

# Sex-Dependent Neurotoxicity of a Mixture of Low-Dose EDCs

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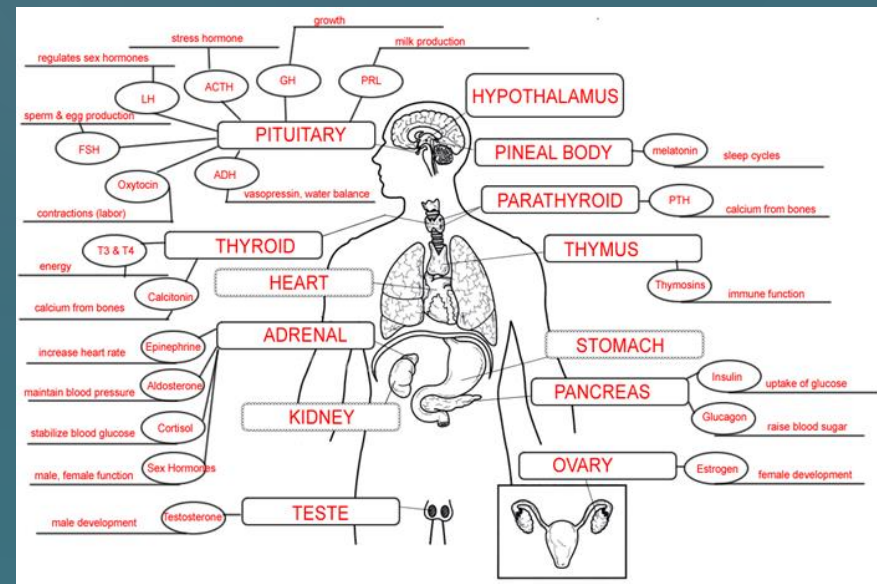
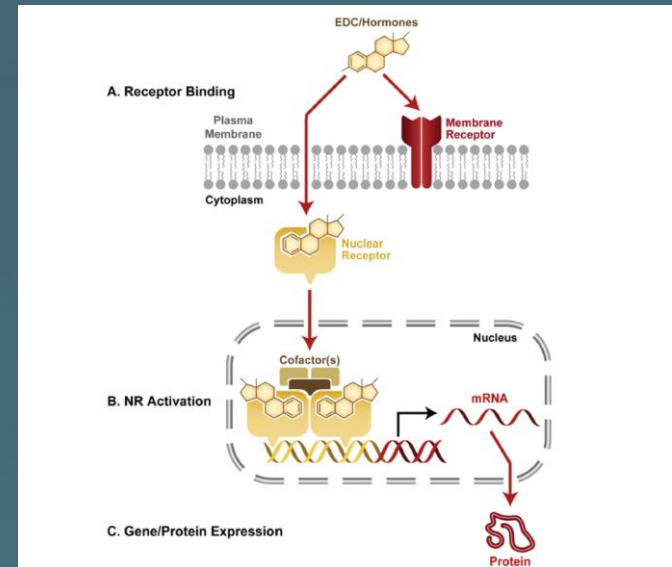
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# Endocrine Systems Coordinate Development

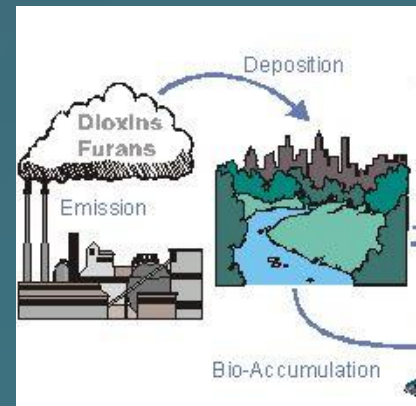
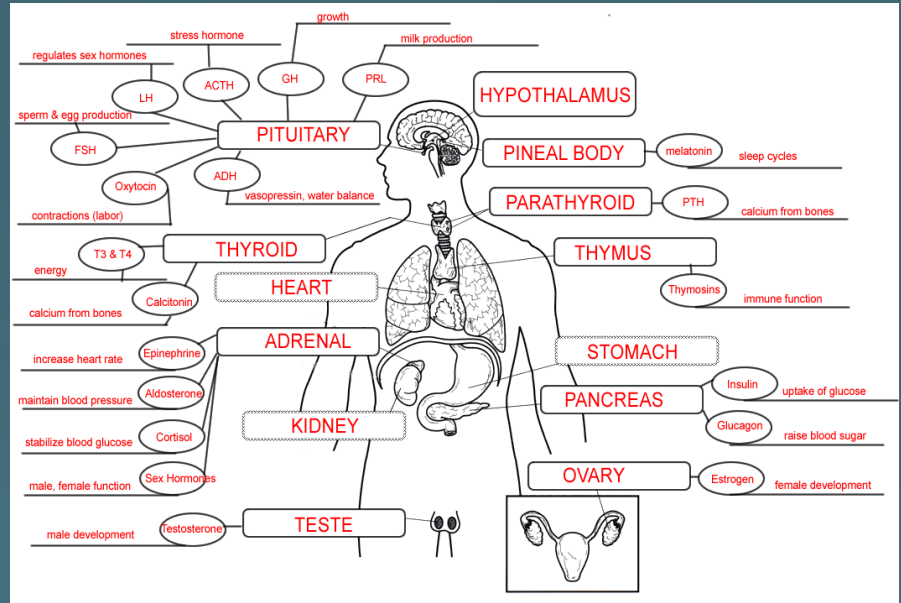
The endocrine system is the body's chemical communication system and aids in maintaining stasis through change, either developmental or environmental.

Developmentally, it plays an essential role in communicating and organizing growth, sex-specific reproductive and brain development.



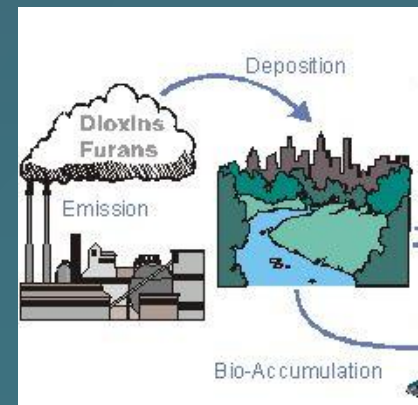
# Endocrine Disrupting Chemical

EDC is any chemical that interferes with the hormone activity, including the production, secretion, transportation, metabolism, binding action, and/or excretion of endogenous hormones.

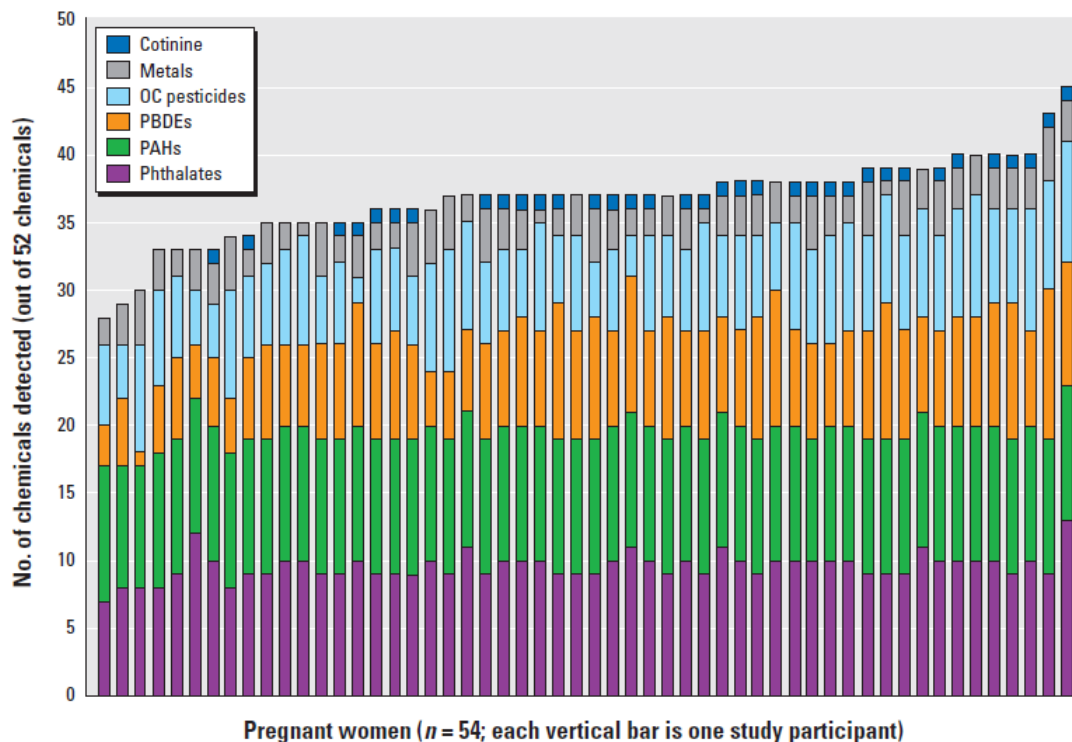
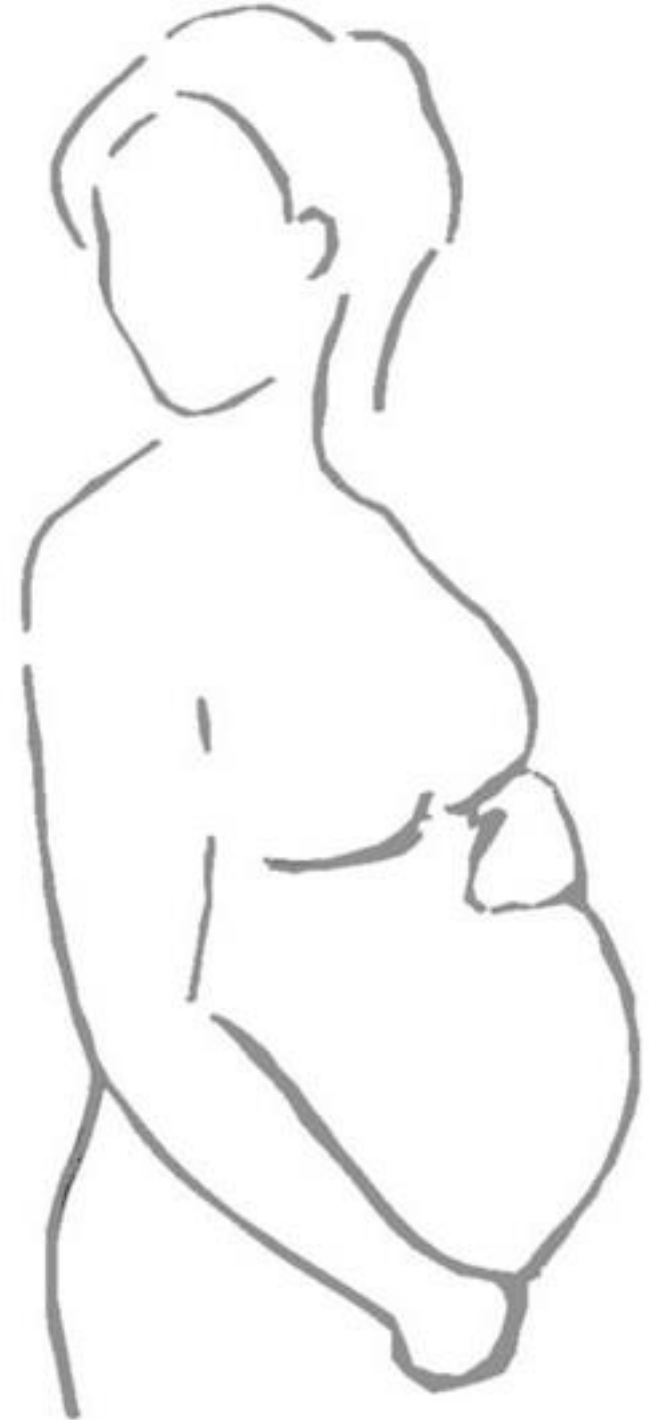


# Endocrine Disrupting Chemicals

Over a 1,000 xenobiotics have been recognized to have an endocrine active properties.




# Maternal Exposure



**Figure 3.** Number of chemicals detected by chemical class in U.S. pregnant women, NHANES subsample B [metals, cotinine, organochlorine (OC) pesticides, phthalates, brominated flame retardants (PBDEs), and PAHs], 2003–2004 ( $n = 54$ ). Each vertical bar represents one study participant. Other subsamples showed similar results.

