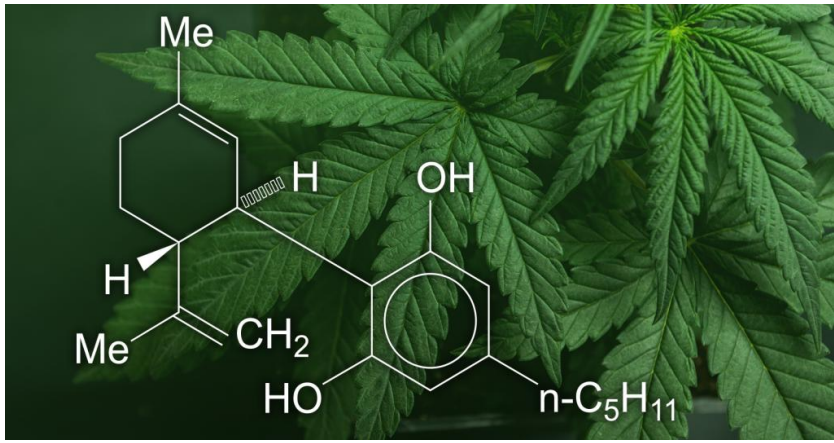
EVIDENCE ON THE DEVELOPMENTAL TOXICITY OF Cannabis (Marijuana) Smoke and Δ^9-THC

Developmental and Reproductive Toxicant Identification Committee Meeting

December 11, 2019

California Environmental Protection Agency
Office of Environmental Health Hazard Assessment
Reproductive and Cancer Hazard Assessment Branch



Identity of Cannabis Smoke and Δ^9-THC

- Cannabis smoke is a complex mixture of several thousand chemicals
- Δ^9-THC is one of at least 60 different cannabinoid compounds contained in *Cannabis* plants and is present in cannabis smoke
- Δ^9-THC is the most potent psychoactive compound present in cannabis

Exposure and Use Information

- Smoking
- Vaping
- Dabbing
- Edibles

Pharmacokinetics

Absorption

- At multiple sites within the aerodigestive tract

Distribution

- Brain, fetus, breast milk and meconium
- 11-OH-THC and THC-COOH, have been detected in umbilical cord tissue

Pharmacokinetics (continued)

Metabolism

- Phase I and Phase II enzymes are expected to be involved in the metabolism of cannabis smoke and Δ^9-THC

Excretion

- Δ^9-THC and its metabolites are excreted via the feces and urine, and to a lesser extent, through sweat, saliva, breast milk, and hair

Outline

- Overview of the endocannabinoid system
- Developmental toxicity
- Somatic outcomes
  - Human studies
  - Animal studies
- Neurodevelopmental outcomes
  - Human studies
  - Animal studies
- Epigenetic and other mechanistic data
- Summary

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- Dr. Yassaman Niknam
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Endocannabinoid System Overview

- Maintenance of pregnancy
- Reproductive Function
- Somatic Development

Cannabinoid Receptors:

CB_1R
- Nervous system
- Peripheral tissues

CB_2R
- Immune system

CB_3R (GPR55)—G-protein receptor 55
- Skeletal and bone tissue

Endocannabinoid System Overview (cont'd)

Bind Endocannabinoids (eCBs)

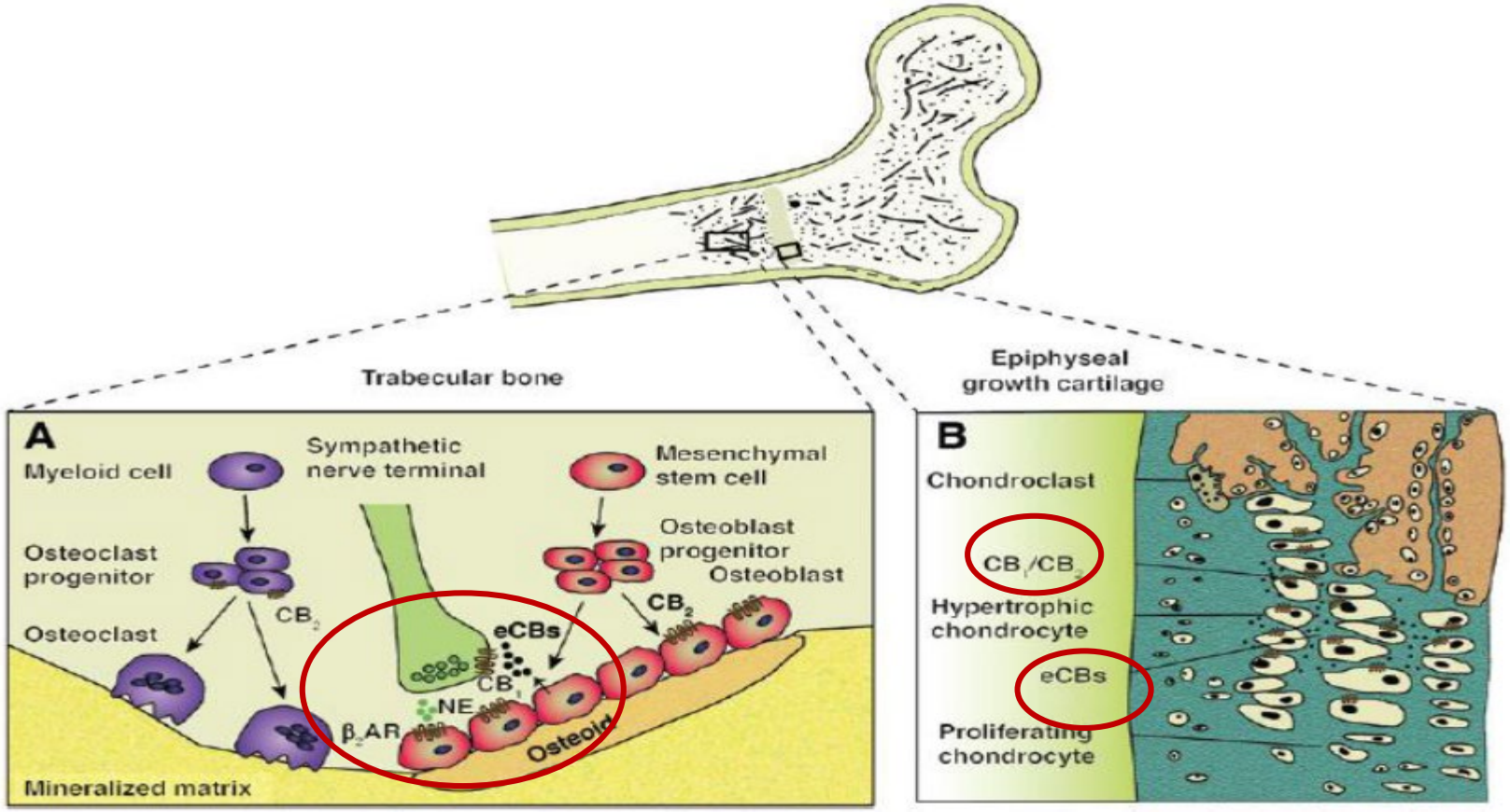
- AEA (anandamide)-partial agonist
- 2-AG (2-arachidonylglycerol)-full agonist
- Synthesized on demand and broken down by Monoacylglycerol lipase (MAGL) and fatty acid hydroxylase (FAAH)

Mechanistic Pathways are Important in:

- Development of the embryo and facilitating successful embryo implantation
- Bone growth and differentiation
- Development of the immune system, and
- Development of the nervous system

Bone Growth and Development

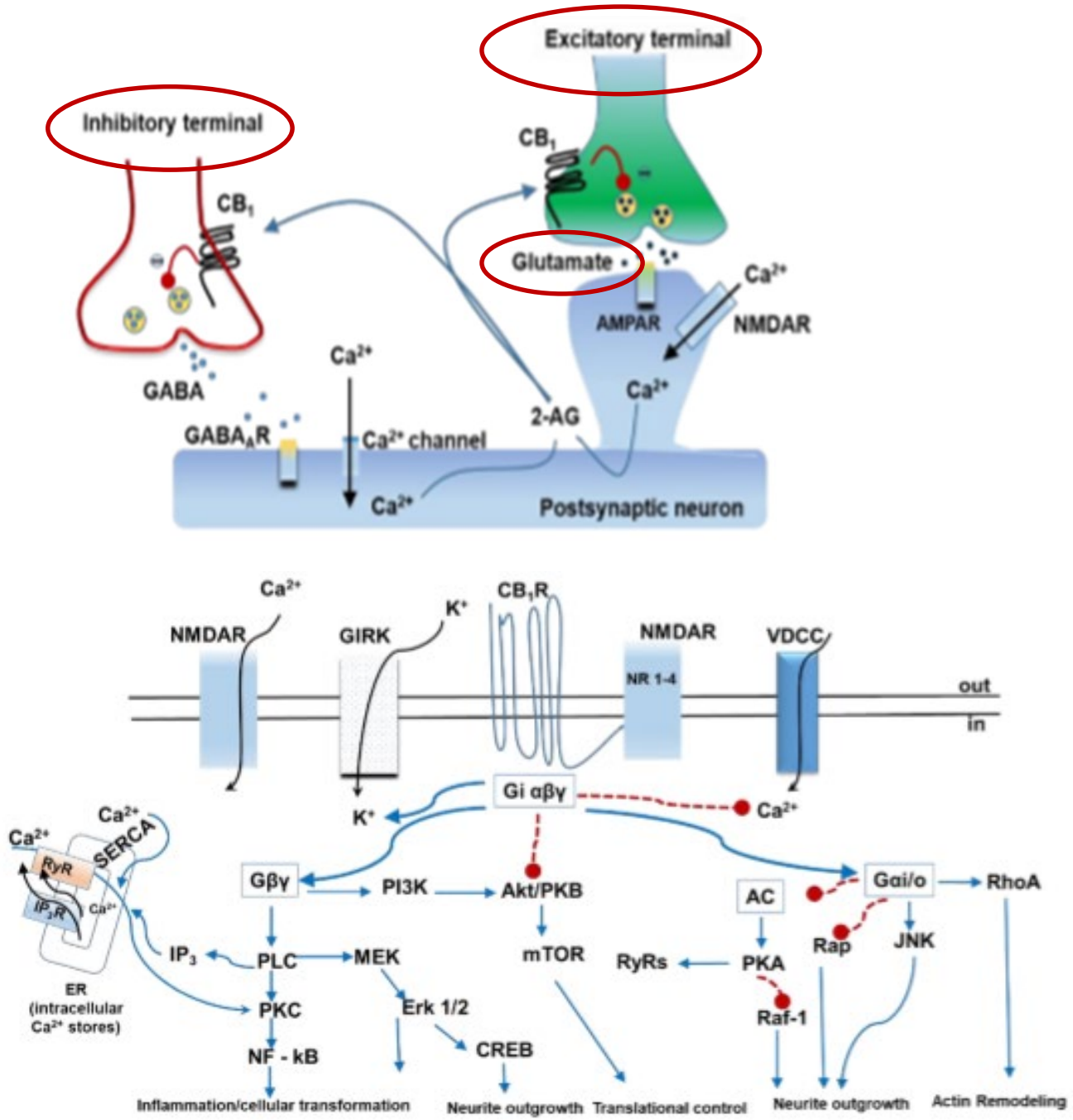
- Osteoblasts and osteoclasts
- CBRs are expressed in osteoblasts, osteocytes, and osteoclasts and regulate bone mass by negative modulation



