



The brain and tobacco smoke: There's more to it than nicotine

**Neurodevelopment and Improving Children's Health
following Environmental tobacco Smoke exposure**

A Children's Environmental Health and Disease Prevention Research Center
at
Duke University

**NIH / NIEHS award P01ES022831
USEPA grant RD-83543701**

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

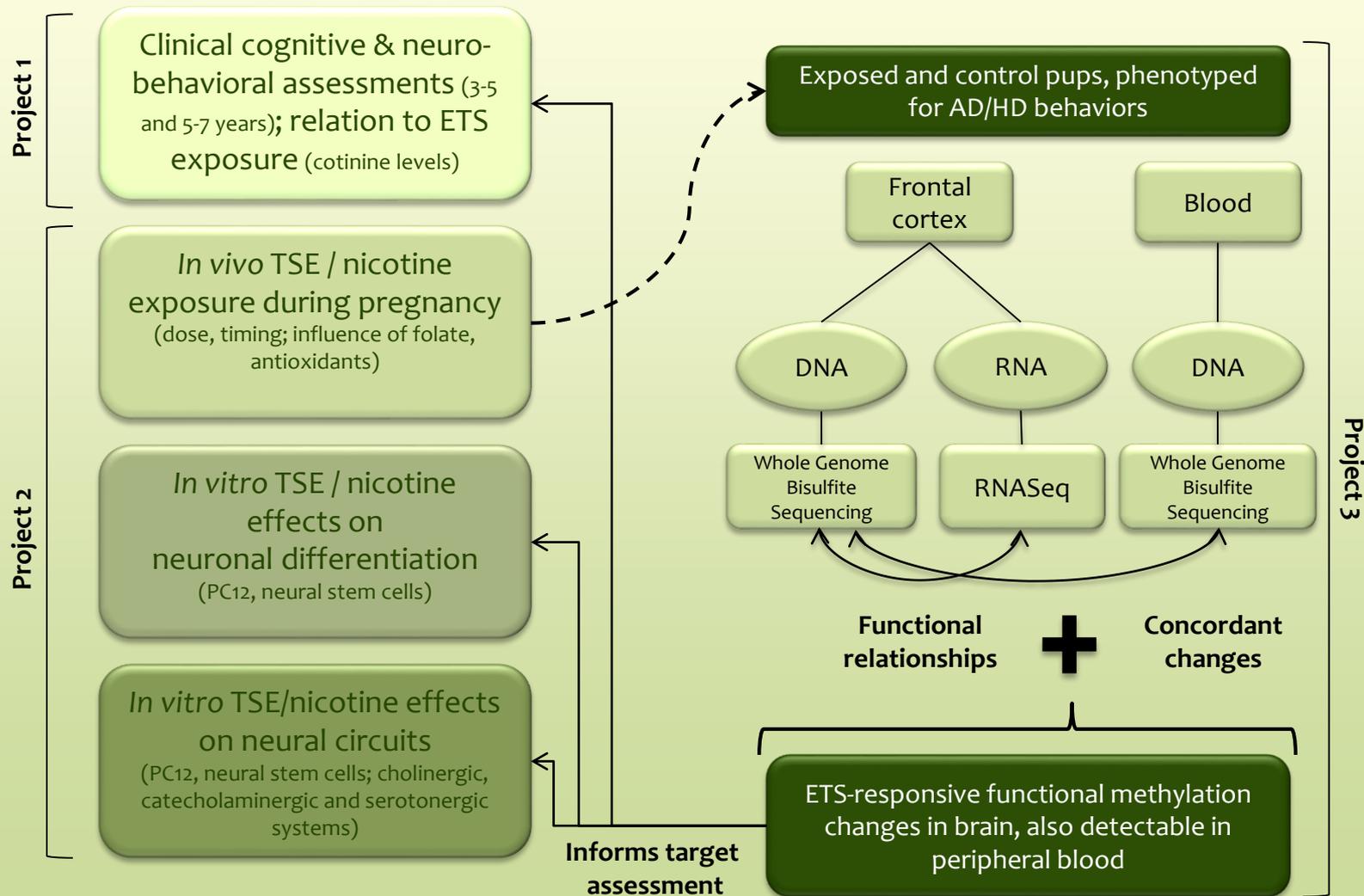
- >400,000 babies in the US are exposed prenatally every year
 - Nicotine is the most commonly used drug in pregnancy
 - NHANES: 23% of pregnant women inaccurately report
 - Does not account for other tobacco products (e.g., hookah, e-cigs)
- Second-hand smoke
 - Nearly 20% of adults and teenagers smoke
 - ~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 in US) (CDC)
- Early life exposure to tobacco: two-fold increased risk of ADHD





Neurotoxic Effects on Attention Deficit and Hyperactivity in Rodent Models

NICHES Project 2

**Mechanisms of Neurobehavioral Dysfunction from
Developmental Nicotine & Tobacco**



Ed Levin, PhD



Brandon Hall, PhD



Marty Cauley

Exposure Protocol



Implant

3 day
recovery

Preconception

≤ 5 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



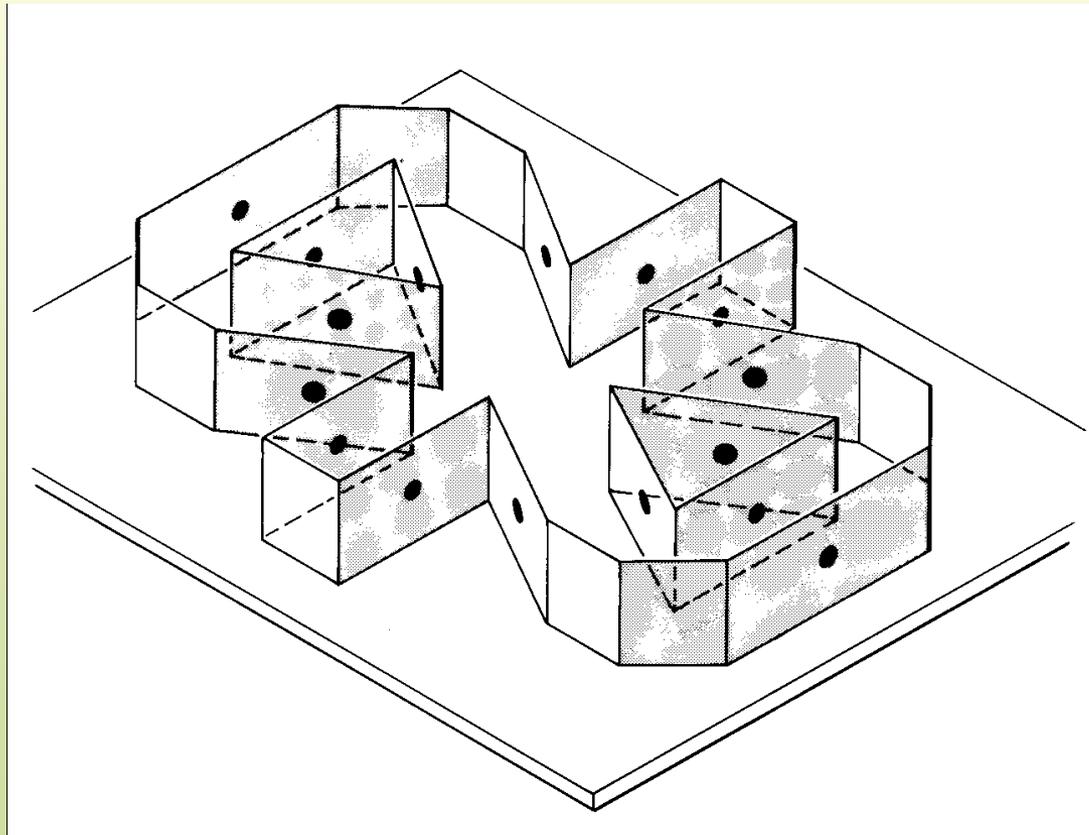
<http://digitalsculpture250.blogspot.com>

