The importance of controlling for stress in reproducibility of studies focused on endocrine endpoints

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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?
Endocrine disrupters and child health
Possible developmental early effects of endocrine disrupters on child health

State of the Science of Endocrine Disrupting Chemicals - 2012

Environment Special: The oceans—why 70% of our planet is in danger
The Facebook Movie: The secret history of social networking

How the first nine months shape the rest of your life
The new science of fetal origins

By Annie Murphy Paul
Pregnancy = Stress

10 WAYS TO DEAL WITH IT

Effects of Stress on Baby During Pregnancy

Stress and Preterm Labor Multifactorial

Stress and pregnancy: Effects on child behavioural and cognitive outcomes | Encyclopedia on E...

www.child-encyclopedia.com

www.FitBottomedMamas.com
The HPA Axis

- Stress disrupts the organization and function of the HPA axis:
  - Altered MR and GR expression in the hippocampus
  - Elevated basal glucocorticoid release
  - Insensitivity to steroid feedback
- The amygdala initiates stress-related coping strategies and behaviors.
- The automatic nervous system, inflammatory cytokines, and metabolic hormones are also involved.
- This axis is sexually dimorphic and sensitivity differs by SEX and AGE.

Stress and the Brain

Dashed bars = sensitive to hormone disruption
Solid bars = periods of rapid growth and sensitivity
Red bars = periods of sensitive and rapid decline

Brain Development (Too Simplified)

Four Major Steps:
- Induction
- Neuro/Glia-genesis
- Migration
- Connection and Myelination
Mapping Infant Brain Myelination with Magnetic Resonance Imaging

Sean C. L. Deoni,1 Evelyne Mercure,2,3 Anna Blasi,2,3 David Gasston,1 Alex Thomson,2 Mark Johnson,3 Steven C. R. Williams,1 and Declan G. M. Murphy2
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From Howdeshell, KL. (2002) Environmental Health Perspectives 110(S3):337-348
“Critical Periods” Extend into Adulthood
Imprinting

• **Konrad Lorenz** and his greylag geese (filial imprinting)

• Critical period 12-48 hrs after hatching in geese
  – Later in more altricial species

• Visually imprint on the largest moving object near them (mom)
  – Olfactory and other cues also play a roll
  – Most common in precocial species

• Filial Imprinting is *irreversible*, establishes species preferences (and helps ID the parent)

• *Can be altered by stress*
Thyroid hormone initiates the imprinting period. Thyroid hormone administration can reopen a "closed" sensitive period. Isolation lengthens but stress shortens the imprinting period.
Brain regions that show increased neuronal activation when the bird hears song are represented in yellow. Nuclei important for song production are in orange.

Broca's area is particularly involved in (but not limited to) speech production, whereas Wernicke's area is involved in speech perception and recognition, as well as other language-related tasks.

Nature Reviews Neuroscience 11, 747-759 (November 2010)
“Critical Periods” Extend into Adulthood

National Center for Children in Poverty, Improving the Odds for Young Children, 2008
Fig. 1. Estimated volumes of brain structures in normal volunteers are plotted against age. The volumes in the figures are presented as standardized residuals (removing variability associated with volume of the supratentorial cranial vault). They are, from left, volumes of frontal cortex, thalamus, nucleus accumbens, and cerebral white matter. Note the rapid age related change (and striking individual differences) in the childhood and adolescent age range.
Rolling Summary

• Sensitivity to stress is AGE and SEX specific.
• Critical periods for brain development extend across the lifespan.
  – The brain is always in a “critical period.”
Critical Period for Human Social Development?

