Development of a Biologically Based Pharmacokinetic (BBPK) Model for the Hypothalamic-Pituitary Thyroid Axis in the Maturing Rat for the Dose Response Assessment of Developmental Neurotoxicity

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Jeffrey Fisher, Ph.D., Duncan Ferguson, V.M.D., Ph.D and John Wagner, Ph.D.

University of Georgia

Interdisciplinary Toxicology Program

Multifaceted Objectives of Research

Modeling (Jeff Fisher, Eva McLanahan, Libby Myers)

1) To better understand relationships between administered dose and HPT axis disturbances in the immature rat and neurodevelopmental toxicity:

A. Biologically based models of the HPT axis are under development for different reproductive states of rats, adult male rats, and human.

B. PBPK models for thyroid active chemicals will be linked to the HPT axis models to predict neuro-developmental toxicity endpoints.

Experimental (Duncan Ferguson, John Wagner, Matthew Taylor, Michael Stramiello, Nadia Paolino)

2) Using gestational/neonatal exposure of rats to thyroid disruptive compounds, to:

A. Examine the sensitivity, capacity and development of compensatory mechanisms of thyroid hormone secretion/metabolism by the thyroid, brain, and liver.

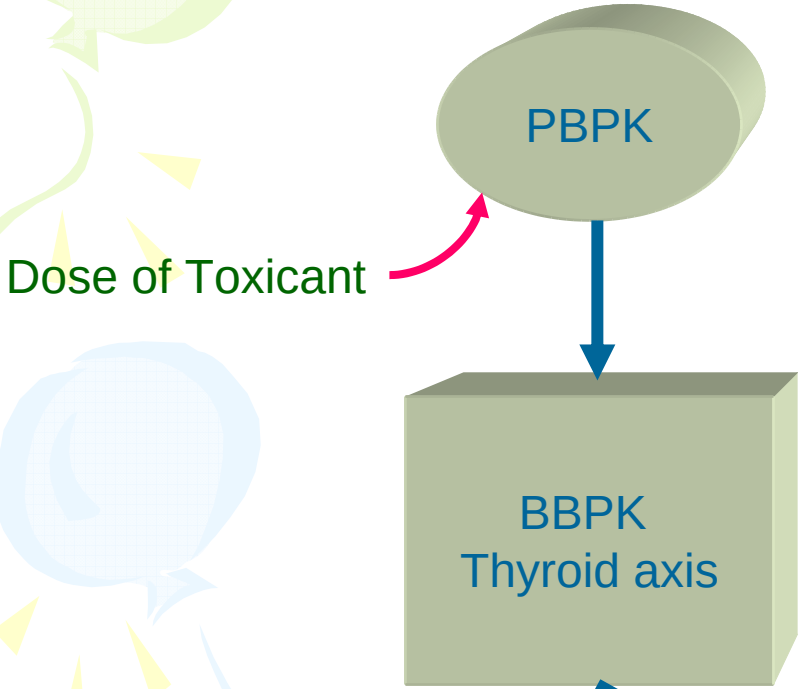
B. Develop quantitative 'dose-response' relationships of serum and tissue markers of thyroid status and correlate with developmental neurotoxicity endpoints.

Approach - Cooperative agreements

- Develop team of interdisciplinary scientists; while working independently, are aiding each other in experimental design, sharing samples and data.

-- UGA, UMass (Tom Zoeller) and USEPA (Kevin Crofton, Mike DeVito, and Mary Gilbert)

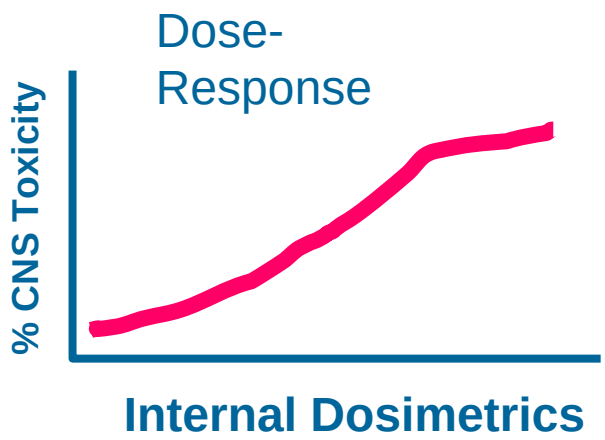
Project Concept for Computational Modeling of Dose-Response in the Fetal/Neonatal Rat



Describes the kinetics of the toxicant and its MOA for disturbing the HPT axis.

Describes the HPT axis and perturbations in the HPT axis from chemical insult.

CNS responses in the brain of the pup or fetus.



Approach for Computational Modeling

…Fill fundamental D-R data gap (HPT disruption →hypothyroidism→ developmental neurotoxicty)

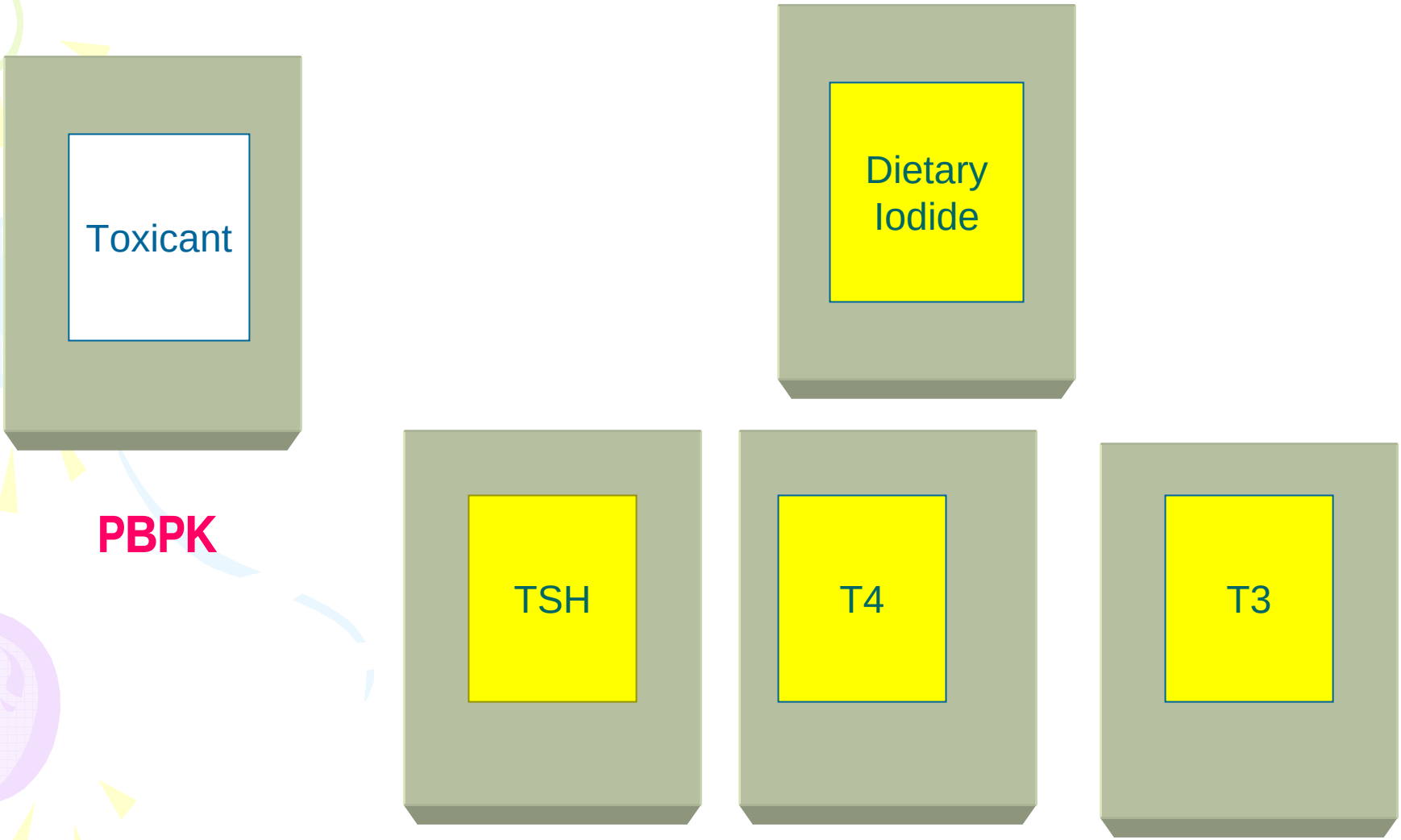
- Use propylthiouracil (PTU), as a probe to establish high to low dose quantitative relationships between disruption of the HPT axis leading to hypothyroidism in the developing rat and neurotoxicity.

…Select two thyroid active chemicals with substantial data

- Perchlorate (iodide blocking at thyroid) and PCB 126 (increased hepatic T4 metabolism). Both environmental chemicals cause hypothyroidism in rats.

Sub models

_____BBPK- HPT axis_____



PBPK

Linking the Sub Models- I see the light.....

•PBPK models
•PBPK dosimetry model with MOA (perchlorate and PCB 126)
--adopt aspects of perchlorate PBPK models

•Pup growth PBPK model using growth equations for organs
--adopt in-house research from deltamethrin
•Utilize in-house PCB 126 kinetic data sets

HPT axis models
•Recalibrate radiolabeled iodide submodel, calibrate radiolabeled T4 and T3, calibrate endogenous TSH submodel

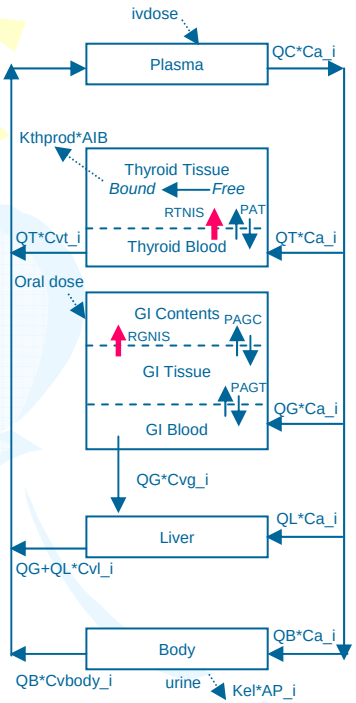
•Develop endogenous ^{127}I, T3, T4 and link with TSH (feedback)

•Articulate compensatory mechanisms for HPT axis such as T3/T4 shift in thyroid hormone production, D2 induction in brain, NIS induction in thyroid, extra-thyroidal D1 decrease

Current Status of Models (two Ph.D. students)

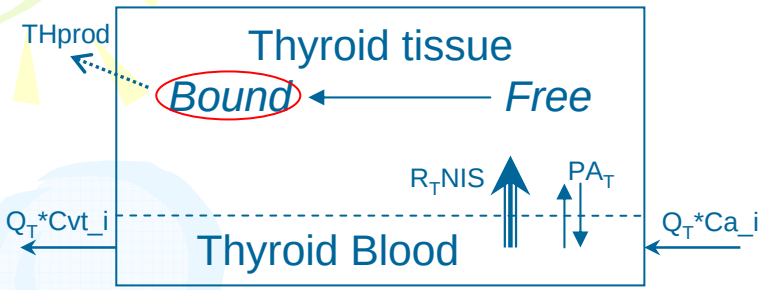
- Develop radiolabel sub-models for iodide and T4 in PND 13 pup and adult rat.
- Examine feedback equations for endogenous serum TSH and T4 concentrations.
- Dietary iodide model for adult human

