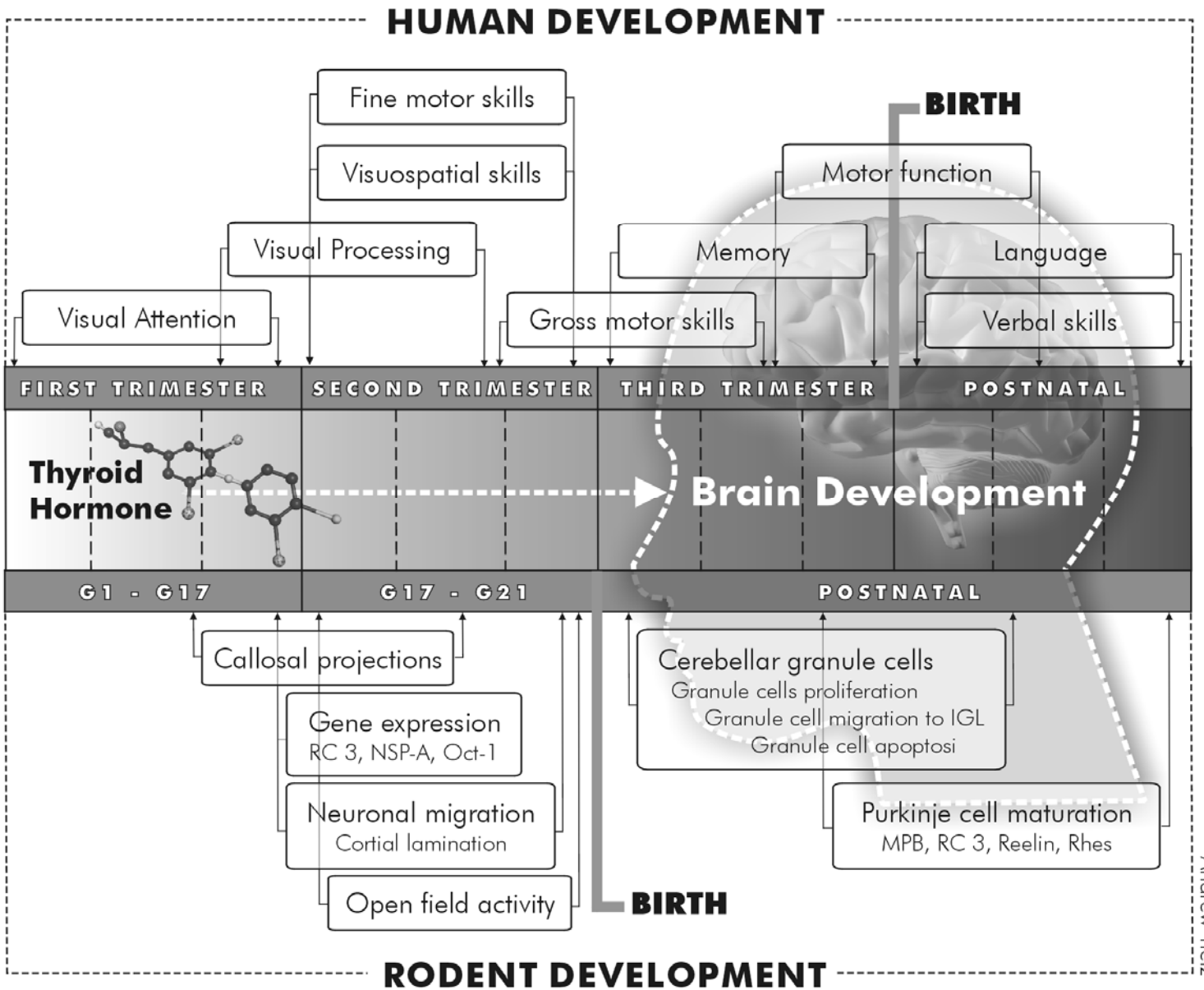
Low-Dose Effects of Thyroid Toxicants on Neurodevelopment

R. T. Zoeller

Thyroid Hormone is Essential for Normal Brain Development

The "critical period" of thyroid hormone action in brain development was defined as that period after birth when TH therapy must be initiated to rescue the infant from cretinism.

Brown AW *et al.* (1939) Hypothyroidism and cretinism in childhood. VI. Influence of thyroid therapy on mental growth. Amer J Dis Child 57:517-523.



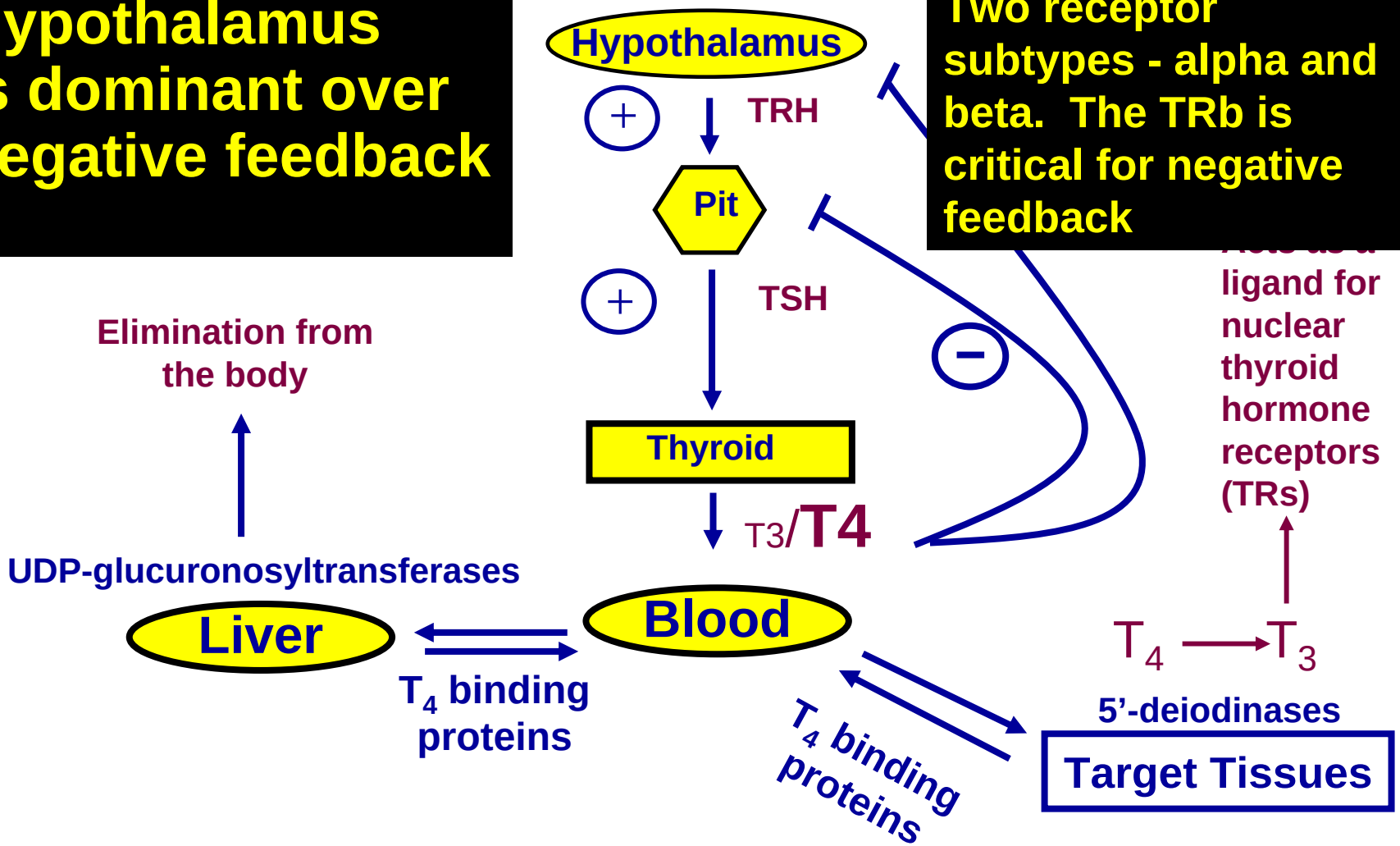
Zoeller and Rovet, 2004. J Neuroendocrinol 16, 809-818.

Environmental factors that interfere with TH signaling may interfere with brain development

How do we identify thyroid toxicants and how to we assess the risk?

Regulation of TH levels in the blood

Hypothalamus Is dominant over negative feedback



Working Hypothesis

- 1) Thyroid hormone produces non-linear dose-dependent effects on endpoints within the developing brain, heart and liver; and some endpoints are more sensitive than others to thyroid hormone insufficiency
- 2) known thyroid toxicants that act at different sites within the HPT axis will produce different dose-response curves on these endpoints
- 3) changes in tissue metabolism of thyroid hormones can account for the differences in dose-responses.

Both PCB Exposure and Methimazole Reduce Serum T4

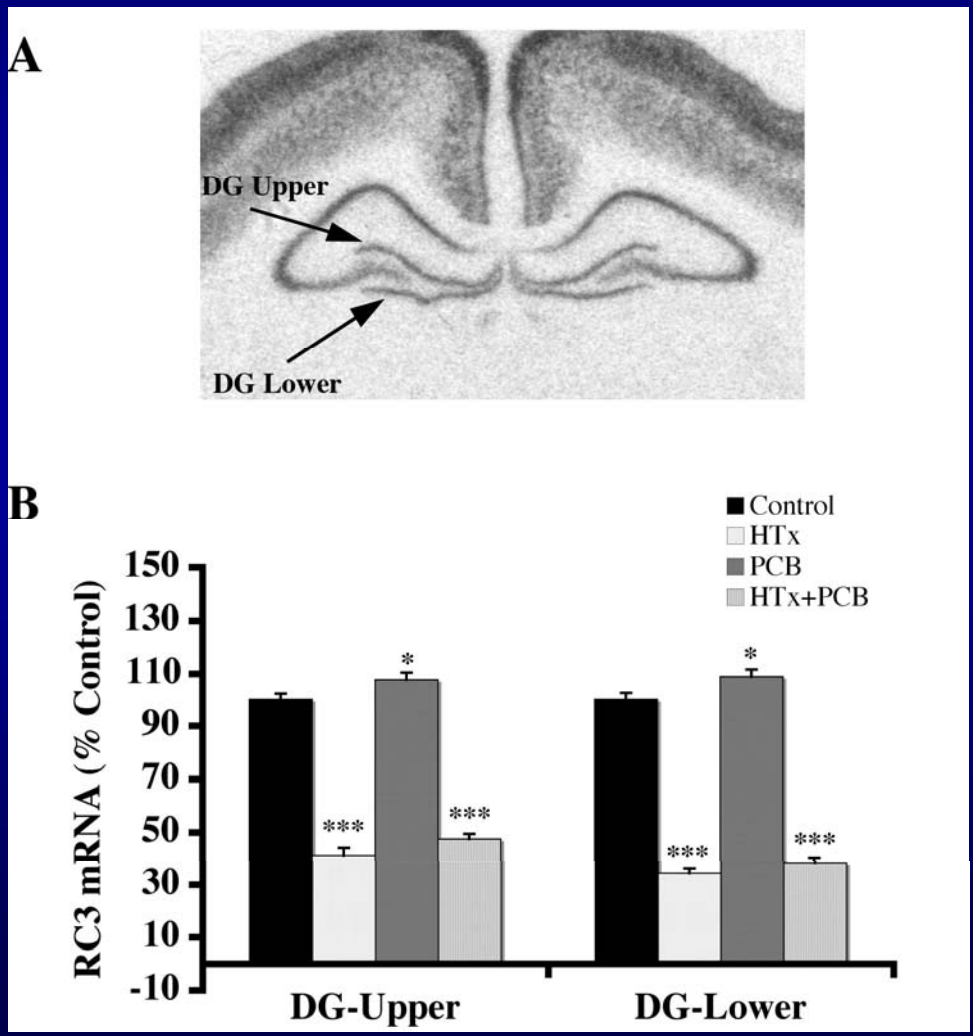
Table1. Serum hormone levels in pups on P15 [24]

