

## Endocrine Reviews

### Central regulation of pituitary-thyroid axis under physiological and pathophysiological conditions

--Manuscript Draft--

<b>Manuscript Number:</b>	ER-13-1087R1
<b>Full Title:</b>	Central regulation of pituitary-thyroid axis under physiological and pathophysiological conditions
<b>Short Title:</b>	Regulation of hypophysiotropic TRH neurons
<b>Article Type:</b>	Review
<b>Corresponding Author:</b>	Csaba Fekete, MD, PhD Institute of Experimental Medicine, Hungarian Academy of Sciences Budapest, HUNGARY
<b>Corresponding Author Secondary Information:</b>	
<b>Corresponding Author's Institution:</b>	Institute of Experimental Medicine, Hungarian Academy of Sciences
<b>Corresponding Author's Secondary Institution:</b>	
<b>First Author:</b>	Csaba Fekete, MD, PhD
<b>First Author Secondary Information:</b>	
<b>Order of Authors:</b>	Csaba Fekete, MD, PhD Ronald M. Lechan, MD, PhD
<b>Order of Authors Secondary Information:</b>	
<b>Abstract:</b>	<p>Thyrotropin-releasing hormone (TRH) is a tripeptide amide that functions as a neurotransmitter, but also serves as a neurohormone that has a critical role in the central regulation of the hypothalamic-pituitary-thyroid (HPT) axis. Hypophysiotropic TRH neurons involved in this neuroendocrine process are located in the hypothalamic paraventricular nucleus and secrete TRH into the pericapillary space of the external zone of the median eminence for conveyance to anterior pituitary thyrotrophs. Under basal conditions, the activity of hypophysiotropic TRH neurons are regulated by the negative feedback effects of thyroid hormone to ensure stable, circulating, thyroid hormone concentrations, a mechanism that involves complex interactions between hypophysiotropic TRH neurons and the vascular system, CSF and specialized glial cells called tanycytes. Hypophysiotropic TRH neurons also integrate other humoral and neuronal inputs that can alter the setpoint for negative feedback regulation by thyroid hormone. This mechanism facilitates adaptation of the organism to changing environmental conditions, including the shortage of food and a cold environment. The thyroid axis is also affected by other adverse conditions such as infection, but the central mechanisms mediating suppression of hypophysiotropic TRH may be pathophysiologic. In this review, we discuss current knowledge about the mechanisms that contribute to regulation of hypophysiotropic TRH neurons under physiological and pathophysiological conditions.</p>

E. Chester Ridgway, M.D.  
Editor-in Chief  
University of Colorado  
School of Medicine  
Aurora, Colorado

September 30, 2013

Dear Dr. Ridgway,

We appreciate the helpful suggestions of the Reviewers and wish to resubmit our manuscript (# ER-13-1087) entitled "**Central regulation of the hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions**" by Csaba Fekete, and Ronald M. Lechan for reconsideration as a publication in *Endocrine Reviews*. Changes in the manuscript and responses to the Reviewers' criticisms are detailed below under each reviewer.

**Reviewer 1:**

**Point 1:** The authors may want to add a short paragraph on the neuroanatomy and possible physiological role of the autonomic innervation....

**Response:** We added a new section to address the autonomic regulation of HPT axis.

**Point 2:** V, p. 35. First sentence: "DIA is an interesting model...". The authors should add for which particular process/function DIA is a model.

**Response:** We altered the first paragraph of Section V to better describe this model.

**Point 3:** VIII. p.41, Cold exposure triggers TRH gene expression in the PVN; the authors discuss the likely role for brainstem catecholaminergic neurons in this response. However, it is unclear what the effect/relevance of this TRH response might be, .....

**Response:** We now discuss the potential relevance of the cold-induced ,transient hyperthyroidism in the regulation of thermogenesis. We also discuss why we think that the hypophysiotropic TRH neurons do not influence BAT *via* the ANS.

**Point 4:** P 16: The text on alpha 1 and alpha 2 tanycytes (lines 8-14) does not match with Fig 7B.

**Response:** We corrected this error in Figure 7.

**Reviewer 2:**

**Point 1:** P13 The sentence concluding the second paragraph ? ; ' different role in the regulation of the thyroid axis in humans » begs the question about the role of CART innervation to the these neurons in regulating feeding in humans and should be clarified.

**Response:** The CART innervation of TRH neurons has not been studied in the human hypothalamus, and even more importantly, due to the serious side effects of CART and the lack of CART agonists or antagonists, it is not possible to perform human experiments to determine the role of CART in the regulation of TRH neurons. However, the fact that CART is expressed in a number of different

neuronal populations in the human hypothalamus compared to rodents is enough to be concerned that the rodent findings may not necessarily be true for humans.

**Point 2:** P15 para1, I don't like the idea that « transmitters interact with each other. ». The molecules don't interact. I think it should be better expressed in terms of the crosstalk between effector systems.

**Response:** We modified this sentence.

**Point 3:** P15 para 2 : specify with species is being referred to for the ref 83.

**Response:** We now specify that these data originate from experiments performed in rats.

**Point 4:** P 15 para 3 a number of the inputs listed as additional - have already been mentioned - GABA , PACAP and glutamate. Rewrite for precision.

**Response:** We corrected this sentence.

**Point 5:** Pp 22 and 18 are a bit contradictory in that on p18 we're told that that (end of para 1) that « most of the regions of the hypothalamus are devoid of D2 » and then on P22 para 1 lines 4-5 that « D2 is also regulated in the hypothalamus at the transcriptional level ». Correct / clarify please.

**Response:** We changed the mentioned sentence on page 22.

**Point 6:** P 20 - To my school of thought 'why' is not a correctly formulated biological question and implies teleology or « purpose » that has no place in current evolutionary theory. The question should be termed in the light of redundance versus specificity. In fact the observations that in many other brain areas TRb1 and TRa1 coexist and coregulate common targets is not considered aberrant - but indeed taken as examples of redundancy and this is no doubt the case for the similar effects of B1 and B2 discussed here.

**Response:** We changed the mentioned sentence.

**Point 7:** P 23 - section on transporters. This part could be usefully reorganised slightly bringing the idea of active transport to the start ( the sentence beginning « Despite their lipophilic?. ») - so that the verb 'extract' makes more sense.

**Response:** We reorganized this part according to the Reviewer's suggestion.

**Point 8:** P24 explain what is meant by 'volume transmission', not obvious.

**Response:** To clarify this point, we now use the term "diffusion".

**Point 9:** Similarly at the end of this paragraph the idea that only hypophysiotropic TRH neurons are affected by changed peripheral TH levels is not only prompted by the previous question ( how T3 reaches the TRH neurons) and the proposed hypothesis - and discussion of this point really needs some highlighting/ more detail of potential hypothesis that could be testable.

**Response:** We added the following sentence to the end of the paragraph: "However, further studies are needed to demonstrate that the T3 content of hypophysiotropic and non-

hypophysiotropic TRH neurons is differentially regulated by changes in peripheral thyroid hormone levels.”

**Point 10:** P 29 and 31 - repetition of the idea that lack of NPY and MC4R abrogate fasting induced drop in T3

**Response:** The reviewer is correct that the data is mentioned in two parts of the manuscript. However, we use these data to support the importance of the the arcuato-paraventricular pathway in the regulation of TRH neurons during fasting on page 29, whereas on page 31 we use the data to demonstrate that the direct effect of leptin on the TRH neurons seems to have negligible effect during fasting despite of the well documented direct action of leptin on these cells. Therefore, repetition of the data on both places is of particular importance.

**Point 11:** Not clear what the overall hypothesis is that is being discussed end P 32 / para 1 p 33.

**Response:** We added a sentence at the beginning of this section to resolve the problem.

**Point 12:** P 36 - suddenly start talking about POMC neurons - well most will know that POMC is processed into  $\alpha$ MSH etc - but given that many readers will be new to the area ( see point 1) then this needs to be explained?

**Response:** We changed the sentence to clarify that proopiomelanocortin (POMC) is the precursor of  $\alpha$ -MSH.

**Point 13:** the discussion of non-critical illness might require some better explanation of the current ideas of 'acute' versus 'prolonged' phases as the therapeutic options differ dramatically according to the phase (Van den Berge's recent data).

**Response:** The Reviewer is correct that the nonthyroidal illness has been separated by some authors into acute and chronic phases. This distinction has been dealt with extensively in a previous *Endocrine Reviews* article published by Boelen et al in 2011. The acute phase is largely believed to be secondary to decreased conversion of T4 to T3 in peripheral tissues (liver, muscle, adipose tissue) due to changes in deiodinases, whereas the chronic phase involves changes both in both the periphery and in the central nervous system. We suspect that central changes are occurring during the acute phase, but this has not been systematically studied.

**Point 14:** Indeed this section (VII) that leads into translational ramifications (IX) (after a short section on cold) is rather superficial and disappointing . Translational aspects of TRH physiology are by no means limited to this pathology. So if there is one area of the reveiw that merits more effort from the authors and a rewrite this is it.

**Response:** We changed the Translational ramification Section.

**Response to minor points:** As recommended, we expanded this section..

**Reviewer 3:**

**Point 1:** It is well written but suffers from too much detail in some areas and not enough in others. First, the focus on the hypothalamic TRH neurons is appropriate but much of the material is the authors' own view of the how the TRH neuron is regulated.

**Response:** The authors made a concerted effort to include all data that are related to the reviewed topics. If there are areas that have not been covered in sufficient detail, it would be helpful to know specifically what those are so we can make the appropriate changes. As noted by the above Reviewer, the detail is helpful to allow the reader to best understand the material.

**Point 2:** I would recommend eliminating many of the darkfield mRNA figures. Many appear to be previously published or too detailed for the general reader.

**Response:** We believe that the darkfield images will help the readers to better understand the topic, therefore, we would like to retain these images.

**Point 3:** The structure of the TRH gene also adds little to the review.

**Response:** The goal of the manuscript is to summarize current knowledge about the function and regulation of hypophysiotropic TRH neurons. The regulation of TRH gene expression is a critical component and it's understanding highly facilitated by the description of the structure of TRH gene. Therefore, we wish to retain this part of the manuscript and the figure that supports it.

**Point 4:** What would be helpful are figures that present how the HPT is regulated by multiple pathways such as by feeding, fasting, thyroid hormone, etc.

**Response:** Fig 10 and 12 summarize the regulation of the TRH neurons by feeding and fasting and by thyroid hormones, respectively.

**Point 5** Finally, the authors argue that in vivo data in TR KO animals are somehow equivalent to in vitro studies of TR over-expression in non-homologous cells. I would suggest rewriting those sections.

**Response:** The only *in vitro* data mentioned in that part of the manuscript is the effect of T3 on hypothalamic cell culture in its early stage when the predominant TR in the cells is TR $\alpha$ . We did not consider these data equal with the knock out studies, but do feel that the cell culture data support the *in vivo* findings. Therefore, we wish to retain this Section in the current form.

E. Chester Ridgway, M.D.  
Editor-in Chief  
University of Colorado  
School of Medicine  
Aurora, Colorado

September 30, 2013

Dear Dr. Ridgway,

We appreciate the helpful suggestions of the Reviewers and wish to resubmit our manuscript (# ER-13-1087) entitled "**Central regulation of the hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions**" by Csaba Fekete, and Ronald M. Lechan for reconsideration as a publication in *Endocrine Reviews*. Changes in the manuscript and responses to the Reviewers' criticisms are detailed below under each reviewer.

**Reviewer 1:**

**Point 1:** The authors may want to add a short paragraph on the neuroanatomy and possible physiological role of the autonomic innervation....

**Response:** We added a new section to address the autonomic regulation of HPT axis.

**Point 2:** V, p. 35. First sentence: "DIA is an interesting model...". The authors should add for which particular process/function DIA is a model.

**Response:** We altered the first paragraph of Section V to better describe this model.

**Point 3:** VIII. p.41, Cold exposure triggers TRH gene expression in the PVN; the authors discuss the likely role for brainstem catecholaminergic neurons in this response. However, it is unclear what the effect/relevance of this TRH response might be, .....

**Response:** We now discuss the potential relevance of the cold-induced ,transient hyperthyroidism in the regulation of thermogenesis. We also discuss why we think that the hypophysiotropic TRH neurons do not influence BAT *via* the ANS.

**Point 4:** P 16: The text on alpha 1 and alpha 2 tanycytes (lines 8-14) does not match with Fig 7B.

**Response:** We corrected this error in Figure 7.

**Reviewer 2:**

**Point 1:** P13 The sentence concluding the second paragraph ? ; ' different role in the regulation of the thyroid axis in humans » begs the question about the role of CART innervation to the these neurons in regulating feeding in humans and should be clarified.

**Response:** The CART innervation of TRH neurons has not been studied in the human hypothalamus, and even more importantly, due to the serious side effects of CART and the lack of CART agonists or antagonists, it is not possible to perform human experiments to determine the role of CART in the regulation of TRH neurons. However, the fact that CART is expressed in a number of different

neuronal populations in the human hypothalamus compared to rodents is enough to be concerned that the rodent findings may not necessarily be true for humans.

**Point 2:** P15 para1, I don't like the idea that « transmitters interact with each other. ». The molecules don't interact. I think it should be better expressed in terms of the crosstalk between effector systems.

**Response:** We modified this sentence.

**Point 3:** P15 para 2 : specify with species is being referred to for the ref 83.

**Response:** We now specify that these data originate from experiments performed in rats.

**Point 4:** P 15 para 3 a number of the inputs listed as additional - have already been mentioned - GABA , PACAP and glutamate. Rewrite for precision.

**Response:** We corrected this sentence.

**Point 5:** Pp 22 and 18 are a bit contradictory in that on p18 we're told that that (end of para 1) that « most of the regions of the hypothalamus are devoid of D2 » and then on P22 para 1 lines 4-5 that « D2 is also regulated in the hypothalamus at the transcriptional level ». Correct / clarify please.

**Response:** We changed the mentioned sentence on page 22.

**Point 6:** P 20 - To my school of thought 'why' is not a correctly formulated biological question and implies teleology or « purpose » that has no place in current evolutionary theory. The question should be termed in the light of redundancy versus specificity. In fact the observations that in many other brain areas TRb1 and TRa1 coexist and coregulate common targets is not considered aberrant - but indeed taken as examples of redundancy and this is no doubt the case for the similar effects of B1 and B2 discussed here.

**Response:** We changed the mentioned sentence.

**Point 7:** P 23 - section on transporters. This part could be usefully reorganised slightly bringing the idea of active transport to the start ( the sentence beginning « Despite their lipophilic?. ») - so that the verb 'extract' makes more sense.

**Response:** We reorganized this part according to the Reviewer's suggestion.

**Point 8:** P24 explain what is meant by 'volume transmission', not obvious.

**Response:** To clarify this point, we now use the term "diffusion".

**Point 9:** Similarly at the end of this paragraph the idea that only hypophysiotropic TRH neurons are affected by changed peripheral TH levels is not only prompted by the previous question ( how T3 reaches the TRH neurons) and the proposed hypothesis - and discussion of this point really needs some highlighting/ more detail of potential hypothesis that could be testable.

**Response:** We added the following sentence to the end of the paragraph: "However, further studies are needed to demonstrate that the T3 content of hypophysiotropic and non-

hypophysiotropic TRH neurons is differentially regulated by changes in peripheral thyroid hormone levels.”

**Point 10:** P 29 and 31 - repetition of the idea that lack of NPY and MC4R abrogate fasting induced drop in T3

**Response:** The reviewer is correct that the data is mentioned in two parts of the manuscript. However, we use these data to support the importance of the the arcuate-paraventricular pathway in the regulation of TRH neurons during fasting on page 29, whereas on page 31 we use the data to demonstrate that the direct effect of leptin on the TRH neurons seems to have negligible effect during fasting despite of the well documented direct action of leptin on these cells. Therefore, repetition of the data on both places is of particular importance.

**Point 11:** Not clear what the overall hypothesis is that is being discussed end P 32 / para 1 p 33.

**Response:** We added a sentence at the beginning of this section to resolve the problem.

**Point 12:** P 36 - suddenly start talking about POMC neurons - well most will know that POMC is processed into  $\alpha$ MSH etc - but given that many readers will be new to the area ( see point 1) then this needs to be explained?

**Response:** We changed the sentence to clarify that proopiomelanocortin (POMC) is the precursor of  $\alpha$ -MSH.

**Point 13:** the discussion of non-critical illness might require some better explanation of the current ideas of 'acute' versus 'prolonged' phases as the therapeutic options differ dramatically according to the phase (Van den Berge's recent data).

**Response:** The Reviewer is correct that the nonthyroidal illness has been separated by some authors into acute and chronic phases. This distinction has been dealt with extensively in a previous *Endocrine Reviews* article published by Boelen et al in 2011. The acute phase is largely believed to be secondary to decreased conversion of T4 to T3 in peripheral tissues (liver, muscle, adipose tissue) due to changes in deiodinases, whereas the chronic phase involves changes both in both the periphery and in the central nervous system. We suspect that central changes are occurring during the acute phase, but this has not been systematically studied.

**Point 14:** Indeed this section (VII) that leads into translational ramifications (IX) (after a short section on cold) is rather superficial and disappointing . Translational aspects of TRH physiology are by no means limited to this pathology. So if there is one area of the review that merits more effort from the authors and a rewrite this is it.

**Response:** We changed the Translational ramification Section.

**Response to minor points:** As recommended, we expanded this section..

**Reviewer 3:**

**Point 1:** It is well written but suffers from too much detail in some areas and not enough in others. First, the focus on the hypothalamic TRH neurons is appropriate but much of the material is the authors' own view of the how the TRH neuron is regulated.



**Response:** The authors made a concerted effort to include all data that are related to the reviewed topics. If there are areas that have not been covered in sufficient detail, it would be helpful to know specifically what those are so we can make the appropriate changes. As noted by the above Reviewer, the detail is helpful to allow the reader to best understand the material.

**Point 2:** I would recommend eliminating many of the darkfield mRNA figures. Many appear to be previously published or too detailed for the general reader.

**Response:** We believe that the darkfield images will help the readers to better understand the topic, therefore, we would like to retain these images.

**Point 3:** The structure of the TRH gene also adds little to the review.

**Response:** The goal of the manuscript is to summarize current knowledge about the function and regulation of hypophysiotropic TRH neurons. The regulation of TRH gene expression is a critical component and it's understanding highly facilitated by the description of the structure of TRH gene. Therefore, we wish to retain this part of the manuscript and the figure that supports it.

**Point 4:** What would be helpful are figures that present how the HPT is regulated by multiple pathways such as by feeding, fasting, thyroid hormone, etc.

**Response:** Fig 10 and 12 summarize the regulation of the TRH neurons by feeding and fasting and by thyroid hormones, respectively.

**Point 5** Finally, the authors argue that in vivo data in TR KO animals are somehow equivalent to in vitro studies of TR over-expression in non-homologous cells. I would suggest rewriting those sections.

**Response:** The only *in vitro* data mentioned in that part of the manuscript is the effect of T3 on hypothalamic cell culture in its early stage when the predominant TR in the cells is TR $\alpha$ . We did not consider these data equal with the knock out studies, but do feel that the cell culture data support the *in vivo* findings. Therefore, we wish to retain this Section in the current form.

Central regulation of the hypothalamic-pituitary-thyroid axis under physiological and  
pathophysiological conditions

Csaba Fekete<sup>1,2</sup>, Ronald M. Lechan<sup>2,3</sup>

<sup>1</sup>Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary.

<sup>2</sup>Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, Tupper Research Institute, Tufts Medical Center, Boston, MA, USA.

<sup>3</sup>Department of Neuroscience, Tufts University School of Medicine, Boston, MA, USA.

**Abbreviate title:** Regulation of hypophysiotropic TRH neurons

**Key terms:** hypophysiotropic TRH neurons, negative feedback regulation, fasting, infection

**Word count:** 14803

**Corresponding Author:** Csaba Fekete, MD, PhD

Department of Endocrine Neurobiology  
Institute of Experimental Medicine  
Hungarian Academy of Sciences  
43 Szigony St.

Budapest, Hungary 1083

Phone: 36-1-210-9947

Fax: 36-1-210-9961

E-mail: [fekete.csaba@koki.mta.hu](mailto:fekete.csaba@koki.mta.hu)

This work was supported by Grants from the Hungarian Science Foundation (OTKA K81845), Seventh EU Research Framework Programme (Health-F2-2010-259772), Lendület Award of the Hungarian Academy of Sciences and the Dr. Gerald J. and Dorothy R. Friedman New York Foundation for Medical Research

Disclosure summary: The authors have nothing to disclose

## **Abstract**

Thyrotropin-releasing hormone (TRH) is a tripeptide amide that functions as a neurotransmitter, but also serves as a neurohormone that has a critical role in the central regulation of the hypothalamic-pituitary-thyroid (HPT) axis. Hypophysiotropic TRH neurons involved in this neuroendocrine process are located in the hypothalamic paraventricular nucleus and secrete TRH into the pericapillary space of the external zone of the median eminence for conveyance to anterior pituitary thyrotrophs. Under basal conditions, the activity of hypophysiotropic TRH neurons are regulated by the negative feedback effects of thyroid hormone to ensure stable, circulating, thyroid hormone concentrations, a mechanism that involves complex interactions between hypophysiotropic TRH neurons and the vascular system, CSF and specialized glial cells called tanycytes. Hypophysiotropic TRH neurons also integrate other humoral and neuronal inputs that can alter the setpoint for negative feedback regulation by thyroid hormone. This mechanism facilitates adaptation of the organism to changing environmental conditions, including the shortage of food and a cold environment. The thyroid axis is also affected by other adverse conditions such as infection, but the central mechanisms mediating suppression of hypophysiotropic TRH may be pathophysiologic. In this review, we discuss current knowledge about the mechanisms that contribute to regulation of hypophysiotropic TRH neurons under physiological and pathophysiological conditions.

## I. Introduction

## II. Organization of the central machinery regulating the hypothalamic-pituitary-thyroid axis

- a. Thyrotropin-releasing hormone (TRH) as central regulator of the hypothalamic-pituitary-thyroid axis
- b. Molecular characterization of the TRH gene
- c. Processing of preproTRH
- d. Inactivation of TRH
- e. Anatomical characteristics of hypophysiotropic TRH neurons
- f. Neuronal inputs of hypophysiotropic TRH neurons
- g. Tanycytes as regulators of the hypothalamic-pituitary-thyroid axis
- h. Involvement of the autonomic nervous system in the regulation of the hypothalamic-pituitary-thyroid axis

## III. Negative feedback regulation of hypophysiotropic TRH neurons

- a. Classical view of negative feedback regulation
- b. Involvement of type 2 and type 3 deiodinases, thyroid hormone transporters pyroglutamyl-peptidase II in the negative feedback regulation of the hypophysiotropic TRH neurons
  1. Role of deiodinases
  2. Role of thyroid hormone transporters
  3. Role of PPII

## IV. Central regulation of the hypothalamic-pituitary-thyroid axis during fasting

- a. Role of the arcuato-paraventricular pathway in the regulation of the HPT axis during fasting
- b. Direct action of leptin on hypophysiotropic TRH neurons
- c. Involvement of tanycytes in the regulation of the HPT axis during fasting
- d. Role of other neuronal pathways in the regulation of the HPT axis during fasting

## V. Effects of dehydration-induced anorexia on the hypothalamic-pituitary-thyroid axis

## VI. Regulation of the HPT axis in high fat diet-induced obese animals

## VII. Central regulation of the HPT axis during infection and prolonged critical illness

- a. Role of neuronal pathways in the regulation of the hypophysiotropic TRH neurons during infection
- b. Tanycytes as the key regulators of hypophysiotropic TRH neurons during infection
- c. Regulation of hypophysiotropic TRH neurons during prolonged critical illness

## VIII. Regulation of hypophysiotropic TRH neurons by cold exposure and suckling

## IX. Translational ramifications

## X. Conclusions

## XI. Acknowledgement

## **I. Introduction**

The hypothalamic-pituitary-thyroid (HPT) axis primarily functions to maintain normal, circulating levels of thyroid hormone that is essential for the biologic function of all tissues including brain development, regulation of cardiovascular, bone and liver function, food intake and energy expenditure among many others (1). Key to this regulatory system are a group of neurons that reside in the hypothalamic paraventricular nucleus (PVN), produce thyrotropin-releasing hormone (TRH) and integrate a wide variety of humoral and neuronal signals to regulate the HPT axis. In the present review, we will summarize current knowledge about the anatomy and physiology of these so called “hypophysiotropic” TRH neurons involved in the central regulation of the HPT axis under physiological and specific, pathophysiological conditions.

## **II. Organization of the central machinery regulating the hypothalamic-pituitary-thyroid axis**

### *a. Thyrotropin-releasing hormone (TRH) as a central regulator of the hypothalamic-pituitary-thyroid axis*

TRH is a tripeptide amide (pGlu-His-ProNH<sub>2</sub>) (2) discovered simultaneously by the groups of Schally and Guilleman in 1969 (3, 4). In these pioneering studies, extracts from more than 250,000 porcine or sheep hypothalami containing only a few milligrams of TRH was shown to have TSH releasing activity. The extracted material contained only three amino acids: glutamic acid, histidine and proline (2), and subsequently was shown to require cyclization of the glutamyl residue and amidation of the proline residue to achieve TSH releasing activity (2).

TRH regulates the synthesis, release and biological activity of TSH (5-7). This effect is mediated *via* the type 1 TRH receptor (TRHR1) (8). At first, TRH stimulates the release of

presynthesized TSH (6), and then increases the synthesis of both TSH subunits, the  $\alpha$ -glycoprotein hormone subunit ( $\alpha$ -GSU), common to all three glycoprotein hormones of the anterior pituitary, and the TSH-specific  $\beta$  subunit (5). Binding of TRH to TRHR1 results in activation of phospholipase C, calcium mobilization and activation of protein kinase C. This cascade leads to the synthesis of  $\alpha$ -GSU through effects on the pituitary LIM homeodomain factor (P-LIM), cAMP response element binding protein (CREB) and CREB binding protein (CBP) transcription factors (5). In contrast, the synthesis of the TSH- $\beta$  subunit is mediated by the pituitary specific transcription factor-1 (Pit-1) and CBP transcription factors (5). TRH also has important role in regulating the glycosylation of TSH by altering the oligosaccharide composition and structure of its 3,N-linked carbohydrate chains, important for the folding, assembly, secretion, metabolic clearance and ultimately increasing the biological activity of TSH (6, 9-11). Indeed, TRH deficiency in both mouse models and man results in decreased TSH bioactivity and low peripheral thyroid hormone levels (12, 13).

*b. Molecular characterization of the TRH gene*

The first partial sequence of the preproTRH gene was cloned from frog skin by Richter et al. (14), and the nearly full length cDNA of preproTRH isolated from rat hypothalamic  $\lambda$ gt11 library (15). A single copy of the preproTRH gene is present in the rat (chromosome 4), mouse (chromosome 6) and human (chromosome 3) genomes (16-19). The structure of the rat preproTRH gene is summarized in Figure 1. In all species, the gene contains three exons and two introns (16). In rats, the size of the introns are 750 and 450 base pairs, respectively (16). The first exon encodes the 5' untranslated region of the mRNA, and the second exon encodes the signal peptide and a portion of the amino terminus of the proTRH peptide. The third exon encodes the remaining part of the amino-terminal peptide, five copies of the TRH sequence separated by non-TRH peptide sequences and the carboxyl terminal peptide

followed by the 3' untranslated region of the mRNA (16). The promoter of the gene contains a TATA box 28 base pairs upstream of the transcriptional initiation site (16). In addition, a series of regulatory elements has been identified in the proximal promoter of the gene. The human preproTRH gene contains three, negative, thyroid hormone response elements (TRE) (20). The so called "site 4" is located -55 to -60 base pairs from the transcriptional initiation site. This thyroid hormone receptor (TR) binding half site binds both thyroid hormone receptor (TR) homodimers and TR-retinoid X receptor (RXR) heterodimers (20-22). Two other functional TREs are present in the first exon between +14 - +19 and +37 - +42, respectively (20). These sites can only bind TR monomers, but are also necessary for the regulation of the gene by thyroid hormone (20). Site 4 of the human preproTRH gene is also thought to function as a cAMP response element (CRE), resulting in competition between cAMP and TR for binding (23, 24). In the rat preproTRH gene, however, site 4 does not bind cAMP and functions exclusively as a TRE (21). A CRE-2 site identified 5' to site 4 at -101 to -94 seems to function as the primary cAMP response element in this gene (21). Glucocorticoid response element (GRE), activator protein 1 (AP-1), Krüppel-like factor 4 (Klf4), SP1 and STAT3 binding sites have also been identified in the proximal promoter of the preproTRH gene (21, 24, 25).