CBRs and Neurodevelopment

- CBRs expressed in the hippocampus, striatum, and cerebral cortex (and other areas)
- EC system plays a role in the hypothalamic-pituitary-adrenocortical axis (HPA)
- Expression of CBRs changes and roles during development differ than those of mature nervous system
- Activation of CBRs during neurodevelopment affects areas including:
  - Neurite outgrowth and growth cone steering considerations
  - Synaptic plasticity
  - Behavior
  - Locomotor activity

EC Molecular Mechanisms of Neurodevelopment

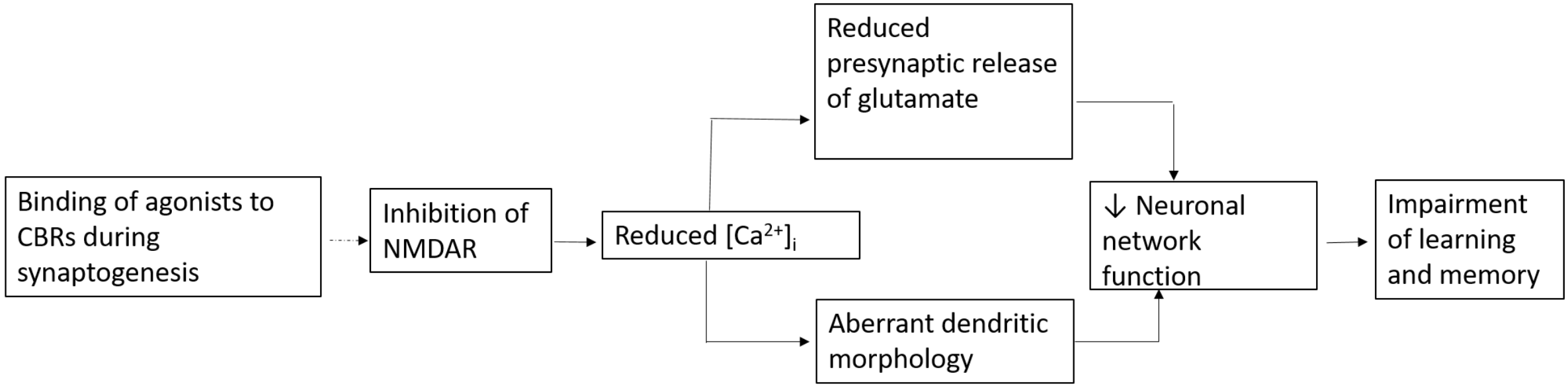


Top: eCBs as retrograde messengers

Bottom: EC system mediated pathways critical in neurodevelopment

Figure modified from: (Kano et al. 2009; Keimpema et al. 2011; Sanchez-Blazquez et al. 2014; Zhuang et al. 2005)

Adapted Impaired Learning and Memory AOP for CBR Agonists



Adapted from OECD

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  - Human studies: Dr. Allegra Kim
  - Animal studies
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Study Selection

- Analytic designs with individual exposure and outcome assessment
- Exposure assessment
  - Biological assay
  - Quantified if self-report
- Addressed potential confounding by tobacco, alcohol

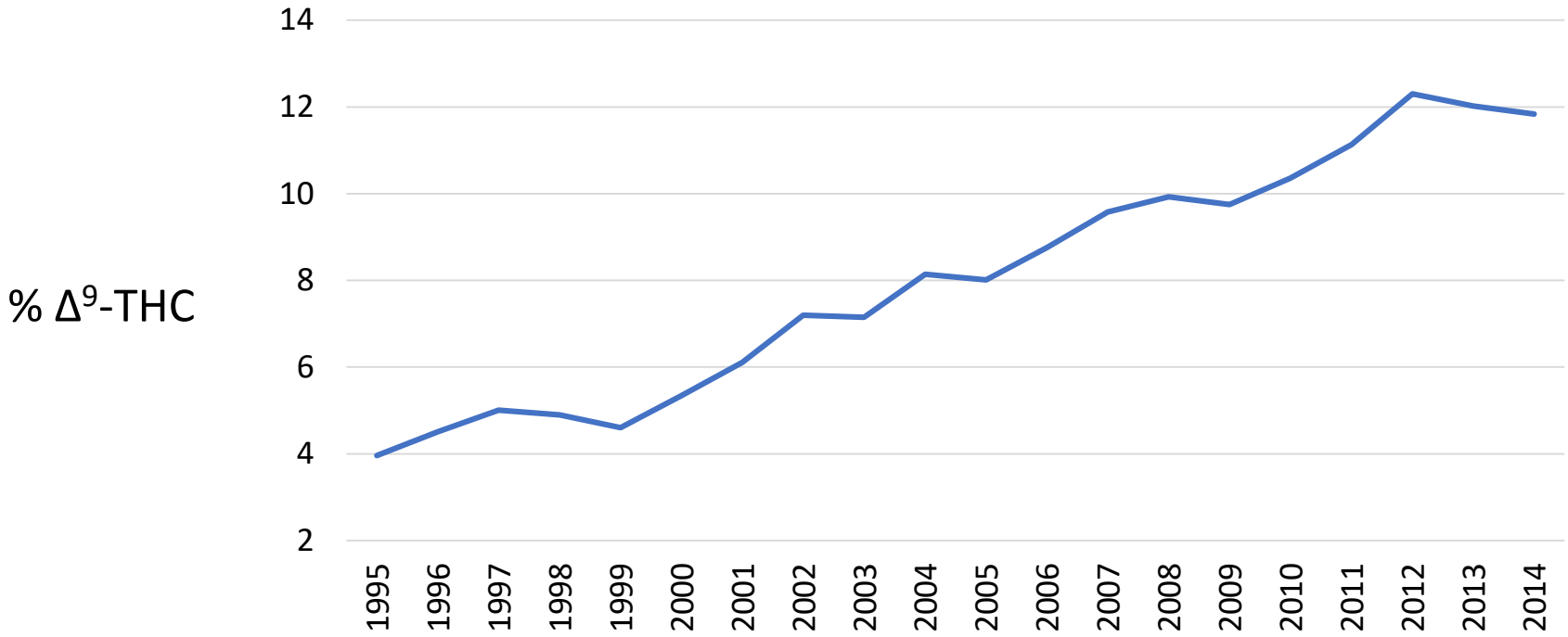
57 studies with birth and somatic developmental outcomes

68 studies with neurodevelopmental outcomes

Assessment of Prenatal Cannabis Exposure

- Exposure assessment from self-report
- Biological assays
- Quantification
- Prevalence
- Low levels
- Timing; windows of susceptibility

Average Δ^9-THC concentration in cannabis specimens, 1995-2014



Source: ElSohly et al., 2016

Longitudinal Cohort Studies

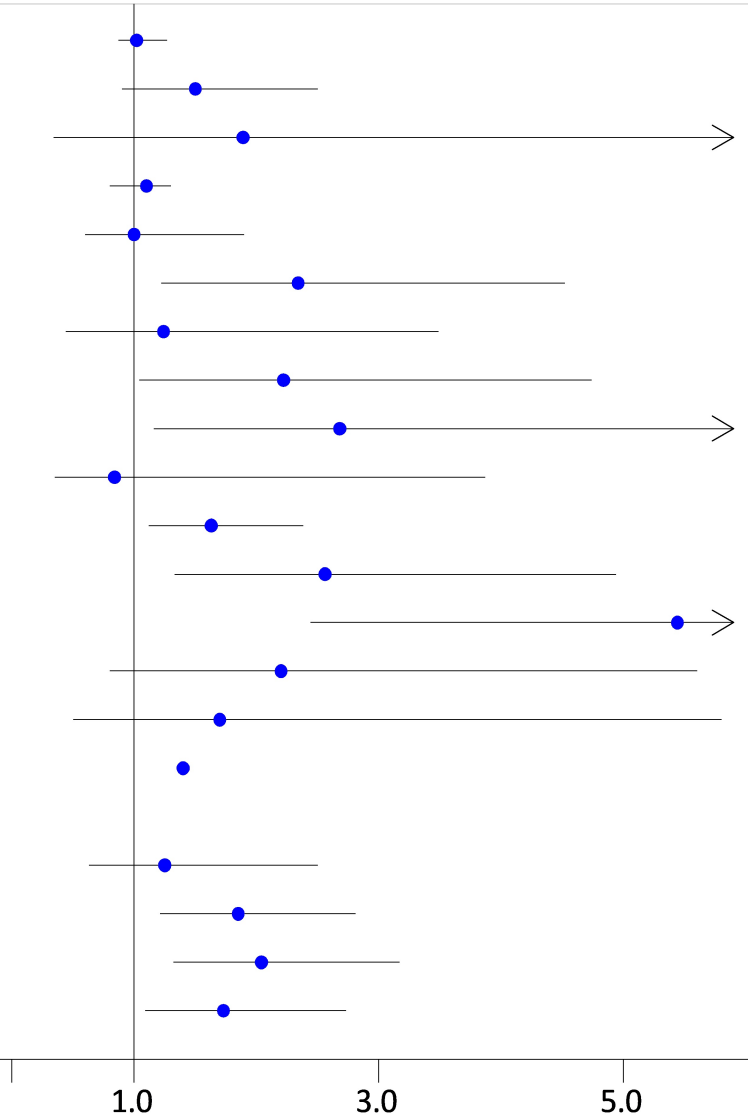
Ottawa Prenatal Prospective Study (OPPS)	• 1978 - 1985 • 698 healthy pregnant women • Followed to 18-22 years
Maternal Health Practices and Child Development Study (MHPCD; Pittsburgh, PA)	• 1982 – 1985 • 763 live-born singletons • Followed to 22-33 years
Generation R (Rotterdam, The Netherlands)	• 2002 – 2006 • 7,452 pregnant women • Followed to 7-10 years