21 days gestation

Postnatal

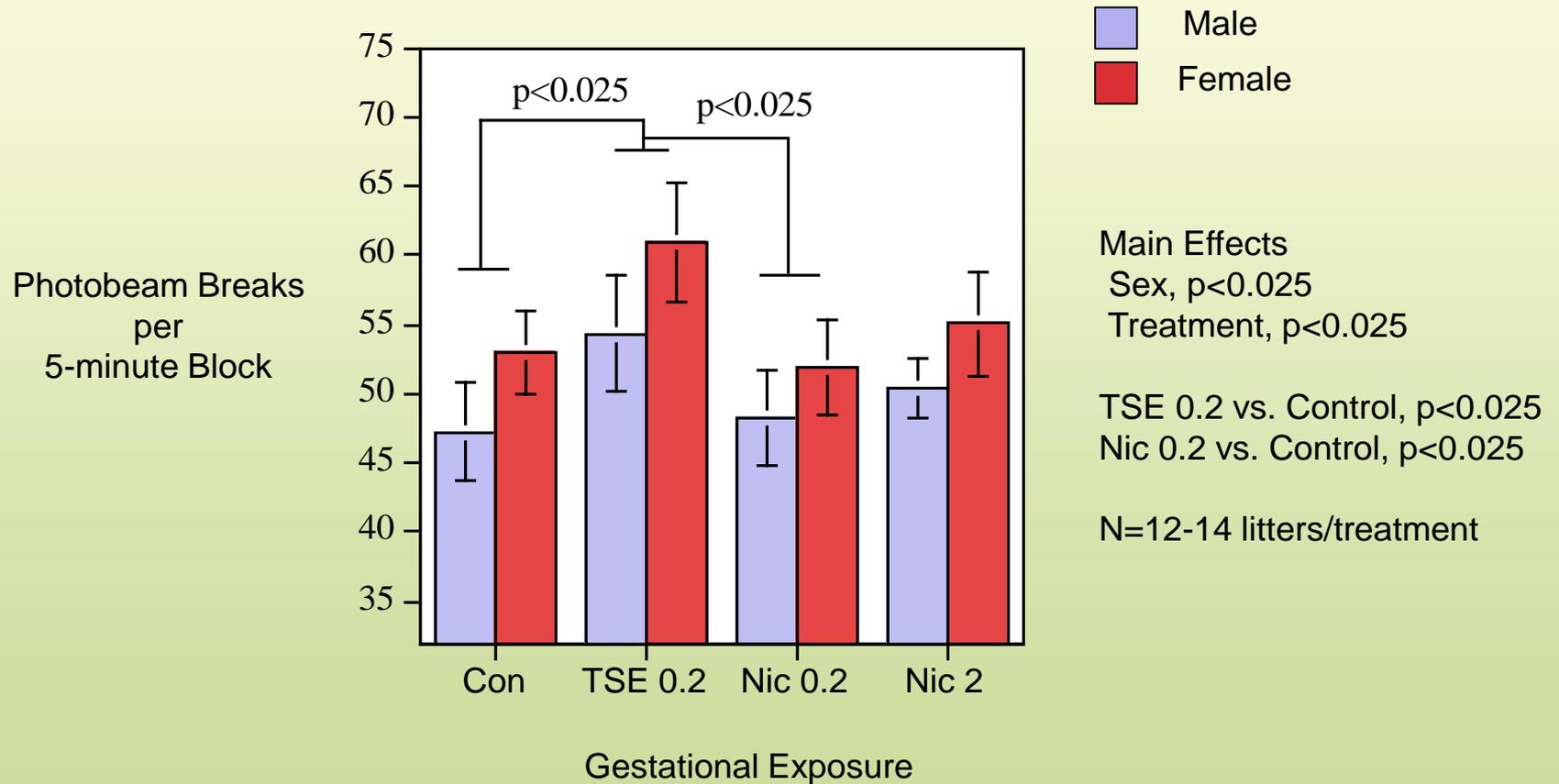
12 +/- 2 days

Locomotor Activity Figure 8 Apparatus



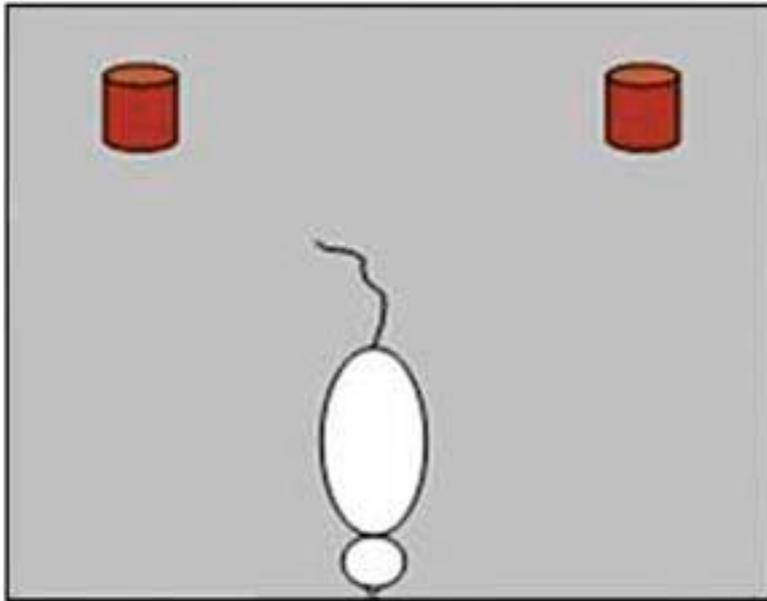
Adolescent Locomotor Activity

TSE > nicotine



Novel Object Recognition

