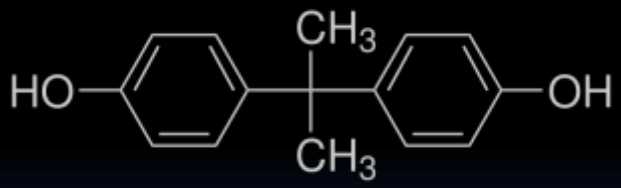
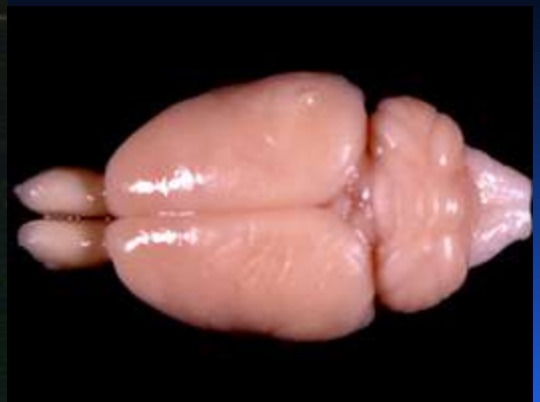
BRAIN SEX DIFFERENCES DURING GESTATION: THE ROLE OF ENDOCRINE DISRUPTING COMPOUNDS





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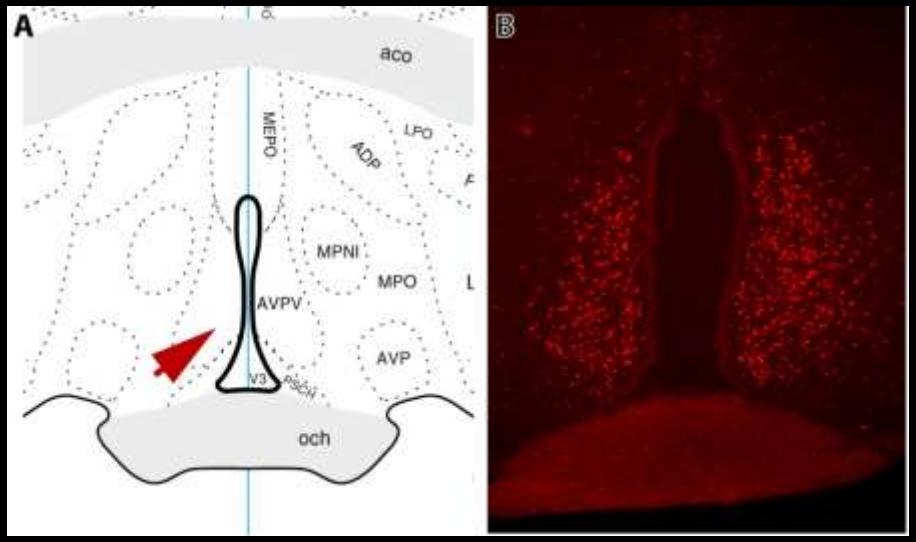
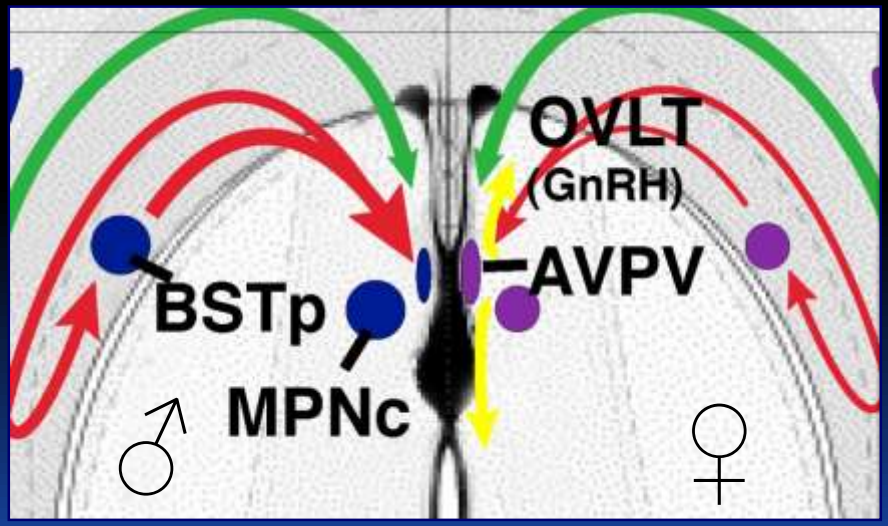
Many Behaviors are Sexually Dimorphic and Influenced by Environment

- Sexual dimorphisms emerge prenatally, are shaped by adolescent experience, and fully manifest in adulthood.
- Behavioral differences include reproductive strategies, social affiliation, agonistic behaviors, parental care, ingestive behaviors, and group dynamics.
- Sex differences in the neural pathways which mediate these behaviors are largely organized by steroid hormones.

- **So if experience can shape the brain and behavior, can chemicals do the same?**





Adapted from an image by Eva Polston, Howard University

Brain sexual dimorphisms are *region* specific and *age* specific.

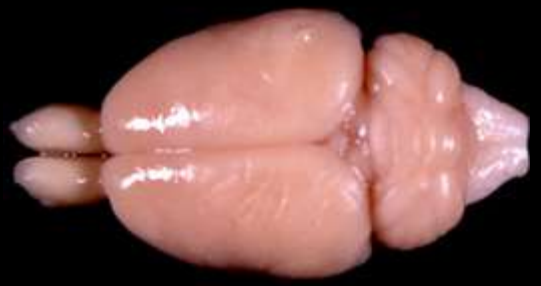
Some brain sexual dimorphisms are *species* specific.

Brain regions can be dimorphic in size, content, and neural projections.

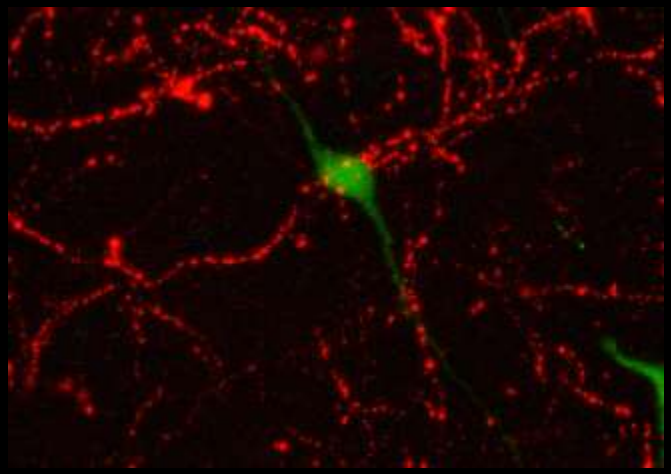
These dimorphisms are organized by steroid hormones in discrete developmental windows.