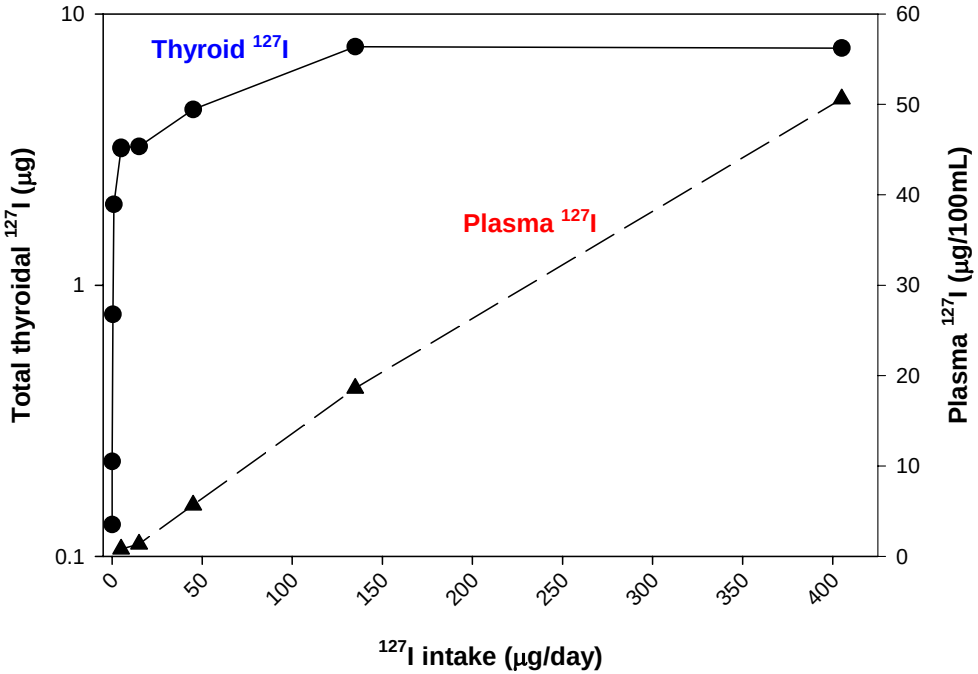
^{125}I Model Structure



Total Iodide

Approach for Iodide Binding in Thyroid





$$\frac{dIB}{dt} = \frac{V\max_b t\_i * Cf\_i}{Km_b\_i + Cf\_i}$$ Binding equation

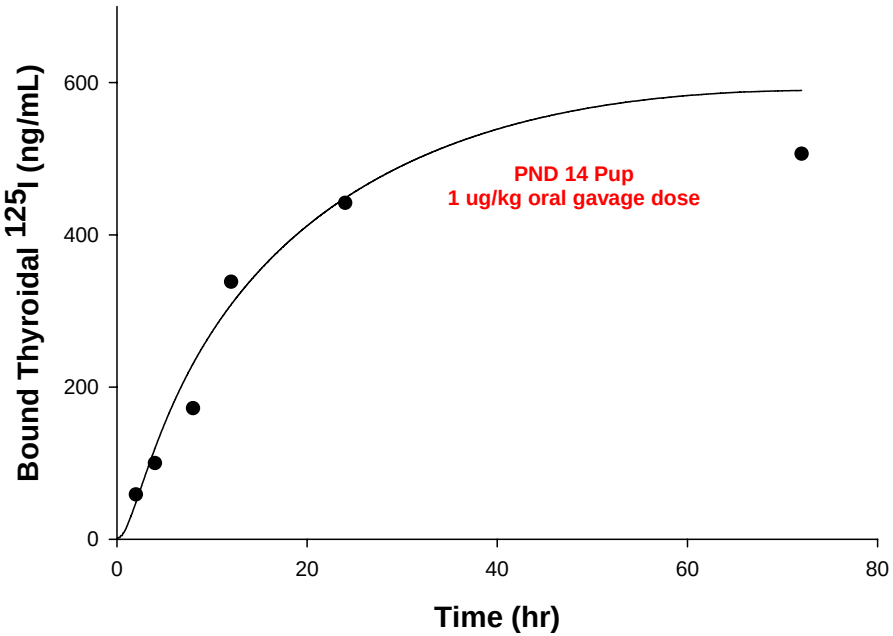
$$V\max_b t\_i = \frac{V0_{bind} * K_{aib}}{Kaib + CIB\_i}$$ Binding inhibition equation

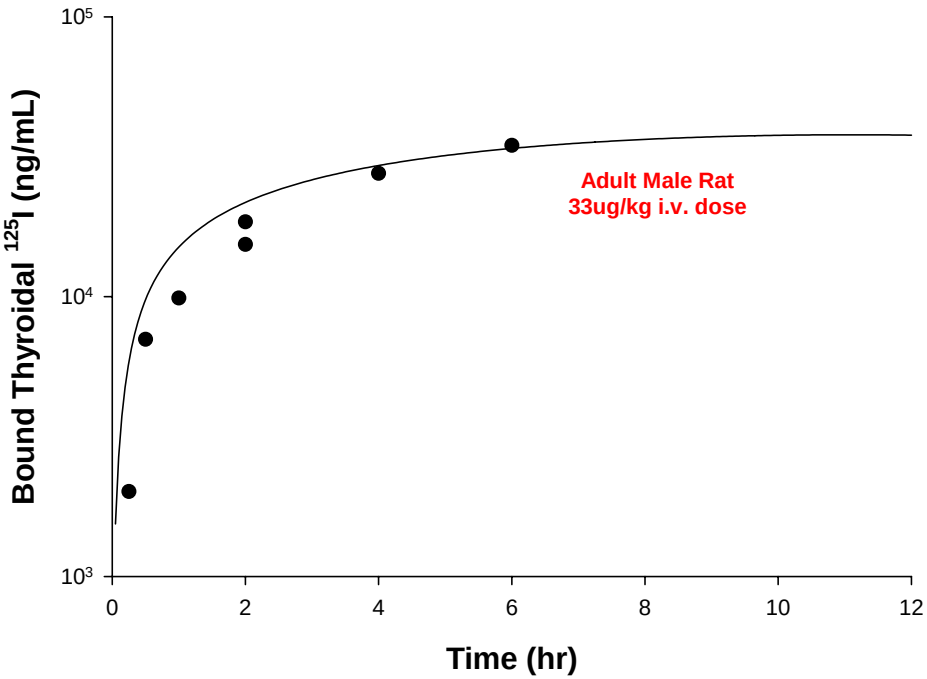
$$\frac{dIB\_i}{dt} = \frac{dIB}{dt} - \frac{dTHprod}{dt}$$ Rate of change in amount bound