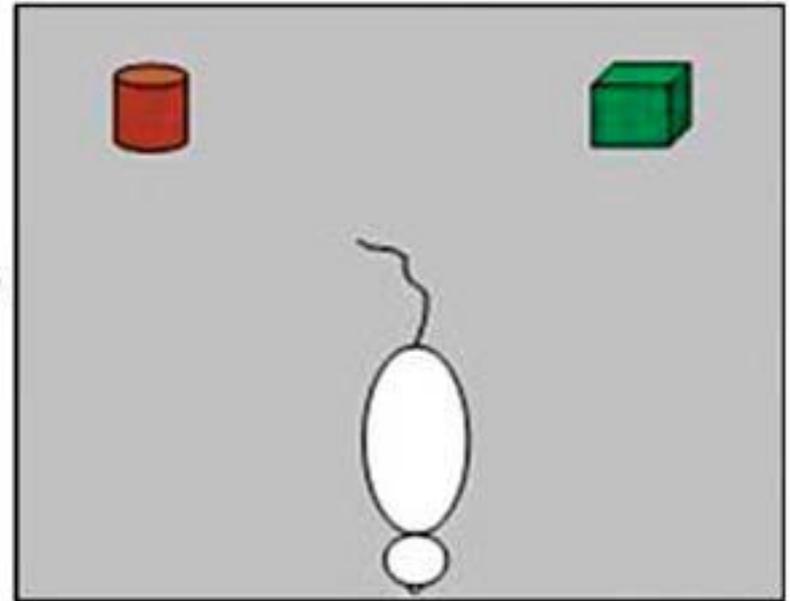
a Sample-object exposure



One placement
(e.g., 10 min)

Delay
(e.g., 1 h)
→

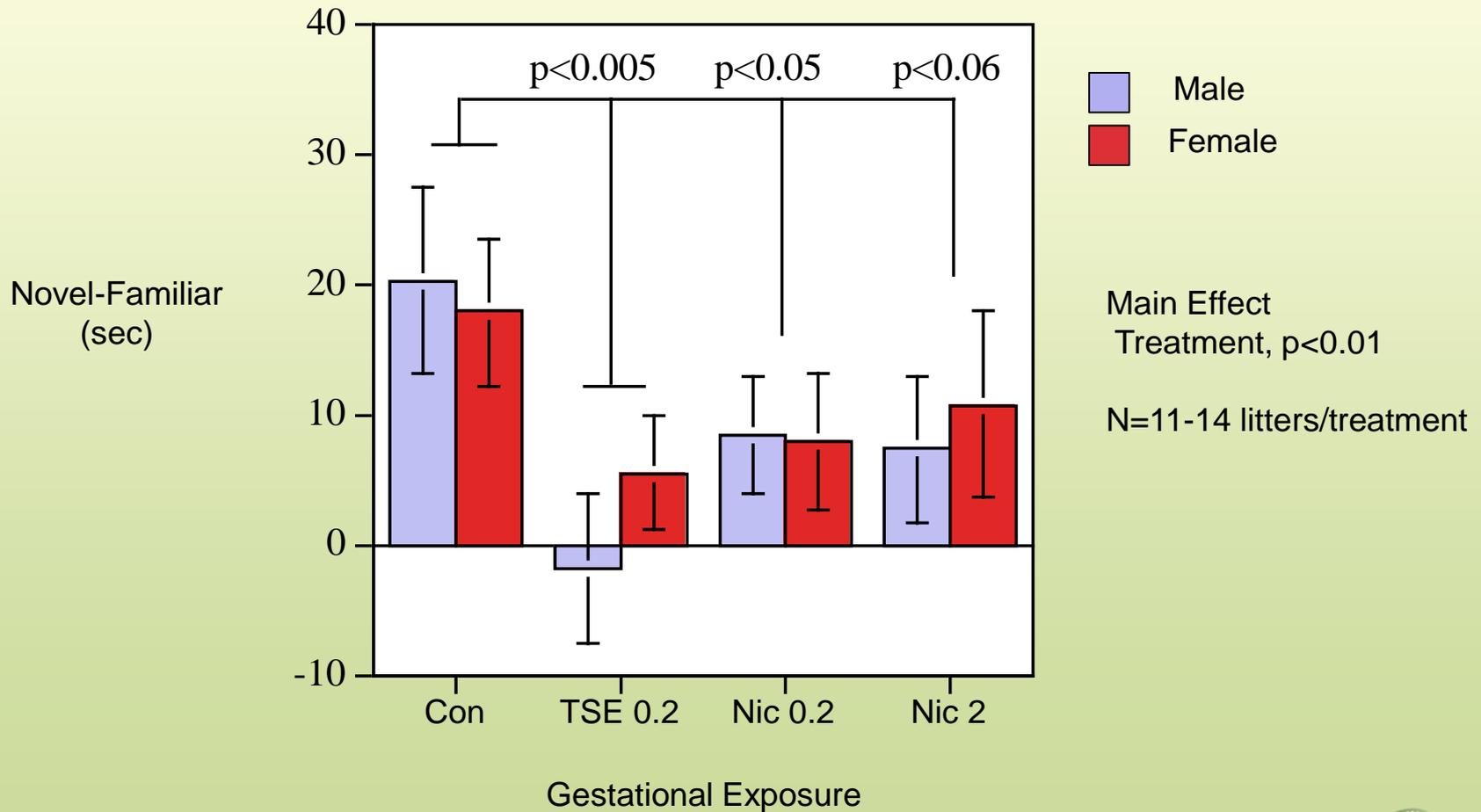
b Novel-object test



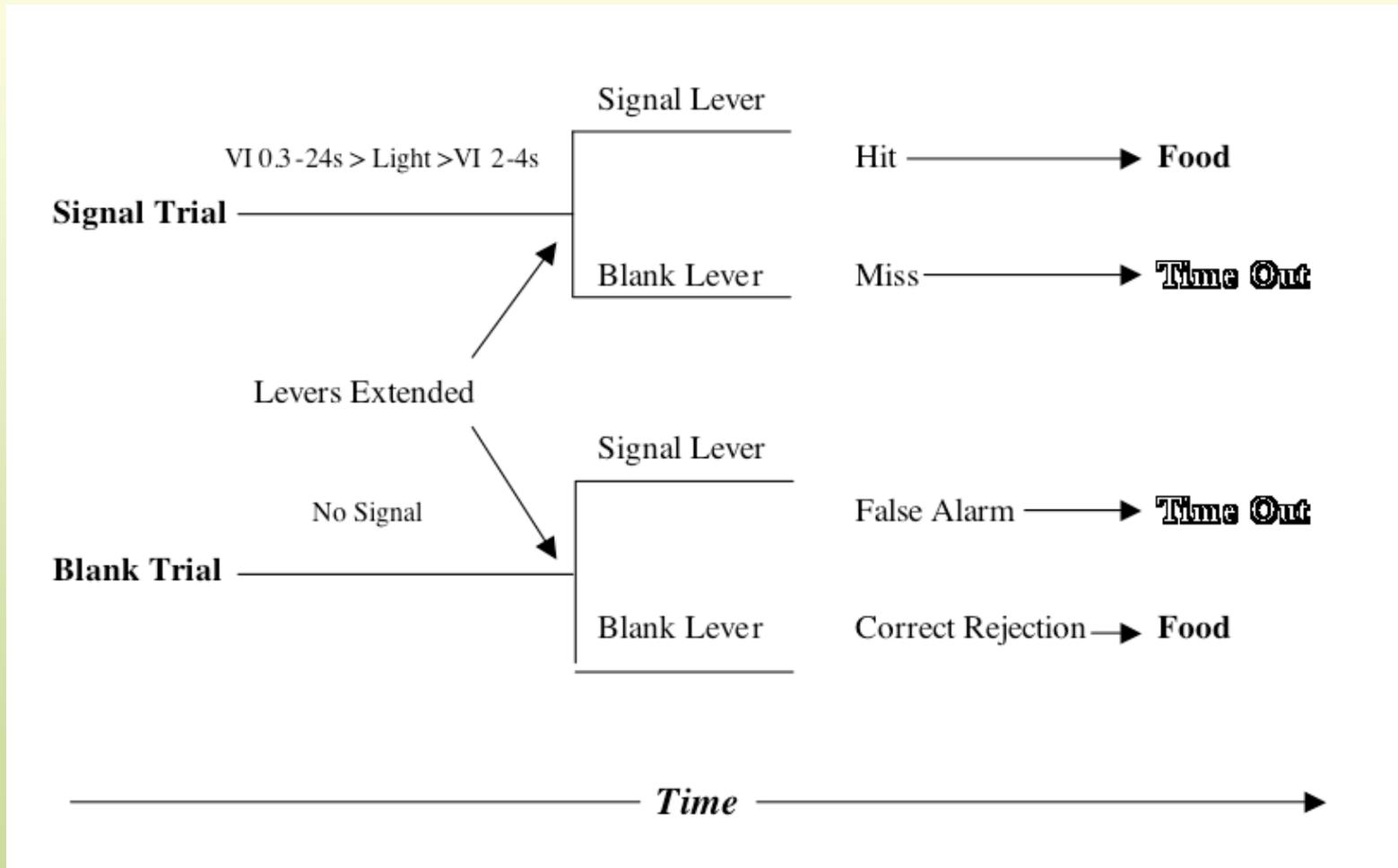
One placement
(e.g., 2–5 min)