Birth and Somatic Outcomes

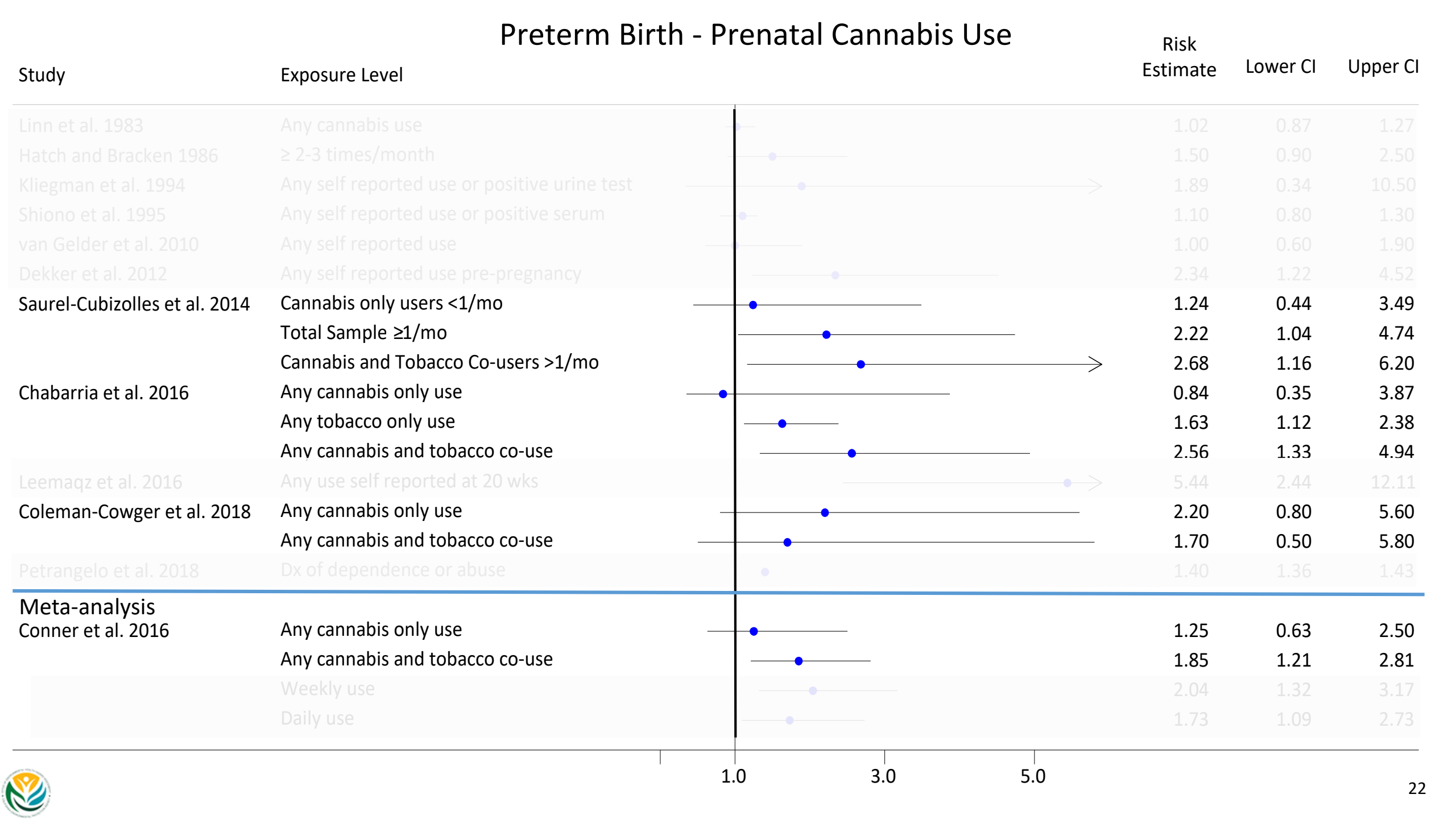
- Preterm birth, gestation length
- Birth weight, low birth weight, small for gestational age , intrauterine growth restriction (IUGR)
- Birth length
- Head circumference
- Ponderal index, BMI, adiposity
- Birth defects
- Viability and mortality: spontaneous abortion, still birth, perinatal mortality, sudden infant death syndrome (SIDS)
- Postnatal growth

Preterm Birth - Prenatal Cannabis Use

Study	Exposure Level	Odds Ratio	Lower CI	Upper CI
Linn et al. 1983	Any cannabis use	1.02	0.87	1.27
Hatch and Bracken 1986	≥ 2-3 times/month	1.50	0.90	2.50
Kliegman et al. 1994	Any self reported use or positive urine test	1.89	0.34	10.50
Shiono et al. 1995	Any self reported use or positive serum	1.10	0.80	1.30
van Gelder et al. 2010	Any self reported use	1.00	0.60	1.90
Dekker et al. 2012	Any self reported use pre-pregnancy	2.34	1.22	4.52
Saurel-Cubizolles et al. 2014	Cannabis only users <1/mo	1.24	0.44	3.49
	Total Sample >1/mo	2.22	1.04	4.74
	Cannabis and Tobacco Co-users >1/mo	2.68	1.16	6.20
Chabarria et al. 2016	Any cannabis only use	0.84	0.35	3.87
	Any tobacco only use	1.63	1.12	2.38
	Any cannabis and tobacco co-use	2.56	1.33	4.94
Leemaqz et al. 2016	Any use self reported at 20 wks	5.44	2.44	12.11
Coleman-Cowger et al. 2018	Any cannabis only use	2.20	0.80	5.60
	Any cannabis and tobacco co-use	1.70	0.50	5.80
Petrangelo et al. 2018	Dx of dependence or abuse	1.40	1.36	1.43
Conner et al. 2016	Any cannabis only use	1.25	0.63	2.50
	Any cannabis and tobacco co-use	1.85	1.21	2.81
	Weekly use	2.04	1.32	3.17
	Daily use	1.73	1.09	2.73



Preterm Birth - Prenatal Cannabis Use



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Meta-analysis				
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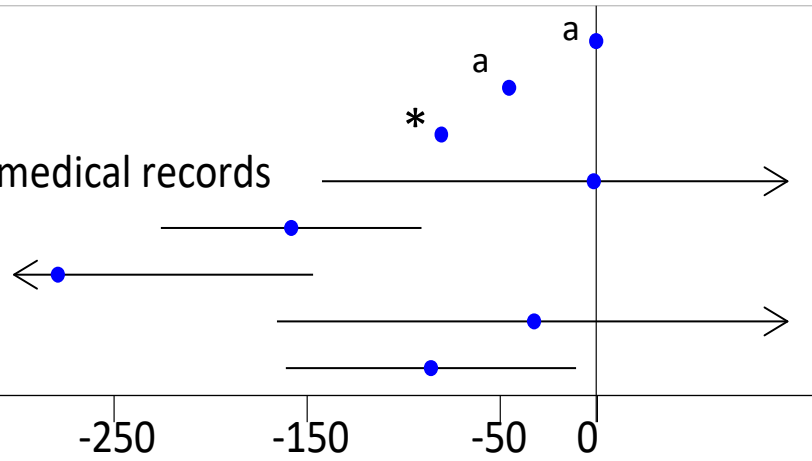
Change in Birth Weight (g) - Prenatal Cannabis Use

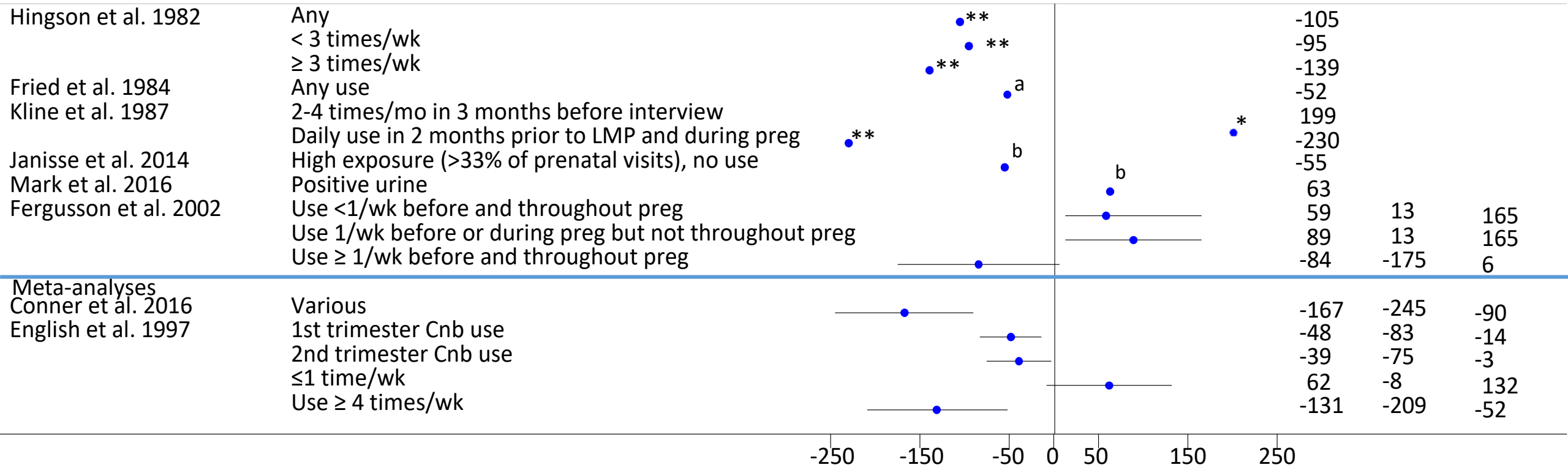
Study	Exposure Level	Beta Coefficient (g)	Lower CI	Upper CI
Hatch and Bracken 1986	≤ once/mo	1.0		
	≥ 2-3 times/mo	-44		
Zuckerman et al. 1989	Positive urine assay	-79		
Schempf and Strobino 2008	Any use by self report, urine assay, or medical records	-0.2	-140.6	140.2
El Marroun et al. 2009	Any use in early pregnancy	-156.6	-224	-89.2
	Any continued use	-277.3	-409.2	-145.4
van Gelder et al. 2010	Any Cnb use - Non smoker	-31	-164	101
Massey et al. 2018	Any use during pregnancy	-84.37	-159.45	-9.28