### *c. Processing of preproTRH*

The 26 kDa rat preproTRH protein is composed of 255 amino acids (26). The N-terminal 25 amino acids comprise the signal peptide that directs the newly synthesized protein into the lumen of the rough endoplasmic reticulum (RER) after its synthesis on ribosomes (26). This sequence is cleaved during the passage into the RER (27), leaving a proTRH protein that contains 5 copies of TRH progenitor sequences, Gln-His-Pro-Gly, four non-TRH peptides located between the TRH progenitor sequences, and C-terminal and N-terminal flanking



peptides (27). The proTRH protein of other species also contains multiple copies of TRH progenitor sequences (27), 6 copies in the human and 7 copies in the frog (27). TRH progenitor peptides are flanked by paired basic amino acids, Lys-Arg or Arg-Arg, that serve as signals for endoprotease enzymes (27). The N-terminal flanking peptide is further cleaved at the pair of Arg amino acids located in the 51-52 position of the preproTRH molecule. Therefore, the processing of the rat proTRH results in 5 copies of TRH and 7 non-TRH peptides (27). Since the non-TRH peptide located between the 4<sup>th</sup> and 5<sup>th</sup> TRH progenitors can be further cleaved, and incomplete processing at Arg-Arg residues following the 3<sup>rd</sup> and 4<sup>th</sup> TRH progenitor sequences can lead to C-terminally extended TRH peptides (27, 28), the actual number of peptides derived from proTRH processing can be even higher. In addition to mature TRH, other proTRH derived peptides such as proTRH 160-169 and proTRH 177-199, also have biological activity (27). Therefore, the large number of peptides derived from proTRH and evidence for differential processing of proTRH in different regions of the brain may serve to increase the diversity of how TRH neurons influence their targets.

The cleavage of proTRH at paired basic amino acid residues is primarily performed by two enzymes, prohormone convertase 1/3 (PC1/3) and prohormone convertase 2 (PC2) (27). However, both PC enzymes can cleave proTRH at multiple sites. In PC1/3 KO mice, the concentration of the TRH tripeptide is reduced by almost 80%, but only by 44% in PC2 KO mice. Therefore, PC1/3 may be more important in the processing of TRH (29). The lack of PC2 has more profound effects on the concentration of some of the non-TRH proTRH peptides (29). After cleavage, the basic amino acid residues are removed by carboxipeptidase E (CPE) (27). The TRH progenitor, Gln-His-Pro-Gly, is then amidated by peptidylglycine alpha amidating monooxygenase (PAM) using the C-terminal glycine as amide donor (27). Finally, the N-terminal glycine is cyclized to pyroglutamate (27), catalyzed by N-glutaminy cyclase (30), resulting in the mature form of TRH.

The processing of the proTRH protein takes place in the trans-Golgi network (TGN) and in the regulated secretory pathway (RSP) (27). The first cleavage of proTRH occurs in the TGN (27) where PC1 processes the prohormone at the second or third TRH precursor resulting in a 9.5 kDa or 15 kDa N-terminal peptide and a 16.5 kDa or 10 kDa C-terminal, intermediate peptide (27). This initial cleavage is also critical for the targeting of proTRH-derived peptides into the regulated secretory pathway and the appropriate sorting of these peptides into secretory vesicles (31). After completion of the initial cleavage, the N- and C-terminal peptides of proTRH are sorted into different vesicles of the regulated secretory pathway (31). Prevention of the initial cleavage by mutation of the paired basic residues, however, directs proTRH protein into the constitutive secretory pathway (31). The C-terminal intermediate protein is further cleaved in the TGN at residues 201-202, but all other cleavage steps and the maturation of the TRH precursor takes place in the immature and mature secretory vesicles (31).

#### *d. Inactivation of TRH*

Inactivation of secreted TRH in the brain is primarily catalyzed by a membrane-bound ectoenzyme, pyroglutamyl peptidase II (PPII) (32-34). PPII is a type II integral membrane protein comprised of a small, N-terminal, intracellular region and a large, extracellular domain containing the active site of the enzyme (32). PPII has stringent substrate specificity as it can only degrade peptides that are no longer than 4 amino acids with a pGlu-His-X structure, where X can be Pro, Ala, Trp, Pro-NH<sub>2</sub>, Pro-Gly or Pro-β-NA (32). PPII produces the dipeptide, His-ProNH<sub>2</sub>, from TRH, which is further degraded by dipeptidyl aminopeptidase IV, or spontaneously cyclizes to His-Pro-diketopiperazine (32). PPII activity can be detected in most brain regions where the axons of TRH neurons terminate, but some mismatch is observed (32). PPII is primarily synthesized by neurons, but it is also produced

by tanycytes, a specialized glial cell type, in the hypothalamus (32, 35). Inhibition of PPII activity markedly increases the amount of TRH released from brain tissue slices, supporting the importance of this peptidase in the metabolism of TRH (32).

In serum, TRH is degraded by a soluble enzyme that was formerly called thyroliberinase (35), but subsequently shown to be a product of the PPII gene produced in the liver by proteolytic cleavage of membrane bound PPII (36). Two, broad specificity cytosolic peptidases, pyroglutamyl peptidase I and prolyl endopeptidase, can also degrade TRH. However, as there is no evidence for the presence of these enzymes in the extracellular space and only a small proportion of the extracellular TRH is internalized, these enzymes do not play a major role in the inactivation of released TRH (32).

*e. Anatomical characteristics of hypophysiotropic TRH neurons*

TRH-synthesizing neurons are present in several brain regions, but only hypophysiotropic TRH neurons located in the PVN are involved in the central regulation of the HPT axis (37). This nucleus is a critical vegetative center of the hypothalamus and located symmetrically at the upper third of the third ventricle.

The PVN contains a magnocellular and a parvocellular division. The magnocellular division houses oxytocin and vasopressin neurons that project to the posterior pituitary. The parvocellular division is further divided into anterior, periventricular, medial, ventral, dorsal and lateral parvocellular subdivisions (38). In rats, TRH neurons are found in all parvocellular subdivisions (Fig. 2 A-C) (39), but the hypophysiotropic TRH neurons are located only in the medial and periventricular subdivisions at the mid and caudal levels of the PVN (Figs. 2 D-F, 3 A-F) (40-42). In mice, hypophysiotropic TRH neurons are located only at the mid level of the PVN (Fig. 3 G-L), intermingled with the magnocellular neurons (43, 44). The periventricular subdivision of the PVN does not contain TRH neurons, and the

medial parvocellular subdivision at the caudal levels of the PVN houses non-hypophysiotropic TRH neurons (43, 44). In humans, the PVN also contains a large population of TRH neurons especially in its medial part, but the location of hypophysiotropic TRH neurons is not yet known (45, 46).

Hypophysiotropic TRH neurons are functionally different from the non-hypophysiotropic TRH neurons in the PVN. Only hypophysiotropic TRH neurons project to the external zone of the median eminence (Fig. 4) where their axon terminals release TRH into the extracellular space of this blood-brain barrier-free circumventricular organ (37). TRH is then conveyed to the anterior pituitary *via* the hypophysial portal circulation where TRH regulates the secretion of TSH from thyrotrophs and prolactin from lactotrophs (37, 47). In addition to TRH, hypophysiotropic neurons also express a second neuropeptide, cocaine- and amphetamine regulated transcript (CART) (42, 48). CART is simultaneously released into the hypophysial portal circulation and has been shown to inhibit the effect of TRH on prolactin secretion, but has no effect on TRH-induced release of TSH (49). Hypophysiotropic TRH neurons also express the vesicular glutamate transporter 2 (VGLUT2), establishing the glutamatergic phenotype of these cells (50). Since TRH axon terminals in the median eminence contain a large number of small, clear vesicles (51), it is likely that glutamate is co-released with TRH, but its physiological significance in the median eminence is currently unknown.

In contrast to the hypophysiotropic TRH neurons, non-hypophysiotropic TRH synthesizing neurons are widely distributed in the central nervous system (39). Currently relatively little information is known about the projection fields and function of these neuronal groups, but some may be involved in the regulation of energy homeostasis. Non-hypophysiotropic TRH neurons in the anterior parvocellular subdivision of the PVN, for example, are densely innervated by inputs containing feeding-related peptides including axons containing AGRP,  $\alpha$ -MSH, CART, galanin and GALP (42, 52, 53). In addition, these cells project to feeding-

related nuclei such as the arcuate and dorsomedial nuclei and the amygdala (54). In the perifornical region, TRH is co-synthesized with another anorexigenic peptide, urocortin 3 (UCN3) (55). These neurons have a prominent projection field to the hypothalamic ventromedial nucleus (54, 55), known to be involved in the regulation of food intake (56). TRH neurons in the preoptic area influence energy homeostasis by regulating thermogenesis (57).

*f. Neuronal inputs of hypophysiotropic TRH neurons*

Hypophysiotropic TRH neurons are embedded in a dense network of neuronal axons in the PVN. These axons form numerous synaptic associations on the surface of TRH neurons and modulate the activity of these cells (58). Integration of these inputs together with humoral signals that can reach TRH neurons through the rich vascular supply of the median eminence or the PVN (59, 60), ensures fine tuning of the activity of the HPT axis and its adaptation to changing environmental conditions. Currently, three, main, neuronal groups are known to send synaptic inputs to the hypophysiotropic TRH neurons: the hypothalamic arcuate nucleus, the hypothalamic dorsomedial nucleus (DMN), and catecholamine-producing neurons in the brainstem (Fig. 5, 6) (58, 61).

The hypothalamic arcuate nucleus plays key role in the regulation of energy homeostasis by relaying humoral signals to second order neuronal groups in the brain (56). Two, major, feeding-related neuronal populations are involved, the medially-located, orexigenic neurons that synthesize neuropeptide Y (NPY), agouti related protein (AGRP) and gamma-amino butyric acid (GABA), and laterally located anorexigenic neurons that produce alpha-melanocyte-stimulating hormone ( $\alpha$ -MSH) and CART (56). Both neuronal groups are responsive to peripheral, feeding-related signals such as changes in leptin, ghrelin, insulin and glucose (56), but are oppositely regulated (56).

Numerous AGRP- and NPY-immunoreactive (IR) axon varicosities form juxtaposition with virtually all TRH neurons in the PVN (Fig. 5 A) and establish a symmetric type of synaptic association with these cells, indicative of an inhibitory function (52, 62, 63). Since the neurons that synthesize AGRP are found only in the arcuate nucleus, the innervation of the TRH neurons by AGRP-IR axons must originate exclusively from this nucleus (63). NPY, however, is synthesized by a number of neuronal populations, but the two major sources for the NPY-IR innervation of the PVN are the arcuate nucleus and catecholaminergic neurons in the brainstem (64). The arcuate nucleus neurons provide approximately 75% of the NPY innervation to TRH neurons in the PVN (64, 65), while the adrenergic NPY neurons of the brainstem contribute the remaining 25% (64).

Anorexigenic  $\alpha$ -MSH/CART neurons of the arcuate nucleus also innervate TRH neurons in the PVN (Fig. 5 A) (58), but establish fewer synapses than observed for axons containing AGRP/NPY (52).  $\alpha$ -MSH is synthesized in two brain regions, the hypothalamic arcuate nucleus and the nucleus tractus solitarius (NTS) of the brainstem. Only  $\alpha$ -MSH-containing neurons in the arcuate nucleus co-express CART (42), and therefore, the co-localization of these two peptides can be used as a marker for  $\alpha$ -MSH axons originating from the arcuate nucleus. As all  $\alpha$ -MSH-IR axon varicosities on the surface of the TRH neurons contain CART, the arcuate nucleus is the exclusive source of the  $\alpha$ -MSH-IR innervation to TRH neurons in the PVN (42). Not all CART-IR axons in juxtaposition to the TRH neurons contain  $\alpha$ -MSH, however, indicating that the arcuate nucleus is not the only source for the CART-IR innervation of these cells (42). Indeed, only a relatively small portion of the CART-IR varicosities on the surface of TRH neurons derive from the arcuate nucleus. CART-synthesizing neurons that innervate the PVN can also be found in the lateral hypothalamus, perifornical area, zona incerta, C1-3 adrenergic neuronal groups and the medial subnucleus of the NTS (66). As unilateral transection of the ascending brainstem

pathways to the PVN results in an approximately 60% reduction of the CART innervation of hypophysiotropic TRH neurons, it would appear that the brainstem gives rise the majority of the CART input to these cells (67).

TRH neurons in the PVN of the human hypothalamus also receive inputs from the infundibular nucleus, the analogue of the rodent arcuate nucleus (46). Similar to the rodent, the human infundibular nucleus contains separate populations of AGRP/NPY and  $\alpha$ -MSH-synthesizing neurons (46). In addition, TRH neurons in the human PVN are densely innervated by axons containing these peptides (46), suggesting that the arcuate (infundibulo)-paraventricular pathway is evolutionarily conserved and thereby, of importance in the regulation of hypophysiotropic TRH neurons (46). There is a major difference between the human and the rodent pathways, however. While CART and  $\alpha$ -MSH are co-expressed by the arcuate nucleus neurons in the rodent, CART is not present in  $\alpha$ -MSH neurons of the human infundibulum (68). Moreover, CART can be detected in approximately 30% of AGRP/NPY neurons (68), indicating that CART may have a somewhat different role in the regulation of the thyroid axis in humans.

The DMN also plays important role in the regulation of energy homeostasis and vegetative functions (69). Like the feeding-related neurons of the arcuate nucleus, DMN neurons sense circulating energy homeostasis-related hormones such as leptin (70), but these signals also influence the DMN indirectly *via* the arcuate nucleus (71). In addition, the DMN is a critical node in the circuit regulating food-entrainable circadian rhythms and the stress response (69, 72), integrating these signals and relaying the information to other neuronal populations such as the sympathetic nervous system and the PVN (69). The DMN also contributes to the regulation of the hypothalamic-pituitary-thyroid axis, as bilateral destruction of the DMN increases 24h release of T3 (73), suggesting a net inhibitory effect. Indeed, anterograde tract tracing studies have demonstrated (Fig. 5 B) that the vast majority of the TRH neurons in the

PVN receive input from the DMN, primarily establishing symmetric type synaptic associations with the TRH neurons characteristic of an inhibitory function (74). Little is known about how the DMN regulates hypophysiotropic TRH neurons, however, but it has been hypothesized that it may be involved in circadian regulation of the hypophysiotropic TRH neurons (58).

Brainstem catecholaminergic cells groups are involved in the regulation of a wide variety of physiological functions including attention, sleep / wakefulness, learning, memory, emotion, reproduction, neuroendocrine processes and central responses to stress (75). These neurons can be subdivided into adrenergic and noradrenergic subtypes based on their transmitter content. Both neuronal populations produce dopamine-beta hydroxylase (DBH), the noradrenaline-synthesizing enzyme, but only adrenergic neurons express phenylethanolamine N-methyltransferase (PNMT), the enzyme that converts noradrenaline to adrenaline (76).

Hypophysiotropic TRH neurons receive a dense catecholaminergic innervation (77), perhaps comprising the largest input to these neurons. Catecholaminergic axons establish asymmetric type synaptic specializations on the surface of TRH neurons (77), indicative of an activating effect and consistent with the observation that noradrenaline stimulates the transcription of TRH gene (78). It has been shown by triple-labeling immunofluorescence (Fig. 5 C) that approximately two thirds of the catecholaminergic innervation of TRH neurons originate from adrenergic neurons, while the noradrenergic neuronal groups give rise to the remaining one third (61). Adrenergic neurons are located exclusively in the C1-3 regions of the medulla, and since the axons of all three adrenergic regions have highly similar distribution patterns in the PVN (79), it is likely that each adrenergic group contributes to the innervation of hypophysiotropic TRH neurons (61).



In addition to the classical transmitters, subpopulations of the adrenergic neurons that innervate the TRH neurons in the PVN also synthesize peptidergic transmitters. Approximately, 50% of the adrenergic innervation to TRH neurons co-contain CART (80), and more than 70% co-contain NPY (64). A large proportion of these adrenergic terminals contain pituitary adenylyl cyclase-activating polypeptide (PACAP) (81). Currently, it is unknown how the co-release of multiple transmitters modulates the effect of adrenergic neurons on their targets, but it is likely that the capacity to release a large array of transmitters from the same terminals provides substantial flexibility for the ability of adrenergic neurons to differentially respond to diverse physiological and pathophysiological conditions.

The brainstem has six noradrenergic cell groups (A1-6), but only the A1, A2 and A6 noradrenergic cell groups project to the PVN (82). The A1 noradrenergic cell group innervates primarily the magnocellular part of the PVN in rats (83), suggesting that this cell group may be only a minor source of the noradrenergic innervation to TRH neurons. The A2 and A6 noradrenergic cell groups, however, densely innervate the periventricular and medial parvocellular subdivisions of the PVN where the hypophysiotropic TRH neurons reside (83), making it likely that these two noradrenergic cell populations are the primary sources of the innervation of hypophysiotropic TRH neurons.

In addition to the above mentioned inputs, the TRH neurons in the PVN receive galanin-, TRH-, corticotrophin-releasing hormone (CRH)-, somatostatin- and endocannabinoid receptor-containing inputs (50, 53, 81, 84-87). Non-synaptic contacts between serotonergic axons and the TRH neurons have also been described (88). However, very little is known about the involvement of these inputs in the regulation of hypophysiotropic TRH neurons. The known inputs of the hypophysiotropic TRH neurons are summarized in Figure 6.

*g. Tanycytes as regulators of the hypothalamic-pituitary-thyroid axis*

Tanycytes are specialized glial cells lining the ventrolateral walls and the floor of the third ventricle between the rostral and caudal limits of the median eminence (Fig. 7 A, B) (89). Characteristic of these cells are a small cell body located in the ependymal layer, and a long, basal process that projects either into to the median eminence or the arcuate, ventromedial or dorsomedial nuclei (90). Based on their location, morphology, cytochemistry and ultrastructure, tanycytes can be classified into four subtypes,  $\alpha 1$  and  $\alpha 2$  tanycytes that line the ventrolateral walls of the third ventricle, and  $\beta 1$  and  $\beta 2$  tanycytes that line the floor and lateral extensions of the third ventricle (Fig. 7 B) (89, 90). Alpha 1 tanycytes are located approximately in the middle third of the ventricular wall and project into the ventromedial and dorsomedial nuclei where their end feet processes terminate on neurons (89, 90). Alpha 2 tanycytes are located ventral to  $\alpha 1$  tanycytes and project their processes into the arcuate nucleus, terminating on neurons and around capillaries. The most ventrally located  $\alpha 2$  tanycytes, however, send their processes to the most lateral portion of the tuberoinfundibular sulcus (89, 90).

Beta 1 tanycytes line the lateral invaginations of the infundibular recess, and their processes arch toward the tuberoinfundibular sulcus and terminate on the surface of the pars tuberalis of the pituitary (89, 90). The  $\beta 2$  tanycytes line the floor of the infundibular recess, and their processes travel through the median eminence to terminate around the portal capillaries in the external zone of the median eminence (89, 90).

In addition to their distinct anatomical locations, the various tanycyte subtypes also differ in their chemical signatures, suggesting that they have independent functions. Alpha and  $\beta 1$  tanycytes express the glucose transporter-1, a blood-brain barrier (BBB) marker (91). This observation is in keeping with the morphological observations that  $\alpha$  tanycytes surround capillaries in the arcuate and ventromedial nuclei and  $\beta 1$  tanycytes create the barrier that

separates median eminence from the mediobasal hypothalamus (91). However,  $\alpha$  but not  $\beta$  tanycytes express somatostatin sst2a receptors, whereas  $\beta$ 1 tanycytes are reactive for N-cadherin, while only  $\alpha$ 1 tanycytes express adenosine triphosphatase enzyme (90). In addition,  $\beta$ 2 tanycytes lack the glucose transporter-1 but express Rab 4, a protein involved in vesicular transport (90, 91).

At the ultrastructural level, a common characteristic of tanycytes is the presence of both early and late endosomes near the apical surface of the cell bodies, suggesting that all types of tanycytes actively incorporate substances from the CSF (90). However, as tight junctions are absent between  $\alpha$  and  $\beta$ 1 tanycytes (90), substances from the CSF can also enter the neuropil directly through the ventrolateral portions of the third ventricular wall. In contrast,  $\beta$ 2 tanycytes are bound together by both zonula adherens and tight junctions, forming an impermeable barrier between the CSF and the median eminence (90).

In addition to functioning as barrier cells, it is becoming increasingly clear that tanycytes are involved in neuroendocrine regulation (90). The role of tanycytes in the regulation of the hypothalamic-pituitary-gonadal axis has been long known (90). Changes in estrogen levels induce cytoskeletal remodeling of tanycytes resulting in retraction of tanycyte end processes from the capillaries during the GnRH surge, allowing GnRH axons to secrete their products into the portal circulation (92). The role of tanycytes in the regulation of the HPT axis has been more recently recognized (89). Tanycytes express thyroid hormone receptors (Fig. 7 C, D), and changes in circulating thyroid hormone levels result in plastic remodeling of tanycyte end feet processes (28, 93), perhaps also to regulate the entry of TRH released by the hypophysiotropic terminals into the portal circulation (93). In addition, the tanycytes express the TRH degrading enzyme, pyroglutamyl peptidase II (PPII, Fig. 7 H) (35), which is regulated in parallel to circulating thyroid hormone levels (35). Thus, hyperthyroidism results in upregulation of tanycyte PPII, contributing to inhibition of TSH secretion by reducing the

amount of TRH reaching the portal system. In support of this hypothesis, inhibition of PPII in the median eminence of hyperthyroid animals significantly increases the amount of secreted TRH (35).

Tanycytes are also thought to be involved in feedback regulation of the HPT axis through their expression of type 2 iodothyronine deiodinase (D2, Fig. 7 E-G), an enzyme that catalyzes 5' deiodination of T4 resulting in the generation of the active form of thyroid hormone, triiodothyronine (T3) (94). In most regions of the brain, D2 is expressed by astrocytes, but in the hypothalamus, D2 is primarily expressed by tanycytes (95-97). Most regions of the hypothalamus including the PVN are devoid of D2 activity (98).

Tanycyte D2 activity is precisely regulated at transcriptional and posttranslational levels (58, 99). Tanycytes synthesize ubiquitin ligase and deubiquitinase enzymes, WSB1 and USP33, respectively, that can quickly and reversibly regulate the activity of D2 (100). In addition, tanycytes are richly replete in the thyroid hormone transporters, MCT8 and OATP1C1 (Fig. 7 C, D) (51, 101, 102), that facilitate the entry of T4 from the circulation or CSF, and release generated T3 into the neuropil or CSF. Under special conditions, tanycytes also express the thyroid hormone degrading enzyme, type 3 iodothyronine deiodinase enzyme (D3) (103). Precise transcriptional and posttranslational regulation of D2 and D3 in tanycytes provides for a powerful mechanism to tightly control hypothalamic T3 availability that may contribute to regulation of the HPT axis.

#### *h. Involvement of the autonomic nervous system in the regulation of the hypothalamic-pituitary-thyroid axis*

In addition to the stimulation of TSH secretion of the anterior pituitary by TRH, the central nervous system can also regulate thyroid function *via* the autonomic nervous system. The thyroid gland is innervated by both adrenergic nerve fibers of the sympathetic nervous system and the cholinergic axons originating from the vagus nerve (104, 105). Both sympathetic and

parasympathetic nerves densely innervate the blood vessels of the thyroid gland, but axon terminals of these autonomic systems can also be found around the thyroid follicles (104, 105), indicating that not only the blood flow, but also the activity of thyroid follicles could be under direct control of autonomic inputs. Retrograde, virus mediated tract tracing studies has verified the existence of both the sympathetic and parasympathetic innervation of the thyroid gland, showing that two days after the injection of pseudorabies virus directly into the thyroid gland, sympathetic preautonomic neurons in the intermediolateral column of the spinal cord and the parasympathetic preautonomic neurons in the dorsal vagal complex were retrogradely labeled (106).

...Relatively little data is available about how the autonomic inputs to the thyroid gland regulate thyroid function. However, the sympathetic input seems to have an inhibitory action as electrical stimulation of the cervical sympathetic trunk decreases thyroid blood flow (107). Noradrenaline also inhibits the stimulatory effect of TSH on the thyroid cells *in vitro* (108) and decreases thyroid hormone secretion *in vivo* (109).

In contrast, electric stimulation of thyroid nerve, that carries parasympathetic inputs to the thyroid gland, results in increased thyroid blood flow that can be prevented by atropine pre-treatment (107). In addition, transection of inferior laryngeal nerve that also carries parasympathetic input to the thyroid gland results in a fall of circulating T4 levels, supporting the stimulatory effect of the parasympathetic inputs on the activity of thyroid gland (110).

In addition to the classical transmitters, the neuropeptides, NPY and vasoactive intestinal peptide (VIP), are also present in axons innervating the thyroid gland (107). NPY is present in the sympathetic innervation of the thyroid gland and similar to norepinephrine, inhibits thyroidal blood flow (111). In contrast, VIP increases the thyroid blood flow and thyroid hormone secretion (107).

In addition to the primary preautonomic neuronal groups, multisynaptic connection of the the suprachiasmatic nucleus and energy homeostasis related neuronal groups of the hypothalamus including the PVN and the arcuate nucleus with the thyroid gland have also been demonstrated (106), suggesting that these hypothalamic cell groups may also be involved in the autonomic regulation of thyroid gland. As central melanocortin and NPY signaling contributes to the metabolism of thyroid hormone in the liver by regulating sulfotransferases (112), it is conceivable that the autonomic nervous system is also involved in regulating the peripheral metabolism of thyroid hormones in addition to their synthesis, although the mechanism remains uncertain.

### **III. Negative feedback regulation of hypophysiotropic TRH neurons**

#### *a. Classical view of negative feedback regulation*

Negative feedback regulation of hypophysiotropic TRH neurons is an important regulatory mechanism to ensure stability of circulating thyroid hormone levels (113). When circulating thyroid hormone levels are increased, TRH gene expression is decreased in hypophysiotropic neurons, whereas the converse is true in association with hypothyroidism (Fig. 8) (58). Regulation of TRH transcription by thyroid hormone is relatively rapid, as the exogenous administration of thyroid hormone can suppress transcription of the TRH gene in the PVN within 5 h (114). This regulatory mechanism is a unique feature of hypophysiotropic TRH neurons, as thyroid hormone does not regulate TRH gene expression in non-hypophysiotropic TRH neurons (113). Thyroid hormone is sensed directly by the hypophysiotropic TRH neurons since implantation of crystalline T3 immediately adjacent to the PVN in hypothyroid animals results in marked inhibition of TRH mRNA on that side, but has no effect on TRH neurons on the contralateral side (115).

Hypophysiotropic TRH neurons express the TR $\alpha$ 1, TR $\beta$ 1 and TR $\beta$ 2 isoforms of the thyroid hormone receptors, although the TR $\beta$ 1 isoform is present in relatively low abundance (116). The presence of all three thyroid hormone receptor isoforms in the same cell type may seem to be redundant. However, all isoforms have different roles in the regulation TRH gene expression in the hypophysiotropic neurons. In TR $\beta$  knock out mice, in which TR $\alpha$ 1 is the only functional thyroid hormone receptor, T3 treatment results in a significant increase of TRH gene expression in the PVN, while the lack of TR $\alpha$ 1 in TR $\alpha$  knock out mice enhances the T3-induced decrease in TRH mRNA in the PVN (117). These data suggest that T3 positively regulates the TRH gene *via* TR $\alpha$ 1. This view is also supported by the stimulatory effect of T3 on the TRH gene expression in cell cultures of developing hypothalamic cells at the 12 DIV stage when the TR $\alpha$ 1 is the predominant TR isoform (118). This TR $\alpha$ 1 mediated stimulatory effect of T3 can be overridden by the TR $\beta$ -mediated inhibitory effect of T3 in wild type mice and also in later stages of cultured hypothalamic cells (117, 118). Since a positive TRE has not been identified in the promoter of TRH gene, it is not clear whether the TR $\alpha$ 1-mediated stimulatory effect exerted directly on the TRH promoter or through indirect effects (118). In contrast to the TR $\alpha$  knock out mice, the T3 induced inhibition of TRH gene expression is completely absent in the PVN of both TR $\beta$  and TR $\beta$ 2 knock out mice (117, 119). Therefore, it has been suggested that negative feedback regulation of the TRH gene is mediated exclusively by the TR $\beta$ 2 isoform (119). This view was challenged by Guissouma et al. (120) using siRNA-mediated knock down of the different TR $\beta$  isoforms in the hypothalamus of mouse pups. The results of these experiments revealed that siRNA-mediated knock down of either TR $\beta$ 2 or TR $\beta$ 1 prevents the T3 dependent inhibition of the activity of a TRH-luciferase construct transfected into the hypothalamus, suggesting that both TR $\beta$ 1 and TR $\beta$ 2 contribute to negative feedback regulation of the TRH gene. It is not clear, however, how the two TR $\beta$  isoforms interact in this regulatory process.