# Exposures at Birth

## Examining Endocrine Disruptors Measured in Newborn Dried Blood Spots and Early Childhood Growth in a Prospective Cohort

Edwina H. Yeung , Erin M. Bell, Rajeshwari Sundaram, Akhgar Ghassabian, Wanli Ma, Kurunthachalam Kannan, Germaine M. Louis

First published: 20 December 2018 | <https://doi.org/10.1002/oby.22332> | Cited by: 1

Measurable levels were detected in 99.9% of samples for PFOS, 99.5% for PFOA and 90% for BPA, reflecting ubiquitous exposure in newborns.

Median (IQR) concentrations PFOS, PFOA, and BPA were 7.8 ng/ml (3.4 to 14.6), 1.7 ng/ml (1.1, 2.4) and 1.1 ng/ml (0.7, 1.6), with NICU babies showing elevated BPA with a median of 8.4 ng/ml.

# Male Reproductive Syndrome and Phthalate Mixtures

Increasingly, studies demonstrate enhanced male reproductive dysfunction from combinations of anti-androgens **even those acting by different mechanisms and administered at sub-threshold doses** (Howdeshell et al. 2007; 2008; 2015; Rider et al. 2010).

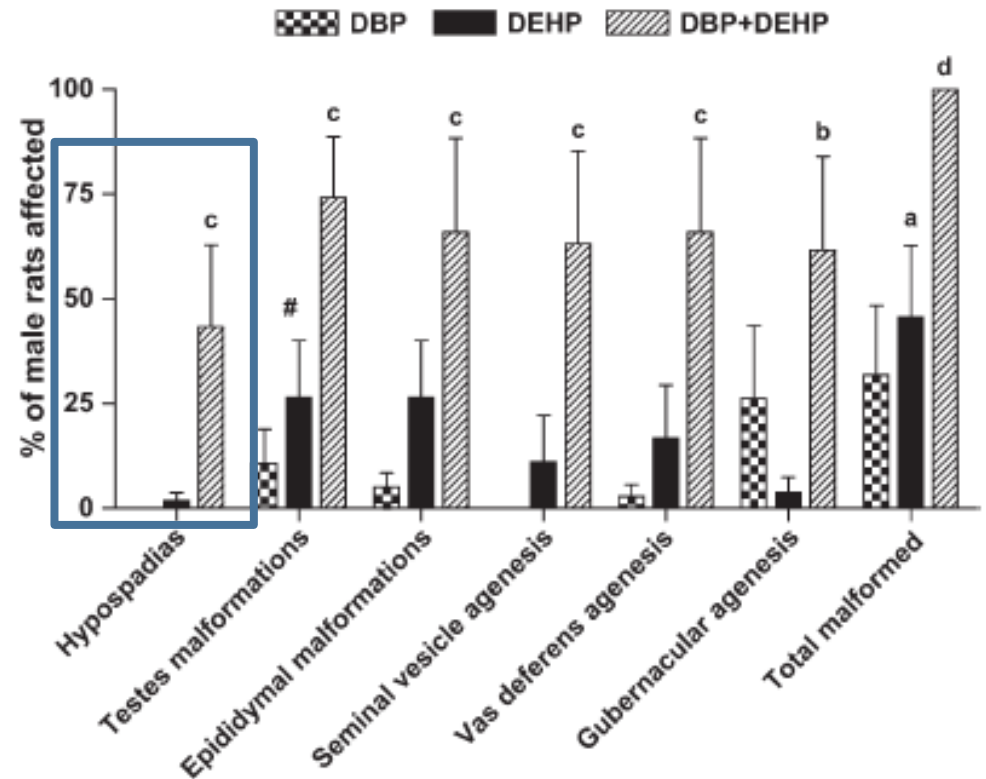
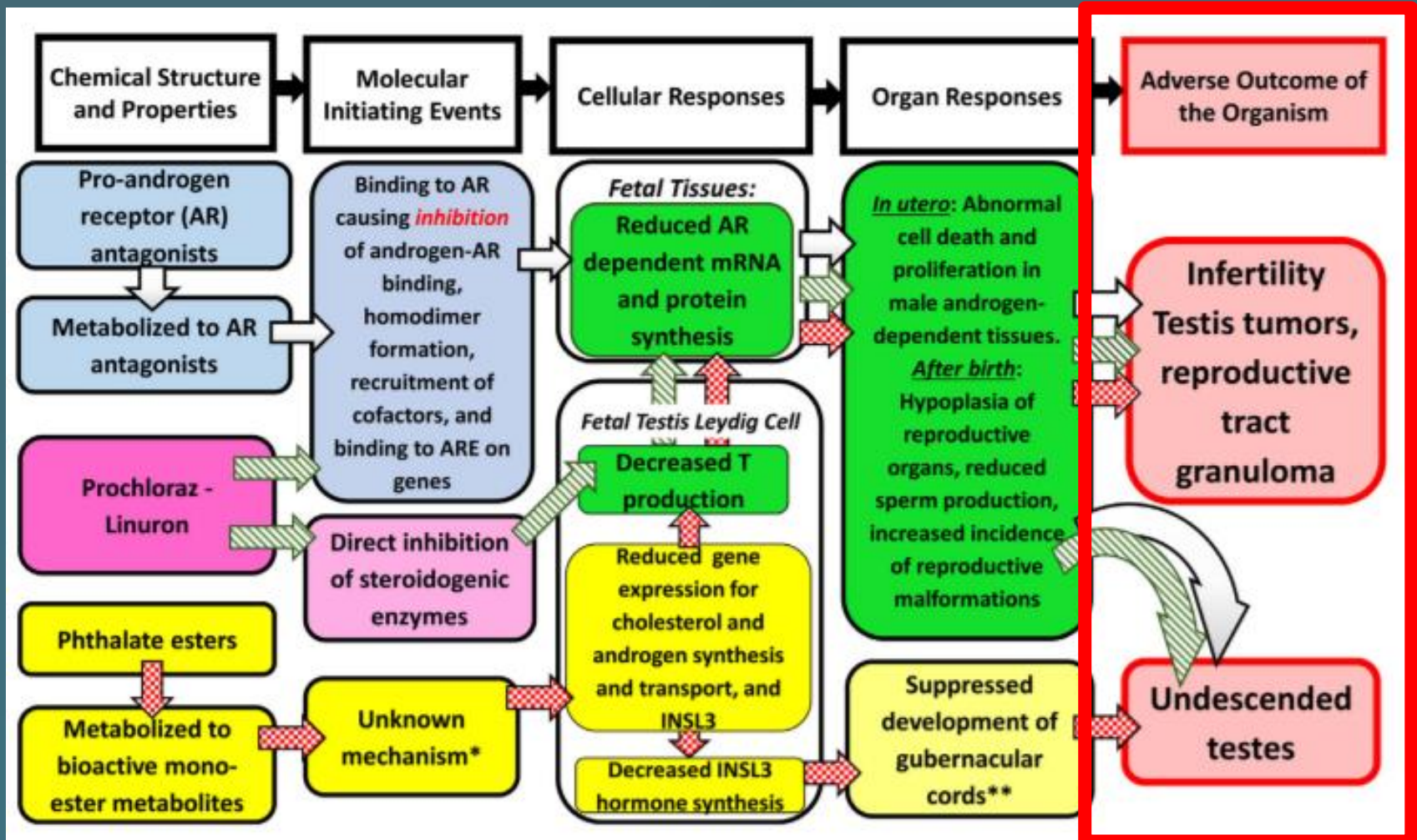


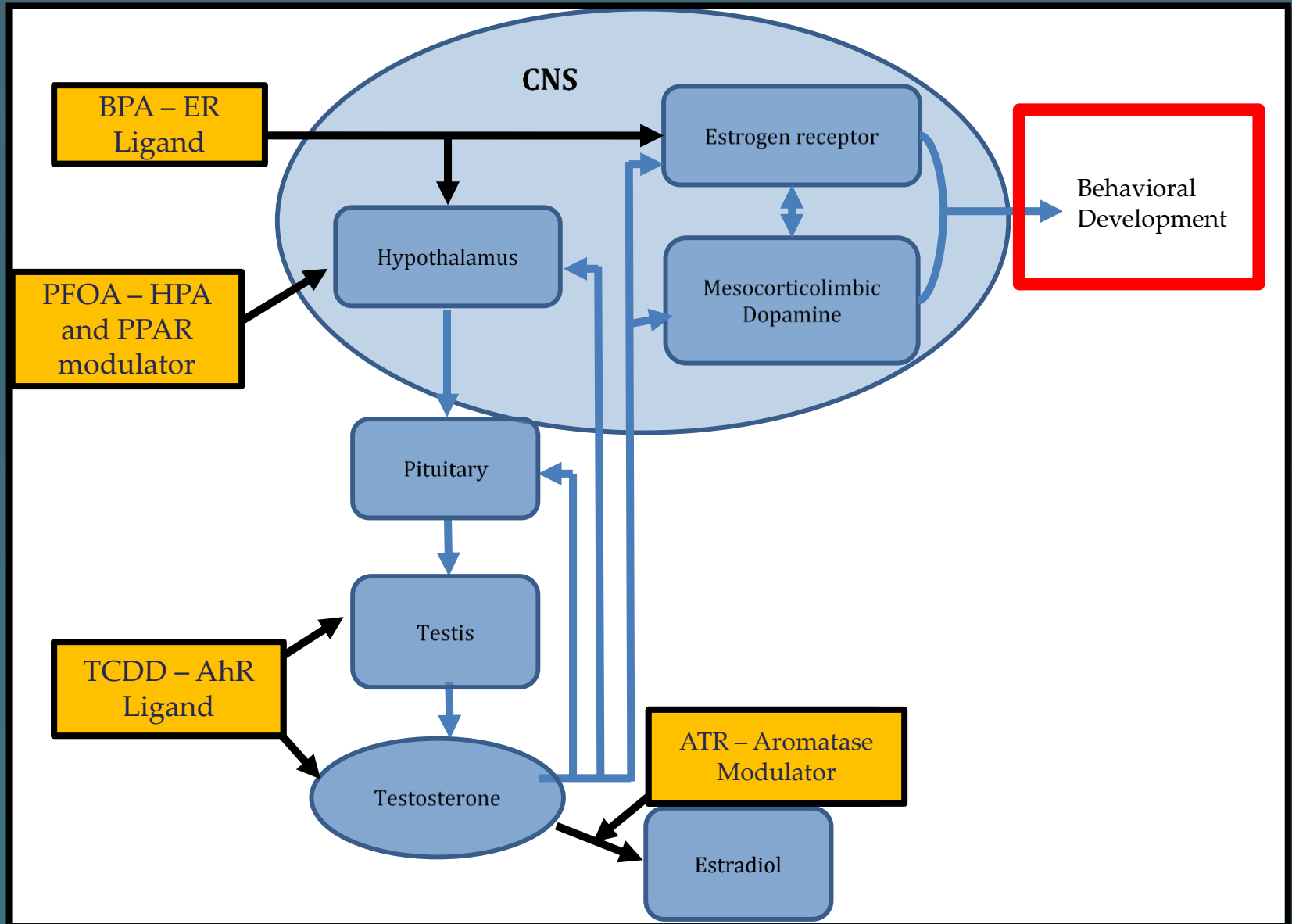
FIG. 2. External and internal reproductive abnormalities in adult male rats prenatally exposed to corn oil (control), DBP (500 mg/kg/day), and/or DEHP (500 mg/kg/day) on GD14-18. <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.005$ , <sup>c</sup> $p < 0.001$ , <sup>d</sup> $p < 0.0001$ , and <sup>#</sup> $p = 0.07$  versus controls.  $n = 6$  litters per treatment, except DBP + DEHP with  $n = 4$  litters. Values are litter mean  $\pm$  SEM. Control males did not exhibit reproductive abnormalities.

