

GRT, March 2024

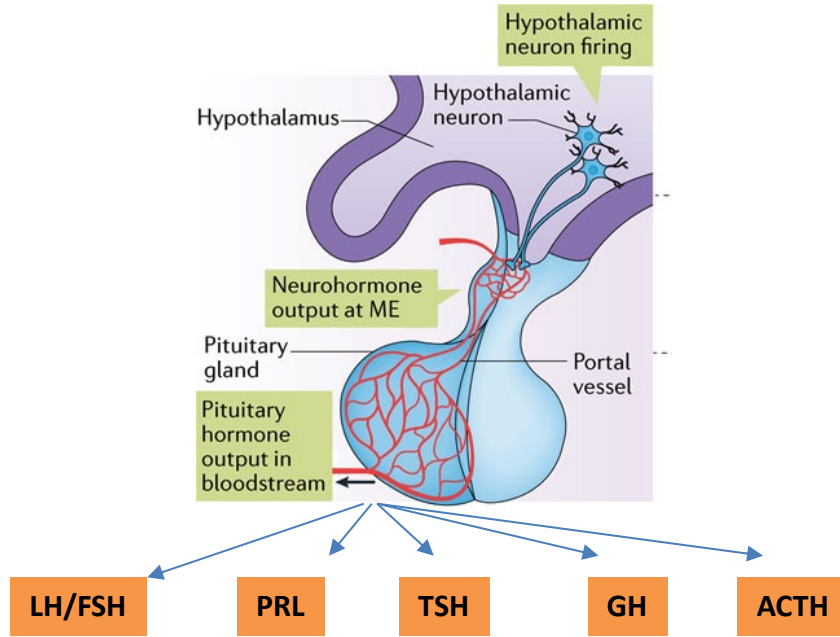
Thyroid control by TSH :
Do we care about TSH secretion patterns?

Patrice Mollard

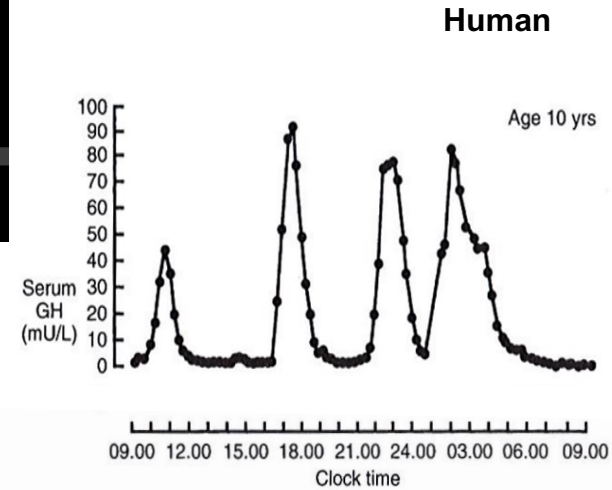
*CNRS-INSERM
Montpellier University*



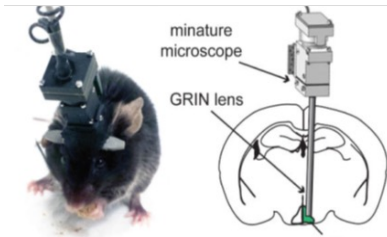
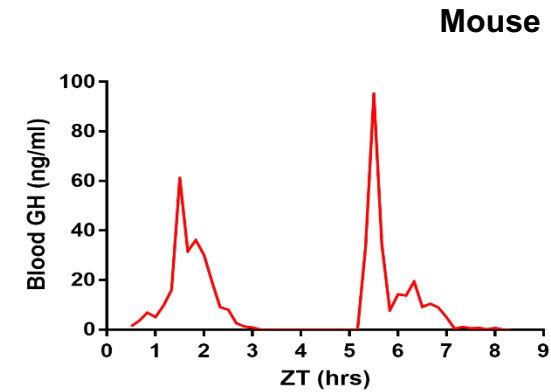
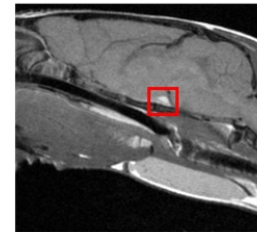
Decipher the logic of the brain-endocrine system dialogue



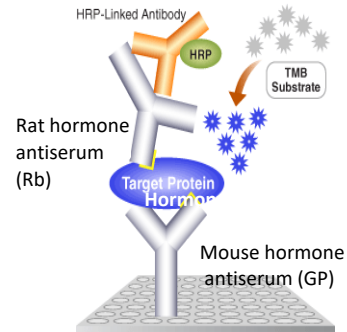
Le Tissier et al. *Nat. Rev. Endoc.* 2017



Robinson and Hindmarsh, 1999

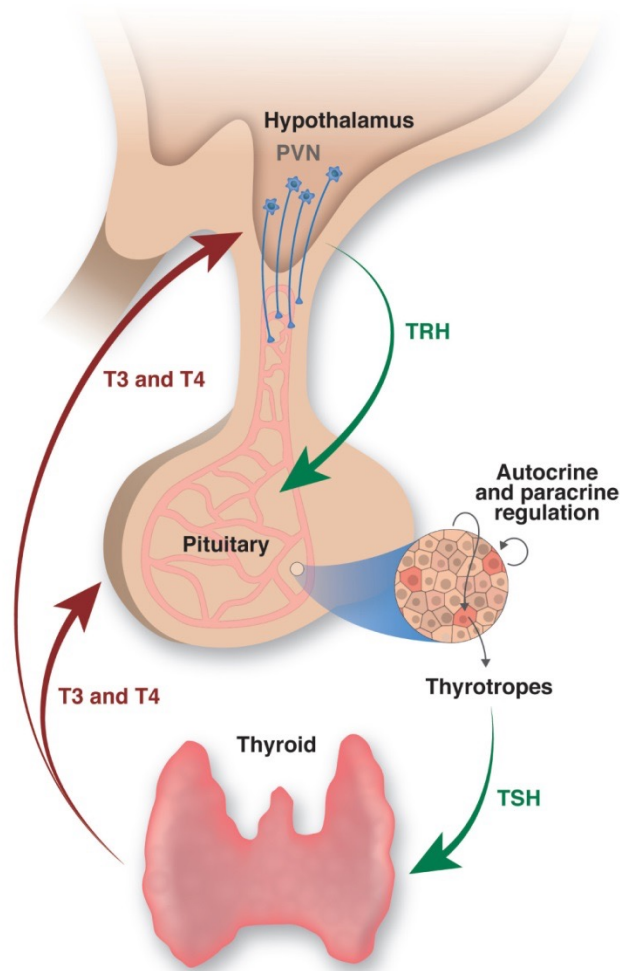


Cellular in vivo imaging

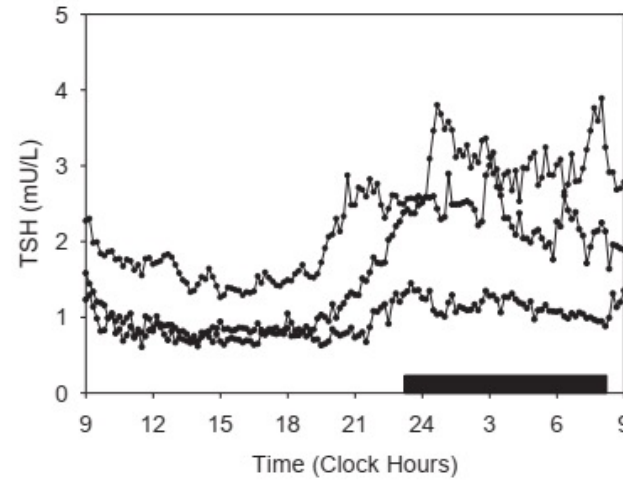


Mouse Hormone ELISA

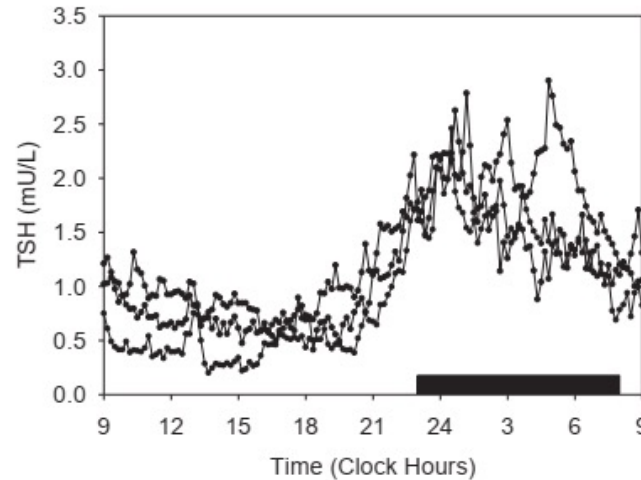
Hypothalamus-Pituitary-Thyroid axis: What do TSH pulse patterns mean?



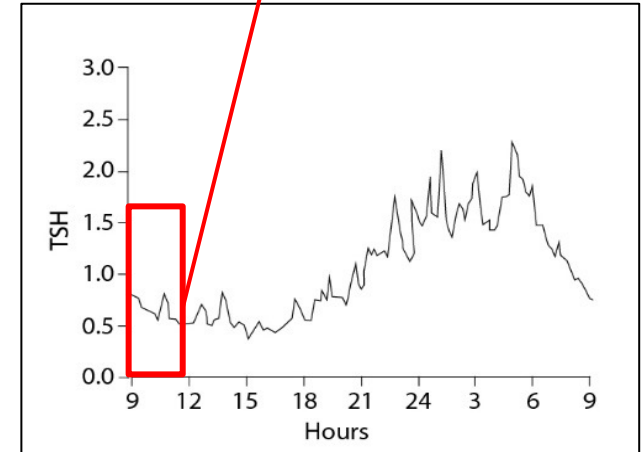
Serum TSH profiles in 3 Healthy Women.