The two TR $\beta$  isoforms, however, unequivocally play different roles in ligand-independent stimulation of the TRH gene (117, 120). While the TRH mRNA level is markedly increased in the PVN of TR $\beta$ 2 knock out mice independent of thyroid status (119), TRH expression is significantly decreased in both hypo- and euthyroid TR $\beta$  knock out mice (117). These data suggest that the TR $\beta$ 1 isoform is critical for ligand-independent stimulation of the TRH promoter and supported by evidence that siRNA-mediated knock down of the TR $\beta$ 1 isoform significantly decreases activity of the TRH promoter in the mouse hypothalamus (120).

In addition to the regulation of TRH at the transcriptional level, thyroid hormone also influences posttranslational processing of proTRH. Increased levels of circulating thyroid hormone lead to the downregulation of two, major, proTRH processing enzymes, PC1/3 and PC2, but selective to the PVN, resulting in the accumulation of intermediate processing products (121). Thyroid hormone may also affect expression of neuropeptide receptors in hypophysiotropic TRH neurons, such as the melanocortin 4 receptor (122), influencing the sensitivity of TRH neurons to their excitatory and inhibitory inputs.

*b. Involvement of type 2 and type 3 deiodinases, thyroid hormone transporters and pyroglutamyl-peptidase II in negative feedback regulation of hypophysiotropic TRH neurons*

1. Role of deiodinases

The concept that the circulating level of T3 is solely responsible for negative feedback regulation of hypophysiotropic TRH by acting directly on these neurons was challenged by Kakucska et al. (123), showing that restoration of circulating levels of T3 to normal in hypothyroid rats without the administration of T4 does not normalize TRH gene expression in the PVN (Fig. 9). Only if very high, hyperthyroid levels of T3 were achieved in the circulating blood was it possible to decrease TRH mRNA levels in the PVN into the normal,



euthyroid range (123). These data indicate that in addition to T3, circulating T4 is also necessary for appropriate feedback control of hypophysiotropic TRH neurons. However, since T4 functions primarily as a prohormone, its conversion to T3 within the CNS must be an essential part of the feedback regulatory mechanism.

As noted previously, the conversion of T4 to T3 in the brain is catalyzed by D2 (94), and in the hypothalamus, this enzyme is expressed primarily in tanycytes (95-98, 124). Although D2 is also present in less abundance in astrocytes in the median eminence and arcuate nucleus region (95), selective ablation of D2 from astrocytes in transgenic mice has no significant effect on feedback regulation of the hypophysiotropic TRH neurons (125), indicating that astrocytes have little or no role in the regulation of this response. Presumably, therefore, tanycyte D2 is responsible for generating the additional T3 required to maintain normal homeostasis in the thyroid axis, although this has not yet been directly tested experimentally. Under certain conditions, the thyroid hormone inactivating enzyme, type 3 iodothyronine deiodinase (D3), is also present in tanycytes (126, 127). It is unknown, however, whether this enzyme contributes to the regulation of hypothalamic T3 concentrations.

In most brain regions, the primary role of D2 is to maintain local T3 concentrations if circulating levels of T4 and T3 decline (128). In the cortex, for example, hypothyroidism upregulates D2 activity to produce more T3, while hyperthyroidism downregulates D2 activity (94). Therefore, the local T3 concentration in the cortex is unchanged even if the circulating T4 concentrations vary over a relatively wide range (128). D2 is also regulated by thyroid hormone in tanycytes at the transcriptional level (97, 129), however, the increased gene expression is not accompanied by a rise of D2 activity in these cells (130). While hypothyroidism results in a more than 4-fold increase of D2 activity in the cortex (131), it has no effect on D2 activity in the mediobasal hypothalamus (130). Similarly, no increase in D2 activity has been observed in the mediobasal hypothalamus in association with iodine

deficiency, contrary to other regions in the brain (132). The posttranscriptional attenuation of thyroid hormone-induced regulation of D2 activity in the MBH suggests that the main role of D2 in this region is not to maintain a constant, local, T3 concentration, but rather to allow the hypothalamus to sense changes in peripheral thyroid hormone levels using T3 as a regulatory signal. This feature is important because stable hypothalamic T3 concentrations would reduce the sensitivity of feedback regulation mechanism of TRH neurons in the PVN.

Despite all the above mentioned data supporting the role of D2 expression of tanycytes in the feedback regulation of the hypophysiotropic TRH neurons, D2 KO mice are euthyroid and their HPT axis has seemingly intact negative feedback regulation (133). This data could question the role of D2 in the feedback regulation of the HPT axis. However, it is important to note that during the embryonic period, thyroid hormone levels have a major influence on the development of the setpoint for feedback regulation of thyroid hormone on the HPT axis (134). In D2 KO animals, D2 activity is already absent from the tanycytes during the embryonic period. Therefore, it is likely that during development, compensatory mechanisms are brought into play that allow feedback regulation of the HPT axis to proceed normally, even in the absence of the thyroid hormone activating capacity of tanycytes. This hypothesis is supported by the observation that thyroid hormone availability during the neonatal period can highly influence the setpoint for negative feedback regulation of the HPT axis (135). Namely, exogenous administration of T4 during the first 12 days after birth results in a permanent decrease in circulating TSH and thyroid hormone concentrations that persists into adulthood despite the normal TSH secretory capacity of animals in response to exogenous TRH administration (135). Were the setpoint of negative feedback regulation to be altered (lowered) during development by chronically low T3 levels in hypothalamus of D2 KO mice, the HPT axis of the adult D2 KO mice would be expected to function normally despite low T3 availability in the hypothalamus of adult D2 KO mice. However, it is likely that ablation of

D2 expression in adult animals would have far more profound impact on the parameters of the HPT axis.

Some hypophysiotropic axon terminals in the median eminence contain D3 (51), indicating that these neurons can regulate T3 availability intracellularly independent of the thyroid hormone concentration in the neuropil by degrading T3. Importantly, however, the vast majority of the TRH-containing hypophysiotropic terminals do not contain D3 and therefore, do not have an internal mechanism for degrading T3 once taken up into the neuron (51).

## 2. Role of thyroid hormone transporters

Despite their lipophilic nature, the transport of thyroid hormone through cell membranes requires active transport (136). Currently, two, main, thyroid hormone transporters are known to be involved in thyroid hormone transport in the brain, OATP1C1 and MCT8, members of the organic anion transporting polypeptide (OATP) and the monocarboxylate transporter (MCT) families, respectively (136). OATP1C1 has a similar high affinity for T4 and T3 and is abundantly expressed in endothelial cells of brain blood vessels, the choroid plexus and tanycytes (102, 137). The activity of the HPT axis is not affected by the lack of OATP1C1 in knockout mice (138), however, suggesting that this transporter does not play a crucial role in feedback regulation of TRH neurons. In contrast, the MCT8 transporter, which is preferentially expressed in neurons including hypophysiotropic TRH neurons (51, 139), and tanycytes (139), has preferential affinity for T3 (139). In MCT8 knockout mice, TRH gene expression increases in the PVN (140).

The location of tanycytes at the blood-brain and CSF-brain barriers, and their high expression of thyroid hormone transporters, place them in a strategic position to extract T4 from the bloodstream or CSF. The former could be accomplished through their end feet processes terminating on portal capillaries or on blood vessels in the arcuate nucleus, and the

latter *via* apical specializations after T4 has traversed the choroid plexus (101, 102, 141). While increased circulating levels of T3 in MCT8 KO mice, and the high expression of this transporter in tanycytes implicate MCT8 as an essential component of the feedback regulation mechanism on hypophysiotropic TRH neurons, the considerable distance between tanycytes and the cell bodies of hypophysiotropic TRH neurons in the PVN raises questions as to how locally synthesized T3 can be transported from tanycytes to the perikarya of TRH neurons. It was initially hypothesized that T3 released from tanycytes into the CSF or neuropil can reach TRH neurons by diffusion through the brain extracellular space (97). This hypothesis, however, does not address how it is possible that only hypophysiotropic TRH neurons are regulated by the changes of peripheral thyroid hormone levels, whereas non-hypophysiotropic TRH neurons in the hypothalamus (including the PVN) that also express thyroid hormone receptors, are not affected (116).

Despite the relatively large distance between the tanycytes and the perikarya of the hypophysiotropic TRH neurons, there is one location where the two cell types are closely associated. This is the external zone of the median eminence where the axon terminals of hypophysiotropic TRH neurons are intertwined with end-feet processes of  $\beta 2$  tanycytes (142). MCT8 is present on the surface of practically all axon terminals in the external zone of the median eminence, including the terminals of hypophysiotropic TRH neurons (51). This observation makes it likely that T3 secreted from the tanycytes can be taken up by hypophysiotropic TRH terminals *via* MCT8 and then transported to the perikarya of TRH neurons by retrograde, axonal transport. In support of this hypothesis, early studies by Dratman et al. (143) presumed axonal transport of T3, based on the presence of radiolabeled T3 in neuronal processes and migration of autoradiographic signal among brain areas during the first 48h after iv administration of radiolabeled T3. In addition, rapid retrograde axonal transport of other bioactive molecules such as neurotrophins, has already been proven (144,

145). To demonstrate that retrograde transport of T3 from the median eminence actually occurs, and changes of peripheral thyroid hormone levels results in different intranuclear T3 concentration in the hypophysiotropic and non-hypophysiotropic TRH neurons, however, will require further study.

The median eminence is a unique brain region, as its T3 content derives from at least two, different sources. Being outside the blood brain barrier (90), T3 circulating in the peripheral blood can readily enter the extracellular space of the median eminence through fenestrated portal capillaries without the need for a specific transport mechanism. However, tanycytes may also contribute to the T3 content in the median eminence given their high concentration of D2 (90) and hence, ability to convert T4 to T3. As nearly a twice normal circulating level of T3 is required to normalize the TRH mRNA level in the PVN if only T3 is administered to rats after inhibition of thyroid hormone synthesis (123), the contribution of T3 derived from tanycytes may contribute substantially to the T3 content of the median eminence in euthyroid animals. Since only hypophysiotropic TRH neurons project to the median eminence, this hypothesis would also provide an explanation for why hypophysiotropic and non-hypophysiotropic TRH neurons are differentially regulated by thyroid hormone. However, further studies are needed to demonstrate that the T3 content of hypophysiotropic and non-hypophysiotropic TRH neurons is differentially regulated by changes of peripheral thyroid hormone levels.

### 3. Role of PPII

In addition to modulating feedback regulation of TRH neurons by influencing T3 availability in the median eminence, tanycytes can also influence of the amount of TRH that reaches portal capillaries in the median eminence. Tanycytes synthesize the TRH degrading enzyme, PPII, which is highly regulated by circulating levels of thyroid hormone (35).

Peripheral administration of T4 markedly increases PPII mRNA synthesis and activity in tanycytes (35). Tanycyte end-feet processes surround the hypophysiotropic terminals in the external zone of the median eminence and PPII is a membrane bound protein with a large extracellular C-terminal region that contains the exopeptidase and catalytic motifs (146). Therefore, PPII synthesized by tanycytes is in anatomical position to degrade secreted TRH from TRH-containing axon terminals in the extracellular space of the median eminence (35). In support of this hypothesis, inhibition of PPII by *Hermodice carunculata* protease inhibitor (HcPI) increases TRH release from median eminence explants, whereas *in vivo*, a single peripheral injection of HcPI results in a significant increase in circulating TSH levels in animals exposed to a cold environment compared to the vehicle injected animals (35).

A summary of the mechanisms contributing to feedback regulation of hypophysiotropic TRH neurons is illustrated in Figure 10.

#### **IV. Central regulation of the hypothalamic-pituitary-thyroid axis during fasting**

The HPT axis plays critical role in the regulation of energy expenditure by affecting basal metabolic rate and through the actions of thyroid hormone to stimulate mitochondrial oxygen consumption and increase thermogenesis (147). It is not surprising, therefore, that alternations in energy availability would be intimately linked to control of the HPT axis. During fasting, for example, circulating thyroid hormone levels decline associated with low or normal TSH levels and inhibition of TRH gene expression in the PVN characteristic of central hypothyroidism (Fig. 11 A, B) (148-150). Presumably, fasting-induced hypothyroidism is a homeostatic mechanism to conserve energy stores until food is once again available. The mechanism by which hypophysiotropic TRH neurons sense alterations in peripheral energy stores is orchestrated by leptin, a white adipose tissue-derived circulating hormone, (148-150), as fasting-induced central hypothyroidism can be completely prevented by the

exogenous administration of leptin (Fig. 11 C) (148). The primary target site for leptin that mediates its effect on the HPT axis is the arcuate nucleus, because ablation of this nucleus abolishes both fasting- and leptin-induced regulation of the HPT axis (151). The circuitry and peptide mediators involved are discussed in detail below and illustrated in Figure 12.

*a. Role of the arcuato-paraventricular pathway in the regulation of the HPT axis during fasting*

Two antagonistic, neuronal populations in the arcuate nucleus, the orexigenic NPY/AGRP/GABA neurons and the anorexigenic  $\alpha$ -MSH/CART neurons, are primarily responsible for sensing and relaying information to hypophysiotropic TRH neurons about the concentration of leptin in the bloodstream (56). Both leptin-sensitive neuronal groups directly target hypophysiotropic TRH neurons and establish synaptic specializations with their perikarya and dendrites in the PVN (58). Similar to leptin, central administration of  $\alpha$ -MSH or CART to fasted animals completely prevents fasting-induced inhibition of TRH gene expression in the PVN (Fig. 13) (42, 52), and can increase TRH release from hypothalamic explants (52, 152).  $\alpha$ -MSH has also been shown to depolarize TRH neurons in the PVN and increase their firing rate (153). In contrast, central administration of NPY or AGRP to fed animals induces a state of central hypothyroidism similar to that observed in fasted animals (Fig. 14), despite that the animals markedly increase their food intake secondary to the potent orexigenic effect of NPY (152, 154, 155). NPY has also been shown to hyperpolarize the TRH neurons in the PVN and decrease the firing rate of these neurons (153).

The  $\alpha$ -MSH/CART and NPY/AGRP/GABA arcuate neurons interact at multiple levels to regulate hypophysiotropic TRH neurons. First, both  $\alpha$ -MSH and AGRP are ligands of the melanocortin 3 and 4 receptors (MC3R and MC4R) (156), with  $\alpha$ -MSH functioning as an agonist and AGRP as a high affinity antagonist (156). The MC4R is expressed by

hypophysiotropic TRH neurons (24), and as the inhibitory effect of AGRP on TRH gene expression is abolished in MC4R KO mice (157), melanocortin signaling to TRH neurons must be mediated primarily by the MC4R. All TRH neurons that are innervated by  $\alpha$ -MSH-containing axon varicosities are also innervated by AGRP-containing terminals (Fig. 5 A) (52), indicating direct, functional interactions between these two peptides. The interaction of the two peptides in the regulation of the TRH neurons is further supported by *in vitro* studies, demonstrating that AGRP prevents the stimulatory effect of  $\alpha$ -MSH on TRH release (152).

A second mechanism may involve post-receptor effects arising from the interaction between  $\alpha$ -MSH and NPY. The MC4R is coupled to G<sub>s</sub> proteins and when activated by  $\alpha$ -MSH, induces the adenylyl cyclase–protein kinase A cascade (158). The resultant increase in cAMP and hence, CREB phosphorylation, regulates TRH gene expression by the binding of phosphoCREB (pCREB) to cAMP response elements (CREs) present in the TRH gene (21, 159). NPY also regulates cAMP synthesis, but as NPY receptors couple to G<sub>i</sub> or G<sub>0</sub> proteins, NPY inhibits adenylyl cyclase, reducing cAMP accumulation (160). In this manner, NPY could inhibit  $\alpha$ -MSH-induced CREB phosphorylation in hypophysiotropic TRH neurons by reducing the intracellular cAMP concentration. In fact, pretreating animals with centrally administered NPY markedly reduces the ability of icv injected  $\alpha$ -MSH to induce CREB phosphorylation in TRH neurons (161).

The inhibitory effects of NPY on hypophysiotropic TRH neurons are mediated primarily by Y<sub>1</sub> and Y<sub>5</sub> receptors, as the central administration of either selective Y<sub>1</sub> or Y<sub>5</sub> receptor agonists are equally effective in inhibiting hypophysiotropic TRH gene expression and the HPT axis (162). In addition, Y<sub>1</sub> receptor mRNA is expressed in TRH neurons in the PVN (163), and the Y<sub>5</sub> receptor has been localized to the PVN (164), although its specific expression in hypophysiotropic TRH neurons has not been studied. Since the fasting-induced fall of leptin stimulates NPY/AGRP neurons and inhibits  $\alpha$ -MSH/CART neurons (56), the



increased inhibitory tone of orexigenic peptides and simultaneous decrease in the stimulatory input from anorexigenic neurons of arcuate nucleus origin play a critical role in the development of fasting-induced central hypothyroidism. This hypothesis is supported by evidence showing that fasting-induced central hypothyroidism is prevented in mice lacking both NPY and the MC4R (112).

Currently, little is known about the interactions between CART and melanocortins or NPY, partly hindered by the fact that the CART receptor(s) has not yet been identified. Central administration of CART increases CREB phosphorylation at least in the CRH neurons in the PVN, but pCREB is absent from hypophysiotropic TRH neurons after this treatment (165). Therefore, it is unknown whether the effect of CART on the hypophysiotropic TRH neurons involves the interaction with the NPY and AGRP signaling at the level of second messengers, or only at the level of the TRH promoter.

In addition to leptin, insulin and glucose also have anorexigenic effects when administered centrally (56). Circulating levels of these substances decrease during fasting and are sensed by the feeding-related neurons of the arcuate nucleus (56), raising the possibility that similar to leptin, insulin and glucose are also involved in fasting-induced regulation of hypophysiotropic TRH neurons. However, while central administration of leptin completely prevents fasting-induced inhibition of the TRH gene expression, central administration of insulin or glucose has no effect on TRH gene expression in fasted rats (166). These observations suggest that changes in insulin and glucose levels do not have a critical role in the fasting-induced regulation of the HPT axis, and indicate that leptin, insulin and glucose have different effects on feeding-related neuronal groups in the arcuate nucleus. Indeed, while leptin can completely reverse fasting-induced changes in NPY, AGRP, POMC and CART gene expression in arcuate nucleus neurons, centrally administered insulin only affects NPY and POMC gene expression, and glucose only affects NPY gene expression (166).

Therefore, it would appear that all four feeding-related peptides of arcuate nucleus origin are necessary to appropriately regulate hypophysiotropic TRH neurons during fasting through the arcuato-paraventricular pathway (166).

Similar to the rodent arcuate nucleus, the human infundibular nucleus also contains neurons that co-express AGRP and NPY, and neurons that synthesize  $\alpha$ -MSH (46). These neuronal populations give rise to axonal projections that directly innervate TRH neurons in the PVN (46). In contrast to the rodent, however, CART is not co-synthesized with  $\alpha$ -MSH, but is present in a subpopulation of the AGRP- and NPY-synthesizing neurons in the infundibular nucleus (68). The significance of this difference is uncertain, but raises the possibility that CART may have a different role in the regulation of the HPT axis in the human brain compared to that observed in rodents (68).

*b. Direct action of leptin on hypophysiotropic TRH neurons*

Despite the observation that regulation of the hypophysiotropic TRH neurons by fasting and leptin is lost in arcuate nucleus ablated animals (151), there has been an ongoing debate about whether leptin can exert a direct effect on hypophysiotropic TRH neurons (24, 37, 58, 153, 167). The presence of the leptin receptor in TRH neurons was shown in primary cultures of fetal rat hypothalamic cells (167), but the approach used did not differentiate between hypophysiotropic TRH neurons and the many other TRH-synthesizing neuronal populations present in the hypothalamus. In addition, it is not clear whether the expression of the leptin receptor observed in fetal cultures is sustained in adult animals or only exists transiently during development. Harris et al. (24), however, have convincingly demonstrated that leptin administration induces the expression of suppressor of cytokine signaling 3 (SOCS3), an accepted marker of leptin receptor signaling, in a small subset (10%) of TRH neurons in the PVN. Furthermore, signal transducers and activators of transcription-3 (STAT3), a second

messenger utilized by the leptin receptor, was shown to directly bind to the TRH promoter in mouse hypothalamus after peripheral administration of leptin (24). These data support the notion that a subgroup of hypophysiotropic TRH neurons in the PVN can be directly targeted by leptin, a view further supported by *in vitro* patch clamp electrophysiology data, demonstrating a direct excitatory effect of leptin on TRH neurons in the PVN (153).

However, it remains uncertain under what physiologic conditions direct vs indirect leptin signaling to TRH neurons is utilized physiologically. During fasting, it seems clear that the arcuato-paraventricular pathway is most important pathway that mediates the effect of leptin on hypophysiotropic TRH neurons. This is based on the observations that arcuate nucleus ablation prevents the effect of fasting and leptin administration on TRH gene expression in the PVN (148). In addition, while central administration of leptin results in both STAT3 and CREB phosphorylation in TRH neurons of the PVN (168), the administration of the melanocortin antagonist, SHU9119, completely prevents the stimulatory effect of centrally administered leptin on TRH release and TSH secretion (168), indicating that leptin influences hypophysiotropic TRH neurons primarily *via* the melanocortin system. Furthermore, in NPY/MC4R double KO mice lacking both NPY and melanocortin signaling, fasting-induced suppression of the HPT axis is completely prevented (112). These data, however, do not exclude the possibility that a direct effect of leptin on hypophysiotropic TRH neurons may be of secondary importance by increasing the expression of MC4R in these neurons (169) and hence, their melanocortin sensitivity. In this manner, a direct effect of leptin may sensitize hypophysiotropic TRH neurons to the arcuate nucleus inputs, rather than directly influence the activity in these cells. A direct effect of leptin on hypophysiotropic TRH neurons, however, may be of importance in maintaining the activity of the HPT axis in association with obesity (170), and is discussed in greater detail in section VI.

*c. Involvement of tanycytes in the regulation of the HPT axis during fasting*

In addition to neuronal groups projecting onto hypophysiotropic TRH neurons and a direct action of leptin, hypothalamic tanycytes may also participate in the central regulation of the HPT axis during fasting. Fasting results in an approximately 2-fold increase in D2 hybridization signal in the mediobasal hypothalamus (130) and a 1.6-fold increase of D2 activity (130). These changes are independent of the fasting-induced fall in peripheral thyroid hormone levels (130), as hypothyroidism has a relatively minimal effect on D2 activity in the MBH, and T4 treatment does not prevent the fasting-induced increase in MBH D2 activity (130). Changes of leptin or corticosterone levels, alone, have no effect on D2 activity in the MBH of fed animals (171), but either removal of the adrenal glands or preventing leptin levels from falling during fasting prevents the increase in D2 activity in the MBH in fasting animals (171). It is hypothesized, therefore, that the fall in leptin levels in fasted animals has a permissive role to enable the stimulatory effect of corticosterone on D2 activity (171). It has been further suggested that the fasting-induced increase of D2 activity in the MBH may contribute to inhibition of the HPT axis by increasing local tissue levels of thyroid hormone (172). This hypothesis is supported by a report that third ventricular administration of a deiodinase inhibitor, iopanoic acid, prevents the fasting-induced increase in hypothalamic T3 concentrations and blunts inhibition of TRH gene expression in the PVN (172). However, iopanoic acid has broad target specificity, inhibiting all deiodinase enzymes and blocking thyroid hormone transporters (173, 174). Therefore, it cannot be unequivocally stated that the observed effect of iopanoic acid treatment is due to inhibition of D2 activity in the MBH, especially because the fasting-induced fall in thyroid hormone levels is very similar in wild type and D2 KO mice (175). An alternative hypothesis is that increased T3 production by tanycytes stimulates NPY neurons in the arcuate nucleus (175), indirectly inhibiting TRH neurons in the PVN. However, since thyroid hormone has no inhibitory effect on

hypophysiotropic TRH neurons in TR $\beta$ 2 KO mice, but the fasting-induced suppression of TRH mRNA in the PVN is completely intact in these animals (119), tanycyte generated T3 during fasting likely has a minimal role in the regulation of the HPT axis. In addition, fasting inhibits TRH neurons in the PVN of Siberian hamsters regardless of whether they are kept under short or long day photoperiods, even though fasting has opposite effects on tanycyte D2 activity during long and short day photoperiods (176).

*d. Role of other neuronal pathways in the regulation of the HPT axis during fasting*

In addition to the arcuate nucleus, TRH neurons also receive inputs from other neuronal groups known to be involved in the regulation of energy homeostasis including the hypothalamic dorsomedial nucleus and neuronal groups in the brainstem (58). Axons arising from all subdivisions of the DMN densely innervate the vast majority of TRH neurons in the PVN, (74). Approximately two thirds of the PVN projecting DMN neurons are located in the medial part of ventral subdivision of the DMN, however, with neurons in the compact subdivision contributing only approximately 10% (71).

The physiologic importance of the DMN in regulating hypophysiotropic TRH neurons remains uncertain, but may be implicit in the knowledge that the DMN also has an important role in the regulation of energy homeostasis (177). Electrolytic and chemical (ibotenic acid) lesions of the DMN results in hypophagia due to an altered setpoint of body weight regulation (177). The DMN likely has both orexigenic and anorexigenic neuronal populations, however, as DMN lesions also increase food intake during the first hour of refeeding after a period of fasting, and attenuate CCK-induced decrease in food intake (177).

As the DMN contains leptin-, insulin- and ghrelin-receptive and glucosensing neurons (70, 177-179), some of these neurons may function to transmit humoral signals to TRH neurons in the PVN. However, it is unlikely that the DMN functions independently of the arcuate

nucleus in the regulation of hypophysiotropic TRH neurons, at least during fasting, as animals deficient in arcuate nucleus NPY and melanocortin peptides lose the ability to induce central hypothyroidism in response to fasting (112, 151).

Neurons of the DMN, however, receive feeding-related inputs from the arcuate nucleus, and are densely innervated by both AGRP- and  $\alpha$ -MSH-containing axons (71, 180). Furthermore, the majority of DMN neurons that project to the PVN receive melanocortin input from the arcuate nucleus (71). This circuitry indicates that the DMN may serve as a relay station from the arcuate nucleus to the PVN, contributing to the effects of melanocortin signaling on the hypophysiotropic TRH neurons (71).

In addition to the regulation of food intake, the DMN is also involved in circadian regulation of hypothalamic systems (181). The DMN receives both direct and indirect inputs from the SCN, and ablation of the DMN reduces the circadian rhythm of wakefulness, feeding, locomotor activity, and the hypothalamic-pituitary-adrenal (HPA) axis (181). The DMN, therefore, may integrate feeding and circadian rhythm-related signals involved in the regulation of the HPT axis. In fact, the DMN is known to be a critical regulator of food-entrainable circadian rhythm of several hypothalamic systems (182). Fasting blunts the nocturnal increase of TSH secretion in humans (183), but it is unclear yet whether the DMN is involved in this process.

Hypophysiotropic TRH neurons also receive dense input from the brainstem that contains feeding related peptides such as NPY and CART (64, 80). These inputs, however, do not seem to have a role in the fasting-induced regulation of TRH neurons, because transection of ascending brainstem pathways does not affect fasting-induced inhibition of TRH gene expression in hypophysiotropic neurons (unpublished personal observations).

## **V. Effects of dehydration-induced anorexia on the hypothalamic-pituitary-thyroid axis**

Dehydration induced by replacement of drinking water with 2.5% NaCl, gradually decreases daily food intake by 80-90% and is associated with significant weight loss despite the fact that the animals have free access to food (184). This experimental model, commonly referred to as dehydration induced anorexia (DIA), has been used by researchers to explore central mechanisms involved in the regulation of anorexia (184). Of interest, while food restriction inhibits all components of the HPT axis, DIA is accompanied by an increase in TRH gene expression in the PVN, elevated circulating TSH levels, yet unaltered T4 and decreased T3 concentrations in the peripheral blood (185). The increase in TSH concentration is likely secondary to increased TRH release, while the low T3 level may be secondary to increased peripheral T3 metabolism or clearance.

The effect of DIA on TRH neurons in the PVN, however, is not uniform (186). Similar to fasting, TRH mRNA is decreased in the mid level of the PVN (186), raising the possibility that these neurons are regulated by the arcuato-paraventricular pathway. Hypophysiotropic TRH neurons in the caudal part of the PVN, however, are stimulated by DIA, while inhibited in food restricted animals (186), indicating that any inhibitory effect of the arcuato-paraventricular pathway is overridden by other factors. Since the TSH concentration is increased in DIA animals (185), it is likely that stimulation of the caudal TRH cell group and inhibition of the mid level TRH cell population in the PVN results in a net increase of TRH release into the hypophysial portal circulation.

