

**Maternal Bisphenol A Programs
Offspring Metabolic Syndrome**

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Metabolic Syndrome

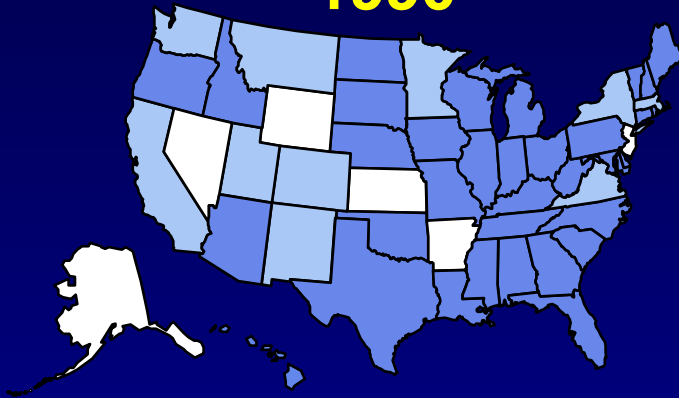


- **Traits:**
 - Obesity
 - Hypertension
 - Type 2 diabetes mellitus
 - Dyslipidemia
- **Mortality:** Leading cause of death in the United States
- **Obesity:** U.S. adults 65% overweight, 31% obese, Childhood obesity 20%
- **Hypertension:** 29% of U.S. population
- **Diabetes:** 27% of U.S. population

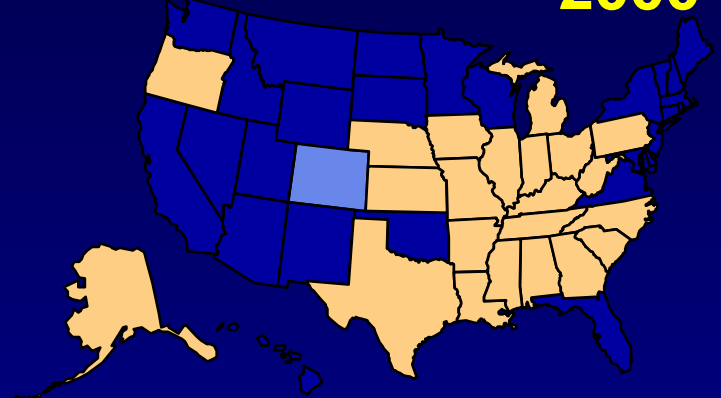
Obesity Trends* Among U.S. Adults From 1990 to 2010

(BRFSS; *BMI ≥ 30 , or about 30 lbs. overweight for 5'4" person)

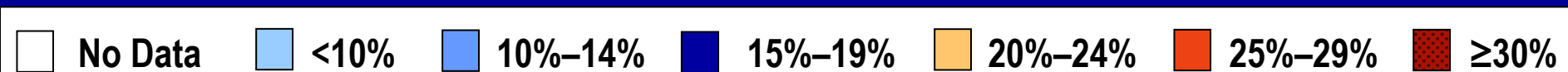
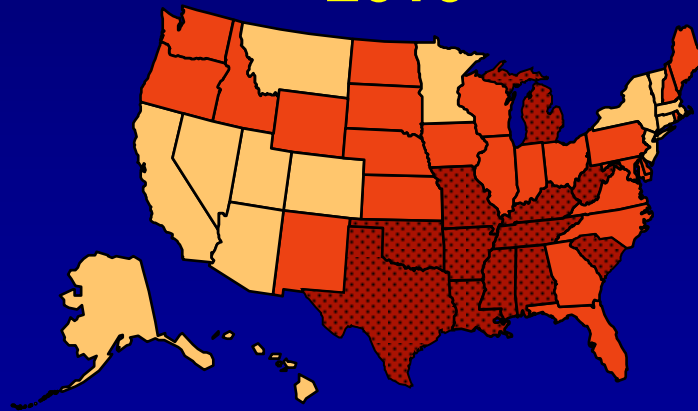
1990



2000



2010



Etiology of Obesity



**Food Availability
High Fat Diets**



**Reduced Energy
Expenditure**



**Propensity for
Obesity**



**Developmental
Programming**

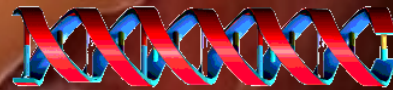
Developmental Programming

Fetal Nutrition, Stress
? Environmental Toxins

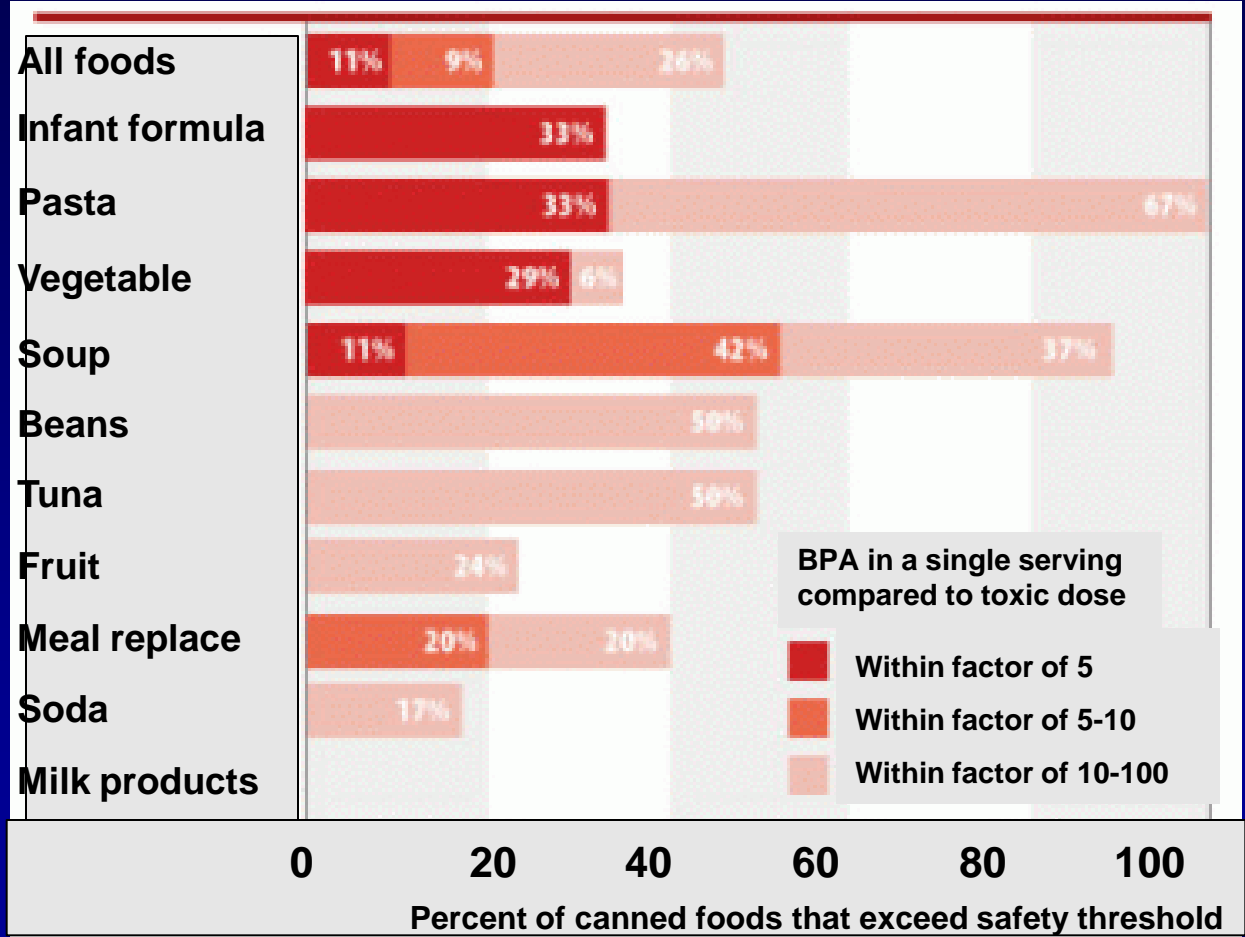
Altered cell number
and differentiation



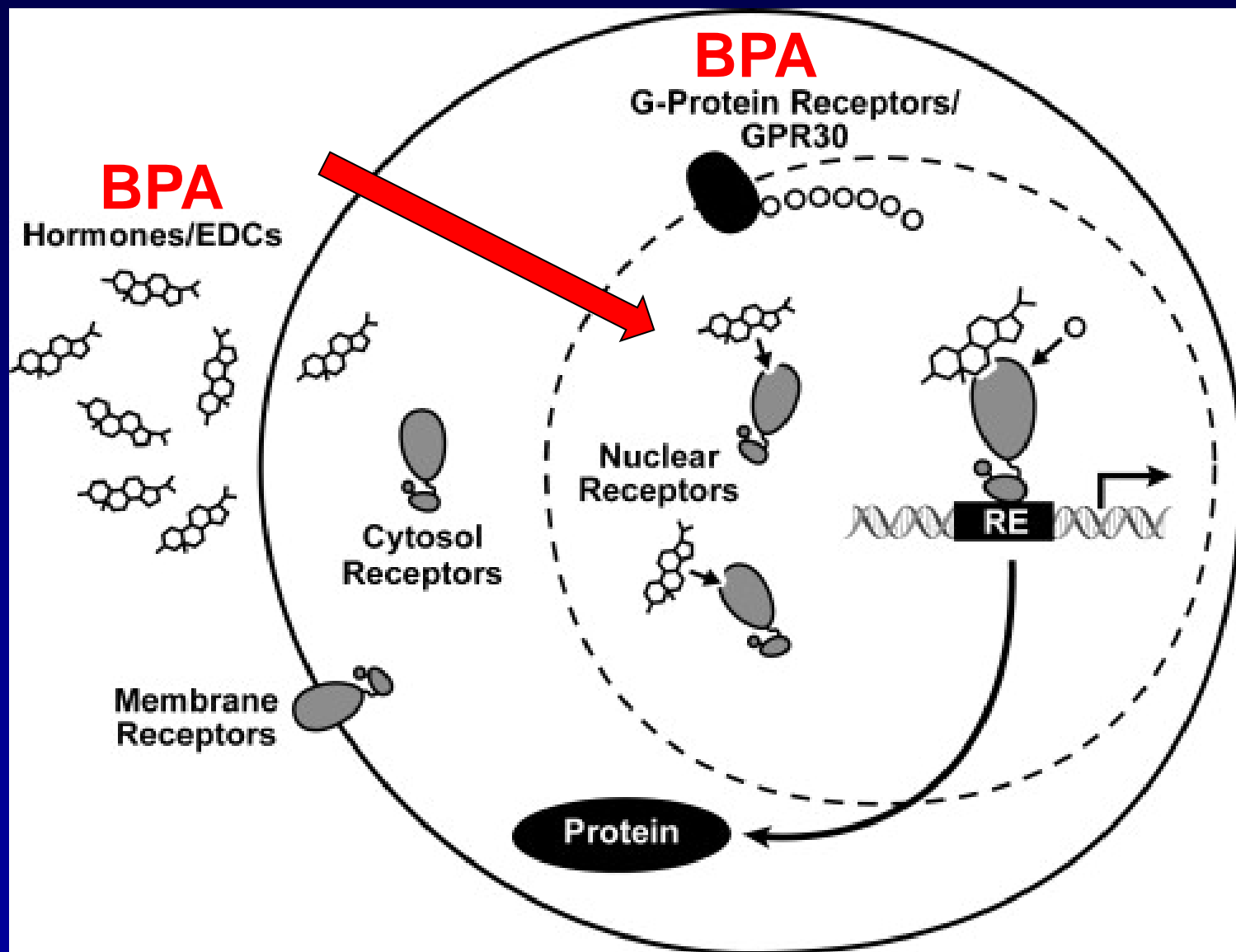
Modified gene expression
altered function