# Molecular Mechanisms of Mixtures





# SHARED PHYSIOLOGY: Multiple Hits Hypothesis



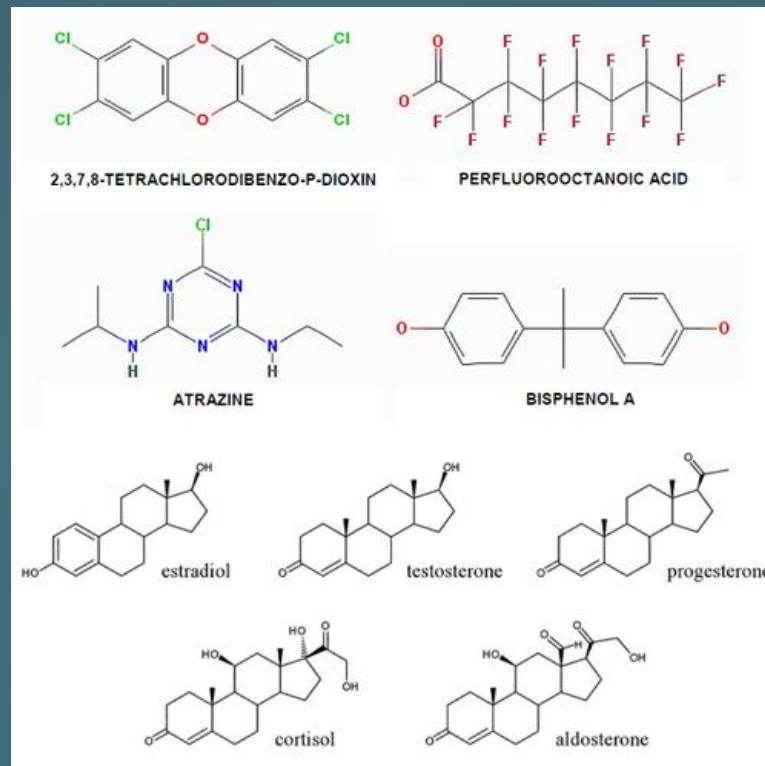
# EDCs and Neurotoxicity

EDC	Atrazine (ATR)	Bisphenol A (BPA)	Perfluorooctanoic acid (PFOA)	2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)
Category	Broad spectrum herbicide	Widely used plastic	Representative perfluorinated compound	Prototypic member of a large family of pollutants (dioxin-like compounds)
Physiological Targets	HPA axis modulator; alters aromatase activity, known developmental and reproductive toxicant.	ER ligand, possible ligand for other receptors; alters pubertal development, reproductive tissue activity.	PPARα ligand interacting with estrogen to influence metabolic homeostasis, HPA axis modulator.	AhR ligand; known to modulate ER expression, reproductive and developmental toxicant.
Rodent Behavioral and Neuroendocrine Outcomes	Decreased locomotor activity Decreases dopamine Kills brain dopamine neurons and non-neuronal cells.	Spatial learning and memory in young mice Hyperactivity Increased reward seeking behavior Impulsivity and Neophobia Altered development of dopaminergic systems Neocortical development by accelerating neuronal differentiation/migration Impairs hippocampal neurogenesis	Changes in exploratory behavior in both male and female offspring, and increased home cage global activity only in males Increased pro-inflammatory cytokines Increased glial response in rat brain and enduring changes in synapsins and synaptophysin Decreased TH activity, in vitro PC12 neuronal cells, altering dopamine activity	Induces deficits in fear memory Abnormal social behavior Alters development of brain neuroendocrine systems as well as monoamines in rat cerebellum Developmental myelination and re-myelination in rat brain and disrupts neural precursor cell proliferation.

# Multiple Hits hypothesis for Adult Behavioral Toxicity

We hypothesized:

1. Developmental exposure to the mixture will uniquely produce behavioral dysfunction. Single EDCs would not alter behavioral function.



# Methods

Dams were treated from gestational day 7-weaning.

One pup / litter / sex / behavioral endpoint.

Soy-free food was used.

EDCs were administered orally via treats.



# Endocrine Disrupting Chemicals

Atrazine

(ATR – 10mg/kg/day),

Perfluorooctanoic acid

(PFOA – 0.1 mg/kg/day),

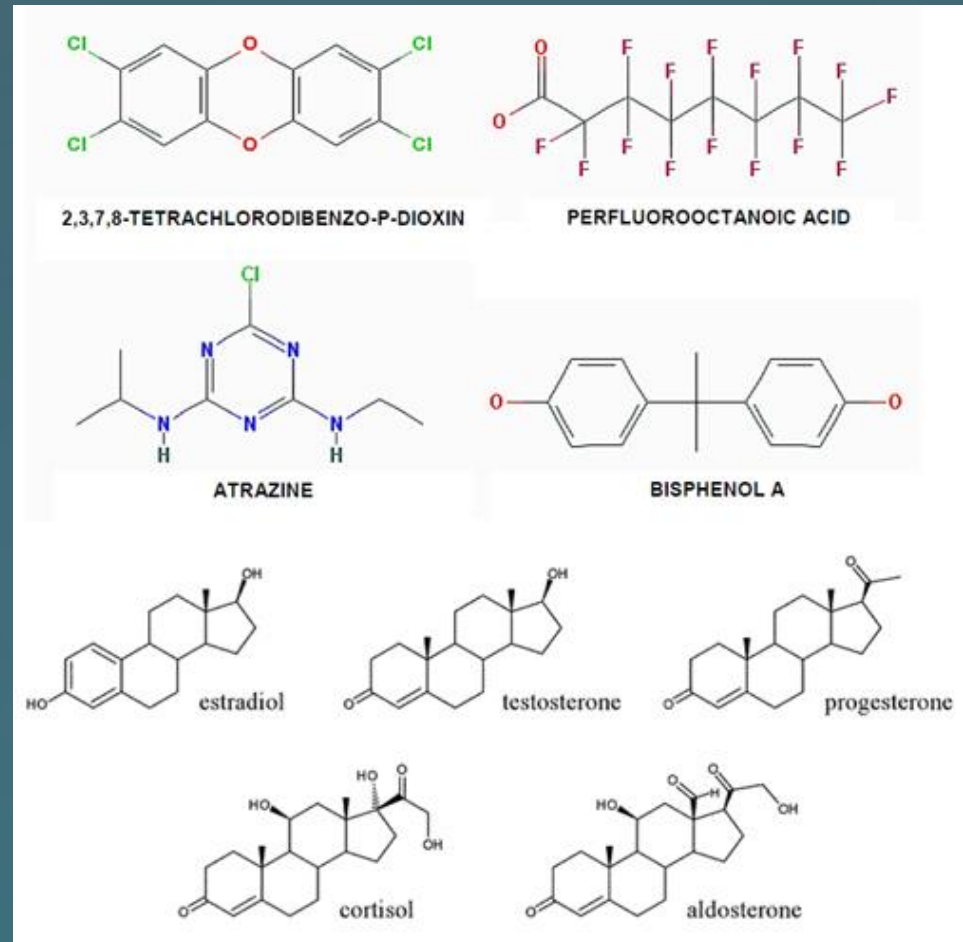
Bisphenol-A

(BPA - 50µg/kg/day)

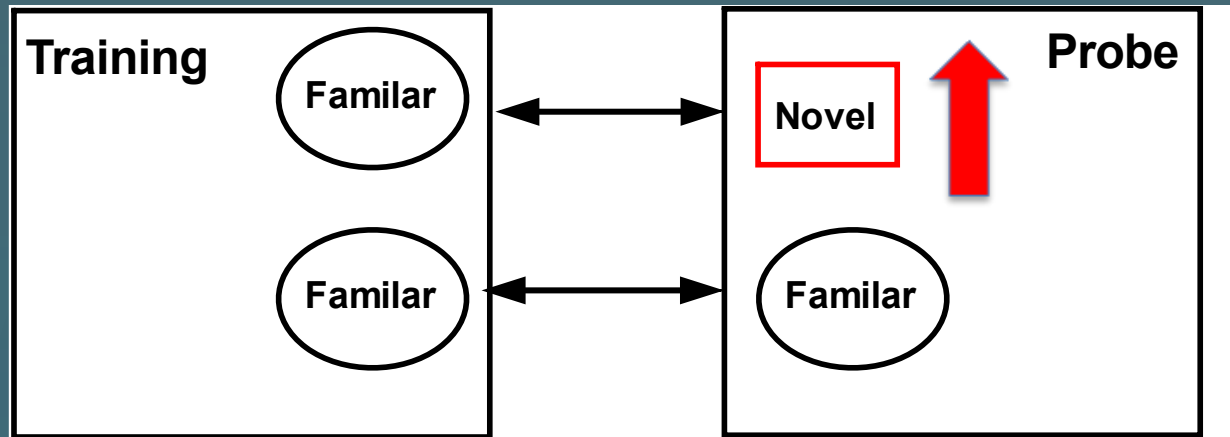
2,3,7,8-Tetrachlorodibenzo  
-p-dioxin

(TCDD – 0.035µg/kg/day)

Mixture (MIX) – All of the Above



# Novel Object Recognition



## Short Term Memory:

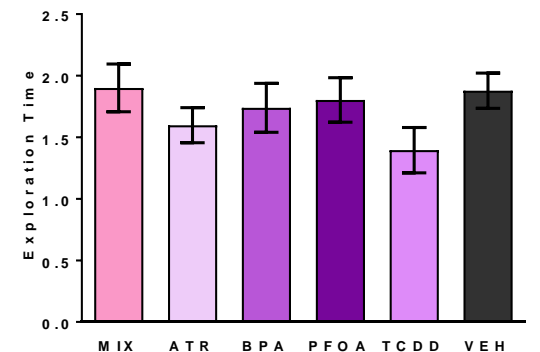
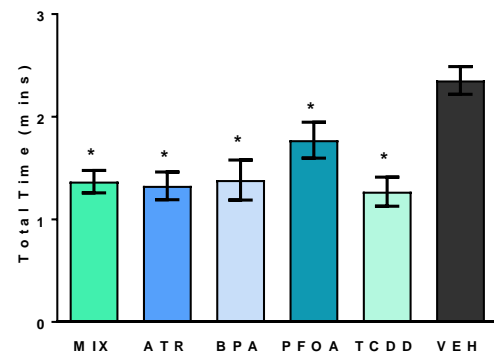
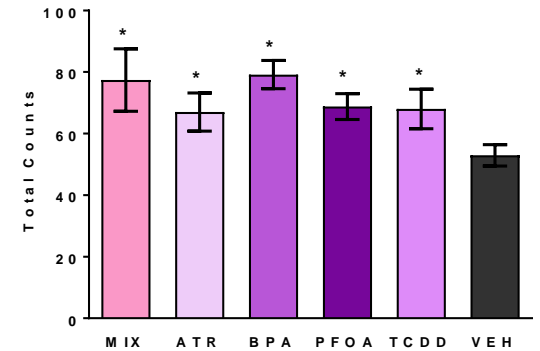
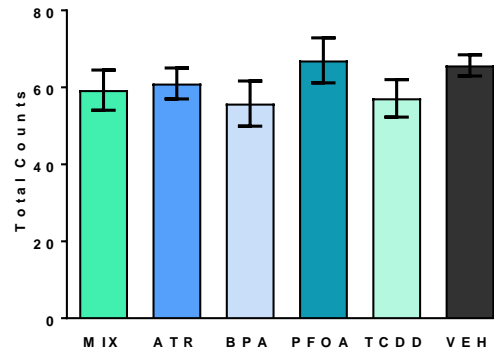
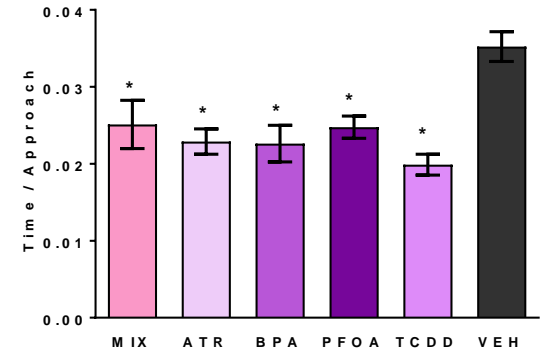
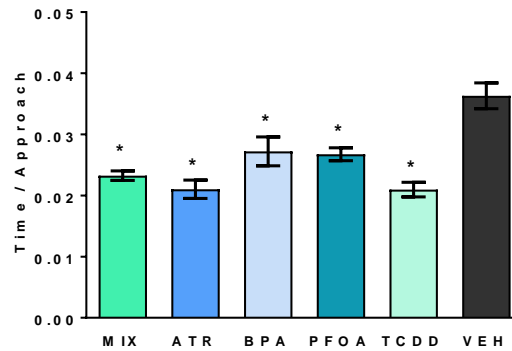
Time Spent with  
Novel Object  
Increases Based  
on Memory of  
Familiar Object

Fagan Test of  
Infant  
Intelligence



# Phase 1: Novel Object Habituation

Both sexes showed altered exploration patterns during object habituation, however males were more severely effected.