Bevins & Besheer, *Nature Protocols*, 2006

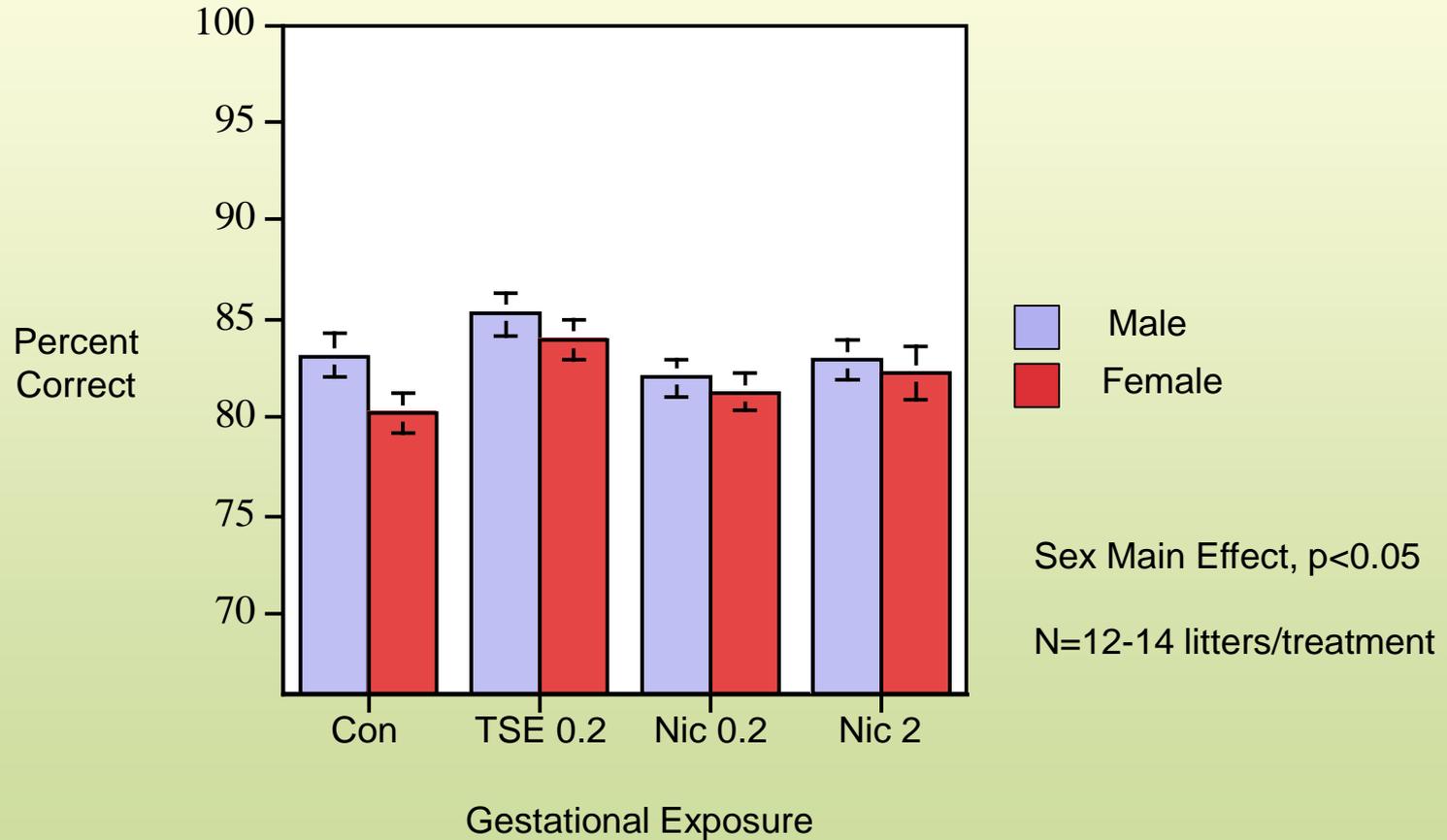
Novel Object Recognition



Operant Signal Detection Task



Operant Signal Detection Task



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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journal homepage: www.elsevier.com/locate/neutera



Effects of tobacco smoke on PC12 cell neurodifferentiation are distinct from those of nicotine or benzo[a]pyrene

 CrossMark

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- 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

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Toxicology
www.toxsci.oxfordjournals.org