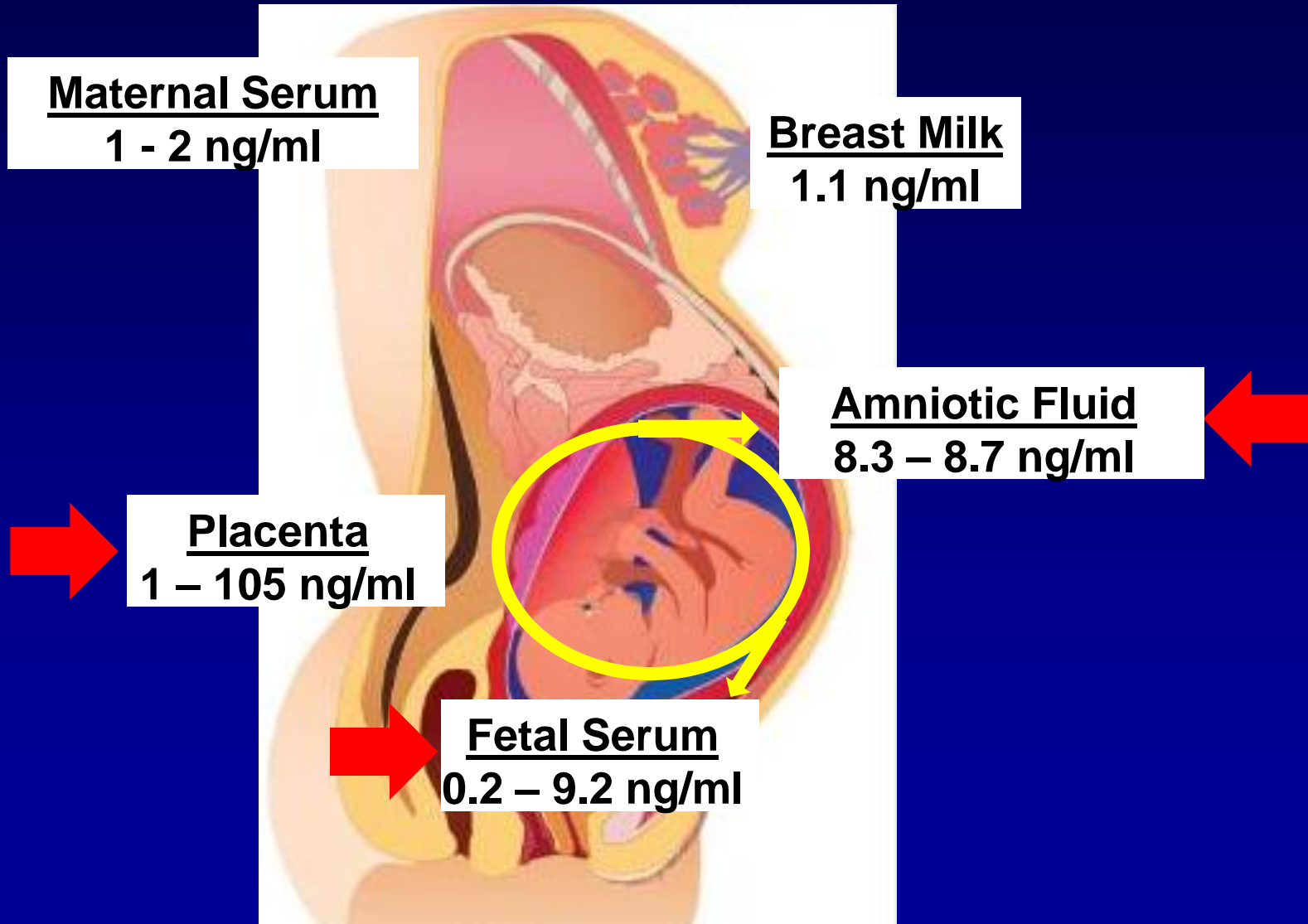
Bisphenol A (BPA)



BPA: Endocrine Disruptor



NHANES: BPA Levels during Pregnancy



Models of BPA-Induced Fetal Programming

- ***In vivo* maternal BPA exposure**
 - Offspring phenotype
 - *In utero* effects on fetal appetite and adipose development
- ***In vitro* fetal BPA exposure**
 - Effect on neural stem cells
 - Effect on adipose tissue development

Maternal Bisphenol A (BPA)

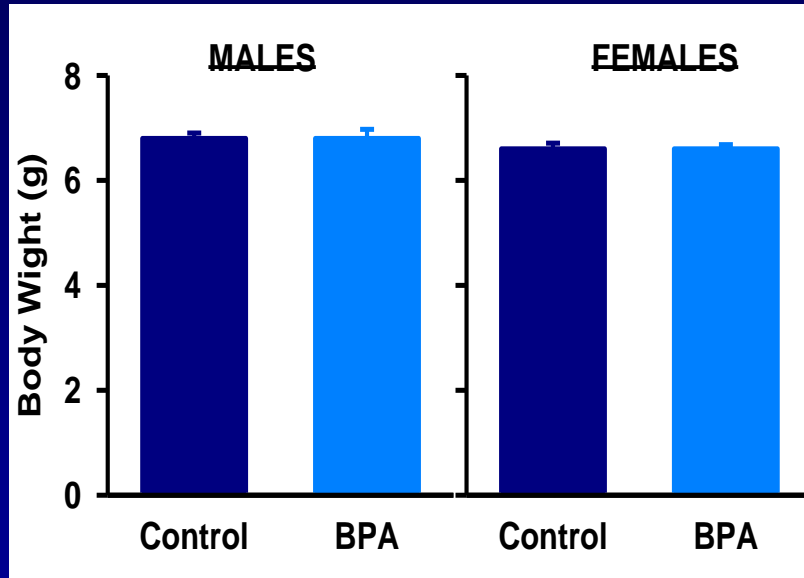


OFFSPRING

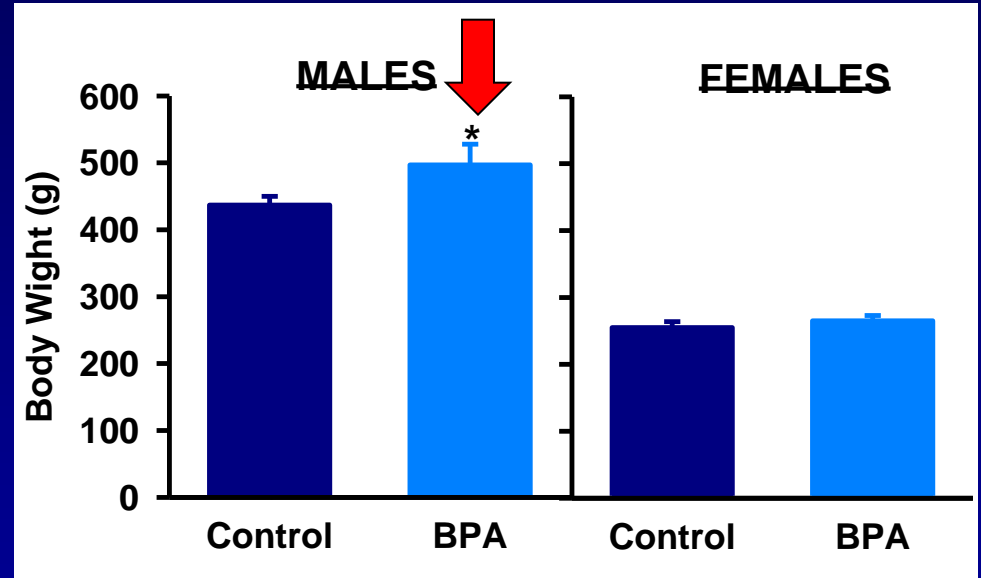
- **Litter size:** Culled to 4 males and 4 females at birth
- **Nursing:** All pups nursed by same dams until p21
- **Weaning:** At p21 to ad libitum food and BPA-free water