Serum TSH profiles in 3 Healthy Men.



Single TSH measurement
to assess thyroid
function

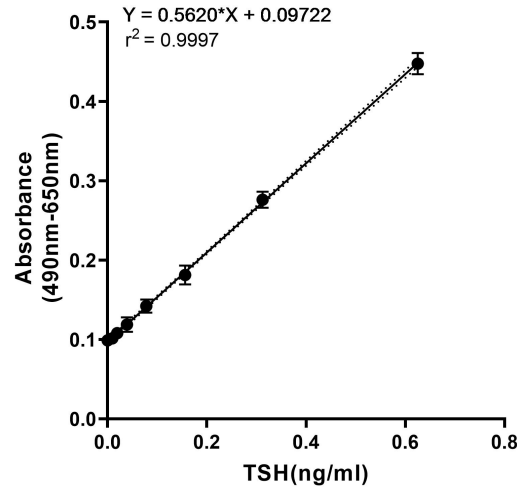
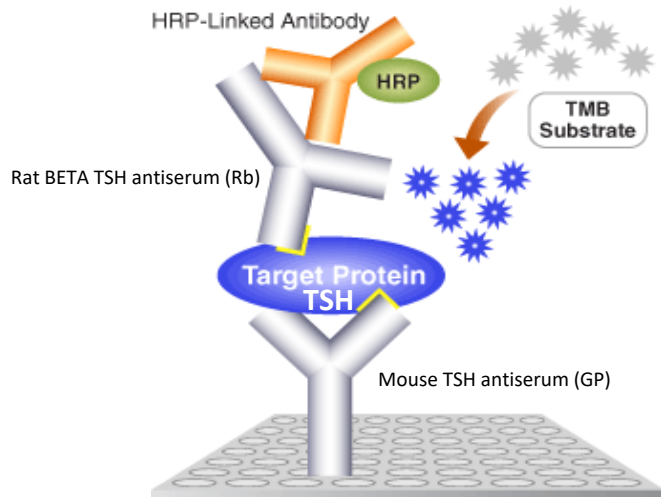
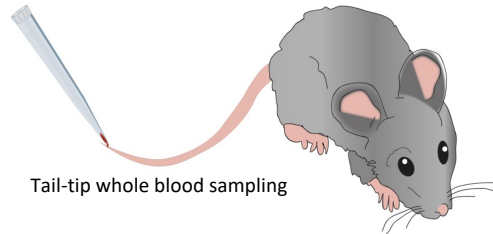


- 1/ Identify TSH secretion patterns in an animal model (mice)
- 2/ Compare the action of TSH pulses versus sustained TSH changes on thyroid signalling and TH release

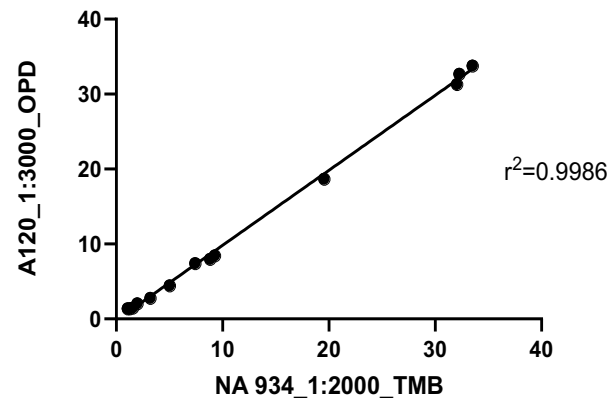
Roelfsema et al., JCEM 2009

Detect mouse TSH in a few blood microlitres

Ultrasensitive in-house mTSH ELISA



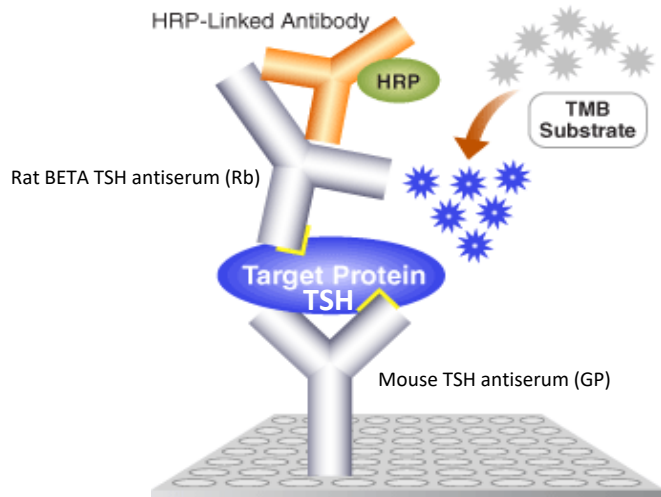
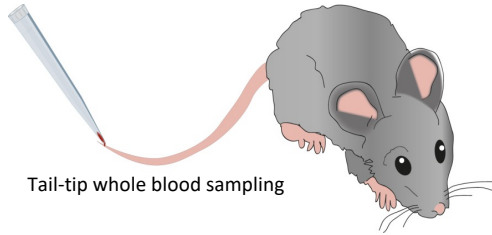
Samples run with two different protocols



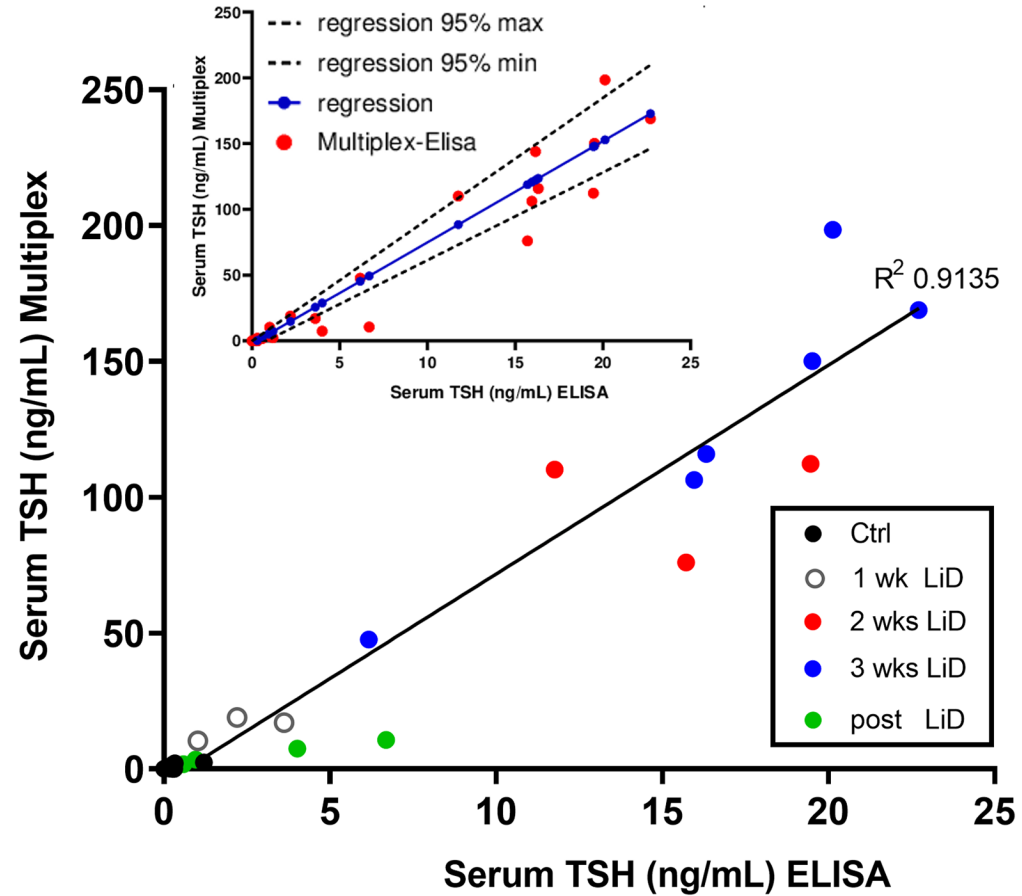
Repeatability precision	Mean ± SEM	CV (%)
Inter-assay CV (Whole blood) (Quality control)	6.970 ± 0.256	12.2
Intra-assay CV (Whole blood + 1.25ng mTSH)	1.106 ± 0.015	3.286
Intra-assay CV (Whole blood + 0.625ng mTSH)	0.6126 ± 0.0136	5.434
Intra-assay CV (Diluted Whole blood 1:20)	0.657 ± 0.007	3.290
LOB/LOD/LOQ		
Blank (OD values) (n=11)	0.09905 ± 0.00659	
LOB (mean blank + 1.645*(SD blank))	LOB = 0.1350	
LOD = LOB + 1.645*(SD low concentration sample)	LOD = 0.1721ng/ml	
Pipetting variability		
Whole blood sample 1	3.885 ± 0.038	3.133
Whole blood sample 2	6.629 ± 0.074	2.487
Accuracy/recovery		
2.5ng/ml		82.9%
0.15625 ng/ml		86.473%
0.039 ng/ml		92.685%

