospholipid-derived neuromodulatory lipids synthesized on-demand from membrane arachidonic acid
    - 2-Arachidonyl glycerol (2-AG)
    - Anandamide (AEA)

- **CANNABINOID RECEPTOR 1&2**
  - Two G-protein-coupled receptors (GPCRs)

- **BIOSYNTHETIC ENZYMES**
  - N-acetylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD)
  - Diacylglycerol lipase (DAGL)

- **BIODEGRADATIVE ENZYMES**
  - Fatty acid amide hydrolase (FAAH)
  - Monoacylglycerol lipase (MAGL)

ECS: Soup or Sub?

The ECS is a modulator of essential biological processes, including cellular communication, survival and death, energy homeostasis, immune response, and reproduction.

The ECS consists of the following:
- Cannabinoid receptors (CB₁ and CB₂)
- Cannabinoid agonists
- Biosynthetic and biodegradative enzymes

Extra-cannabinoid receptors with affinity for cannabinoid binding:
- Transient Receptor Potential Cation Channel V1 (TRPV1)
- GGP 55 & GPR 18
- 5-HT₁ₐ, 5-HT₂ₐ, 5-HT₃ₐ
- GABA & Glycine
- Peroxisome Proliferator-Activated Receptors (PPARs)
- Opioid

Parker LA. Cannabinoids and the Brain 2017.
The Brain and the Endocannabinoid System (ECS)
Brain development proceeds through a highly organized multistep process that is genetically determined, epigenetically modified, and environmentally edited (Tau and Peterson, 2010).

Brain development in infants and children is driven by brain volume, which includes the genesis of brain cells and numerous synaptic creation.

From childhood to adolescence, brain development shifts from producing large numbers of neurons to sculpting efficient pathways:

- Inefficient connections between neurons are “pruned” or eliminated.
- Useful neurons and synapses are identified and strengthened (Whitford et al, 2007).

Lee TT, Gorzalka BB. Int Rev Neurobiol 2015;125:49-84.
Both structural and neurochemical changes occur in the developing brain that results in maturation of functional properties.

MRI studies show brain development in different areas of the cortex do not occur simultaneously.

- Cortical areas that serve simple tasks first develop followed by maturation of progressively higher domains.

Prefrontal Cortex (PFC) Development

- Development of the prefrontal cortex (PFC) is dependent upon the correct development of lower regions that occur earlier.

- The PFC undergoes maturation during childhood, with a reduction of synaptic and neuronal density, a growth of dendrites, and an increase in white matter.

- Cognitive functions (including working memory and planning) reach a relative plateau of maturity near the age of 12.

- Higher cognitive functions, including language and intelligence, continue to develop into the third decade of life.

GABA-ergic Development in the Adolescent Brain

- Basolateral amygdala fibers develop projections to the GABA-ergic interneurons in the PFC in adolescence; pyramidal cells provide extensive connections to the dorsolateral PFC.

- Parvalbumin (PV) cells (GABA-containing interneurons) control the firing rate of nearby pyramidal neurons and modulate the development of executive functions associated with PFC.

- Delayed maturation of the PV interneuron system impairs the development of mature executive function performance.

- The input of the GABA-ergic interneurons to the PFC declines from adolescence to adulthood.

Dopamine (DA) Development in the Adolescent Brain

The DA balance between the PFC and subcortical structures of the mesolimbic system evolves during adolescence

- DA undergoes significant reorganization during adolescence and early adulthood
- PFC DA peaks in early adolescence and then declines until early adulthood
- Subcortical DA synthesis and turnover, and projections to the PFC, decline in adolescence
- Pyramidal cells undergo significant pruning and the density of afferents to the PFC increases; catechol-o-methyl-transferase (COMT) activity surges

The ECS in Early Stages of Life

The ECS plays an important role in the development of the brain in early life.

- Critical levels of AEA are required for successful passage of the embryo through the oviduct and implantation in the uterus.

- The CB₁ receptor regulates neural progenitor differentiation into neurons and glial during fetal life.

- Postnatal blockade of the CB₁ receptor impairs the start of suckling.