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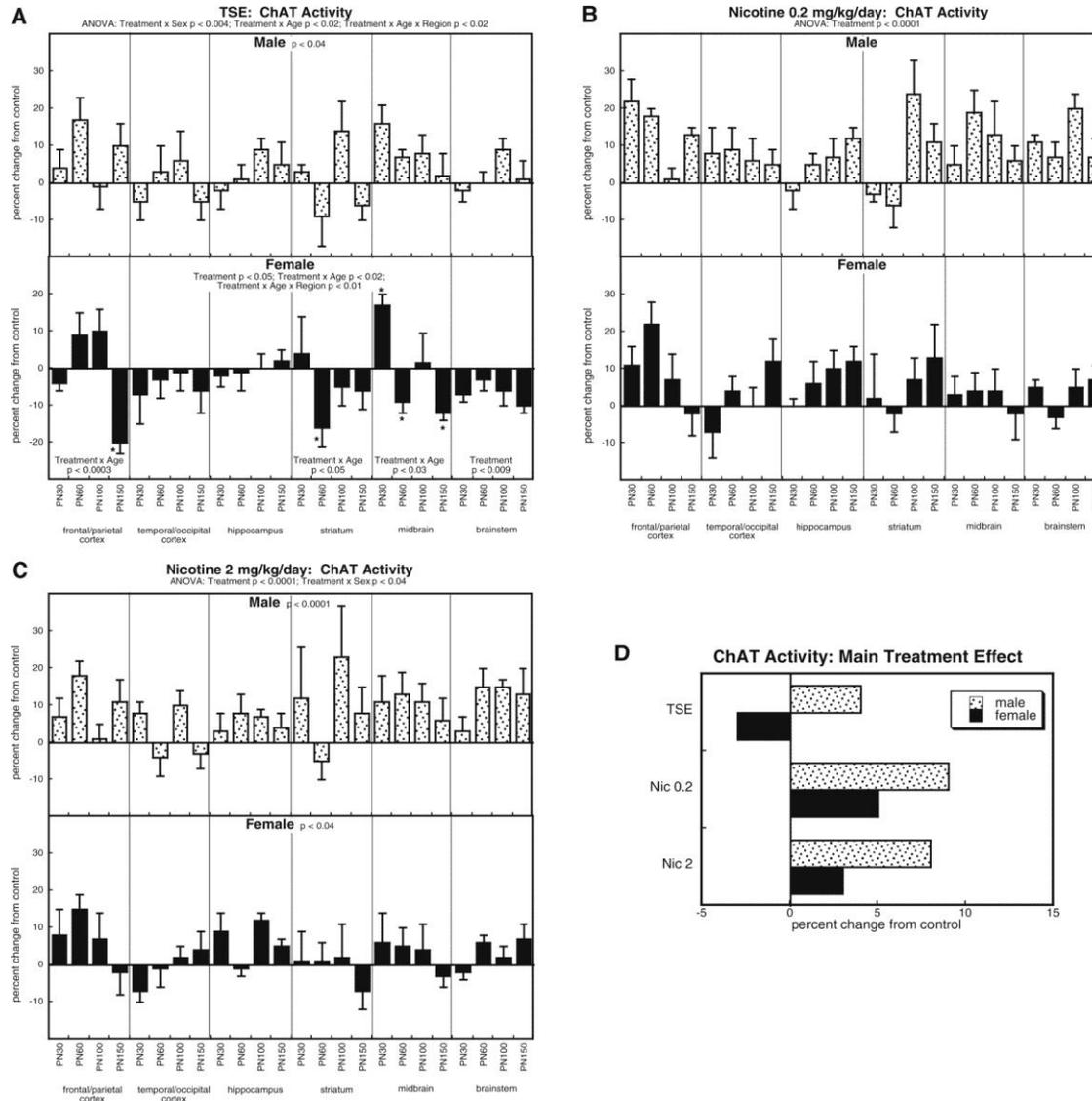
Developmental Neurotoxicity of Tobacco Smoke Directed Toward Cholinergic and Serotonergic Systems: More Than Just Nicotine

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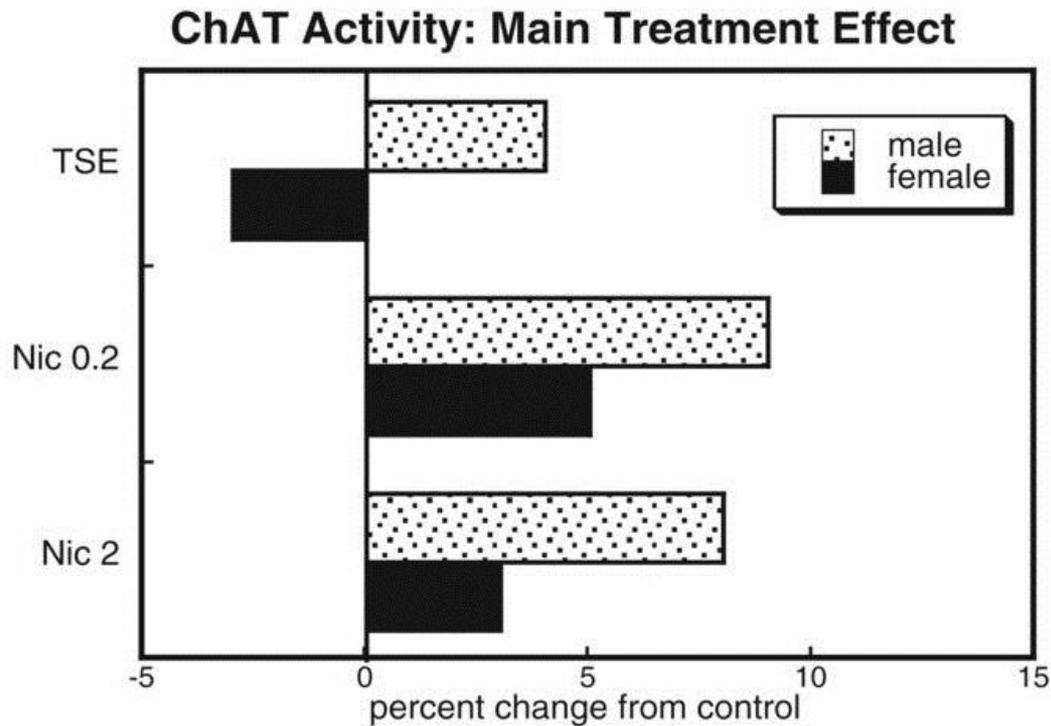
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Effect of Exposure on Choline Acetyltransferase Activity