mTSH Elisa versus TSH luminex assay

Ultrasensitive in-house mTSH ELISA

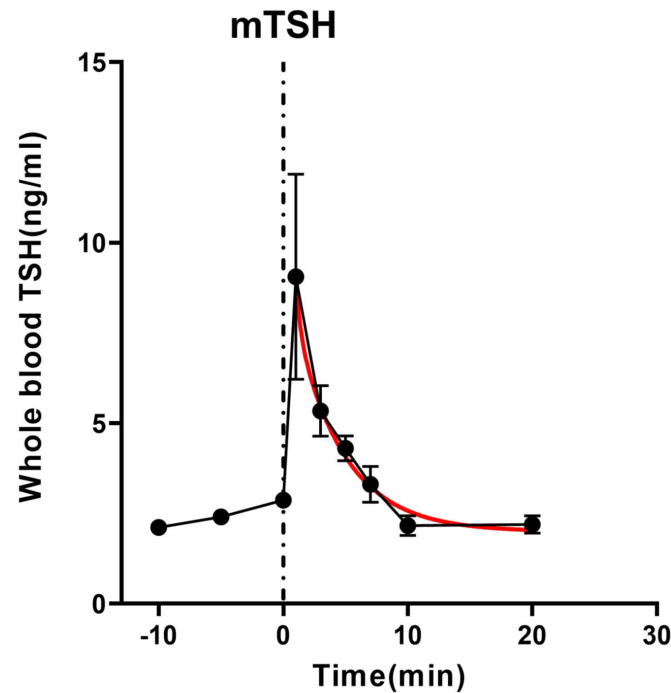
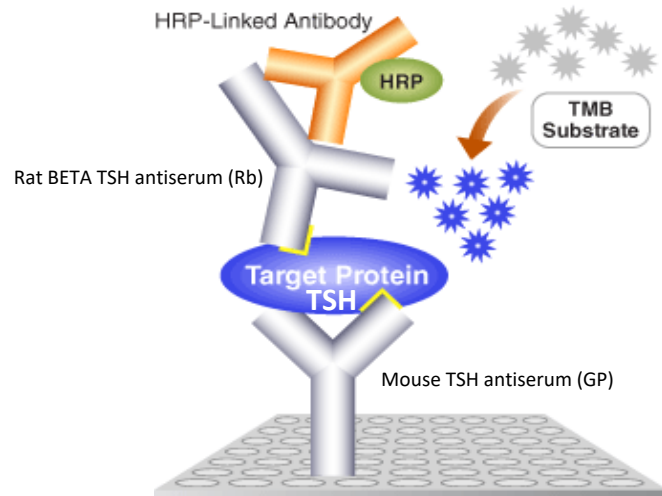
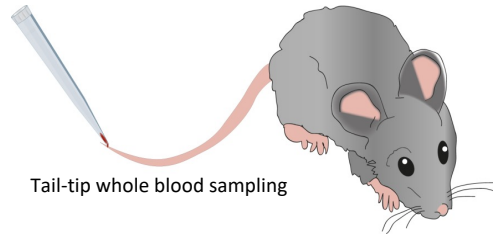


Luminex assays
Dan Bernard, McGill

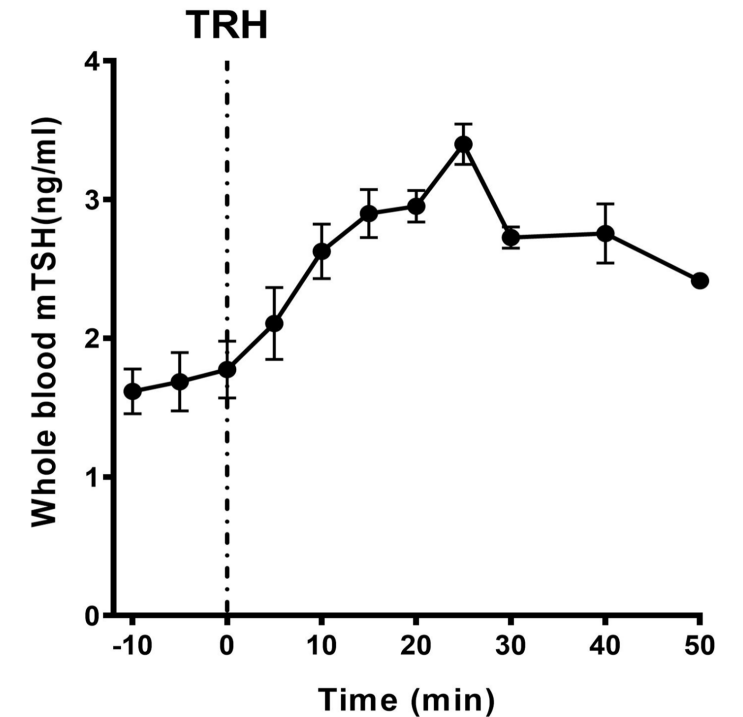


mTSH Elisa: in vivo detection of TSH changes

Ultrasensitive in-house mTSH ELISA



i.v. mouse TSH injection
(12 ng/ml of mouse blood)

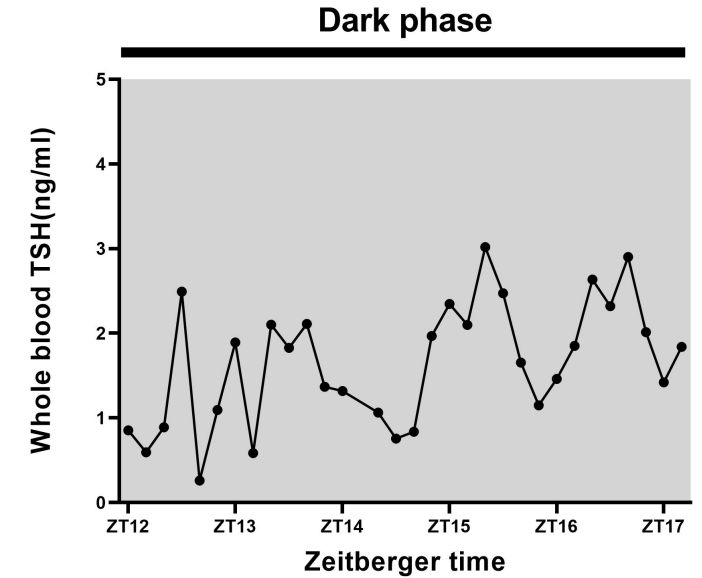
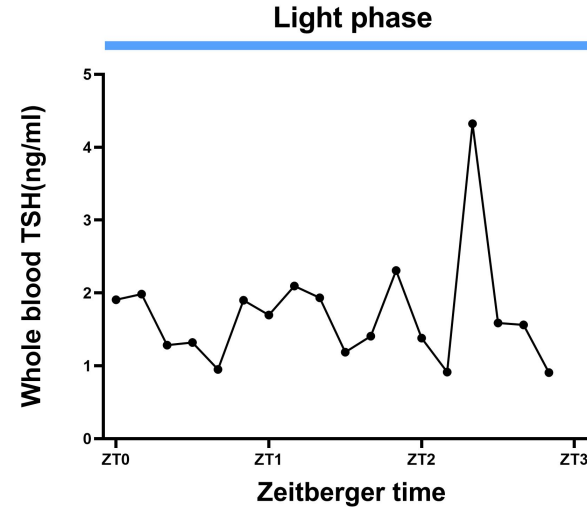
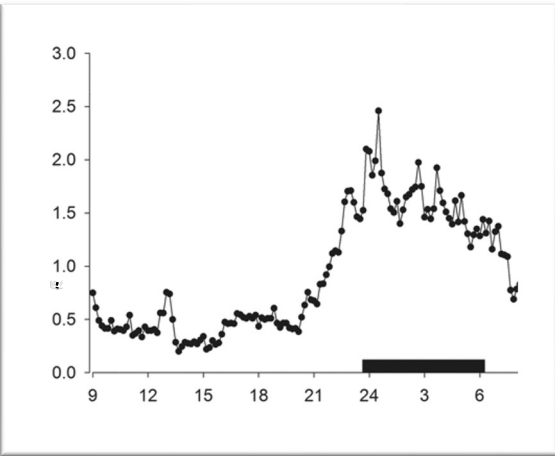


i.p. TRH injection

Ultradian mouse TSH secretion patterns over the day-night period

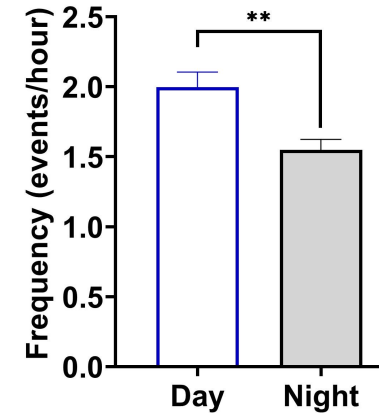
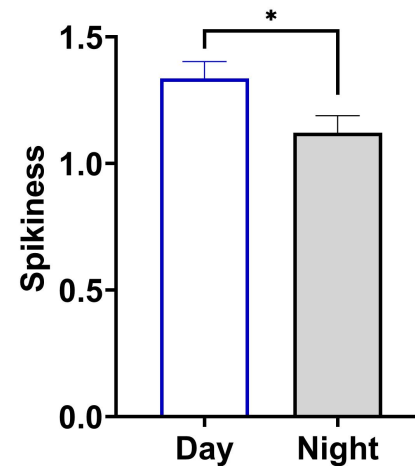
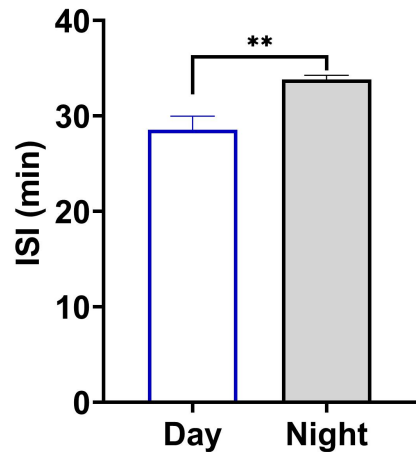
mouse