Prenatal to Adolescent Development of the CB$_1$ Receptor

- During prenatal development, the CB$_1$ receptor is highly expressed in the mesocorticolimbic brain structures (Wand et al, 2004, 2006)

- CB$_1$ receptors are found at glutamate, GABA, and dopamine terminals, and modulate chemical communication between neurons

- The orderly development of these neurons through the ECS and possible disruption by exogenous cannabis may have important influence on the developing brain and future behavior

Cannabis Is the Most Commonly Used Illicit Drug in Pregnancy

- An estimated 3.8% of the global population (182.5 million) have used cannabis

- In the US, an estimated 8.4% of the population (22.2 million) have used cannabis with over 42% of people >12

- Among adolescents, 7.4% (1.8 million) frequent smokers of cannabis and now surpass tobacco smoking in total users

- ~5.2% of pregnant women in the US (115,000) use cannabis

Phytocannabinoids

The Science of Marijuana, 2nd ed" 2008; Oxford University Press.
Is THC a Neurotoxin?

YES

1. Toxic effects on cultured neurons blocked by CB1 receptor antagonists (Campbell, 2001)
2. Chronic exposure in vivo reduces mean volume and density of hippocampal neurons and their nuclei, synaptic density, and dendritic length (Landfield et al, 1988)
3. Chronic exposure of immature rats induce more irreversible residual effects on behavior than chronic exposure of mature rats (Scallet, 1991)

Preclinical evidence

NO

1. CB1-dependent neuroprotective effects of cannabinoids (Hampson et al, 1998; Shen and Thayer, 1998)

Disruption of the ECS Occurs Through Maternal Cannabis Use

- **In utero**, human fetuses of cannabis-smoking mothers have lower body weight compared to controls (Hurd et al, 2005)

- Maternal exposure to cannabis reported *in utero* decrease of human fetal D2 DA receptors in the amygdala, but no changes in CB₁ mRNA or D1 DA receptors (Wang et al, 2004)

- Preclinical studies report that prenatal and postnatal maternal exposure to cannabis disrupted the normal DA-dependent motor functions and HPA axis response to stress in adult offspring (Ramos et al, 2002)

The Ottawa Prenatal Prospective Study (OPPS)

**STUDY DESIGN**
- 291 expectant women enrolled in prospective study; ~20% of subjects used cannabis during pregnancy
- Assessed during pregnancy and at least annually for first 6 years with standardized global measures and neuropsychological (NP) outcomes

**RESULTS**
- Cannabis-exposed neonates had increased startle response and altered visual responses
- By age four these same subjects showed more distinct changes in NP testing, increased behavioral problems, decreased visual performance, and poor attention and memory
- Delay in executive functions reported in older children
Maternal Health Practices and Child Development Study (MHPCD)

STUDY DESIGN
• 519 mothers and live born infants evaluated at 4- and 7-months gestation and postpartum
• Offspring assessed up to young adulthood

RESULTS
• Prenatal marijuana exposure (PME) more likely to lead to adolescent cannabis use (38% before age 15)
• At age 22 less likely to have completed HS (37.2% vs. 54.5%), less likely to be employed (52.1% vs. 67.6%), more likely to have been arrested (56% vs. 27.3%)

The Dunedin Study

**STUDY DESIGN**

- Prospective study of 1037 individuals from birth to 38 years
- Cannabis use recorded at ages 18, 21, 26, 32, and 38
- Neuropsych testing at age 13 before the use of cannabis and at age 38 after a pattern of persistent use

**RESULTS**

- Impairment was greatest among adolescent-onset users
- Broad neuropsych decline across all domains tested with executive function and processing speed most impacted

The Generation R Study

STUDY DESIGN

- 9778 expectant women enrolled between 2002–06 with offspring to be followed from fetal life to adulthood
- Identify early environmental and genetic causes leading to abnormal growth and development

RESULTS

- 7452 expectant women provided information on substance use and neuroimaging data reported in 2016
- In utero exposure to cannabis associated with thicker frontal cortex

What Do These Studies Tell Us About Cannabis and Development of the Nervous System?

- Newborns show increased tremors with exaggerated startle and disrupted sleep
- Adolescents may have impaired executive function and higher risk for psychiatric disorders and substance abuse
In Utero and Perinatal Exposure of Cannabis on Offspring Adult Cognition

The ECS strongly influences the maturation of the PFC.

- Longitudinal studies have reported that prenatal and perinatal exposure to cannabis may cause cognitive and executive function impairment as adults.

- Preclinical studies using rats of prenatal and perinatal exposure to cannabis have reported executive function impairment and disruptions in emotional behavior; behavioral changes appear more prominent in adult male rats but not in females.