Treatment	T_4(μg/dL)	GH(ng/mL)
Control	5.851 ± 0.38	8.230 ± 2.8
HTx	0.4[a]	0.5[a]
PCB	1.365 ± 0.42[b]	14.65 ± 5.4
HTx+PCB	0.4[a]	0.5[a]

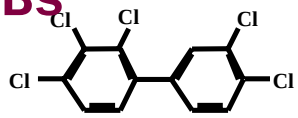
[a] Values below detection limit

[b] Significantly different from control

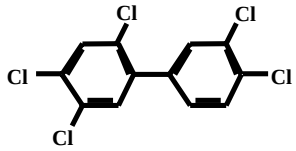
PCB Exposure and MMI Do Not Produce the Same Effect on TH Signaling in the Brain



Mono-*ortho* chlorinated PCBs

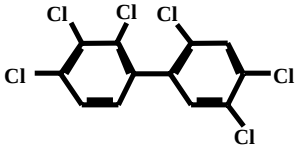


PCB 105

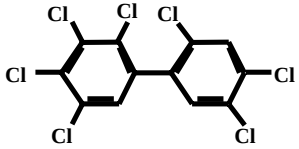


PCB 118

Di-*ortho* chlorinated PCBs

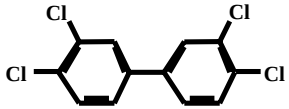


PCB 138

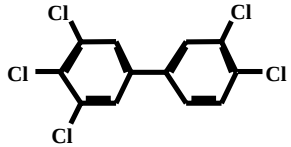


PCB 153

Non-*ortho* chlorinated PCBs



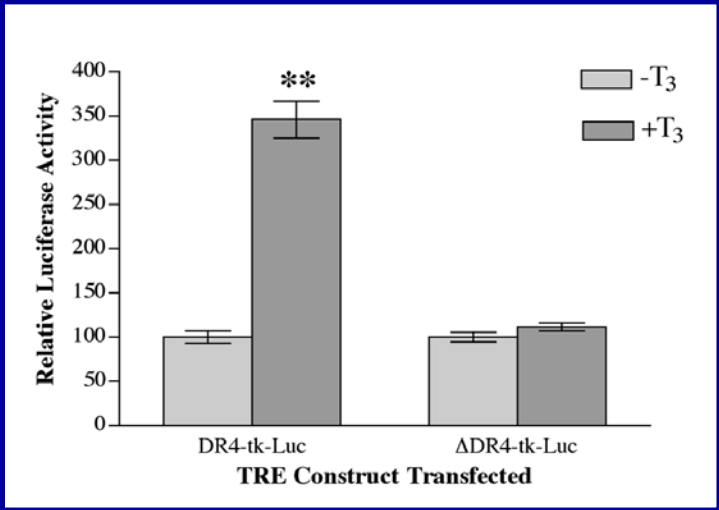
PCB 77

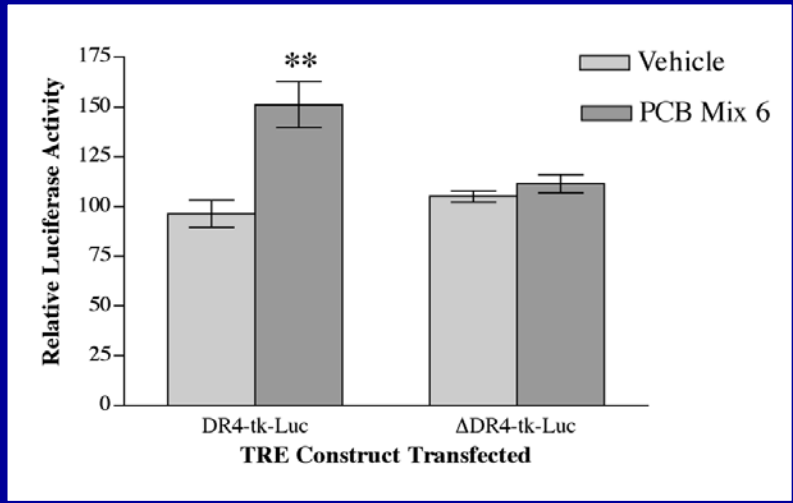


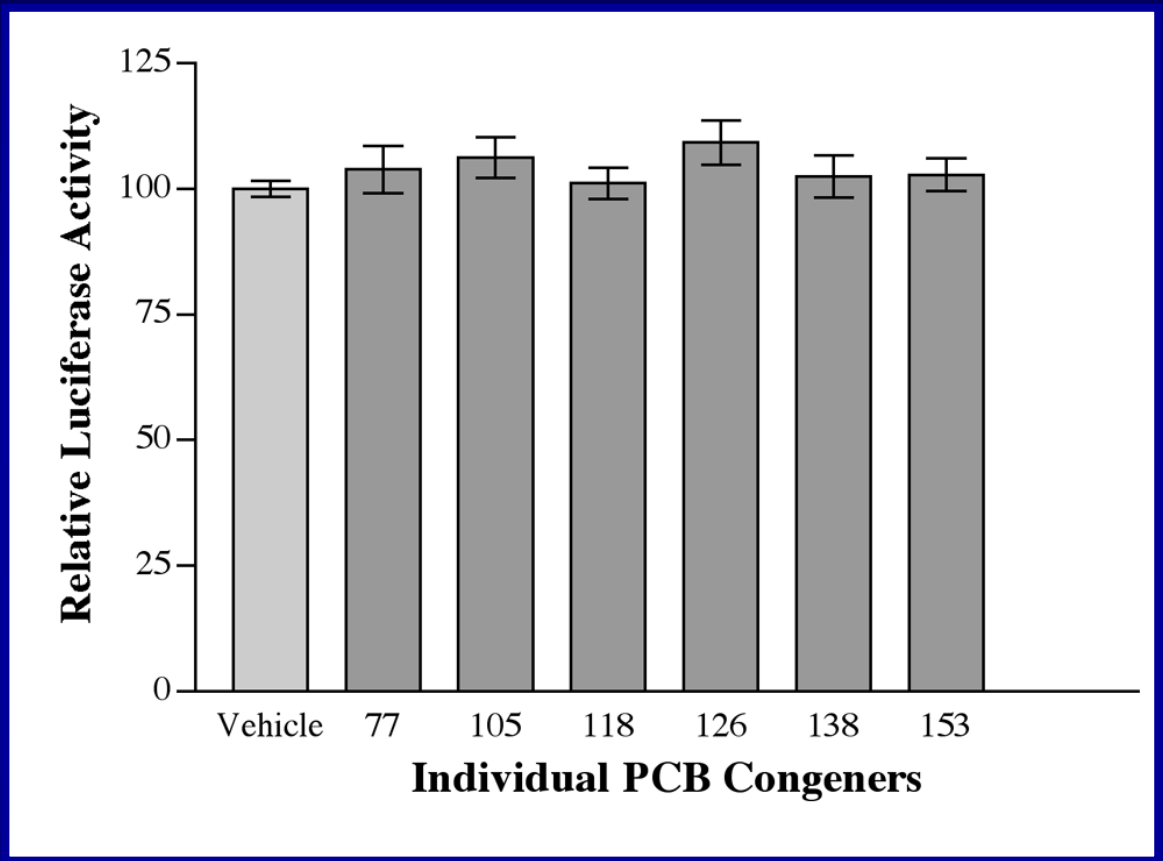
PCB 126

To identify specific PCB congeners that may act as TH agonists, we developed a mixture of 6 PCBs that represent three classes.

