The brain and tobacco smoke: There’s more to it than nicotine
Causes of ADHD

• Genetics (*DRD4, DRD5, TPH2, ADHD1-4*, etc.)

• Brain injury

• Lead or other environmental exposures

• Premature delivery

• Low birth weight

• Alcohol or tobacco use during pregnancy
Prenatal smoke exposure

• >400,000 babies in the US are exposed prenatally every year
  – Nicotine is the most commonly used drug in pregnancy
  – NHANES: 23% of pregnant women inaccurately report
  – Does not account for other tobacco products (e.g., hookah, e-cigs)

• Second-hand smoke
  – Nearly 20% of adults and teenagers smoke
  – ~50% of pre-school aged children in SF found exposed to SHS

2014 Surgeon General’s Report on Smoking
2012 Arch Pediatr Adolesc Med 166:851
NICHEs goal: to understand the epigenetic mechanisms that underlie / mediate neurodevelopmental vulnerability to environmental toxicants; initial focus on tobacco smoke and ADHD

- Approximately 25% of women reported smoking before and 13.8% continued smoking during pregnancy (CDC)

- ADHD affects 14.4% of children in North Carolina (second highest is US) (CDC)

- Early life exposure to tobacco: two-fold increased risk of ADHD
Clinical cognitive & neuro-behavioral assessments (3-5 and 5-7 years); relation to ETS exposure (cotinine levels)

In vivo TSE / nicotine exposure during pregnancy (dose, timing; influence of folate, antioxidants)

In vitro TSE / nicotine effects on neuronal differentiation (PC12, neural stem cells)

In vitro TSE/nicotine effects on neural circuits (PC12, neural stem cells; cholinergic, catecholaminergic and serotonergic systems)

Exposed and control pups, phenotyped for AD/HD behaviors

Functional relationships + Concordant changes

ETS-responsive functional methylation changes in brain, also detectable in peripheral blood

Project 1

Project 2

Project 3

NiCHES

Children's Environmental Health and Disease Prevention Research Center

Frontal cortex

DNA

RNA

RNASeq

Blood

DNA

Whole Genome Bisulfite Sequencing

Whole Genome Bisulfite Sequencing

Informs target assessment
Neurotoxic Effects on Attention Deficit and Hyperactivity in Rodent Models

NICHES Project 2

Mechanisms of Neurobehavioral Dysfunction from Developmental Nicotine & Tobacco

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Exposure Protocol

Preconception

- Implant
- 3 day recovery
- ≤ 5 days

Postnatal

- 12 +/- 2 days

TSE with 0.2 mg/kg/day nicotine
- 0.2 mg/kg/day nicotine
- 2.0 mg/kg/day nicotine
- DMSO vehicle control

N=12-14 litters per exposure

21 days gestation

http://digitalsculpture250.blogspot.com
Locomotor Activity
Figure 8 Apparatus
Adolescent Locomotor Activity
TSE > nicotine

Main Effects
- Sex, p<0.025
- Treatment, p<0.025

TSE 0.2 vs. Control, p<0.025
Nic 0.2 vs. Control, p<0.025

N=12-14 litters/treatment
Novel Object Recognition

(a) Sample-object exposure
One placement (e.g., 10 min)

(b) Novel-object test
One placement (e.g., 2–5 min)

Delay (e.g., 1 h)

Novel Object Recognition

Gestational Exposure to Tobacco Smoke or Nicotine:

- Novel Object Recognition Test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Novel-Familiar (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td></td>
</tr>
<tr>
<td>TSE 0.2</td>
<td></td>
</tr>
<tr>
<td>Nic 0.2</td>
<td></td>
</tr>
<tr>
<td>Nic 2</td>
<td></td>
</tr>
</tbody>
</table>

- Male
- Female

Main Effect: Treatment, p<0.01
N=11-14 litters/treatment
Operant Signal Detection Task

Signal Trial
- VI 0.3-24s > Light > VI 2-4s
  - Levers Extended
  - Blank Lever

Blank Trial
- No Signal
  - Blank Lever

Signal Lever
- Hit → Food
- Miss → Time Out

Blank Lever
- False Alarm → Time Out
- Correct Rejection → Food

Time
Operant Signal Detection Task

Gestational Exposure to Tobacco Smoke Extract or Nicotine: Visual Signal Detection Attention Task: Percent Correct

Female
Male
Sex Main Effect, p<0.05
N=12-14 litters/treatment
Summary

• Developmental exposure to tobacco smoke extract causes adolescent locomotor hyperactivity. This is not apparent in adulthood.