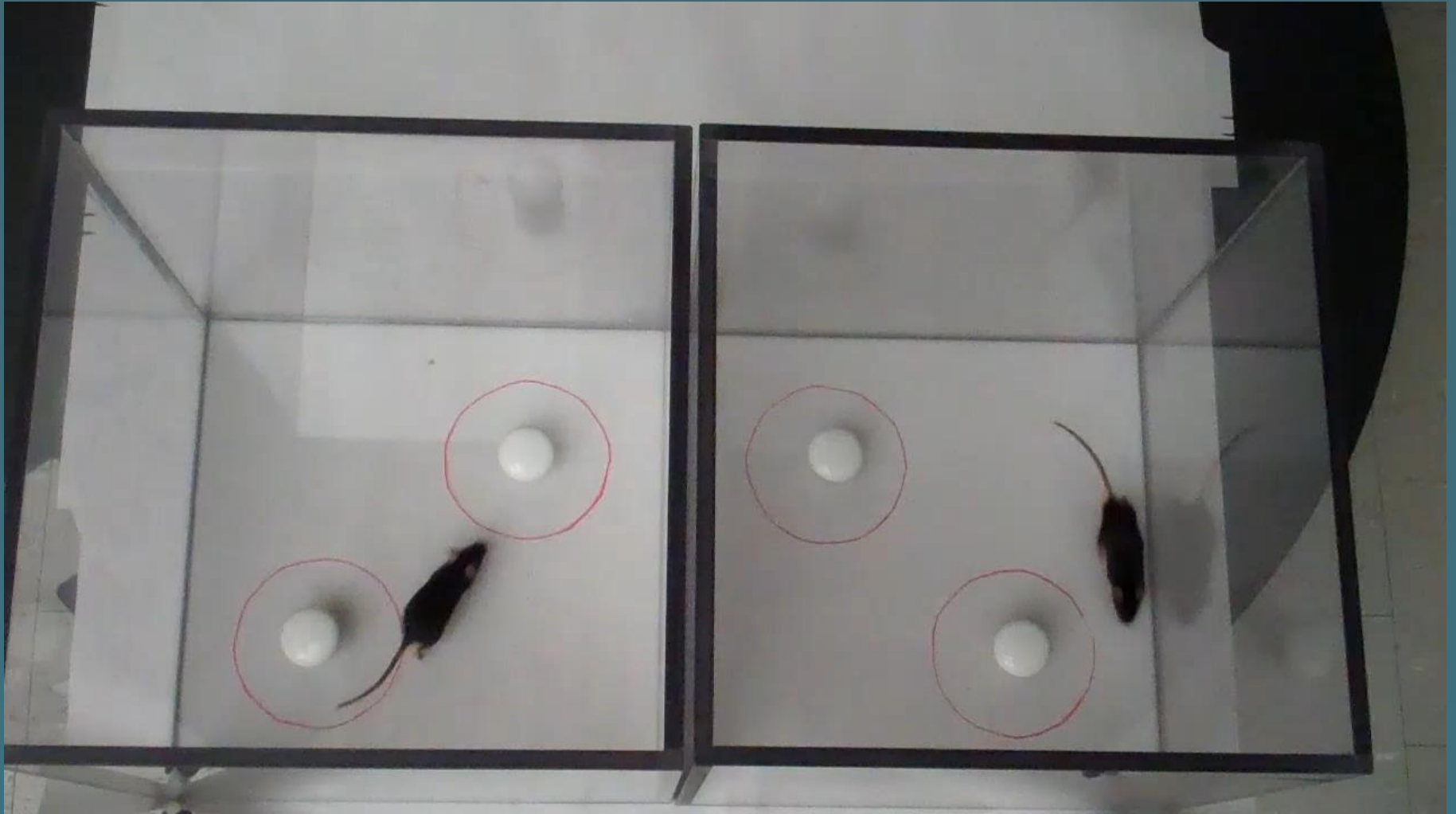


# Long Investigation Bout





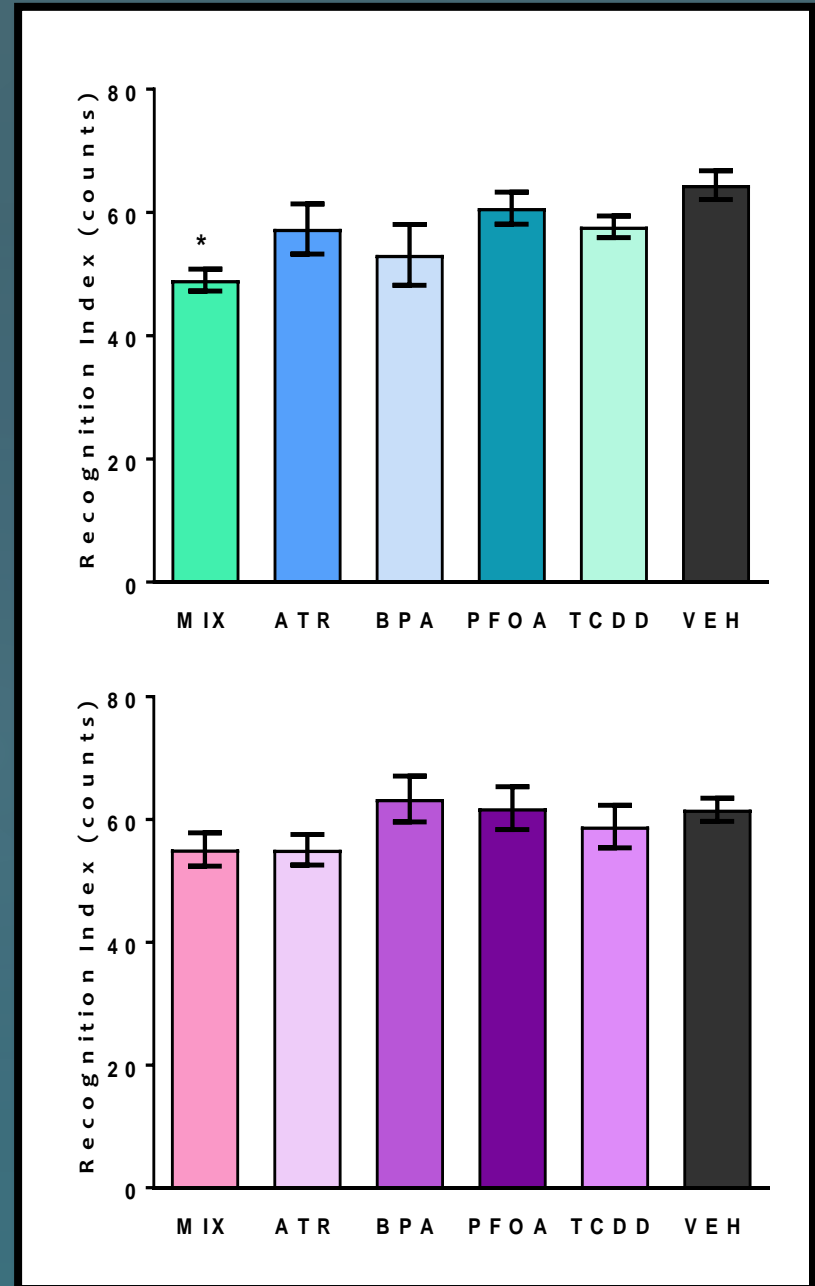
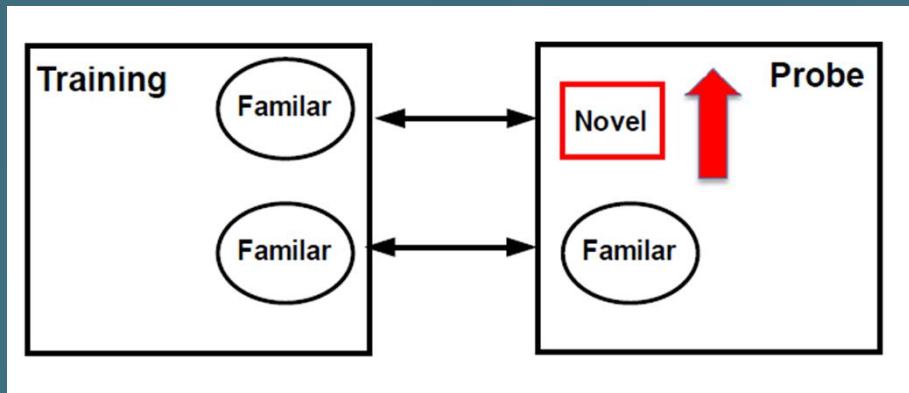
# Short Investigation Bout



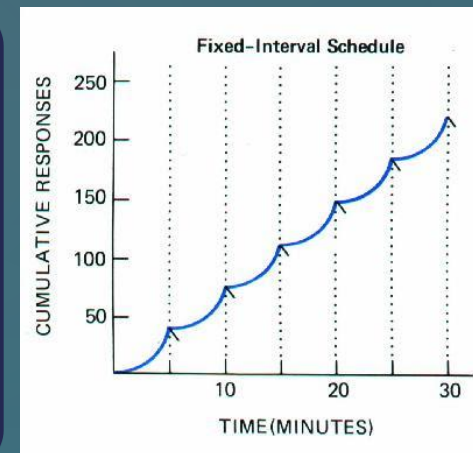
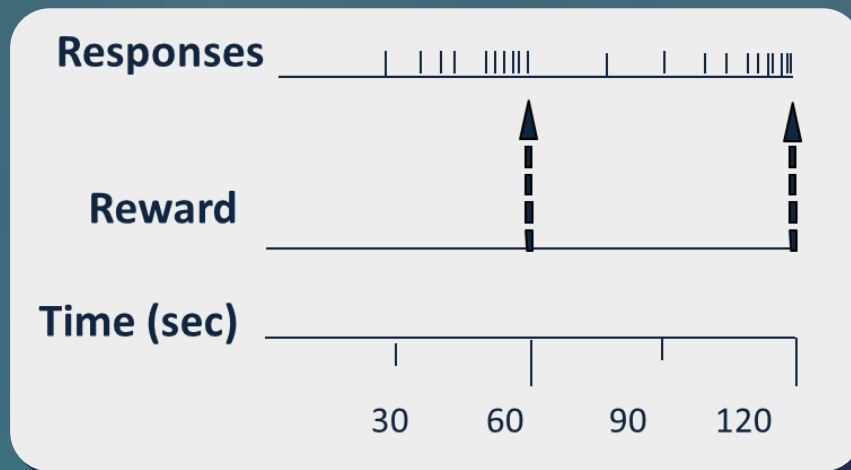
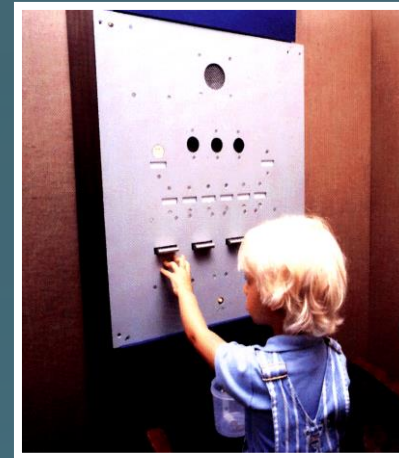
## Phase 2: Novel Object Recognition

Only males showed evidence of decreased ability to recognize the novel object.

MIX exposed males showed significantly decreased recognition index values compared to controls and all other single EDCs (Sobolewski et al. 2014).