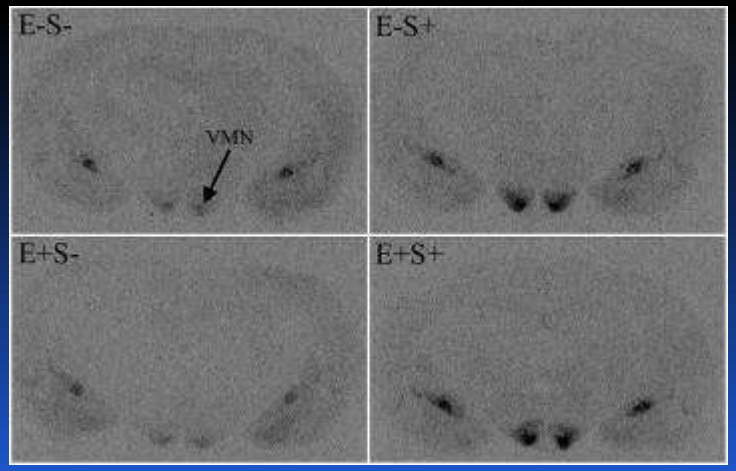
When? How? Are they vulnerable to endocrine disruption?

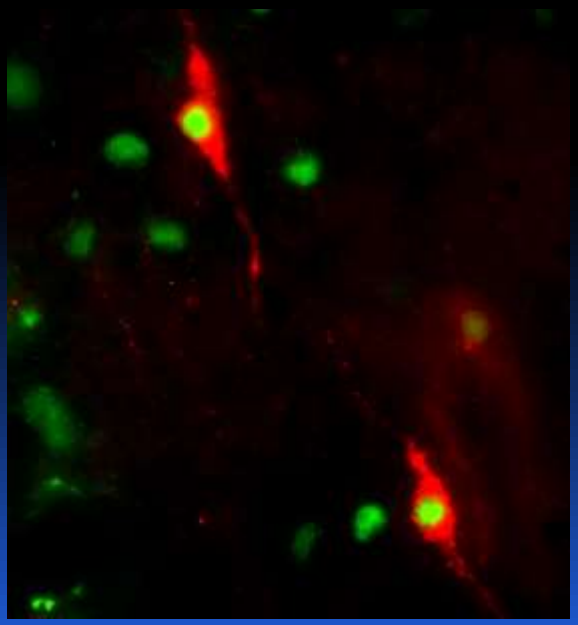
Research Questions



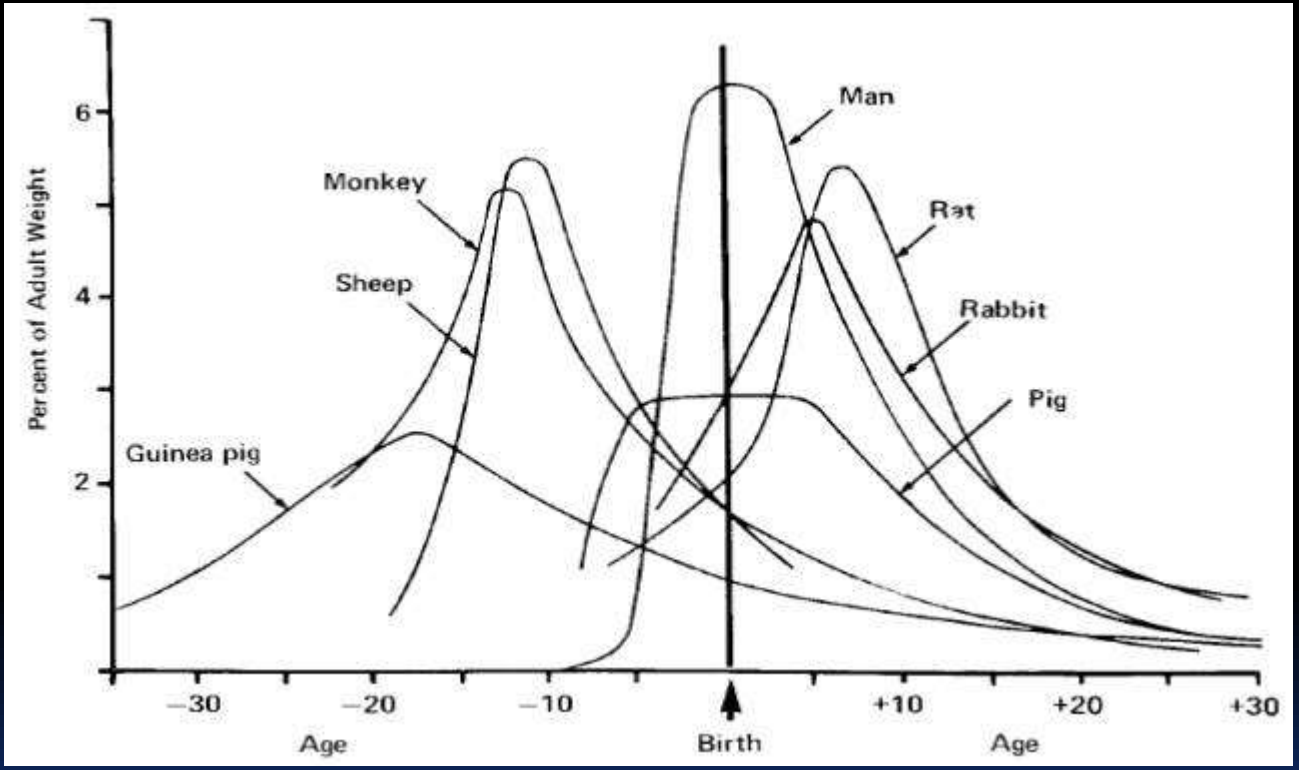
- How are male and female brains different?
- What roles do steroid hormones play in the differentiation of brain neuroanatomy and behavior?
- Can environmental endocrine disruptors (EDCs) interfere with sexually dimorphic brain organization and behaviors?