- Breast feeding transfer cannabinoids and can have significant impact on the maturation of the PFC (Scheyer et al, 2019).

In utero Cannabis Exposure Effects in the Forebrain and Ventral Tegmental Area (VTA)

• Dopamine strongly influences the developmental differentiation and maturation of circuits of the forebrain

• Maternal cannabis use has strong effects on the developing fetal mesocorticolimbic system and possible behavioral effects on the offspring

• Alterations of dopamine D2 receptor gene expression after in utero cannabis exposure in mesocorticolimbic structures have been reported in human fetal tissue

• In contrast, there is less agreement on the effects of maternal cannabis use on the VTA where dopamine forebrain projections originate

Anandamide (AEA) and Vulnerability to Drug Addiction

- Adolescence is a critical neurodevelopmental period with major changes to the mesolimbic DA pathway that is modulated by the ECS to regulate reward-associated behaviors (Gee et al, 2018)

- The ECS achieves peak influence and activity throughout the brain in adolescence (Meyer et al, 2018)

- The expression of CB$_1$ and AEA both peak during adolescence (Hill and Tasker, 2012)
  - CB$_1$ is at highest expression in the PFC and striatum
  - AEA increases during early life and fluctuate during adolescence in the nucleus accumbens and striatum (Ellgren et al, 2008)

- Similar fluctuations in the catabolic enzyme FAAH occur in adolescence and may account for the changes in AEA

Single Nucleotide Polymorphism (SNP) and the FAAH Gene

- An SNP in the FAAH gene (C385A) results in greater proteolytic degradation of FAAH and increased levels of AEA (Chiang et al, 2004)
  - FAAH C385A carriers found in 38% of individuals of European descent
  - Substitution of proline for threonine residue at position 129 and enhances connectivity between PFC and the amygdala and in adolescence increased response of fear-related behaviors (Gee et al, 2016)
  - FAAH C385A carriers also display increased striatal activity and impulsivity
  - Several studies have linked this FAAH SNP to drug use including the likelihood to use cannabis (Tyndale et al, 2012) although the progression to dependence is controversial (Parsons and Hurd, 2015)

Genetic Vulnerability to Cognitive Impairment in Adolescent Cannabis Use

- Individual genetic vulnerability to cannabis exposure may contribute to some behavioral and cognitive disturbances

- The expression of the *CNR1* gene is different in different brain cells and THC potentially could initiate cell-type-specific responses and behaviors (Han et al, 2012)

- Astrocyte *CNR1* stimulation (but not neuron) by THC activates inflammatory signaling and glutamate release and cognitive changes (Chen et al, 2012)
  - Genetic variants that occurs only in an astrocyte or neuron might express different behaviors (Hurd et al, 2019)
  - Similar effect was reported for a rare, highly penetrant mutation, a dominant-negative form of Disrupted in Schizophrenia I (DN-DISC1) (Ballinger et al, 2015)

Cannabis Access Is Increasing and the Product Has Become More Potent

- In 2020, 11 states and Washington, DC, permit recreational marijuana and 33 states and Washington, DC, allow medical marijuana.

- CBD, a nonpsychoactive cannabinoid can be derived from the cannabis plant or hemp, and is legally available if the dried product derived from hemp contains no more than 0.3% THC.

- THC potency has consistently increased over the past quarter century; in 1996 confiscated cannabis contained ~4% THC; in 2017 the average THC potency was 17.1% (ElSohly et al, 2016).

- Ratio of THC to CBD has increase ten-fold from 12 in 2008 to 104 in 2017 (Chandra et al, 2019).

THC Exposure and Epigenetic Effects

THC exposure on the adult brain may modulate gene expression without altering the genetic code

- 24 human male donors (12 cannabis-users daily > 6 mos and 12 non-users) between 18–40 provided semen samples for analysis
- DNA methylation differed in users compared to non-users by >10% at CpG sites (cytosine-guanine nucleotide)
- Seminal studies of 16 sexually-mature rats (8 placebo and 8 THC treated) also showed increased DNA methylation in treated animals

The Early Belief That Neurogenesis Occurred Only *In Utero*

Ramon Y Cabral

“Father of Modern Neuroscience”
Neurogenesis

Adult neurogenesis is where functional neurons are continuously generated in the nervous system after embryonal development and throughout adult life.

