Does Mother Nature Always Know Best?
The Contribution of Chemosensory Fetal Programming on Adolescent Alcohol and Nicotine Acceptance as a Consequence of Prenatal Alcohol Exposure

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Learning Objectives

• Understand the relationship between fetal alcohol exposure, chemosensory plasticity (a normally adaptive process) and the enhanced risk for initial alcohol intake and choice behavior in adolescence

• Understand the underlying mechanisms contributing to the behavioral preference for alcohol odor and the perception and acceptability of alcohol’s flavor, as a consequence of fetal exposure

• Understand the potential chemosensory-based mechanism(s) by which fetal alcohol exposure increases the later initial risk for nicotine acceptability
References:


References (continued):


Youngentob SL, Glendinning JI (2009). Fetal ethanol exposure increases ethanol intake by making it smell and taste better. Proc Natl Acad Sci USA. Mar 31;106(13) :5359-64.

Alcoholism Generator

Adapted from Miller & Spear (ACER, 2006)
Flavor Perception

odor

taste

oral irritation
Fetal Programming

Mennella et al. (Pediatrics, 2004)
Fetal Programming

Dominguez et al. (Alcohol, 1998)
Fetal Alcohol Exposure

Koren et al. (CMAJ, 2003)
Overarching Hypotheses

* Fetal alcohol experience alters the developmental trajectory of one or more of the neural systems involved in the preference for alcohol odor and the perception and acceptability of alcohol’s flavor.

* These changes are important contributors to the initial risk of intake and continued abuse in adolescence because “alcohol smells and tastes better”.

Fetal Programming

Maternal Treatment

- Ethanol
- Pair-Fed
- Free Choice

Youngentob et al. (Behav Neurosci, 2007a)
Innate Odor-Mediated Response

Youngentob (Chem Senses 2005)
<table>
<thead>
<tr>
<th>Parameter</th>
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<tbody>
<tr>
<td>1: Respiratory Frequency</td>
</tr>
<tr>
<td>2: Average Expiratory Volume</td>
</tr>
<tr>
<td>3: Average Inspiratory Volume</td>
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<tr>
<td>4: Number of Expiratory Sniffs</td>
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<tr>
<td>5: Number of Inspiratory Sniffs</td>
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<tr>
<td>6: Average Expiratory Sniff Duration</td>
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<td>7: Average Inspiratory Sniff Duration</td>
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<tr>
<td>8: Average Expiratory Flow Rate</td>
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<td>9: Average Inspiratory Flow Rate</td>
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<td>10: Total Expiratory Volume</td>
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<td>11: Total Inspiratory Volume</td>
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<tr>
<td>12: Aver. Peak Expiratory Flow Rate</td>
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<tr>
<td>13: Aver. Peak Inspiratory Flow Rate</td>
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<td>14: Total Apneic Period (ms)</td>
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Raw Data

Yougentob (Chem Senses 2005)
Effects of Fetal Ethanol Exposure on Olfactory Functioning

P15

P90

Neural Response

Ethanol Intake

Youngentob et al. (Behav Neurosci, 2007a)
Fetal exposure alters the behavioral response to EtOH odor and EtOH intake.

a: mean (± 2-D standard errors) composite sniffing indexes for the maternal treatment groups.

b: Illustrates the grams of absolute ethanol consumed per kilogram of body weight as a function of maternal treatment. The data are the means ± s.e.m.

Increased EtOH intake can be attributed to an enhanced odor response.

a: The slope of the predictive relationship between the response to ethanol odor and ethanol intake.

b: The height of the column is the net difference in absolute ethanol intake between ethanol and chow animals. Solid black insert is the contribution to the total effect directly attributable to the response to ethanol odor (~21%).
Ontogeny of the Odor-Mediated Effect

Eade et al. (ACER, 2010)
<table>
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<tr>
<th>Functional Annotation</th>
<th>GNAO1</th>
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<th>LRRC55</th>
<th>FOLH1</th>
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Specific Effects on Neurotransmission

A

$\Delta$ Ct (Cycles to threshold rel to 18S)

B

Mean Fold Changes in EtOH Treated vs Ctrl

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<tr>
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Overview of Olfactory Bulb Gene and Exon Level Expression Changes