Adapted from Pedraza *et al.*, 2006

and Nagataki *et al.*, 1966

Bound ^{125}I in Thyroid





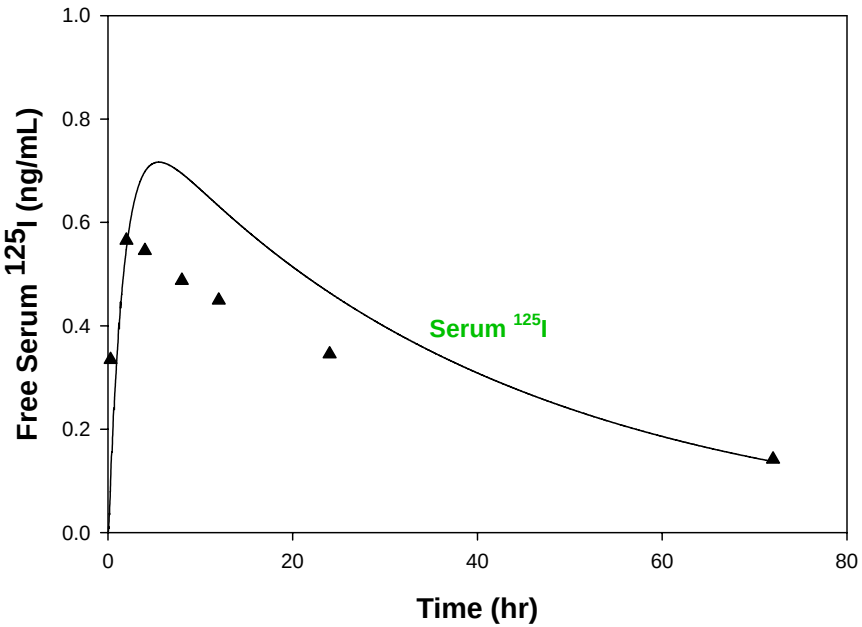
Clewell *et al.*, 2003

Yu *et al.*, 2002

^{125}I PBPK Model Predictions

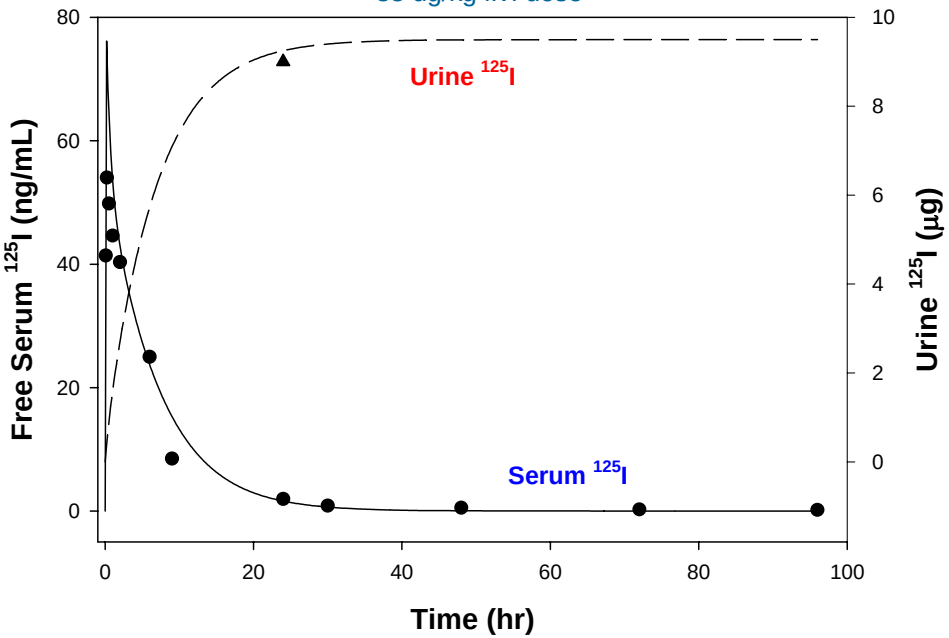
PND 14 Pup

1 ug/kg oral gavage dose



Adult Male Rat

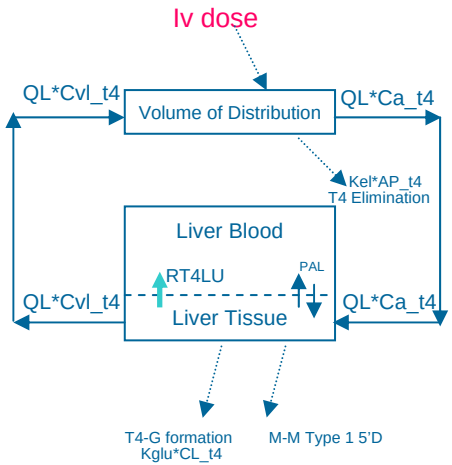
33 ug/kg i.v. dose

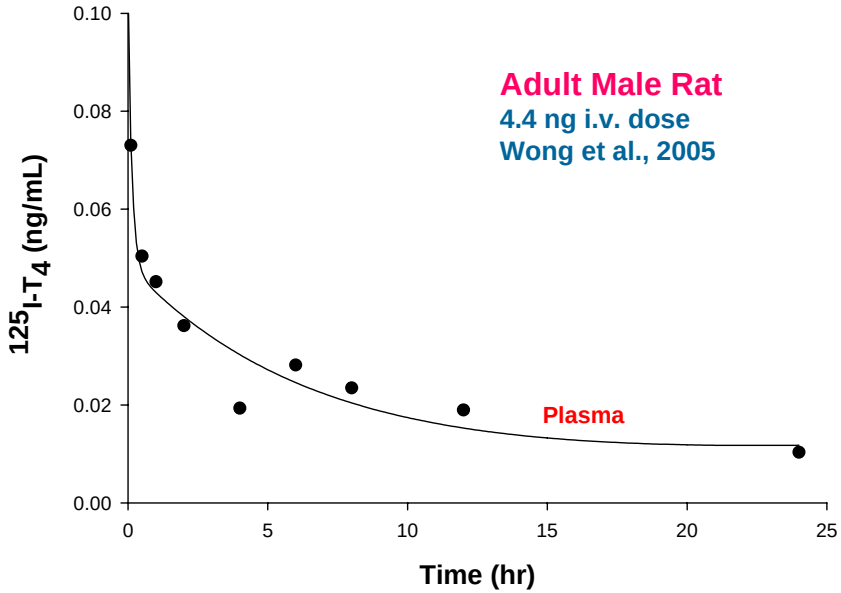


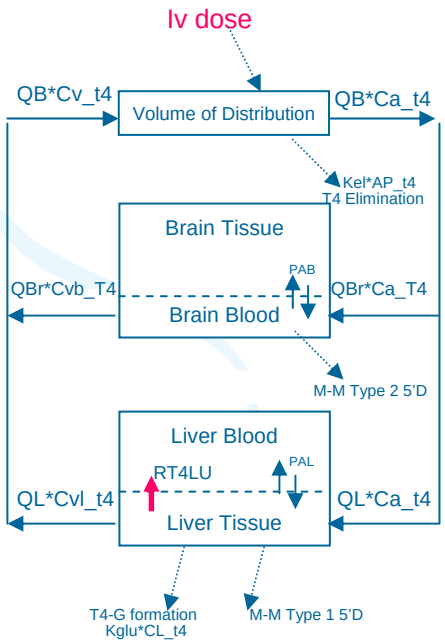
Clewell *et al.*, 2003

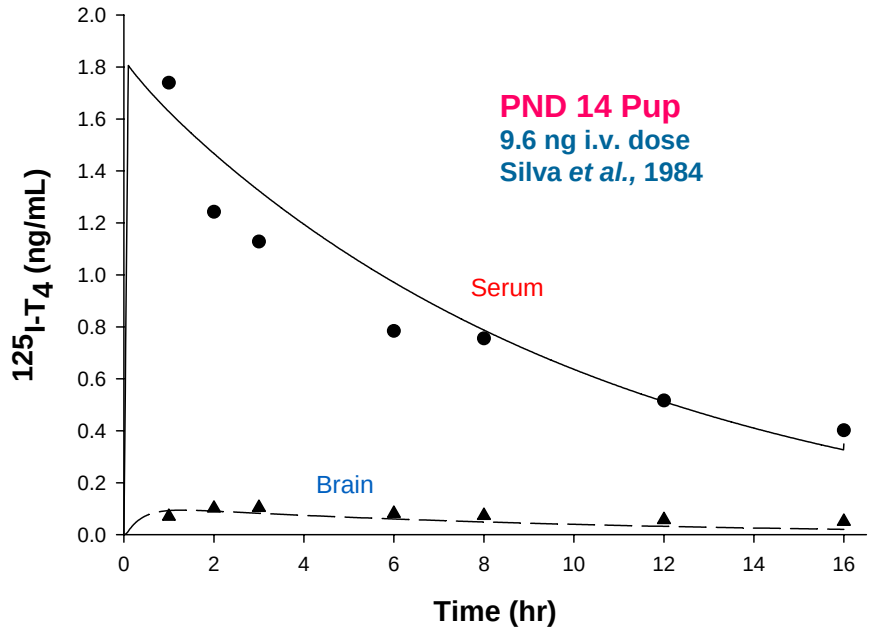
Yu *et al.*, 2002

[^{125}I]-T4 PBPK Model Structures and Predict









Thyroid (negative feedback loop)

↑TSH,↑T4 production

$$V_d\left(\frac{dT_4}{dt}\right)=\left(k_0+\frac{k_{0,\max}*[TSH]}{[TSH]+k_{\mathrm{TSH}}}\right)-\left(k_{cat}*[UDPGT]*[T4]\right)-\left(k_{cat}{}'*[Deiod]*[T4]\right)$$

$$V_{pl}\left(\frac{dTSH}{dt}\right)=\left(\frac{k_{0,\mathrm{TSH}}*k_{T4}}{k_{\mathrm{T4}}+[T4]}\right)-\left(k_{\mathrm{elim,TSH}}*[TSH]\right)$$

Negative feedback, ↓T4,↑TSH

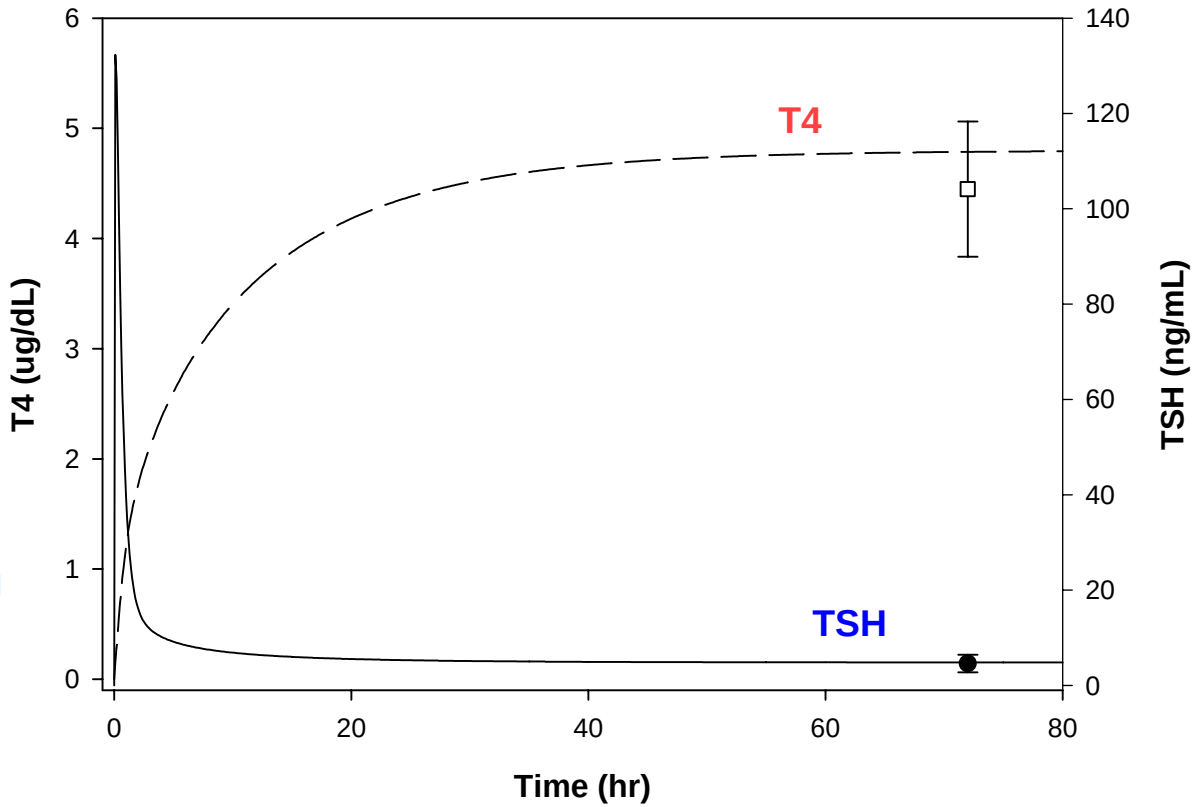
Where

k0= basal production rate of T4 (in absence of TSH)

k0,max=maximal rate of T4 secretion to plasma (under TSH stimulation)

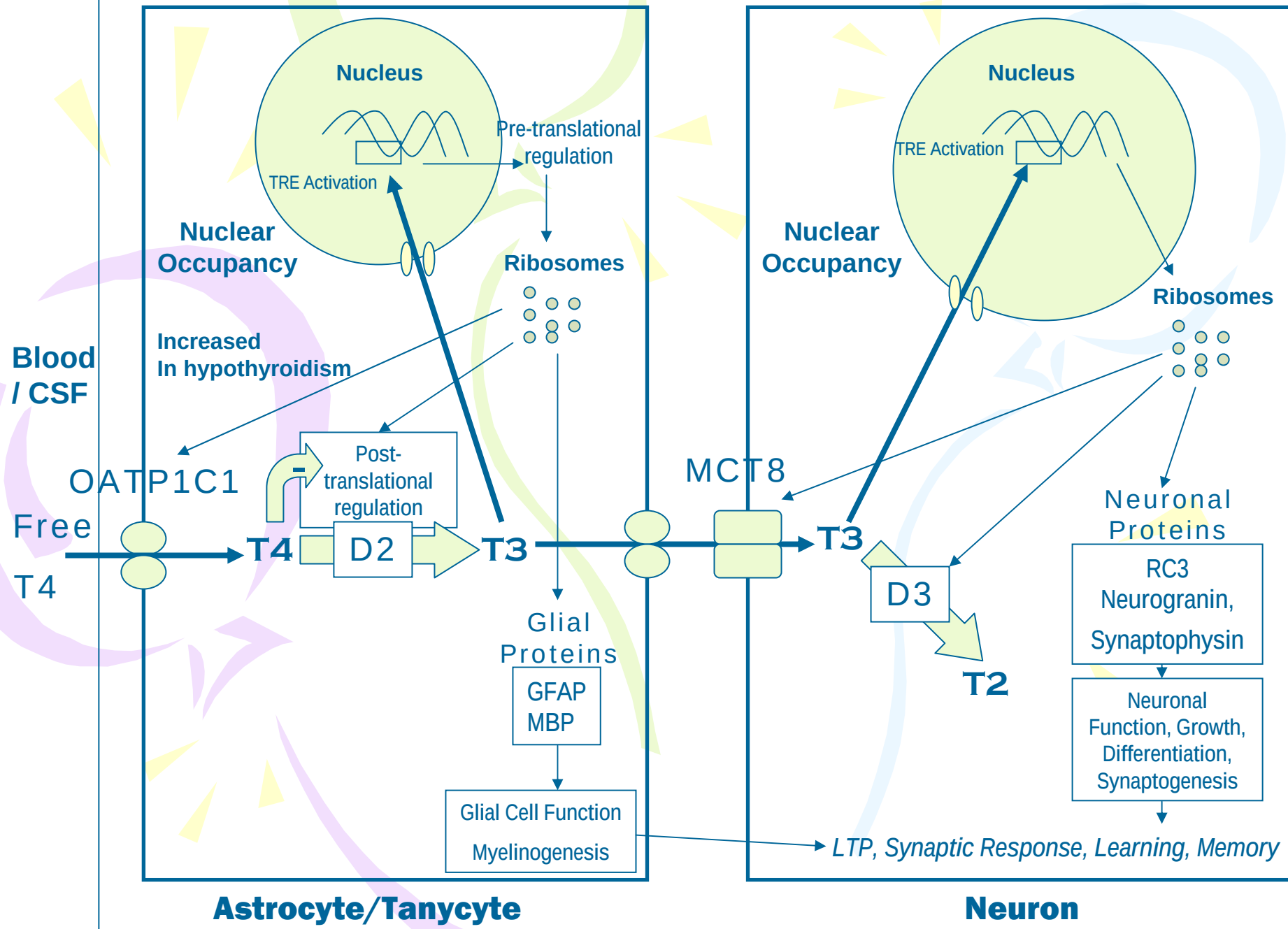
k0,TSH=rate of TSH production (T4 conc. approaches 0)