human



Mice were placed in light-inverted cabinets

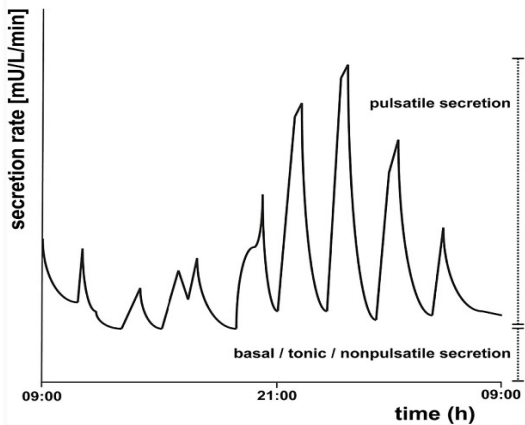
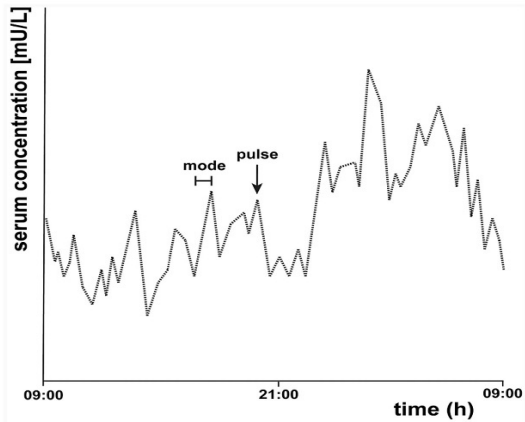
Roelfsema et al., JCEM 2009



Guillou et al., Endocrinology 2023, PMID: 37934802

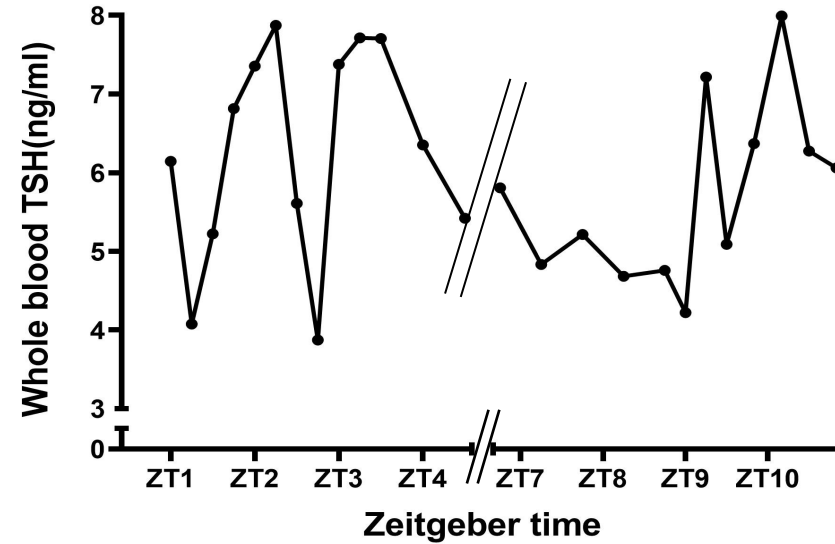
Ultradian mouse TSH secretion patterns over the day period

human

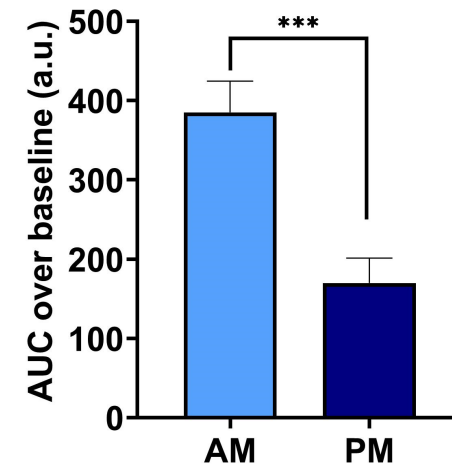
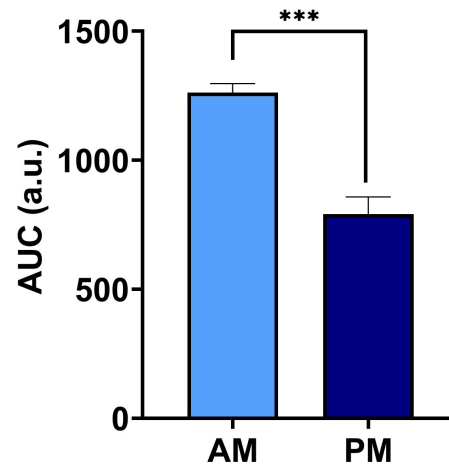