Maternal BPA: Offspring Body Weights

-1 Day

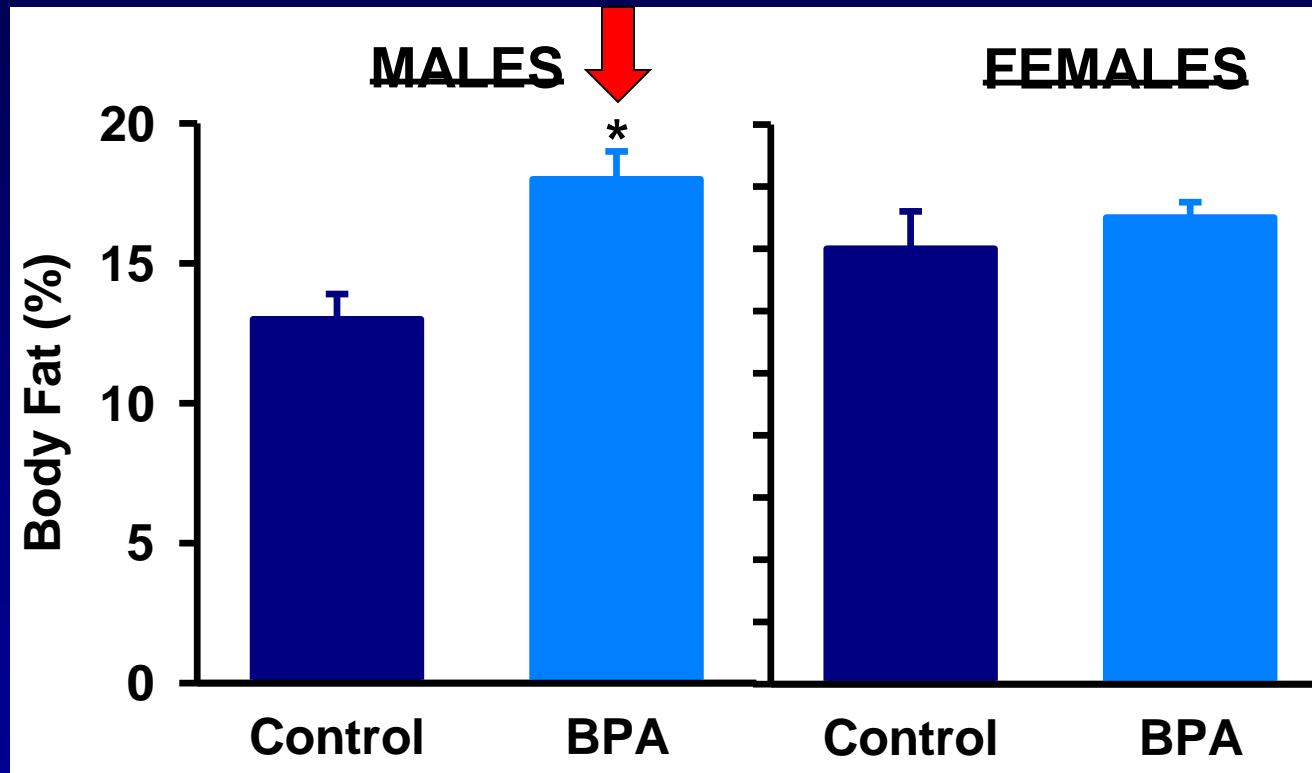


6 Month



Increased Body Weights in Males

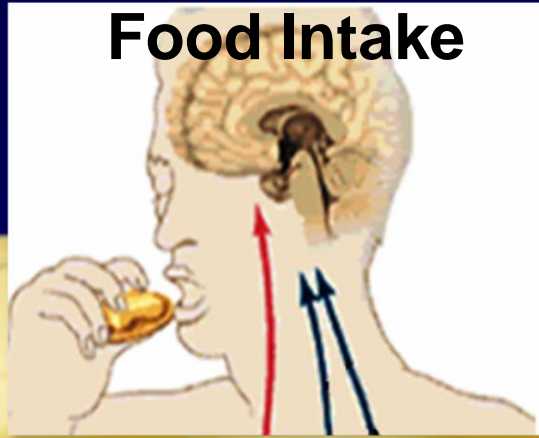
Maternal BPA: Body Fat of 6 Month Offspring



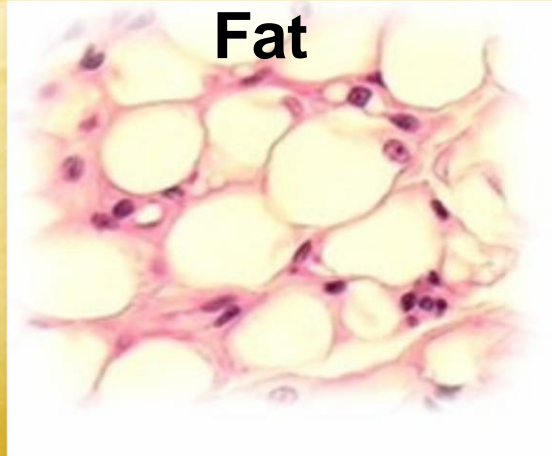
Increased Body Fat in Males

Major Contributors of Obesity

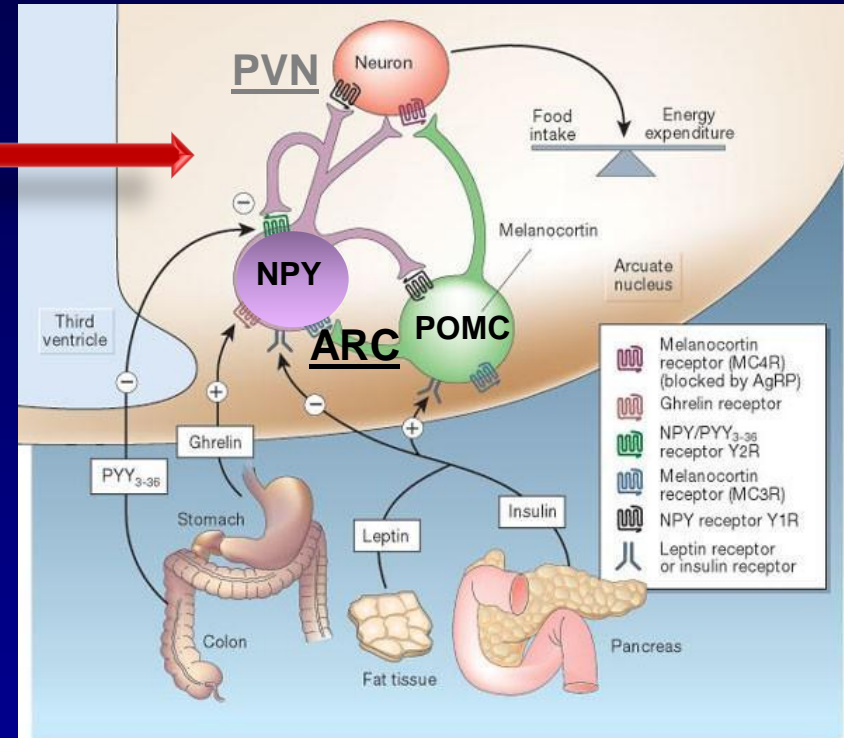
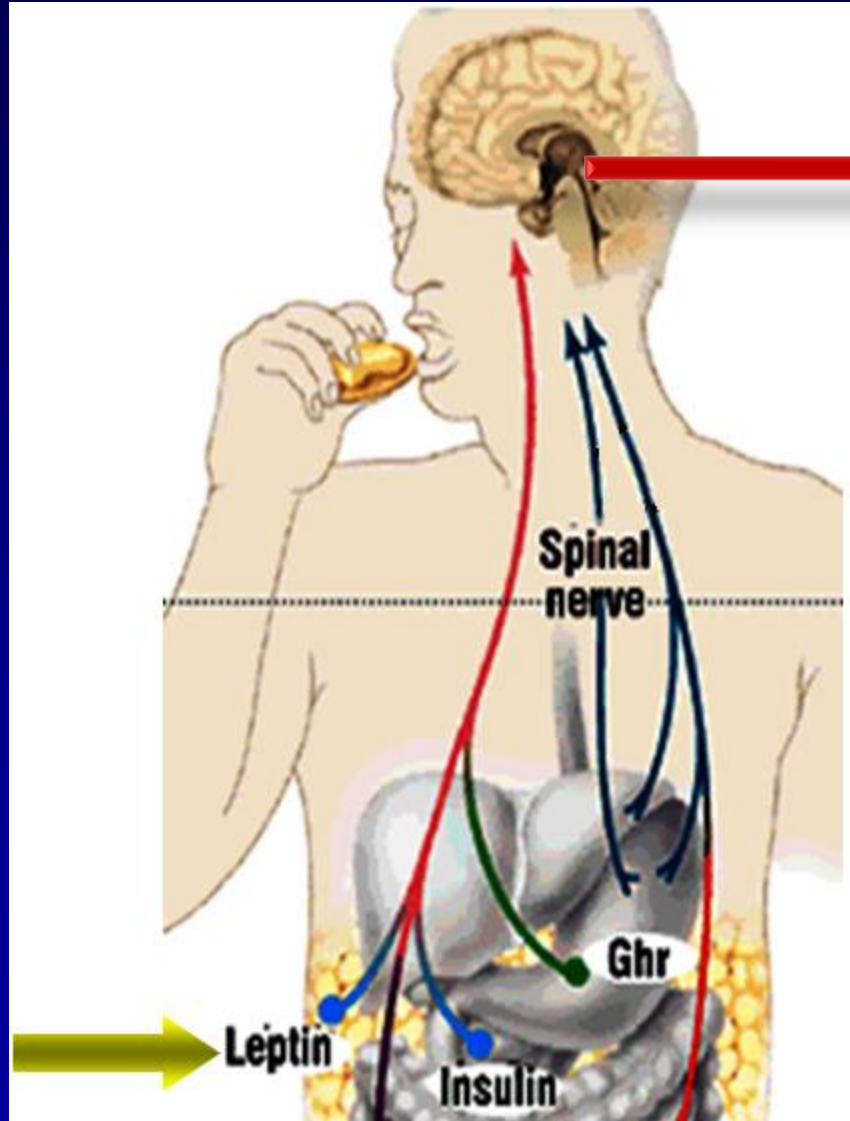
Food Intake