CI not provided: *p<0.04, [a] p=0.40

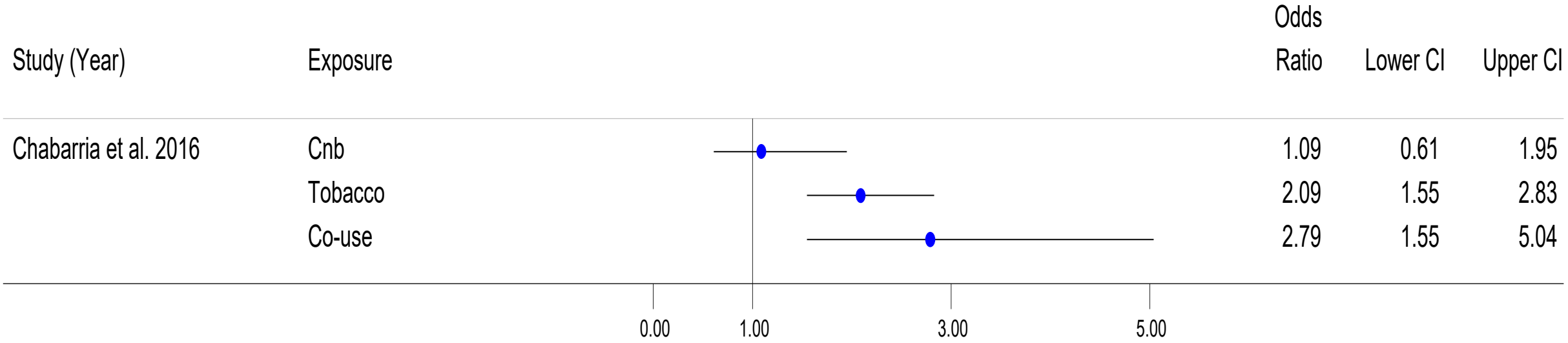
Difference in Mean Birth Weight (g) - Prenatal Cannabis Use

Study (Year)	Exposure Level	Mean Difference (g)	Lower CI	Upper CI
Hingson et al. 1982	Any	-105		
	< 3 times/wk	-95		
	≥ 3 times/wk	-139		
Fried et al. 1984	Any use	-52		
Kline et al. 1987	2-4 times/mo in 3 months before interview	199		
	Daily use in 2 months prior to LMP and during preg	-230		
Janisse et al. 2014	High exposure (>33% of prenatal visits), no use	-55		
Mark et al. 2016	Positive urine	63		
Fergusson et al. 2002	Use <1/wk before and throughout preg	59	13	165
	Use 1/wk before or during preg but not throughout preg	89	13	165
	Use ≥ 1/wk before and throughout preg	-84	-175	6
Meta-analyses				
Conner et al. 2016	Various	-167	-245	-90
English et al. 1997	1st trimester Cnb use	-48	-83	-14
	2nd trimester Cnb use	-39	-75	-3
	≤1 time/wk	62	-8	132
	Use ≥ 4 times/wk	-131	-209	-52

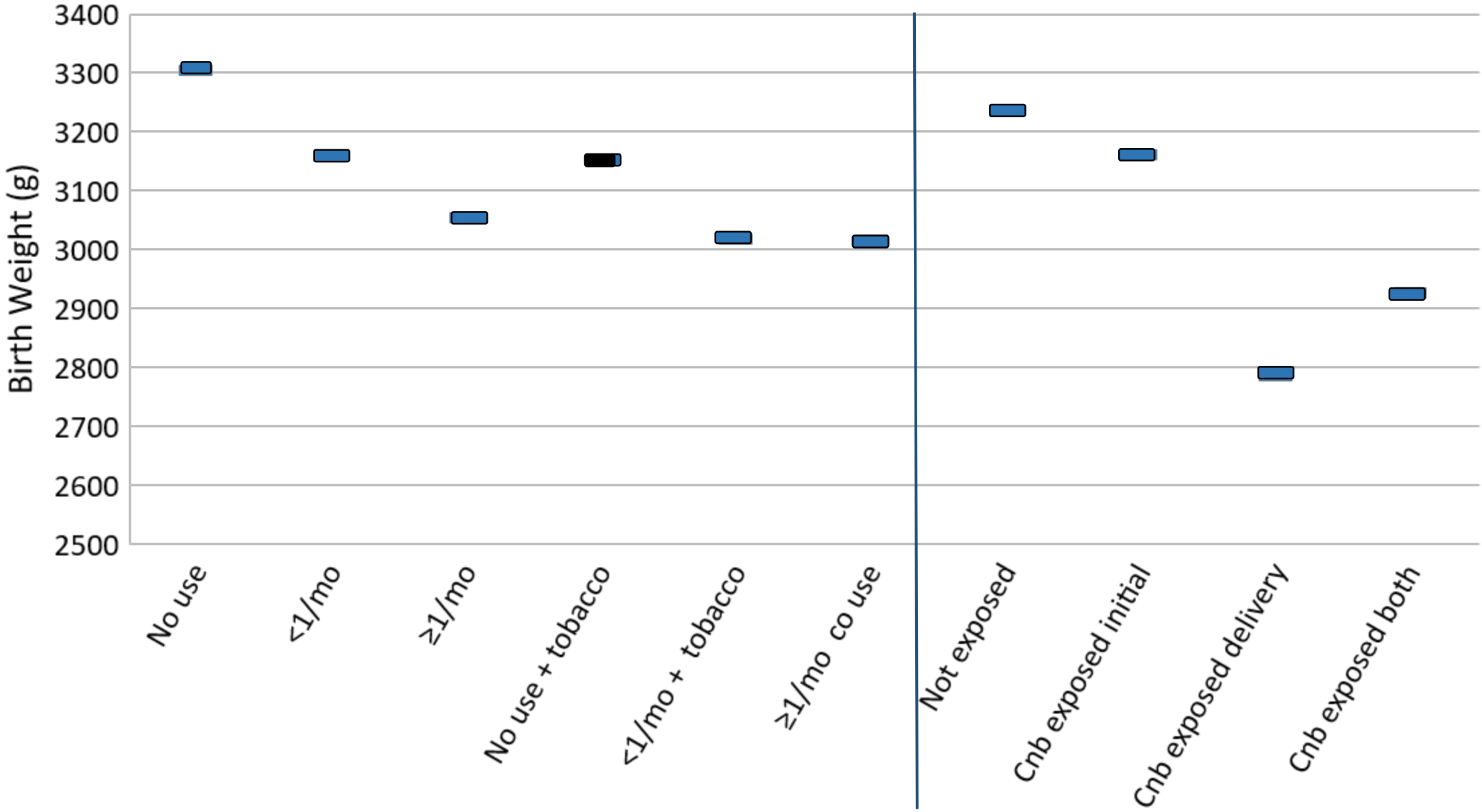


CI not provided: *p<0.05, ** p<0.01, [a] p=0.59, [b] no other statistics provided

Birth Weight <25th Percentile: Odds Ratio for Prenatal Cannabis Use



Birth Weight (g) – Prenatal Cannabis Exposure



Saurel-Cubizolles et al. 2014

Howard et al. 2019

Birth Length - Prenatal Cannabis Exposure

14 studies

- ↓ length – 5 studies (Tennes et al . 1985, Zuckerman et al. 1989, Day et al. 1991, Gray et al. 2010, Howard et al., 2019)
- Mixed findings – 1 study (Fergusson et al. 2002)
- No associations – 8 studies (Hingson et al. 1982, Knight et al. 1994, Cornelius et al. 1995, Fried et al. 1999, Quinlivan and Evans 2002, Shankaran et al. 2004, Lozano et al. 2007, Coleman-Cowger et al. 2018)

Viability and Mortality

- Spontaneous abortion and stillbirth combined: 1 study

  OR=12.1 (1.03, 141.8) for prenatal cannabis only (Coleman–Cowger et al., 2018)

- Stillbirth: 4 studies (including 3 without adjustment for tobacco)
  - OR=1.50 (1.39, 1.62) (Petrangelo et al., 2018)
  - Unadjusted associations: OR=2.34 (1.13, 4.81) (Varner et al. 2014), OR=1.74 (1.03, 2.93) (Conner et al. 2016)
  - 1 study: "excesses" among weekly and daily users (Linn et al. 1983)

- SIDS: 2 studies
  - No associations with maternal prenatal cannabis use (Scragg et al, 2001; Klonoff-Cohen and Lam-Kruglick, 2001)
  - Paternal exposure during conception period OR=2.2 (1.2, 4.2) and pregnancy OR=2.0 (1.0, 4.1) (Klonoff-Cohen and Lam-Kruglick, 2001)