- **Rene Spitz**
  - Austrian-American Psychiatrist
  - First to researcher to use direct observation for research on child development
  - 1940s: "anaclitic depression" or "hospitalism"

- **Key Findings**
  - Emotional and maternal deprivation have profound effects on attachment and social cognition.
  - Infants similar at 4 mo then the deprived infants begin to fall behind
  - Social deprivation after 5 mo leads to irreversible damage
  - 1952 Psychogenic Disease in Infancy
Social Rearing in Primates

- Harry and Margaret Harlow (1960's)
- Reared rhesus macaques for in various forms of isolation
  - Ranged from 6mo up to 15 years
  - Some were in cages where they could see peers not but physically interact.
  - Some were completely deprived (solo housed in a small room)
- Biggest effects in those deprived at a younger age
  - Fearful, failure to play, stereotypic behaviors, failure to socially interact
  - Most failed to completely recover
- Disrupted stress axis and immune function
A critical period for social isolation in the rat

Dorothy F. Einon, M. J. Morgan

First published: March 1977  Full publication history
DOI: 10.1002/dev.420100205  View/save citation
Cited by (CrossRef): 192 articles  Citation tools

Post-weaning social isolation of male rats reduces the volume of the medial amygdala and leads to deficits in adult sexual behavior

Bradley M. Cooke, Winyoo Chowanadisai, S. Marc Breedlove*

Department of Psychology, University of California, 3201 Tolman Hall, UC, Berkeley, CA 94720-1650, USA
Received 2 March 2000; received in revised form 12 July 2000; accepted 12 July 2000
Females react differently than males to social isolation

Date: October 11, 2016
Source: eLife
Summary: While male and female mice have similar responses to physical stress, research suggests females, not males, feel stressed when alone.

Social Isolation Worsens Cancer, Mouse Study Suggests

Date: September 29, 2009
Source: University of Chicago Medical Center
Summary: Using mice as a model to study human breast cancer, researchers have demonstrated that a negative social isolation causes increased tumor growth. The work shows -- for the first time -- that social isolation is associated with altered gene expression in mouse mammary glands, and that these changes are accompanied by larger tumors. This novel finding may begin to explain how the environment affects human susceptibility to other chronic diseases: central obesity, type 2 diabetes, hypertension.
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  – Effects can be species, sex, and STRAIN specific.
Microglial colonization is sexually dimorphic in some regions and microglia help shape the developing brain.

From
Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease
Marco Prinz & Josef Priller
Nature Reviews Neuroscience 15, 300–312 (2014) | doi:10.1038/nrn3722
BPA And EE Increase Rat Hippocampal Microglial Numbers at P12

Rebuli et al. (2016) General and Comparative Endocrinology
The Immune Connection.....

Bilbo et al. (2017) Experimental Neurology
Bilbo et al. (2017) Experimental Neurology
The Placenta and Prenatal Stress

Prenatal Stress-Induced Increases in Placental Inflammation and Offspring Hyperactivity Are Male-Specific and Ameliorated by Maternal Antiinflammatory Treatment

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The Immune Connection.....
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• There are profound interactions between stress and the immune system including heightened inflammation in the placenta and developing brain
  – Interactions between stress and immune action can be sex specific
  – Effects can include deficits in sociality, hyperactivity and dysregulated HPA function
  – Males appear to be especially vulnerable.
Your Mice Are Likely Freezing

Figure 1

Mouse Models Used To Study Human Diseases Are Influenced by Housing Temperature. Depicted here is the wide variety of phenotypes that have been reported to differ when mice are housed under standard temperatures (~22 °C) versus thermoneutral temperatures (~30 °C) (followed by references [7, 10, 12, 27, 28, 29, 37, 40, 42, 43, 44, 46, 47, 63, 66, 67, 70, 71, 84, 89, 93, 94]). Several other conditions or phenomena that are modeled in mice and are likely to be influenced by housing temperature, but for which there is little or no published information are also listed (followed by ?). Abbreviations: GEM, genetically engineered mice; HSP, heat shock proteins.
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• **Mice are particularly sensitive to thermogenic stress.** Under 30ºC, deleterious effects may be exacerbated by cold.
Bisphenol A (BPA)

- Chemical building block of polycarbonate and epoxy resins.
- The U.S. Centers for Disease Control (CDC) have estimated that 95% of Americans have detectable levels of BPA in their bodies.
  - 1 – 4 ng/ml (higher in children)
- 5 mg/kg bw/day NOAEL (FDA, 2008)
  - No-Observed-Adverse-Effect-Level: highest tested dose or conc. of substance at with no adverse effect is found in exposed test organisms
BPA: Health and Human Risk

“The main problem encountered was a body of literature that reported conflicting results and used experimental protocols that prevented the comparison of results from one experiment to another.”