Van Der Spoel et al., Front. Endoc. 2021

mouse

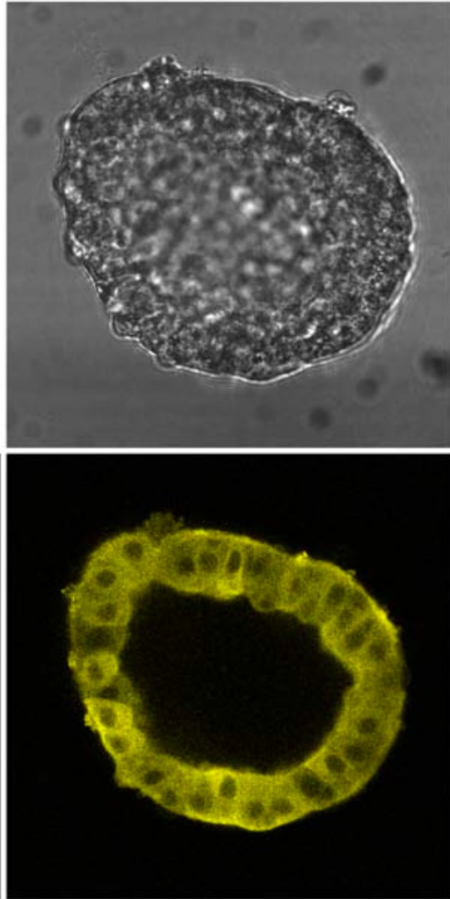


Light phase

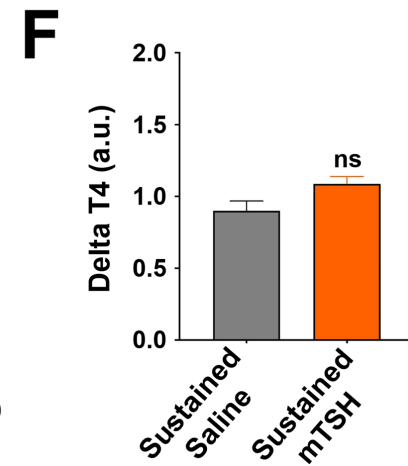
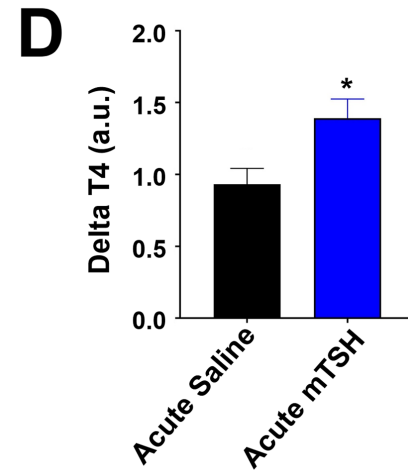
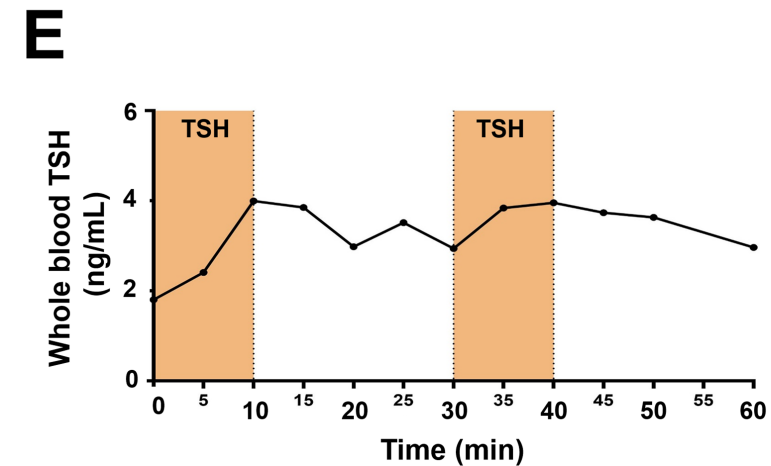
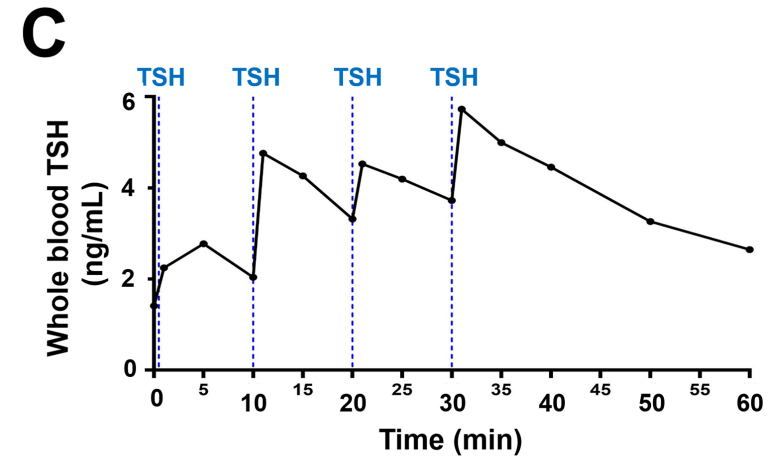
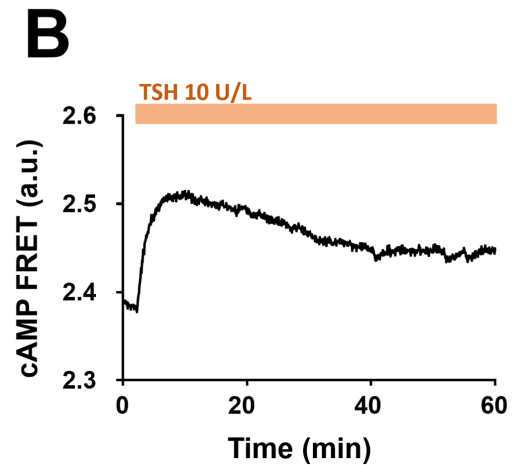
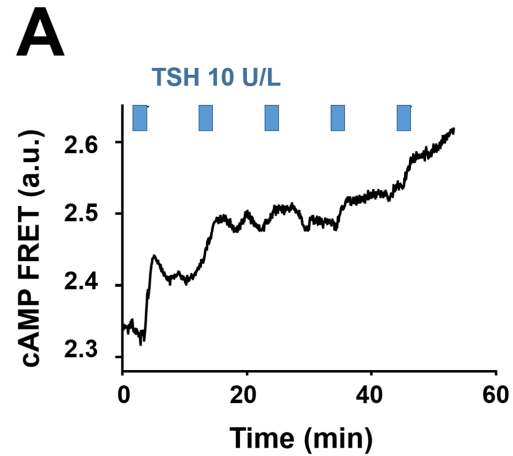


Guillou et al., Endocrinology 2023, PMID: 37934802

TSH Pulses Finely Tune Thyroid Hormone Release and TSH Receptor Transduction



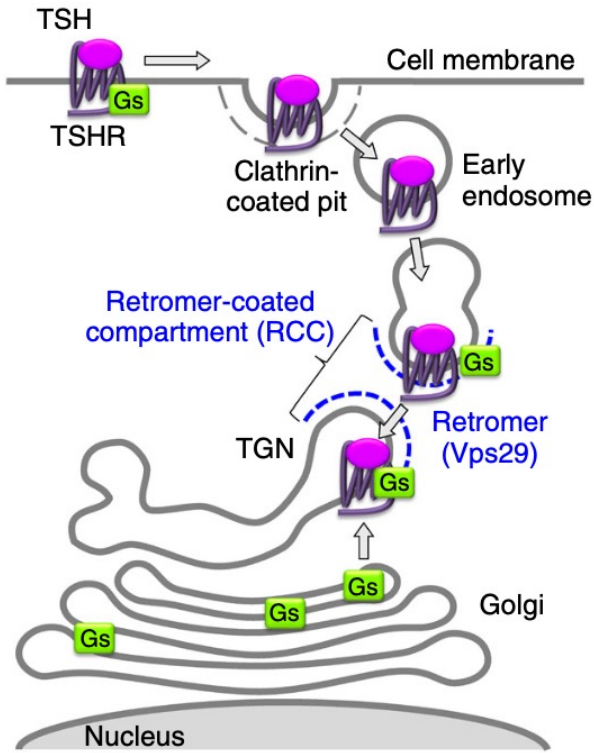
Fluorescent cAMP sensor
in mouse thyroid follicles



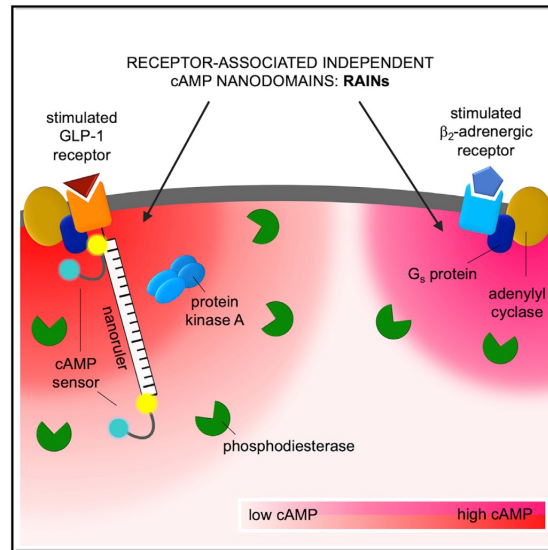
TSH pulses, cAMP metabolism/micro-domains and HPT diseases?

Internalized TSH receptors en route to the TGN induce local G_s -protein signaling and gene transcription

NATURE COMMUNICATIONS | 8: 443 | DOI: 10.1038/s41467-017-00357-2 |



Anton et al., Cell 2022



PDEs: spatial cAMP buffers!

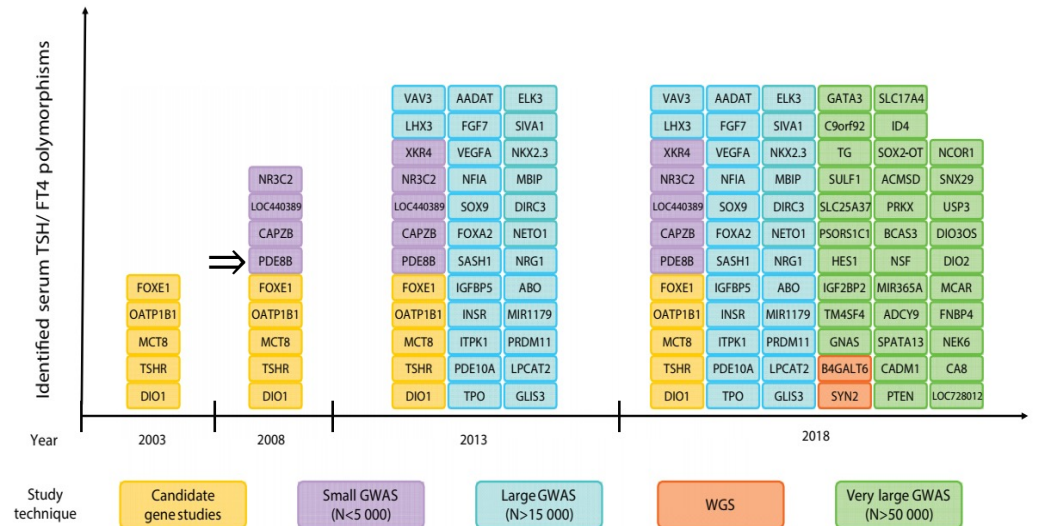
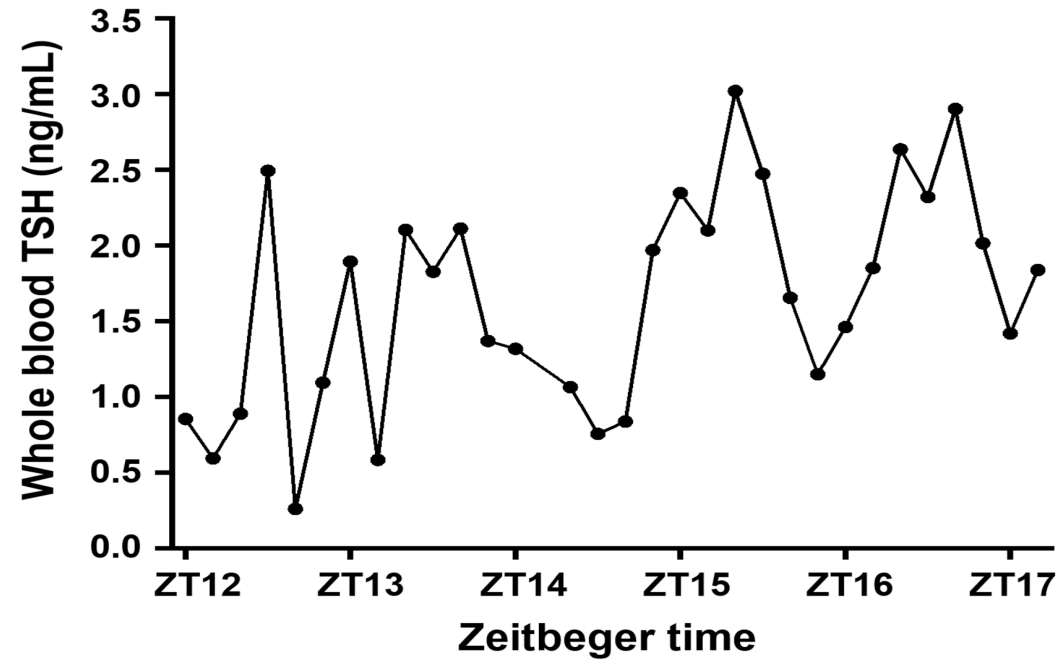
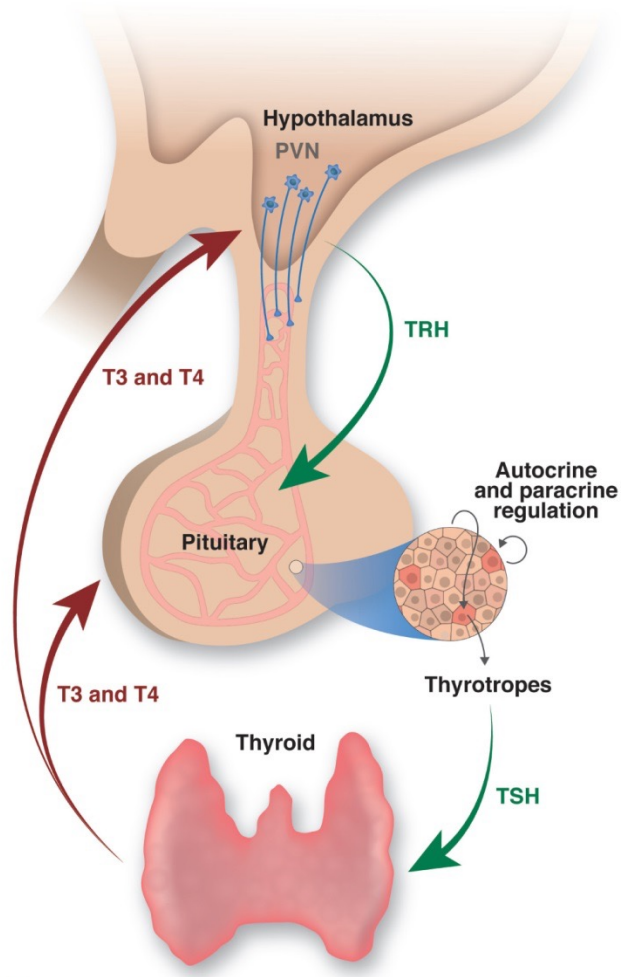