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Effects of Cannabis Smoke and Δ^9-THC on Developmental Toxicity in Animals

- Early embryo development and implantation
- Whole animal developmental toxicity studies
- Evidence for effects on immune system development and bone growth
- Neurodevelopmental toxicity

Early Effects of Δ^9-THC on Embryo Development and Implantation

- Embryos express CB1R and CB2R, beginning at the 2-cell stage
- Δ^9-THC delayed mouse embryo development *in vitro* and *in vivo*
- Implantation in WT mice *in vivo*:
  - THC alone = no effect
  - THC + Cytochrome P450 inhibitor = profoundly decreased implantation rate
  - THC + Cytochrome P450 inhibitor + CB1 R inhibitor = recovered implantation rate
- Implantation in *CB1*$^{-/-}$ X *CB2*$^{-/-}$ mice *in vivo*:
  - THC + Cytochrome P450 inhibitor = normal implantation rate

(Paria et al., 1992; 1995; 1998; 2001)

Whole Animal Developmental Toxicity Studies

- **38 published whole-animal developmental toxicity studies of prenatal exposure to cannabis smoke or Δ^9-THC**

- **Inadequacies of study design and reporting affecting confidence in reported observations included**:
  - Inadequate or marginal sample size
  - Failure to analyze data on a per litter basis

Inhalation Exposure to Cannabis Smoke

- **7/9 experiments in rodents from 5 published studies reported at least a single significant adverse outcome, most commonly:**
  - Delayed postnatal developmental landmarks (4)*
  - Decreased birth weights and decreased postnatal weight gain (4)
- **Confidence in the data limited by:**
  - Statistical analysis by dose group, rather than per litter basis
  - Where analysis was performed on a per litter basis, statistical significance was not achieved

*number of experiments in which specified outcome was reported

Oral Exposure to Δ^9-THC

- **20/27 experiments in rodents or rabbits from 18 published studies reported at least a single significant adverse effect on offspring, most commonly:**
  - Increased fetal, perinatal, or postnatal offspring mortality (8)*
  - Decreased fetal or birth weights (7)
  - Altered hormone levels or decreased fertility in F1 males (6)
- **Confidence in the data limited by:**
  - Statistical analysis by dose group, rather than per litter basis
  - Failure to note numbers of pregnant animals per dose group, or to account for all animals at final analysis

*number of experiments in which specified outcome was reported

Fleischman et al., 1980; oral Δ^9-THC

- 3 experiments in rats plus 1 in mice
  - Rat experiments tested doses of 0, 12.5, 25, or 50 mg/kg-day Δ^9-THC
  - Mouse experiment tested doses of 0, 150, 300, or 600 mg/kg-day Δ^9-THC
- Treatment and evaluation schedules:
  - Dosing on GD 6-15 with evaluation every 3 days, starting on GD 8 (rat and mouse)
  - Dosing on GD 5-7, 6-8, 7-9, 8-10, or 9-11 with evaluation on GD 14 (rat)
  - Dosing on GD 6-9 with evaluation on GD 12 or GD 16 (rat)
- Decreased live fetuses/litter for both rats and mice
  - Rats: significant at all doses
  - Mice: No fetuses at 600 mg/kg-day, apparent dose-response at 150 and 300 mg/kg-day
- Confidence reduced by lumping of data for animals sacrificed on different days
  - Exposure to the same daily dose, but not the same total dose
  - Not exposed during the same potential windows of sensitivity

Injection Exposure to Δ^9-THC

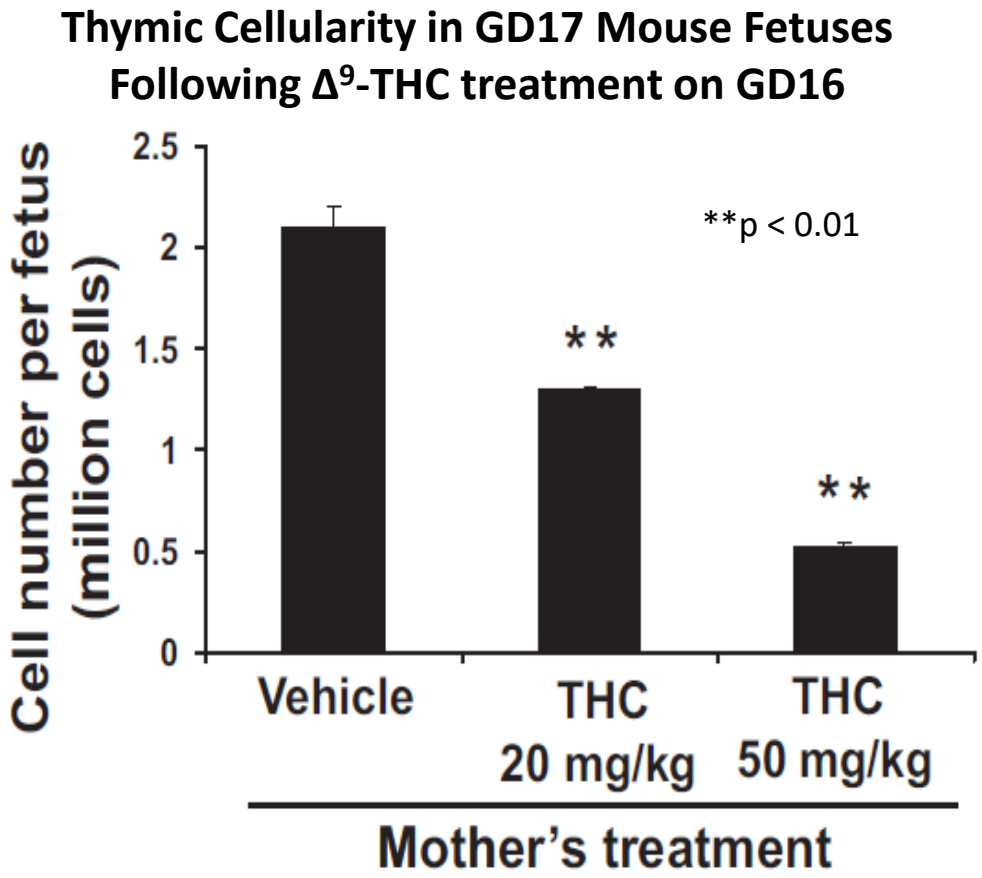
- **14/16 experiments in rodents or rabbits from 13 published studies reported at least a single significant adverse effect on offspring**
  - Decreased fetal or birth weights (9)*
  - Increased fetal, perinatal, or postnatal offspring mortality (8)

- **Single study in Rhesus monkeys (Asch & Smith, 1986)**
  - 5 females/group, Δ^9-THC at 2.5 mg/kg-day i.m. throughout gestation
  - Controls – 5/5 live births
  - Treated – 3/5 early spontaneous abortion; 1/5 stillbirth; 1/5 live birth

*number of experiments in which specified outcome was reported

Effects of Δ^9-THC on Development of the Immune System

- Mouse fetal thymocytes express high levels of CB1 and CB2 receptors
- **Total thymic cellularity in GD 17 mouse fetuses following Δ^9-THC given ip on GD 16** (a sensitive window for immune system development)
- Caspase-dependent apoptosis caused thymic atrophy and altered T cell subpopulations following Δ^9-THC on GD 16
- *In vivo* pretreatment with antagonists attenuated Δ^9-THC-induced changes
- Significant functional immune dysregulation at 5 weeks postnatal age following GD 16 exposure to Δ^9-THC

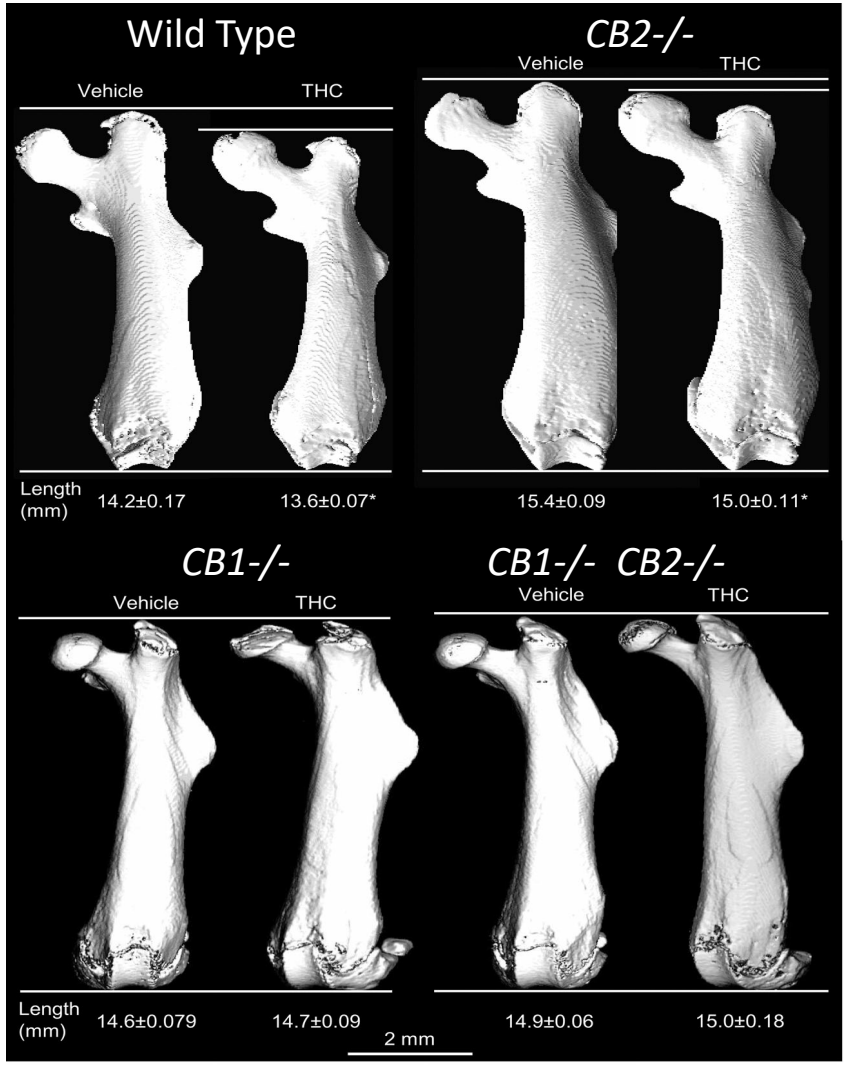


(Lombard et al., 2011)