Mixture of 6 PCB Congeners Activates The TR in GH3 Cells

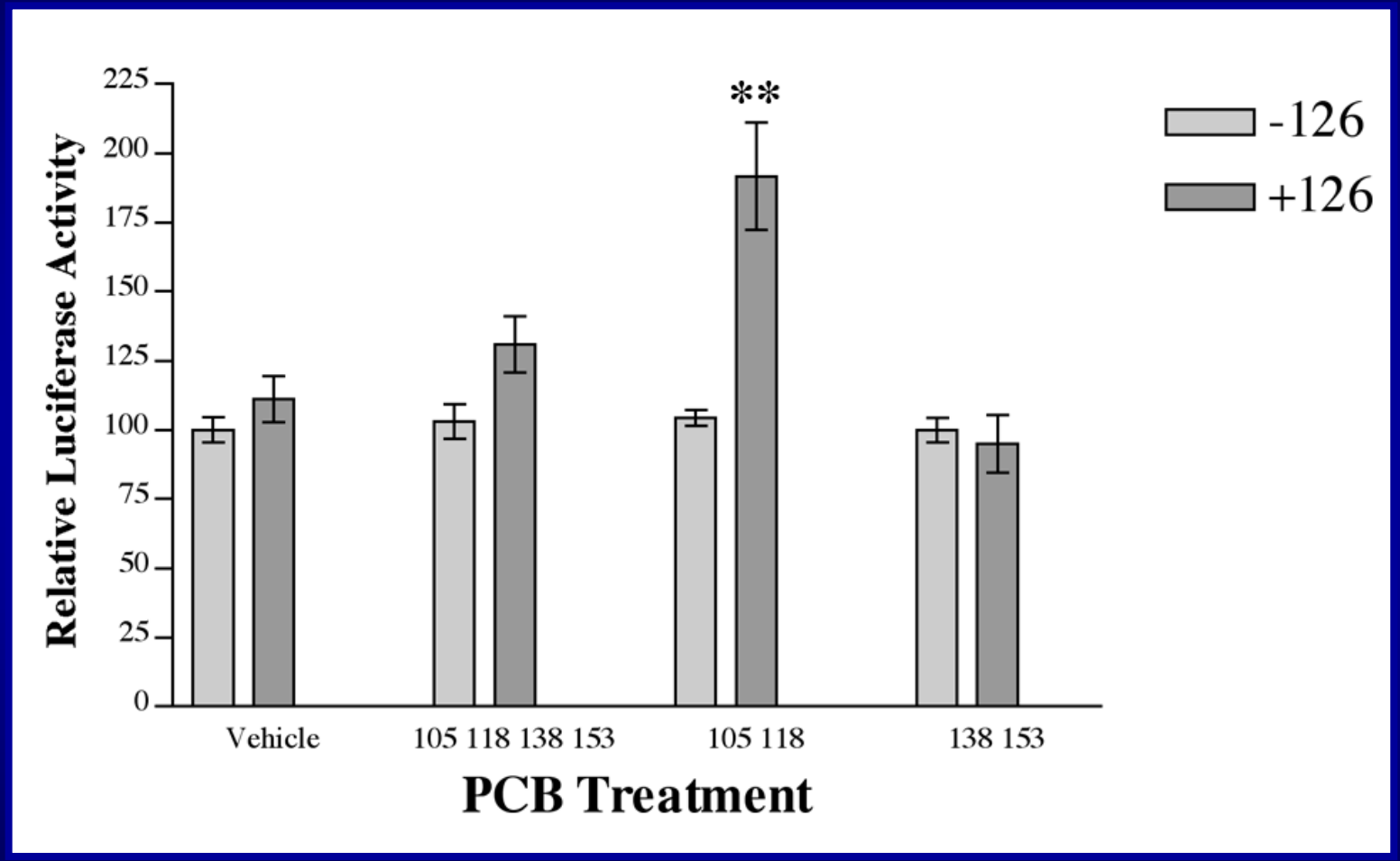




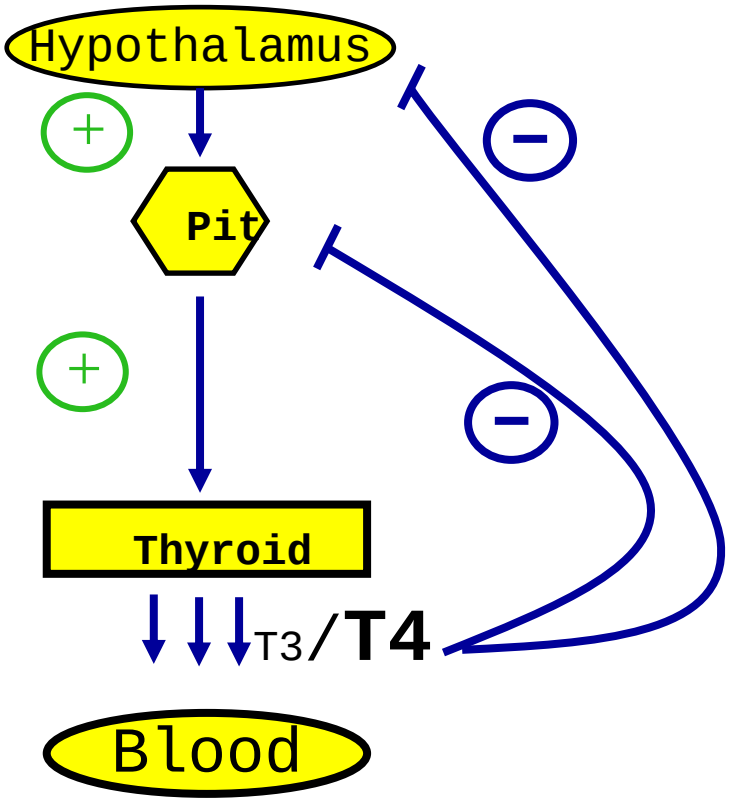


None of the individual PCB congeners exerts a TH-like effect on TRE-driven relative luciferase activity

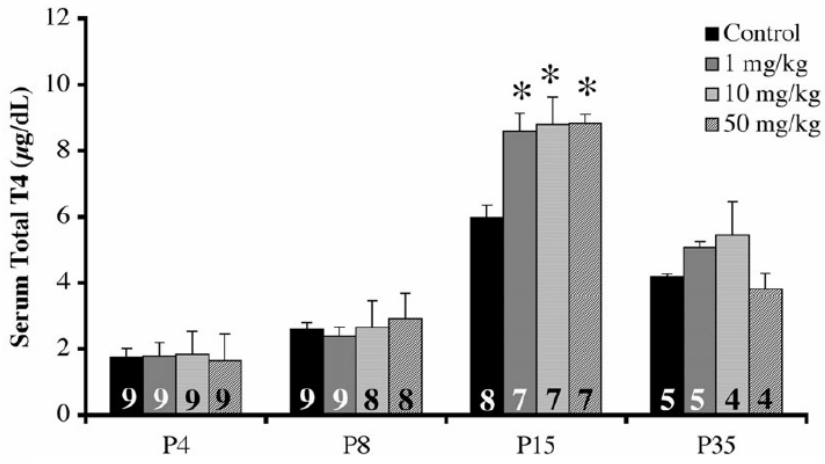
PCB 126 is required for the mono-ortho PCBs to increase TRE-driven relative luciferase activity



BPA antagonizes TR-β mediated negative feedback *in vivo*

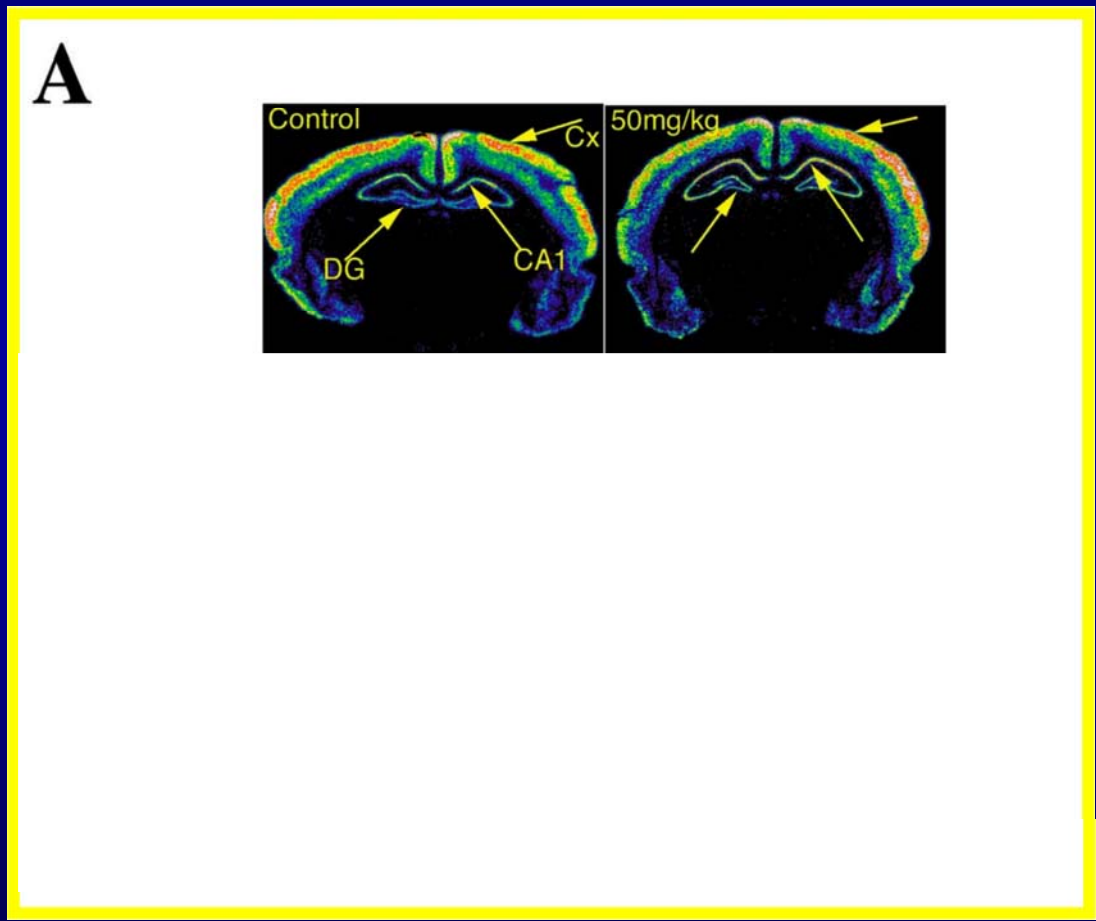


Negative feedback in the rat is not functional for the first week.



Elevated levels of TH increase RC3/Neurogranin expression on P15

- But a TRβ-selective agonist would not affect tissues/processes regulated by the TRα.



Endocrinology 146(2): 607-612

Toxicants

- PTU -- used to produce a graded series of groups characterized by different T4 levels (Mary Gilbert).
- Perchlorate -- Acts by a different mechanism of action (NIS inhibitor rather than TPO inhibitor (PTU)) (with Jeff Fisher).
- PBDE -- PHAH that is predicted to act in a manner similar to that of PCBs (with Kevin Crofton).