Figure 1. Identified serum TSH and/or FT4 associated loci over time, using different study techniques. Variants found to be significant in candidate gene studies were included when associations were replicated in at least one independent population (N > 50) or in case of in vitro evidence for functionality. Abbreviations: GWAS, genome-wide association study; N, number of subjects analyzed in the study; WGS, whole-genome sequencing.

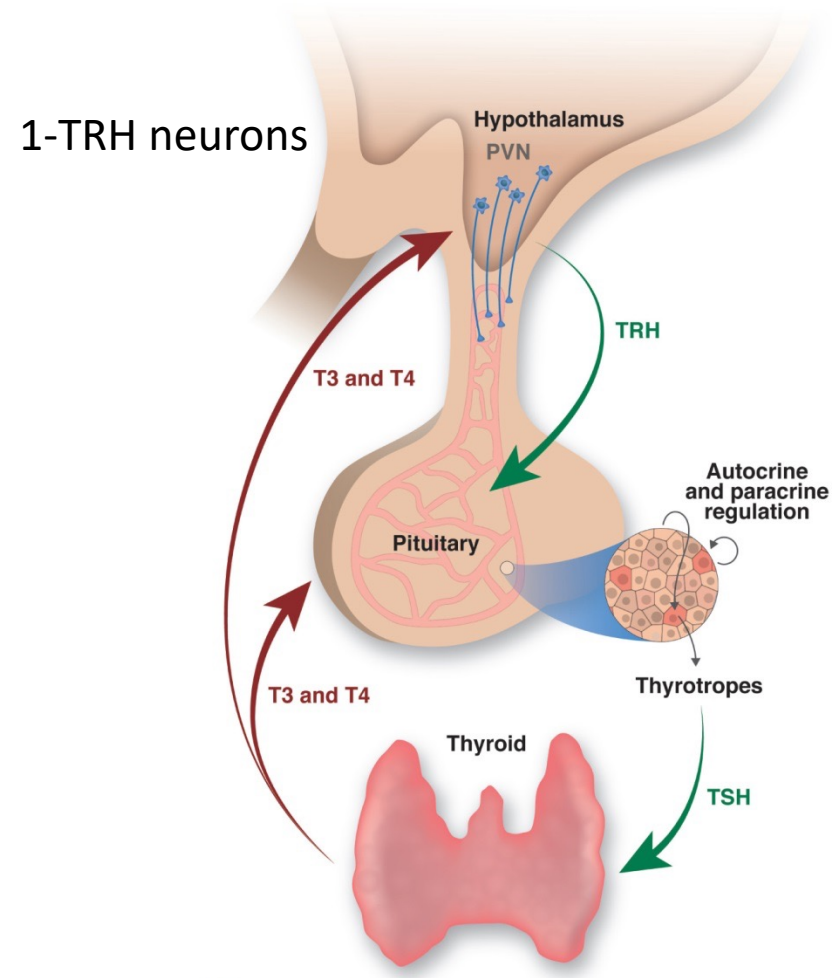
J Clin Endocrinol Metab, June 2020, 105(6):1707–1721

Origin of TSH pulse patterns?

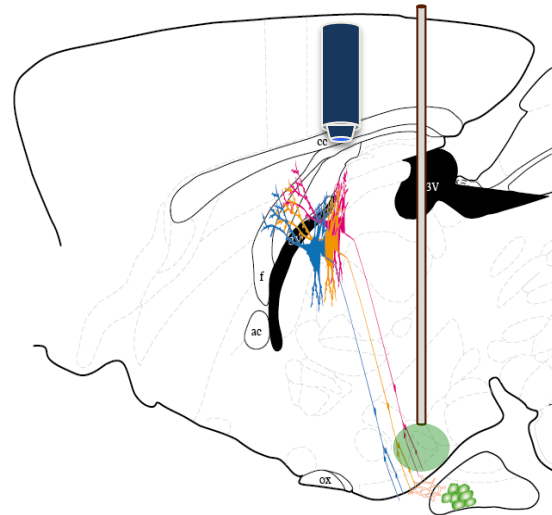


Origin of TSH pulse patterns? 1-TRH?

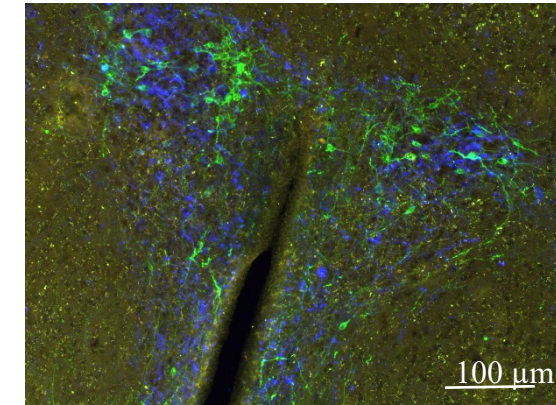
TRH-Cre mouse model
From Brad Lowell, Harvard