Effects of Δ^9-THC on Linear Bone Growth

Femur Length in Female Pups Exposed to Δ^9-THC from 5 to 11 Weeks Postnatal Age:

- Decreased femoral length in WT or CB2-/- female pups
- No effect on femur length in CB1-/- or double mutant mice
- Δ^9-THC may interact with the CB1 receptor in affecting linear bone growth.
- Δ^9-THC also associated with decreased weight gain, but not fat weight, in female mice having functional CB1 receptors



(Wasserman et al., 2015)

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Neurodevelopmental Studies in Humans

Neurodevelopmental Outcomes in Association with Prenatal Cannabis Exposure in Humans

Central Nervous System (CNS) Maturation

7 Studies
- 4 OPPS
- 3 MHPCD

Visual Perception and Functioning

6 Studies
- 4 OPPS
- 1 MHPCD
- 1 Other

Attention

12 studies
- 5 OPPS
- 4 MHPCD
- 1 Gen R
- 3 Other

Intelligence/ Achievement

13 studies
- 7 OPPS
- 5 MHPCD
- 1 Other

Neurodevelopmental Outcomes - Prenatal Cannabis Exposure




Infancy	1-5 years	6-12 years	13-18+ years	
↓ delayed maturation of visual system	↑ attention problems (girls)	↓ sustained attention	↓ sustained attention	CNS Maturation
		↑ sustained attention	↑ impulsivity	Attention
↑ sleep problems		↑ impulsivity and hyperactivity		
	↓ language comp-rehension	↓ language comprehension	↓ spelling scores	Intelligence
↓habituation to light in neonates		↓ verbal and quantitative reasoning	↓ school achievement	
	↓ memory	↓ learning and memory	↑ metacognition	
		↓ intelligence scores		
	↑global perception thresholds	↓ visual planning, integration, visual analysis, and synthesis	↓ slower processing speed, poorer inter-hemispheric motor coordination	Visual Functioning and Processing

Central Nervous System Maturation - Prenatal Cannabis Exposure

Cohort	*Neonate*	*1-5 years*	*6-12 years*	*13-18+ years*
Ottawa Cohort *(OPPS)*	*2- 3 days* Fried 1980 *3-6 days* Fried and Makin 1987 *30 days* Fried 1982	*3 years* Tansley et al. 1986		
Pittsburgh Cohort *(MHPCD)*	*1-2 days* Scher et al. 1988 *1 month* Scher et al. 1998	*18 months* Scher et al. 1998 *3 years* Dahl et al. 1995		

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*Statistically significant

Central Nervous System Maturation - Prenatal Cannabis Exposure

Cohort	*Neonate*	*1-5 years*	*6-12 years*	*13-18+ years*
Ottawa Cohort (OPPS)	*2-3 days* ↓ habituation and response to light *3-6 days* ↓ habituation to light ↑ startles, tremors, and irritability *30 days* Habituation, startles, tremors, and irritability normalized by 30 days	*3 years* ↑ variability of binocular indices		
Pittsburgh Cohort (MHPCD)	*1-2 days* ↑ body movements ↓total quiet sleep ↓trace alternant quiet sleep *1 month* ↑ P1 wave latency	*18 months* ↑ P1 wave latency *3 years* ↓ sleep efficiency ↑ arousals and awake time		

*Only statistically significant results are shown

Neurodevelopmental Outcomes - Prenatal Cannabis Exposure




Infancy	1-5 years	6-12 years	13-18+ years	
↓ delayed maturation of visual system	↑ attention problems (girls)	↓ sustained attention ↑ sustained attention ↑ impulsivity and hyperactivity	↓ sustained attention ↑ impulsivity	CNS Maturation Attention
↑ sleep problems	↓ language comp-rehension	↓ language comprehension ↓ verbal and quantitative reasoning	↓ spelling scores ↓ school achievement	Intelligence
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Attention - Prenatal Cannabis Exposure

Cohort	*1-5 years*	*6-12 years*	*13-18+ years*
Ottawa Cohort (OPPS)		*6 years* Fried et al. 1992a *9-12 years* Fried et al. 1998 *6-9 years* O'Connell and Fried 1991	*13-16 years* Fried and Watkinson 2001 *18-22 years* Smith et al. 2004
Pittsburgh Cohort (MHPCD)		*6 years* Leech et al. 1999 *10 years* Richardson et al. 2002 Goldschmidt et al. 2000	*14 years* Goldschmidt et al. 2012
Gen R	*18 months* El Marroun et al. 2011		
Other	*4 years* Noland et al. 2005		*High-School* Rose-Jacobs et al. 2017

Attention - Prenatal Cannabis Exposure

Cohort	*1-5 years*	*6-12 years*	*13-18+ years*
Ottawa Cohort (OPPS)		*6 years* Fried et al. 1992a *9-12 years* Fried et al. 1998 *6-9 years* O'Connell and Fried 1991	*13-16 years* Fried and Watkinson 2001 *18-22 years* Smith et al. 2004
Pittsburgh Cohort (MHPCD)		*6 years* Leech et al. 1999 *10 years* Richardson et al. 2002 Goldschmidt et al. 2000	*14 years* Goldschmidt et al. 2012
Gen R	*18 months* El Marroun et al. 2011		
Other	*4 years* Noland et al. 2005		*High-School* Rose-Jacobs et al. 2017

*Statistically significant

Attention - Prenatal Cannabis Exposure

Cohort	*1-5 years*	*6-12 years*	*13-18+ years*
Ottawa Cohort (OPPS)		*<u>6 years</u>* ↑ impulsivity/hyperactivity ↓sustained attention *<u>9-12 years</u>* ↑impulsivity	*<u>13-16 years</u>* ↓ sustained attention *<u>18-22 years</u>* ↑ impulsivity ↑ prefrontal cortex activity
Pittsburgh Cohort (MHPCD)		*<u>6 years</u>* ↑ impulsivity ↑ sustained attention *<u>10 years</u>* ↑impulsivity ↑ hyperactivity/impulsivity ↓ attention	*<u>14 years</u>* ↑ attention problems
Gen R	*<u>18 months</u>* ↑ attention problems (girls)		
Other			*<u>High-School</u>* ↑ behavioral regulation

*Only statistically significant results are shown

Neurodevelopmental Outcomes - Prenatal Cannabis Exposure




Infancy	1-5 years	6-12 years	13-18+ years	
↓ delayed maturation of visual system	↑ attention problems (girls)	↓ sustained attention ↑ sustained attention ↑ impulsivity and hyperactivity	↓ sustained attention ↑ impulsivity	CNS Maturation Attention
↑ sleep problems ↓habituation to light in neonates	↓ language comp-rehension ↓ memory	↓ language comprehension ↓ verbal and quantitative reasoning ↓ learning and memory ↓ intelligence scores	↓ spelling scores ↓ school achievement ↑ metacognition	Intelligence
	↑global perception thresholds	↓ visual planning, integration, visual analysis, and synthesis	↓ slower processing speed, poorer inter-hemispheric motor coordination	Visual Functioning and Processing

Intelligence and Academic Achievement – Prenatal Cannabis Use

Cohort	*1-5 years*	*6-12 years*	*13-18+ years*
Ottawa Cohort (OPPS)	*1-2 years* Fried and Watkinson 1988 *3-4 years* Fried and Watkinson 1990	*5-6 years* Fried et al. 1992b *6-9 years* O'Connell and Fried 1991 *9-12 years* Fried et al. 1997 Fried et al. 1998	*13-16 years* Fried et al. 2003
Pittsburgh Cohort (MHPCD)	*3 years* Day et al. 1994b	*6 years* Goldschmidt et al. 2008 *10 years* Goldschmidt et al. 2004 Richardson et al. 2002	*14 years* Goldschmidt et al. 2012
Other			*High-school* Rose-Jacobs et al. 2017

Intelligence and Academic Achievement – Prenatal Cannabis Use

Cohort	1-5 years	6-12 years	13-18+ years
Ottawa Cohort (OPPS)	*1-2 years* Fried and Watkinson 1988 *3-4 years* Fried and Watkinson 1990	*5-6 years* Fried et al. 1992b *6-9 years* O'Connell and Fried 1991 *9-12 years* Fried et al. 1997 Fried et al. 1998	*13-16 years* Fried et al. 2003
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Other		*Statistically significant	*High-school* Rose-Jacobs et al. 2017

Intelligence and Academic Achievement – Prenatal Cannabis Use

Cohort	*1-5 years*	*6-12 years*	*13-18+ years*
Ottawa Cohort (OPPS)	<u>*1-2 years*</u> ↓ language comprehension <u>*3-4 years*</u> ↓ memory ↓ vocabulary test scores	<u>*6-9 years*</u> ↓ language comprehension <u>*9-12 years*</u> ↓ phonological scores ↓ abstract reasoning and mental flexibility	<u>13-16 years</u> ↓ abstract design and Peabody spelling scores
Pittsburgh Cohort (MHPCD)	<u>*3 years*</u> ↓ verbal reasoning and short term memory in African American children	<u>*6 years*</u> ↓ composite intelligence, verbal and quantitative reasoning <u>*10 years*</u> ↓ academic achievement via ↓ psychological status ↓ learning and memory	<u>14 years</u> ↓ school achievement
Other	*Only statistically significant results		<u>*High-school*</u> ↑ metacognition