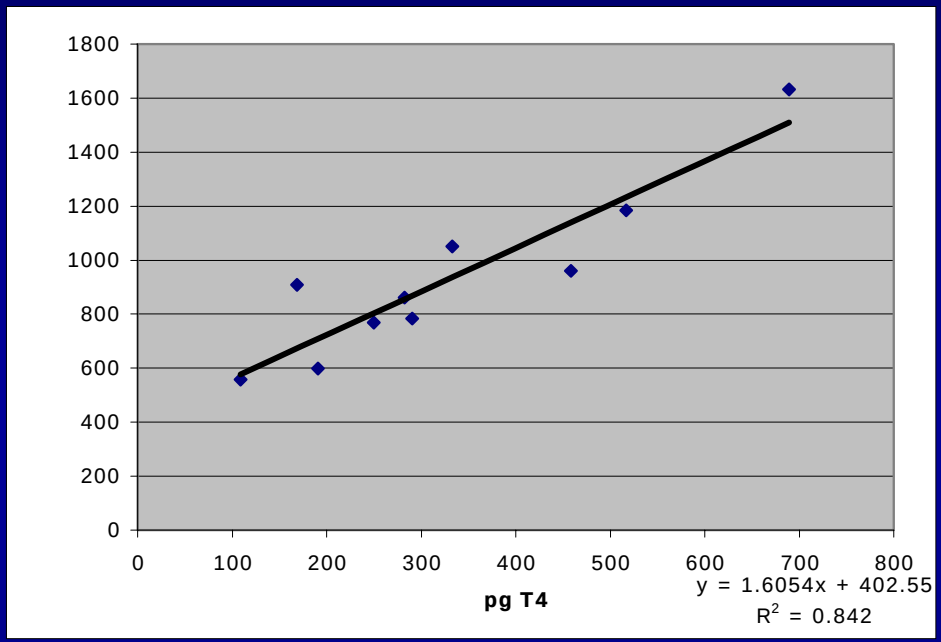
Specific Aims

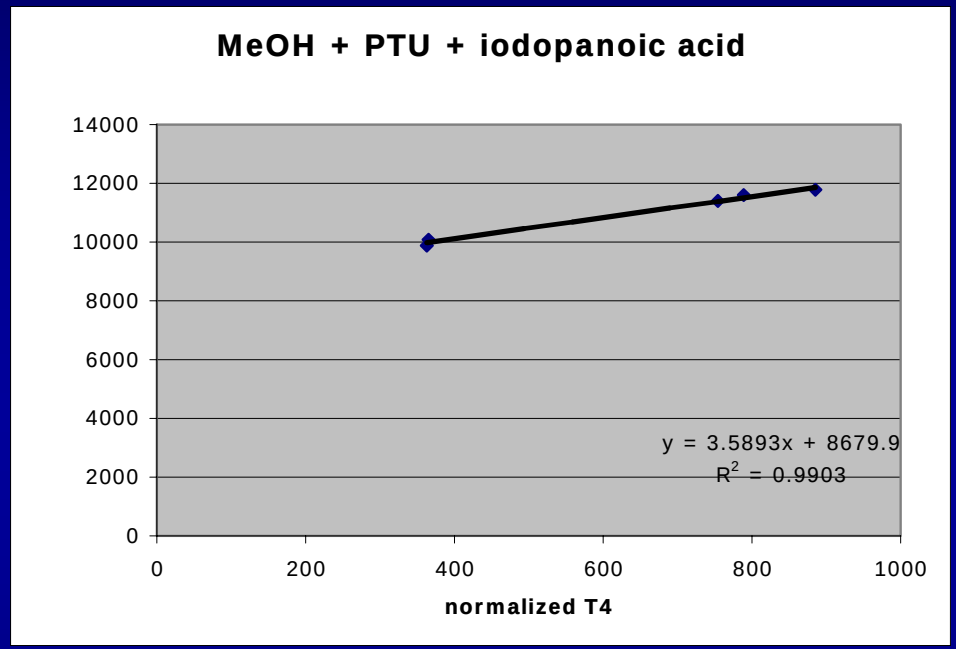
- **1. To determine the relationship between dose of thyroid hormone and response of several developmentally important endpoints in brain, liver, and heart.**
  - RIA of total serum T_4 and T_3, free T_4 and T_3-index, TSH, circulating transthyretin, thyroxine-binding globulin and thyroglobulin.
  - Endpoints of thyroid hormone action will include the expression of genes known to be thyroid hormone responsive in the developing brain, heart and liver.
  - Endpoints of developmental events will include specific measurements within the cerebral cortex, hippocampus and cerebellum in the brain, and size and weight of heart and liver.

Specific Aims

- RIA of total serum T_4 and T_3, free T_4 and T_3-index, TSH, circulating transthyretin, thyroxine-binding globulin and thyroglobulin and tissue-levels of T3/T4.

Tissue T4/T3





There is a very strong correlation between the amount of tissue extracted and the amount of T4 measured. We should validate using HPLC.

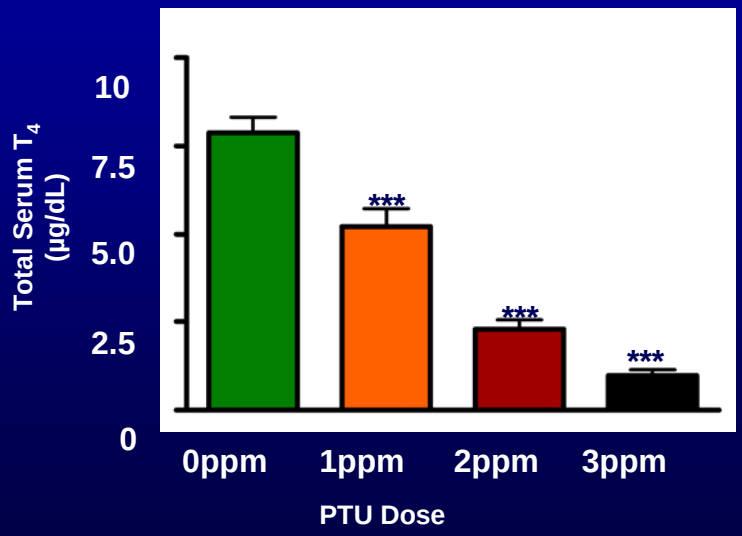
Experimental Design (Mary Gilbert)

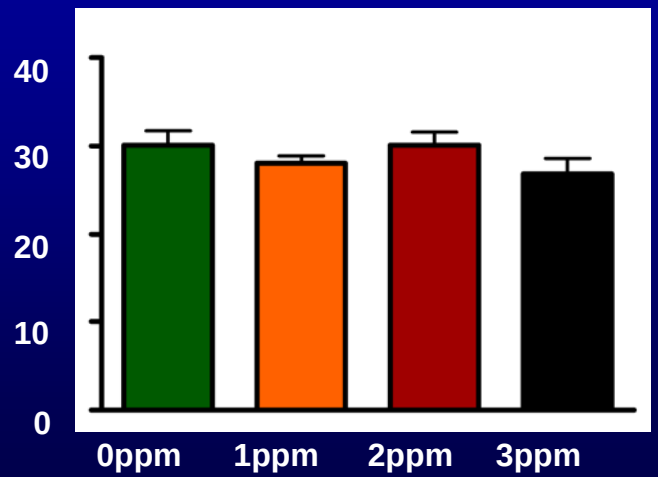
PTU Treatment of Pregnant Rats Initiated on G6

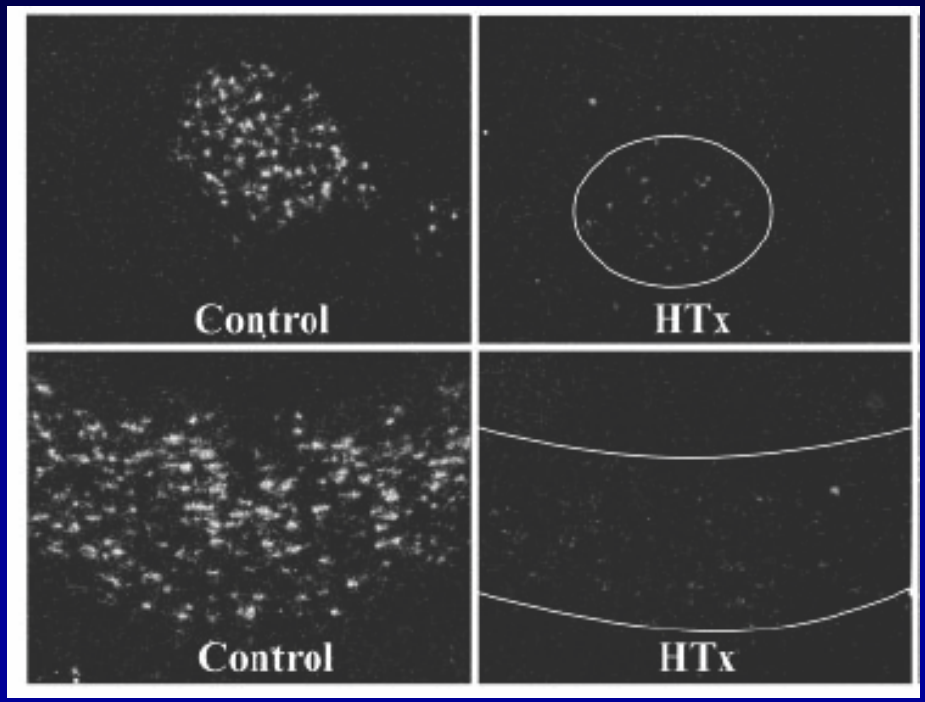
Control | 1ppm PTU | 2ppm PTU | 3ppm PTU

Animals Sacrificed on P15, trunk blood collected for T_4 analysis
Brains Harvested for ISH and IHC

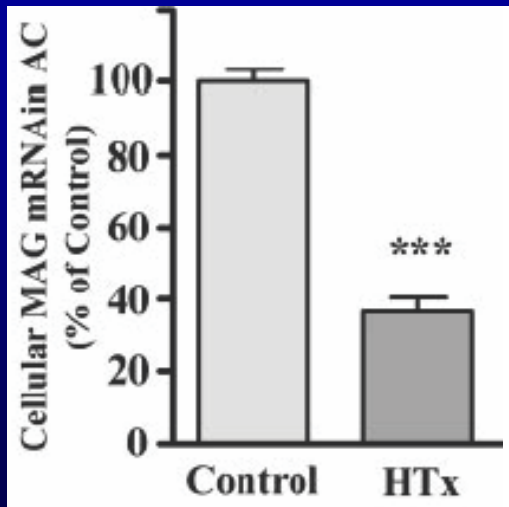
Developmental PTU exposure reduces T_4 levels but does not alter body weight