Fat



Appetite Regulation

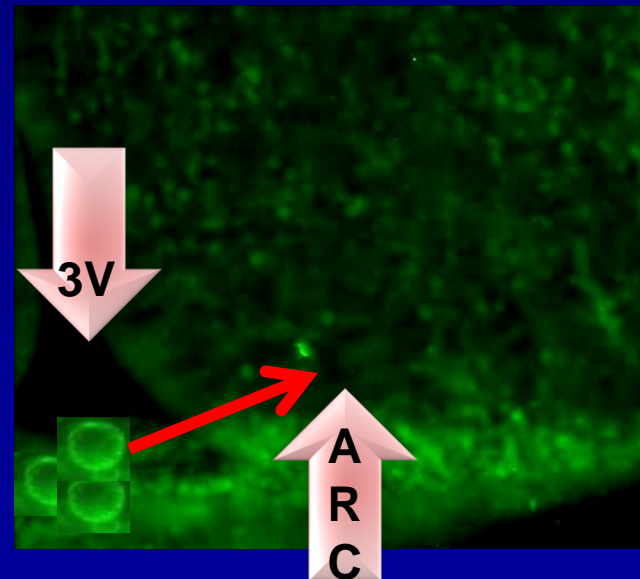
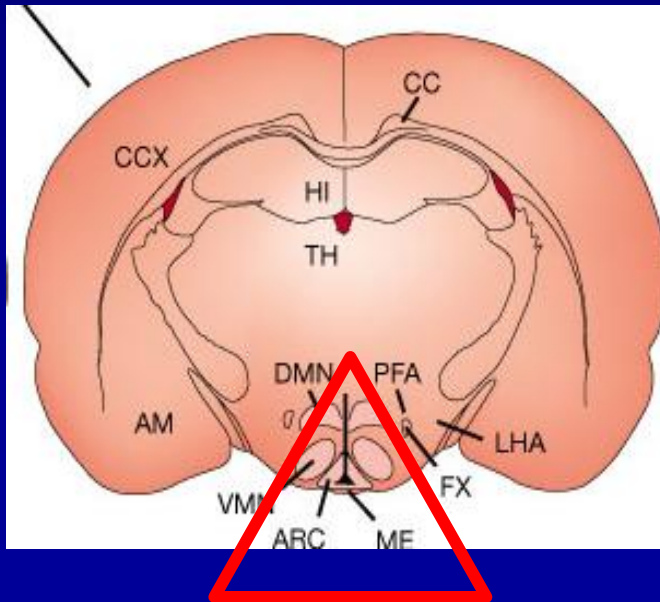


ARC Nucleus Development

- ARC cells arise from Neural Stem Cells in periventricular region
- Appetite (NPY) and Satiety (POMC) neurons populate the ARC during fetal life and this continues to develop during postnatal life

Appetite Regions

Neural Stem Cells

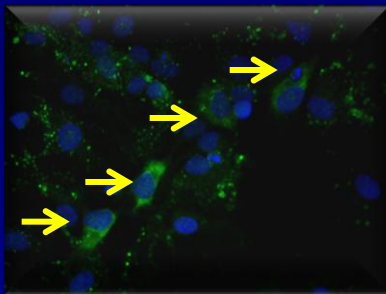


Hypothesis of Enhanced Appetite

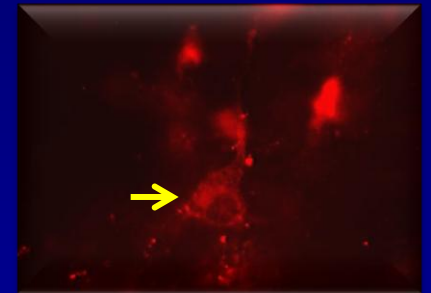
Fetal BPA exposure



↑ Appetite (NPY) neurons



↓ Satiety (POMC) neurons

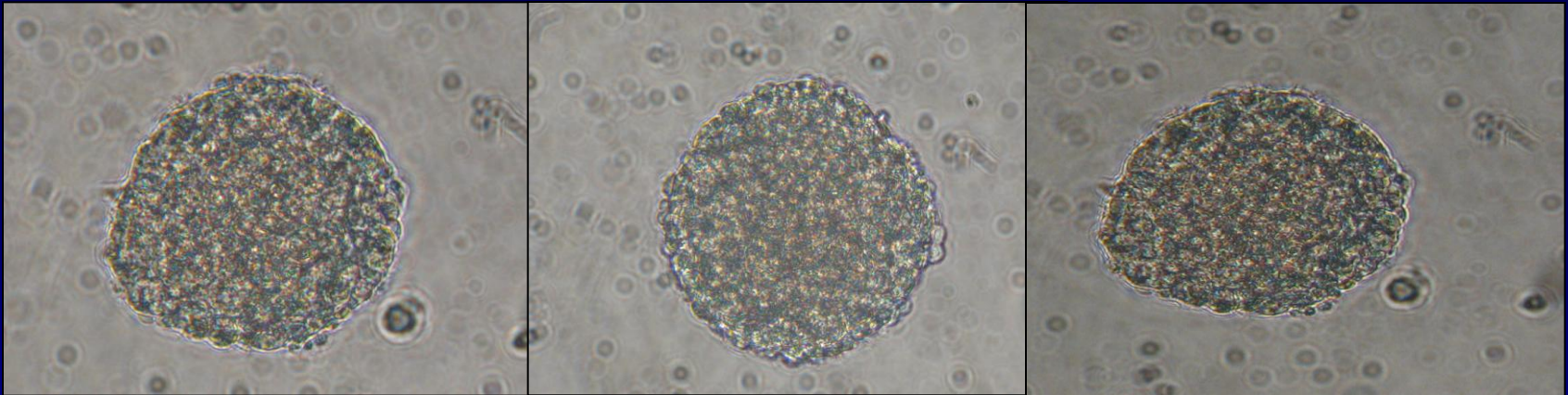


Increased Food Intake



Hypothalamic Neurospheres

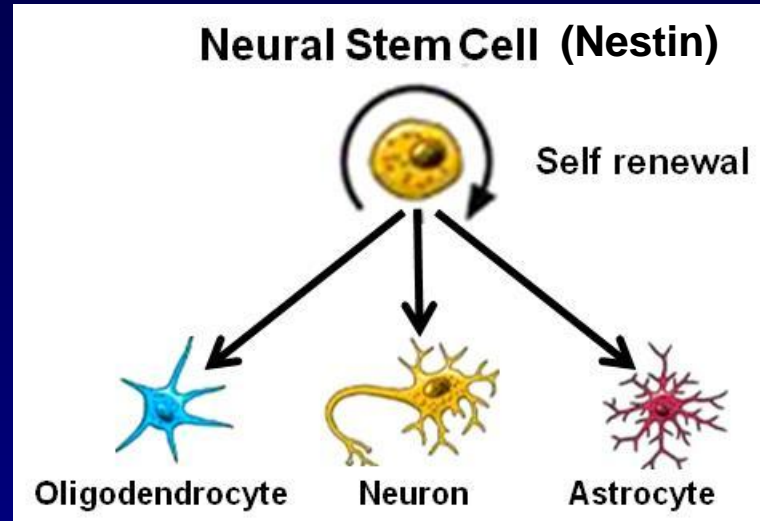
Undifferentiated



Early Differentiation

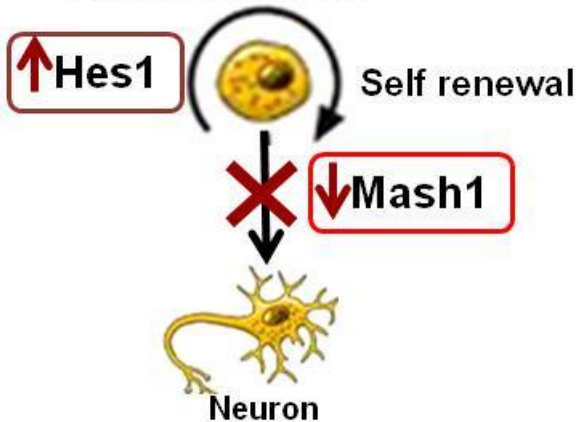


NSC Proliferative & Differentiation Factors



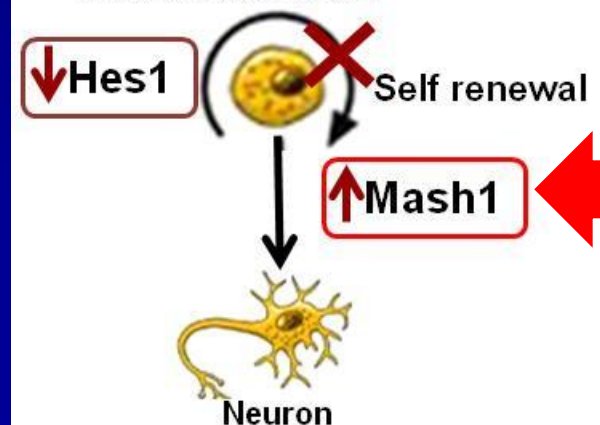
Proliferation

Neural Stem Cell



Differentiation

Neural Stem Cell



Methods: NSC

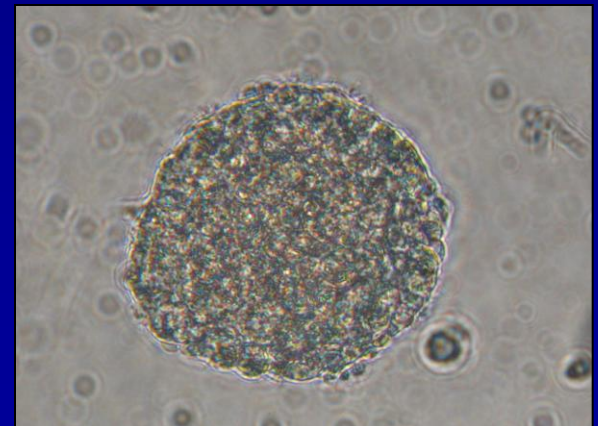
In Utero BPA Exposure

Hypothalamic NSCs from 1 day old newborns of Control and BPA treated dams.