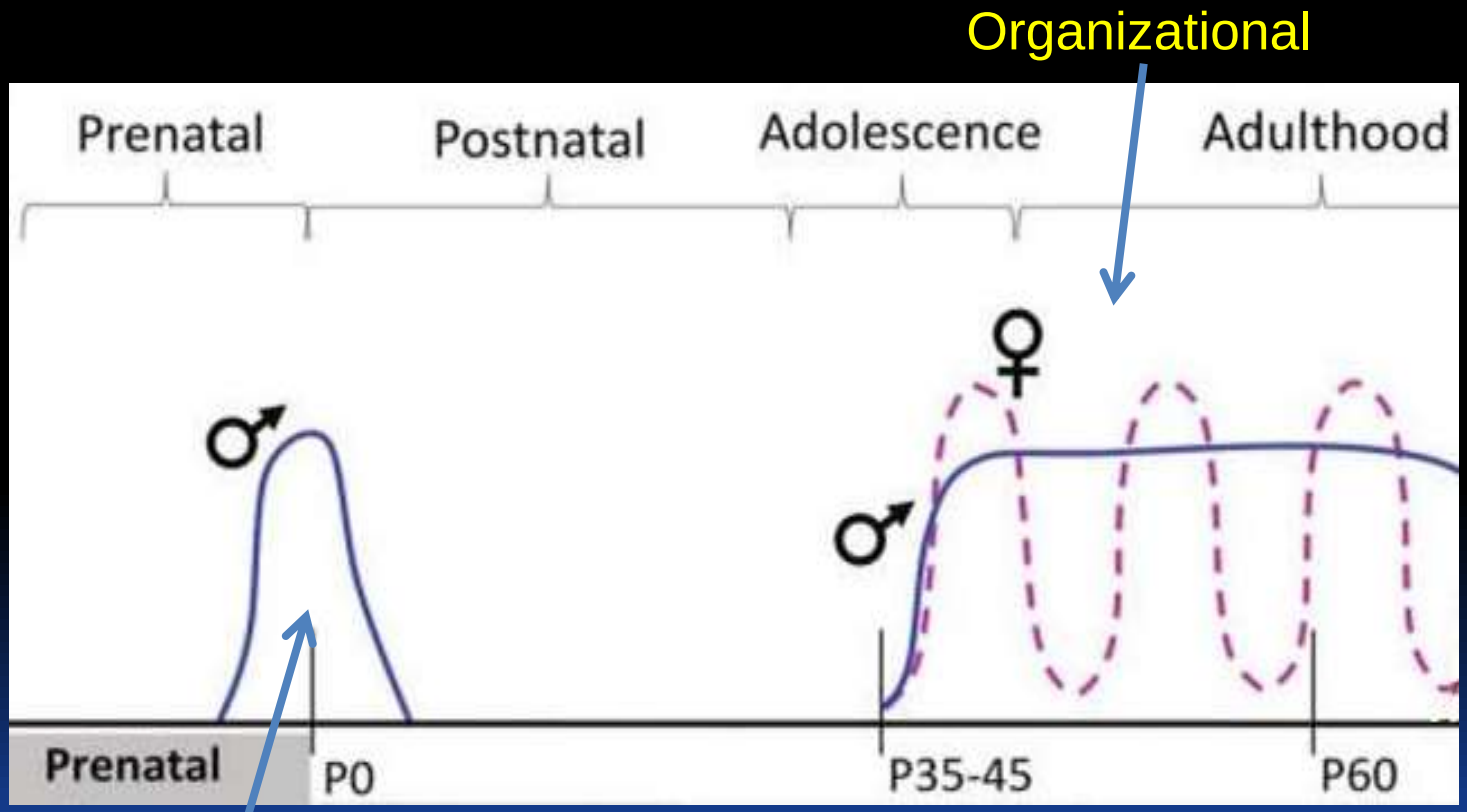


Many Neural Events in Rodents are Prenatal in Humans



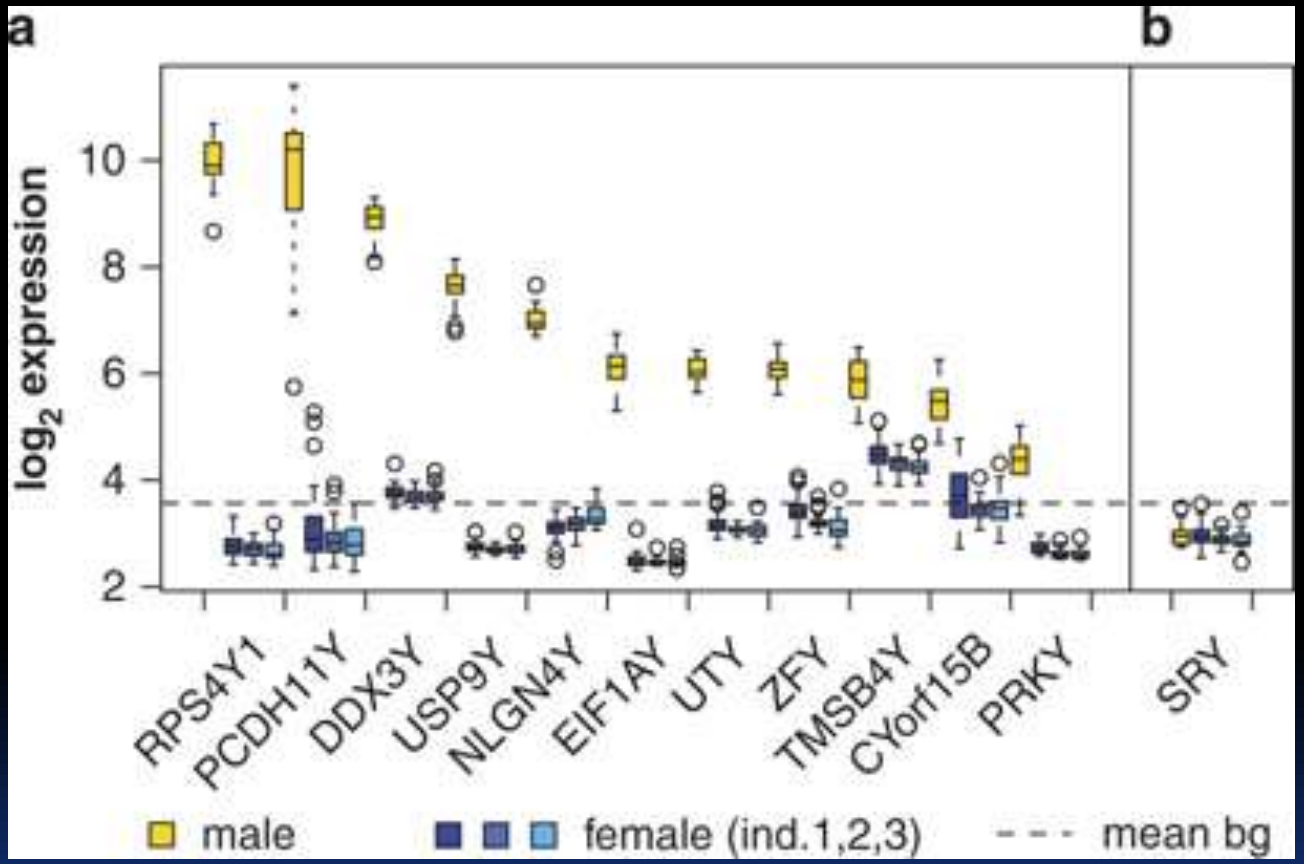
Dobbing, J.; Sands, J. Comparative aspects of the brain growth spurt. *Early Hum. Dev.* **1979**, *3*(1), 79-83.