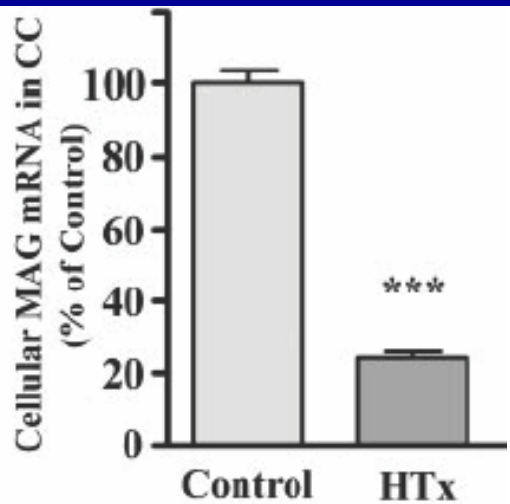


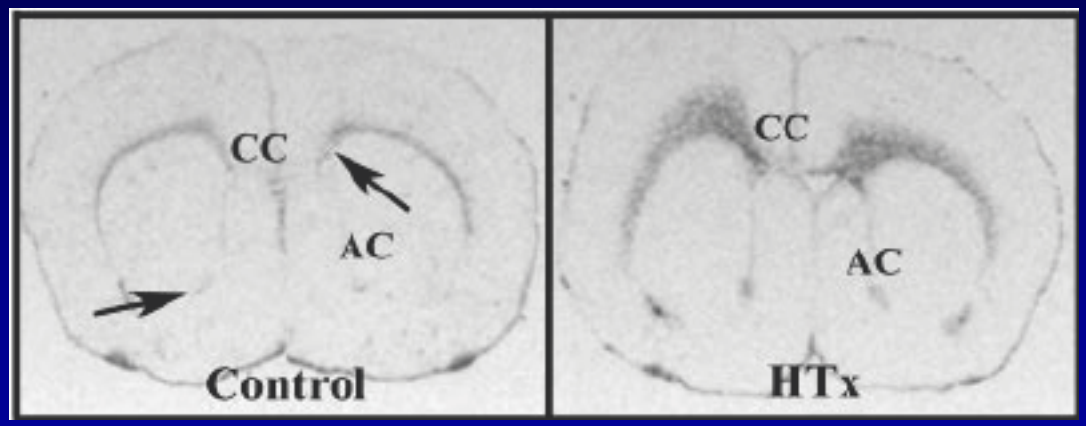




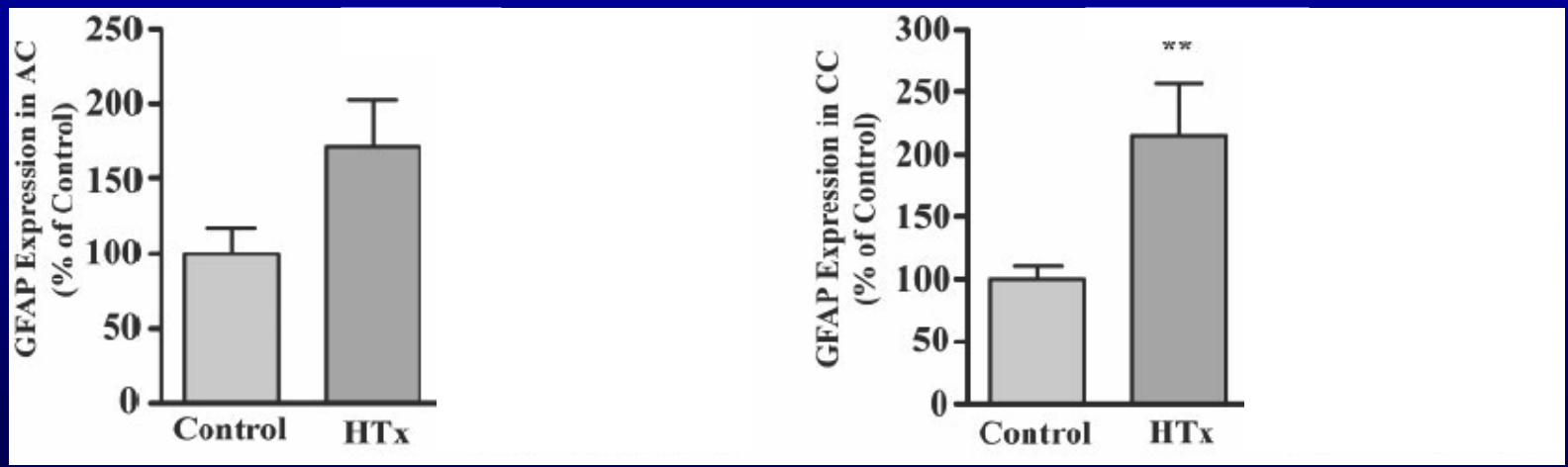
TH increases the number of oligodendrocytes



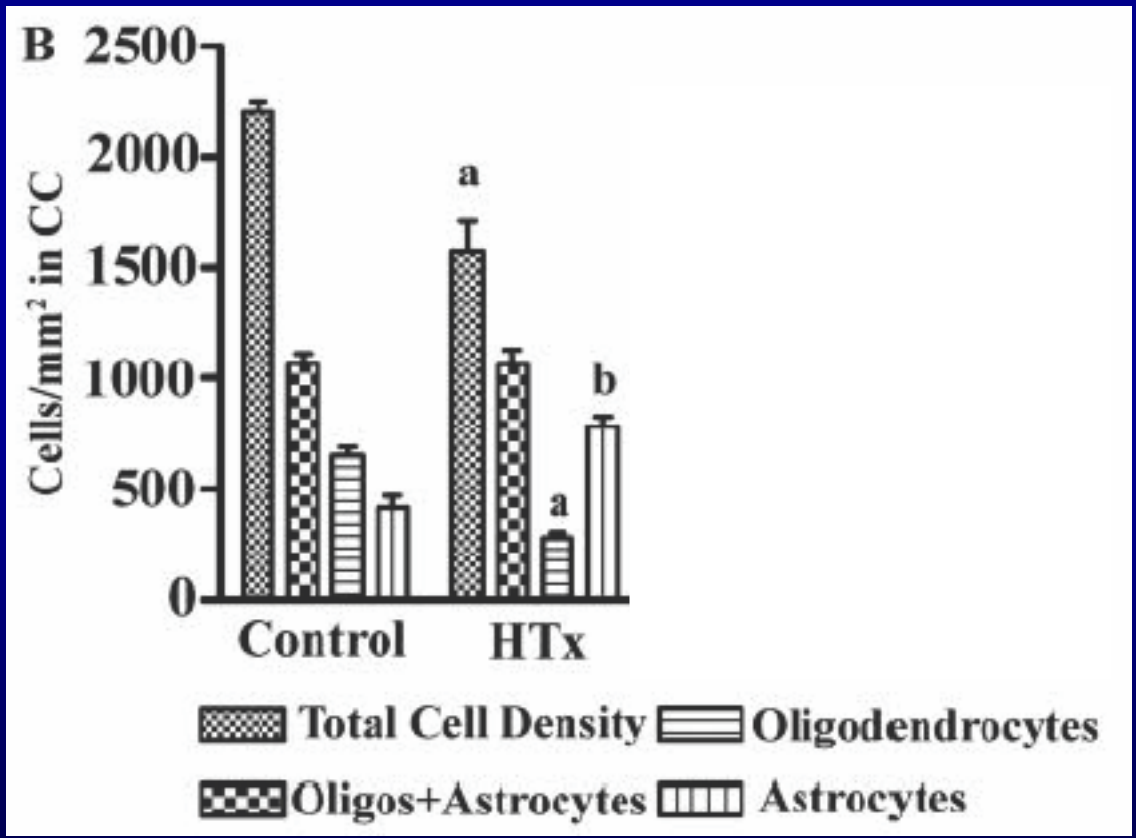




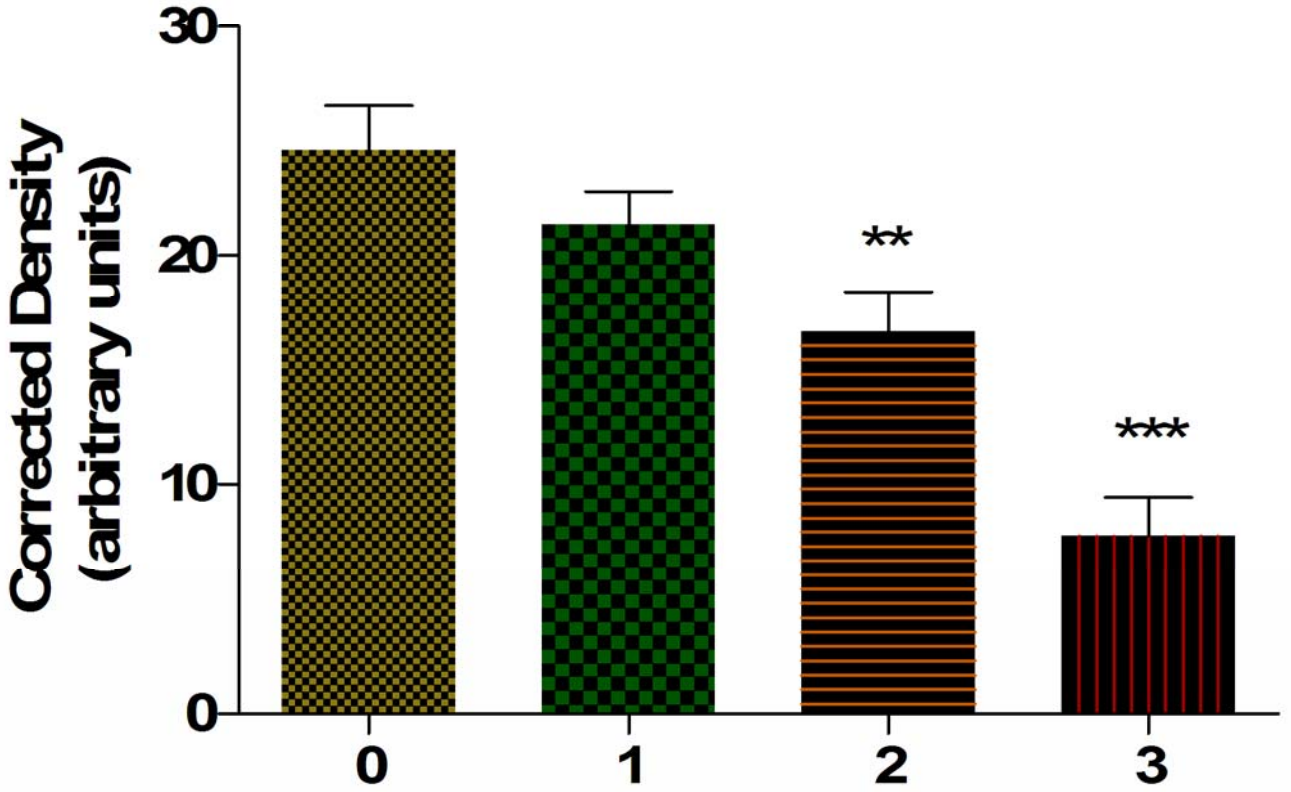
TH decreases the number of astrocytes



TH controls the ratio of oligodendrocytes to astrocytes



Effect of PTU on MAG mRNA Levels in the Corpus Callosum on P15



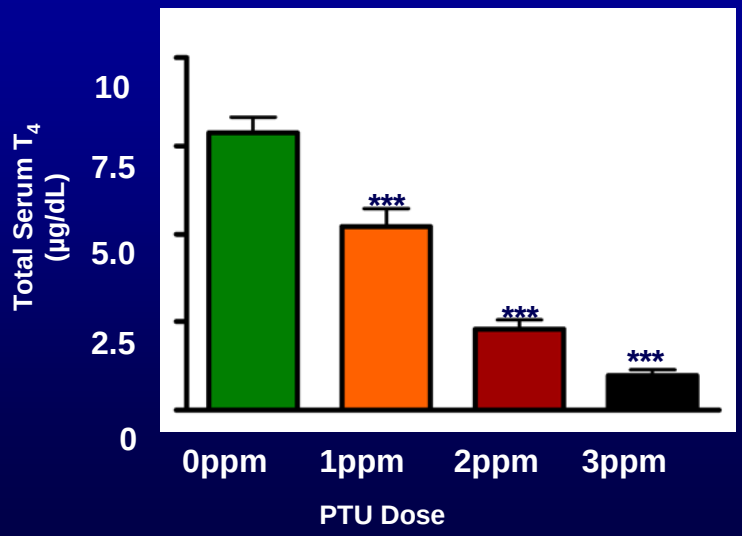
Experimental Design (Mary Gilbert)