* Dysregulation of neurotransmission and olfactory signaling genes at both the gene and exon level

* Specific spans of exons for a small list of candidate genes that provide evidence of alternative splicing
The Reinforcing Properties of EtOH Contribute to the Odor-Mediated Effect of Fetal Exposure

Youngentob et al. (EBM, 2012)
The Reinforcing Properties of EtOH Contribute to the of Fetal Exposure on EtOH Intake

Youngentob et al. (EBM, 2012)
The “At Risk” Period of Adolescence
The Social Interaction

Observer

Demonstrator
Ethanol Re-exposure Model

Ethanol Group
- 2 Males
  - Observer
  - Demonstrator
- 2 Females
  - Observer
  - Demonstrator

Water Control
- 2 Males
  - Observer
  - Demonstrator
- 2 Females
  - Observer
  - Demonstrator

Pretreatment
- Ethanol
- Pair-fed
- Free Choice

No Ethanol Exposure = PF/H2O, FC/H20
Fetal Exposure Only = ET/H20
Adolescent Exposure Only = PF/ET, FC/ET
Fetal + Adolescent Exposure = ET/ET

Eade et al. (BBF, 2009)
Adolescent Ethanol Exposure Augments the Effect of Prior Fetal Experience
A Cumulative Effect
Combined fetal ethanol exposure plus adolescent odor re-exposure leads to behavioral alterations in adulthood only in females.

ET/ET = fetal ethanol animals with ethanol odor re-exposure through social transmission in adolescence. Control/ET = pair-fed and free choice animals with ethanol odor exposure through social transmission in adolescence.

While ethanol odor-specific exposure through social interaction is important, additional factors such as the pairing of retronasal and hematogenic olfaction with ethanol’s intoxicating properties appear necessary to achieve adult persistence in both sexes.

Eade and Youngentob (ACER, 2010)
Prenatal Ethanol Exposed Animals are Attracted to an Intoxicated Peer
Prenatal Ethanol Exposed Animals are Attracted to an Intoxicated Peer
We Measure Oro-sensory Responses with a Gustometer
Fetal exposure increases the acceptability of ethanol and QHCl, but not sucrose.

\[ \text{Lick ratio} \]

\[ \text{[EtOH]} \% \]

\[ \text{[QHCl]} \text{ (mM)} \]

\[ \text{[Sucrose]} \text{ (mM)} \]

\[ \text{EtOH-fed} \]

\[ \text{Pair-fed} \]

\[ \text{Chow-fed} \]

\[ \beta = 0.553 \]

**a:** The slope of the relationship between the animals’ orosensory response index to QHCl and the animals’ response index to ethanol.

**b:** The height of the column is the net difference in the EtOH response index between ethanol and chow animals. Black insert: the contribution to the net ethanol effect that can be ascribed to fetal ethanol’s effect on the response to ethanol’s bitter taste (~29%).

Youngentob and Glendinning (PNAS, 2009)
Fetal exposure increases the acceptability of (a) ethanol and (b) Capsaicin, but not (c) AITC.

A: the slope of the relationship between the rats’ orosensory response index to capsaicin and the same rats’ response index to ethanol.

B: the height of the column is the net difference in the ethanol response index between ethanol-exposed and control animals. The solid black represents the amount of the difference attributable to TrpV1-mediated oral irritation by ethanol.
Peripheral Oro-sensation

Yarmolinsky et al.(Cell, 2009)
Peripheral Oro-sensation

Taste Receptor Cells

Taste bud

Nociceptive fibers: e.g., Trpv1, Trpa1 expressing

Chandrashekar et al. (Nature, 2006)
Gustatory Gene Expression

CVs, Fol, FF, Pal Harvested ET, FCL

RNA Purification

Gene Specific Primer Reverse Transcription

qRT-PCR Analysis of 37 genes

Prestia et al. (In preparation)
Oro-sensory Gene Expression

P35-Adolescence
P90-Adulthood

Bitter taste receptors (T2rs)