One, major difference between the peptide expression profile of the food restricted and DIA animals is increased CRH expression in lateral hypothalamic neurons in DIA animals (184). These CRH neurons are known to project to the PVN (184) and may contribute to the innervation of TRH neurons in the PVN (86). In support of this hypothesis, CRH stimulates TRH biosynthesis, and administration of a CRH-R2 antagonist prevents DIA-induced effects on TRH gene expression in the PVN (187).

In addition to the caudal part of the PVN, DIA also stimulates TRH neurons in the anterior parvocellular subdivision of the PVN (186). These neurons are not involved in the regulation of the HPT axis (58). Nevertheless, since the central administration of TRH inhibits food intake (188) and TRH neurons in the anterior PVN project to brain nuclei that are involved in the regulation of food intake (54), it is possible that the increased activity of TRH neurons in the anterior PVN contributes to the anorexigenic effect of DIA.

## **VI. Regulation of the HPT axis in high fat diet-induced obese animals**

Consumption of high fat diet (HFD) results in increased caloric intake, increased weight gain and also a stimulation of the HPT axis, characterized by increased TRH mRNA and protein levels in the PVN and TSH and thyroid hormone levels in the circulation (170).

Since the HFD-induced obese (DIO) animals have elevated leptin levels, and the HFD does not stimulate the HPT axis in leptin deficient *ob/ob* mice (170), the effect of HFD on the HPT axis seems to be mediated by leptin (170). However, expression of the proopiomelanocortin (POMC, the precursor of  $\alpha$ -MSH) gene in the arcuate nucleus of DIO rats remains unchanged (189), suggesting that HFD decreases leptin sensitivity of anorexigenic neurons in this nucleus. This concept is supported by decreased STAT3 phosphorylation and increased SOCS3 and PTP1B synthesis in the arcuate nucleus of HFD animals after leptin treatment (190, 191).

Therefore, in contrast to fasting, leptin would appear to regulate the HPT axis independently from POMC neurons. Since central leptin administration results in STAT3 phosphorylation in hypophysiotropic TRH neurons in DIO rats, it is hypothesized that the HPT axis is regulated by a direct action of leptin on these neurons (170). However, leptin-induced regulation of AGRP synthesis in the arcuate nucleus and the leptin sensitivity of neurons in the dorsomedial nucleus remain intact in DIO animals (189, 190), necessitating the



need for additional studies to exclude the possibility that the effect of leptin on hypophysiotropic TRH neurons in DIO animals is mediated indirectly by these neuronal groups.

## **VII. Central regulation of the HPT axis during infection and prolonged critical illness**

Similar to fasting, infection and other acute and chronic disorders can lead to inhibition of the HPT axis. In man, this condition is commonly referred to as the non-thyroidal illness syndrome (192) and is discussed in further detail in section IX. Characteristic of this syndrome is a decline in circulating T3, and in more severe cases, a general inhibition of the HPT axis due to the development of central hypothyroidism (192-194).

### *a. Role of neuronal pathways in the regulation of hypophysiotropic TRH neurons during infection*

A commonly used model for infection is the peripheral administration of bacterial lipopolysaccharide (LPS). However, while both fasting and LPS administration have a similar effect to induce central hypothyroidism, the mechanisms by which these two conditions influence the hypophysiotropic TRH neurons are different. As discussed previously, the fall in the circulating level of leptin orchestrates the inhibitory effect of fasting on hypophysiotropic TRH neurons *via* the arcuate-paraventricular pathway (37, 58). In contrast, LPS treatment increases circulating leptin levels (58, 195), stimulates POMC and CART gene expression in arcuate nucleus neurons, and does not increase NPY expression (195, 196), changes that would predict activation of hypophysiotropic TRH neurons rather than suppression as observed (37, 58, 194, 197). Presumably, therefore, a more potent, inhibitory mechanism becomes activated during infection that overrides any stimulatory action of leptin,  $\alpha$ -MSH or CART on hypophysiotropic TRH neurons.

One possibility to explain the effect of LPS on hypophysiotropic TRH neurons is activation of adrenergic neurons in the medulla, as these neurons are known to mediate the effect of LPS on CRH neurons in the PVN and result in a marked increase in circulating levels of corticosterone (198). As described above, medullary adrenergic neurons directly innervate hypophysiotropic TRH neurons (77). Unilateral transection of ascending brainstem pathways or ablation of the catecholaminergic input of the PVN which markedly attenuates LPS-induced c-Fos expression in the PVN and the increase in CRH gene expression (198, 199), however, results in an even more profound inhibition of TRH gene expression in the PVN (199). Thus, brainstem inputs would appear to stimulate rather than inhibit hypophysiotropic TRH neurons during infection, and in keeping with the activating effects of catecholamines on hypophysiotropic TRH (78).

*b. Tanycytes as key regulators of hypophysiotropic TRH neurons during infection*

LPS has a potent effect on tanycyte D2 gene expression and enzymatic activity, resulting in an approximately four-fold increase of D2 mRNA level and activity in the MBH within 12 hours of administration (Fig. 15 A, B) (197, 200). It has been hypothesized, therefore, that LPS may inhibit hypophysiotropic TRH neurons by increasing local T3 levels in the median eminence, where the processes of  $\beta$  tanycytes and the axon terminals of the hypophysiotropic TRH neurons are closely associated (51, 99). In support of this hypothesis, LPS-induced inhibition of hypophysiotropic TRH neurons is abolished in the D2 knock out mice (201). While D2 activity rapidly increases in tanycytes and is maximal 9-12h after LPS treatment, D2 activity gradually increases in other responsive tissues such as the cortex and the anterior pituitary, and becomes maximal at approximately 24h (99, 202). This difference in the D2 response favors distinct, tissue-specific mechanisms for activation of D2. The increase in D2 activity in the cortex and anterior pituitary is inversely related to peripheral thyroid hormone

levels and can be prevented by clamping circulating T4 to euthyroid levels (99, 197). This indicates that in the cortex and anterior pituitary, D2 activation is secondary to LPS-induced reduction in peripheral thyroid hormone levels. The T4 clamp, however, has no effect on the LPS-induced increase in D2 activity in the tanycytes (197).

The mechanism by which LPS stimulates D2 activity in tanycytes remains uncertain. One attractive possibility includes a direct action of LPS on tanycytes *via* their expression of Toll-like receptor 4 (TLR4) (203), as lack of TLR4 in TLR4 knock out mice prevents the LPS induced non-thyroidal illness syndrome (204). The main second messenger utilized by TLR4 and many of the cytokine receptors is the NF- $\kappa$ B system (205), and, the promoter of the D2 gene is highly responsive to NF- $\kappa$ B (99, 206). The human D2 gene contains two, high affinity, NF- $\kappa$ B binding sites at positions -683bp (called no.2) and -198bp (no. 5), 5' to the transcriptional starting site (206). Although both sites have a similar binding affinity by EMSA assay, site-directed mutagenesis and promoter assay indicates that only site no. 5 possesses transactivation potency in the presence of the p65 subunit of NF- $\kappa$ B, increasing D2 promoter activity 150-fold (206). However, LPS-induced activation of I $\kappa$ B $\alpha$  mRNA, a marker of NF- $\kappa$ B signaling, is observed only in a subset of  $\alpha$  tanycytes and only 12h after LPS administration (Fig. 15 C-E), significantly later than the peak of the LPS-induced increase in D2 mRNA in these cells (142, 202). Thus, LPS-induced NF- $\kappa$ B activation *via* TLR4 or other cytokine receptors seem less likely the initial stimulus that increases D2 activity in tanycytes in response to LPS treatment, and rather may be involved in the maintenance of increased D2 (142).

I $\kappa$ B $\alpha$  mRNA, however, markedly increases in the pars tuberalis of the pituitary gland 3h after LPS treatment (Fig. 15 D) (142), a portion of the pituitary that is immediately adjacent to tanycyte end-feet processes in the external zone of the median eminence and in the sulcus tuberoinfundibularis (207). The pars tuberalis contains a large number of TSH-producing

cells, but in contrast to thyrotropes in the anterior pituitary, TSH-producing cells of the pars tuberalis are not affected by TRH or thyroid hormone (207). Since the synthesis of TSH $\beta$  subunit increases significantly in these cells 9h after LPS treatment (Fig. 15 F, G) (142), and the TSH receptor is expressed in tanycytes (103), TSH secretion from the pars tuberalis may be involved in mediating the effects of LPS on the tanycytes. Indeed, TSH has been shown to increase D2 activity in these cells *via* activation of cAMP (208). In addition, CREB phosphorylation, a marker of increased cAMP production, is evident in tanycytes 9h after LPS administration (Fig. 15 H) when D2 synthesis peaks, but absent from tanycytes at 6h after LPS administration when D2 synthesis is already increased (142).

The effect of LPS on the tanycytes, therefore, may be mediated by at least three factors. The first factor is responsible for early increase in D2, but remains unknown. TSH released from the pars tuberalis may then contribute to enhancing D2 activation shortly thereafter, followed by other factors that maintain D2 activity by stimulating NF- $\kappa$ B signaling in tanycytes. While changes in circulating levels of corticosterone have been suggested to have a role in the stimulation of D2 activity in fasted animals (171), a corticosterone clamp in adrenalectomized animals does not prevent the LPS-induced increase of D2 activity (202) nor influence LPS-induced inhibition of TRH synthesis (194).

### *c. Regulation of hypophysiotropic TRH neurons during prolonged critical illness*

The effect of prolonged critical illness on the HPT axis has been studied in a rabbit model using third-degree burn injury (209, 210). As in infection, prolonged critical illness results in low T4, T3 and TSH concentration in the circulation and decreased TRH mRNA level in the PVN (209, 210). In addition, it is associated with increased D2 gene expression in the MBH (210), indicating that similar to infection, burn injury may inhibit the HPT axis by inducing local hypothalamic hyperthyroidism caused by the increased thyroid hormone activating

capacity of tanycytes. Comparison of the T3 content in whole hypothalamic blocks of healthy and ill rabbits did not support this hypothesis, however, as T3 levels were not found to be higher in the experimental animals (210). Nevertheless, the approach did not take into account that burn-induced tissue hyperthyroidism may take place in only focal regions of the hypothalamus such as the median eminence and paraventricular nucleus, which may be sufficient to inhibit hypophysiotropic TRH neurons.

Prolonged illness is also accompanied by a nutritional deficit, raising the possibility that the decreased calorie intake also contributes to the development of central hypothyroidism during prolonged critical illness (209). Early parenteral nutrition, however, does not influence the illness-induced decrease in TRH gene expression and the decline in circulating level of T4 (209). The expected decline in the circulating level of T3 was prevented, however, suggesting that the nutritional deficit may have its greatest influence on thyroid hormone metabolism in peripheral tissues (209).

### **VIII. Regulation of hypophysiotropic TRH neurons by cold exposure and suckling**

Thyroid hormone has a critical role in the regulation of both obligatory and adaptive thermogenesis (211). In the presence of hypothyroidism, obligatory thermogenesis, the heat dissipated due to the biochemical processes necessary for the maintenance of life, is reduced by approximately 30% (211). Hypothyroidism also interferes with adaptive thermogenesis, required to maintain normal body temperature in a cold environment (211). It is not surprising, therefore, that cold exposure serves as an important stimulus for TRH gene regulation in the PVN to increase thyroid hormone levels (212). The increase in TRH mRNA by cold exposure is transient, however, increasing by 100% within 1h of the cold exposure, followed by a decline to normal levels during the second hour (213). Peak TRH release from the median eminence also occurs 40 minutes after the cold exposure (213), and is facilitated

by a cold-induced increase in the synthesis of prohormone convertase enzymes, PC1/3 and PC2, involved in the processing of proTRH (214).

The physiological role of this early, transient increase in thyroid hormone levels is not completely clear. Cold exposure markedly increases D2 activity in BAT (94). As a result, thyroid hormone receptors are fully occupied by T3 in the BAT after 4h of cold exposure (211) when the thyroid hormone levels have already normalized after a transient increase. In addition, administration of low doses of T4 to thyroidectomized rats can completely normalize the thermogenesis without normalizing the thyroid hormone concentration in the circulation (215), suggesting that even low circulating levels of thyroid hormone are sufficient for normal thermogenesis. We hypothesize, therefore, that the cold-induced transient increase in thyroid hormone may be necessary to initiate thermogenesis in BAT before D2 activity is sufficiently increased to maintain BAT thermogenesis, even without increased thyroid hormone levels in the circulation. This hypothesis, however, requires further clarification.

As central administration of TRH stimulates thermogenesis in BAT *via* the activation of the sympathetic nervous system (57), the cold-induced increase in TRH gene expression in the PVN might be hypothesized to increase heat production in BAT simultaneously with stimulation of the HPT axis. Indeed, TRH has been shown to stimulate the BAT after its administration into the DMN, preoptic area, hypothalamic ventromedial nucleus (VMN), anterior hypothalamus or hindbrain (57, 216). However, as none of these brain regions are innervated by hypophysiotropic TRH neurons (unpublished observations), the effect of TRH on BAT is likely mediated by other groups of TRH-synthesizing neurons.

The effect of cold on the activity of hypophysiotropic TRH neurons is thought to be mediated primarily by the brainstem catecholaminergic neurons, because noradrenaline stimulates TRH synthesis (78) and inhibition of noradrenaline prevents cold-induced activation of the HPT axis (217). In addition, the HPT axis does not respond to cold during

the first, ten, postnatal days in rodents during which time catecholaminergic axons are still migrating to TRH neurons in the PVN (1). Furthermore, inhibition of adrenergic receptors blocks cold-induced TRH synthesis and release (214, 218).

Cold stimulates TRH gene expression, biosynthesis and proteolytic processing *via*  $\beta$  adrenergic receptors, and by increasing cAMP production (214). Both adrenergic and noradrenergic brainstem neurons densely innervate hypophysiotropic TRH neurons in the PVN (61), but the A2 noradrenergic cell group is the only catecholaminergic population in which cold-induced c-Fos activation can be observed. The release of TRH is also stimulated by cold *via*  $\alpha$  adrenergic receptors (214, 218). This effect is independent of the regulation of TRH synthesis (214, 218).

Similar to cold exposure, suckling also stimulates TRH gene expression (213, 219). TRH mRNA is increased in the PVN during the first days of lactation, but then declines after the 8<sup>th</sup> day (219). Acute suckling also results in a 2-fold increase in TRH mRNA in the PVN after 30 min of suckling, that gradually decreases to presuckling levels after 6h (213, 219). This response is associated with increased prolactin levels in the circulation, but surprisingly, with unaltered TSH and thyroid hormone levels (219, 220). The mechanism for suckling-induced activation of hypophysiotropic TRH neurons is currently unclear, but the differential response of the anterior pituitary to TRH is likely mediated by several factors. Corticosterone may be important in the prevention of TRH-induced TSH secretion, as corticosterone-clamped, adrenalectomized animals increase TSH in response to suckling (219). A decline in dopamine release in response to suckling may also contribute to the increased sensitivity of lactotrophs to TRH (47). CART, co-synthesized in hypophysiotropic TRH neurons, has been shown to modulate the effect of TRH on lactotrophs, preventing TRH from releasing prolactin without influencing TSH release during cold exposure and in association with hypothyroidism (220, 221). As opposed to cold exposure and hypothyroidism when CART is increased in

hypophysiotropic neurons (220), CART remains unchanged during suckling in hypophysiotropic neurons, potentially favoring the release of prolactin (220).

### **IX. Translational ramifications**

Because of the similarities between the anatomical organization of the hypophysiotropic TRH-synthesizing neuronal system in experimental animals and man (see above), it is reasonable to assume that regulation of the HPT axis in man may have similarities to that observed in rodents. In support of this hypothesis, the important role of TRH in the regulation of anterior pituitary TSH secretion in man is suggested by the presence of central hypothyroidism associated with mutations in the TRH receptor gene. Namely, individuals homozygous for TRH receptor mutations or compound heterozygotes demonstrate low levels of total and free T4, and have inappropriately low or normal TSH levels (222, 223). In addition, their TSH secretion is not influenced by exogenous administration of TRH (222, 223).. Hence, although these individuals are able to produce TSH, the biological activity of their TSH is decreased, and they regulate TSH secretion at a lower set point, presumably due to deficiency in TRH signaling, resulting in lower than normal circulating levels of T4 (222, 223).

As in rodents, feedback regulation of the HPT axis in man would also appear to require the MCT8 thyroid hormone transporter. This is apparent in the Allan-Herndon-Dudley syndrome, secondary to mutations in MCT8, characterized by elevations in T3, a low T4 level and a circulating TSH level in the upper normal range (224, 225). Although one might have anticipated a greater elevation in TSH if MCT8 has an important role in the transport of thyroid hormone to hypophysiotropic TRH neurons in man, these patients can have a blunted response to exogenously administered TRH (226), indicating that anterior pituitary thyrotropes may still retain the ability to transport T3 through other mechanisms, diminishing



the direct stimulatory effect of TRH. Negative feedback inhibition of the HPT axis by thyroid hormone would also appear to be primarily mediated by TR $\beta$  in man, as individuals with mutations in TR $\beta$  show major abnormalities in circulating thyroid hormone levels and have inappropriately elevated TSH level (227). In contrast, whereas mutation of TR $\alpha$  is associated with clinical signs of severe hypothyroidism, it results in slightly low circulating levels of T4 and normal levels of T3 and TSH (228). This observation is also consistent with the concept that TR $\alpha$ 1 may have an activating effect on the synthesis of TRH in hypophysiotropic TRH neurons (see above, Section III)

Leptin is also likely to have an important role in the regulation of the thyroid axis in man, as suggested by the close correlation between the circadian rhythm of leptin secretion and TSH (229). Circulating levels of both hormones are the highest between midnight and early morning and lowest in the early to mid-afternoon (229). In addition, subjects with congenital leptin deficiency, due to mutations in the leptin gene, have impaired thyroid function characterized by low levels of T4, inappropriately normal levels of TSH, dysregulation of TSH pulsatility and circadian rhythms, and/or an exaggerated response to exogenously administered TRH that is a characteristic feature of central hypothyroidism (229, 230). The thyroid phenotype, however, tends to be heterogeneous among the various families described (229, 230). In the Pakistani family with leptin deficiency reported by Montague et al (231), leptin replacement therapy significantly increased their T3 and T4 levels that were maintained at these higher levels with continued leptin treatment (232). Leptin also appears to orchestrate the suppression in circulating thyroid hormone levels during fasting and other disorders associated with a reduction in caloric intake in man such as hypothalamic amenorrhea and eating disorders. Leptin administration to normal individuals undergoing a 3 day fast restored fasting-induced effects on TSH pulsatility and increased T4 levels (233). In addition, leptin administration to individuals undergoing a more prolonged period of a less severe caloric

restriction prevented the fall in T3 and T4 levels (234), although a similar response was not observed in a separate study working with obese patients (235). Leptin and thyroid hormone levels also tend to be low in individuals with hypothalamic amenorrhea induced by strenuous exercise and eating disorders (236). These alterations can be reversed with leptin replacement (237). Although there has been a reluctance to use leptin to treat patients with anorexia nervosa due to its suppressive effects on appetite and to increase energy expenditure, a correlation between low thyroid hormone and leptin levels is also well described in this disorder (238). Presumably, as in rodents, this response is homeostatic to reduce energy expenditure. Whether leptin is also responsible for increases in thyroid hormone levels observed in obese individuals is uncertain, but a correlation between increased leptin levels and higher T3 and TSH levels has been observed in obese patients (239).

A somewhat more complex issue is whether leptin is involved in the development of the so-called “nonthyroidal illness syndrome” or “euthyroid sick syndrome”, observed in approximately 40-70% of patients with severe illness in intensive care settings (240, 241). Low circulating levels of thyroid hormones but seemingly inappropriately low or normal TSH levels, diminished TSH pulsatility and decreased TRH gene expression in the paraventricular nucleus are observed, suggesting the presence of central hypothyroidism, but the changes are completely reversible when the patient recovers (193, 240, 241). The decrease in thyroid hormone levels may be a homeostatic response to conserve energy stores under these adverse conditions and consistent with the physiology described above in experimental animals and human subjects associated with fasting. In support of this hypothesis, recent studies by Casaer et al (242) and Langouche et al (243) have demonstrated that early nutrient restoration to patients with critical illness in an intensive care unit setting is associated with greater complications and a more prolonged hospitalization. A very low T4 level, however, is predictive of poor outcomes in the intensive care unit setting, and perhaps an even better

indicator of the severity of illness than the APACHE II score (244, 245). When T4 falls to less than 4 µg/dl, the risk of death rises to ~50%, and when T4 falls to <2 µg/dl, mortality increases even more to ~80% (246-248). Thus, when T4 begins to decline, it remains unclear whether this is the progression of an adaptive response and does not require therapy, or whether it is indicative of a maladaptive response and should be vigorously treated to restore circulating thyroid hormone levels to normal. Evidence that the mechanisms for central hypothyroidism associated with infection is vastly different than that elucidated associated with fasting, cited in section VII, would give credence to the concern that the nonthyroidal illness syndrome associated with severe illness may not be a homeostatic mechanism mediated by leptin but, in fact, maladaptive and pathophysiologic (246). Namely, central hypothyroidism may be an epiphenomenon of infection-induced upregulation of type D2 deiodinase in tanocytes causing hypothalamic hyperthyroidism and not part of an intended, homeostatic, physiologic response to reduce metabolic rate and energy expenditure to promote survival.

A number of small clinical trials have attempted to determine whether thyroid hormone replacement in intensive care unit patients has any beneficial or detrimental effects on overall outcomes (246, 247, 249). Most of these studies have shown T4 or T3 to be safe and well tolerated (250, 251). Improvement in cardiac hemodynamic parameters including cardiac output, end diastolic volume and stroke volume, and a reduction in peripheral arterial resistance have been observed in patients receiving T3 following coronary artery bypass surgery and/or in patients with dilated cardiomyopathy (249, 252-255), but at the expense of further suppression in circulating levels of TSH. However, other studies have not found a benefit as summarized by Kaptein et al (256). It could be argued, however, that because of the associated rise in D1 and D3 in peripheral tissues in these patients (257), neither T4 nor T3 is appropriate therapy due to the effects of these enzymes to increase the conversion of T4

to reverse T3 (rather than T3) or degrade T3, respectively (246). The appropriate studies to determine whether correcting the HPT axis to normal in patients with severe illness, therefore, have not been conducted. A novel approach for the treatment of the nonthyroidal illness syndrome in critically ill patients has recently been suggested by Van den Berghe et al (258, 259). Using a continuous infusion of TRH together with a growth hormone secretagogue restored thyroid hormone levels and TSH pulsatility in these patients, and also improved catabolic parameters. However, carefully controlled studies are needed to determine whether this approach is associated with improved outcomes and reduced mortality.

## **X. Conclusion**

Maintenance of circulating levels of thyroid hormones is controlled by what is classically called a “simple” negative feedback mechanism exerted on hypophysiotropic TRH neurons in the PVN. This regulatory mechanism, however, is vastly more complex, being modulated by glial-neuronal and neural-neural interactions as well as other circulating, humoral substances such as leptin. Given that hypophysiotropic TRH neurons are innervated by axons containing multiple peptides whose origin and physiological significance has not yet been determined (see Fig. 6), it is likely that the regulatory mechanisms involved are even more complex. Presumably, this finely tuned machinery provides flexibility to the HPT axis, enabling continuous adaptation to the constantly changing internal and external milieu. In most situations, alterations in the setpoint for negative feedback regulation would appear to be adaptive, contributing to the optimization of energy homeostasis. Elucidation of the basic mechanisms involved in central regulation of the thyroid axis, however, raise questions as to whether under certain circumstances, such as prolonged critical illness, regulation of the HPT axis may be maladaptive. Further research to elucidate the neurobiology involved in central regulation of hypophysiotropic TRH neurons will be instrumental in understanding how these

disorders give rise to alterations in the HPT axis and to facilitate the appropriate approach to therapy.

## **XI. Acknowledgement**

Special thanks to Erzsébet Farkas for drawing the schematic illustrations.

## Figure Legends

**Figure 1.** Schematic illustration of the organization of the rat preproTRH gene.

**Figure 2.** Distribution of TRH-synthesizing neurons in the rat PVN. (A-C) Low power micrographs illustrate the TRH neurons at three rostrocaudal levels of the PVN. Schematic drawings (D-F) illustrate the subdivisions of the PVN where hypophysiotropic TRH neurons are localized (gray). AP, anterior parvocellular subdivision; DP, dorsal parvocellular subdivision; LP, lateral parvocellular subdivision; MN, Magnocellular part of PVN; MP, medial parvocellular subdivision, PV, periventricular parvocellular subdivision, III, third ventricle (From Fekete and Lechan (58), copyright 2007, with permission from Elsevier.)

**Figure 3.** Darkfield photomicrographs showing proTRH mRNA expression in the anterior-, mid- and posterior levels of the PVN in (A-C) control and (D-F) hypothyroid rats, and (G-I) control and (J-L) hypothyroid mice. Note dramatic increase of in silver grains denoting proTRH mRNA in the mid and caudal level of the hypothyroid rat PVN (E, F), while hypothyroidism increases proTRH mRNA only in mid-level neurons in mice (K). III, third ventricle. (G-L are from Kádár et al. (44), copyright 2010, with permission from Wiley-Liss Inc.)

**Figure 4.** Distribution of TRH-immunoreactive (IR) terminals in the mouse median eminence. TRH-IR axons densely innervate the external zone of the median eminence. III, third ventricle; ME, median eminence

**Figure 5.** Innervation of the TRH neurons in the rat PVN from neurons originating in the (A) arcuate nucleus, (B) DMN and (C) catecholaminergic neurons in the brainstem. In (A) TRH

neurons (blue) are contacted by axon terminals containing  $\alpha$ -MSH (*red; arrowhead*) and AGRP (*green; arrows*). In (B), axon varicosities containing the anterogradely transported marker protein, PHA-L (black) are juxtaposed to TRH-synthesizing neurons (brown) after iontophoretic administration of the tracer into the DMN. In (C), both noradrenergic- (*red, open arrows*) and adrenergic-containing (*yellow, white arrows*) axons establish contacts with the TRH neurons. (Modified from Fekete et al. (52), Mihaly et al. (74), copyright 2001, with permission from Elsevier; and Fuzesi et al. (61), copyright 2009, with permission from Elsevier)

**Figure 6.** Schematic drawing summarizing known inputs to TRH neurons in the PVN. The inputs with identified origins are depicted by a neuron sending its axon to the TRH neuron. Inputs with currently unknown origins are labeled with axon terminals on the surface of the TRH neuron.

**Figure 7.** (A,B) Organization of tanycyte subtypes in the mediobasal hypothalamus. (A) Vimentin-immunolabeled (*red*) coronal section with DAPI counterstaining (*blue*) shows the distribution of tanycytes and their processes. The schematic diagram (B) illustrates the location of tanycyte subtypes in the wall and floor of the third ventricle. All tanycyte subtypes synthesize (C) MCT8, and (D) OATP1C1 thyroid hormone transporters. In (E) silver grains denoting type 2 deiodinase mRNA are accumulated over the cells lining the wall of the third ventricle, the tuberoinfundibular sulci (*arrow heads*) and around blood vessels in the arcuate nucleus (*arrows*). Higher power micrographs shows the association of D2 mRNA with (F) the tuberoinfundibular sulcus (*arrow heads*) and a blood vessel (*arrows*) in the arcuate nucleus, and (G) in the external zone of the median eminence. (H) Tanycyte expression of pyroglutamyl peptidase II (PPII) mRNA. III, third ventricle; Arc, arcuate

nucleus, DMN, hypothalamic dorsomedial nucleus; ME, median eminence; VMN, ventromedial nucleus. (Modified from Sanchez et al. (35); Kalló et al. (51); Fekete et al. (124), copyright 2000, with permission from Elsevier; (E) courtesy of Dr. Gábor Wittmann)

**Figure 8.** *In situ* hybridization autoradiograms showing the effect of hypo- and hyperthyroidism on proTRH mRNA level in the medial parvocellular subdivision of the PVN. A substantial increase in silver grain accumulation is observed in the (A) hypothyroid animal compared to the (B) fed control. In contrast, hyperthyroidism results in a marked reduction of proTRH mRNA level in the PVN (C). (From Dyess et al (115), and from Fekete and Lechan (58), copyright 2007, with permission from Elsevier.)