Steady state prediction vs observation for serum T4 and TSH concentrations in adult male rat



Experimental Work

Duncan Ferguson


Nucleus
Pre-translational regulation
TRE Activation
Nuclear Occupancy
Ribosomes
Increased In hypothyroidism
Blood / CSF
OATP1C1
Free T4
Post-translational regulation
T4
D2
T3
Glial Proteins
GFAP MBP
Glial Cell Function
Myelinogenesis
MCT8
T3
D3
T2
Neuronal Proteins
RC3 Neurogranin, Synaptophysin
Neuronal Function, Growth, Differentiation, Synaptogenesis
LTP, Synaptic Response, Learning, Memory
Astrocyte/Tanycyte
Neuron

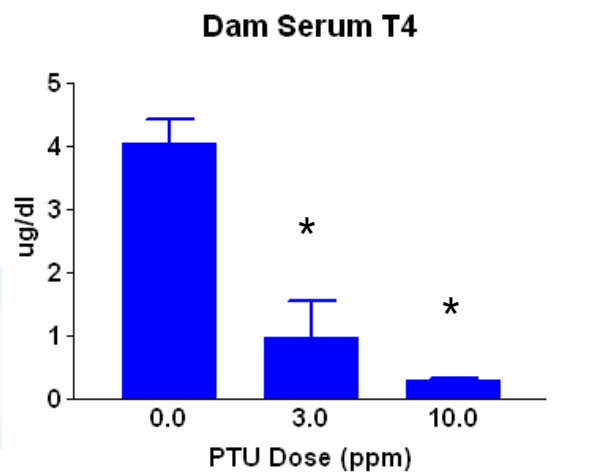
Dosing Protocol

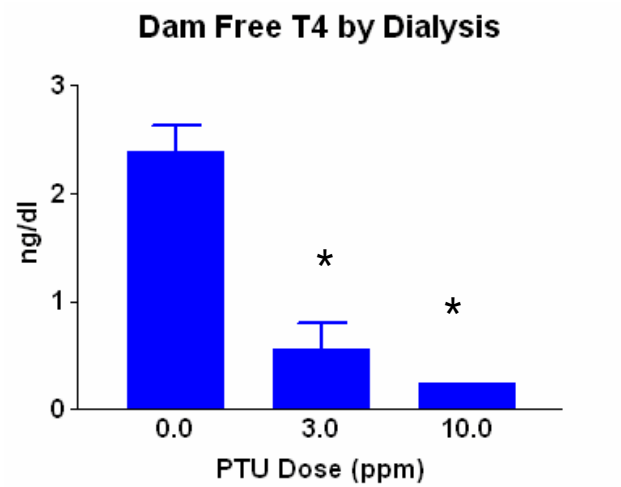
- Dosing of timed pregnant Sprague-Dawley dams started at GD2 and continued through PND21-PND30 depending upon sacrifice schedule
- Current dose levels are 0, 3, and 10 mg/L (ppm) PTU in the drinking water.
- Water intake recorded and animals weighed q48h
- Gender of offspring determined in the third week after birth, and female pups culled midway through that week

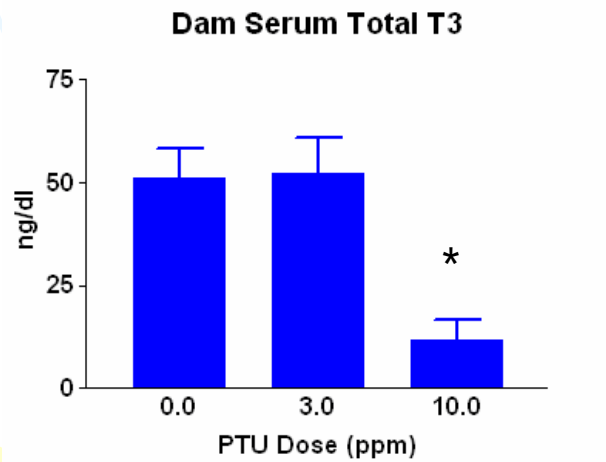
Timeline

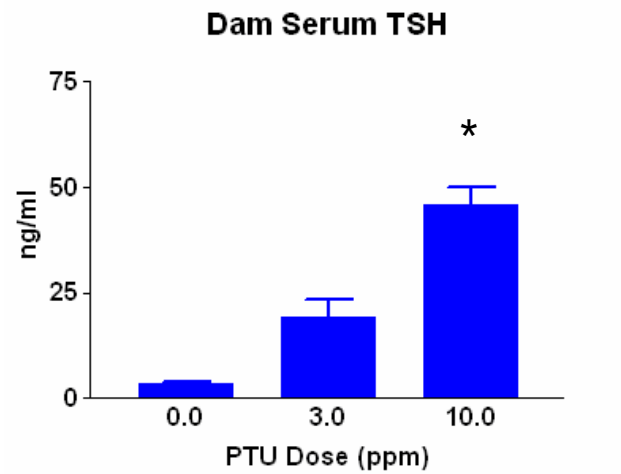
- Pups were sacrificed from PND21-PND31.
- Dams were sacrificed on PND31, when the pups were weaned.
- Adults were sacrificed starting 2 months after weaning (average PND100)
- Female pups were culled on approximately PND 24; 2 males per litter studied at each timepoint
- 14 litters
  - 0 ppm (n= 5)
  - 3 ppm (n=5)
  - 10 ppm (n=4)
- Additional analyses for D2 activity were performed on Hooded Long-Evans rats (1 dam and 1 PND21 pup) dosed at 0 (n=12),1 (n=13),2 (n=13) and 3 (n=12) ppm PTU from GD6 to PND21.

Serum Thyroid Hormone and TSH Concentrations: Dams

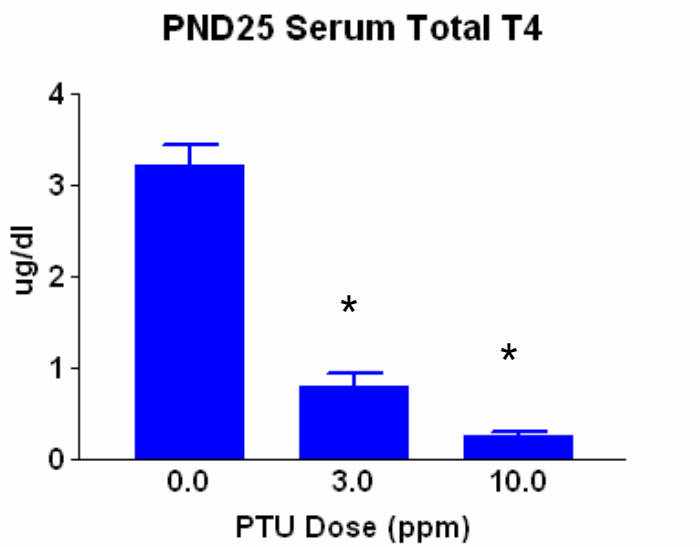


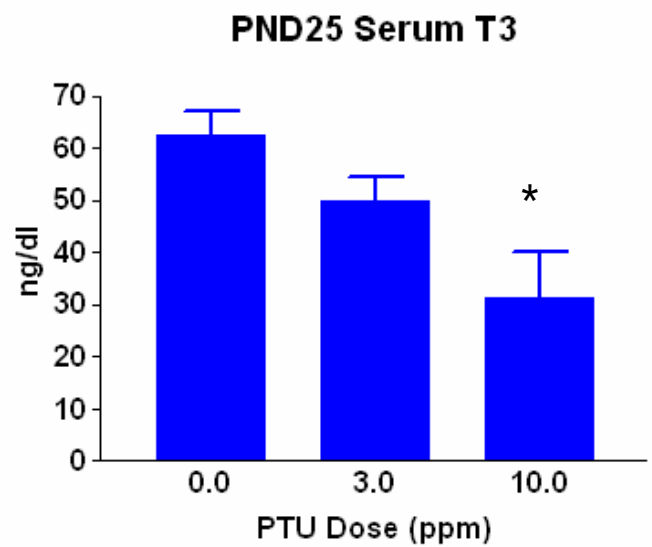


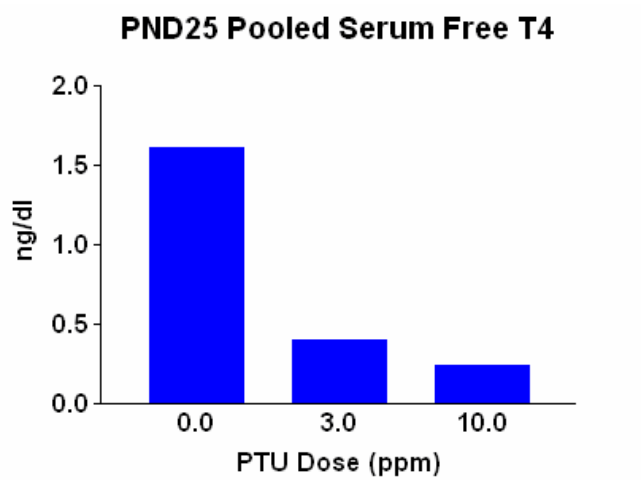




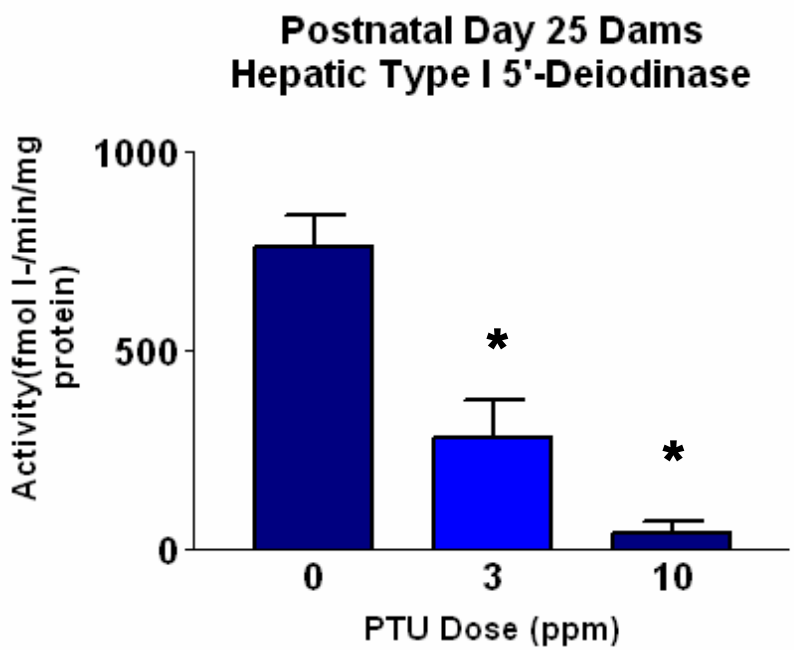
Serum Thyroid Hormone Concentrations: PND25