- Culture media - complete medium

Analysis

- Immunostaining of 10 micron sections:
 - **Nestin** – NSC marker
 - **Hes1** - proliferative factor

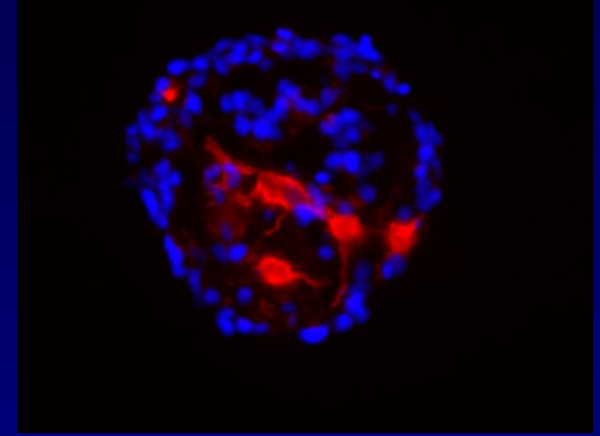
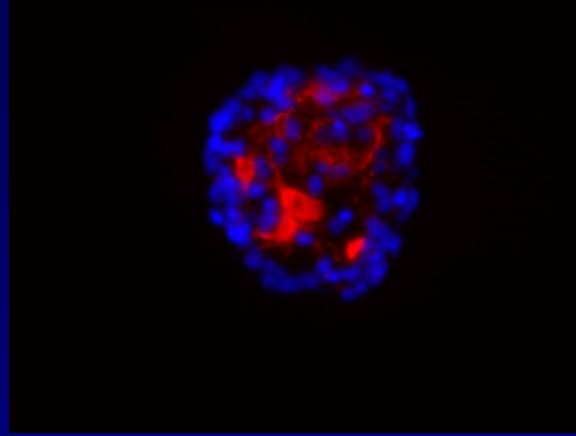


NSC Immunostaining: 1 Day Newborn

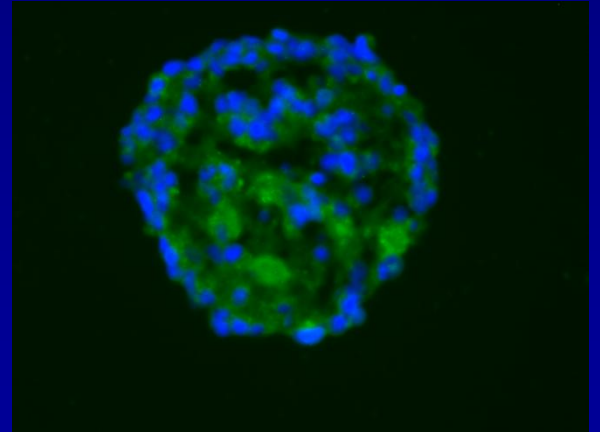
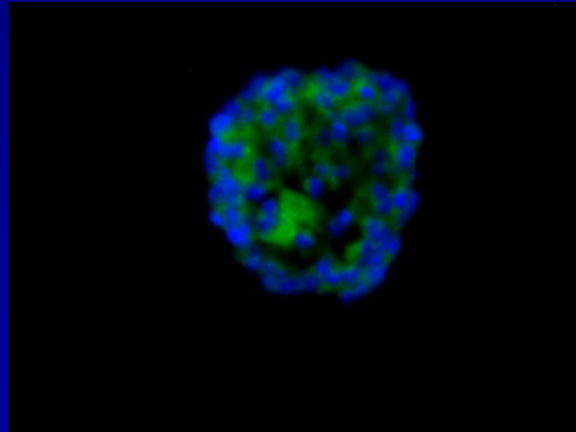
Control

BPA

**DAPI (nuclear)
+ Nestin (NSC)**



**DAPI (nuclear)
+ Hes1 (Prolif)**



Cultured for 7 days in complete medium; 10 micron sections

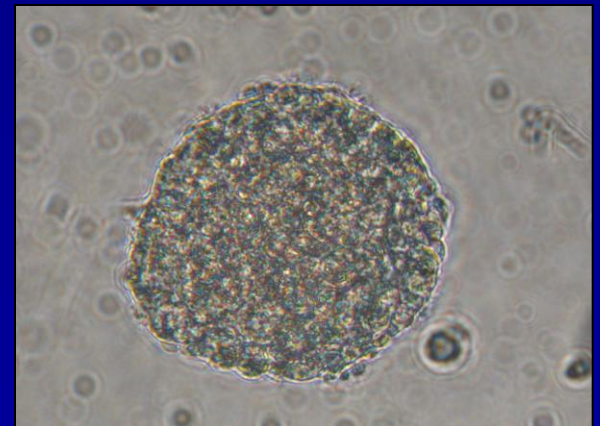
Methods: *In Vitro* BPA Exposure

BPA Treatment of Neural Stem Cells

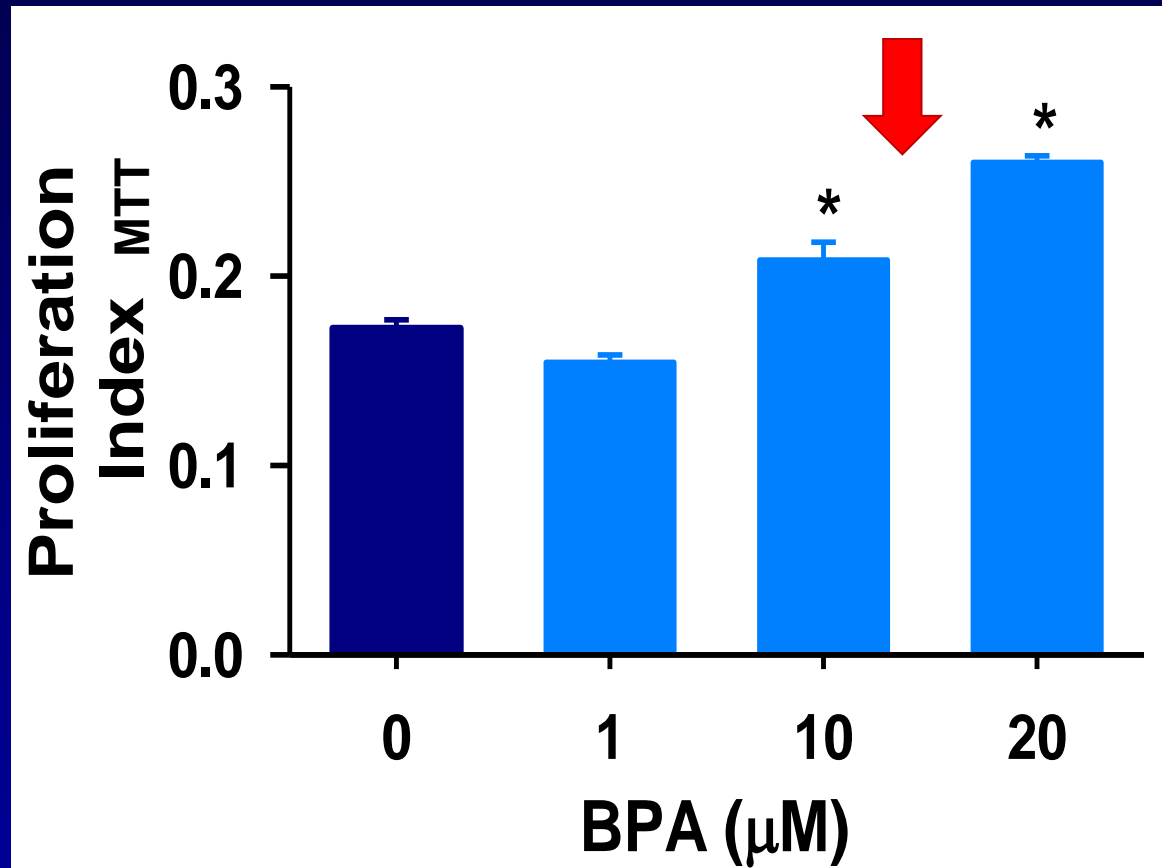
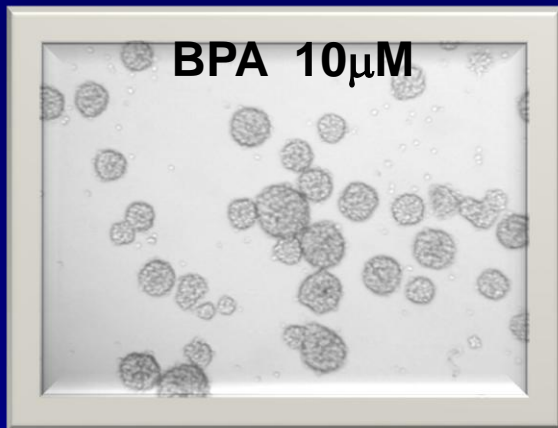
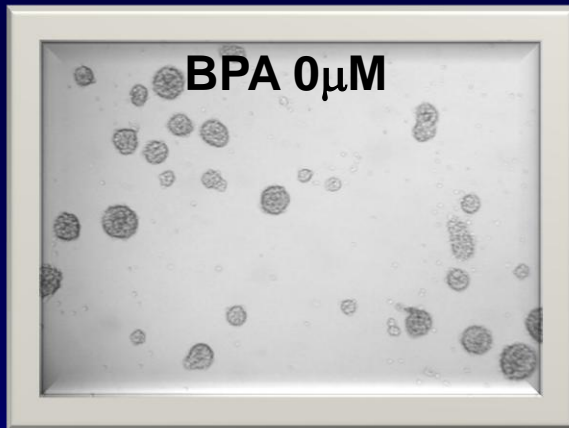
- Hypothalamic NSCs: 1 day old newborns
- BPA treatment:
 - Neurospheres Cultures: Self renewal or differentiated
 - Dose - 1, 10, 20 μM x 5 days

Analysis

- MTT assay: NSC proliferation
- Protein expression (Western Blot):
 - **Nestin** – NSC marker
 - **Hes1** - proliferative factor
 - **Tuj1** - neuronal marker
 - **GFAP** - astrocyte
 - **Mash1** - neurogenic factor