**Figure 9.** *In situ* hybridization autoradiographs of proTRH mRNA in the paraventricular nucleus (PVN) of (A) hypothyroid, (B) euthyroid and (C,D) hypothyroid animals receiving a constant infusion of (C) 0.5  $\mu\text{g}$  or (D) 0.75  $\mu\text{g}$  of T3/100 gm/bw/d. Mean plasma triiodothyronine (T3) levels ( $\pm\text{SEM}$ ) are shown for each group at the bottom of the photomicrographs. Note that only the higher dose of T3 that raised plasma T3 levels into the supranormal range was capable of suppressing proTRH mRNA to euthyroid levels. (E) Regression analysis of above experiment. Interrupted line represents the mean  $\ln(\text{proTRH mRNA})$  for euthyroid animals, and its intercept with the regression line estimates the plasma T3 concentration required to suppress proTRH mRNA to euthyroid levels. Ninety-five percent confidence intervals for each intercept are bracketed. Open dots denote values for hypothyroid animals and hypothyroid animals infused with graded doses of T3. Closed dots denote values for euthyroid controls. (Modified from Kakucska et al (123), and from Fekete and Lechan (58), copyright 2007, with permission from Elsevier.)

**Figure 10.** Schematic illustration of the machinery involved in negative feedback regulation of the HPT axis by thyroid hormone.



**Figure 11.** Darkfield illumination photomicrographs of proTRH mRNA in the hypothalamic PVN in (A) fed, (B) fasted, and (C) fasted animals receiving leptin. Note the marked reduction in *silver grains* over neurons in the PVN in the fasted animals but restoration to normal in the fasted animals receiving leptin. III, third ventricle (From Fekete et al (155), and from Fekete and Lechan (58), copyright 2007, with permission from Elsevier.)

**Figure 12.** Schematic drawing summarizing the regulation of TRH neurons by fasting.

**Figure 13.** Darkfield illumination micrographs of proTRH mRNA in the medial and periventricular parvocellular subdivisions of the hypothalamic PVN in (A, D) fed, (B, E) fasted and (C,F) fasted animals receiving an intracerebroventricular infusion of either (C)  $\alpha$ -MSH or (F) CART every 6 hr for 64 hr. Note the reduction in the accumulation of silver grains over the PVN in fasted animals compared with the fed controls. Both  $\alpha$ -MSH and CART administration prevent the fasting-induced fall of proTRH mRNA. III, Third ventricle (From Fekete et al (42, 52), and from Fekete and Lechan (58), copyright 2007, with permission from Elsevier.)

**Figure 14.** Darkfield illumination photomicrographs of proTRH mRNA in the medial parvocellular subdivision of the PVN in (A) control, (B) AGRP-, and (C) NPY-treated animals. Note marked reduction in silver grains over neurons in the PVN in both the AGRP- and NPY-infused groups. III, Third ventricle (From Fekete et al (155), and from Fekete and Lechan (58), copyright 2007, with permission from Elsevier.)

**Figure 15.** (A,B) Regulation of type 2 deiodinase gene expression in tanycytes by LPS. Low-power darkfield micrographs of the caudal part of the hypothalamus showing the D2

expression in (A) control animals and (B) 12h after LPS administration. Note marked increase in the density of silver grains, particularly in the external zone of the median eminence. (C-E) Darkfield illumination photomicrographs of I $\kappa$ -B $\alpha$  mRNA expression in the MBH in animals receiving (C) saline or LPS (D) 3 h, or (E) 12 h before sacrifice. Note the marked accumulation of silver grains over the pars tuberalis (PT) in (D) 3 h after the administration of LPS. I $\kappa$ -B $\alpha$  mRNA is only seen in a subset of  $\alpha$  tanycytes 12 h after LPS administration (*arrows* in E). (F, G, *arrows*) Expression of TSH $\beta$  mRNA in the pars tuberalis (PT) of animals receiving (F) saline or (G) LPS 9h before sacrifice. Note increased expression of TSH $\beta$  mRNA in the PT after LPS administration. Ventricular borders are demarcated with *dotted lines*. Double-labeled fluorescent image (H) illustrates the presence of phospho-CREB (green, yellow, *arrows*) in the nucleus of tanycytes (red) 9h after administration of LPS. III, Third ventricle; ME, median eminence; PT, pars tuberalis (Modified from fekete et al. (99); and Sanchez et al. (142).)

## References

1. **Lechan RM, Hollenberg A, Fekete C** 2009 Hypothalamic-Pituitary-Thyroid axis: Organization, Neural/Endocrine Control of TRH. In: Squire LR ed. Encyclopedia of Neuroscience. Oxford: Academic Press; 75-87
2. **Reichlin S** 1989 TRH: historical aspects. *Ann N Y Acad Sci* 553:1-6
3. **Boler J, Enzmann F, Folkers K, Bowers CY, Schally AV** 1969 The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidyl-proline amide. *Biochem Biophys Res Commun* 37:705-710
4. **Burgus R, Dunn TF, Desiderio D, Guillemin R** 1969 [Molecular structure of the hypothalamic hypophysiotropic TRF factor of ovine origin: mass spectrometry demonstration of the PCA-His-Pro-NH<sub>2</sub> sequence]. *C R Acad Sci Hebd Seances Acad Sci D* 269:1870-1873
5. **Hashimoto K, Zanger K, Hollenberg AN, Cohen LE, Radovick S, Wondisford FE** 2000 cAMP response element-binding protein-binding protein mediates thyrotropin-releasing hormone signaling on thyrotropin subunit genes. *J Biol Chem* 275:33365-33372
6. **Persani L** 1998 Hypothalamic thyrotropin-releasing hormone and thyrotropin biological activity. *Thyroid* 8:941-946
7. **Harris AR, Christianson D, Smith MS, Fang SL, Braverman LE, Vagenakis AG** 1978 The physiological role of thyrotropin-releasing hormone in the regulation of thyroid-stimulating hormone and prolactin secretion in the rat. *J Clin Invest* 61:441-448
8. **Rabeler R, Mittag J, Geffers L, Ruther U, Leitges M, Parlow AF, Visser TJ, Bauer K** 2004 Generation of thyrotropin-releasing hormone receptor 1-deficient mice as an animal model of central hypothyroidism. *Mol Endocrinol* 18:1450-1460
9. **Grossmann M, Szkudlinski MW, Tropea JE, Bishop LA, Thotakura NR, Schofield PR, Weintraub BD** 1995 Expression of human thyrotropin in cell lines with different glycosylation patterns combined with mutagenesis of specific glycosylation sites. Characterization of a novel role for the oligosaccharides in the in vitro and in vivo bioactivity. *J Biol Chem* 270:29378-29385
10. **Magner JA, Kane J, Chou ET** 1992 Intravenous thyrotropin (TSH)-releasing hormone releases human TSH that is structurally different from basal TSH. *J Clin Endocrinol Metab* 74:1306-1311
11. **Taylor T, Weintraub BD** 1989 Altered thyrotropin (TSH) carbohydrate structures in hypothalamic hypothyroidism created by paraventricular nuclear lesions are corrected by in vivo TSH-releasing hormone administration. *Endocrinology* 125:2198-2203
12. **Shibusawa N, Yamada M, Hirato J, Monden T, Satoh T, Mori M** 2000 Requirement of thyrotropin-releasing hormone for the postnatal functions of pituitary thyrotrophs: ontogeny study of congenital tertiary hypothyroidism in mice. *Mol Endocrinol* 14:137-146
13. **Beck-Peccoz P, Amr S, Menezes-Ferreira MM, Faglia G, Weintraub BD** 1985 Decreased receptor binding of biologically inactive thyrotropin in central hypothyroidism. Effect of treatment with thyrotropin-releasing hormone. *N Engl J Med* 312:1085-1090
14. **Richter K, Kawashima E, Egger R, Kreil G** 1984 Biosynthesis of thyrotropin releasing hormone in the skin of *Xenopus laevis*: partial sequence of the precursor deduced from cloned cDNA. *Embo J* 3:617-621
15. **Lechan RM, Wu P, Jackson IM, Wolf H, Cooperman S, Mandel G, Goodman RH** 1986 Thyrotropin-releasing hormone precursor: characterization in rat brain. *Science* 231:159-161
16. **Lee SL, Stewart K, Goodman RH** 1988 Structure of the gene encoding rat thyrotropin releasing hormone. *J Biol Chem* 263:16604-16609
17. **Yamada M, Satoh T, Monden T, Mori M** 1999 Assignment of the thyrotropin-releasing hormone gene (TRH) to human chromosome 3q13.3-->q21 by in situ hybridization. *Cytogenet Cell Genet* 87:275
18. **Roller ML, Camper SA** 1995 Localization of the thyrotropin-releasing hormone gene, *Trh*, on mouse chromosome 6. *Mamm Genome* 6:443-444

19. **Yamada M, Wondisford FE, Radovick S, Nakayama Y, Weintraub BD, Wilber JF** 1991 Assignment of human preprothyrotropin-releasing hormone (TRH) gene to chromosome 3. *Somat Cell Mol Genet* 17:97-100
20. **Hollenberg AN, Monden T, Flynn TR, Boers ME, Cohen O, Wondisford FE** 1995 The human thyrotropin-releasing hormone gene is regulated by thyroid hormone through two distinct classes of negative thyroid hormone response elements. *Mol Endocrinol* 9:540-550
21. **Diaz-Gallardo MY, Cote-Velez A, Carreon-Rodriguez A, Charli JL, Joseph-Bravo P** 2010 Phosphorylated cyclic-AMP-response element-binding protein and thyroid hormone receptor have independent response elements in the rat thyrotropin-releasing hormone promoter: an analysis in hypothalamic cells. *Neuroendocrinology* 91:64-76
22. **Satoh T, Yamada M, Iwasaki T, Mori M** 1996 Negative regulation of the gene for the preprothyrotropin-releasing hormone from the mouse by thyroid hormone requires additional factors in conjunction with thyroid hormone receptors. *J Biol Chem* 271:27919-27926
23. **Wilber JF, Xu AH** 1998 The thyrotropin-releasing hormone gene 1998: cloning, characterization, and transcriptional regulation in the central nervous system, heart, and testis. *Thyroid* 8:897-901
24. **Harris M, Aschkenasi C, Elias CF, Chandrankunnel A, Nillni EA, Bjoorbaek C, Elmquist JK, Flier JS, Hollenberg AN** 2001 Transcriptional regulation of the thyrotropin-releasing hormone gene by leptin and melanocortin signaling. *J Clin Invest* 107:111-120
25. **Perez-Monter C, Martinez-Armenta M, Miquelajauregui A, Furlan-Magaril M, Varela-Echavarria A, Recillas-Targa F, May V, Charli JL, Perez-Martinez L** 2011 The Kruppel-like factor 4 controls biosynthesis of thyrotropin-releasing hormone during hypothalamus development. *Mol Cell Endocrinol* 333:127-133
26. **Sevarino KA, Goodman RH, Spiess J, Jackson IM, Wu P** 1989 Thyrotropin-releasing hormone (TRH) precursor processing. Characterization of mature TRH and non-TRH peptides synthesized by transfected mammalian cells. *J Biol Chem* 264:21529-21535
27. **Nillni EA, Sevarino KA** 1999 The biology of pro-thyrotropin-releasing hormone-derived peptides. *Endocr Rev* 20:599-648
28. **Lechan RM, Qi Y, Berrodin TJ, Davis KD, Schwartz HL, Strait KA, Oppenheimer JH, Lazar MA** 1993 Immunocytochemical delineation of thyroid hormone receptor beta 2-like immunoreactivity in the rat central nervous system. *Endocrinology* 132:2461-2469
29. **Cyr NE, Stuart RC, Zhu X, Steiner DF, Nillni EA** 2012 Biosynthesis of proTRH-derived peptides in prohormone convertase 1 and 2 knockout mice. *Peptides* 35:42-48
30. **Schilling S, Kohlmann S, Bauscher C, Sedlmeier R, Koch B, Eichentopf R, Becker A, Cynis H, Hoffmann T, Berg S, Freyse EJ, von Horsten S, Rossner S, Graubner S, Demuth HU** 2011 Glutaminyl cyclase knock-out mice exhibit slight hypothyroidism but no hypogonadism: implications for enzyme function and drug development. *J Biol Chem* 286:14199-14208
31. **Perello M, Stuart R, Nillni EA** 2008 Prothyrotropin-releasing hormone targets its processing products to different vesicles of the secretory pathway. *J Biol Chem* 283:19936-19947
32. **Charli JL, Vargas MA, Cisneros M, de Gortari P, Baeza MA, Jasso P, Bourdais J, Perez L, Uribe RM, Joseph-Bravo P** 1998 TRH inactivation in the extracellular compartment: role of pyroglutamyl peptidase II. *Neurobiology (Bp)* 6:45-57
33. **Heuer H, Schafer MK, Bauer K** 1998 The thyrotropin-releasing hormone-degrading ectoenzyme: the third element of the thyrotropin-releasing hormone-signaling system. *Thyroid* 8:915-920
34. **Sanchez E, Charli JL, Lechan RM** 2013 Pyroglutamyl-peptidase II. In: Rawlings ND, Salvesen G eds. *Handbook of Proteolytic Enzymes*. 3rd ed. London: Academic Press; 414-419
35. **Sanchez E, Vargas MA, Singru PS, Pascual I, Romero F, Fekete C, Charli JL, Lechan RM** 2009 Tanycyte pyroglutamyl peptidase II contributes to regulation of the hypothalamic-pituitary-thyroid axis through glial-axonal associations in the median eminence. *Endocrinology* 150:2283-2291

36. **Schmitmeier S, Thole H, Bader A, Bauer K** 2002 Purification and characterization of the thyrotropin-releasing hormone (TRH)-degrading serum enzyme and its identification as a product of liver origin. *Eur J Biochem* 269:1278-1286
37. **Lechan RM, Fekete C** 2006 The TRH neuron: a hypothalamic integrator of energy metabolism. *Prog Brain Res* 153:209-235
38. **Swanson LW, Sawchenko PE** 1983 Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Annu Rev Neurosci* 6:269-324
39. **Lechan RM, Segerson TP** 1989 Pro-TRH gene expression and precursor peptides in rat brain. Observations by hybridization analysis and immunocytochemistry. *Ann N Y Acad Sci* 553:29-59
40. **Ishikawa K, Taniguchi Y, Inoue K, Kurosumi K, Suzuki M** 1988 Immunocytochemical delineation of thyrotrophic area: origin of thyrotropin-releasing hormone in the median eminence. *Neuroendocrinology* 47:384-388
41. **Merchenthaler I, Liposits Z** 1994 Mapping of thyrotropin-releasing hormone (TRH) neuronal systems of rat forebrain projecting to the median eminence and the OVLT. Immunocytochemistry combined with retrograde labeling at the light and electron microscopic levels. *Acta Biol Hung* 45:361-374
42. **Fekete C, Mihaly E, Luo LG, Kelly J, Clausen JT, Mao Q, Rand WM, Moss LG, Kuhar M, Emerson CH, Jackson IM, Lechan RM** 2000 Association of cocaine- and amphetamine-regulated transcript-immunoreactive elements with thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus and its role in the regulation of the hypothalamic-pituitary-thyroid axis during fasting. *J Neurosci* 20:9224-9234
43. **Biag J, Huang Y, Gou L, Hintiryan H, Askarinam A, Hahn JD, Toga AW, Dong HW** 2012 Cyto- and chemoarchitecture of the hypothalamic paraventricular nucleus in the C57BL/6J male mouse: a study of immunostaining and multiple fluorescent tract tracing. *J Comp Neurol* 520:6-33
44. **Kadar A, Sanchez E, Wittmann G, Singru PS, Fuzesi T, Marsili A, Larsen PR, Liposits Z, Lechan RM, Fekete C** 2010 Distribution of hypophysiotropic thyrotropin-releasing hormone (TRH)-synthesizing neurons in the hypothalamic paraventricular nucleus of the mouse. *J Comp Neurol* 518:3948-3961
45. **Fliers E, Noppen NW, Wiersinga WM, Visser TJ, Swaab DF** 1994 Distribution of thyrotropin-releasing hormone (TRH)-containing cells and fibers in the human hypothalamus. *J Comp Neurol* 350:311-323
46. **Mihaly E, Fekete C, Tatro JB, Liposits Z, Stopa EG, Lechan RM** 2000 Hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in the human hypothalamus are innervated by neuropeptide Y, agouti-related protein, and alpha-melanocyte-stimulating hormone. *J Clin Endocrinol Metab* 85:2596-2603
47. **Freeman ME, Kanyicska B, Lerant A, Nagy G** 2000 Prolactin: structure, function, and regulation of secretion. *Physiol Rev* 80:1523-1631
48. **Broberger C** 1999 Hypothalamic cocaine- and amphetamine-regulated transcript (CART) neurons: histochemical relationship to thyrotropin-releasing hormone, melanin-concentrating hormone, orexin/hypocretin and neuropeptide Y. *Brain Res* 848:101-113
49. **Fekete C, Lechan RM** 2006 Neuroendocrine implications for the association between cocaine- and amphetamine regulated transcript (CART) and hypophysiotropic thyrotropin-releasing hormone (TRH). *Peptides* 27:2012-2018
50. **Hrabovszky E, Wittmann G, Turi GF, Liposits Z, Fekete C** 2005 Hypophysiotropic thyrotropin-releasing hormone and corticotropin-releasing hormone neurons of the rat contain vesicular glutamate transporter-2. *Endocrinology* 146:341-347
51. **Kallo I, Mohacsik P, Vida B, Zeold A, Bardoczi Z, Zavacki AM, Farkas E, Kadar A, Hrabovszky E, Arrojo EDR, Dong L, Barna L, Palkovits M, Borsay BA, Herczeg L, Lechan RM, Bianco AC, Liposits Z, Fekete C, Gereben B** 2012 A novel pathway regulates thyroid hormone availability in rat and human hypothalamic neurosecretory neurons. *PLoS One* 7:e37860

52. **Fekete C, Legradi G, Mihaly E, Huang QH, Tatro JB, Rand WM, Emerson CH, Lechan RM** 2000 alpha-Melanocyte-stimulating hormone is contained in nerve terminals innervating thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus and prevents fasting-induced suppression of prothyrotropin-releasing hormone gene expression. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 20:1550-1558
53. **Wittmann G, Sarkar S, Hrabovszky E, Liposits Z, Lechan RM, Fekete C** 2004 Galanin- but not galanin-like peptide-containing axon terminals innervate hypophysiotropic TRH-synthesizing neurons in the hypothalamic paraventricular nucleus. *Brain Res* 1002:43-50
54. **Wittmann G, Fuzesi T, Singru PS, Liposits Z, Lechan RM, Fekete C** 2009 Efferent projections of thyrotropin-releasing hormone-synthesizing neurons residing in the anterior parvocellular subdivision of the hypothalamic paraventricular nucleus. *J Comp Neurol* 515:313-330
55. **Wittmann G, Fuzesi T, Liposits Z, Lechan RM, Fekete C** 2009 Distribution and axonal projections of neurons coexpressing thyrotropin-releasing hormone and urocortin 3 in the rat brain. *J Comp Neurol* 517:825-840
56. **Schwartz MW, Woods SC, Porte D, Jr., Seeley RJ, Baskin DG** 2000 Central nervous system control of food intake. *Nature* 404:661-671
57. **Shintani M, Tamura Y, Monden M, Shiomi H** 2005 Thyrotropin-releasing hormone induced thermogenesis in Syrian hamsters: site of action and receptor subtype. *Brain Res* 1039:22-29
58. **Fekete C, Lechan RM** 2007 Negative feedback regulation of hypophysiotropic thyrotropin-releasing hormone (TRH) synthesizing neurons: role of neuronal afferents and type 2 deiodinase. *Front Neuroendocrinol* 28:97-114
59. **Ambach G, Palkovits M** 1974 Blood supply of the rat hypothalamus. II. Nucleus paraventricularis. *Acta Morphol Acad Sci Hung* 22:311-320
60. **Ambach G, Palkovits M, Szentagothai J** 1976 Blood supply of the rat hypothalamus. IV. Retrochiasmatic area, median eminence, arcuate nucleus. *Acta Morphol Acad Sci Hung* 24:93-119
61. **Fuzesi T, Wittmann G, Lechan RM, Liposits Z, Fekete C** 2009 Noradrenergic innervation of hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in rats. *Brain Res* 1294:38-44
62. **Toni R, Jackson IM, Lechan RM** 1990 Neuropeptide-Y-immunoreactive innervation of thyrotropin-releasing hormone-synthesizing neurons in the rat hypothalamic paraventricular nucleus. *Endocrinology* 126:2444-2453
63. **Legradi G, Lechan RM** 1999 Agouti-related protein containing nerve terminals innervate thyrotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus. *Endocrinology* 140:3643-3652
64. **Wittmann G, Liposits Z, Lechan RM, Fekete C** 2002 Medullary adrenergic neurons contribute to the neuropeptide Y-ergic innervation of hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in the rat. *Neurosci Lett* 324:69-73
65. **Legradi G, Lechan RM** 1998 The arcuate nucleus is the major source for neuropeptide Y-innervation of thyrotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus. *Endocrinology* 139:3262-3270
66. **Fekete C, Wittmann G, Liposits Z, Lechan RM** 2004 Origin of cocaine- and amphetamine-regulated transcript (CART)-immunoreactive innervation of the hypothalamic paraventricular nucleus. *J Comp Neurol* 469:340-350
67. **Fekete C, Sarkar S, Lechan RM** 2005 Relative contribution of brainstem afferents to the cocaine- and amphetamine-regulated transcript (CART) innervation of thyrotropin-releasing hormone synthesizing neurons in the hypothalamic paraventricular nucleus (PVN). *Brain Res* 1032:171-175
68. **Menyhert J, Wittmann G, Lechan RM, Keller E, Liposits Z, Fekete C** 2007 Cocaine- and amphetamine-regulated transcript (CART) is colocalized with the orexigenic neuropeptide Y and agouti-related protein and absent from the anorexigenic alpha-melanocyte-stimulating

- hormone neurons in the infundibular nucleus of the human hypothalamus. *Endocrinology* 148:4276-4281
69. **Bernardis LL, Bellinger LL** 1998 The dorsomedial hypothalamic nucleus revisited: 1998 update. *Proc Soc Exp Biol Med* 218:284-306
  70. **Scott MM, Lachey JL, Sternson SM, Lee CE, Elias CF, Friedman JM, Elmquist JK** 2009 Leptin targets in the mouse brain. *J Comp Neurol* 514:518-532
  71. **Singru PS, Fekete C, Lechan RM** 2005 Neuroanatomical evidence for participation of the hypothalamic dorsomedial nucleus (DMN) in regulation of the hypothalamic paraventricular nucleus (PVN) by alpha-melanocyte stimulating hormone. *Brain Res* 1064:42-51
  72. **Fuller PM, Lu J, Saper CB** 2008 Differential rescue of light- and food-entrainable circadian rhythms. *Science* 320:1074-1077
  73. **Bellinger LL, Bernardis LL, McCusker RH, Campion DR** 1985 Plasma hormone levels in growth-retarded rats with dorsomedial hypothalamic lesions. *Physiol Behav* 34:783-790
  74. **Mihaly E, Fekete C, Legradi G, Lechan RM** 2001 Hypothalamic dorsomedial nucleus neurons innervate thyrotropin-releasing hormone-synthesizing neurons in the paraventricular nucleus. *Brain Res* 891:20-31
  75. **Itoi K, Sugimoto N** 2010 The brainstem noradrenergic systems in stress, anxiety and depression. *J Neuroendocrinol* 22:355-361
  76. **Wittmann G** 2008 Regulation of hypophysiotrophic corticotrophin-releasing hormone- and thyrotrophin-releasing hormone-synthesising neurones by brainstem catecholaminergic neurones. *J Neuroendocrinol* 20:952-960
  77. **Liposits Z, Paull WK, Wu P, Jackson IM, Lechan RM** 1987 Hypophysiotrophic thyrotropin releasing hormone (TRH) synthesizing neurons. Ultrastructure, adrenergic innervation and putative transmitter action. *Histochemistry* 88:1-10
  78. **Cote-Velez A, Perez-Martinez L, Diaz-Gallardo MY, Perez-Monter C, Carreon-Rodriguez A, Charli JL, Joseph-Bravo P** 2005 Dexamethasone represses cAMP rapid upregulation of TRH gene transcription: identification of a composite glucocorticoid response element and a cAMP response element in TRH promoter. *J Mol Endocrinol* 34:177-197
  79. **Cunningham ET, Jr., Bohn MC, Sawchenko PE** 1990 Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *J Comp Neurol* 292:651-667
  80. **Wittmann G, Liposits Z, Lechan RM, Fekete C** 2004 Medullary adrenergic neurons contribute to the cocaine- and amphetamine-regulated transcript-immunoreactive innervation of thyrotropin-releasing hormone synthesizing neurons in the hypothalamic paraventricular nucleus. *Brain Res* 1006:1-7
  81. **Legradi G, Hannibal J, Lechan RM** 1997 Association between pituitary adenylate cyclase-activating polypeptide and thyrotropin-releasing hormone in the rat hypothalamus. *J Chem Neuroanat* 13:265-279
  82. **Sawchenko PE, Swanson LW, Grzanna R, Howe PR, Bloom SR, Polak JM** 1985 Colocalization of neuropeptide Y immunoreactivity in brainstem catecholaminergic neurons that project to the paraventricular nucleus of the hypothalamus. *J Comp Neurol* 241:138-153
  83. **Cunningham ET, Jr., Sawchenko PE** 1988 Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. *J Comp Neurol* 274:60-76
  84. **Fekete C, Wittmann G, Liposits Z, Lechan RM** 2002 GABA-ergic innervation of thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. *Brain Res* 957:251-258
  85. **Toni R, Jackson IM, Lechan RM** 1990 Thyrotropin-releasing-hormone-immunoreactive innervation of thyrotropin-releasing-hormone-tuberoinfundibular neurons in rat hypothalamus: anatomical basis to suggest ultrashort feedback regulation. *Neuroendocrinology* 52:422-428
  86. **Liao N, Vaudry H, Pelletier G** 1992 Neuroanatomical connections between corticotropin-releasing factor (CRF) and somatostatin (SRIF) nerve endings and thyrotropin-releasing

- hormone (TRH) neurons in the paraventricular nucleus of rat hypothalamus. *Peptides* 13:677-680
87. **Deli L, Wittmann G, Kallo I, Lechan RM, Watanabe M, Liposits Z, Fekete C** 2009 Type 1 cannabinoid receptor-containing axons innervate hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons. *Endocrinology* 150:98-103
  88. **Kiss J, Halasz B** 1990 Ultrastructural analysis of the innervation of TRH-immunoreactive neuronal elements located in the periventricular subdivision of the paraventricular nucleus of the rat hypothalamus. *Brain Res* 532:107-114
  89. **Lechan RM, Fekete C** 2007 Infundibular tanycytes as modulators of neuroendocrine function: hypothetical role in the regulation of the thyroid and gonadal axis. *Acta Biomed* 78 Suppl 1:84-98
  90. **Rodriguez EM, Blazquez JL, Pastor FE, Pelaez B, Pena P, Peruzzo B, Amat P** 2005 Hypothalamic tanycytes: a key component of brain-endocrine interaction. *Int Rev Cytol* 247:89-164
  91. **Peruzzo B, Pastor FE, Blazquez JL, Schobitz K, Pelaez B, Amat P, Rodriguez EM** 2000 A second look at the barriers of the medial basal hypothalamus. *Exp Brain Res* 132:10-26
  92. **Prevot V, Bellefontaine N, Baroncini M, Sharif A, Hanchate NK, Parkash J, Campagne C, de Seranno S** 2010 Gonadotrophin-releasing hormone nerve terminals, tanycytes and neurohaemal junction remodelling in the adult median eminence: functional consequences for reproduction and dynamic role of vascular endothelial cells. *J Neuroendocrinol* 22:639-649
  93. **Yamamura T, Yasuo S, Hirunagi K, Ebihara S, Yoshimura T** 2006 T(3) implantation mimics photoperiodically reduced encasement of nerve terminals by glial processes in the median eminence of Japanese quail. *Cell Tissue Res* 324:175-179
  94. **Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR** 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev* 23:38-89
  95. **Diano S, Leonard JL, Meli R, Esposito E, Schiavo L** 2003 Hypothalamic type II iodothyronine deiodinase: a light and electron microscopic study. *Brain Res* 976:130-134
  96. **Guadano-Ferraz A, Obregon MJ, St Germain DL, Bernal J** 1997 The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. *Proc Natl Acad Sci U S A* 94:10391-10396
  97. **Tu HM, Kim SW, Salvatore D, Bartha T, Legradi G, Larsen PR, Lechan RM** 1997 Regional distribution of type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. *Endocrinology* 138:3359-3368
  98. **Riskind PN, Kolodny JM, Larsen PR** 1987 The regional hypothalamic distribution of type II 5'-monodeiodinase in euthyroid and hypothyroid rats. *Brain Res* 420:194-198
  99. **Fekete C, Gereben B, Doleschall M, Harney JW, Dora JM, Bianco AC, Sarkar S, Liposits Z, Rand W, Emerson C, Kacs Kovics I, Larsen PR, Lechan RM** 2004 Lipopolysaccharide induces type 2 iodothyronine deiodinase in the mediobasal hypothalamus: implications for the nonthyroidal illness syndrome. *Endocrinology* 145:1649-1655
  100. **Fekete C, Freitas BC, Zeold A, Wittmann G, Kadar A, Liposits Z, Christoffolete MA, Singru P, Lechan RM, Bianco AC, Gereben B** 2007 Expression patterns of WSB-1 and USP-33 underlie cell-specific posttranslational control of type 2 deiodinase in the rat brain. *Endocrinology* 148:4865-4874
  101. **Alkemade A, Friesema EC, Unmehopa UA, Fabriek BO, Kuiper GG, Leonard JL, Wiersinga WM, Swaab DF, Visser TJ, Fliers E** 2005 Neuroanatomical pathways for thyroid hormone feedback in the human hypothalamus. *J Clin Endocrinol Metab* 90:4322-4334
  102. **Roberts LM, Woodford K, Zhou M, Black DS, Haggerty JE, Tate EH, Grindstaff KK, Mengesha W, Raman C, Zerangue N** 2008 Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier. *Endocrinology* 149:6251-6261