# Fixed Interval Schedule of Food Reinforcement



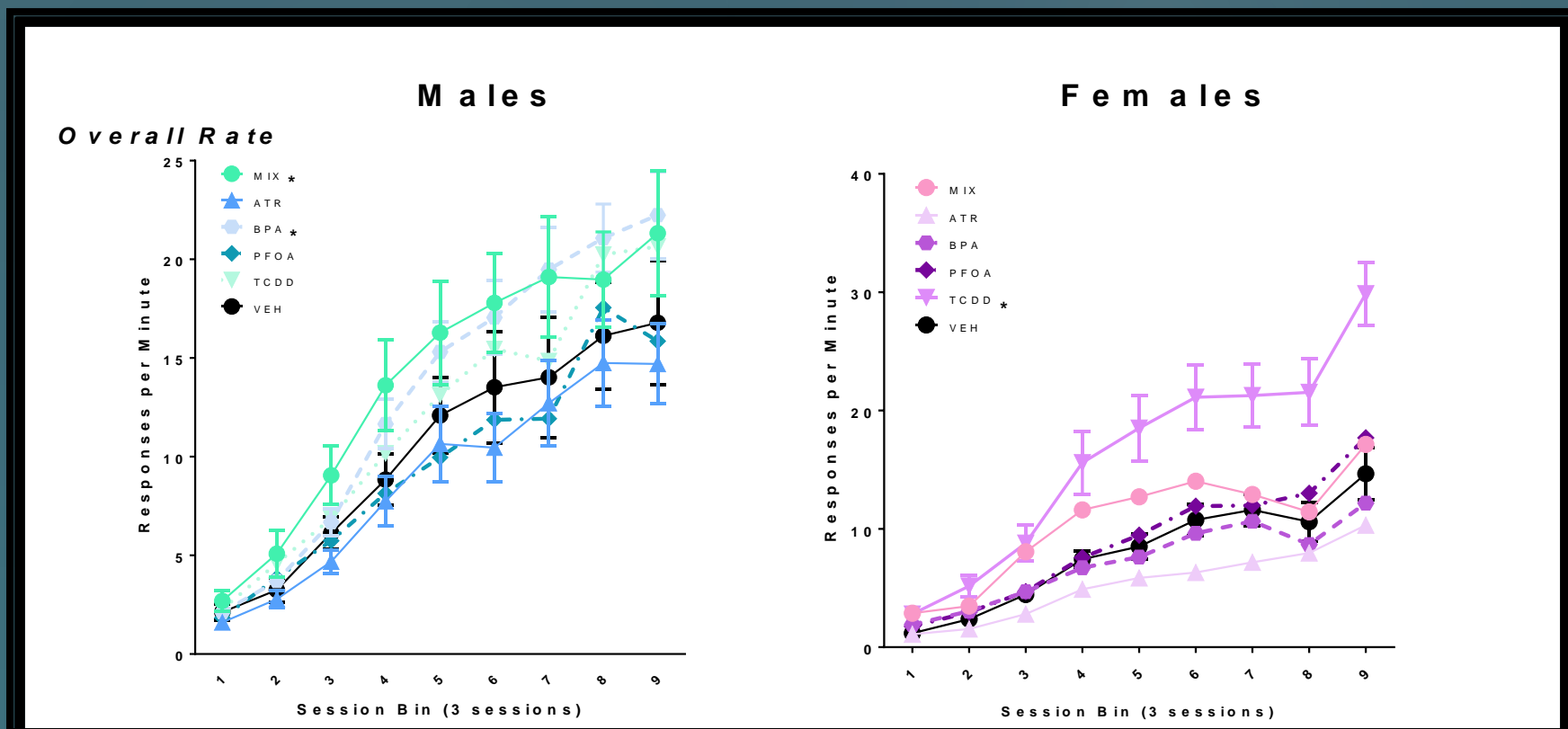
The first response occurring after a fixed interval of time has elapsed (60 sec in this example) results in reward delivery and the initiation of the next fixed interval of time. The schedule produces a pattern of responding that is very low early in the interval but increases as the time to reward approaches. Human example: studying for an exam

# Learning Phenotype: Fixed Interval (FI) Schedule

60

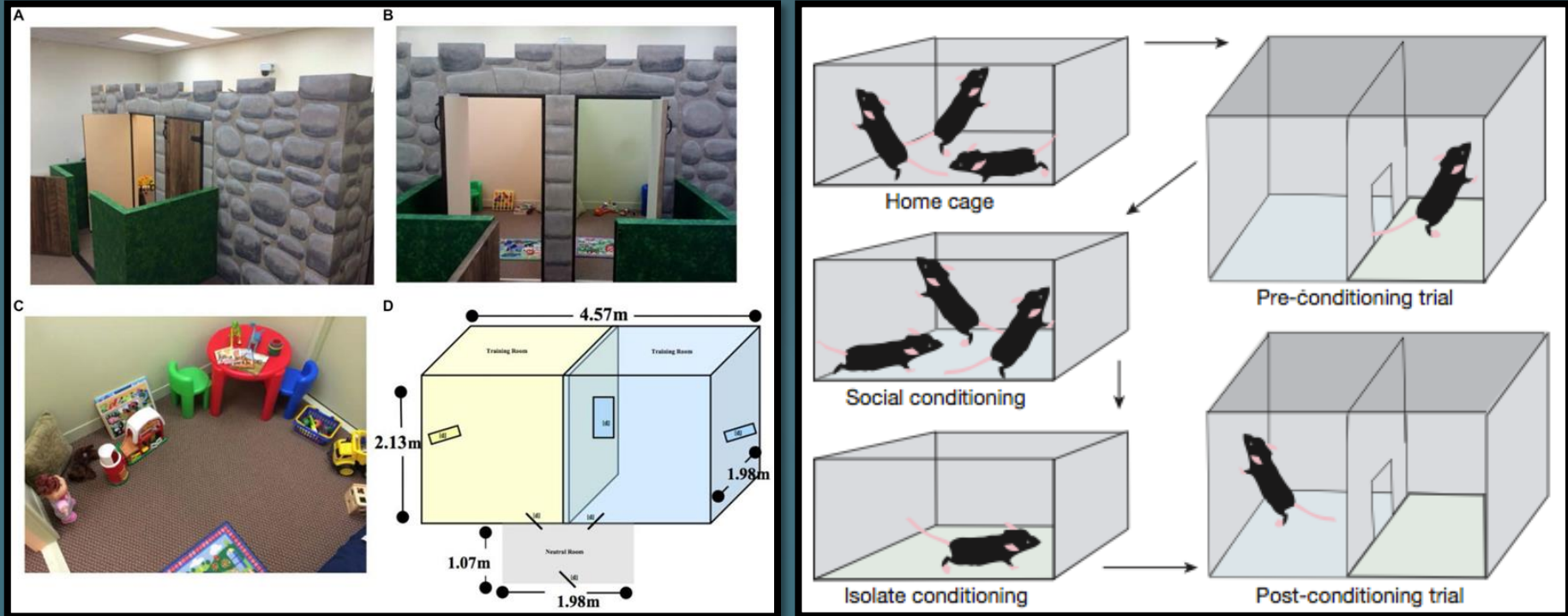


# MIX Increased Correct Lever Response Rates on the Fixed Interval Schedule of Food Reinforcement



Both sexes showed altered behavior under the FI schedule.

# Social Conditioned Place Preference



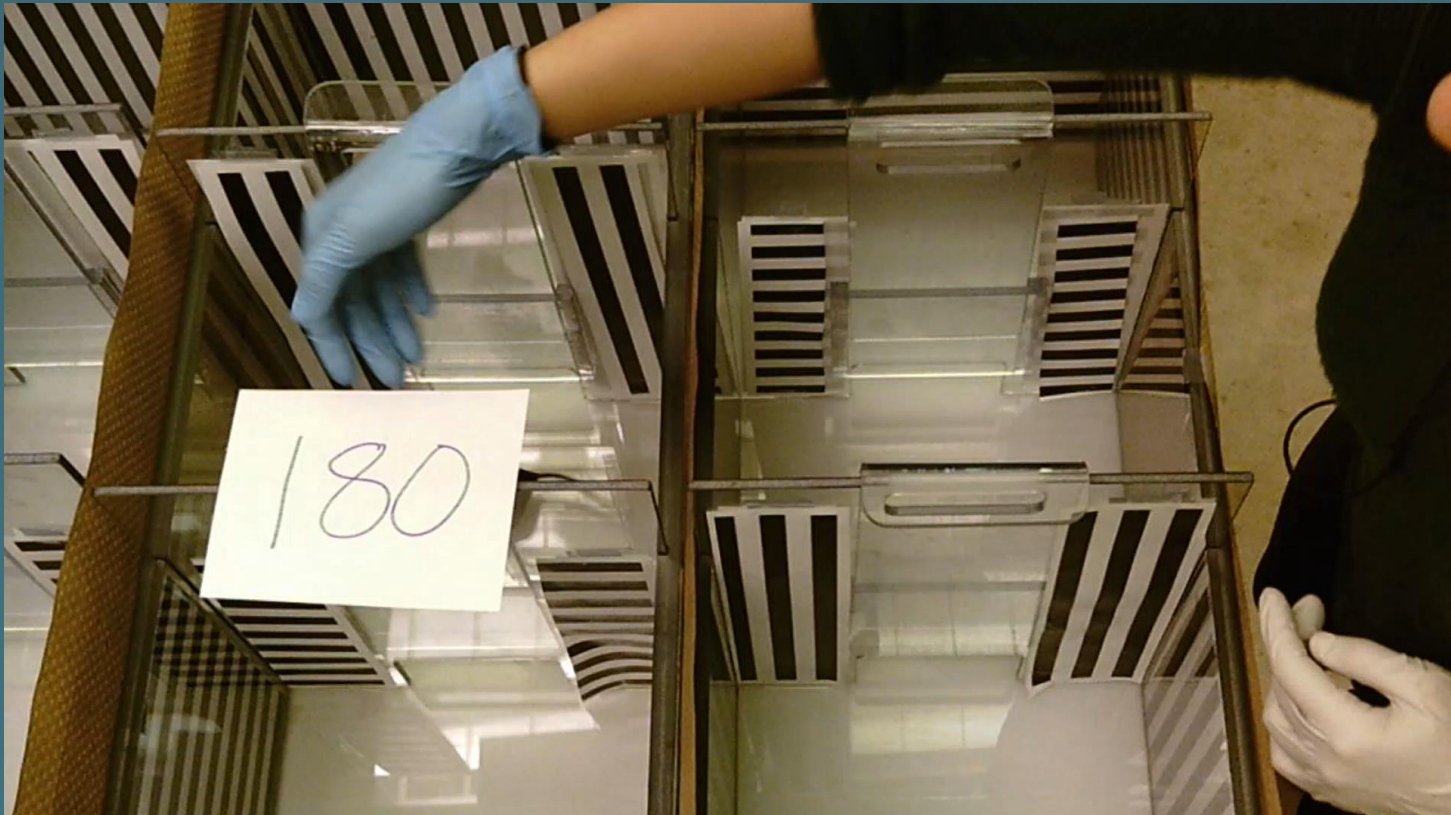
# Social Condition Place Preference (SCPP)

Social

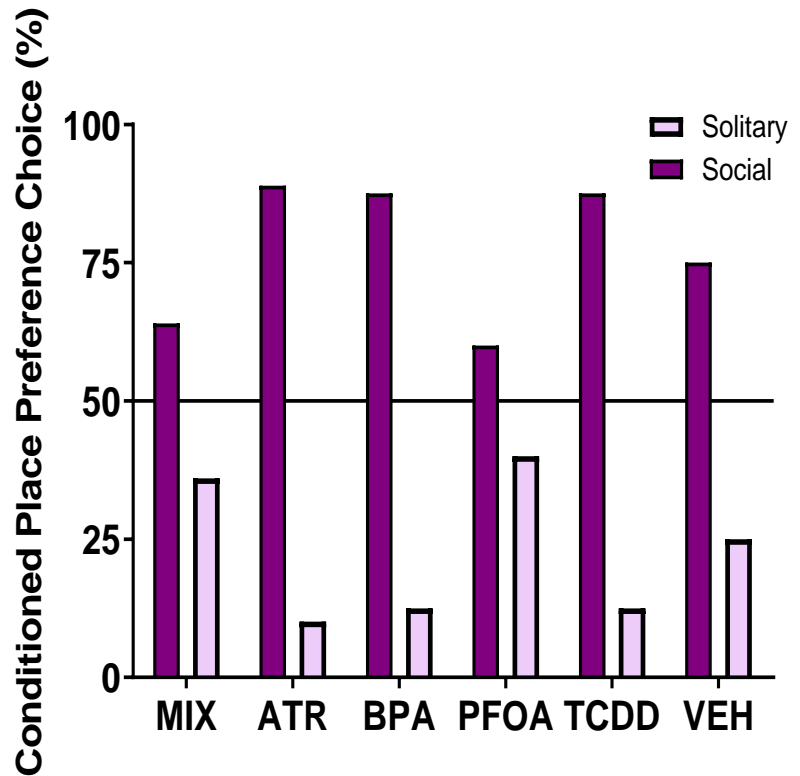
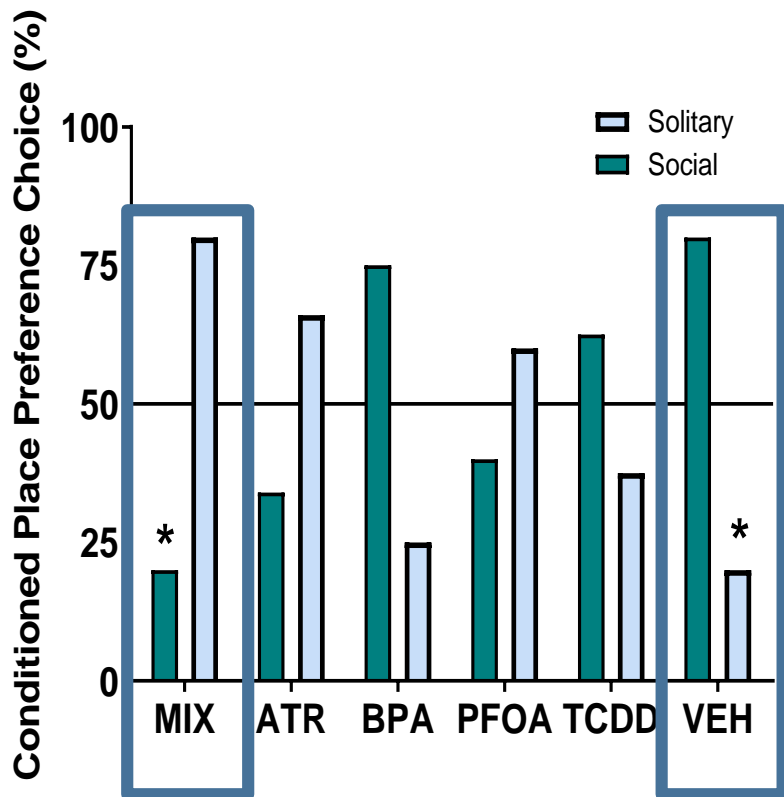