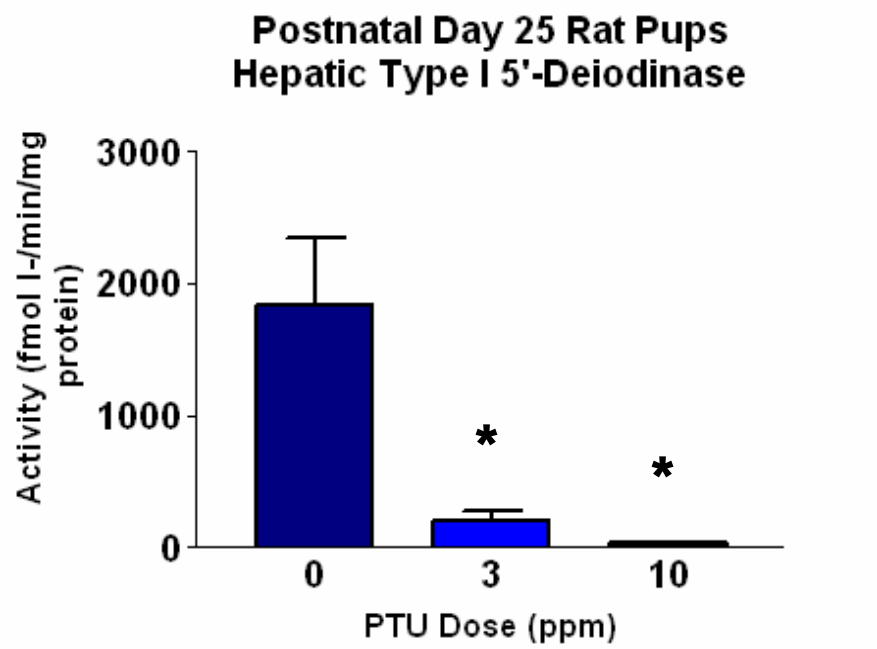




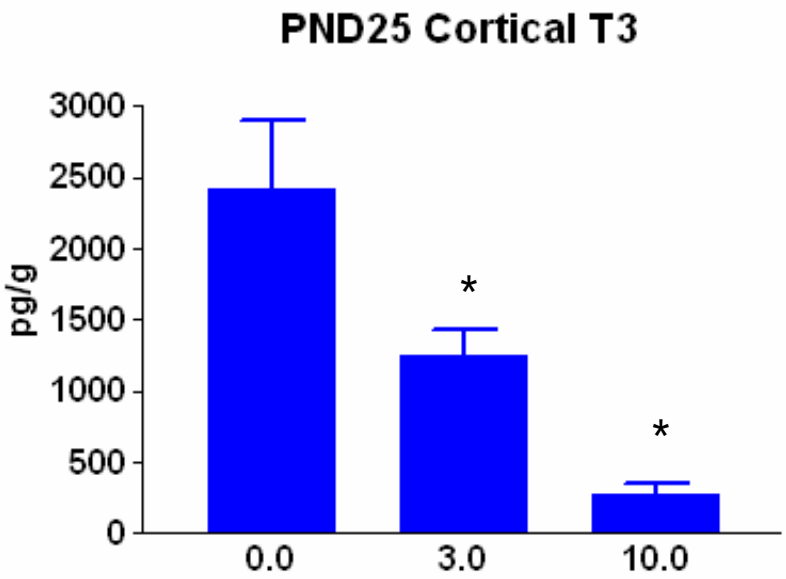


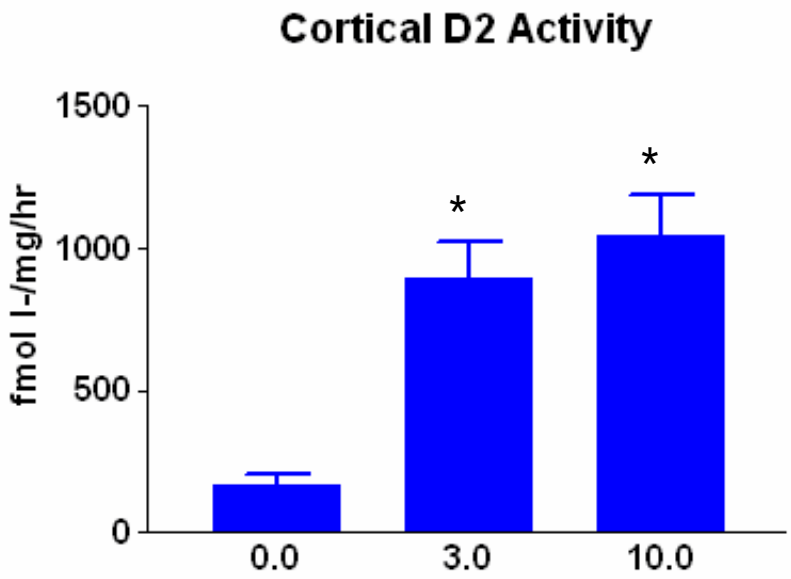
Hepatic D1 Activity

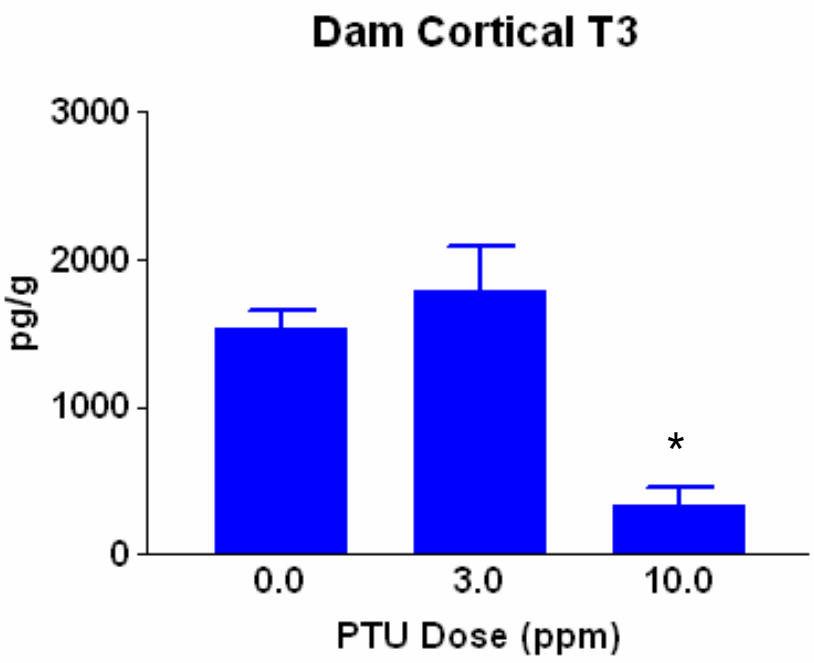


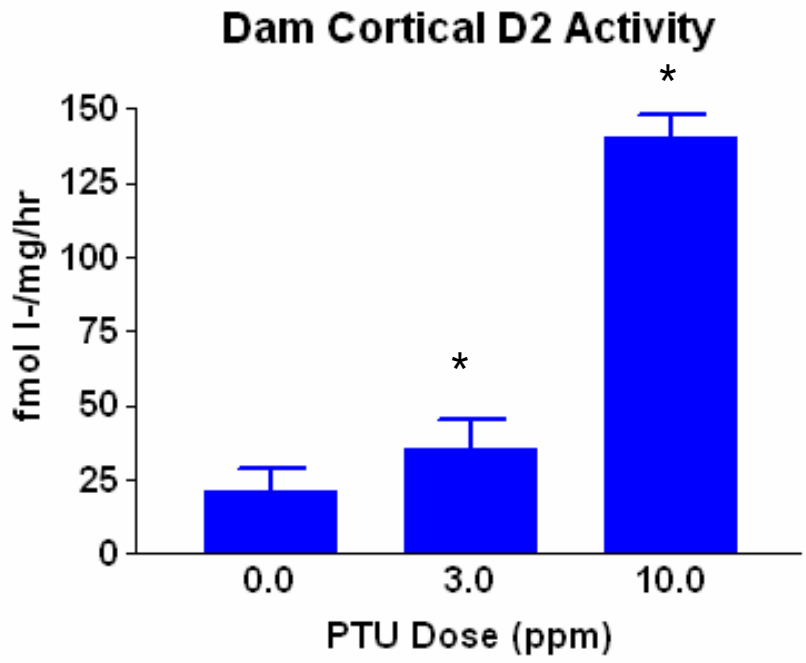


PND25 Cortical T3 and D2 Activity: PND25 vs. Dam

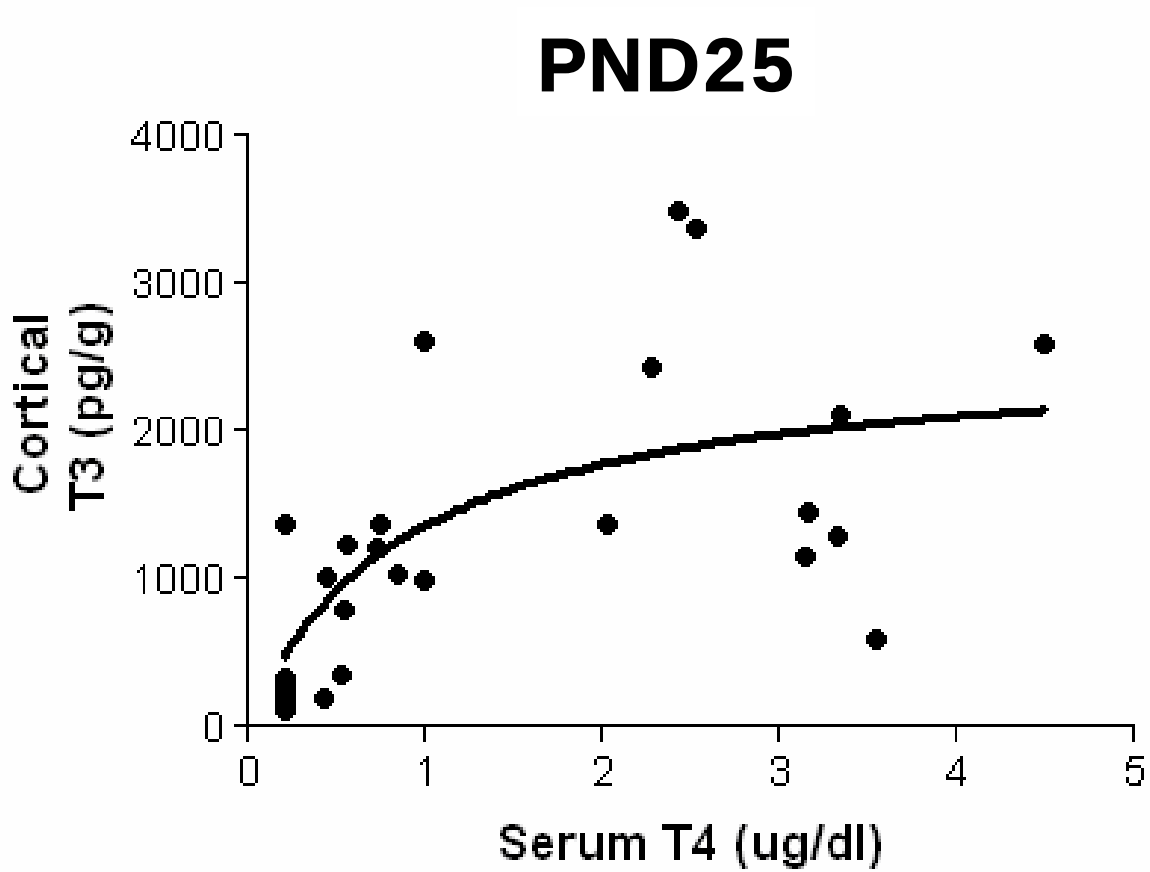






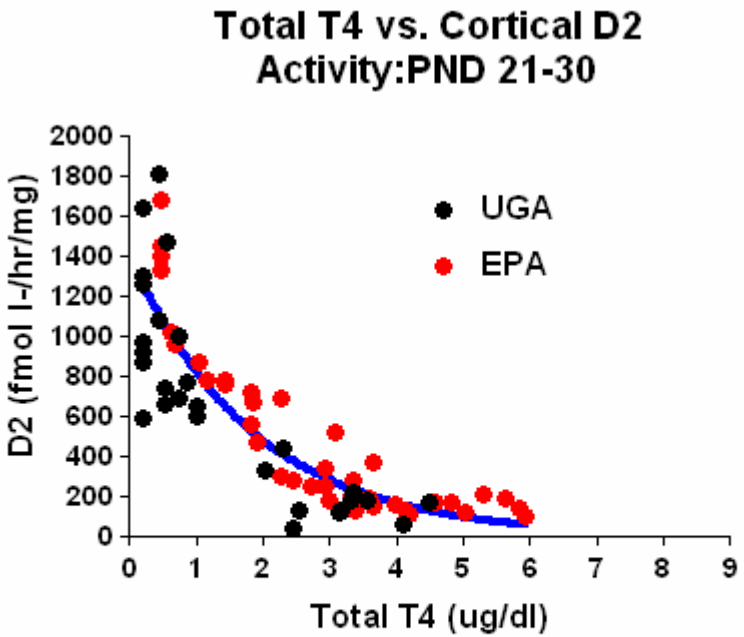


Serum T4 vs. Cortical T3

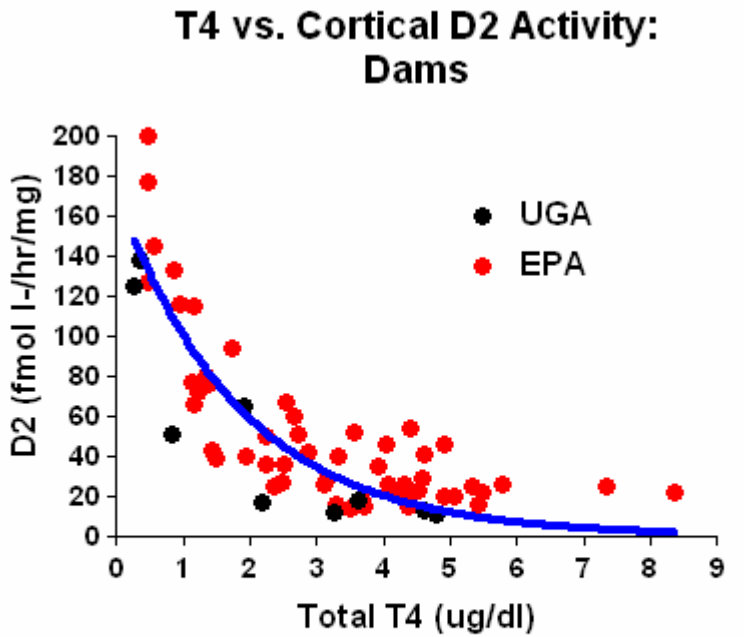


Max = 2564+/550 pg/g