WHO/FAO and NTP reports indicate that there is a need for more research of the effects of BPA, using validated study especially on brain and behavior, methods.
Complied data from TEDX Low-dose Bisphenol A project:
- Comparison of 391 *in vivo* and *in vitro* studies of BPA published before 2009
- Doses below BPA NOAEL of 5 mg/kg bw/day (FDA, 2008)

Source: www.endocrinedisruption.org
Question: Does prenatal BPA exposure alter estrogen receptor (ER) expression in the neonatal (PND 1) rat brain?
Can PRENATAL Exposure to BPA Alter Sex Specific ER Expression at Birth?

ANIMAL MODEL: NCTR Sprague Dawley Rats

COMPOUNDS: Vehicle (control)
*LOW BPA: 2.5 µg/kg bw
*HIGH BPA: 25 µg/kg bw
EE: 5 µg/kg bw
EE: 10 µg/kg bw

EXPOSURE: GD 6 - PND 1 (prenatal only)

ROUTE: oral gavage
included a naïve control

ASSESSMENT: PND 1

*50 mg/kg per day is the NOAEL
*50 µg/kg per day is the dose FDA considers “safe.”
*4 µg/kg per day is the dose EFSA considers "safe."
Brain Regions of Interest
Sex Difference in ERα Expression Eliminated by the Lowest BPA Dose

ERβ Expression in the Same Region is Unaffected

Enhanced ERβ expression in VMN at high dose in both sexes but levels are not above naive (ungavaged) controls......
Loss of ERα sex differences in vehicle controls

Can Prenatal Exposure to BPA Alter ER Expression in the Amygdala?
ERα expression upregulated by EE10 and BPA 2.5 in MePD and PLCo
ERβ expression upregulated by EE and BPA in MePD; inhibited by gavage.
Summary

• **Gavage is Stressful:** Marked and statistically significant differences between vehicle controls and naive (restrained but not gavaged) groups.
  – Likely indicative of a significant interaction between exposure and prenatal stress.
  – Can “mask” expected sex differences and the effects of BPA.
  – Experiment is incomplete – would need additional groups to definitively test!

• Some of these effects of BPA persist into puberty and adulthood and some of them do not (Rebuli et al. (2014) *Tox Sci*).
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    - Males appear to be especially vulnerable.
- Mice are particularly sensitive to thermogenic stress. Under 30ºC, deleterious effects may be exacerbated by cold.
- **Gavage is a potentially significant source of stress** – route of exposure is important for brain endpoints (especially those in the HPA axis and its accessory structures).
Additional Proactive Measures to Reduce Stress

• Try to use alternative dosing methods.
• Include enrichment when possible.
• Match light cycle to actual time of year.
  – Yes, they “know.”
• Minimize predatory odors (cats).
• Ventilated cages can be a source of stress.
• Provide ample nesting material.
  – Ultraclean = stressful! (Remember the foundling hospital babies – your lab animals need a true home cage).
• Rack placement matters! Most animals do not like the top row.
Neuroendocrine Disruption: The New Frontier

• The human brain undergoes tremendous organizational change after birth.
  – We have to extend the DoHAD model to the post-natal period.
  – Adolescence is a particularly ripe and underexplored “critical period” for EDCs and other chemicals.

• We should not neglect the aging brain.
  – Healthy aging is a critical part of environmental health.
  – This is a phase in which proactive interventions might have meaningful benefits.
    • Soy-rich foods
    • Exercise and low inflammatory diets
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