**Training:** 10 min each  
chamber x 7 days  
**Preference Test:** 5 min

Non-social

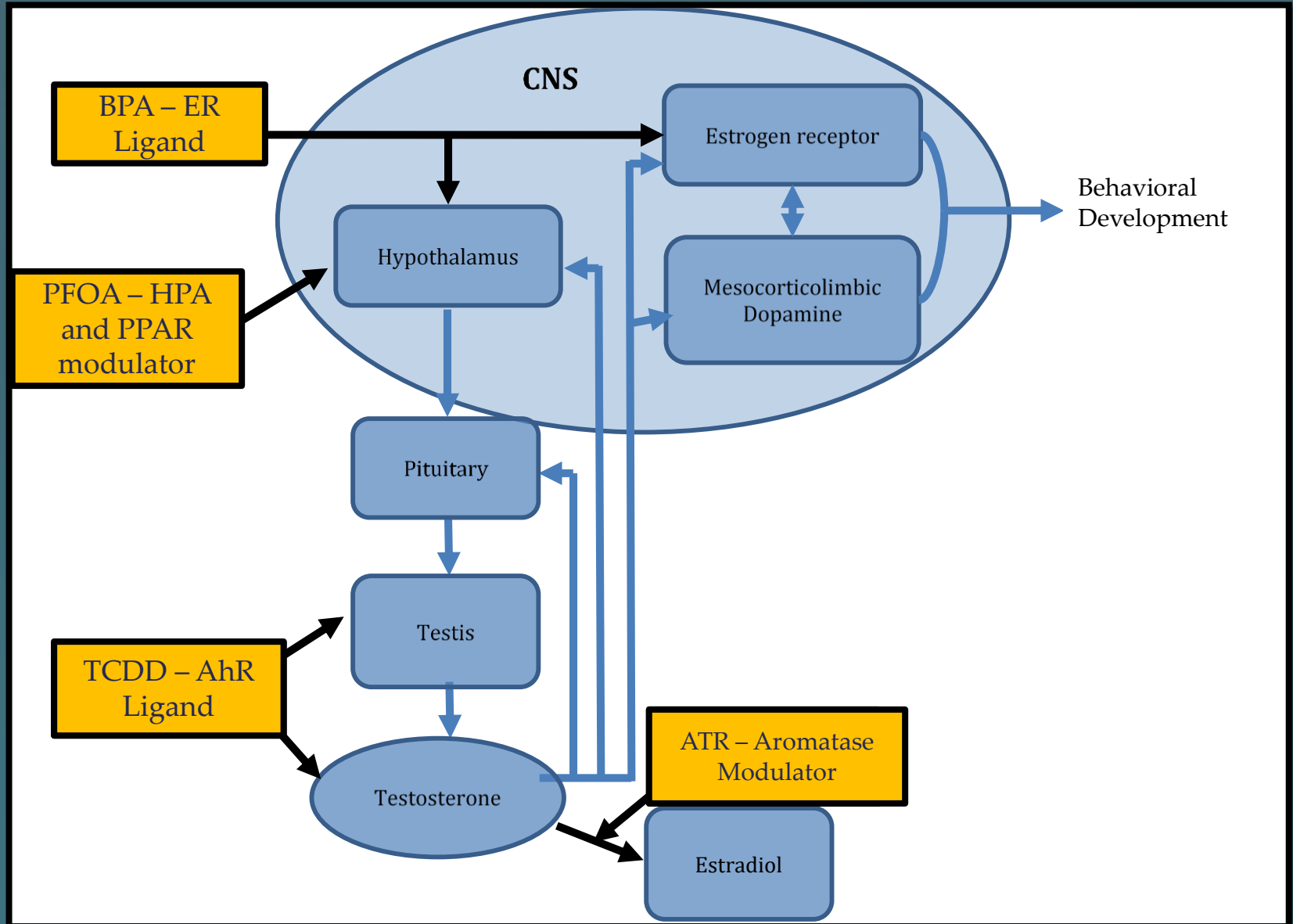


# Social Conditioned Place Preference

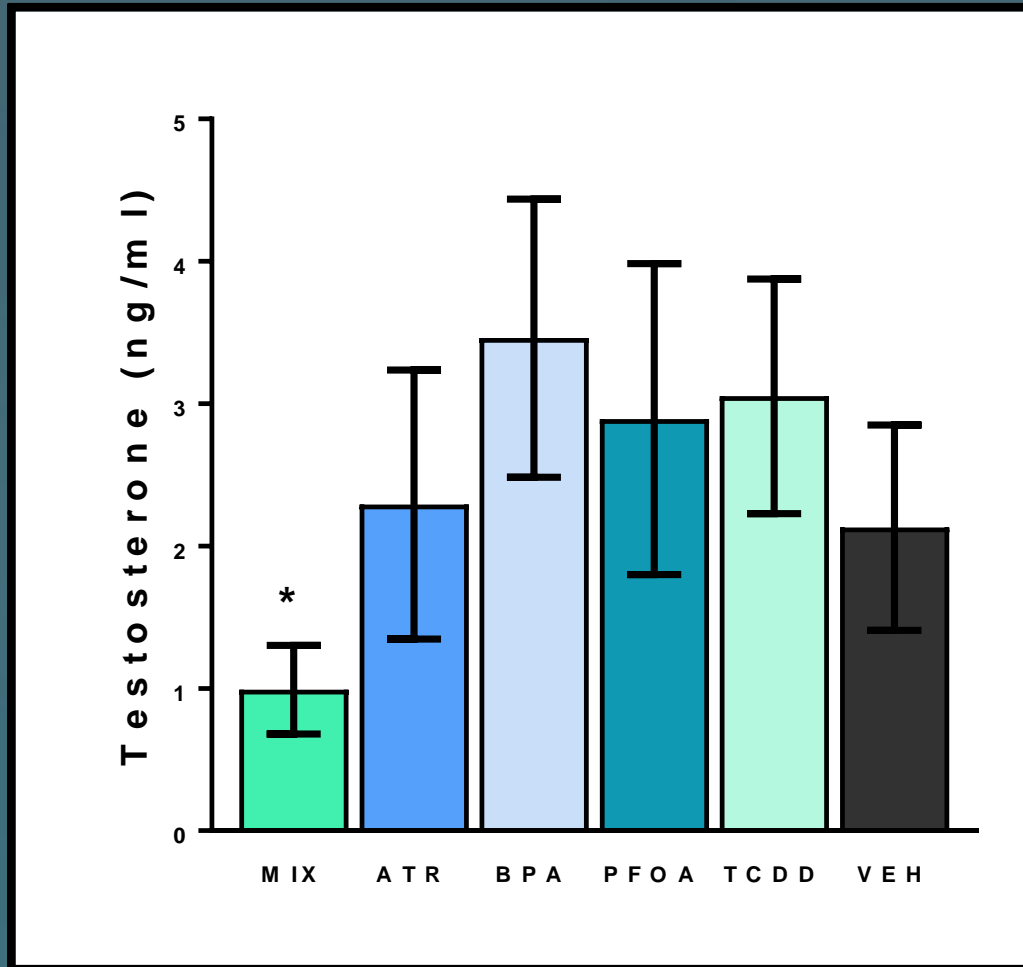




# SHARED PHYSIOLOGY: Multiple Hits Hypothesis



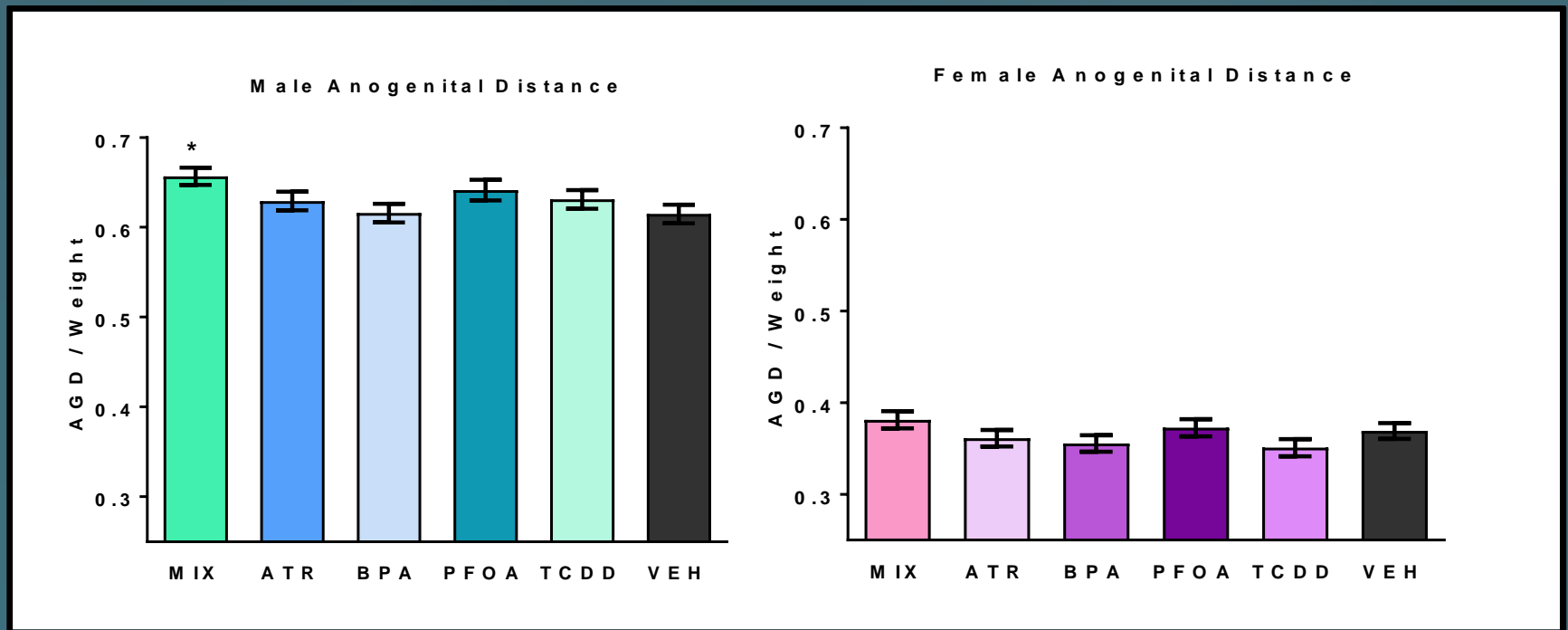
# Adult Serum Steroid Hormone Concentrations



MIX males showed significantly DECREASED steroid hormone levels, for both testosterone and corticosterone in adulthood (N = 7-10 males, \* indicates  $P < 0.05$ ).