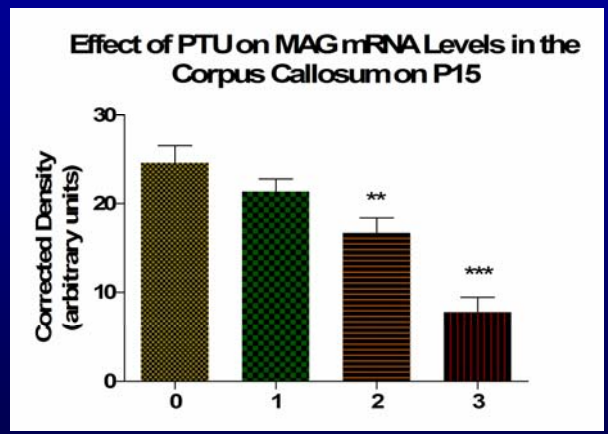
PTU Treatment of Pregnant Rats Initiated on G6

Control | 1ppm PTU | 2ppm PTU | 3ppm PTU

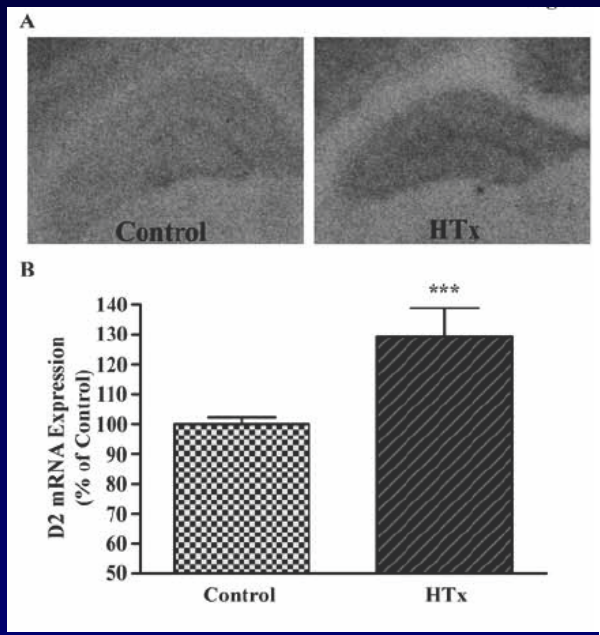
Animals Sacrificed on P15, trunk blood collected for T_4 analysis
Brains Harvested for ISH and IHC

The dose of PTU required to reduce T4 is lower than the dose of PTU required to affect white matter

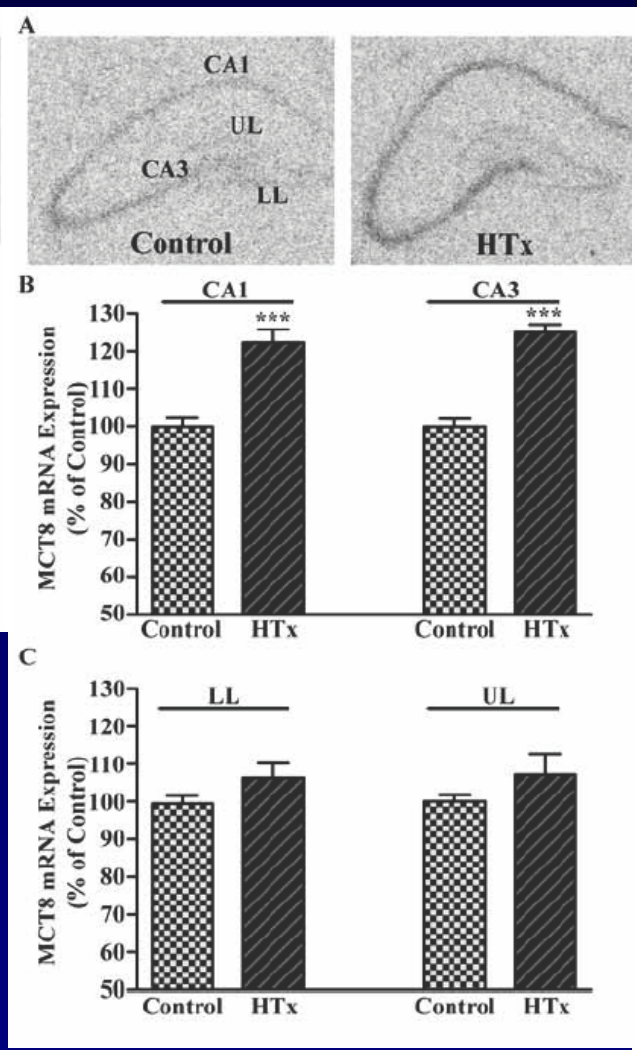




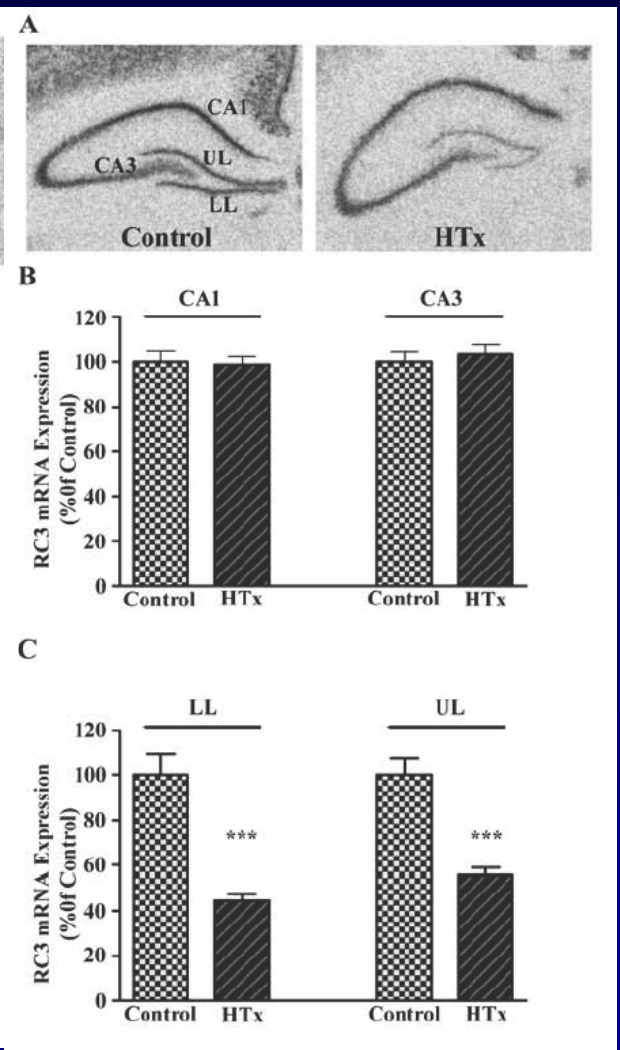
Site-Specific Compensation?



D2



MCT8

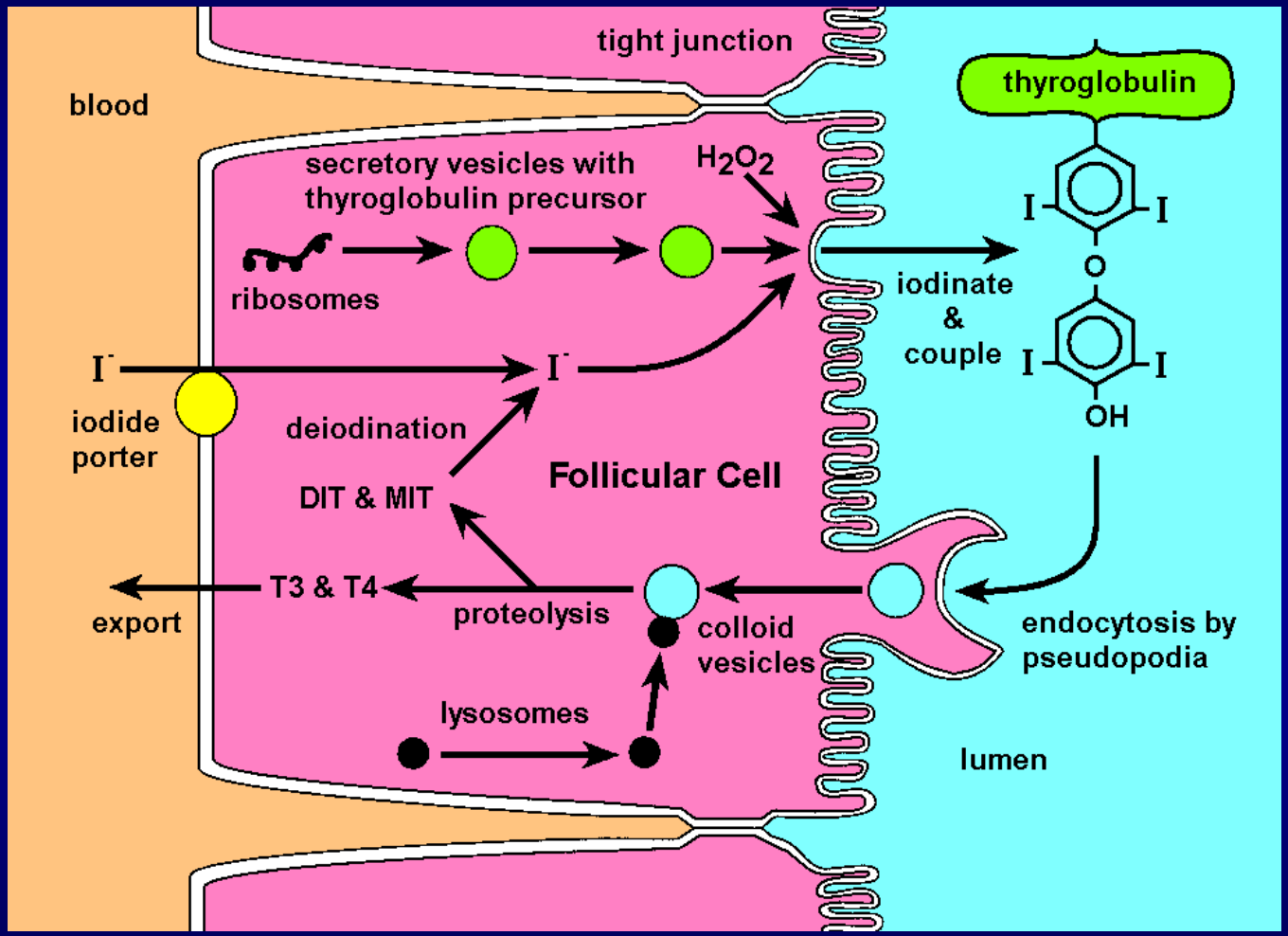


RC3

In Progress

- Gene expression in brain and heart
  - These studies will define the shape of the dose-response using a compound that produces an “idealized” thyroidal response.