103. **Murphy M, Jethwa PH, Warner A, Barrett P, Nilaweera KN, Brameld JM, Ebling FJ** 2012 Effects of manipulating hypothalamic triiodothyronine concentrations on seasonal body weight and torpor cycles in Siberian hamsters. *Endocrinology* 153:101-112
104. **Amenta F, Caporuscio D, Ferrante F, Porcelli F, Zomparelli M** 1978 Cholinergic nerves in the thyroid gland. *Cell Tissue Res* 195:367-370
105. **Melander A, Sundler F, Westgren U** 1975 Sympathetic innervation of the thyroid: variation with species and with age. *Endocrinology* 96:102-106
106. **Kalsbeek A, Fliers E, Franke AN, Wortel J, Buijs RM** 2000 Functional connections between the suprachiasmatic nucleus and the thyroid gland as revealed by lesioning and viral tracing techniques in the rat. *Endocrinology* 141:3832-3841
107. **Klieverik I, Kalsbeek A, Flier JS** 2005 Autonomic innervation of the thyroid gland and its functional implications. In: *Hot Thyroidology: European Thyroid Association*
108. **Juvenal GJ, Pregliasco LB, Krawiec L, Bocanera LV, Silberschmidt D, Pisarev MA** 1997 Long-term effect of norepinephrine on iodide uptake in FRTL-5 cells. *Thyroid* 7:795-800
109. **Boado RJ, Romeo HE, Chuluyan HE, Cageao L, Cardinali DP, Zaninovich AA** 1991 Evidence suggesting that the sympathetic nervous system mediates thyroidal depression in turpentine-induced nonthyroidal illness syndrome. *Neuroendocrinology* 53:360-364
110. **Romeo HE, Diaz MC, Ceppi J, Zaninovich AA, Cardinali DP** 1988 Effect of inferior laryngeal nerve section on thyroid function in rats. *Endocrinology* 122:2527-2532
111. **Michalkiewicz M, Huffman LJ, Dey M, Hedge GA** 1993 Endogenous neuropeptide Y regulates thyroid blood flow. *Am J Physiol* 264:E699-705
112. **Vella KR, Ramadoss P, Lam FS, Harris JC, Ye FD, Same PD, O'Neill NF, Maratos-Flier E, Hollenberg AN** 2011 NPY and MC4R signaling regulate thyroid hormone levels during fasting through both central and peripheral pathways. *Cell Metab* 14:780-790
113. **Segerson TP, Kauer J, Wolfe HC, Mobtaker H, Wu P, Jackson IM, Lechan RM** 1987 Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. *Science* 238:78-80
114. **Sugrue ML, Vella KR, Morales C, Lopez ME, Hollenberg AN** 2010 The thyrotropin-releasing hormone gene is regulated by thyroid hormone at the level of transcription in vivo. *Endocrinology* 151:793-801
115. **Dyess EM, Segerson TP, Liposits Z, Paull WK, Kaplan MM, Wu P, Jackson IM, Lechan RM** 1988 Triiodothyronine exerts direct cell-specific regulation of thyrotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus. *Endocrinology* 123:2291-2297
116. **Lechan RM, Qi Y, Jackson IM, Mahdavi V** 1994 Identification of thyroid hormone receptor isoforms in thyrotropin-releasing hormone neurons of the hypothalamic paraventricular nucleus. *Endocrinology* 135:92-100
117. **Dupre SM, Guissouma H, Flamant F, Seugnet I, Scanlan TS, Baxter JD, Samarut J, Demeneix BA, Becker N** 2004 Both thyroid hormone receptor (TR)beta 1 and TR beta 2 isoforms contribute to the regulation of hypothalamic thyrotropin-releasing hormone. *Endocrinology* 145:2337-2345
118. **Carreon-Rodriguez A, Charli JL, Perez-Martinez L** 2009 T3 differentially regulates TRH expression in developing hypothalamic neurons in vitro. *Brain Res* 1305:20-30
119. **Abel ED, Ahima RS, Boers ME, Elmquist JK, Wondisford FE** 2001 Critical role for thyroid hormone receptor beta2 in the regulation of paraventricular thyrotropin-releasing hormone neurons. *J Clin Invest* 107:1017-1023
120. **Guissouma H, Froidevaux MS, Hassani Z, Demeneix BA** 2006 In vivo siRNA delivery to the mouse hypothalamus confirms distinct roles of TR beta isoforms in regulating TRH transcription. *Neurosci Lett* 406:240-243
121. **Perello M, Friedman T, Paez-Espinosa V, Shen X, Stuart RC, Nillni EA** 2006 Thyroid hormones selectively regulate the posttranslational processing of prothyrotropin-releasing hormone in the paraventricular nucleus of the hypothalamus. *Endocrinology* 147:2705-2716

122. **Decherf S, Seugnet I, Kouidhi S, Lopez-Juarez A, Clerget-Froidevaux MS, Demeneix BA** 2010 Thyroid hormone exerts negative feedback on hypothalamic type 4 melanocortin receptor expression. *Proc Natl Acad Sci U S A* 107:4471-4476
123. **Kakucska I, Rand W, Lechan RM** 1992 Thyrotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus is dependent upon feedback regulation by both triiodothyronine and thyroxine. *Endocrinology* 130:2845-2850
124. **Fekete C, Mihaly E, Herscovici S, Salas J, Tu H, Larsen PR, Lechan RM** 2000 DARPP-32 and CREB are present in type 2 iodothyronine deiodinase-producing tanycytes: implications for the regulation of type 2 deiodinase activity. *Brain Res* 862:154-161
125. **Fonseca TL, Medina MC, Campos MPO, Wittmann G, Werneck-de-Castro JP, Arrojo e Drigo R, Mora-Garzon ME, Ueta CB, Caicedo A, Fekete C, Gereben B, Lechan RM, Bianco AC** 2013 Pituitary-specific inactivation of type II deiodinase (D2) reveals coordination between T4 to T3 conversion in thyrocytes and in tanycytes in the T4-mediated TSH feedback mechanism. *Journal of Clinical Investigation*:in press
126. **Barrett P, Ebling FJ, Schuhler S, Wilson D, Ross AW, Warner A, Jethwa P, Boelen A, Visser TJ, Ozanne DM, Archer ZA, Mercer JG, Morgan PJ** 2007 Hypothalamic thyroid hormone catabolism acts as a gatekeeper for the seasonal control of body weight and reproduction. *Endocrinology* 148:3608-3617
127. **Ross AW, Helfer G, Russell L, Darras VM, Morgan PJ** 2011 Thyroid hormone signalling genes are regulated by photoperiod in the hypothalamus of F344 rats. *PLoS One* 6:e21351
128. **Broedel O, Eravci M, Fuxius S, Smolarz T, Jeitner A, Grau H, Stoltenburg-Didinger G, Plueckhan H, Meinhold H, Baumgartner A** 2003 Effects of hyper- and hypothyroidism on thyroid hormone concentrations in regions of the rat brain. *Am J Physiol Endocrinol Metab* 285:E470-480
129. **Anguiano B, Quintanar A, Luna M, Navarro L, Ramirez del Angel A, Pacheco P, Valverde C** 1995 Neuroendocrine regulation of adrenal gland and hypothalamus 5' deiodinase activity. II. Effects of splanchnicotomy and hypophysectomy. *Endocrinology* 136:3346-3352
130. **Diano S, Naftolin F, Goglia F, Horvath TL** 1998 Fasting-induced increase in type II iodothyronine deiodinase activity and messenger ribonucleic acid levels is not reversed by thyroxine in the rat hypothalamus. *Endocrinology* 139:2879-2884
131. **Leonard JL, Kaplan MM, Visser TJ, Silva JE, Larsen PR** 1981 Cerebral cortex responds rapidly to thyroid hormones. *Science* 214:571-573
132. **Serrano-Lozano A, Montiel M, Morell M, Morata P** 1993 5' Deiodinase activity in brain regions of adult rats: modifications in different situations of experimental hypothyroidism. *Brain Res Bull* 30:611-616
133. **Schneider MJ, Fiering SN, Pallud SE, Parlow AF, St Germain DL, Galton VA** 2001 Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. *Mol Endocrinol* 15:2137-2148
134. **Alonso M, Goodwin C, Liao X, Page D, Refetoff S, Weiss RE** 2007 Effects of maternal levels of thyroid hormone (TH) on the hypothalamus-pituitary-thyroid set point: studies in TH receptor beta knockout mice. *Endocrinology* 148:5305-5312
135. **Dussault JH, Coulombe P, Walker P** 1982 Effects of neonatal hyperthyroidism on the development of the hypothalamic-pituitary-thyroid axis in the rat. *Endocrinology* 110:1037-1042
136. **Jansen J, Friesema EC, Milici C, Visser TJ** 2005 Thyroid hormone transporters in health and disease. *Thyroid* 15:757-768
137. **Sugiyama D, Kusuhara H, Taniguchi H, Ishikawa S, Nozaki Y, Aburatani H, Sugiyama Y** 2003 Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood-brain barrier: high affinity transporter for thyroxine. *J Biol Chem* 278:43489-43495
138. **Mayerl S, Visser TJ, Darras VM, Horn S, Heuer H** 2012 Impact of Oatp1c1 deficiency on thyroid hormone metabolism and action in the mouse brain. *Endocrinology* 153:1528-1537

139. **Heuer H, Maier MK, Iden S, Mittag J, Friesema EC, Visser TJ, Bauer K** 2005 The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone-sensitive neuron populations. *Endocrinology* 146:1701-1706
140. **Trajkovic M, Visser TJ, Mittag J, Horn S, Lukas J, Darras VM, Raivich G, Bauer K, Heuer H** 2007 Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. *J Clin Invest* 117:627-635
141. **Southwell BR, Duan W, Alcorn D, Brack C, Richardson SJ, Kohrle J, Schreiber G** 1993 Thyroxine transport to the brain: role of protein synthesis by the choroid plexus. *Endocrinology* 133:2116-2126
142. **Sanchez E, Singru PS, Wittmann G, Nouriel SS, Barrett P, Fekete C, Lechan RM** 2010 Contribution of TNF-alpha and nuclear factor-kappaB signaling to type 2 iodothyronine deiodinase activation in the mediobasal hypothalamus after lipopolysaccharide administration. *Endocrinology* 151:3827-3835
143. **Dratman MB, Crutchfield FL, Futaesaku Y, Goldberger ME, Murray M** 1987 [125I] triiodothyronine in the rat brain: evidence for neural localization and axonal transport derived from thaw-mount film autoradiography. *J Comp Neurol* 260:392-408
144. **von Bartheld CS, Williams R, Lefcort F, Clary DO, Reichardt LF, Bothwell M** 1996 Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the p75NTR and trkB receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 16:2995-3008
145. **Watson FL, Heerssen HM, Moheban DB, Lin MZ, Sauvageot CM, Bhattacharyya A, Pomeroy SL, Segal RA** 1999 Rapid nuclear responses to target-derived neurotrophins require retrograde transport of ligand-receptor complex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 19:7889-7900
146. **Chavez-Gutierrez L, Matta-Camacho E, Osuna J, Horjales E, Joseph-Bravo P, Maigret B, Charli JL** 2006 Homology modeling and site-directed mutagenesis of pyroglutamyl peptidase II. Insights into omega-versus aminopeptidase specificity in the M1 family. *J Biol Chem* 281:18581-18590
147. **Silva JE** 2003 The thermogenic effect of thyroid hormone and its clinical implications. *Ann Intern Med* 139:205-213
148. **Legradi G, Emerson CH, Ahima RS, Flier JS, Lechan RM** 1997 Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. *Endocrinology* 138:2569-2576
149. **Rondeel JM, Heide R, de Greef WJ, van Toor H, van Haasteren GA, Klootwijk W, Visser TJ** 1992 Effect of starvation and subsequent refeeding on thyroid function and release of hypothalamic thyrotropin-releasing hormone. *Neuroendocrinology* 56:348-353
150. **van Haasteren GA, Linkels E, Klootwijk W, van Toor H, Rondeel JM, Themmen AP, de Jong FH, Valentijn K, Vaudry H, Bauer K, et al.** 1995 Starvation-induced changes in the hypothalamic content of prothyrotrophin-releasing hormone (proTRH) mRNA and the hypothalamic release of proTRH-derived peptides: role of the adrenal gland. *J Endocrinol* 145:143-153
151. **Legradi G, Emerson CH, Ahima RS, Rand WM, Flier JS, Lechan RM** 1998 Arcuate nucleus ablation prevents fasting-induced suppression of ProTRH mRNA in the hypothalamic paraventricular nucleus. *Neuroendocrinology* 68:89-97
152. **Kim MS, Small CJ, Stanley SA, Morgan DG, Seal LJ, Kong WM, Edwards CM, Abusnana S, Sunter D, Ghatei MA, Bloom SR** 2000 The central melanocortin system affects the hypothalamo-pituitary thyroid axis and may mediate the effect of leptin. *J Clin Invest* 105:1005-1011
153. **Ghamari-Langroudi M, Vella KR, Srisai D, Sugrue ML, Hollenberg AN, Cone RD** 2010 Regulation of thyrotropin-releasing hormone-expressing neurons in paraventricular nucleus of the hypothalamus by signals of adiposity. *Mol Endocrinol* 24:2366-2381

154. **Fekete C, Kelly J, Mihaly E, Sarkar S, Rand WM, Legradi G, Emerson CH, Lechan RM** 2001 Neuropeptide Y has a central inhibitory action on the hypothalamic-pituitary-thyroid axis. *Endocrinology* 142:2606-2613
155. **Fekete C, Sarkar S, Rand WM, Harney JW, Emerson CH, Bianco AC, Lechan RM** 2002 Agouti-related protein (AGRP) has a central inhibitory action on the hypothalamic-pituitary-thyroid (HPT) axis; comparisons between the effect of AGRP and neuropeptide Y on energy homeostasis and the HPT axis. *Endocrinology* 143:3846-3853
156. **Cone RD** 2005 Anatomy and regulation of the central melanocortin system. *Nat Neurosci* 8:571-578
157. **Fekete C, Marks DL, Sarkar S, Emerson CH, Rand WM, Cone RD, Lechan RM** 2004 Effect of Agouti-related protein in regulation of the hypothalamic-pituitary-thyroid axis in the melanocortin 4 receptor knockout mouse. *Endocrinology* 145:4816-4821
158. **Jegou S, Cone RD, Eberle AN, Vaudry H** 2013 Melanocortins. In: Kastin AJ ed. *Handbook of biologically active peptides*. 2nd ed. Amsterdam: Academic Press; 838-844
159. **Mayr B, Montminy M** 2001 Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat Rev Mol Cell Biol* 2:599-609
160. **Pedragosa-Badia X, Stichel J, Beck-Sickingler AG** 2013 Neuropeptide Y receptors: how to get subtype selectivity. *Front Endocrinol (Lausanne)* 4:5
161. **Sarkar S, Lechan RM** 2003 Central administration of neuropeptide Y reduces alpha-melanocyte-stimulating hormone-induced cyclic adenosine 5'-monophosphate response element binding protein (CREB) phosphorylation in pro-thyrotropin-releasing hormone neurons and increases CREB phosphorylation in corticotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus. *Endocrinology* 144:281-291
162. **Fekete C, Sarkar S, Rand WM, Harney JW, Emerson CH, Bianco AC, Beck-Sickingler A, Lechan RM** 2002 Neuropeptide Y1 and Y5 receptors mediate the effects of neuropeptide Y on the hypothalamic-pituitary-thyroid axis. *Endocrinology* 143:4513-4519
163. **Kishi T, Aschkenasi CJ, Choi BJ, Lopez ME, Lee CE, Liu H, Hollenberg AN, Friedman JM, Elmquist JK** 2005 Neuropeptide Y Y1 receptor mRNA in rodent brain: distribution and colocalization with melanocortin-4 receptor. *J Comp Neurol* 482:217-243
164. **Morin SM, Gehlert DR** 2006 Distribution of NPY Y5-like immunoreactivity in the rat brain. *J Mol Neurosci* 29:109-114
165. **Sarkar S, Wittmann G, Fekete C, Lechan RM** 2004 Central administration of cocaine- and amphetamine-regulated transcript increases phosphorylation of cAMP response element binding protein in corticotropin-releasing hormone-producing neurons but not in prothyrotropin-releasing hormone-producing neurons in the hypothalamic paraventricular nucleus. *Brain Res* 999:181-192
166. **Fekete C, Singru PS, Sanchez E, Sarkar S, Christoffolete MA, Riberio RS, Rand WM, Emerson CH, Bianco AC, Lechan RM** 2006 Differential effects of central leptin, insulin, or glucose administration during fasting on the hypothalamic-pituitary-thyroid axis and feeding-related neurons in the arcuate nucleus. *Endocrinology* 147:520-529
167. **Nillni EA, Vaslet C, Harris M, Hollenberg A, Bjorbak C, Flier JS** 2000 Leptin regulates prothyrotropin-releasing hormone biosynthesis. Evidence for direct and indirect pathways. *J Biol Chem* 275:36124-36133
168. **Perello M, Stuart RC, Nillni EA** 2006 The role of intracerebroventricular administration of leptin in the stimulation of prothyrotropin releasing hormone neurons in the hypothalamic paraventricular nucleus. *Endocrinology* 147:3296-3306
169. **Ghamari-Langroudi M, Srisai D, Cone RD** 2011 Multinodal regulation of the arcuate/paraventricular nucleus circuit by leptin. *Proc Natl Acad Sci U S A* 108:355-360
170. **Perello M, Cakir I, Cyr NE, Romero A, Stuart RC, Chiappini F, Hollenberg AN, Nillni EA** 2010 Maintenance of the thyroid axis during diet-induced obesity in rodents is controlled at the central level. *Am J Physiol Endocrinol Metab* 299:E976-989

171. **Coppola A, Meli R, Diano S** 2005 Inverse shift in circulating corticosterone and leptin levels elevates hypothalamic deiodinase type 2 in fasted rats. *Endocrinology* 146:2827-2833
172. **Coppola A, Hughes J, Esposito E, Schiavo L, Meli R, Diano S** 2005 Suppression of hypothalamic deiodinase type II activity blunts TRH mRNA decline during fasting. *FEBS Lett* 579:4654-4658
173. **St Germain DL** 1988 The effects and interactions of substrates, inhibitors, and the cellular thiol-disulfide balance on the regulation of type II iodothyronine 5'-deiodinase. *Endocrinology* 122:1860-1868
174. **Westholm DE, Stenehjem DD, Rumbley JN, Drewes LR, Anderson GW** 2009 Competitive inhibition of organic anion transporting polypeptide 1c1-mediated thyroxine transport by the fenamate class of nonsteroidal antiinflammatory drugs. *Endocrinology* 150:1025-1032
175. **Coppola A, Liu ZW, Andrews ZB, Paradis E, Roy MC, Friedman JM, Ricquier D, Richard D, Horvath TL, Gao XB, Diano S** 2007 A central thermogenic-like mechanism in feeding regulation: an interplay between arcuate nucleus T3 and UCP2. *Cell Metab* 5:21-33
176. **Herwig A, Wilson D, Logie TJ, Boelen A, Morgan PJ, Mercer JG, Barrett P** 2009 Photoperiod and acute energy deficits interact on components of the thyroid hormone system in hypothalamic tanycytes of the Siberian hamster. *Am J Physiol Regul Integr Comp Physiol* 296:R1307-1315
177. **Bellinger LL, Bernardis LL** 2002 The dorsomedial hypothalamic nucleus and its role in ingestive behavior and body weight regulation: lessons learned from lesioning studies. *Physiol Behav* 76:431-442
178. **Zigman JM, Jones JE, Lee CE, Saper CB, Elmquist JK** 2006 Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J Comp Neurol* 494:528-548
179. **Wilcox BJ, Corp ES, Dorsa DM, Figlewicz DP, Greenwood MR, Woods SC, Baskin DG** 1989 Insulin binding in the hypothalamus of lean and genetically obese Zucker rats. *Peptides* 10:1159-1164
180. **Broberger C, Johansen J, Johansson C, Schalling M, Hokfelt T** 1998 The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proc Natl Acad Sci U S A* 95:15043-15048
181. **Chou TC, Scammell TE, Gooley JJ, Gaus SE, Saper CB, Lu J** 2003 Critical role of dorsomedial hypothalamic nucleus in a wide range of behavioral circadian rhythms. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 23:10691-10702
182. **Gooley JJ, Schomer A, Saper CB** 2006 The dorsomedial hypothalamic nucleus is critical for the expression of food-entrainable circadian rhythms. *Nat Neurosci* 9:398-407
183. **Romijn JA, Adriaanse R, Brabant G, Prank K, Endert E, Wiersinga WM** 1990 Pulsatile secretion of thyrotropin during fasting: a decrease of thyrotropin pulse amplitude. *J Clin Endocrinol Metab* 70:1631-1636
184. **Watts AG, Sanchez-Watts G, Kelly AB** 1999 Distinct patterns of neuropeptide gene expression in the lateral hypothalamic area and arcuate nucleus are associated with dehydration-induced anorexia. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 19:6111-6121
185. **Jaimes-Hoy L, Joseph-Bravo P, de Gortari P** 2008 Differential response of TRHergic neurons of the hypothalamic paraventricular nucleus (PVN) in female animals submitted to food-restriction or dehydration-induced anorexia and cold exposure. *Horm Behav* 53:366-377
186. **Alvarez-Salas E, Aceves C, Anguiano B, Uribe RM, Garcia-Luna C, Sanchez E, de Gortari P** 2012 Food-restricted and dehydrated-induced anorexic rats present differential TRH expression in anterior and caudal PVN. Role of type 2 deiodinase and pyroglutamyl aminopeptidase II. *Endocrinology* 153:4067-4076
187. **de Gortari P, Mancera K, Cote-Velez A, Amaya MI, Martinez A, Jaimes-Hoy L, Joseph-Bravo P** 2009 Involvement of CRH-R2 receptor in eating behavior and in the response of the HPT axis in rats subjected to dehydration-induced anorexia. *Psychoneuroendocrinology* 34:259-272

188. **Vijayan E, McCann SM** 1977 Suppression of feeding and drinking activity in rats following intraventricular injection of thyrotropin releasing hormone (TRH). *Endocrinology* 100:1727-1730
189. **van den Heuvel JK, van Rozen AJ, Adan RA, la Fleur SE** 2011 An overview on how components of the melanocortin system respond to different high energy diets. *Eur J Pharmacol* 660:207-212
190. **Munzberg H, Flier JS, Bjorbaek C** 2004 Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology* 145:4880-4889
191. **White CL, Whittington A, Barnes MJ, Wang Z, Bray GA, Morrison CD** 2009 HF diets increase hypothalamic PTP1B and induce leptin resistance through both leptin-dependent and -independent mechanisms. *Am J Physiol Endocrinol Metab* 296:E291-299
192. **De Groot LJ** 1999 Dangerous dogmas in medicine: the nonthyroidal illness syndrome. *J Clin Endocrinol Metab* 84:151-164
193. **Fliers E, Guldenaar SE, Wiersinga WM, Swaab DF** 1997 Decreased hypothalamic thyrotropin-releasing hormone gene expression in patients with nonthyroidal illness. *J Clin Endocrinol Metab* 82:4032-4036
194. **Kondo K, Harbuz MS, Levy A, Lightman SL** 1997 Inhibition of the hypothalamic-pituitary-thyroid axis in response to lipopolysaccharide is independent of changes in circulating corticosteroids. *Neuroimmunomodulation* 4:188-194
195. **Kim YW, Kim KH, Ahn DK, Kim HS, Kim JY, Lee DC, Park SY** 2007 Time-course changes of hormones and cytokines by lipopolysaccharide and its relation with anorexia. *J Physiol Sci* 57:159-165
196. **Sergeyev V, Broberger C, Hokfelt T** 2001 Effect of LPS administration on the expression of POMC, NPY, galanin, CART and MCH mRNAs in the rat hypothalamus. *Brain Res Mol Brain Res* 90:93-100
197. **Fekete C, Sarkar S, Christoffolete MA, Emerson CH, Bianco AC, Lechan RM** 2005 Bacterial lipopolysaccharide (LPS)-induced type 2 iodothyronine deiodinase (D2) activation in the mediobasal hypothalamus (MBH) is independent of the LPS-induced fall in serum thyroid hormone levels. *Brain Res* 1056:97-99
198. **Schiltz JC, Sawchenko PE** 2007 Specificity and generality of the involvement of catecholaminergic afferents in hypothalamic responses to immune insults. *J Comp Neurol* 502:455-467
199. **Fekete C, Singru PS, Sarkar S, Rand WM, Lechan RM** 2005 Ascending brainstem pathways are not involved in lipopolysaccharide-induced suppression of thyrotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus. *Endocrinology* 146:1357-1363
200. **Boelen A, Kwakkel J, Thijssen-Timmer DC, Alkemade A, Fliers E, Wiersinga WM** 2004 Simultaneous changes in central and peripheral components of the hypothalamus-pituitary-thyroid axis in lipopolysaccharide-induced acute illness in mice. *J Endocrinol* 182:315-323
201. **Freitas BC, Gereben B, Castillo M, Kallo I, Zeold A, Egri P, Liposits Z, Zavacki AM, Maciel RM, Jo S, Singru P, Sanchez E, Lechan RM, Bianco AC** 2010 Paracrine signaling by glial cell-derived triiodothyronine activates neuronal gene expression in the rodent brain and human cells. *J Clin Invest* 120:2206-2217
202. **Sanchez E, Singru PS, Fekete C, Lechan RM** 2008 Induction of type 2 iodothyronine deiodinase in the mediobasal hypothalamus by bacterial lipopolysaccharide: role of corticosterone. *Endocrinology* 149:2484-2493
203. **Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K, Akira S** 1999 Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* 162:3749-3752
204. **Rocchi R, Kimura H, Tzou SC, Suzuki K, Rose NR, Pinchera A, Ladenson PW, Caturegli P** 2007 Toll-like receptor-MyD88 and Fc receptor pathways of mast cells mediate the thyroid dysfunctions observed during nonthyroidal illness. *Proc Natl Acad Sci U S A* 104:6019-6024