Intelligence and Academic Achievement – Prenatal Cannabis Use

Cohort	1-5 years	6-12 years	13-18+ years
Ottawa Cohort (OPPS)	*1-2 years* ↓ language comprehension *3-4 years* ↓ memory ↓ vocabulary test scores	*6-9 years* ↓ language comprehension *9-12 years* ↓ phonological scores ↓ abstract reasoning and mental flexibility	13-16 years ↓ abstract design and Peabody spelling scores
Pittsburgh Cohort (MHPCD)	*3 years* ↓ verbal reasoning and short term memory in African American children	*6 years* ↓ composite intelligence, verbal and quantitative reasoning *10 years* ↓ academic achievement via ↓ psychological status ↓ learning and memory	14 years ↓ school achievement *Corrected for post-natal cannabis exposure*
Other	*Only statistically significant results		*High-school* ↑ metacognition

Neurodevelopmental Outcomes - Prenatal Cannabis Exposure




Infancy	1-5 years	6-12 years	13-18+ years	
↓ delayed maturation of visual system	↑ attention problems (girls)	↓ sustained attention ↑ sustained attention ↑ impulsivity and hyperactivity	↓ sustained attention ↑ impulsivity	CNS Maturation Attention
↑ sleep problems ↓habituation to light in neonates	↓ language comp-rehension ↓ memory	↓ language comprehension ↓ verbal and quantitative reasoning ↓ learning and memory ↓ intelligence scores	↓ spelling scores ↓ school achievement ↑ metacognition	Intelligence
	↑global perception thresholds	↓ visual planning, integration, visual analysis, and synthesis	↓ slower processing speed, poorer inter-hemispheric motor coordination	Visual Functioning and Processing

Visual Function and Processing - Prenatal Cannabis Exposure

Cohort	*1-5 years*	*6-12 years*	*13-18+ years*
Ottawa Cohort (OPPS)		*6-9 years* Fried and O'Connell 1991 *9-12 years* Fried et al. 1998 Fried and Watkinson 2000	*18-22 years* Smith et al. 2006
Pittsburgh Cohort (MHPCD)			*16 years* Willford et al. 2010b
Other	*4.5 years* Chakraborty et al. 2015		

Visual Function and Processing - Prenatal Cannabis Exposure

Cohort	*1-5 years*	*6-12 years*	*13-18+ years*
Ottawa Cohort (OPPS)		*6-9 years* Fried and O'Connell 1991 *9-12 years* Fried et al. 1998 Fried and Watkinson 2000	*18-22 years* Smith et al. 2006
Pittsburgh Cohort (MHPCD)			*16 years* Willford et al. 2010b
Other	*4.5 years* Chakraborty et al. 2015		

*Statistically significant

Visual Function and Processing - Prenatal Cannabis Exposure

Cohort	*1-5 years*	*6-12 years*	*13-18+ years*
Ottawa Cohort (OPPS)		*9-12 years* ↓ higher order visual analysis, perceptual organization, spatial visualization, abstract conceptualization ↓ visual planning, integration, analysis, and synthesis	*18-22 years* ↓ interhemispheric coordination ↑ visual motor coordination
Pittsburgh Cohort (MHPCD)			*16 years* ↓ interhemispheric motor coordination ↓ processing speed
Other	*4.5 years* ↑ global motion perception thresholds		

*Only statistically significant results are shown

Substance Use - Prenatal Cannabis Exposure

- E-cigarette use (*De Genna et al. 2018a, MHPCD*)
- Initiation and frequency of cannabis use (*Frank et al. 2014; Sonon et al. 2015, MHPCD Sonon et al. 2016 MHPCD, Day et al. 2006, MHPCD*)
- Cannabis and tobacco co-use (*De Genna et al. 2018b, MHPCD*)
- Drug use disorders (*Porath and Fried 2005, OPPS*)

6/7 studies observed significant associations either by a direct or indirect pathway

1/7 study reported no significant associations *(Frank et al. 2014)*

Mood Disorders - Prenatal Cannabis Exposure

- Depression *(Gray et al. 2005; Leech et al. 2006; Goldschmidt et al. 2012; all MHPCD)*
- Anxiety *(Leech et al. 2006, MHPCD)*
- Psychotic symptoms and experiences *(Zammit et al. 2009; Day et al. 2014, MHPCD; Bolhuis et al. 2018, Gen R)*

4/6 studies observed significant associations *(Gray et al. 2005; Goldschmidt et al, 2012; Leech et al. 2006; Bolhuis et al. 2018)*

1/6 reported marginally significant associations (p=0.06) (*Day et al. 2014)*

1/6 study reported no significant association (*Zammit et al. 2009)*

Behavior - Prenatal Cannabis Exposure

9 studies examined various aspect of behavior

- Child behavior problems (*Goldschmidt et al. 2000, MHPCD; Goldschmidt et al. 2016, MHPCD; Eiden et al. 2018b; El Marroun et al. 2018, Gen R; Godleski et al. 2018*)
- Aggression (*El Marroun et al. 2011, Gen R)*
- Early Sexual Behavior (*De Genna et al. 2015, MHPCD*)
- Negative Adult Roles (*Goldschmidt et al. 2016, MHPCD)*
- Emotional Problems *(El Marroun et al. 2018, Gen R; Eiden et al. 2018a)*
- Behavioral Resilience *(Liebschutz et al. 2015)*

8/9 studies observed significant associations through direct or indirect pathways

(*Goldschmidt et al. 2000; Goldschmidt et al. 2016; Eiden et al. 2018a; Eiden et al. 2018b; El Marroun et al. 2018; Godleski et al. 2018; El Marroun et al. 2011; De Genna et al. 2015; Goldschmidt et al 2016*)

1/9 reported no significant associations *(Liebschutz et al. 2015)*

Brain Morphology and Structural Changes - Prenatal Cannabis Exposure

3 studies used MRI (Magnetic Resonance Imaging):

- Ages 6-8, *(El Marroun et al. 2016, Gen R)*
  - Significantly thicker cortices (superior frontal area of left hemisphere)
  - Significantly thicker frontal pole in right hemisphere
  - No significant differences of total brain volume, gray matter volume or white matter volume
- Ages 18-22, *(Wilford et al. 2010b, MPHCD)*
  - No significant association with structure of the caudate nucleus
- Ages 10-14, *(Rivkin et al. 2008)*
  - No association with cortical gray matter, total parenchymal volumes

Executive Functioning - Prenatal Cannabis Exposure

3 studies used functional MRI (fMRI) in 18-22 year olds (*all OPPS*):

- Response inhibition *(Smith et al. 2004)*
- Visual working memory *(Smith et al. 2006)*
- Re-analysis of findings from Smith et al. 2004, 2006 plus 2 additional tasks (working memory and cognition) *(Smith et al. 2016)*

  - ➢ No significant differences in performance of the tasks
  - ➢ Significantly more brain activity, specifically in the left posterior brain region reported for all 4 executive functioning tasks

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- Developmental toxicity
- Somatic outcomes
  - Human studies
  - Animal studies
- Neurodevelopmental outcomes
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Overview of Publications on Neurodevelopmental Effects of Cannabis smoke, Cannabis extract or Δ^9-THC

47 Publications:

- 39 rat studies
- 3 mice studies
- 1 primate study (Rhesus monkeys)
- 4 zebrafish studies

Routes and Substances (mammalian studies):

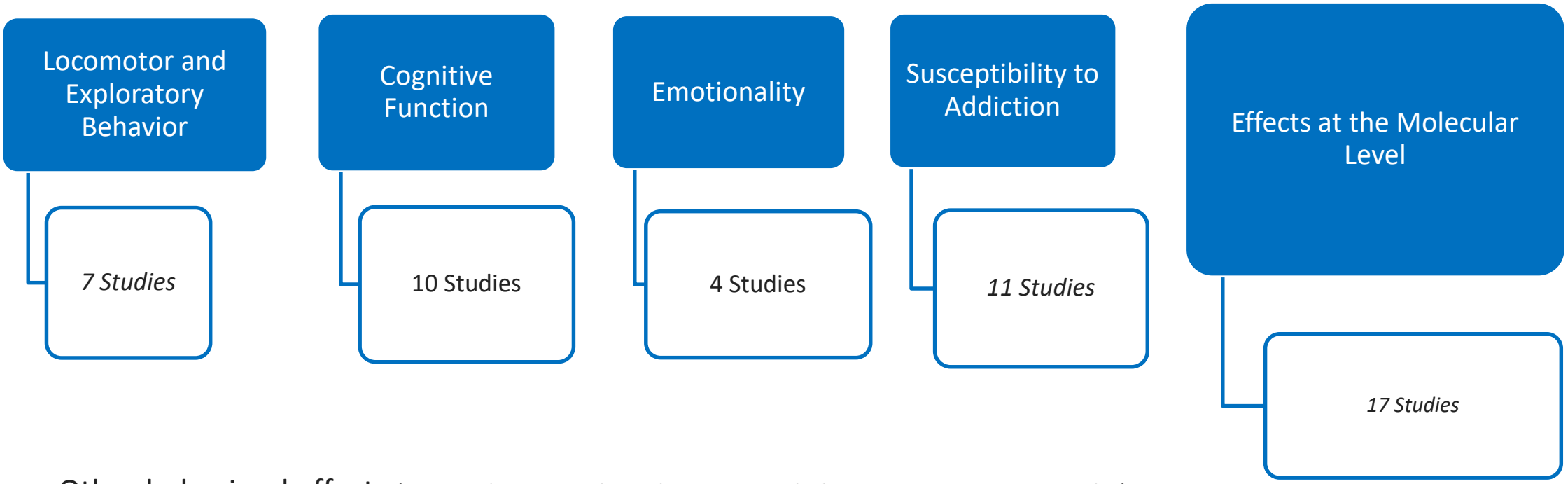
- 3 Inhalation (cannabis smoke)
- 27 Oral (Δ^9-THC; one study hashish)
- 13 Parenteral routes (7 ip, 3 iv, 3 sc; 12 studies Δ^9-THC, one study *C. sativa* extract)

Exposure windows:

- 6 preconceptional only (1 paternal)
- 1 preconceptional and *in utero*
- 17 with *in utero* only dose groups
- 20 *in utero* and postnatal exposure component
- 2 preconceptional, *in utero* and postnatal exposure component
- 2 postnatal only