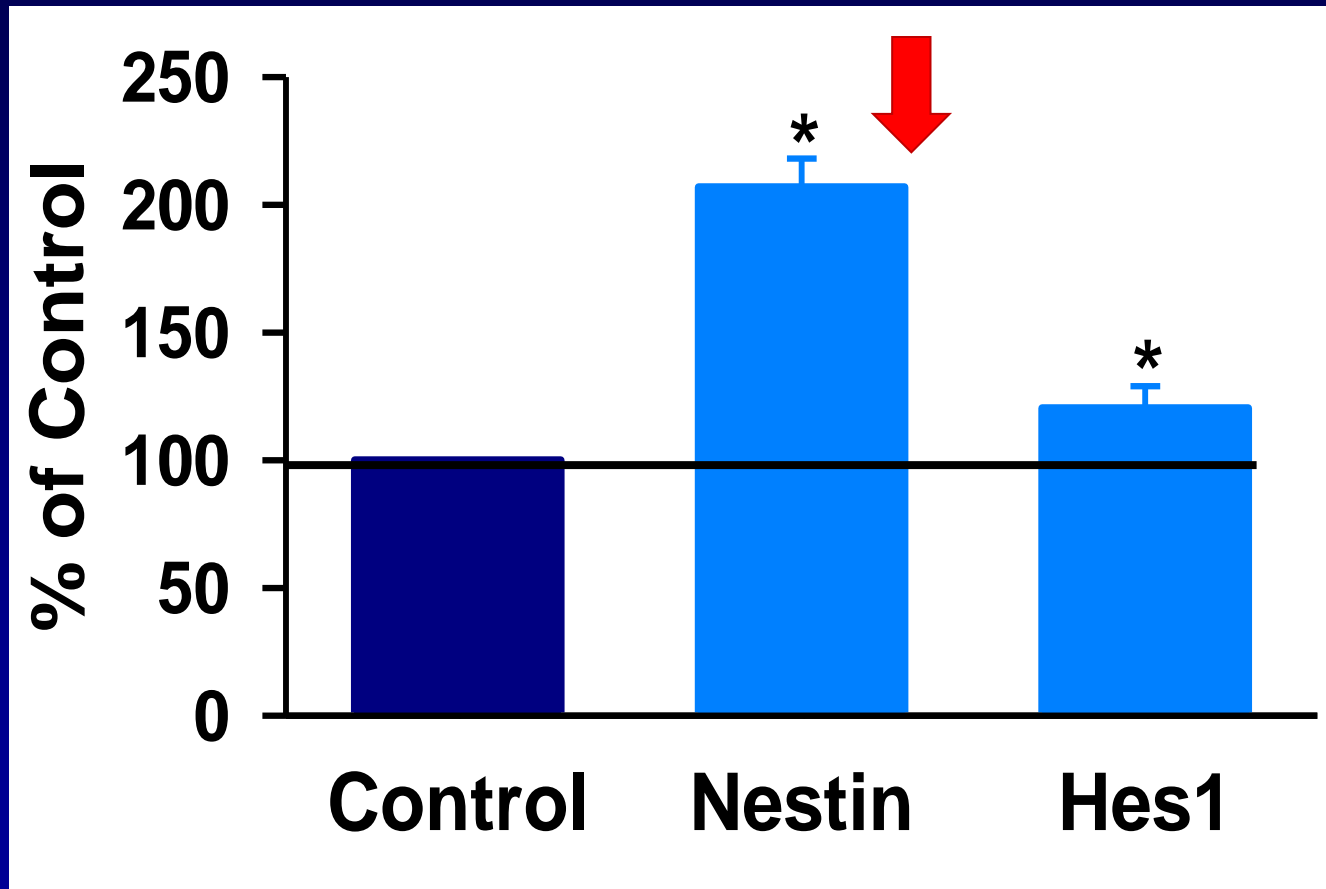


BPA: Neural Stem Cell Proliferation



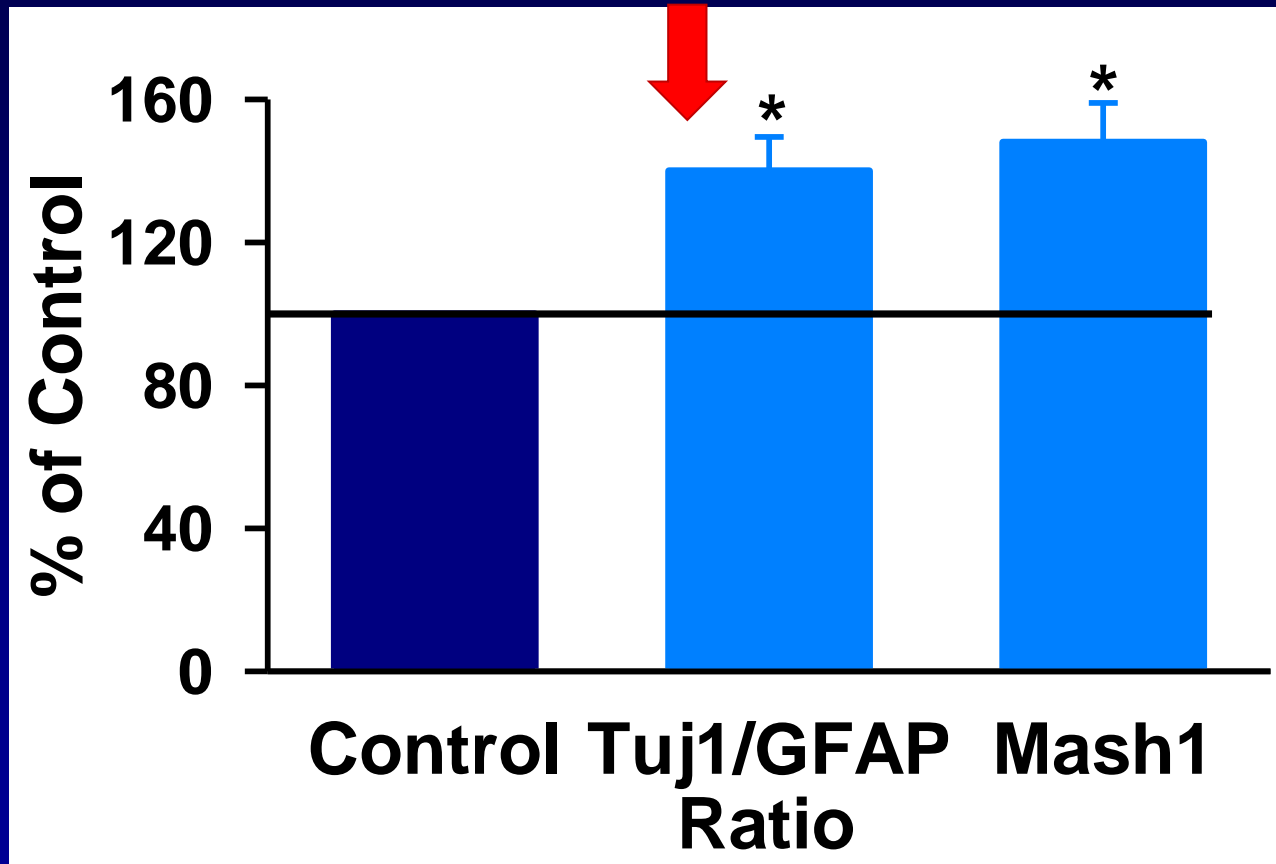
Increased Proliferation

BPA: Neural Stem Cell Proliferation Factors



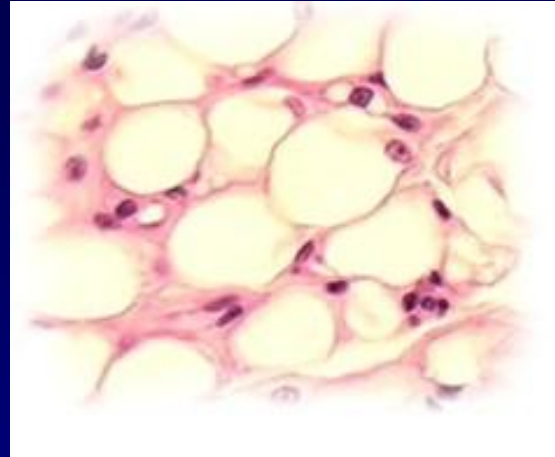
Increased Proliferative Potential

BPA: Neural Stem Cell Differentiation



Increased Neuron to Astrocyte Ratio

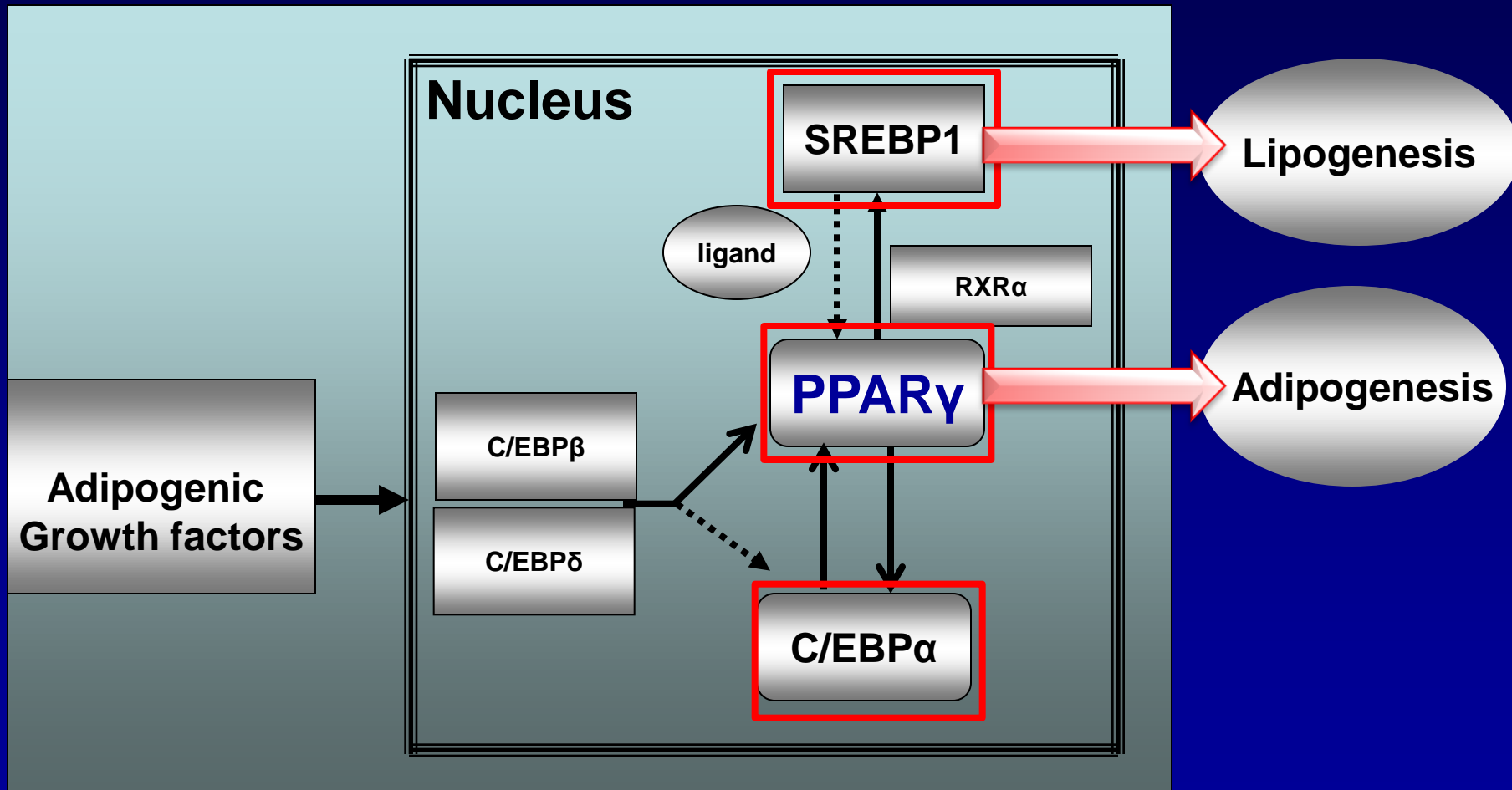
Programmed Adipogenesis



- **Adipose Proliferation and Differentiation**
- **Lipogenesis**

Regulation of Adipogenesis

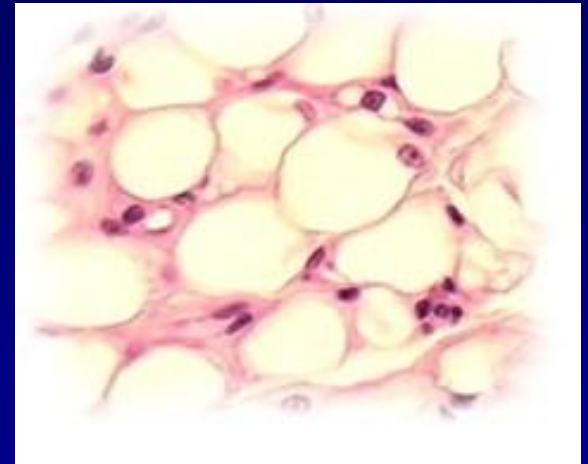
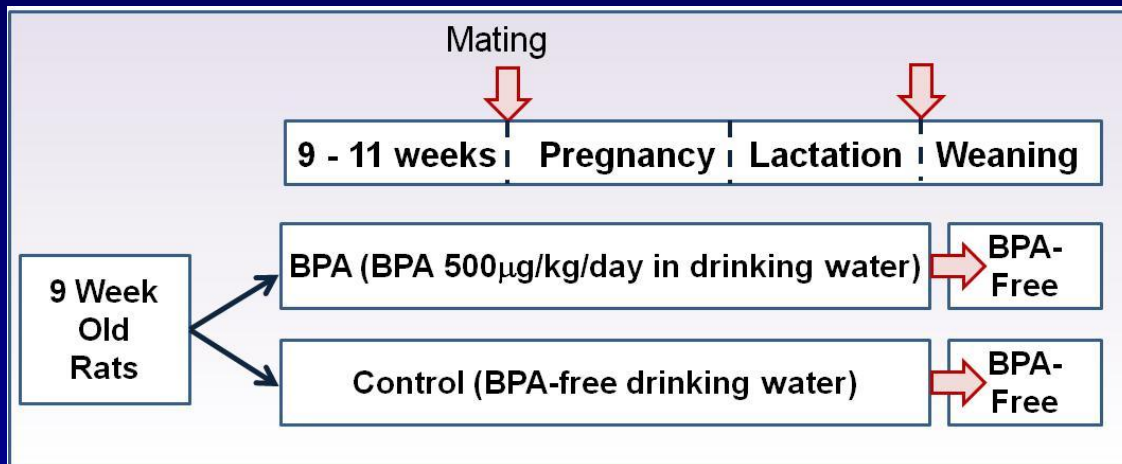
Peroxisome proliferator-activated receptor gamma 2 (PPAR γ 2)



In Utero BPA Exposure

Control and BPA treated dams: 3 week old offspring

Retroperitoneal adipose tissue

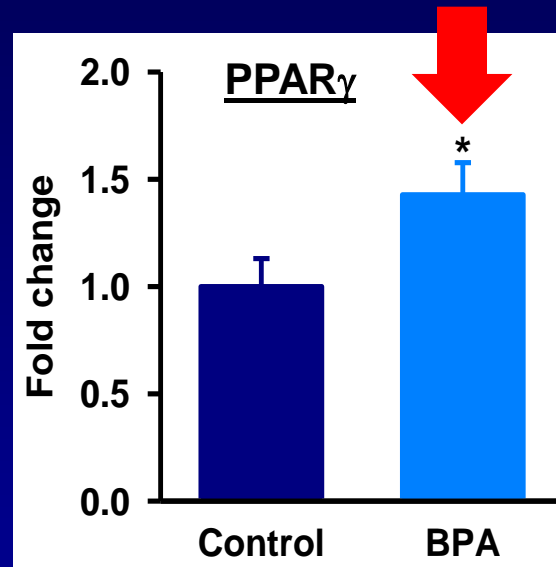
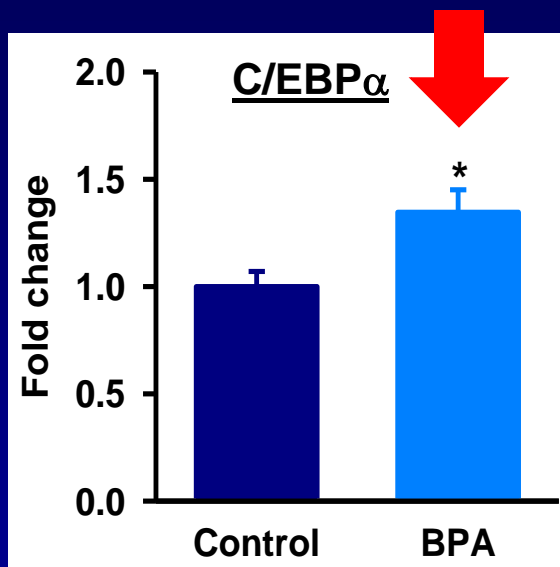


Analysis

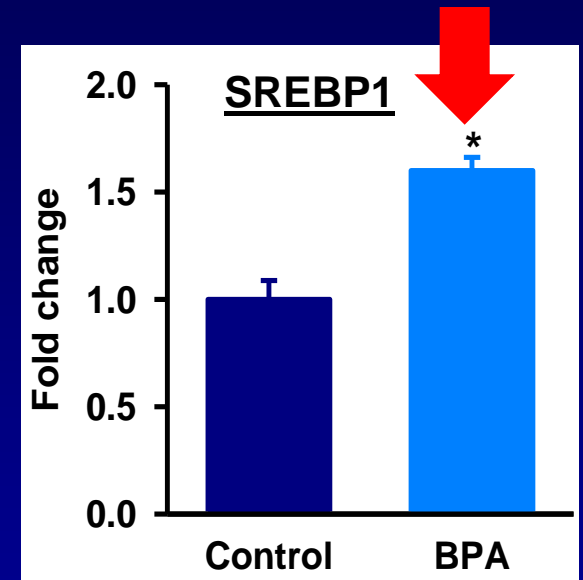