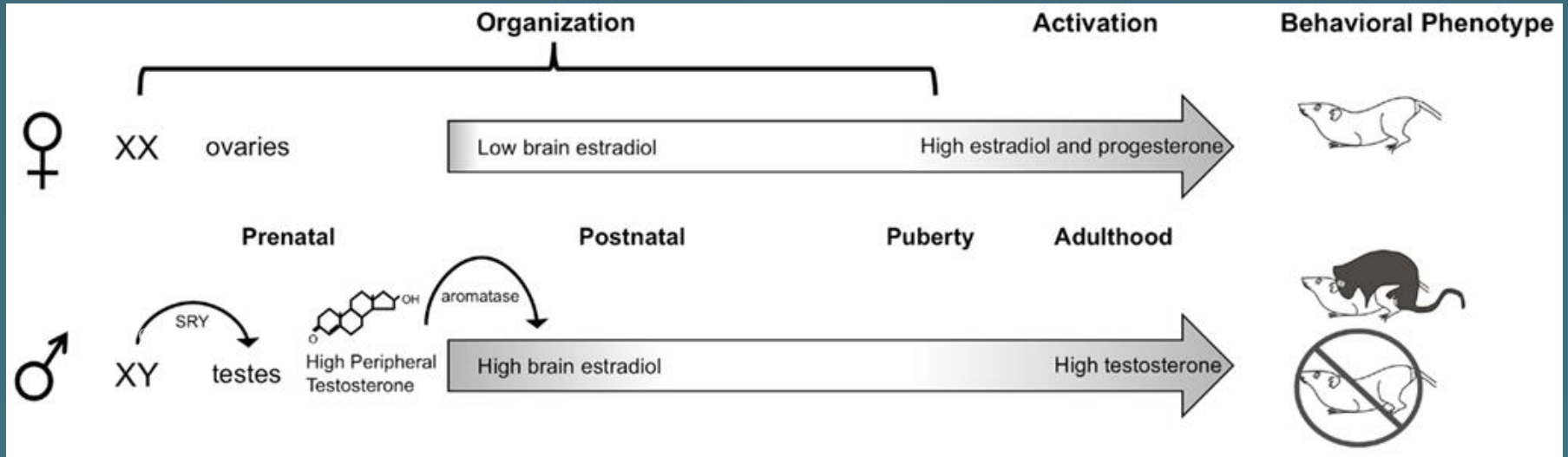
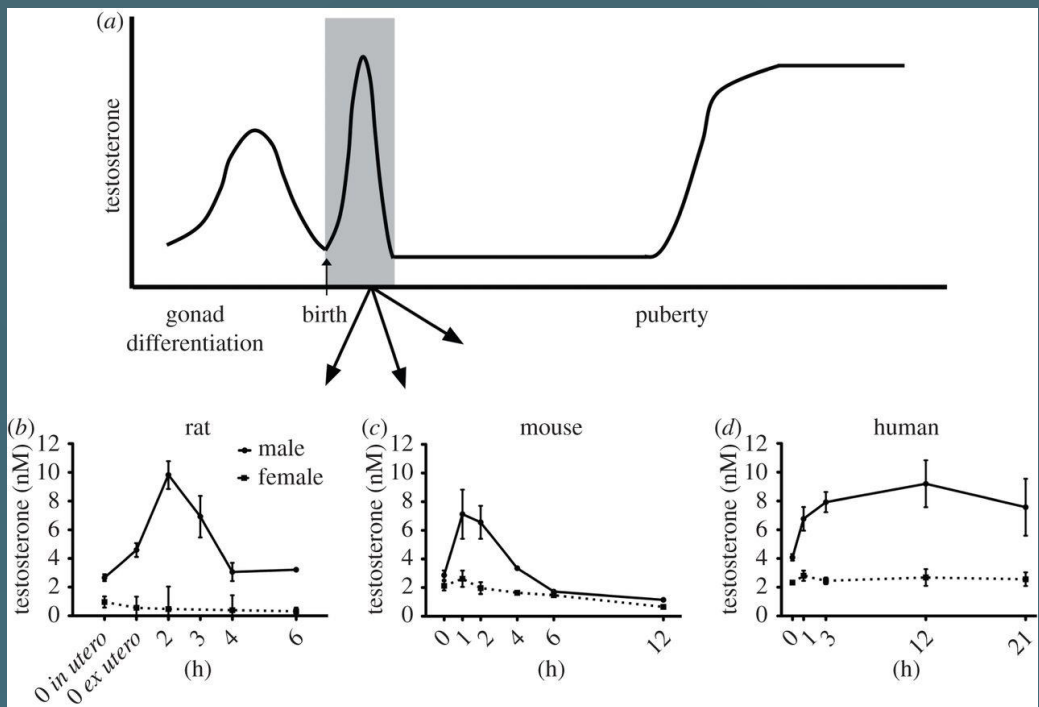
# Reproductive Tract Alterations

Reproductive organs were significantly altered in MIX males with increased anogenital distance at weaning (N = 7-11\* indicated  $p < 0.01$ ).

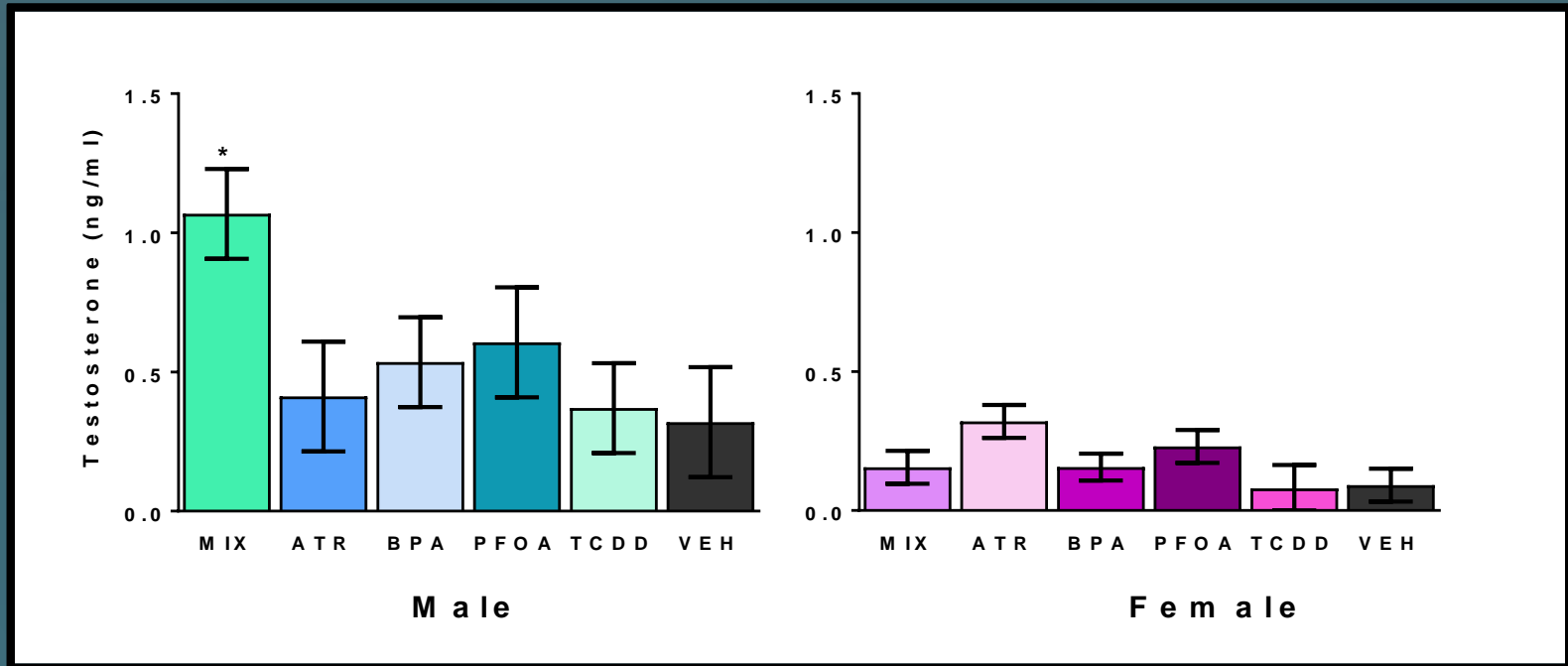


# Perinatal Sex Steroids Organize the Nervous System

(Clarkson and Herbison 2016; Lenz et al. 2012; Phoenix et al. 1959)



# Testosterone at Birth



Our data suggests that gestational exposure to a mixture of low-dose EDCs increased testosterone production at birth, this surge at birth is critical for the developing male fetus to begin masculinized neural and reproductive differentiation (\* indicates  $p < 0.02$ ).

# Male Vulnerability: Perinatal Testosterone and Translation to Human Disease

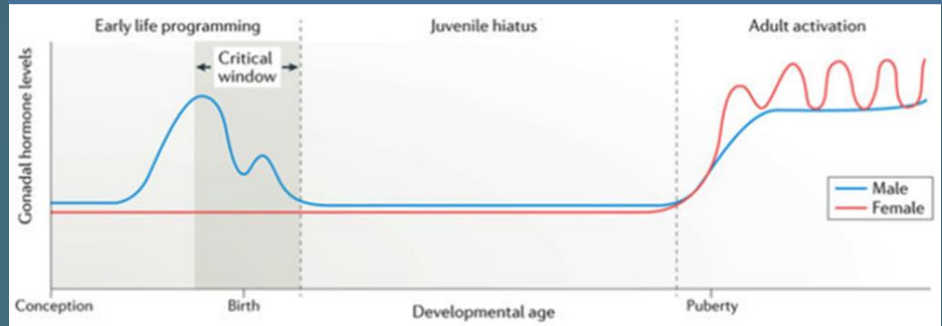
MIX Exposure



?



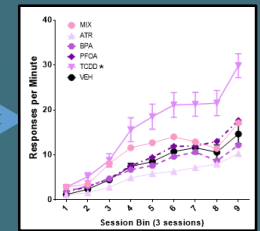
?



?



?

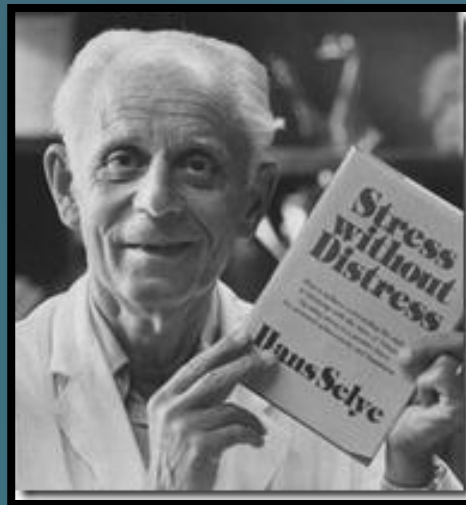


# Behavioral Endocrinology

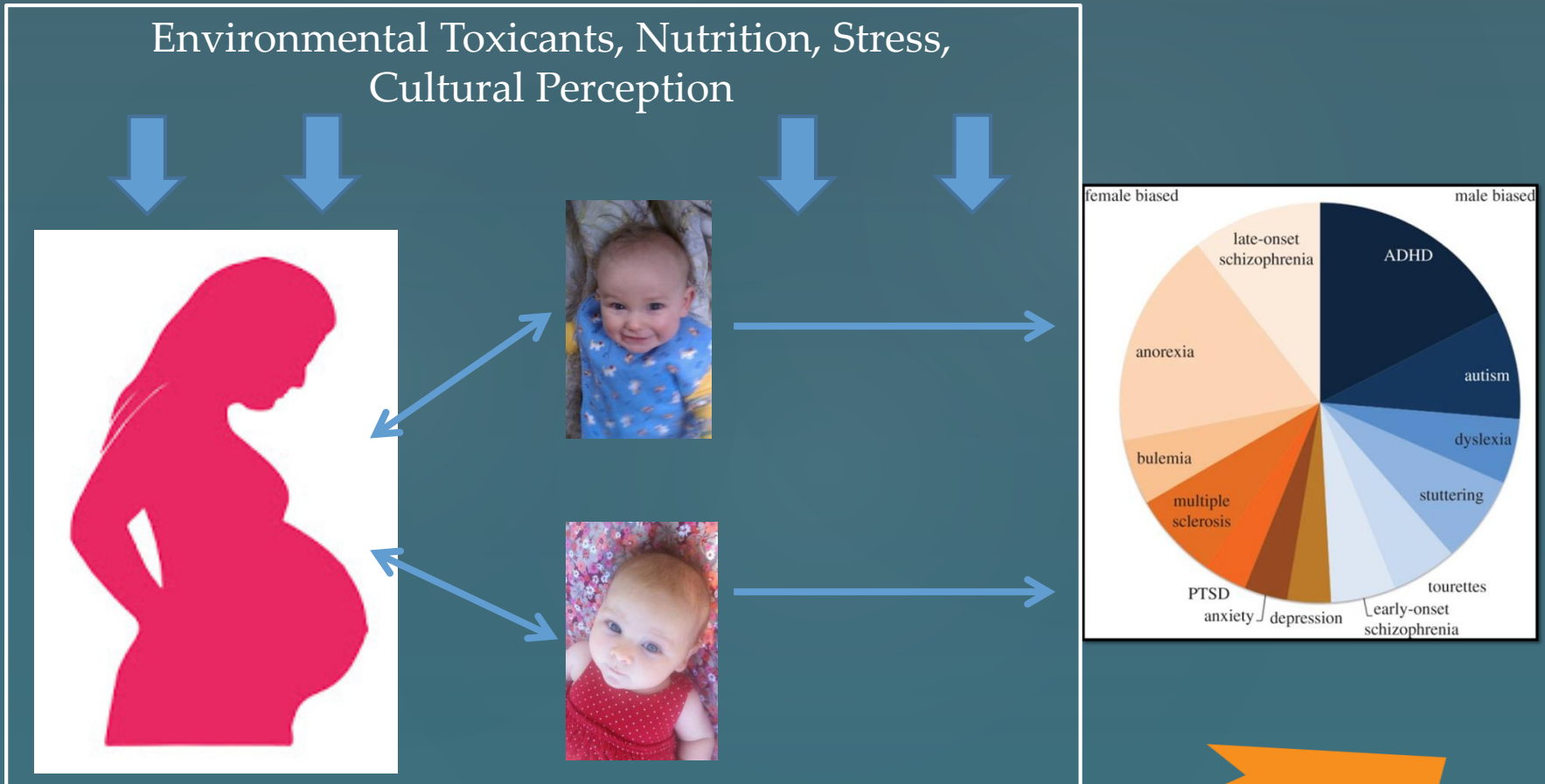
Hormones + Environmental stimulus  $\Leftrightarrow$  behavior