T4 at Half Max =0.88+/-0.52
R = 0.69

Comparison of D2 response to Fall in Total T4: PND21-30 vs. Dams

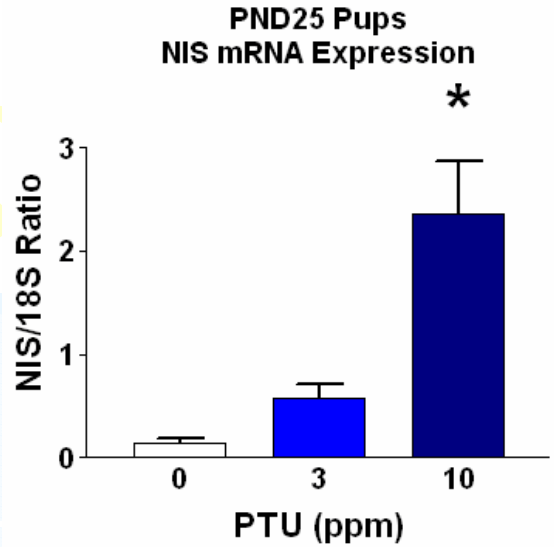


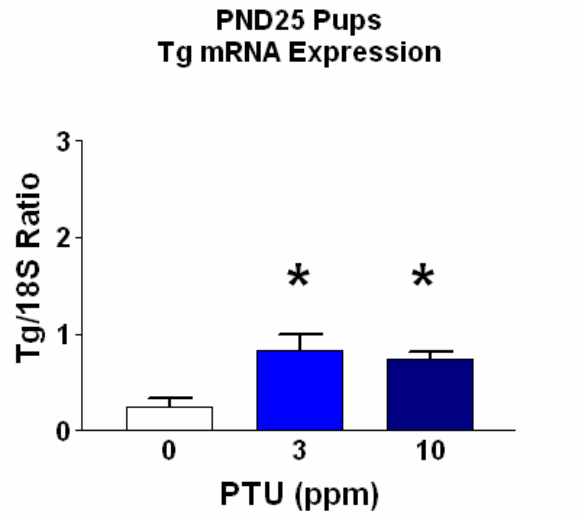
y= C*exp(-k*x)
C = 1401 +/-153(SD)
k = 0.533 +/- 0.107; D2 doubles with 1.3 ug/dl fall
R = 0.88 p<0.0001

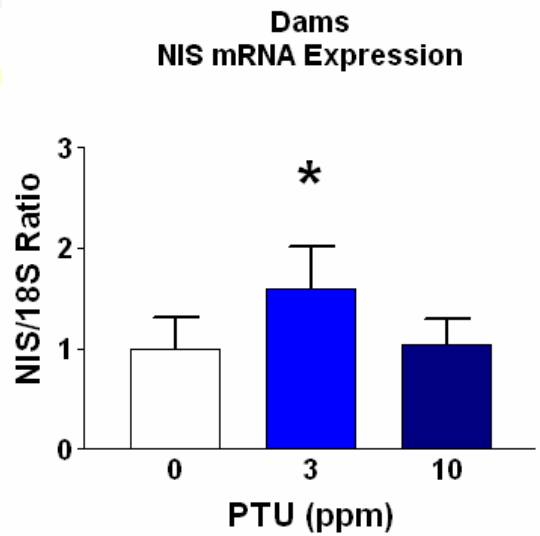


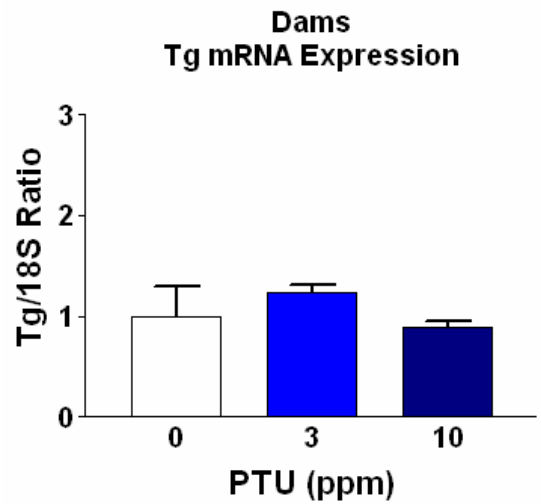
y= C*exp(-k*x)
C = 171 +/- 24(SD)
k = 0.531 +/- 0.108; D2 doubles with 1.3 ug/dl fall
R = 0.86 p<0.0001