- Protein Expression (Western Blot):
 - **PPAR γ** – adipogenic transcription factor
 - **C/EBP α** – adipogenic transcription factor
 - **SREBP1** – lipogenic transcription factor

BPA: Adipocyte Transcription Factors

Adipogenic



Lipogenic



**Increased Adipocyte Differentiation
and Lipogenesis**

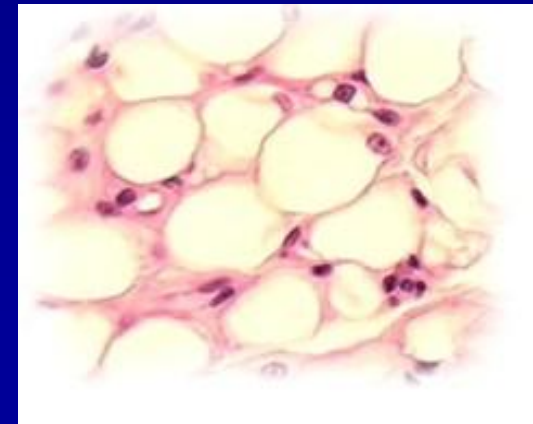
Methods: *In Vitro* BPA Exposure

Adipocytes

- Subcutaneous adipose tissue: 1 day old newborns
- Cultures: pre-adipocytes or differentiated adipocytes
- BPA treatment:
 - Dose - 1, 10, 20 μM
 - Period of treatment - 5 days

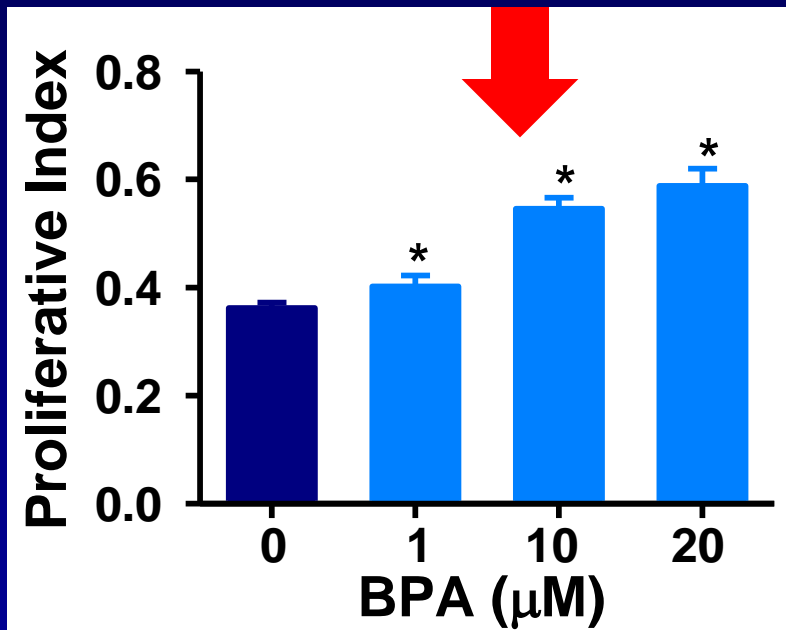
Analysis

- MTT assay: Preadipocyte proliferation
- Oil O Red stain: Preadipocyte lipid storage
- Protein expression (Western Blot):
 - **PPAR γ** – adipogenic transcription factor
 - **C/EBP α** – adipogenic transcription factor
 - **SREBP1** – lipogenic transcription factor