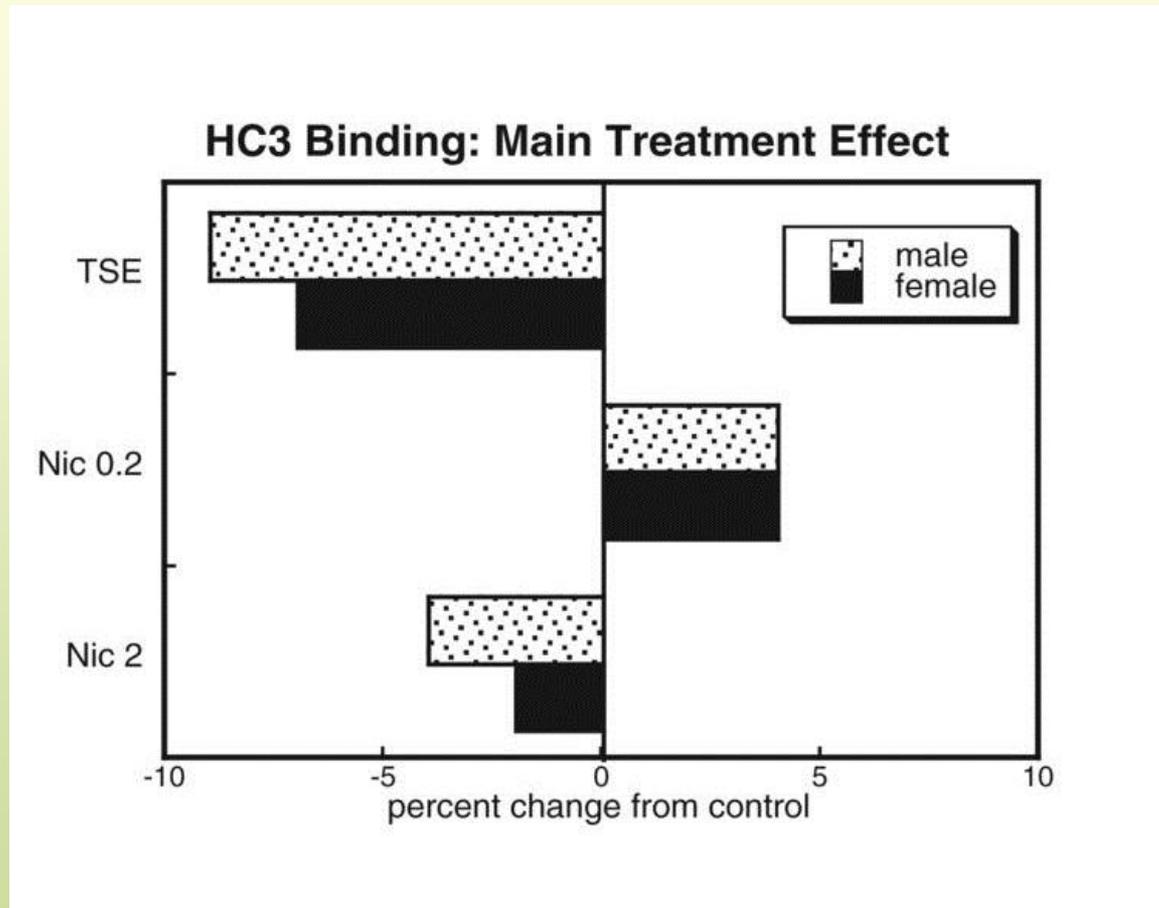


- PN30 (adolescence)
- PN60
- PN100
- PN150
- Frontal/parietal cortex
- Temporal/occipital cortex
- Hippocampus
- Striatum
- Midbrain
- Brainstem

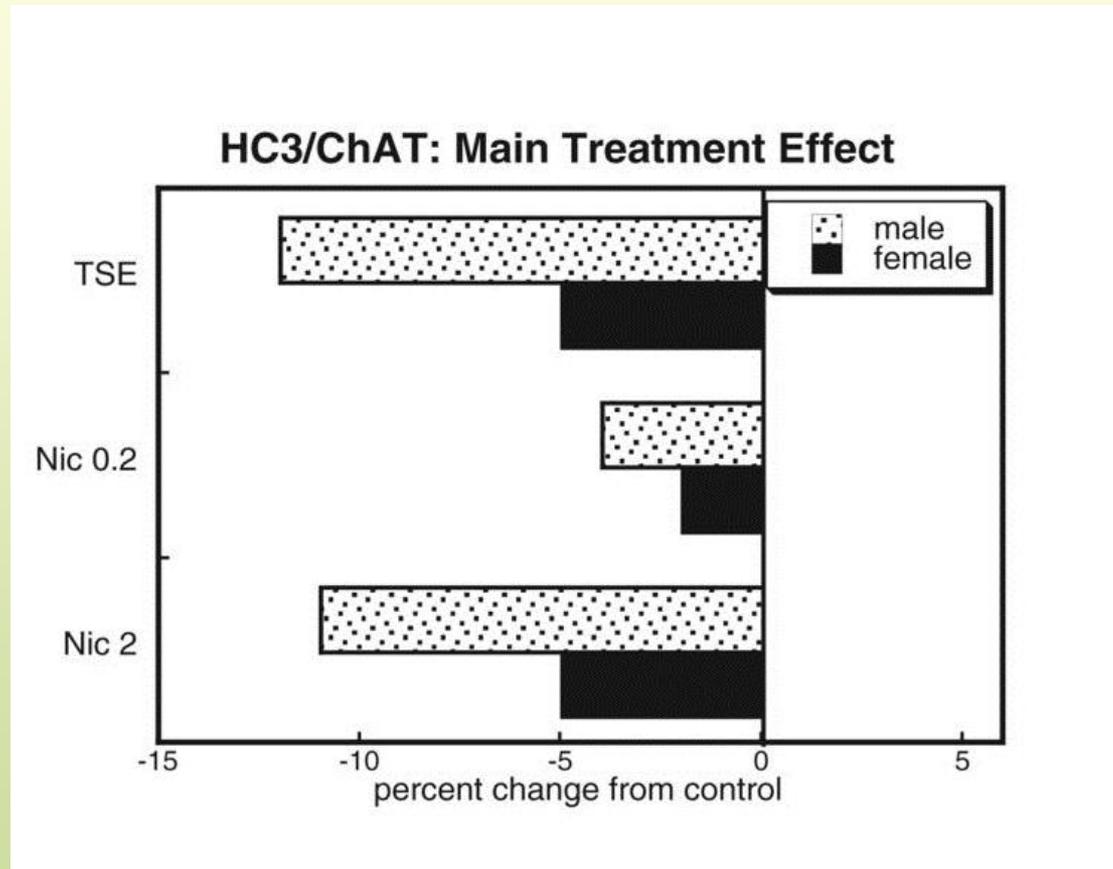
Effect of Exposure on Choline Acetyltransferase Activity