Comparison of Thyroid mRNA response: PND21-30 vs. Dams

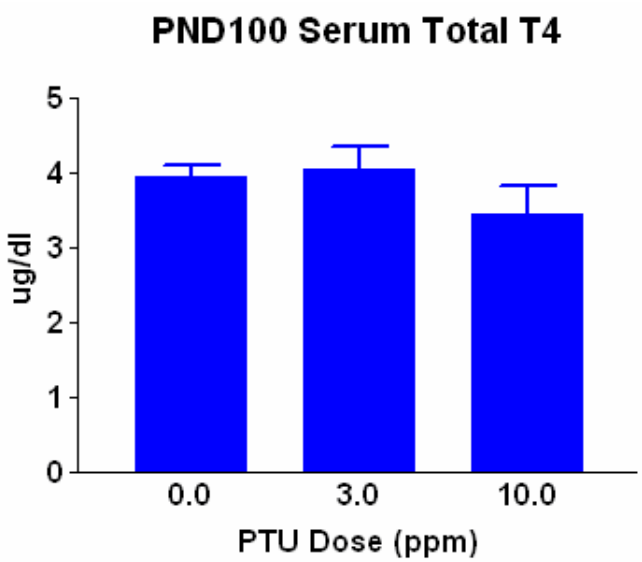


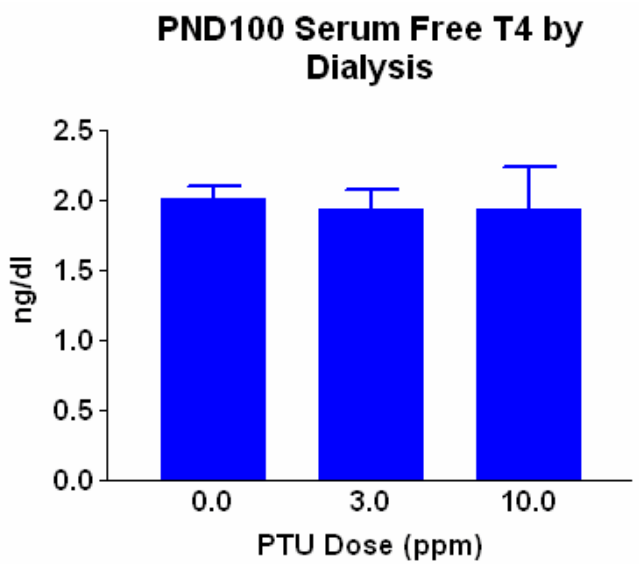


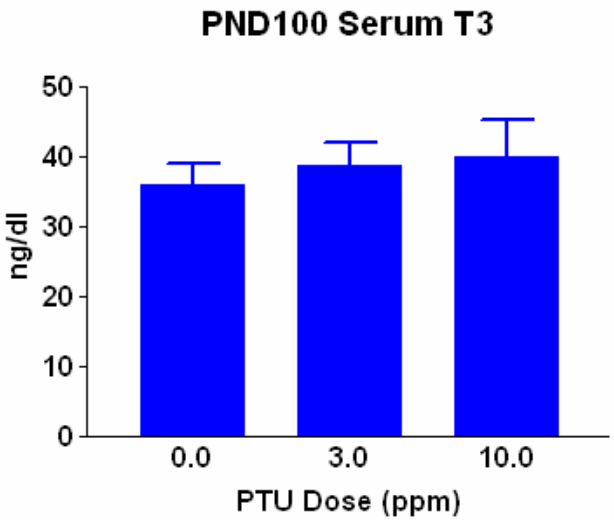




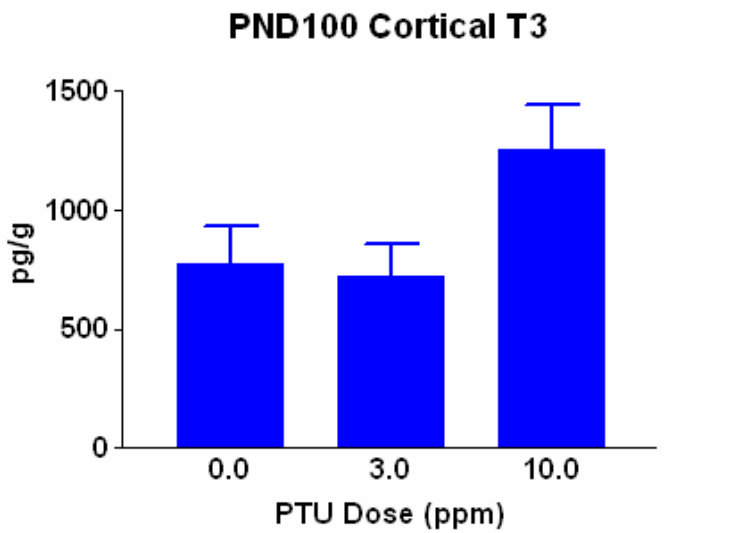
PND100 Serum Hormones

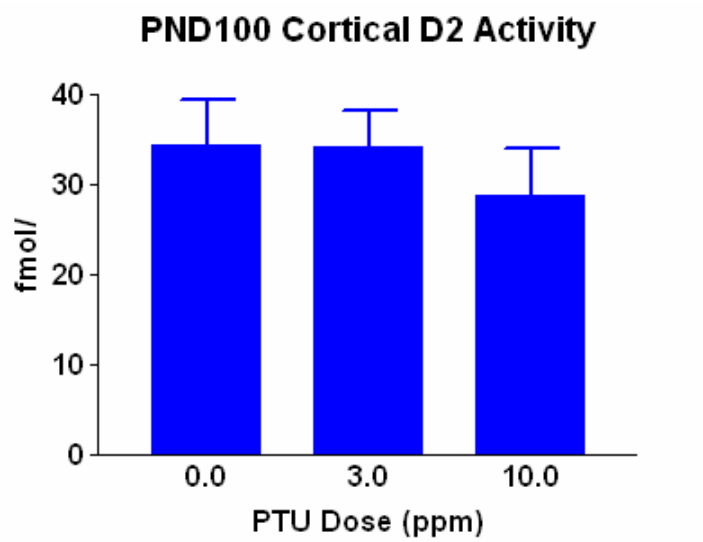


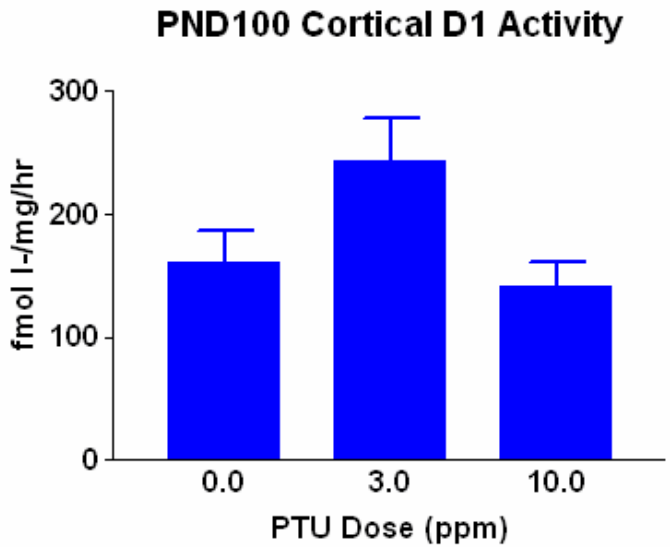




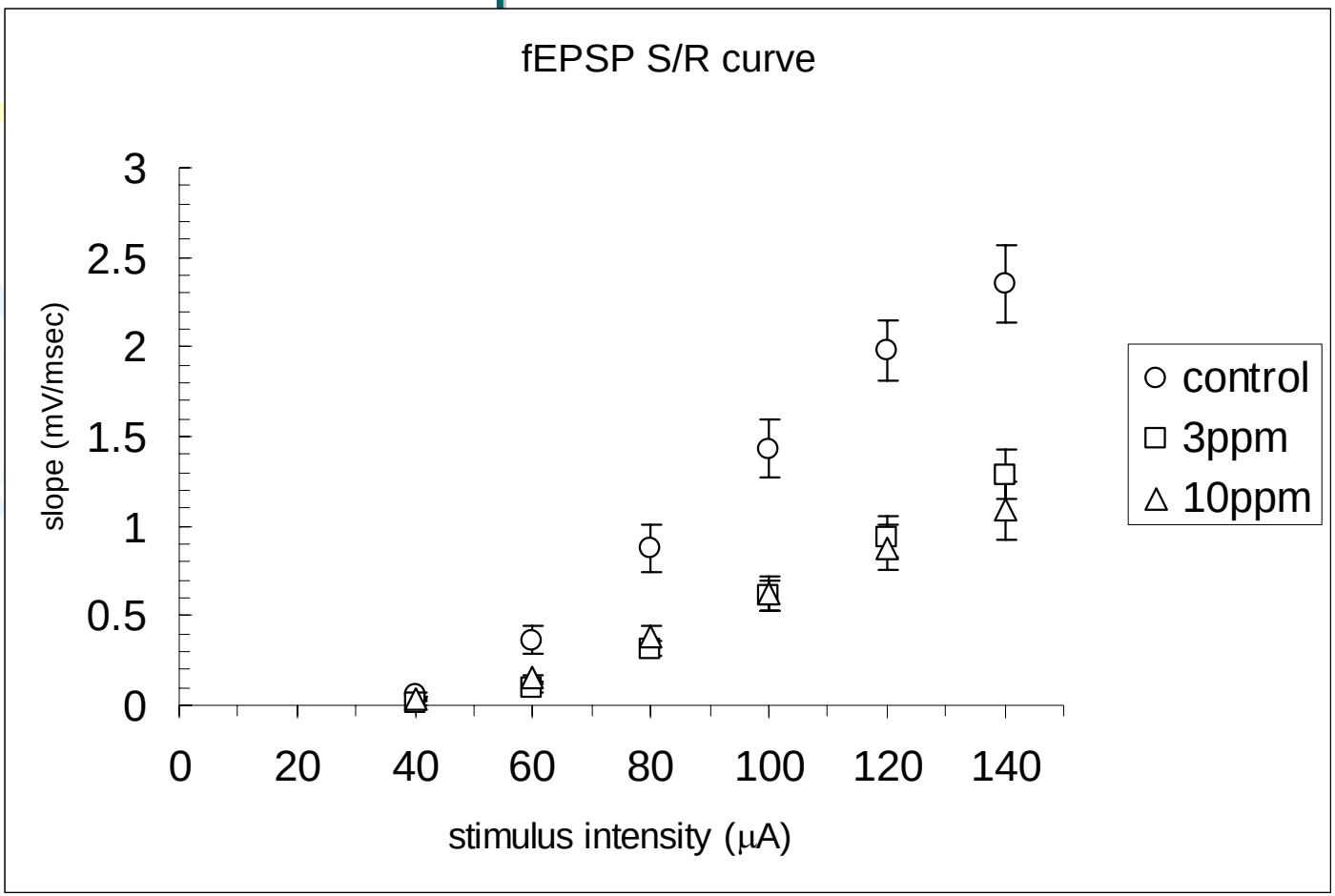
PND100 Cortical T3, and D1 and D2 Activities