Perchlorate inhibits iodide uptake

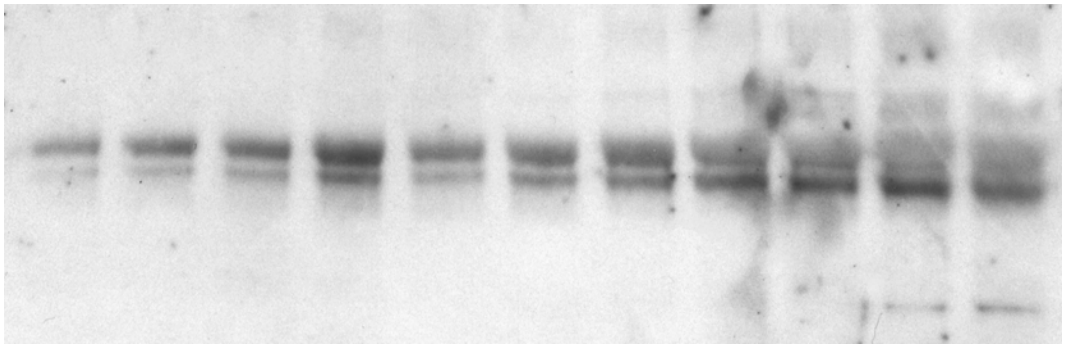


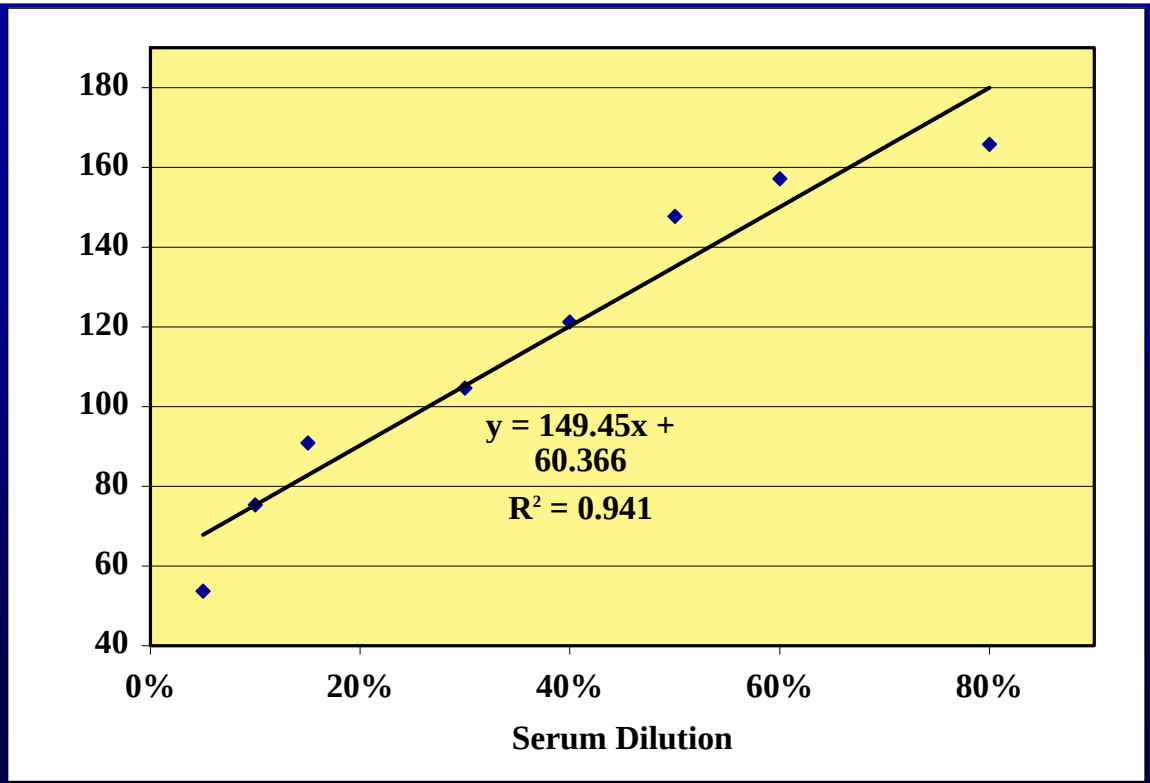
at the Sodium/Iodide Symporter

Effects of Perchlorate Exposure

- Timed pregnant Sprague-Dawley Rats
  - Exposure in Drinking Water
  - Doses: 0, 10, 100, 250, 500, 5000 ppb
  - Duration: G7 - P21 (weaning)

Thyroxine Binding Globulin

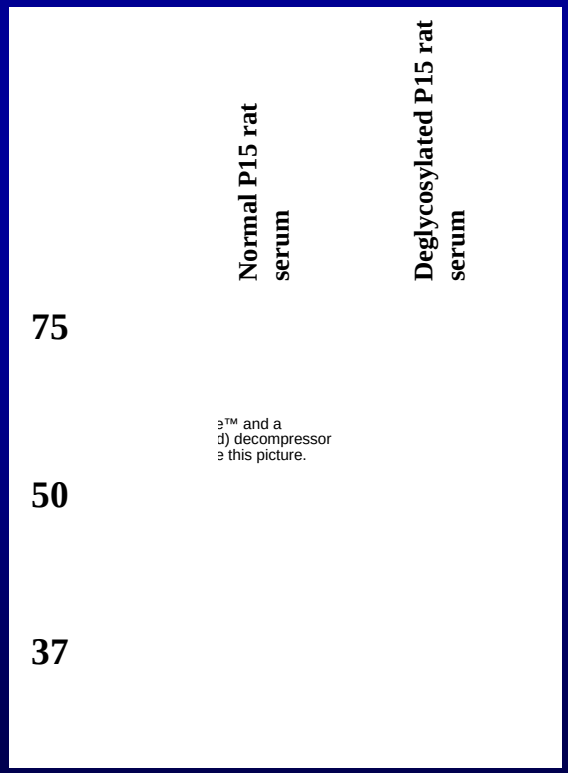




Dilution of normal P8 rat serum shows a linear relationship between film density and TBG content by Western blot.