In males, hormone levels are high around the time of birth and critical for brain sexual differentiation



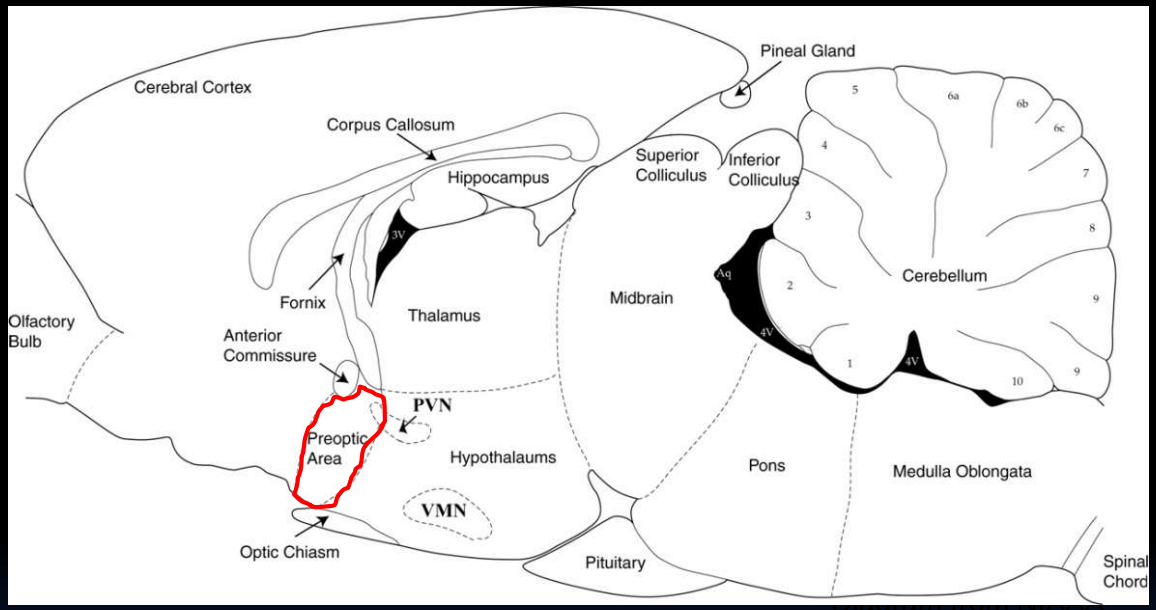
Schwarz and Bilbo (2012)

Expression of Y-linked Genes in Human Prenatal Male Brain



Note that SRY is not detectable. PRKY was expressed only in cortex emphasizing that sex-biased gene expression can be region specific. ZFY is primate-specific. Reinius and Jazin (2009) *Molecular Psychiatry*

Sexually Dimorphic Gene Expression in Neonatal Rat



Assessed gene expression in the PND1 rat preoptic area (POA) via RNAseq.

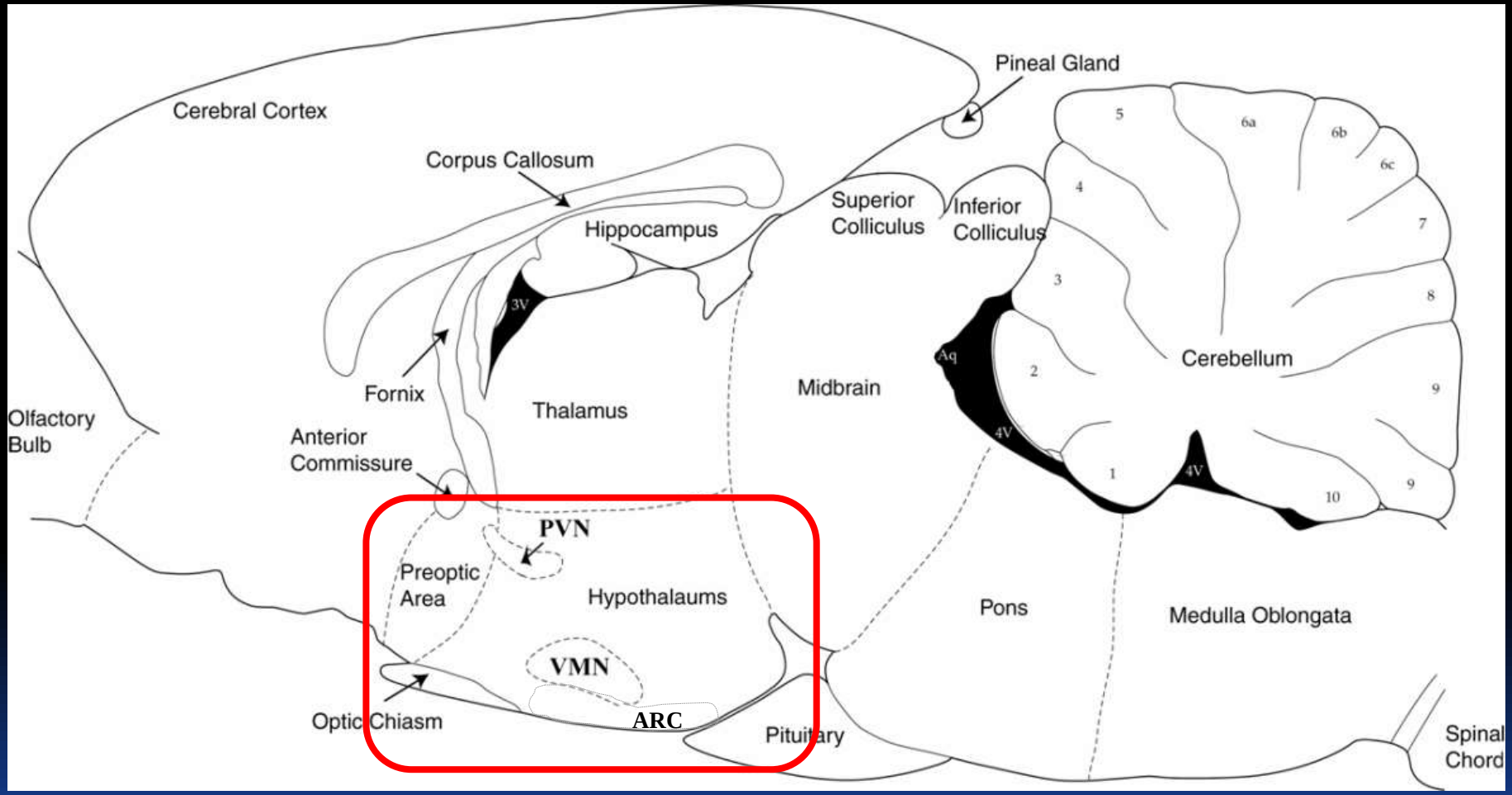

nature neuroscience

Brain feminization requires active repression of masculinization via DNA methylation