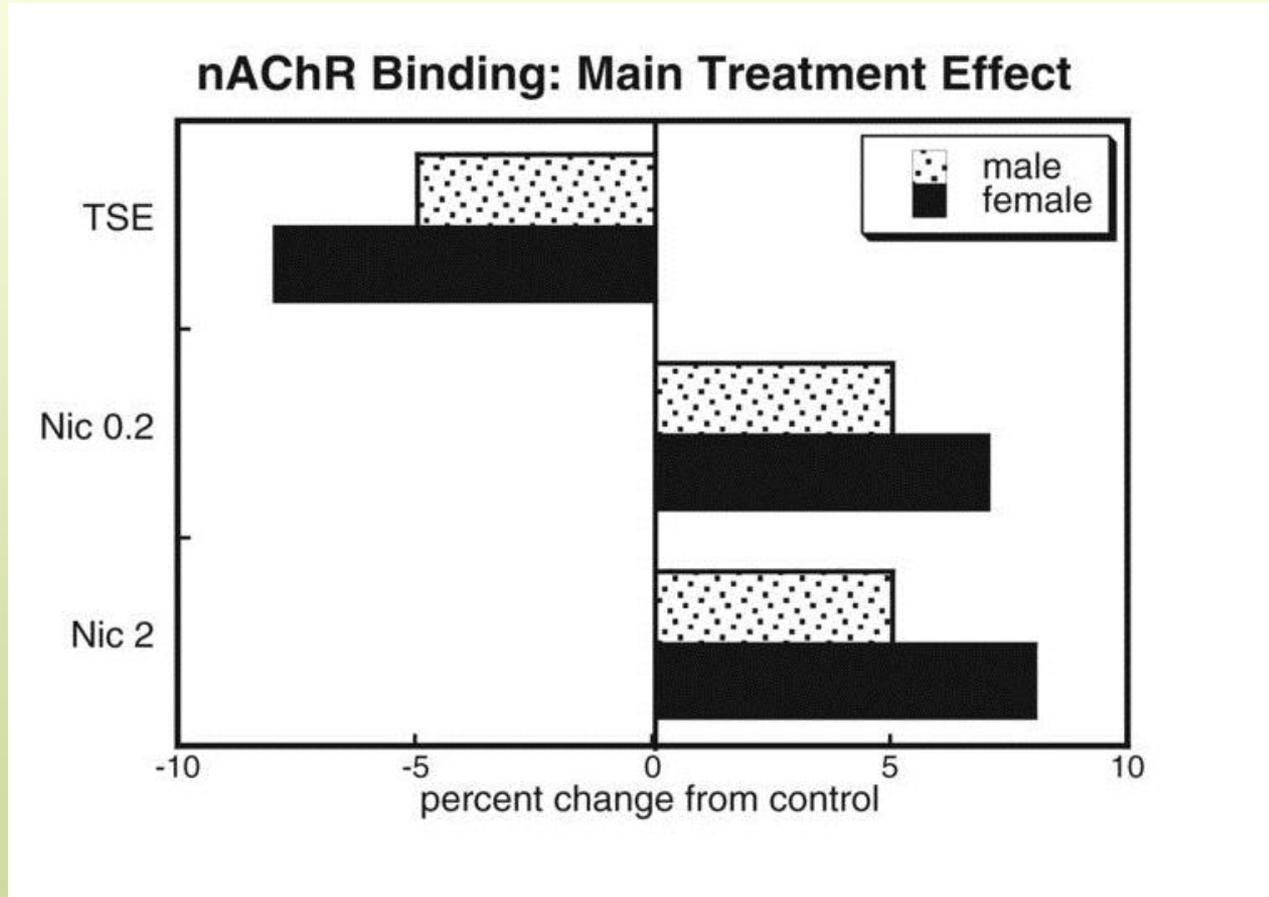
Effect of Exposure on Hemicholinium-3 Binding



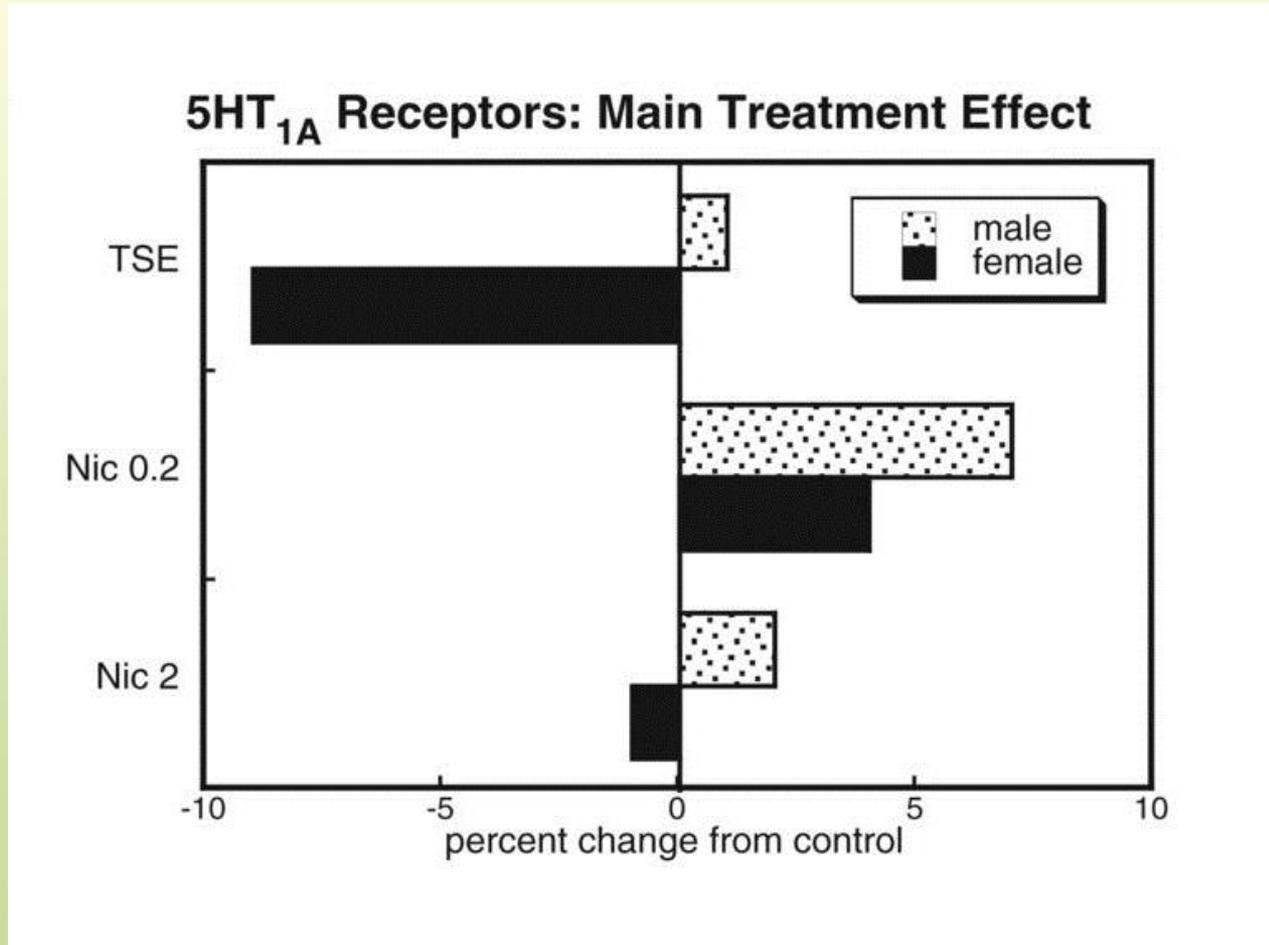
Effect of Exposure on Hemicholinium-3 / Choline Acetyltransferase Ratio