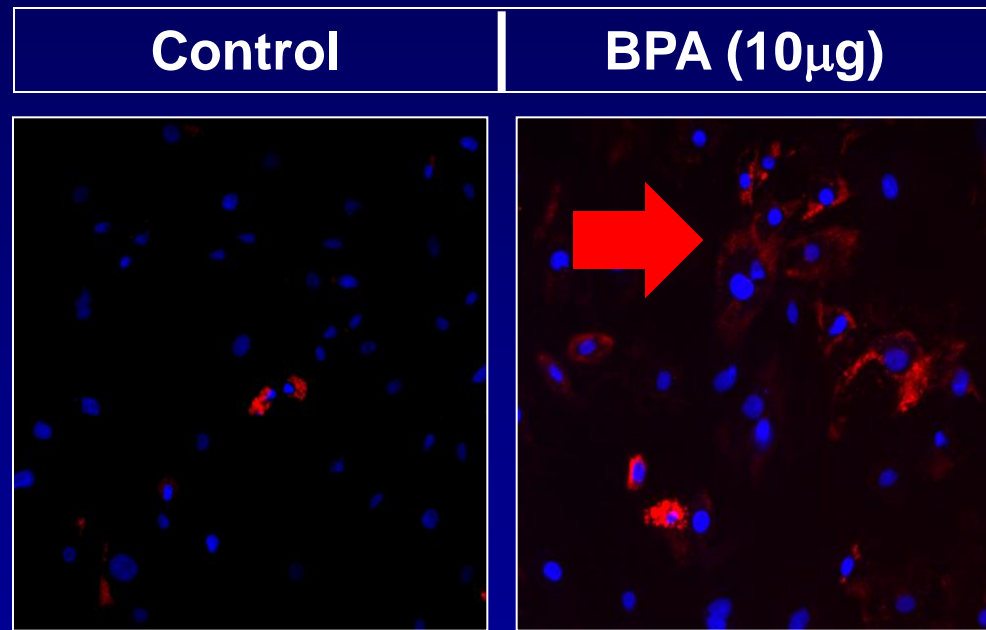


BPA: Preadipocytes

Adipogenesis



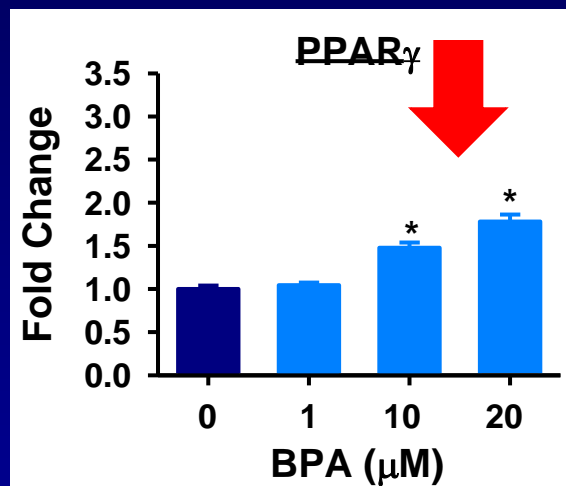
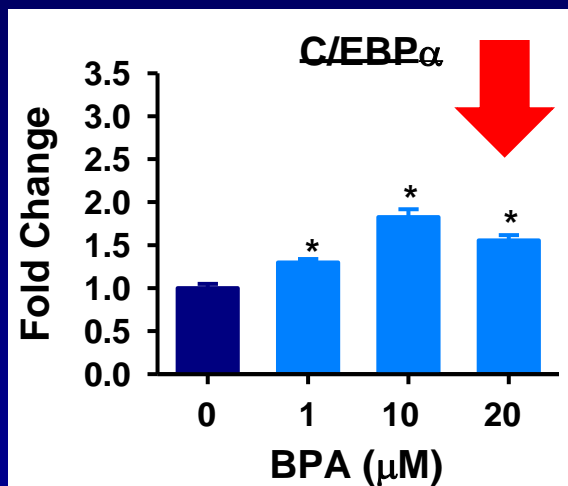
Lipogenesis



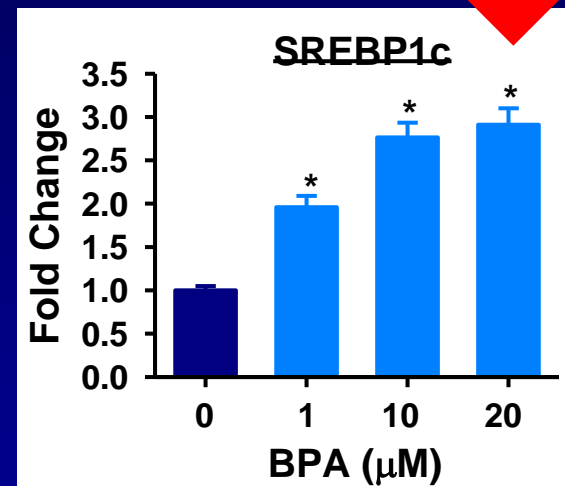
Increased Proliferation and Lipid Storage

BPA: Differentiated Adipocytes Transcription Factors

Adipogenic

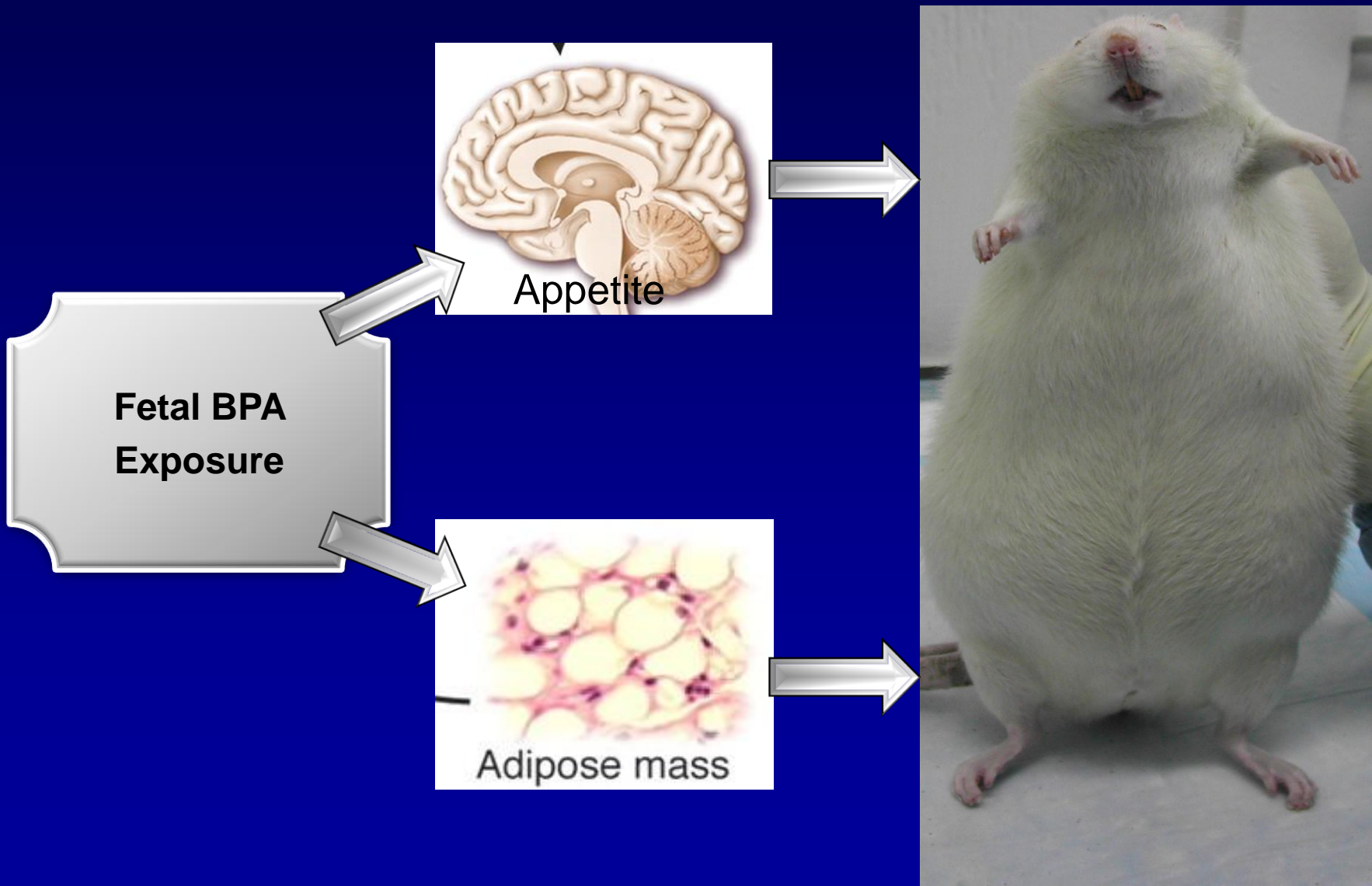


Lipogenic



**Increased Adipocyte Differentiation
and Lipogenesis**

Fetal Programming of Obesity: Effect of BPA



Conclusions

- ***In utero* exposures altered nutrition and/or environmental agents may have marked effect on children and grandchildren**
 - Need to refocus environmental agent effects beyond toxicity
 - Animal studies to explore mechanisms
 - Correlation of animal and human effects
 - Longer term human studies
- In the meantime, **“all things in moderation”**