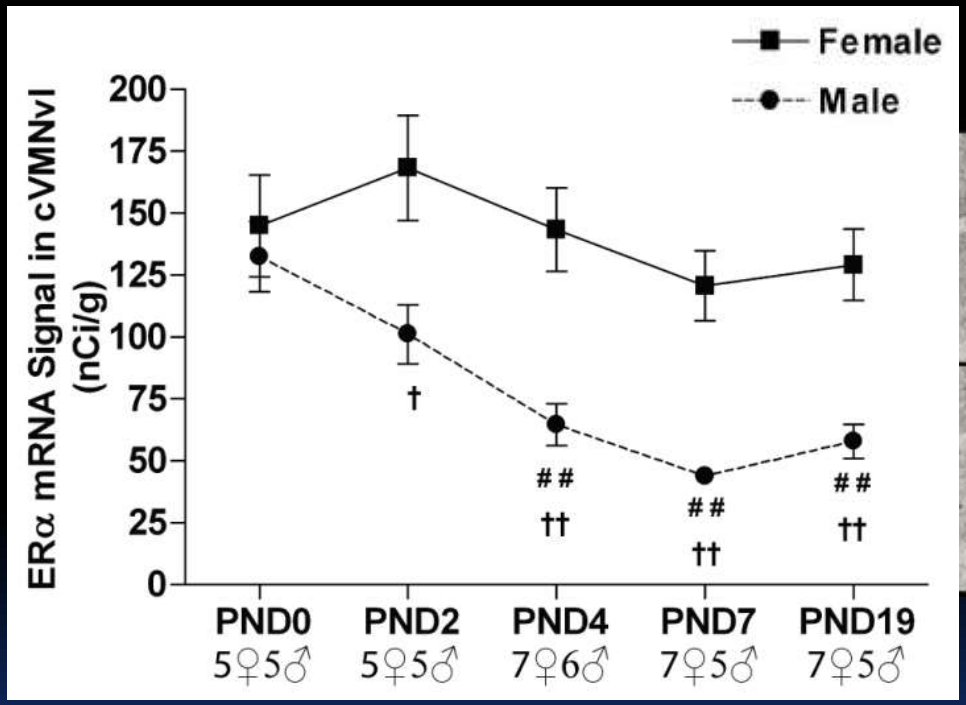
Bridget M Nugent[1,2], Christopher L Wright[2], Amol C Shetty[3], Georgia E Hodes[4], Kathryn M Lenz[2], Anup Mahurkar[3], Scott J Russo[4], Scott E Devine[3] & Margaret M McCarthy[1,2]

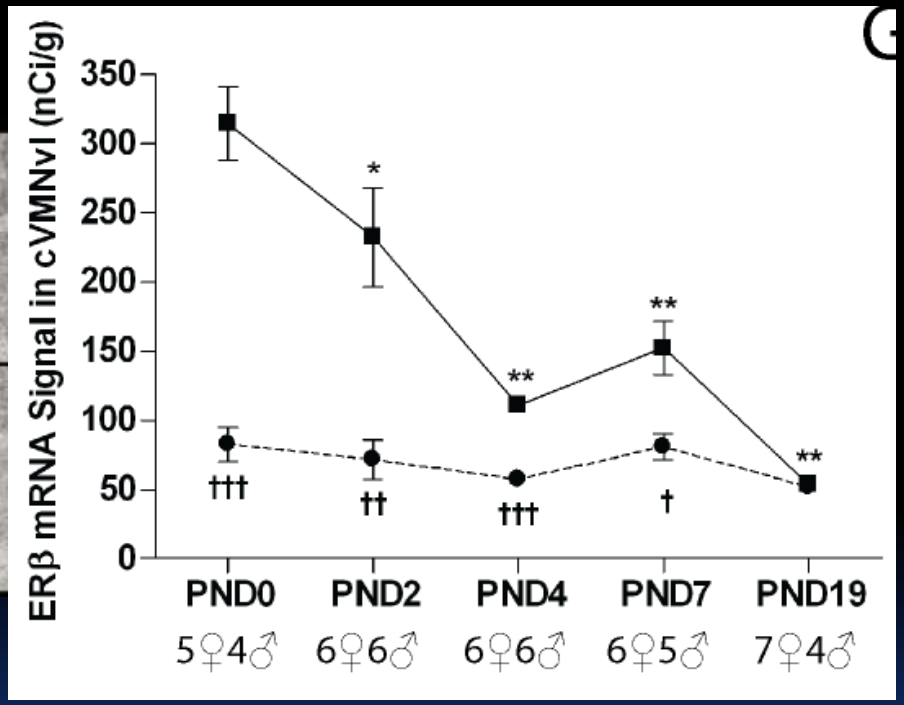
34 genes higher in males
36 genes higher in females

Brain Regions of Interest



Hypothalamic ER Expression Across Postnatal Development is Sexually Dimorphic



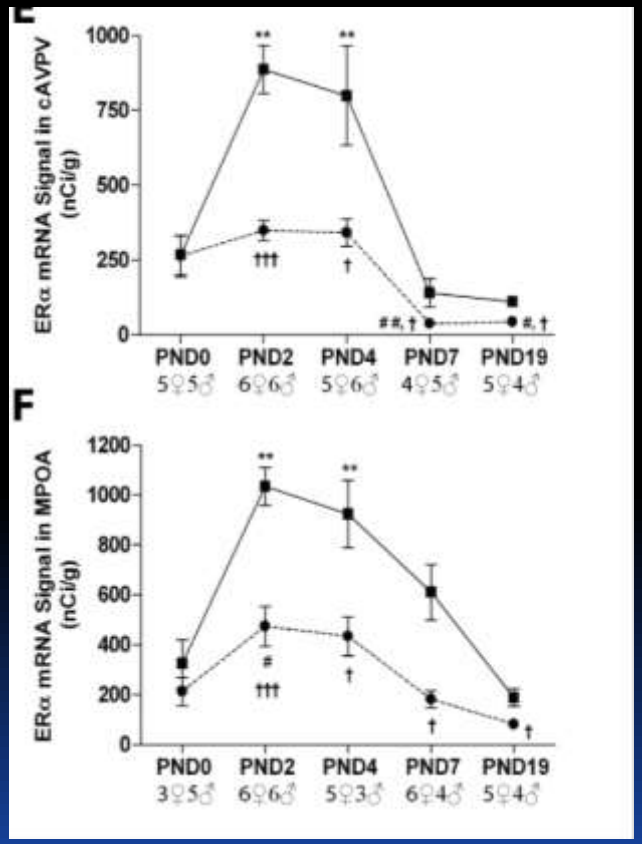


Some sex differences in ER expression emerge across neonatal development while others are lost – *even in the same brain region*.