205. **Zhang G, Ghosh S** 2001 Toll-like receptor-mediated NF-kappaB activation: a phylogenetically conserved paradigm in innate immunity. *J Clin Invest* 107:13-19
206. **Zeold A, Doleschall M, Haffner MC, Capelo LP, Menyherth J, Liposits Z, da Silva WS, Bianco AC, Kacs Kovics I, Fekete C, Gereben B** 2006 Characterization of the nuclear factor-kappa B responsiveness of the human *trh* gene. *Endocrinology* 147:4419-4429
207. **Bockmann J, Bockers TM, Winter C, Wittkowski W, Winterhoff H, Deufel T, Kreutz MR** 1997 Thyrotropin expression in hypophyseal pars tuberalis-specific cells is 3,5,3'-triiodothyronine, thyrotropin-releasing hormone, and pit-1 independent. *Endocrinology* 138:1019-1028
208. **Nakao N, Ono H, Yamamura T, Anraku T, Takagi T, Higashi K, Yasuo S, Katou Y, Kageyama S, Uno Y, Kasukawa T, Iigo M, Sharp PJ, Iwasawa A, Suzuki Y, Sugano S, Niimi T, Mizutani M, Namikawa T, Ebihara S, Ueda HR, Yoshimura T** 2008 Thyrotrophin in the pars tuberalis triggers photoperiodic response. *Nature* 452:317-322
209. **Mebis L, Eerdekens A, Guiza F, Princen L, Derde S, Vanwijngaerden YM, Vanhorebeek I, Darras VM, Van den Berghe G, Langouche L** 2012 Contribution of nutritional deficit to the pathogenesis of the nonthyroidal illness syndrome in critical illness: a rabbit model study. *Endocrinology* 153:973-984
210. **Mebis L, Debaveye Y, Ellger B, Derde S, Ververs EJ, Langouche L, Darras VM, Fliers E, Visser TJ, Van den Berghe G** 2009 Changes in the central component of the hypothalamus-pituitary-thyroid axis in a rabbit model of prolonged critical illness. *Crit Care* 13:R147
211. **Silva JE** 1995 Thyroid hormone control of thermogenesis and energy balance. *Thyroid* 5:481-492
212. **Zoeller RT, Kabeer N, Albers HE** 1990 Cold exposure elevates cellular levels of messenger ribonucleic acid encoding thyrotropin-releasing hormone in paraventricular nucleus despite elevated levels of thyroid hormones. *Endocrinology* 127:2955-2962
213. **Uribe RM, Redondo JL, Charli JL, Joseph-Bravo P** 1993 Suckling and cold stress rapidly and transiently increase TRH mRNA in the paraventricular nucleus. *Neuroendocrinology* 58:140-145
214. **Perello M, Stuart RC, Vaslet CA, Nillni EA** 2007 Cold exposure increases the biosynthesis and proteolytic processing of prothyrotropin-releasing hormone in the hypothalamic paraventricular nucleus via beta-adrenoreceptors. *Endocrinology* 148:4952-4964
215. **Bianco AC, Silva JE** 1987 Intracellular conversion of thyroxine to triiodothyronine is required for the optimal thermogenic function of brown adipose tissue. *J Clin Invest* 79:295-300
216. **Rogers RC, Barnes MJ, Hermann GE** 2009 Leptin "gates" thermogenic action of thyrotropin-releasing hormone in the hindbrain. *Brain Res* 1295:135-141
217. **Krulich L, Giachetti A, Marchlewska-Koj A, Hefco E, Jameson HE** 1977 On the role of the central noradrenergic and dopaminergic systems in the regulation of TSH secretion in the rat. *Endocrinology* 100:496-505
218. **Arancibia S, Tapia-Arancibia L, Astier H, Assenmacher I** 1989 Physiological evidence for alpha 1-adrenergic facilitatory control of the cold-induced TRH release in the rat, obtained by push-pull cannulation of the median eminence. *Neurosci Lett* 100:169-174
219. **van Haasteren GA, van Toor H, Klootwijk W, Handler B, Linkels E, van der Schoot P, van Ophemert J, de Jong FH, Visser TJ, de Greef WJ** 1996 Studies on the role of TRH and corticosterone in the regulation of prolactin and thyrotrophin secretion during lactation. *J Endocrinol* 148:325-336
220. **Sanchez E, Fekete C, Lechan RM, Joseph-Bravo P** 2007 Cocaine- and amphetamine-regulated transcript (CART) expression is differentially regulated in the hypothalamic paraventricular nucleus of lactating rats exposed to suckling or cold stimulation. *Brain Res* 1132:120-128
221. **Raptis S, Fekete C, Sarkar S, Rand WM, Emerson CH, Nagy GM, Lechan RM** 2004 Cocaine- and amphetamine-regulated transcript co-contained in thyrotropin-releasing hormone (TRH) neurons of the hypothalamic paraventricular nucleus modulates TRH-induced prolactin secretion. *Endocrinology* 145:1695-1699

222. **Collu R, Tang J, Castagne J, Lagace G, Masson N, Huot C, Deal C, Delvin E, Faccenda E, Eidne KA, Van Vliet G** 1997 A novel mechanism for isolated central hypothyroidism: inactivating mutations in the thyrotropin-releasing hormone receptor gene. *J Clin Endocrinol Metab* 82:1561-1565
223. **Bonomi M, Busnelli M, Beck-Peccoz P, Costanzo D, Antonica F, Dolci C, Pilotta A, Buzi F, Persani L** 2009 A family with complete resistance to thyrotropin-releasing hormone. *N Engl J Med* 360:731-734
224. **Dumitrescu AM, Liao XH, Best TB, Brockmann K, Refetoff S** 2004 A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. *American journal of human genetics* 74:168-175
225. **Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, Barrett TG, Mancilla EE, Svensson J, Kester MH, Kuiper GG, Balkassmi S, Uitterlinden AG, Koehrle J, Rodien P, Halestrap AP, Visser TJ** 2004 Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* 364:1435-1437
226. **Boccone L, Mariotti S, Dessi V, Pruna D, Meloni A, Loudianos G** 2010 Allan-Herndon-Dudley syndrome (AHDS) caused by a novel SLC16A2 gene mutation showing severe neurologic features and unexpectedly low TRH-stimulated serum TSH. *European journal of medical genetics* 53:392-395
227. **Refetoff S, Weiss RE, Usala SJ** 1993 The syndromes of resistance to thyroid hormone. *Endocr Rev* 14:348-399
228. **Bochukova E, Schoenmakers N, Agostini M, Schoenmakers E, Rajanayagam O, Keogh JM, Henning E, Reinemund J, Gevers E, Sarri M, Downes K, Offiah A, Albanese A, Halsall D, Schwabe JW, Bain M, Lindley K, Muntoni F, Vargha-Khadem F, Dattani M, Farooqi IS, Gurnell M, Chatterjee K** 2012 A mutation in the thyroid hormone receptor alpha gene. *N Engl J Med* 366:243-249
229. **Mantzoros CS, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, Hamnvik OP, Koniaris A** 2011 Leptin in human physiology and pathophysiology. *Am J Physiol Endocrinol Metab* 301:E567-584
230. **Paz-Filho G, Wong ML, Licinio J** 2011 Ten years of leptin replacement therapy. *Obesity reviews : an official journal of the International Association for the Study of Obesity* 12:e315-323
231. **Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S** 1997 Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 387:903-908
232. **Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, Lechler RI, DePaoli AM, O'Rahilly S** 2002 Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest* 110:1093-1103
233. **Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS** 2003 The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J Clin Invest* 111:1409-1421
234. **Rosenbaum M, Murphy EM, Heymsfield SB, Matthews DE, Leibel RL** 2002 Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones. *J Clin Endocrinol Metab* 87:2391-2394
235. **Shetty GK, Matarese G, Magkos F, Moon HS, Liu X, Brennan AM, Mylvaganam G, Sykoutri D, Depaoli AM, Mantzoros CS** 2011 Leptin administration to overweight and obese subjects for 6 months increases free leptin concentrations but does not alter circulating hormones of the thyroid and IGF axes during weight loss induced by a mild hypocaloric diet. *Eur J Endocrinol* 165:249-254



236. **Bruni V, Dei M, Morelli C, Schettino MT, Balzi D, Nuvolone D** 2011 Body composition variables and leptin levels in functional hypothalamic amenorrhea and amenorrhea related to eating disorders. *Journal of pediatric and adolescent gynecology* 24:347-352
237. **Chou SH, Chamberland JP, Liu X, Matarese G, Gao C, Stefanakis R, Brinkoetter MT, Gong H, Arampatzi K, Mantzoros CS** 2011 Leptin is an effective treatment for hypothalamic amenorrhea. *Proc Natl Acad Sci U S A* 108:6585-6590
238. **Misra M, Klibanski A** 2010 Neuroendocrine consequences of anorexia nervosa in adolescents. *Endocrine development* 17:197-214
239. **Reinehr T, Isa A, de Sousa G, Dieffenbach R, Andler W** 2008 Thyroid hormones and their relation to weight status. *Hormone research* 70:51-57
240. **Plikat K, Langgartner J, Buettner R, Bollheimer LC, Woenckhaus U, Scholmerich J, Wrede CE** 2007 Frequency and outcome of patients with nonthyroidal illness syndrome in a medical intensive care unit. *Metabolism* 56:239-244
241. **Bello G, Ceaihsiciuc I, Silva S, Antonelli M** 2010 The role of thyroid dysfunction in the critically ill: a review of the literature. *Minerva Anesthesiol* 76:919-928
242. **Casaer MP, Wilmer A, Hermans G, Wouters PJ, Mesotten D, Van den Berghe G** 2013 Role of disease and macronutrient dose in the randomized controlled EPaNIC trial: a post hoc analysis. *Am J Respir Crit Care Med* 187:247-255
243. **Langouche L, Vander Perre S, Marques M, Boelen A, Wouters PJ, Casaer MP, Van den Berghe G** 2013 Impact of early nutrient restriction during critical illness on the nonthyroidal illness syndrome and its relation with outcome: a randomized, controlled clinical study. *J Clin Endocrinol Metab* 98:1006-1013
244. **Rothwell PM, Lawler PG** 1995 Prediction of outcome in intensive care patients using endocrine parameters. *Crit Care Med* 23:78-83
245. **Chinga-Alayo E, Villena J, Evans AT, Zimic M** 2005 Thyroid hormone levels improve the prediction of mortality among patients admitted to the intensive care unit. *Intensive Care Med* 31:1356-1361
246. **De Groot LJ** 2006 Non-thyroidal illness syndrome is a manifestation of hypothalamic-pituitary dysfunction, and in view of current evidence, should be treated with appropriate replacement therapies. *Crit Care Clin* 22:57-86, vi
247. **Adler SM, Wartofsky L** 2007 The nonthyroidal illness syndrome. *Endocrinol Metab Clin North Am* 36:657-672, vi
248. **Angelousi AG, Karageorgopoulos DE, Kapaskelis AM, Falagas ME** 2011 Association between thyroid function tests at baseline and the outcome of patients with sepsis or septic shock: a systematic review. *Eur J Endocrinol* 164:147-155
249. **Haas NA, Camphausen CK, Kececioglu D** 2006 Clinical review: thyroid hormone replacement in children after cardiac surgery--is it worth a try? *Crit Care* 10:213
250. **Brent GA, Hershman JM** 1986 Thyroxine therapy in patients with severe nonthyroidal illnesses and low serum thyroxine concentration. *J Clin Endocrinol Metab* 63:1-8
251. **Becker RA, Vaughan GM, Ziegler MG, Seraile LG, Goldfarb IW, Mansour EH, McManus WF, Pruitt BA, Jr., Mason AD, Jr.** 1982 Hypermetabolic low triiodothyronine syndrome of burn injury. *Crit Care Med* 10:870-875
252. **Klemperer JD, Klein I, Gomez M, Helm RE, Ojamaa K, Thomas SJ, Isom OW, Krieger K** 1995 Thyroid hormone treatment after coronary-artery bypass surgery. *N Engl J Med* 333:1522-1527
253. **Spratt DI, Frohnauer M, Cyr-Alves H, Kramer RS, Lucas FL, Morton JR, Cox DF, Becker K, Devlin JT** 2007 Physiological effects of nonthyroidal illness syndrome in patients after cardiac surgery. *Am J Physiol Endocrinol Metab* 293:E310-315
254. **Pingitore A, Galli E, Barison A, Iervasi A, Scarlattini M, Nucci D, L'Abbate A, Mariotti R, Iervasi G** 2008 Acute effects of triiodothyronine (T3) replacement therapy in patients with chronic heart failure and low-T3 syndrome: a randomized, placebo-controlled study. *J Clin Endocrinol Metab* 93:1351-1358

255. **Mullis-Jansson SL, Argenziano M, Corwin S, Homma S, Weinberg AD, Williams M, Rose EA, Smith CR** 1999 A randomized double-blind study of the effect of triiodothyronine on cardiac function and morbidity after coronary bypass surgery. *J Thorac Cardiovasc Surg* 117:1128-1134
256. **Kaptein EM, Sanchez A, Beale E, Chan LS** 2010 Clinical review: Thyroid hormone therapy for postoperative nonthyroidal illnesses: a systematic review and synthesis. *J Clin Endocrinol Metab* 95:4526-4534
257. **Boelen A, Kwakkel J, Fliers E** 2011 Beyond low plasma T3: local thyroid hormone metabolism during inflammation and infection. *Endocr Rev* 32:670-693
258. **Van den Berghe G, Baxter RC, Weekers F, Wouters P, Bowers CY, Iranmanesh A, Veldhuis JD, Bouillon R** 2002 The combined administration of GH-releasing peptide-2 (GHRP-2), TRH and GnRH to men with prolonged critical illness evokes superior endocrine and metabolic effects compared to treatment with GHRP-2 alone. *Clin Endocrinol (Oxf)* 56:655-669
259. **Van den Berghe G, Wouters P, Weekers F, Mohan S, Baxter RC, Veldhuis JD, Bowers CY, Bouillon R** 1999 Reactivation of pituitary hormone release and metabolic improvement by infusion of growth hormone-releasing peptide and thyrotropin-releasing hormone in patients with protracted critical illness. *J Clin Endocrinol Metab* 84:1311-1323

Figure 1  
[Click here to download high resolution image](#)

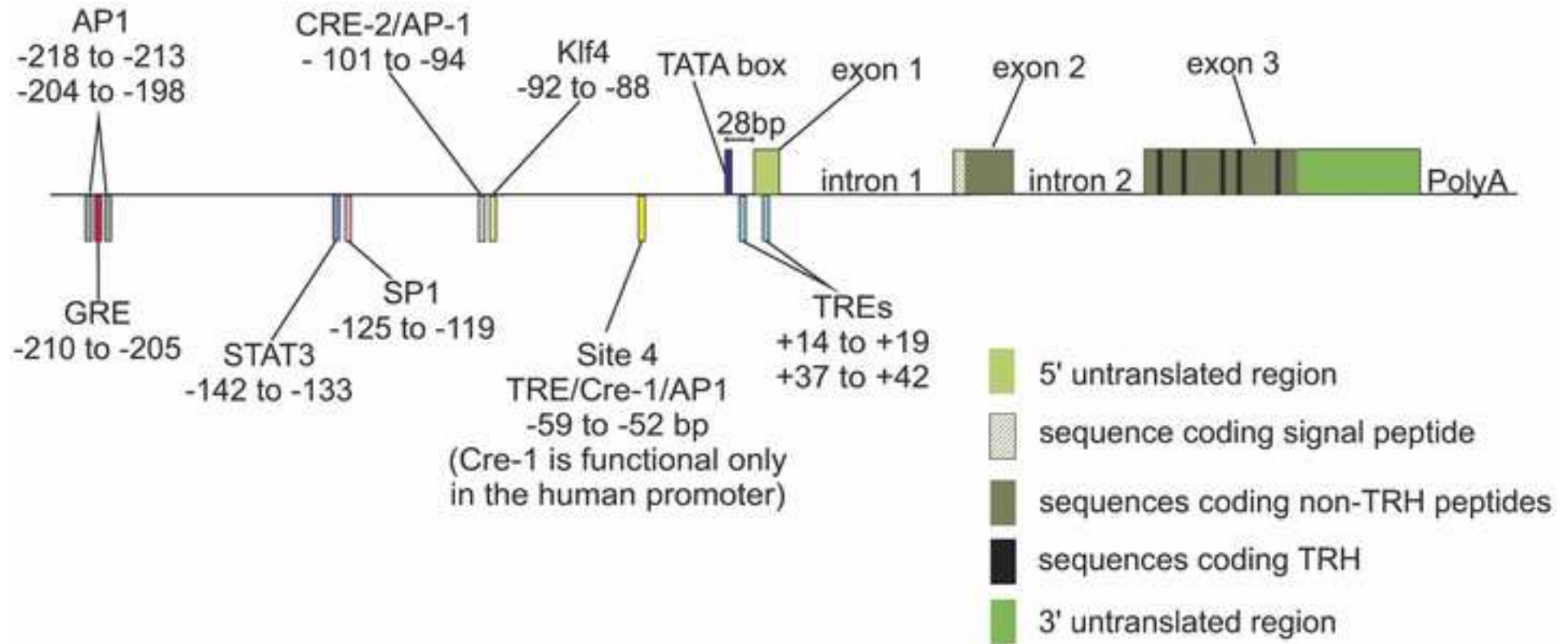


Figure 2  
[Click here to download high resolution image](#)

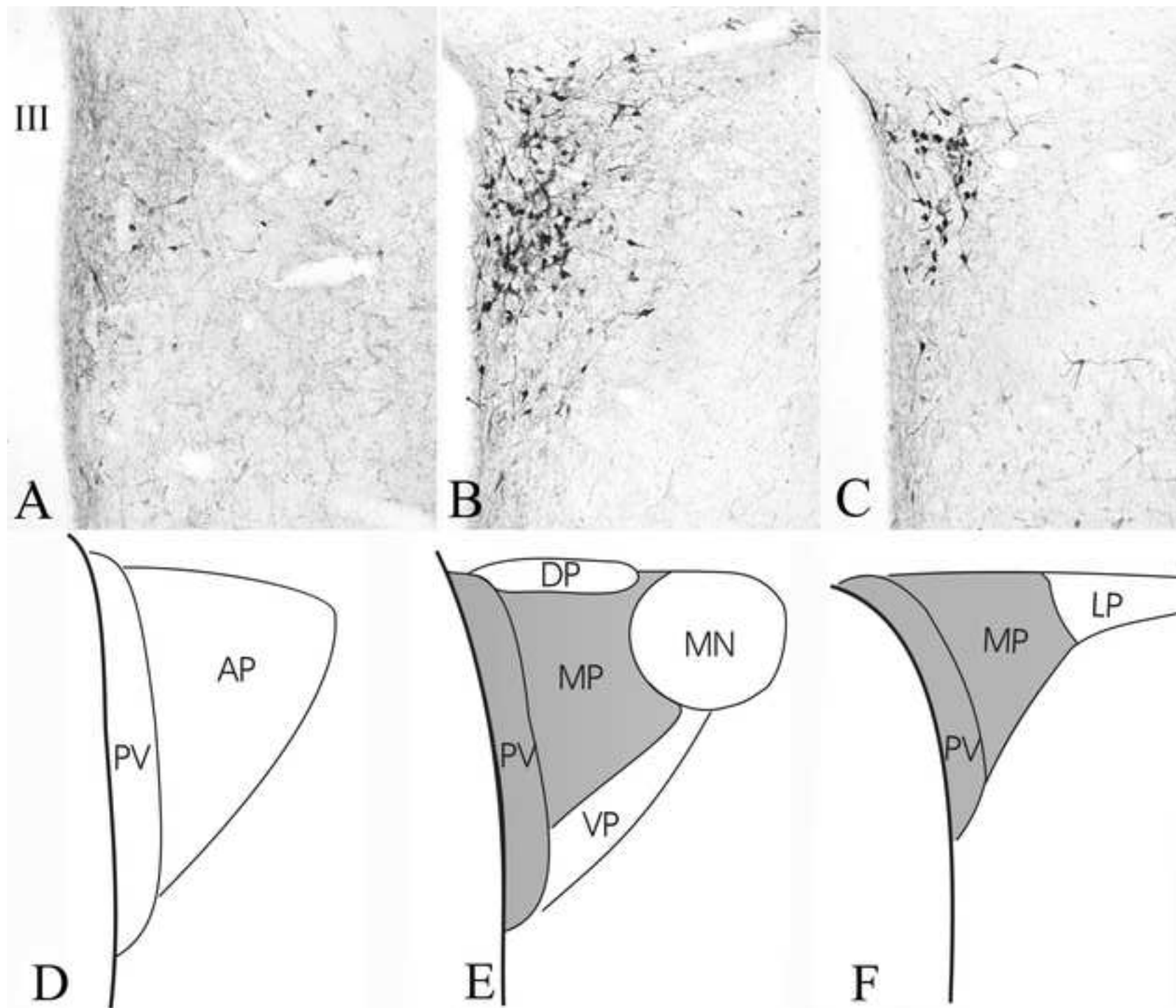


Figure 3  
[Click here to download high resolution image](#)

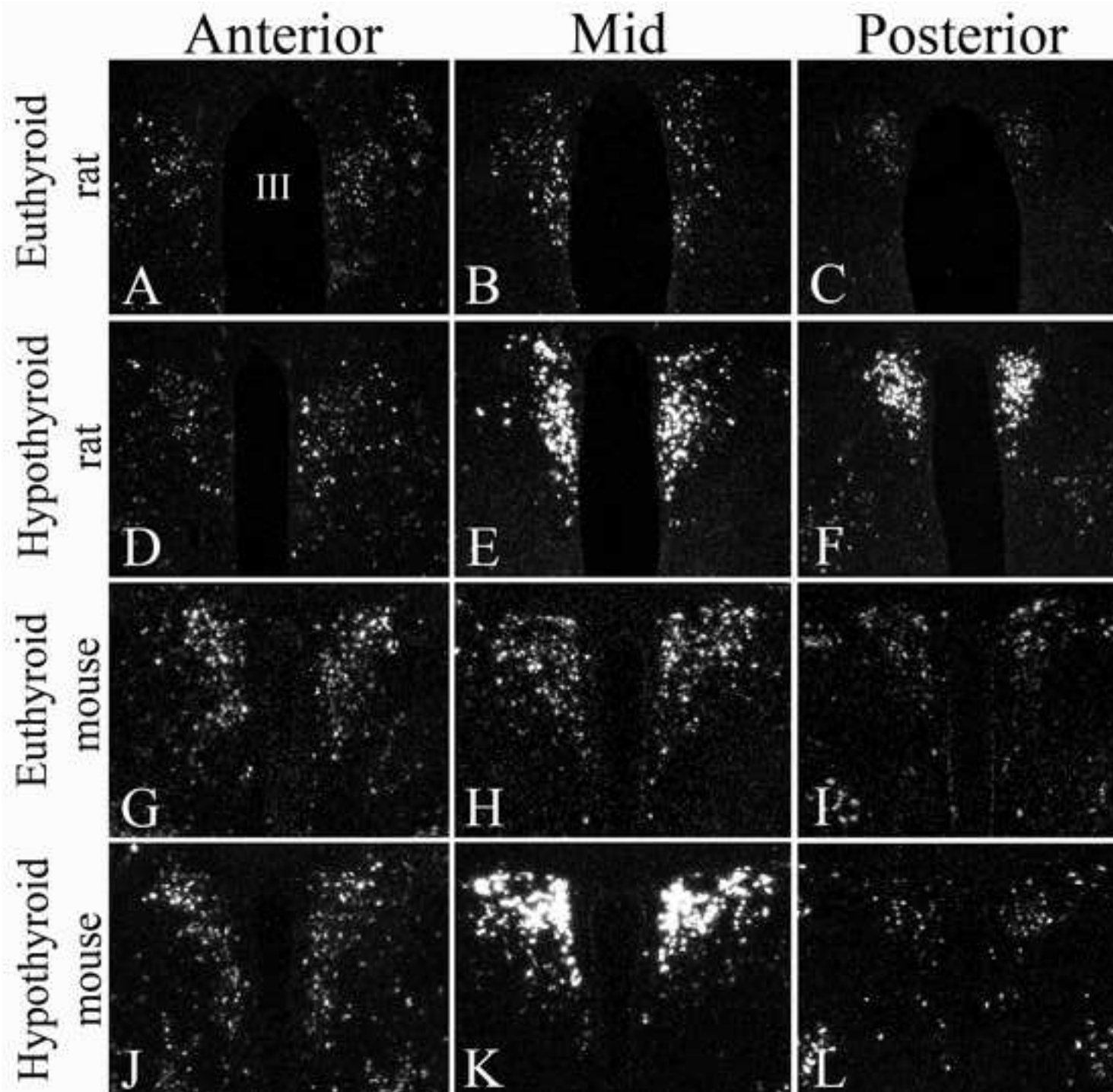




Figure 4  
[Click here to download high resolution image](#)

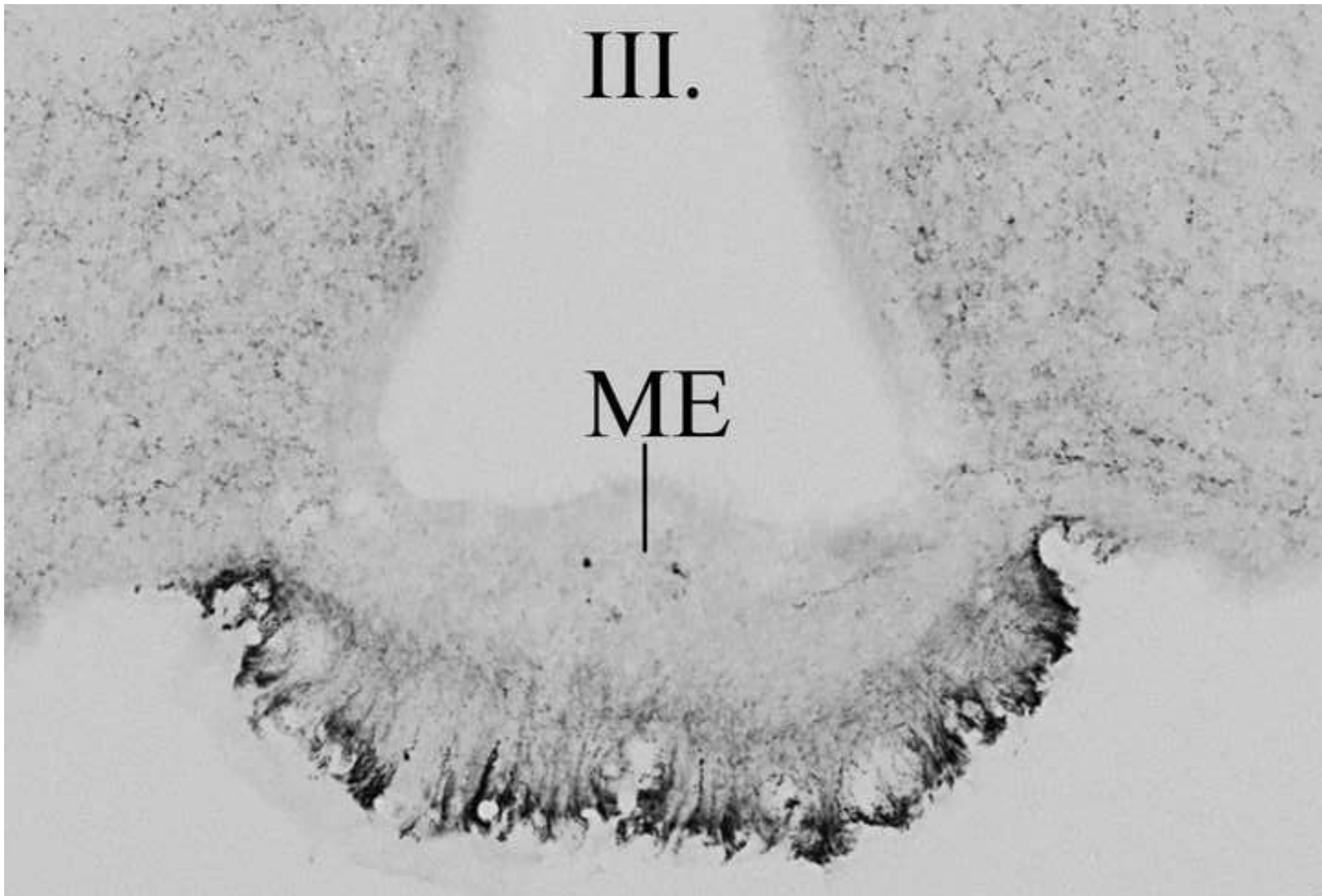


Figure 5  
[Click here to download high resolution image](#)

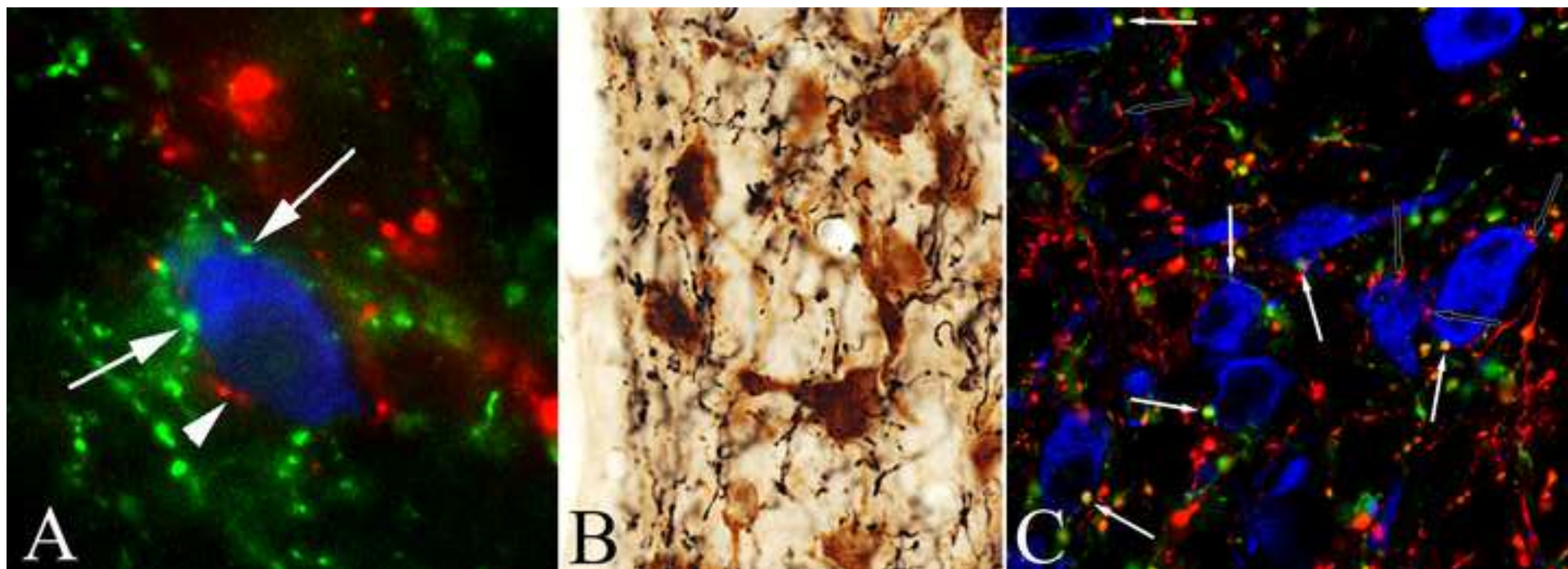


Figure 6  
[Click here to download high resolution image](#)