Hormone: (Greek) – “to urge on”, “to impel”, “that which sets in motion”

(Beylis and Starling, 1902)



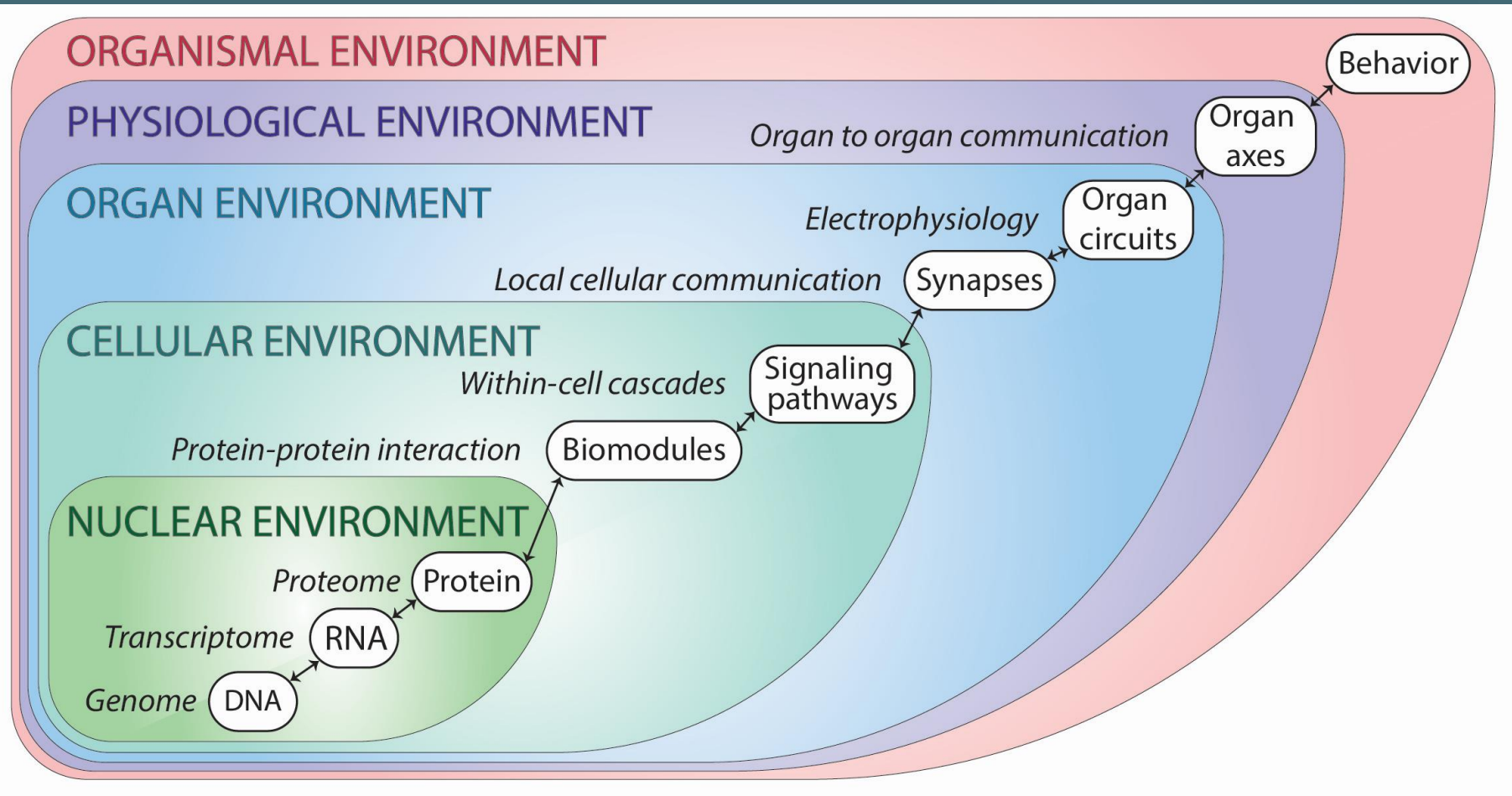
# Sex-dependent risk for neurobehavioral disorders: Interactions between maternal environments, fetal/infant development, and external environmental factors influence



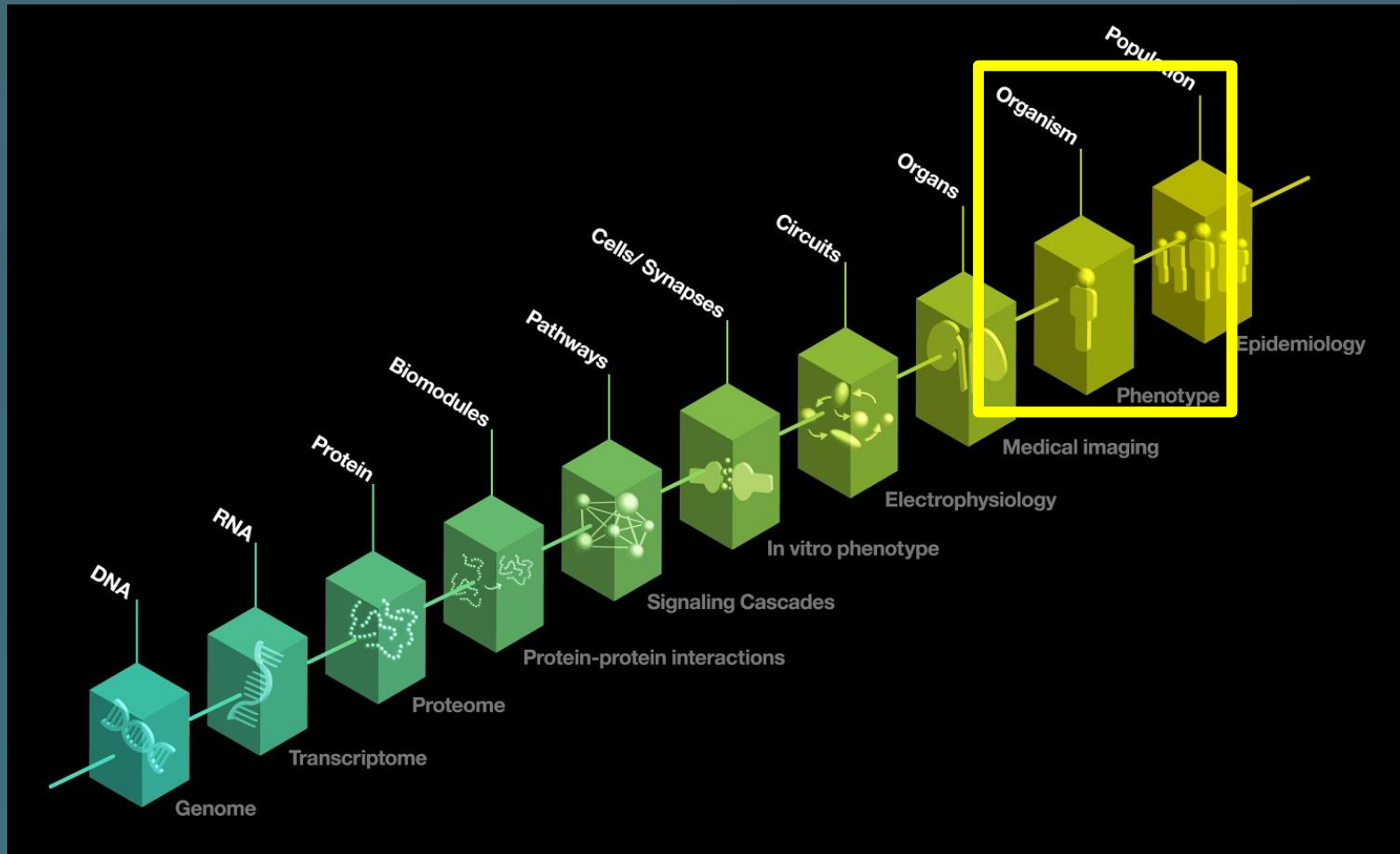
*What happens  
in the womb lasts  
a lifetime*



# Environmental Context Matters across all Scales of Biology



# The How and the Why



“Every scale in biology receives its mechanism from the scale below and its significance from the scale above.”

-- George Bartholomew

# Many Thanks

## Collaborators

Deborah Cory-Slechta

B. Paige Lawrence

Martha Susiarjo

## Sobolewski Lab

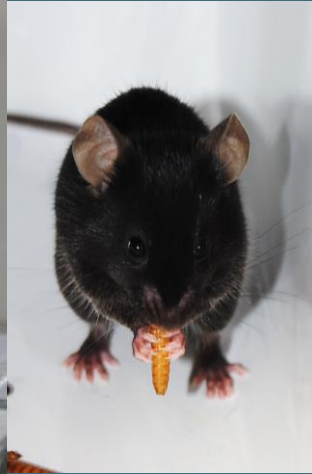
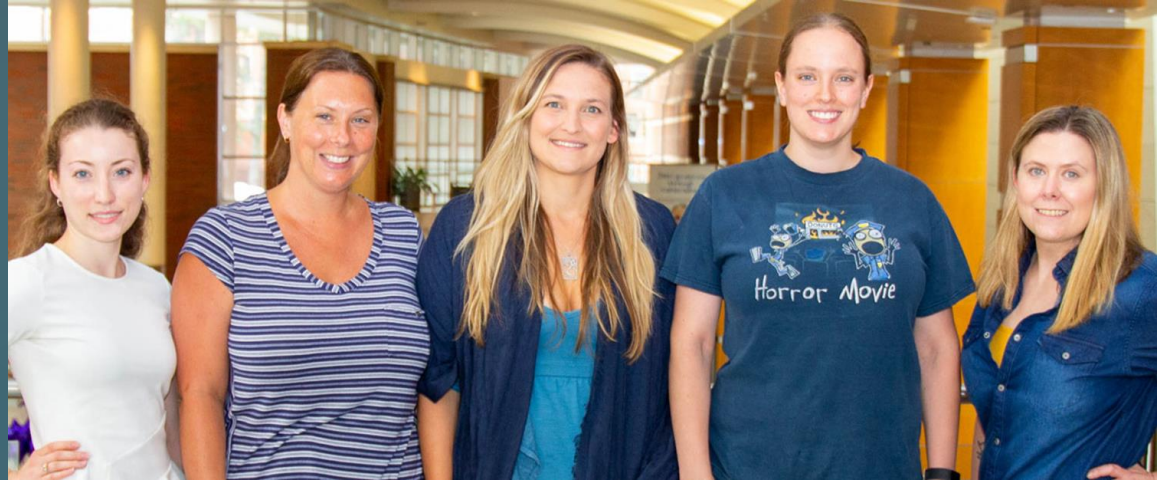
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