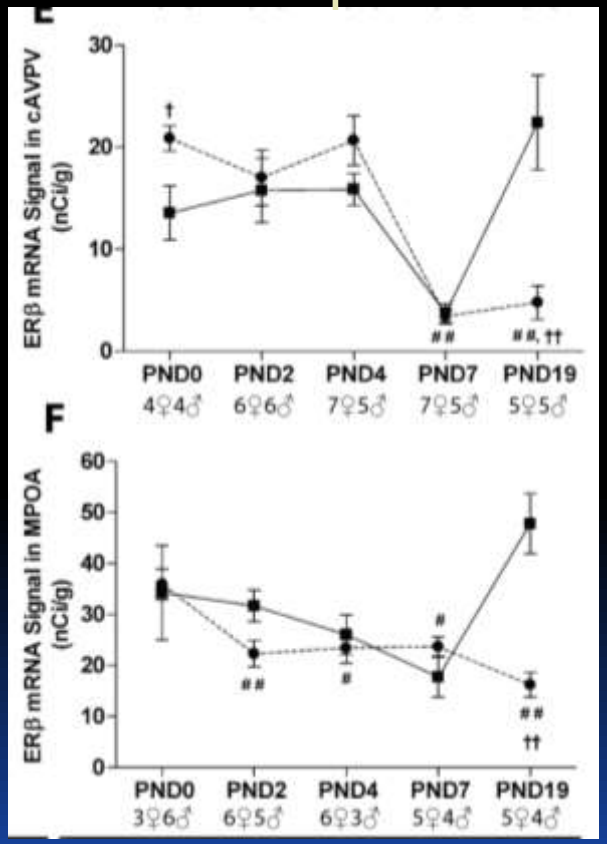
Cao and Patisaul (2011) JCN. 519(15):2954-77

Sex Differences in Hypothalamic ER Expression Across Postnatal Development Are Dynamic

ERα



ERβ



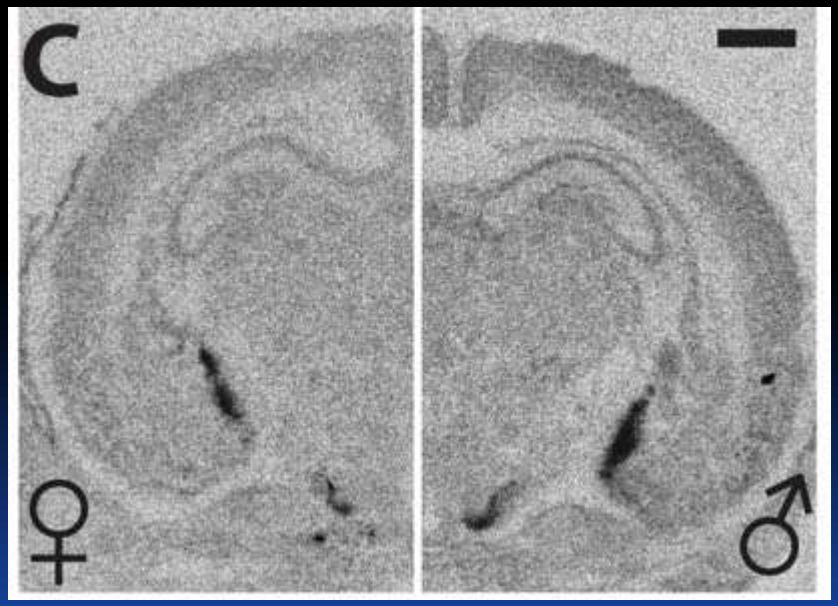
ER expression patterns are very different from each other, even in the SAME brain regions.

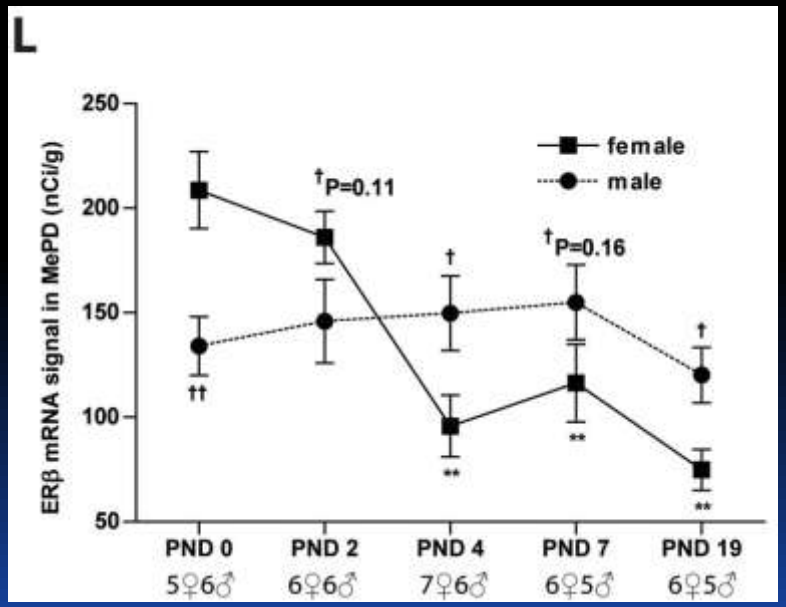
Cao and Patisaul (2011) JCN. 519(15):2954-77

In Some Subregions of the Amygdala, Sexually Dimorphic ERβ Expression Reverses

Expression of ERα, ERβ, and Kiss1 mRNA in the Postnatal Rat Amygdaloid Complex

Gene name	Area examined	PND0	PND 2	PND 4	PND 7	PND 19
ERα	MePD	F = M	F = M	⇓F = M⇓	⇓F = M⇓	F = M
	PLCo	F = M	⇓F = M⇓	⇓F = M⇓	F = M	F = M
	PMCo	F = M	⇓F = M⇓	⇓F = M⇓	⇓F = M⇓	⇓F = M⇓
	AHi	F < M	F = M⇓	F = M⇓	F = M⇓	F = M⇓
ERβ	MePD	F > M	F > M*	⇓F < M	⇓F < M**	⇓F < M
Kiss1	MePD/PMCo/PLCo/AHi	ND	ND	ND	ND	ND





Summary

- Y-linked genes are heavily expressed in the human fetal brain, and at least one shows region-specific expression (only cortex).
  - SRY is not one of them. Thus brain and testis have different expression profiles of Y-linked genes.
- In the rat neonatal POA 60+ genes show sexually dimorphic expression, including many which are not Y-linked.
- Some transcriptional sexual dimorphisms emerge in the first few days of life, some are lost, and some reverse.
- Sex differences in ER expression are most pronounced in regions well known to coordinate sexually dimorphic physiology and behaviors.