Taste transduction Trp receptor

Oro-somatic sensory (Trp) receptors

Genes

- T2r106
- T2r105
- T2r104
- T2r130
- T2r120
- T2r107
- T2r116
- T2r113
- T2r136
- T2r140
- T2r114
- T2r121
- T2r129
- T2r124
- T2r103
- T2r125
- T2r137
- T2r40
- T2r39
- T2r108
- T2r38
- T2r126
- T2r135
- T2r118
- T2r119
- T2r143
- T2r134
- Loc100360076
- Rgd1564521

Sweet & umami taste receptors (T1rs)

- T1r1
- T1r2
- T1r3
- Trpm5
- Trpa1
- Trpv1
- Trpv3
- Trpm8

Prestia et al. (In preparation)
<table>
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Involved in Quinine Sensitivity

* T2r106
* T2r105
* T2r104
* T2r130
* T2r120
* T2r107
* T2r116
* T2r113
* T2r136
* T2r140
* T2r114
* T2r121
* T2r129
* T2r124
  T2r103
  T2r125
Involved in Quinine sensitivity

* T2r106
* T2r105
* T2r104
* T2r130
* T2r120
* T2r107

* T2r116
* T2r113
* T2r136
* T2r140
* T2r114
* T2r121
* T2r129
* T2r124
 T2r103
 T2r125

Located on a quantitative trait locus for increased ethanol consumption

qRT-PCR
Adolescent CV
Log₂ Fold Change
BH-FDR
**Additional T2rs of the Adolescent CV**

**Known Function or Ligand**
- *T2r39*-Isoflavonoids/e.g., bitterness in green tea
- *T2r108*-Cholinergic chemosensory cells in mouse trachea
- *T2r38*-PTC/PROP sensitivity
- *T2r137* Phantogeusia
- *T2r40*

**Unknown Function or Ligand**
- *T2r126*
- *LOC100360076*
- *T2r135*
- *T2r118*
- *T2r119*
- *T2r143*
- *T2r134*
- RGD1564521
Transient Receptor Channels (*Trps*) of the Adolescent CV

**Examples of Ligands**

- *Trpa1* - AITC
- *Trpv1* - Capsaicin
- *Trpv3* - Camphor
- *Trpm8* - Menthol

**Taste Transduction**

- *Trpm5* Calcium signaling for bitter, sweet and umami
Additional Taste Receptors of the Adolescent CV

Sweet and Umami
* $T1r1$
* $T1r2$
* $T1r3$
Additional Taste Receptors of the Adolescent CV

Sweet and Umami

* $T1r1$
* $T1r2$
* $T1r3$
The effects of fetal exposure on the CV ameliorates with time.
Oro-Sensory Gene Expression

Summary

* CV - Bitter, sweet and oral irritation transduction genes
  - Decreased during adolescence
  - Normalized or rebounded by adulthood
  - Results consistent with previous behavioral findings

* The fetal exposure effect was specific to the CV
General Summary

* Our behavioral and genomic data provide evidence for the hypothesis there are epigenetic chemosensory mechanisms by which maternal patterns of alcohol use can be transferred to offspring, and via which, the adolescent system is primed to have the effects of fetal exposure augmented and/or preserved into adulthood.
Lessons Learned from Animal Studies of Fetal Alcohol Exposure
Nicotine and Alcohol have Common Component Chemosensory Qualities

* Both are described as having an irritating and aversive odor

* Their orosensory qualities are both described as irritating and having a bitter taste
Innate Odor-Mediated Response
Prenatal Alcohol Exposure Alters the Odor-Mediated Response to Nicotine

Mantella and Youngentob (PLoS one, 201)
We Measure Oro-sensory Responses with a Gustometer
Oral Acceptability of Nicotine and Sucrose Solutions to Prenatal Alcohol- and Control-Exposed Adolescent Rats

Mantella and Youngentob (PLoS one, 2014)
Effects of Fetal Ethanol Exposure on Latency to First Lick to Nicotine or Sucrose Solutions

Mantella and Youngentob (PLoS one, 2014)
Additional Conclusions

Fetal exposure to alcohol also influences nicotine initial postnatal acceptability, at a minimum, by decreasing the aversive properties of both its smell and taste.

The present findings point to a broader mechanistic concern regarding the consequence of fetal exposure with one substance of abuse and its impact on the potential initial acceptability of others.
Acknowledgements

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- Dr. Sandra Mooney

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