TRH-Cre mouse + AAV9-CAG-dflex-GCamp6



Recording site:
Para Ventricular Nucleus
-0.75mm from Bregma



ir.TRH GCamp

Campos et al., J. Endoc. 2020

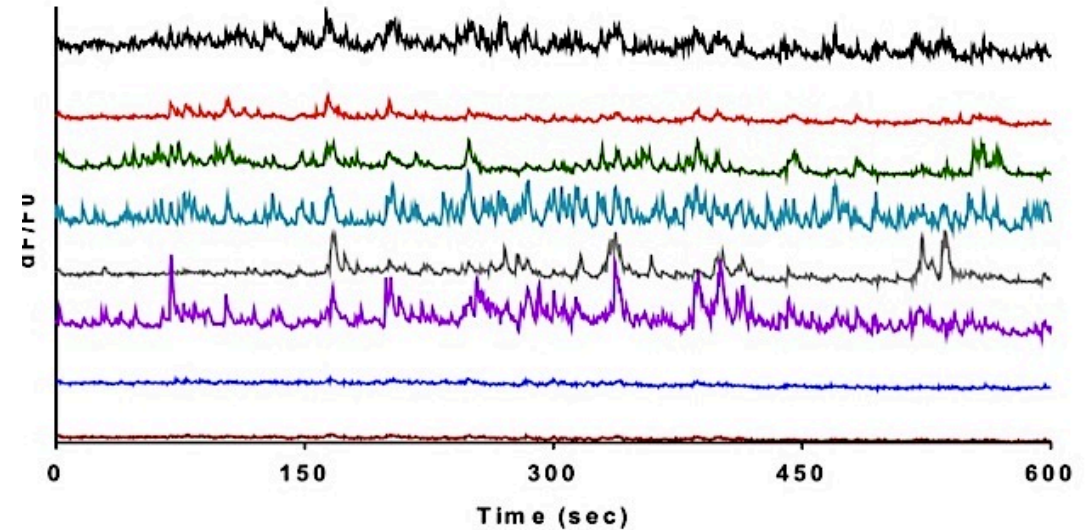
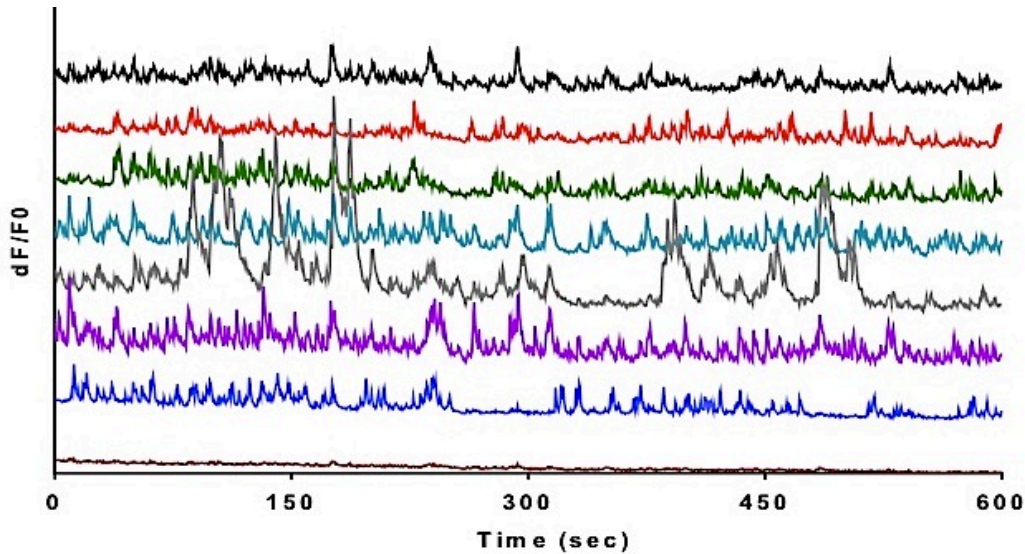
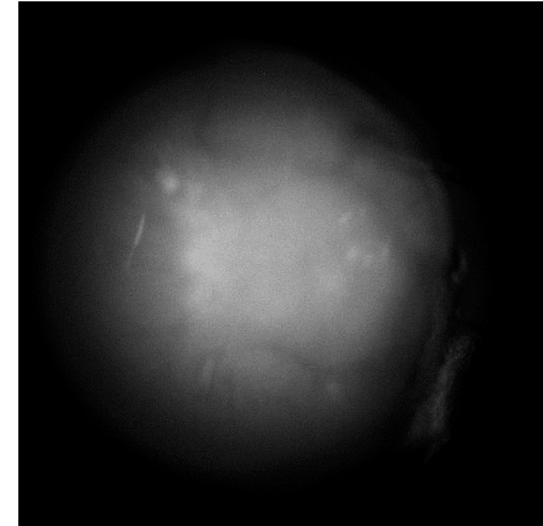
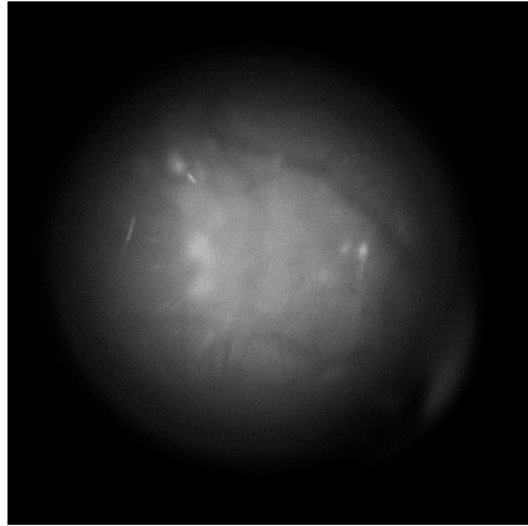
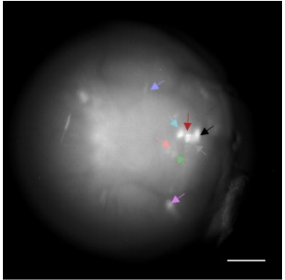
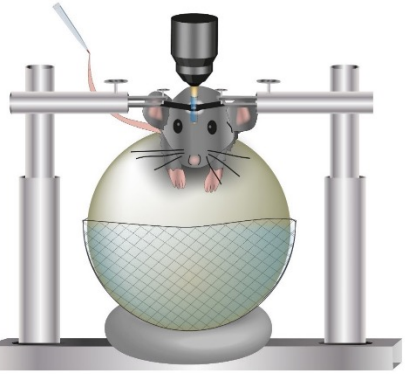
Hypophysiotropic TRH neuron calcium activity in conscious mice

Campos et al., J. Endoc. 2020

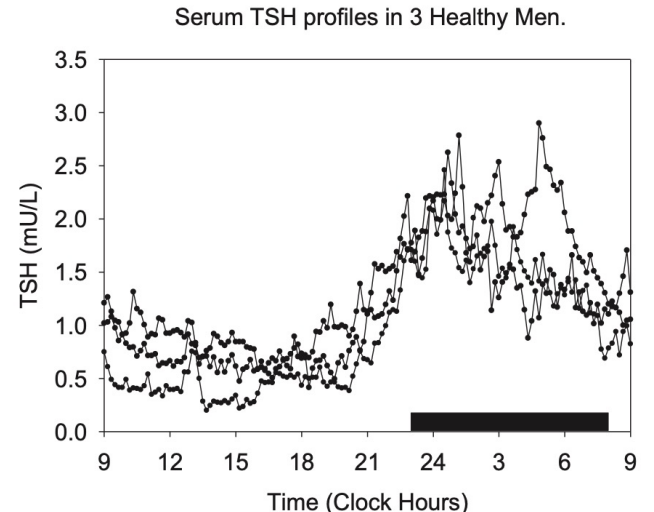
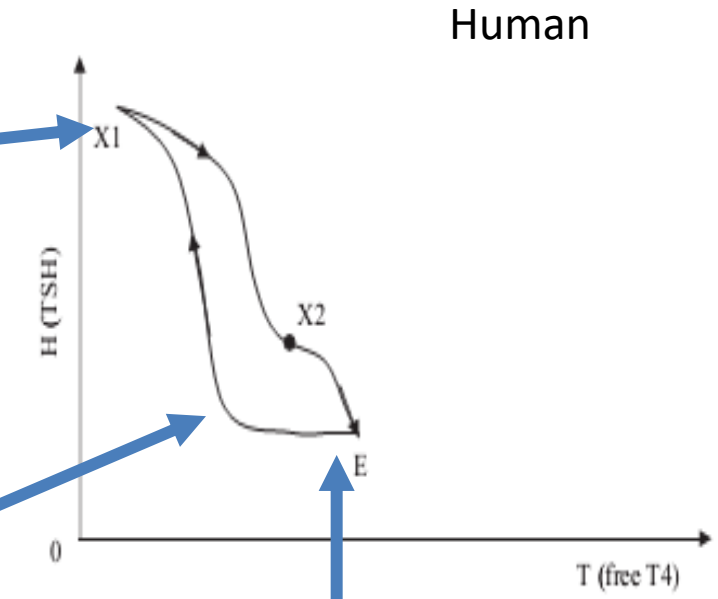
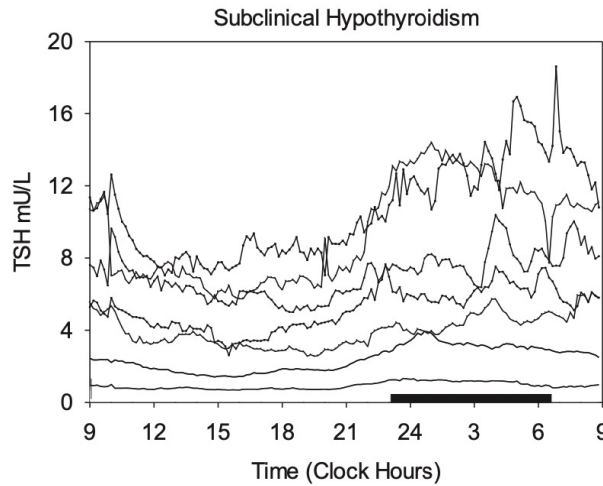
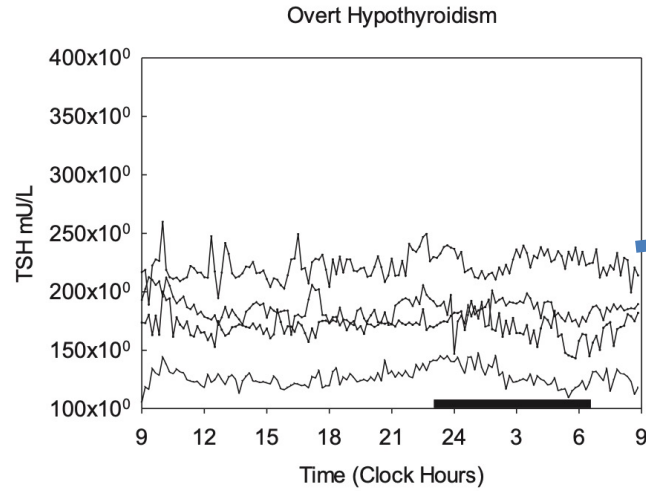
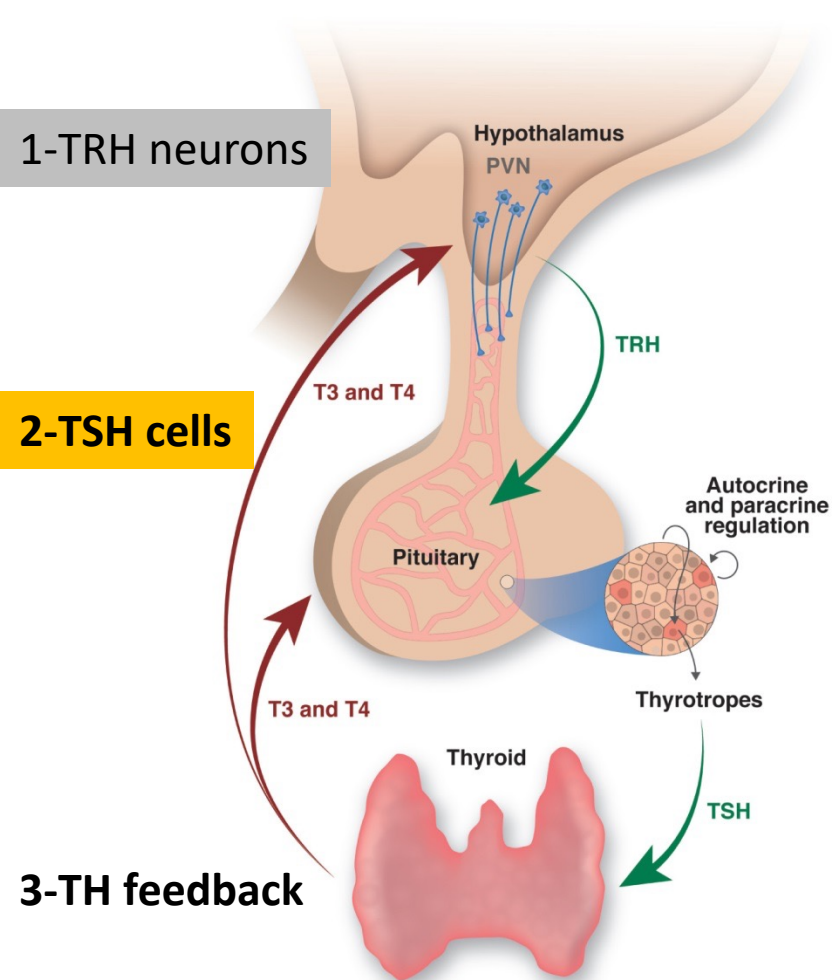
TRH-Cre mouse + AAV9-CAG-dflex-GCamp6