Are these and other brain sex differences vulnerable to endocrine disruption?

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

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

Review

Assessment of sex specific endocrine disrupting effects in the prenatal and pre-pubertal rodent brain

Meghan E. Rebuli[a,b], Heather B. Patisaul[a,b,*]

[a] North Carolina State University, Department of Biological Sciences, Raleigh, NC 27695, United States
[b] W.M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC 27695, United States

Question 1: Does developmental EDC exposure alter sexual dimorphisms in the developing brain?

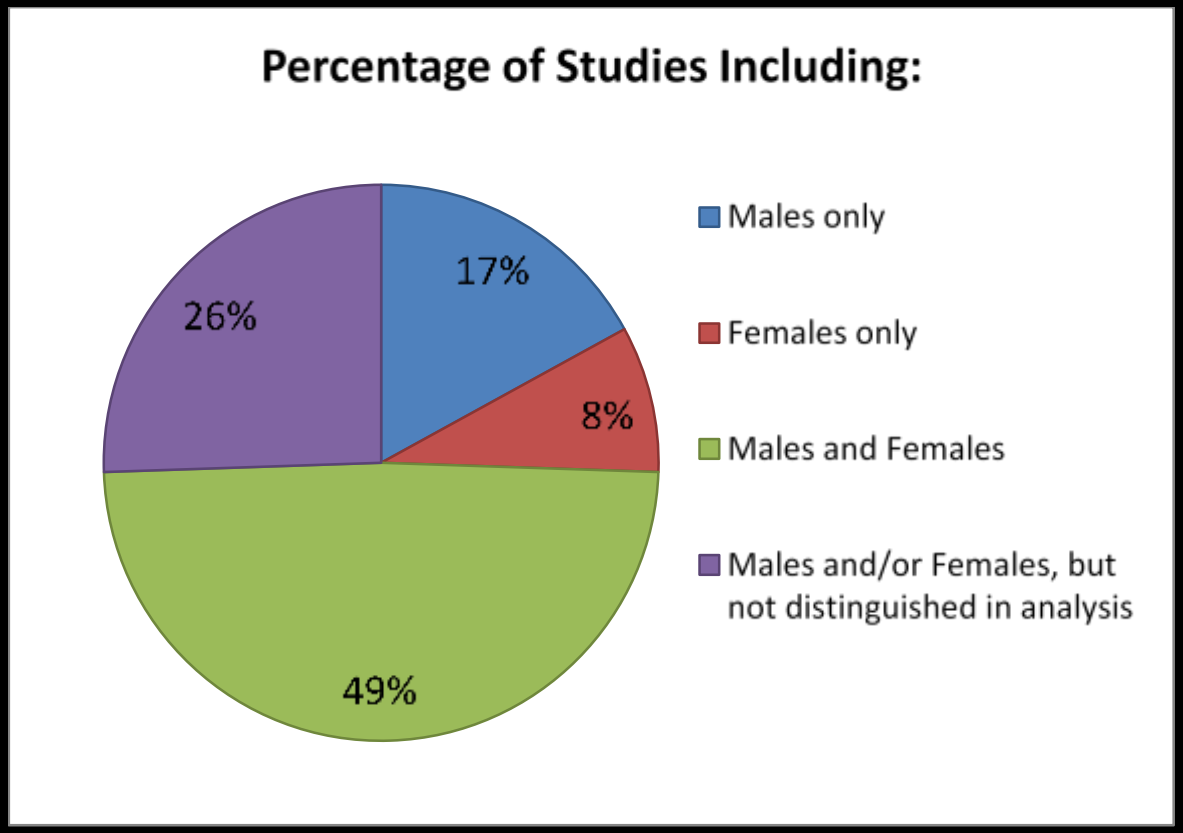
Question 2: In general, does the EDC literature do a reasonable job of accounting for sex?

Search Strategy

- Pubmed search
- Keywords: Endocrine disrupting compound, EDC, endocrine active compound, EAC, brain, neuro, hypothalamus, dimorphic, development
- Did not search by individual chemical names
  - Too many to name (hundreds of EDCs)
  - Considered biased for “legacy” chemicals

Summary of Findings

- Hypothalamus: 19 studies identified; 15 examined and reported sex-specific effects
- Hippocampus: 11 studies identified; 6 males only; 4 did not report sex
- Cortex/Cerebellum/Midbrain: 14 studies identified; 7 differentiated by sex; 3 did not report sex
- Whole and embryonic brain: 2 of 9 studies stratified for sex



Assessment of sex in the EDC studies reviewed here. Nearly half of the studies looked for effects in both sexes but more than a quarter did not distinguish between the sexes at all. Consistent with the literature in general, studies considering only one sex disproportionally focus on males.

Summary of Findings

- Hypothalamus:
  - Altered nuclear volume (SDN, AVPV)
  - Altered gene expression (ERs, PR, GABA receptors) and methylation
  - Altered GnRH and GABA signaling pathways
  - Altered dopaminergic cell numbers
- Hippocampus:
  - Altered neurogenesis and spine density
  - Evidence of thyroid hormone disruption
- Cortex/Cerebellum/Midbrain:
  - Varied targets and pathways: ER, AhR, TH, GABA
- Whole and embryonic brain:
  - Gene expression; neurogenesis and mylenation

EDC Effects on Behavior Reviewed Elsewhere

Published in final edited form as:
J Neuroendocrinol. 2012 January ; 24(1): 144–159. doi:10.1111/j.1365-2826.2011.02229.x.

ENDOCRINE DISRUPTERS: A REVIEW OF SOME SOURCES, EFFECTS, AND MECHANISMS OF ACTIONS ON BEHAVIOR AND NEUROENDOCRINE SYSTEMS

C. Frye[a], E. Bo[b,c], G. Calamandrei[d], L. Calzà[e,f], F. Dessi-Fulgheri[g], M. Fernández[e], L. Fusani[h], O. Kah[i], M. Kajta[m], Y. Le Page[i], H.B. Patisaul[n], A. Venerosi[d], A.K. Wojtowicz[p], and G.C. Panzica[b,c,q]

NeuroToxicology
Volume 33, Issue 6, December 2012, Pages 1420–1426
NEUROTOX 27 Special Issue

27th Int Neurotox Conf

Sex dimorphic behaviors as markers of neuroendocrine disruption by environmental chemicals: The case of chlorpyrifos

A. Venerosi[a], L. Ricceri[a], S. Tait[b], G. Calamandrei[a]

Current Opinion in Pharmacology
Volume 19, December 2014, Pages 134–144
Gastrointestinal • Endocrine and metabolic diseases