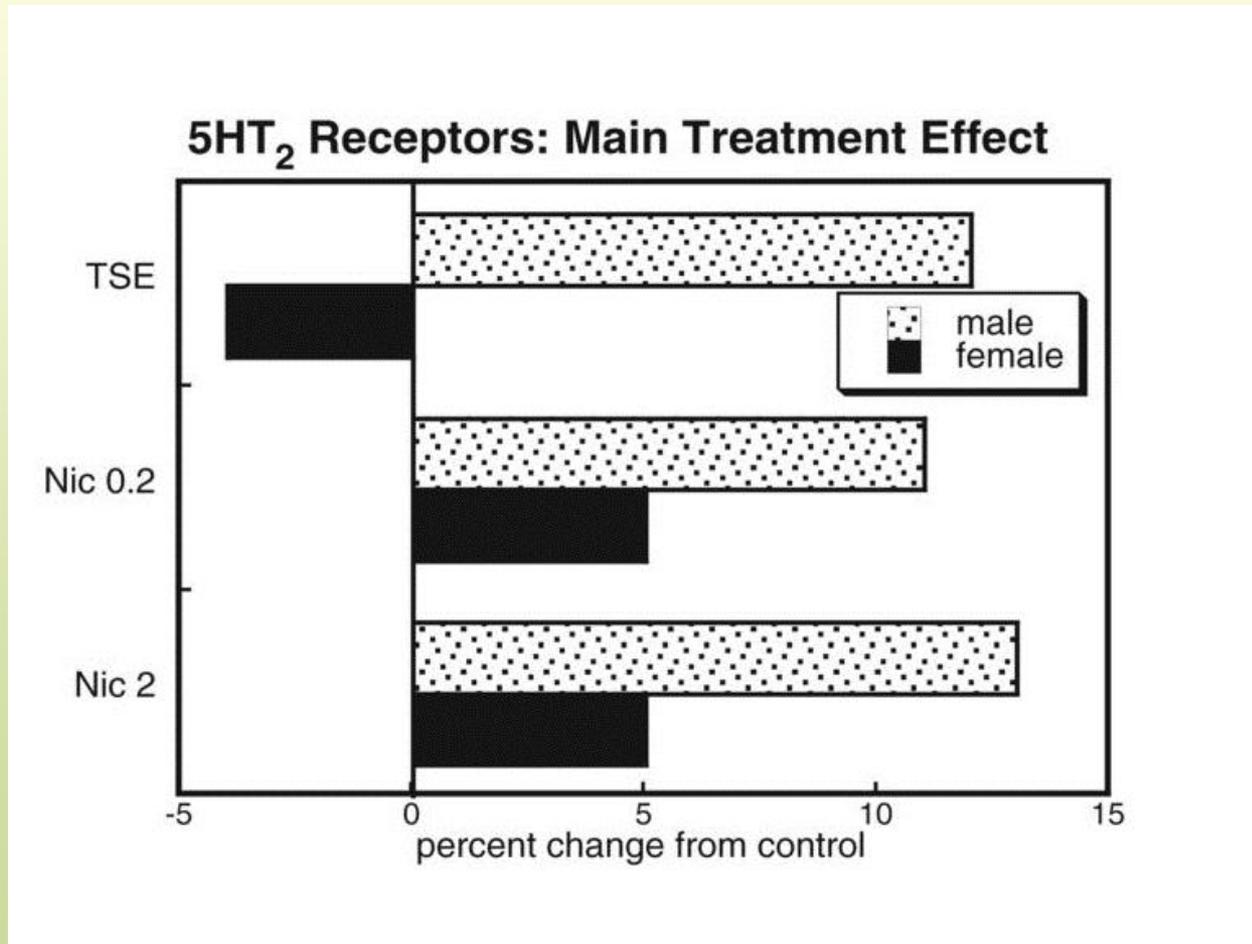
Effect of Exposure on Nicotinic Acetylcholine Receptor Binding



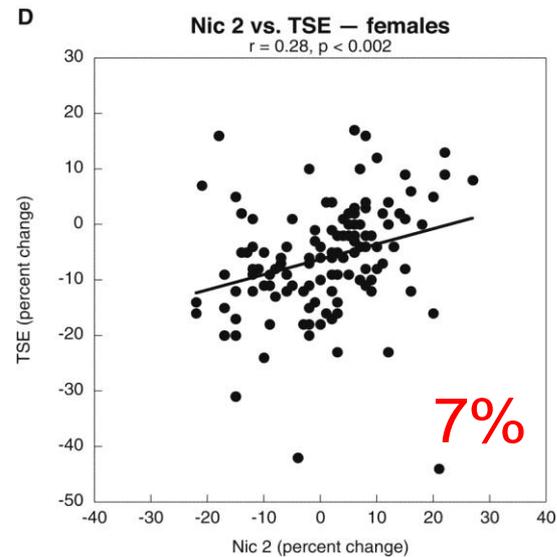
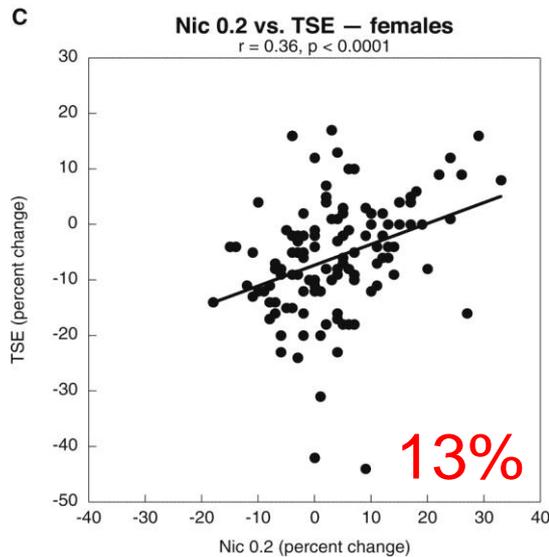
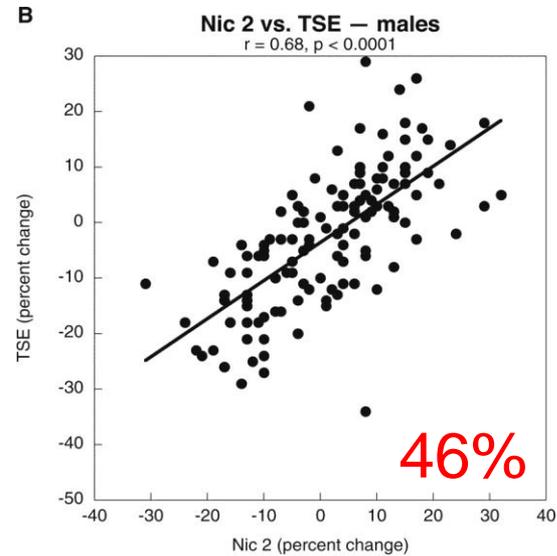
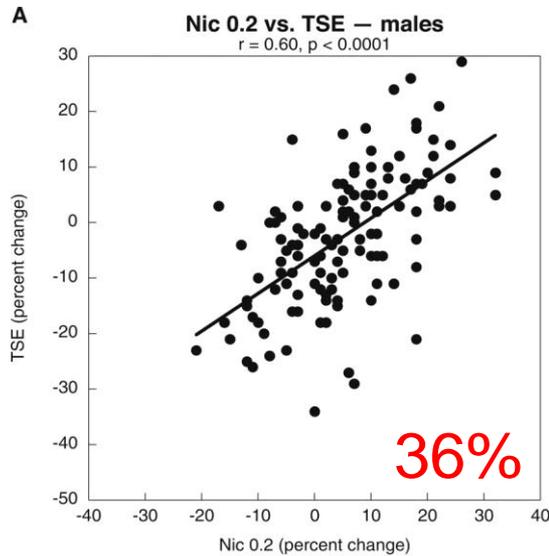
Effect of Exposure on Serotonin Receptor 1A Binding



Effect of Exposure on Serotonin Receptor 5HT₂ Binding



Correlations Across all Parameters



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, worsen ingdecreased 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**