Day1 AM

Day8 PM



TH feedback and TSH patterns upon primary hypothyroidism (human)

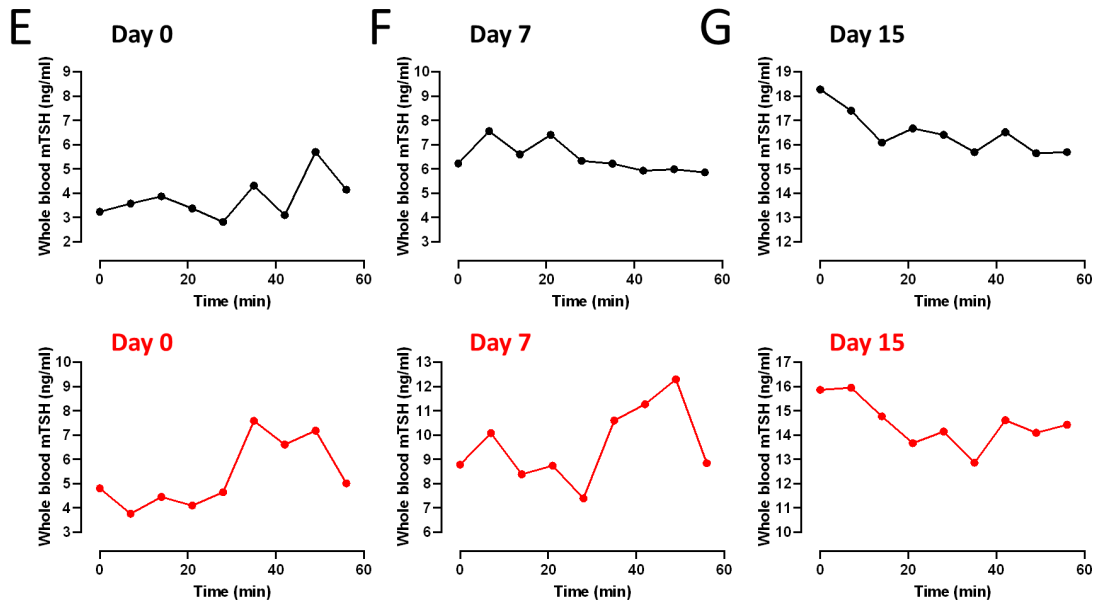
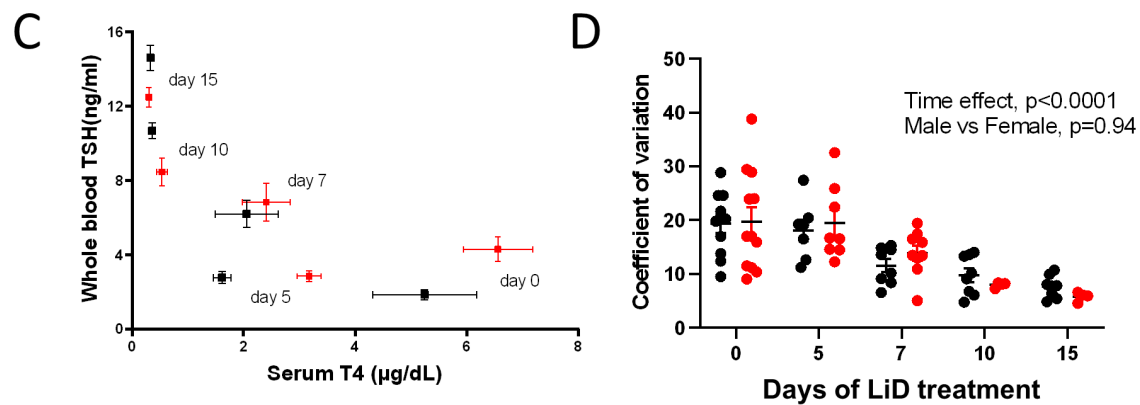
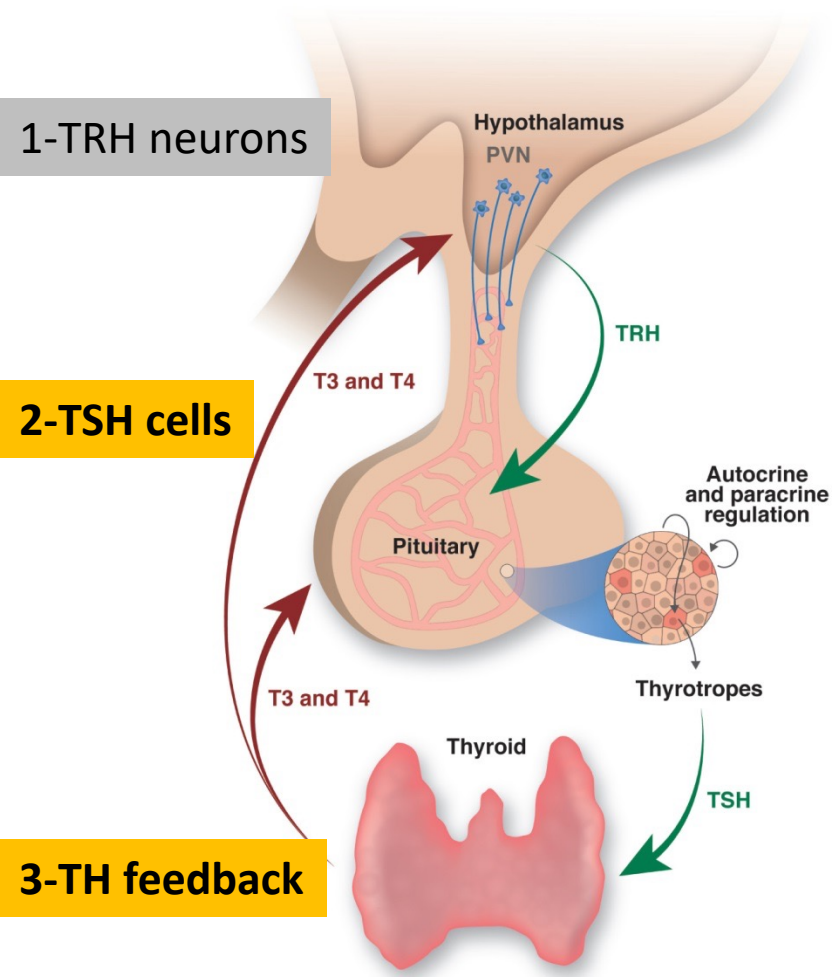


Roelfsema et al., JCEM 2010

TH feedback and TSH patterns upon primary hypothyroidism (mice)

Induction of primary hypothyroidism
(low-iodine diet + PTU)

Male mice
Female mice



On-going project:
Sexual dimorphism of TSH-TH relationships



Acknowledgements

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Pierre Fontanaud (IGF)

Laura Castel

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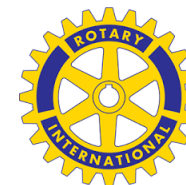
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Thank you for your attention