• Developmental tobacco and nicotine exposure causes impaired novel object recognition.

• No impairment seen in higher motivation radial maze and signal detection task.

• Impairment may be related to motivational state.
Developmental Neurotoxicity of Tobacco Smoke

NICHES Project 2

Mechanisms of Neurobehavioral Dysfunction from Developmental Nicotine & Tobacco

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Developmental Neurotoxicity of Tobacco Smoke

- TSE has more potent effects than does nicotine alone on altering neurodifferentiation
- TSE promotes transition from neural cell replication to neurodifferentiation at the expense of cell numbers
- TSE suppresses the emergence of the acetylcholine phenotype, and instead promotes the monoaminergic phenotype
Developmental Neurotoxicity of Tobacco Smoke
Directed Toward Cholinergic and Serotonergic Systems: More Than Just Nicotine

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Effect of Exposure on Choline Acetyltransferase Activity

- PN30 (adolescence)
- PN60
- PN100
- PN150
- Frontal/parietal cortex
- Temporal/occipital cortex
- Hippocampus
- Striatum
- Midbrain
- Brainstem
Effect of Exposure on Choline Acetyltransferase Activity
Effect of Exposure on Hemicholinium-3 Binding

HC3 Binding: Main Treatment Effect

percent change from control

TSE
Nic 0.2
Nic 2

male
female
Effect of Exposure on Hemicholinium-3 / Choline Acetyltransferase Ratio

![Graph showing the effect of exposure on HC3/ChAT ratio for different treatments: TSE, Nic 0.2, and Nic 2, with male and female data represented.]
Effect of Exposure on Nicotinic Acetylcholine Receptor Binding
Effect of Exposure on Serotonin Receptor 1A Binding
Effect of Exposure on Serotonin Receptor 5HT2 Binding
Correlations Across all Parameters

A. Nic 0.2 vs. TSE — males
   $r = 0.60$, $p < 0.0001$
   36%

B. Nic 2 vs. TSE — males
   $r = 0.68$, $p < 0.0001$
   46%

C. Nic 0.2 vs. TSE — females
   $r = 0.36$, $p < 0.0001$
   13%

D. Nic 2 vs. TSE — females
   $r = 0.28$, $p < 0.002$
   7%
Summary

• TSE effects during neurodevelopment are greater than that of just the nicotine in the mixture

• Sensitivity to TSE effects is evident at secondhand smoke exposure levels

• Unlike nicotine, there was a lack of compensatory ChAT activity with TSE plus decreased hemicholinium-3 binding - suggesting augmented presynaptic activity deficits with TSE

• Postsynaptic nAChRs were increased with nicotine by itself but were decreased with TSE, worsening decreased ACh activity

• Nicotine in TSE explains less than half the effects in males and even less in females; nicotine has a greater impact on males that is exacerbated by the other components in TSE
Conclusions

• Early life exposure to tobacco smoke extract causes increased locomotor activity and deficits in novel object recognition. The lack of deficits in the operant test suggests that the effects of exposure may be relevant to low motivation.

• The complex mixture of compounds in tobacco smoke extract contributes to developmental neurotoxicity above that measured for nicotine.

• Sex differences in the impact of TSE on brain development reflect the greater impact of nicotine on males and exacerbation by the other components in TSE.

• Nicotine alone is responsible for nearly half of the effects of tobacco smoke in males; harmful even at secondhand smoke exposure levels.

  • **Calls into question cigarette alternatives (e-cigarettes)**

  • **Underscores recommendations to avoid secondhand smoke during pregnancy**