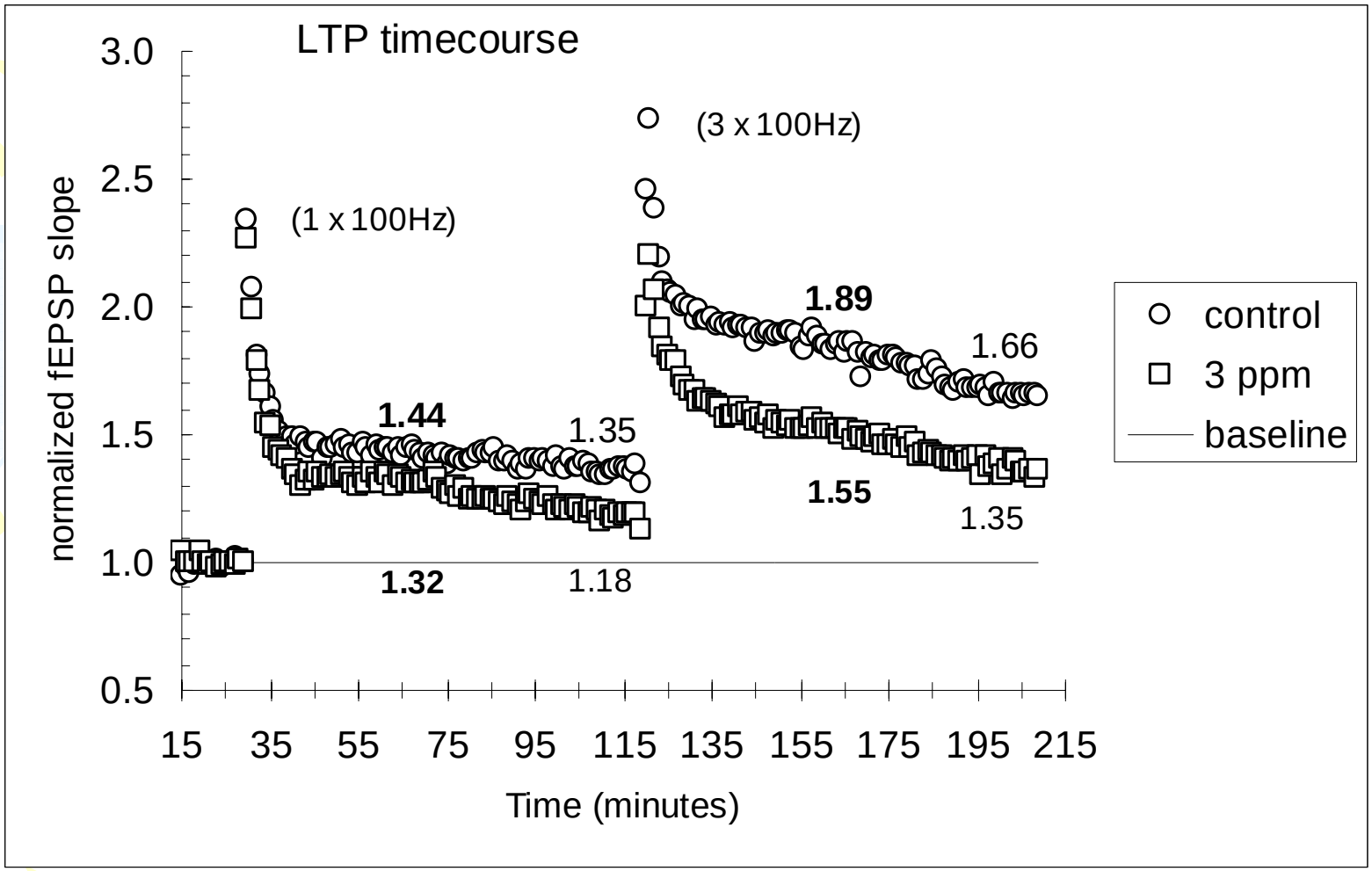




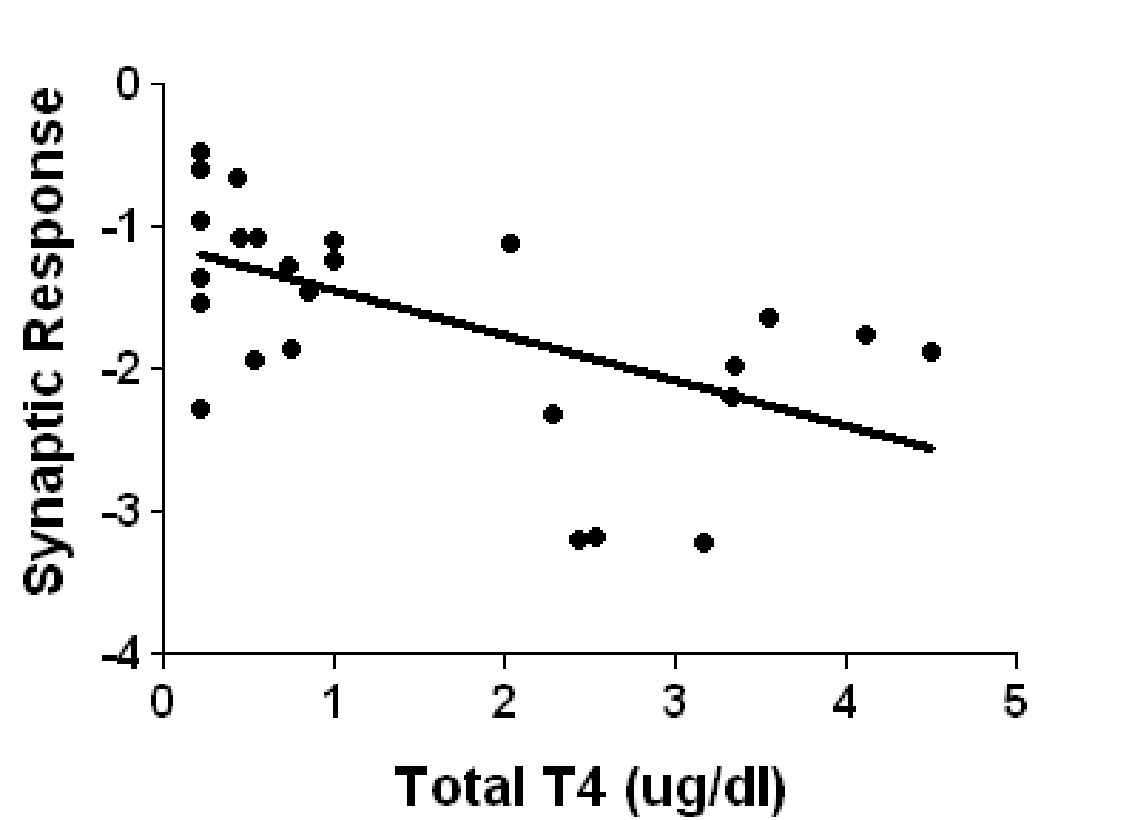
Hippocampal Electrophysiology: Stimulus/Response Curves: PND21-30



Longterm Potentiation: PND100

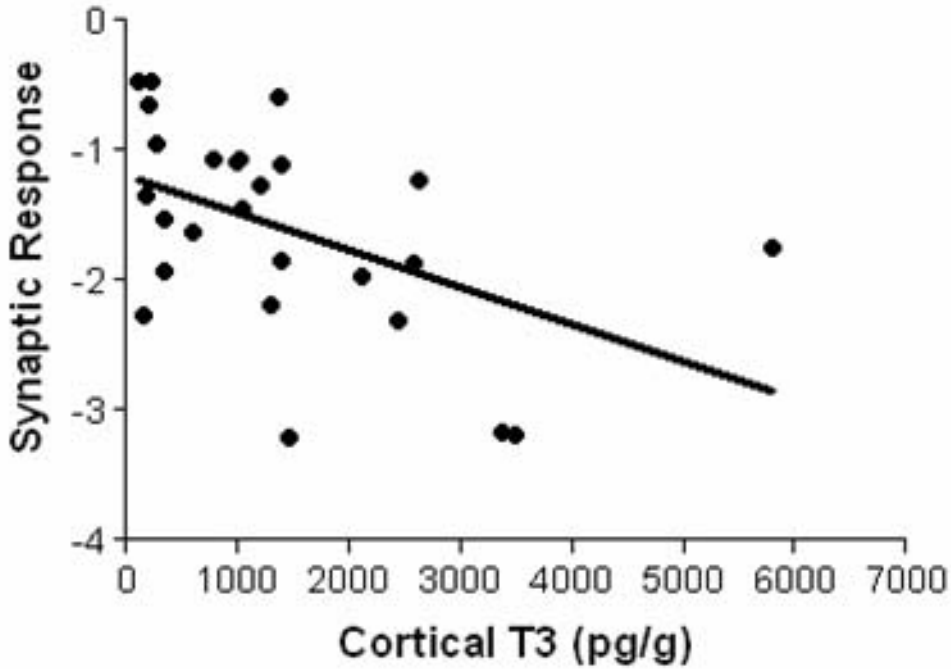


Serum T4 vs. Synaptic Response: PND21-30



y=-0.319x+-1.118
slope=-0.319+/-0.093
y intercept = -1.118+/-0.190
R =0.57 p=0.002

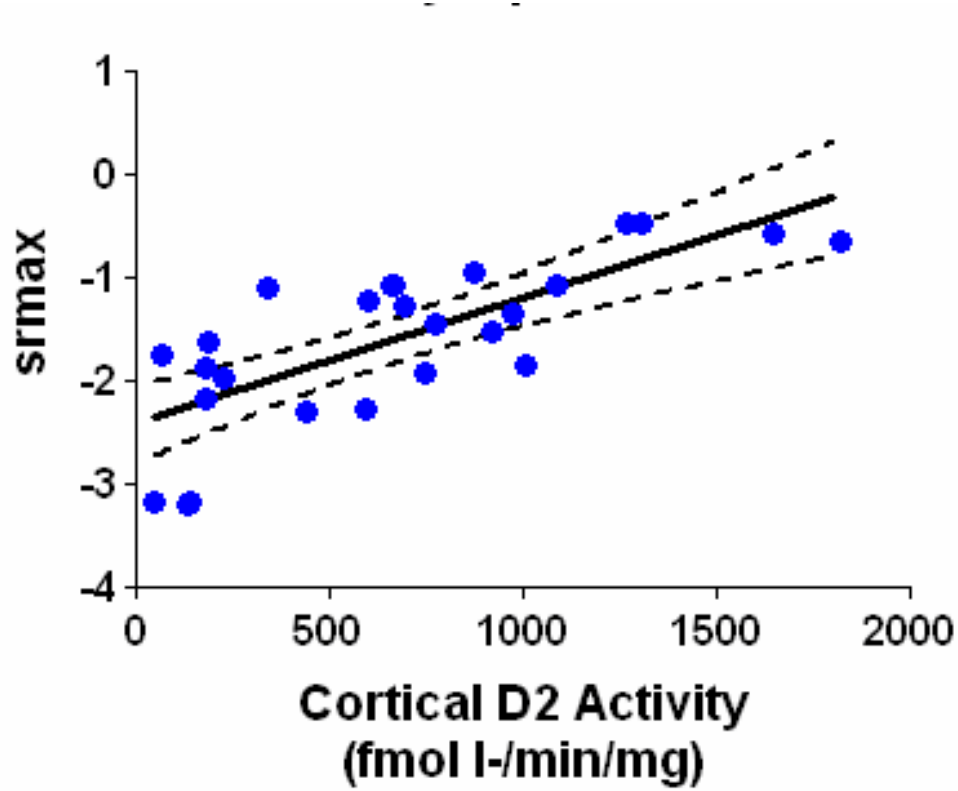
Cortical T3 vs. Synaptic Response: PND21-30



Slope	-0.0002849 ± 0.0001057
Y-intercept	-1.193 ± 0.2035

R=-0.484 p=0.012

Correlation of D2 vs. Maximum Synaptic Response at PND21-30



Slope: 0.00122+/-0.00022
Intercept: -2.41+/-0.18
R= 0.75 p<0.0001

Conclusions: Thyroid Parameters

- Thyroid hormone depletion by gestational/neonatal PTU exposure is ameliorated within the cerebral cortex by D2 induction, whereas hepatic D1 activity is maximally inhibited by 10 ppm PTU.
- Cortical T3 concentrations in PND21-30 pups were maintained in the euthyroid range until a fall of about 75% of serum T4.
- A highly significant negative exponential relationship was observed between serum T4 concentration and D2 activity, with a doubling in D2 with every 1.3 ug/dl fall in T4 in both dams and pups. The relative D2 maximal response was ~8-fold higher in the pups.
- Both cortical D2 and thyroid NIS mRNA induction, likely tissue biomarkers of T4 deficiency/TSH elevation, demonstrate greater sensitivity of the offspring to thyroid hormone deficiency.
- All serum and tissue thyroid parameters returned to normal following 2 months of PTU withdrawal.

Conclusions: Electrophysiology

- Baseline synaptic transmission was significantly reduced in the CA1 region of hippocampal slices obtained from PND21-30 rats under the ongoing influence of PTU exposure.
- Slices obtained from littermates allowed to mature in the absence of PTU until PND90-100 did not exhibit any persisting change in baseline synaptic transmission, however a significant reduction in the magnitude of LTP was observed.
- The decreased ability of the synapses to undergo synaptic plasticity even after the animal has recovered to euthyroid status suggests that although some of the acute impact of hypothyroidism can be restored, the potential remains for significant persisting impairments on the processing of information through neuronal networks.
- D2 enzymatic activity is tightly and positively correlated with synaptic potential at PND25, and may serve as a useful biomarker of thyroid hormone sufficiency in the brain.

Ongoing and Future Work

- Tissue thyroid markers
  - Cortical D3 activity
  - Tissue T4 concentrations
  - In situ hybridization: D2,D3, RC3, GFAP, MCT8, OATP1C1
- Thyroid markers
  - Histomorphometry
  - NIS and Tg immunohistochemistry
- Anatomical
  - Brain histopathology
  - Immunohistochemistry for BDNF, synaptophysin
- Refined dose studies: 0.3,1 and 3 ppm
- Behavioral studies: locomotor and cognitive function as adults