Endocrine-disrupting actions of PCBs on brain development and social and reproductive behaviors

Margaret R Bell

Hormones and Behavior
Volume 59, Issue 3, March 2011, Pages 296–305
Special Issue: Behavioral Epigenetics

Review

The role of Bisphenol A in shaping the brain, epigenome and behavior

Jennifer T. Wolstenholme[a], Emilie F. Rissman[a, b], Jessica J. Connelly[b, c]

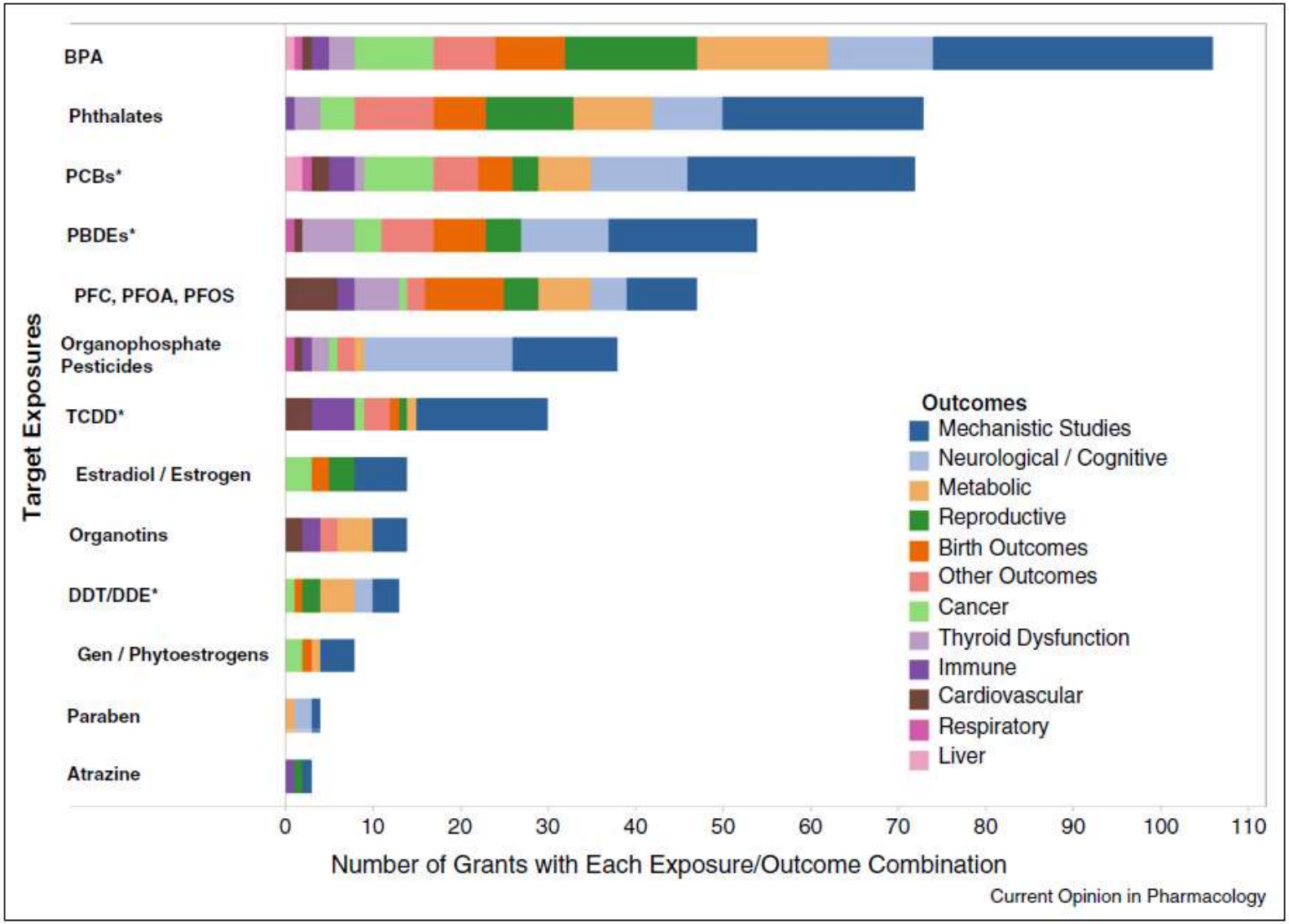
Numerous reviews of ECDs and behavior. Most are chemical-specific.

Summary of Findings

- Significant effects of developmental EDC exposure found in all brain regions examined.
  - Hypothalamus particularly vulnerable but also most intensely studied.
  - Strong evidence for effects in hippocampus but examination of sex-specific effects is greatly needed.
  - Evidence in gestational brain very limited.

- Most identified papers focused on well-known chemicals: BPA, PCBs, PBDEs, genistein and dioxin
  - Likely at least partly an artifact of the search strategy.
  - Also reflects funding patterns……

Figure 1



Filer *et al* (2014) Current Opinion in Pharmacology

Rapidly Emerging Data About Neuroendocrine Disruption from Zebrafish and Other Models

Advanced Morphological — Behavioral Test Platform Reveals Neurodevelopmental Defects in Embryonic Zebrafish Exposed to Comprehensive Suite of Halogenated and Organophosphate Flame Retardants

Pamela D. Noyes, Derik E. Haggard, Greg D. Gonnerman and Robert L. Tanguay[1]

Department of Environmental & Molecular Toxicology, Environmental Health Sciences Center, and the Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, Oregon 97331

Toxicol. Sci. (2015) 145 (1): 177-195.

PNAS

Low-dose exposure to bisphenol A and replacement bisphenol S induces precocious hypothalamic neurogenesis in embryonic zebrafish

Cassandra D. Kinch[a,b,c], Kingsley Ibhazehiebo[b,c], Joo-Hyun Jeong[b,c], Hamid R. Habibi[a], and Deborah M. Kurrasch[b,c,1]

Departments of [a]Biological Sciences and [b]Medical Genetics and [c]Alberta Children's Hospital Research Institute, University of Calgary, Calgary, AB, Canada T2N 4N1

Edited* by Joan V. Ruderman, Harvard Medical School, Boston, MA, and approved November 26, 2014 (received for review September 16, 2014)

Summary

- EDCs are everywhere.
- Limited but strong evidence in rodents that EDCs can disrupt brain sexual differentiation.
- EDC researchers do a reasonably good job of accounting for sex in their studies but could do better, particularly when examining regions outside of the hypothalamus.
- Both synthetic and naturally occurring compounds can produce effects.
  - Soy phytostrogens
- Need more information about emerging and less well known EDCs.
- New, more high-throughput approaches may help more rapidly identify neuroendocrine disruptors and their sex specific consequences.
- Having a common set of key words would make papers easier to identify for systematic review.