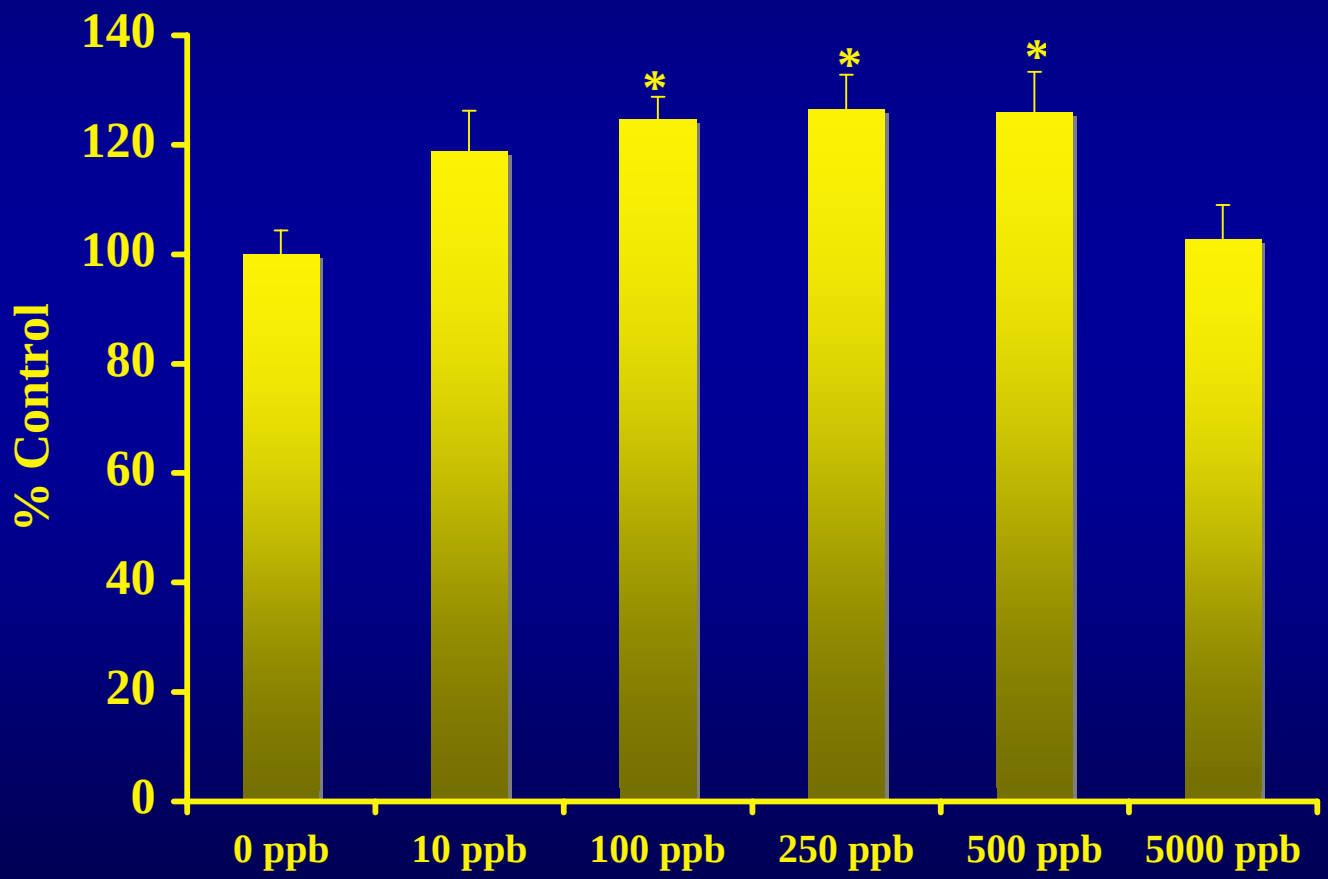
Thyroxine Binding Globulin

Deglycosylation of Serum TBG

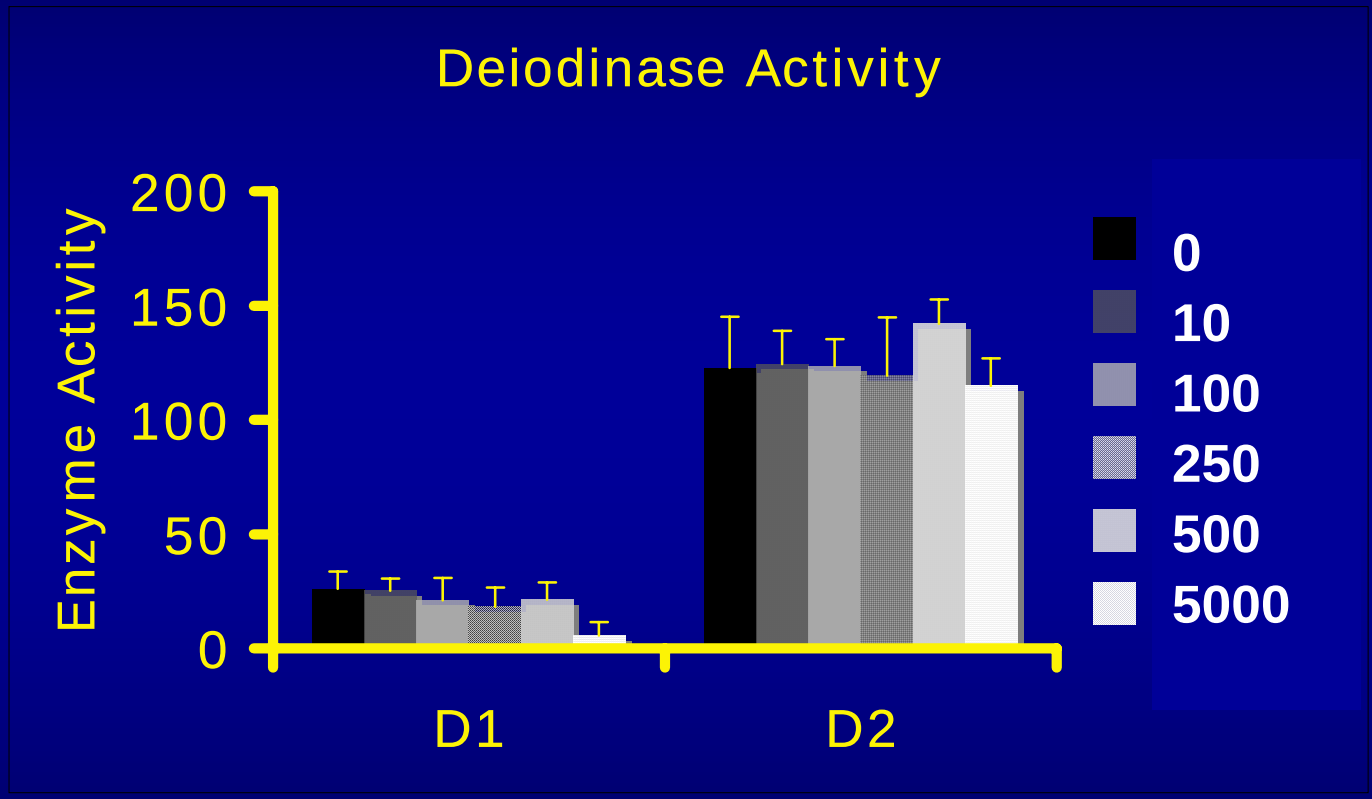


Normal P15 rat serum was deglycosylated using PNGase F to remove all N-linked glycans. The expected size shift of native TBG (54kD) to deglycosylated TBG (44kD) is observed.

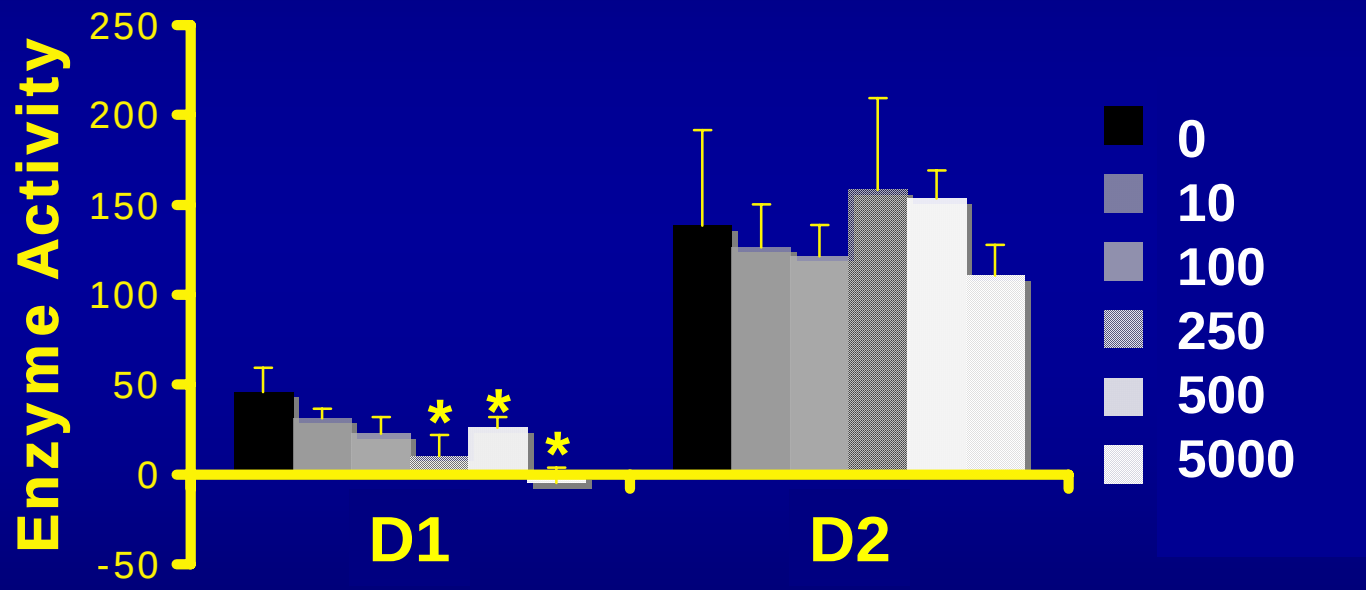
Effect of Perchlorate on serum TBG in P15 Pups



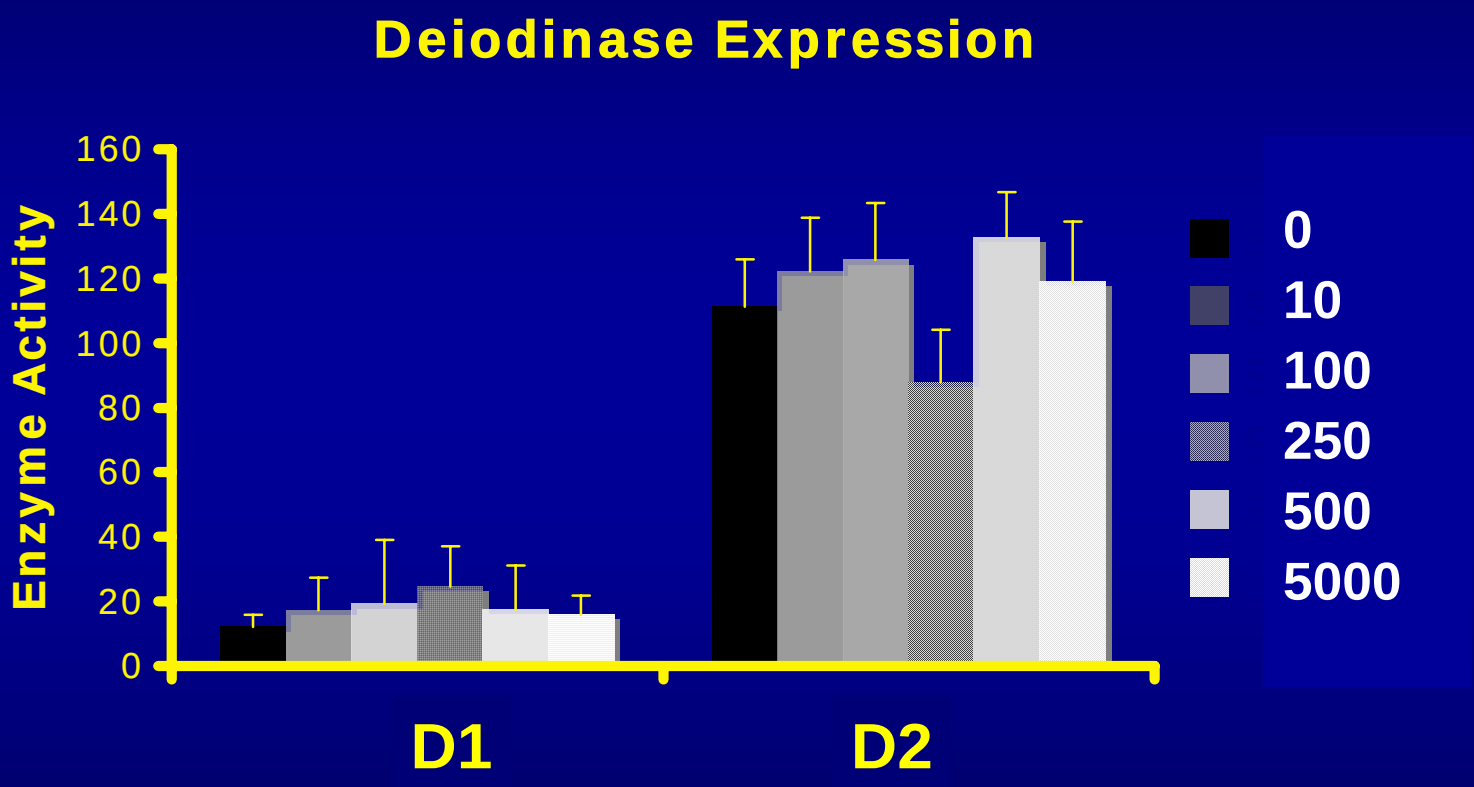
Deiodinase Activity in Cortex of Male And Female Pups on P21



Deiodinase Activity in P21 Males



Deiodinase Expression in P21 Females



In Progress

- Complete serum and tissue hormone analysis in PTU and Perchlorate Experiments
- Complete tissue analysis of TH endpoints
- PBDEs

Acknowledgements

- Ruby Bansal
- Dave Sharlin
- Laurelis Santiago
- Dan Tiege
- Mike DeVito
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