Embryonic Exposure to Cannabinoids and Alcohol Alters Early Development in Zebrafish and May Have Long-term Consequences in Adults

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Relationship with commercial interests:
None
...from a few of cells...
Animal Models of FASD

- Nematode (C. elegans)
- Fruit flies (D. melanogaster)
- Xenopus laevis
- Zebrafish, Medaka
- Chicks
- Mice, Rats (third trimester equivalent is post-natal)
- Guinea pigs (true in utero exposure over three trimester equivalents)
- Non-human Primates such as macaques (“gold standard” but expensive, time consuming, ethics)

[Image: http://www.seriouslyfish.com/species/oryzias-latipes/]
More pregnant women are smoking marijuana to tame morning sickness: study

By Carmen Chai
National Online Journalist, Health  Global News

- In 2002, about 2.4% of women admitted to smoking pot while pregnant.

- By 2014, about 4% said they got high during their pregnancy.
Increasing marijuana use in adults

Potency trends of marijuana
Prenatal exposure of marijuana

- Cognitive
- Behavioral and
- Neuropsychiatric defects

### Neurocognitive and Behavioural Effects

<table>
<thead>
<tr>
<th>Age</th>
<th>Deficits</th>
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<tbody>
<tr>
<td>18 months</td>
<td>Increased aggressive behaviour&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Attention deficits&lt;sup&gt;b&lt;/sup&gt; (females)&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Short-term memory&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Attention deficits&lt;sup&gt;de&lt;/sup&gt;</td>
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<td></td>
<td>- Impulsivity&lt;sup&gt;de&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Impaired vigilance&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>3–6 years</td>
<td>Deficits in:</td>
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<tr>
<td></td>
<td>- Verbal and perceptual skills&lt;sup&gt;ac&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Verbal reasoning&lt;sup&gt;ac&lt;/sup&gt;</td>
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<td>- Visual reasoning&lt;sup&gt;ac&lt;/sup&gt;</td>
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<td>- Verbal and quantitative reasoning&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>9–10 years</td>
<td>Deficits in:</td>
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<td></td>
<td>- Abstract and visual reasoning&lt;sup&gt;abc&lt;/sup&gt;</td>
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<td></td>
<td>- Executive functioning&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Reading&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>- Spelling&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Hyperactivity&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Attention deficits&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>- Impulsivity&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Depressive and anxious symptoms&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>14–16 years</td>
<td>Deficits in:</td>
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<tr>
<td></td>
<td>- Visual-cognitive functioning&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>- Academic achievement&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Information processing speed&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Visual motor coordination&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>- Delinquency&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>17–22 years</td>
<td>Deficits in:</td>
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<tr>
<td></td>
<td>- Executive functioning&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>- Response&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Inhibition&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Visuospatial working memory&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>- Smoking&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>- Substance use&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>- Early initiation of substance use&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup> OPPS, <sup>b</sup> MHPDC, <sup>c</sup> Generation R

Cannabinoids

Gaoni and Mechoulam, 1964
Our study:

• Effect of THC, cannabinol, cannabidiol and alcohol (ethanol) on neurodevelopment and neuromuscular activity in embryos
• Effects on zebrafish locomotion
• What role do endocannabinoids play in communication between cells
• Expose zebrafish embryos to THC, cannabinol and cannabidiol during the gastrulation stage of development and then determine the effect on neuronal function.
Exposure Timelines

- Fertilization
- Cleavage
- Gastrulation
- Segmentation
- Pharyngula
- Hatching

Drug Exposure

Exposure Timelines:
- 0h
- 5h
- 10h
- 24h
- 48h

Effects Studied
Gestational Timeline in Humans

Embryonic exposure to cannabinoids alters morphology

Transverse section through the trunk of a zebrafish embryo

Exposure to cannabinoids alters neuronal growth and branching

Embryonic exposure to cannabinoids alters responses to sound

A) 

B) 

C) 

D) 

Controls

THC
Embryonic exposure to alcohol alters motor neurons

Amin et al (Unpublished)
Embryonic exposure to CBD alters adult behavior/locomotion

Time spent in Thigmotaxis

Distance moved

Amin et al (Unpublished)
Summary

- Zebrafish embryos exposed to THC or CBD experience morphological defects.
- Zebrafish embryos exposed to THC or CBD exhibit reduced hatching, survival and heart rates.
- Zebrafish embryos exposed to THC or CBD exhibit reduced branching patterns of motoneurons and fewer neuromuscular junctions.
- Zebrafish embryos show reduced sensation to sound
Acknowledgements

• Trevor Hamilton (Collaborator at Grant Macewan University).
• Jeffrey Crook (undergraduate student, Grant Macewan University)
• Md Ruhul Amin (PhD student)
• Kazi T, Ahmed (PhD student)
• Janatul Ferdous (MSc student)
Learning Objectives

1. To recognize the effect of cannabinoids on early development.
2. To describe the combined effects of cannabinoids and alcohol on early development and maturation.
3. To identify or review the implications of cannabinoid exposure for pregnancy.
Illicit drug usage during pregnancy is increasing

**USA**

According to National Survey on Drug Use and Health in the United States-

In 2009-2010 - 3.6%
In 2011-2012 - 5.2%.

During the first trimester - 10.7%

**Canada**

In 2008, illicit drug use during pregnancy was about 5%  
(According to Canadian Perinatal Health Report)

In 2013, the percentage was reported as 11%  
(According to Health Canada)
CDC: 1 in 10 pregnant women report alcohol use

By Paula Wolfson | @PWolfsonWTOP
September 24, 2015 5:31 pm

The Centers for Disease Control and Prevention is out with new statistics that show that one in 10 pregnant women in the United States between the ages of 18 and 44 admit to drinking alcohol in the past 30 days. (Thinkstock)
References

- Substance Abuse and Mental Health Services Administration [SAMHSA], 2013)
Managing Potential Bias

- Not required.