Neurodevelopmental Effects Studied After Exposure (Pre-conceptional/Prenatal/Perinatal) to Cannabis smoke, Cannabis extract or Δ^9-THC in Animals

Behavior and Molecular Effects



Other behavioral effects (e.g., auditory startle and stereotypic behaviors, response to novelty)

Locomotor and Exploratory Behavior Effects Reported

Altered spontaneous locomotor and exploratory behaviors (7 studies)
(*Charlebois & Fried, 1980; Levin et al. 2019; Moreno et al. 2003, 2005; Rubio et al. 1995; Navarro et al. 1994; Szutorisz et al. 2016*)

- ↑/↓ Locomotor activity
- ↑ time spent in immobility
- ↑ hyperactivity-infants & adolescents, not adults
- ↓ movements in ♀ not ♂ after pre-conceptional exposure
- More rapid habituation of locomotor activity in ♀

No changes in locomotor activity (4 studies)
(*Abel et al. 1984; Trezza et al. 2008; Brake et al. 1987; Navarro et al 1995*)

Cognitive Function Effects Reported

10 Studies

Memory and learning impairment:

- Long-term memory (inhibitory avoidance test) (*Silva et al. 2012*);
- Olfactory short-term memory (social discrimination task) (*Silva et al. 2012*);
- Reference and working memory (delayed alternation task) *(O'Shea and Mallet 2005)*

- ↓ Maze learning ability (*Gianutsos and Abbatiello 1972; Levin et al. 2019*)
- ↓ Spatial learning (delayed alternation task) (*O'Shea and Mallet. 2005)*
- Impairment of consolidation during retention testing and reversal learning *(Silva et al. 2012)*
- ↓ acquisition of passive avoidance (passive avoidance training) (*Vardaris et al. 1976)*
- No significant effects on spatial learning and memory (spontaneous alternation test) *(Abel et al. 1984, 1990 a & b)*

Cognitive Function Effects Reported

Other cognitive function effects

- Alteration in response to visual stimuli and response to novel stimuli (*Golub 1981)*
- Alteration in time taken to complete tasks and attention deficits (*Campolongo et al. 2007; Silva et al. 2012)*
- Long-lasting impairment in attentional performance (operant visual attention task) (*Levin et al. 2019)*

Emotionality Effects Reported

4 studies

Social interaction:

- ↑ Emotionality (in response to novel conditions: emergence latency and socio-sexual approach tests) (*Navarro et al. 1994*)
- ↑ Social interaction (↓ in emotional reactivity) (*Newsom and Kelly 2008*)
- ↓ Social interaction at PND35 (*Trezza et al. 2008*)
- No effects in emotional reactivity (*Vardaris et al. 1976*)

Anxiety:

- ↑ Ultrasonic vocalizations at PND 12 (separation-induced anxiety); Elevated plus maze PND 80 (generalized anxiety) (*Trezza et al. 2008*)
- ↑ Anxiety measured by open field behavior (*Newsom and Kelly 2008*)

Susceptibility to Addiction Effects Reported

11 studies

- ↑ rate of acquisition of morphine self-administration (*Vela et al. 1998*)
- ↑ sensitivity towards the rewarding effects of morphine or heroin (*DiNieri et al. 2011; Navarro et al. 1995; Rubio et al.1995; 1998; Vela et al. 1998; Singh et al. 2006, Szutor*isz *et al. 2014*).
- ↓ sensitivity to natural rewards (*Pitsilis et al., 2017*)
- Heroin seeking behavior: no differences under normal conditions but ↑during mild stress/drug cessation (*Spano et al. 2007*)
- No differences in ethanol/morphine self-administration (*Economidou et al. 2007; Gonzalez et al. 2003*)

Zebrafish embryos/larvae exposed to Δ^9-THC

Effects linked to Stress and Anxiety (4 Studies)

- ↑ locomotor activity at lower concentrations *(Akhtar et al. 2013; Carty et al. 2018)*
- ↓ locomotor activity at higher concentrations *(Akhtar et al. 2013; Carty et al. 2018)*
- ↓ activity in dark at higher concentrations (reversal of light: dark behavior) *(Carty et al. 2018)*
- ↓ basal activity (average distance traveled) *(Achenbach et al., 2018)*
- ↓ Locomotor responses to sound *(Ahmed et al. 2018)*
- ↑ Nicotinic acetylcholine receptor expression *(Ahmed et al. 2018)*
- ↑ c-fos gene expression *(Carty et al. 2018)*

Other effects

- ↓ survival, ↓ body length, ↓ heart rate *(Ahmed et al. 2018)*
- ↑ bent body/curved primary axis; ↑ yolk sac edema; ↑ pericardial edema *(Akhtar et al. 2013)*

Examples of Effects Reported at the Molecular Level with Δ^9-THC Exposure

17 Studies

(Dalterio et al. 1984; Walters & Carr, 1988; Navarro et al. 1996; Wenger et al. 1997; Vela et al. 1998; Bonnin et al. 1995, 1996; Gomez et al. 2003; Gonzalez et al. 2003; Suarez et al. 2004; Campolongo et al. 2007; Castaldo et al. 2010; Szutorisz et al., 2014, 2016; de Salas-Quiroga et al., 2015; Vargish et al. 2017; Beggiato et al. 2017)

Alterations in Gene expression and Protein levels

Gene ontology categories related to neurodevelopment *(Campolongo et al. 2007)*

(e.g. cortical genes related to glutamatergic and noradrenergic systems;
↓ genes related to myelination; ↑ genes involved in apoptosis)

mRNA and protein levels related to neurotransmitters

(Campolongo et al. 2007; Castaldo et al. 2010; Bonnin et al. 1995, 1996; Navarro et al. 1996)

(e.g. ↓ in cortical extracellular levels of glutamate and noradrenaline)
↑ Tyrosine hydroxylase mRNA
↓ in DOPAC contents in the limbic forebrain)

Involvement of specific brain regions involved with addiction *(Szutorisz et al. 2014, 2016)*

(e.g. altered mRNA levels first in the nucleus accumbens and later in the dorsal striatum)

Effects Reported at the Molecular Level with Δ^9-THC Exposure

Changes related to cannabinoid receptors *(de Salas-Quiroga et al. 2015)*

- Age-dependent
- Hippocampal GABAergic system *(Beggiato et al. 2017)*
  - (e.g. ↓ in [^{3}H] GABA uptake and ↓ CB1 receptor Bmax binding)
- Changes in the density of the μ opioid receptors *(Vela et al. 1998)*

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Epigenetic Effects Reported with Exposure in Human (Cannabis) and Animal Studies (Δ^9-THC, WIN)

Human Studies of Cannabis Use

Adult exposure,* Changes in DNA methylation *(2 studies)

- ↓ methylation in sperm majority of CpG sites; and of PTG1R (prostaglandin I2 receptor)
- ↑ methylation of DRD2, (dopamine receptor 2), and NCAM1, (neural cell adhesion molecule) genes (Gerra et al. 2018)
- ↑ methylation of CSNK1E, (casein kinase 1 epsilon) (Murphy et al., 2018)

Perinatal exposure (1 study)

- ↑ DNA methylation of DRD4 (dopamine receptor D4) (Fransquet et al. 2017).

Prenatal exposure (3 studies)

- ↓ in DRD2 gene expression in the NAc (DiNieri et al. 2011); and in the amygdala basal nucleus in male fetuses (Wang et al. 2004).
- Alterations in levels of opioid receptor and opioid precursor mRNAs (Wang et al. 2006b)

Animal Studies of Δ^9-THC or WIN

Preconception exposure (5 studies):

- Differentially methylated regions in rats Nac , sperm DNA and in GRIN2A (Murphy et al. 2018; Watson et al. 2015)
- ↑ mRNA expression of CBR1 and glutamate receptors in the NAc in adolescent male rats (PND 32) (Szutorisz et al. 2014)
- ↓ mRNA expression of CBR1, DRD2, GRIN2A, GRIA1, and GRIA2 in adult male brain (PND 62) (Szutorisz et al. 2014)
- Sex-specific mRNA expression patterns in rat brains (Szutorisz et al. 2016).

Perinatal exposure (1 study)

- Altered profile of histone methylation marks (2meH3K9 and 3meH3K4) at the DRD2 locus in the NAc in rats.
- ↓ DRD2 mRNA expression and binding sites in the NAc, but not the dorsal striatum (DiNieri et al. 2011).

Prenatal exposure (1 study)

- ↑ L1CAM (cell adhesion protein) mRNA transcripts in brain regions of male rats (Gómez et al. 2003).

Studies with the CB_1R agonist WIN (2 studies, preconception exposure)

- ↑ DNA methylation of male rats (Ibn Lahmar Andaloussi et al. 2019).
- ↑ in OPRM1 (opioid receptor mu 1) and no change in DRD1 or DRD2 gene expression (Vassoler et al. 2013).

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Summary of Developmental Somatic Outcomes

Human Studies
(Cannabis Smoke)

- Viability and mortality
- Preterm birth
- Birth length
- Birth weight

Animal Studies
(Cannabis Smoke or Δ^9-THC)

- Pre-, peri-, and postnatal mortality
- Early embryo development
- Pre- and postnatal growth
- Fetal or birth weight
- Immune development and function

Summary of Neurodevelopmental Outcomes

Human Studies
(Cannabis Smoke)

- Cognitive/Executive Function
  - Learning and Memory
  - Language Comprehension
  - Visual Functioning and Processing
  - Attention and/or Impulsivity
- Early Substance Use
- Mood Disorders (depression, anxiety)
- Behavior (aggression - girls, child behavior problems)
- CNS Maturation

Animal Studies
(Cannabis Smoke, Cannabis Extracts, or Δ^9-THC)

- Cognitive Function
  - Learning and memory
- Susceptibility to Addiction (drug-seeking behavior)
- Involvement of specific brain regions involved with addiction
- Emotional Reactivity: Anxiety and Altered Social Interactions
- Locomotor Activity and Exploratory Behavior
- Changes in Gene expression and Protein levels