Neural stem cells (NSCs) are self-renewing, multipotent cells found within two niches of the CNS:
- **Subventricular Zone (SVZ)** along the lateral ventricular wall
- **Subgranular Zone (SGZ)** of the dentate gyrus in the hippocampus

Generation of functional neurons is a complex and coordinated process that involves proliferation, differentiation, and migration of NSCs into existing circuitry.

Role of the CB$_1$ Receptor in Adult Neurogenesis

- The CB$_1$ receptor serves a key proneurogenic role in the germinal areas of adult neurogenesis (SVZ and SGZ)
  - Chronic stimulation by arachidonyl-2-chloroethylamide (ACEA) produced robust neural proliferation in the SGZ and increased neuroblast rostral migration towards the olfactory bulb
  - In mouse model of epilepsy ACEA increased neuronal proliferation, migration, and differentiation in the hippocampus
    - Valproate had no effect on neurogenesis, but combination with ACEA resulted in marked neurogenesis in the SGZ
  - ACEA reversed impairment of neurogenesis in the SGZ in the rat by alcohol intoxication but not in the SVZ
  - Chronic administration of 2-AG produced neurogenesis in mouse olfactory epithelium and blocked by CB$_1$ antagonist

Role of the CB2 Receptor in Adult Neurogenesis

- The CB2 receptor plays a more complex role in adult neurogenesis
  - CB2 ablation in 4-month-old mice did not alter SGZ neurogenesis; other studies of CB2 knockout mice found reduced basal cell proliferation in embryonic day 17.5 and at 2 months post-natal
  - CB2 agonism stimulated neurogenesis in multiple neuropathological models by normalizing neuronal homeostasis and survival like apoptosis, oxidative stress, and inflammation
  - In adults, CB2 may be more relevant with coping with brain injury rather than maintaining basal neurogenesis
    - HIV-1 associated encephalitis (Avraham et al, 2014)
    - Parkinson’s disease (Shi et al, 2017)
    - Alzheimer’s Disease (Wu et al, 2017)
    - Focal brain ischemia (Bravo-Ferrer et al, 2017)
Inhibitors of endocannabinoid-degrading enzymes (FAAH and MAGL) increase the levels of AEA and 2-AG.

Results to date have been unclear with conflicting results with FAAH inhibition.

Limited work with MAGL inhibition prevented neurogenesis impairment in the SGZ and restored long-term potentiation in the hippocampus; MAGL inhibition also blocked the effect of chronic stress-induced depressive behaviors in rats.

What Should We Advise Clinicians on Cannabis and Cannabinoids?

Many important advances in psychopharmacology have occurred from serendipity; the discoveries with cannabinoids have only begun

Promotional reach of medical marijuana has outpaced the public awareness of clinical research

Clinicians should be conversant on the science of cannabinoids and counsel their patients based on science rather than promotional campaigns

We must decide if the current cannabis products are sufficient or if better solutions should be found

More investment in research to uncover potential uses of cannabinoids to improve health is required
Summary

• The ECS serves a critical role in the differentiation and maturation of the CNS

• Maturation of the brain occurs in orderly steps with most neurotransmitter systems developed in the embryo and early postnatal period; dopamine system maturation occurs later during adolescence

• Four large epidemiological studies find an association between exposure to cannabis in utero, perinatal, and in adolescence, and disruption of neuropsychological function in the adult brain

• Exposure to cannabis at sensitive periods of brain development may disrupt the normal development of the CNS

• Neurogenesis occurs in utero and in a limited number of areas in the brain into adult life

• The ECS is closely linked to neurogenesis
THC binds to the following receptors in the brain:

1. GPR 55
2. Peroxisome Proliferator-Activated Receptors (PPARs)
3. CB₁ and CB₂ receptors
4. All of the above
The endocannabinoid anandamide (AEA) serves a critical role in which of the following developmental steps?

1. Passage of the embryo through the oviduct and implantation in the uterus
2. Pruning of pyramidal cells in the mesocortical tract and maturation of the prefrontal cortex
3. AEA peaks in perinatal period and declines during childhood and adolescence
4. AEA peaks in adulthood after maturation of the endocannabinoid system (ECS)
Posttest Question 3

Large epidemiology studies of cannabis use in expectant mothers result in what effects on offspring?

1. Higher birth defects including facial and limb deformities
2. Higher risk in adolescence for psychiatric disorders and substance abuse
3. Atrial Septal Defect (ASD)
4. Thinning of cortical gray matter
5. 2 and 4