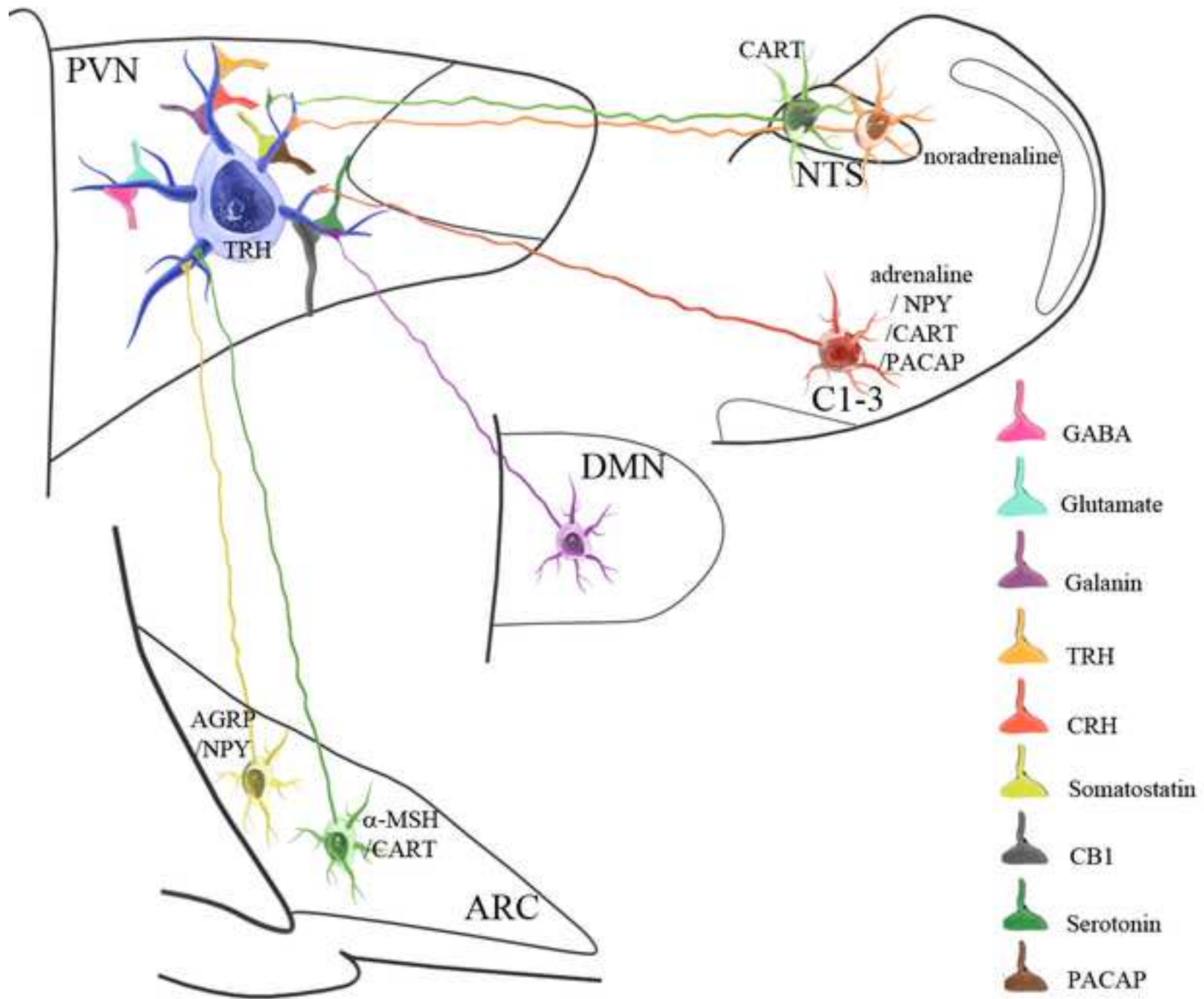




Figure 7  
[Click here to download high resolution image](#)

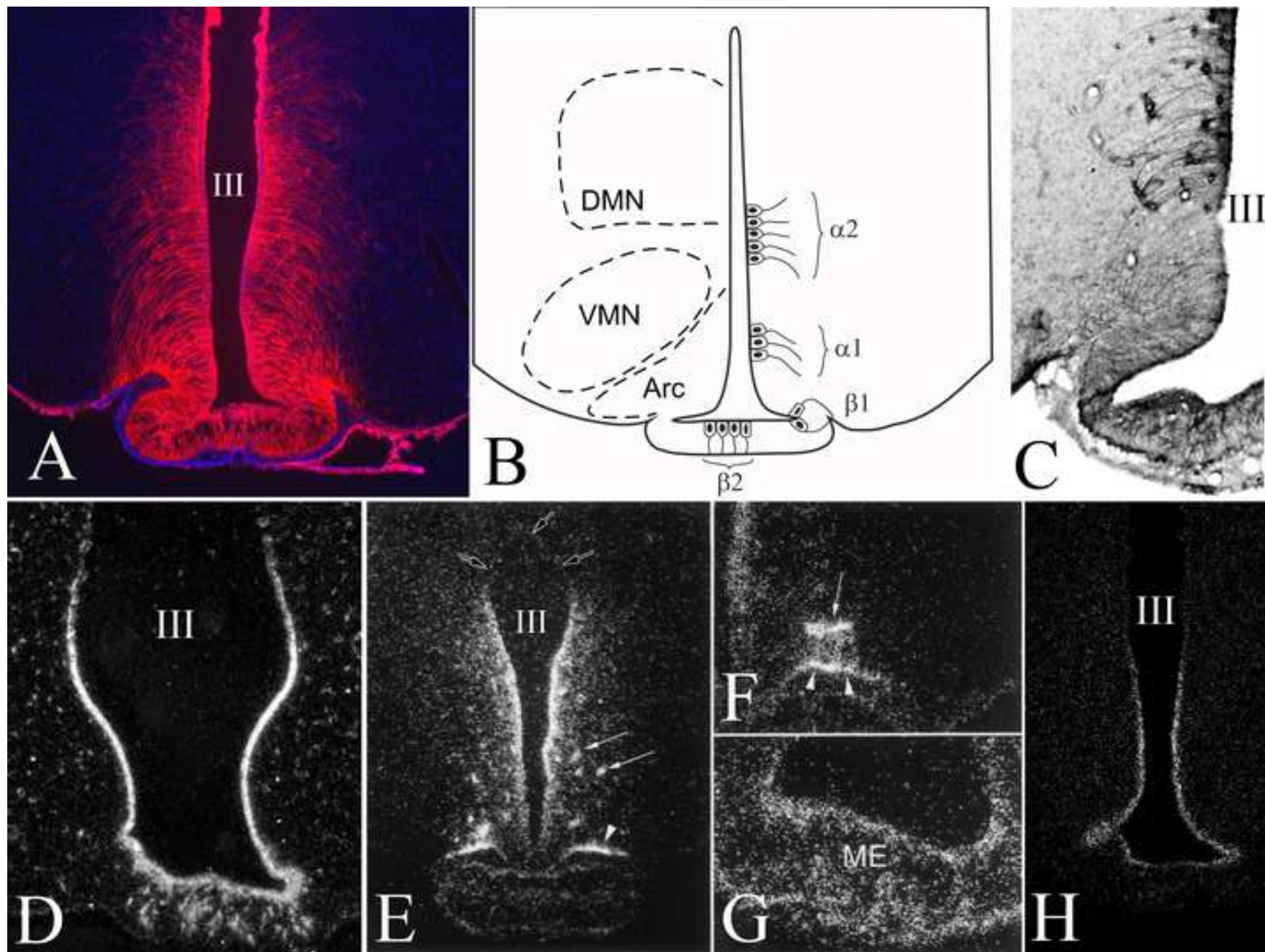


Figure 8  
[Click here to download high resolution image](#)

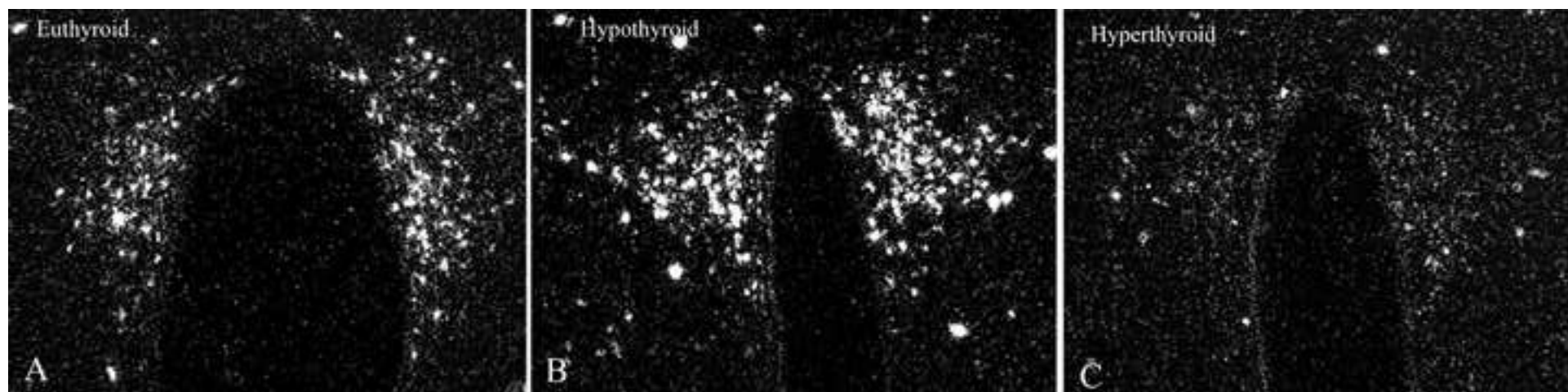


Figure 9  
[Click here to download high resolution image](#)

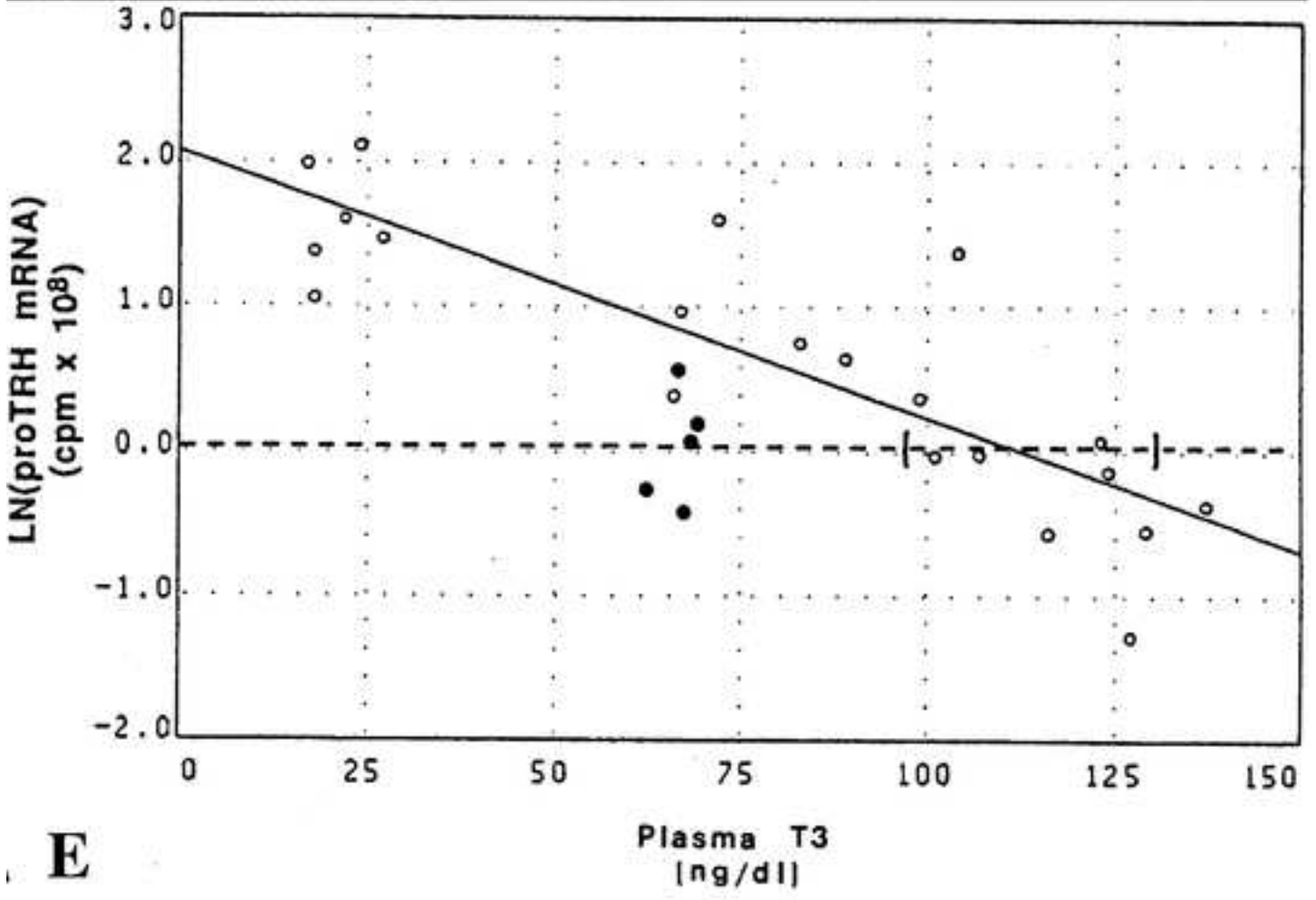
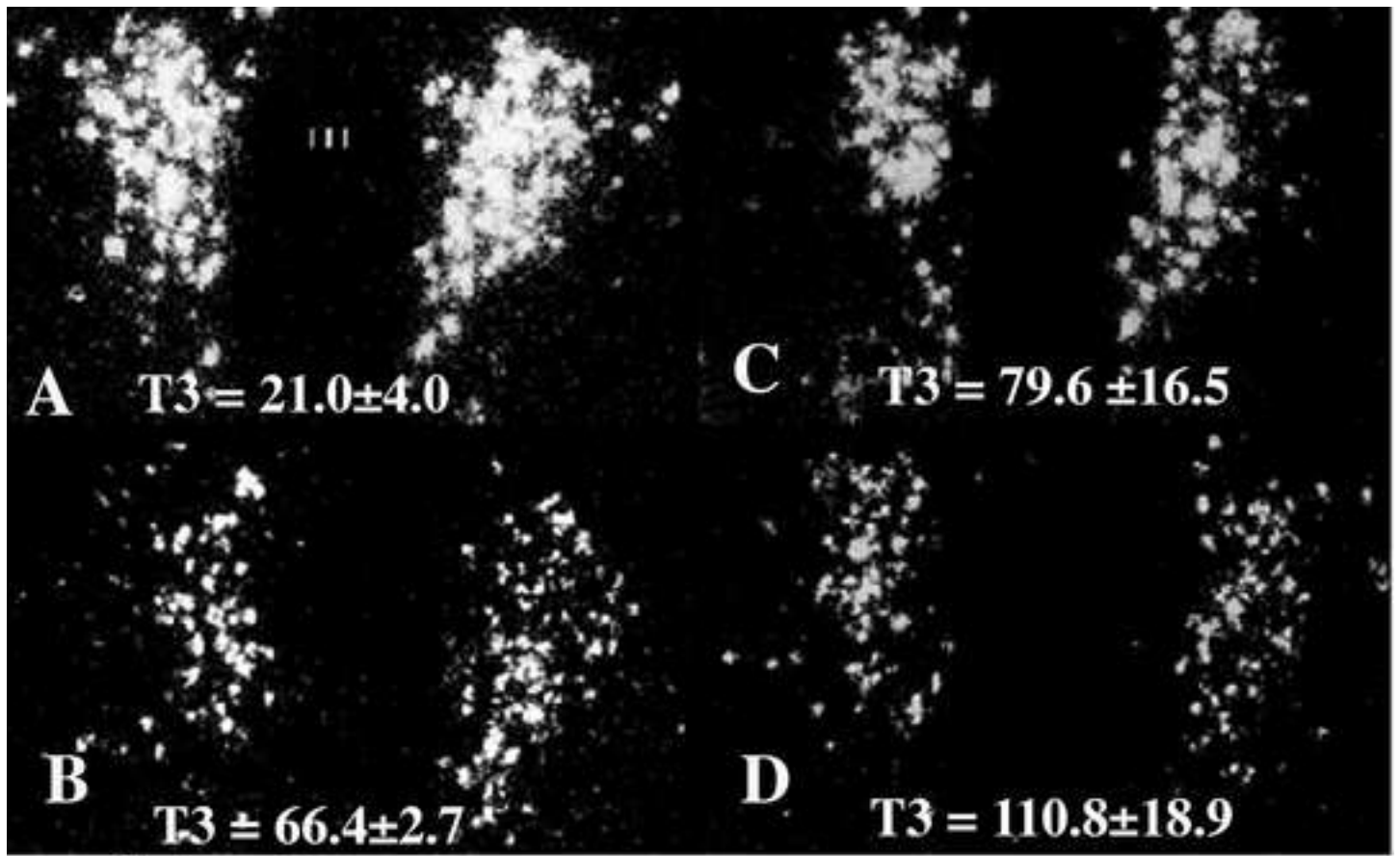




Figure 11  
[Click here to download high resolution image](#)

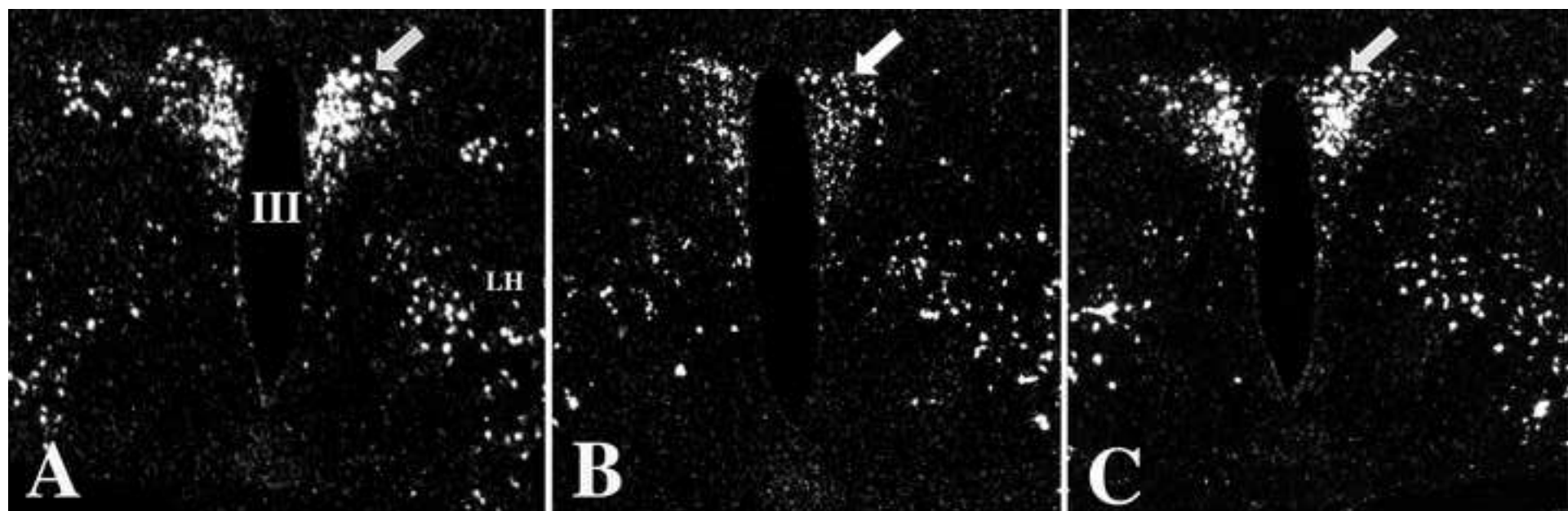


Figure 12  
[Click here to download high resolution image](#)

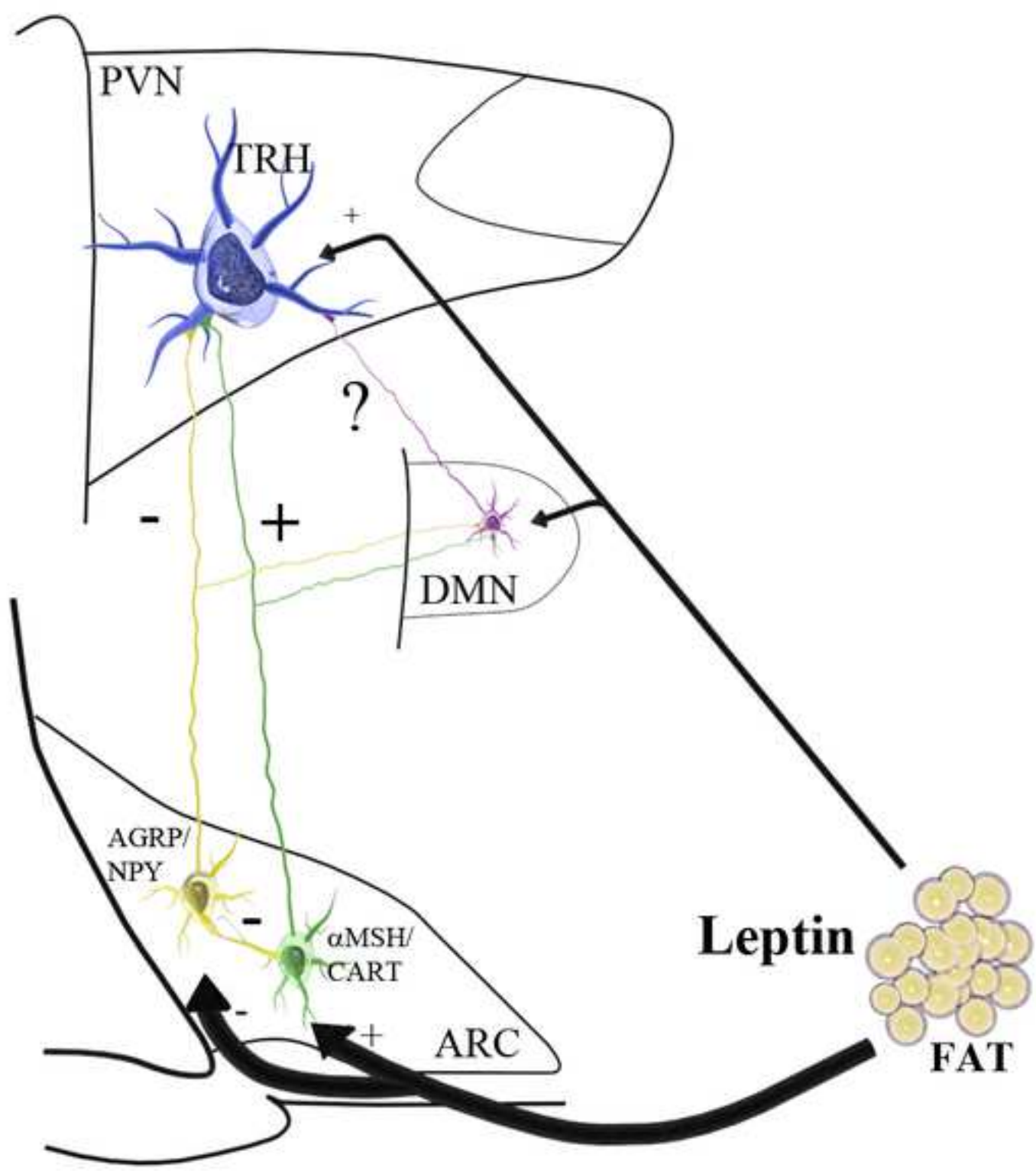


Figure 13  
[Click here to download high resolution image](#)

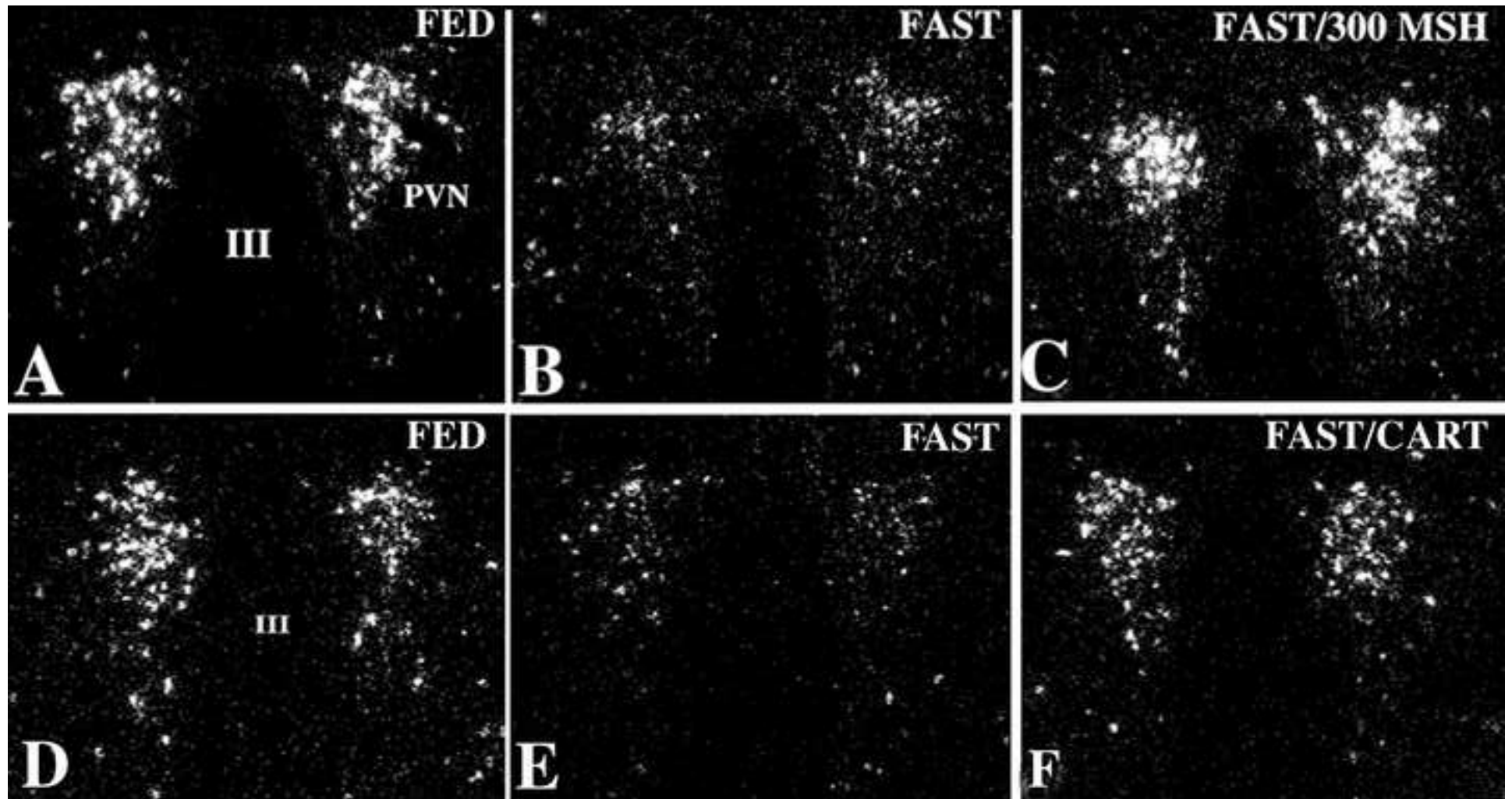


Figure 14  
[Click here to download high resolution image](#)

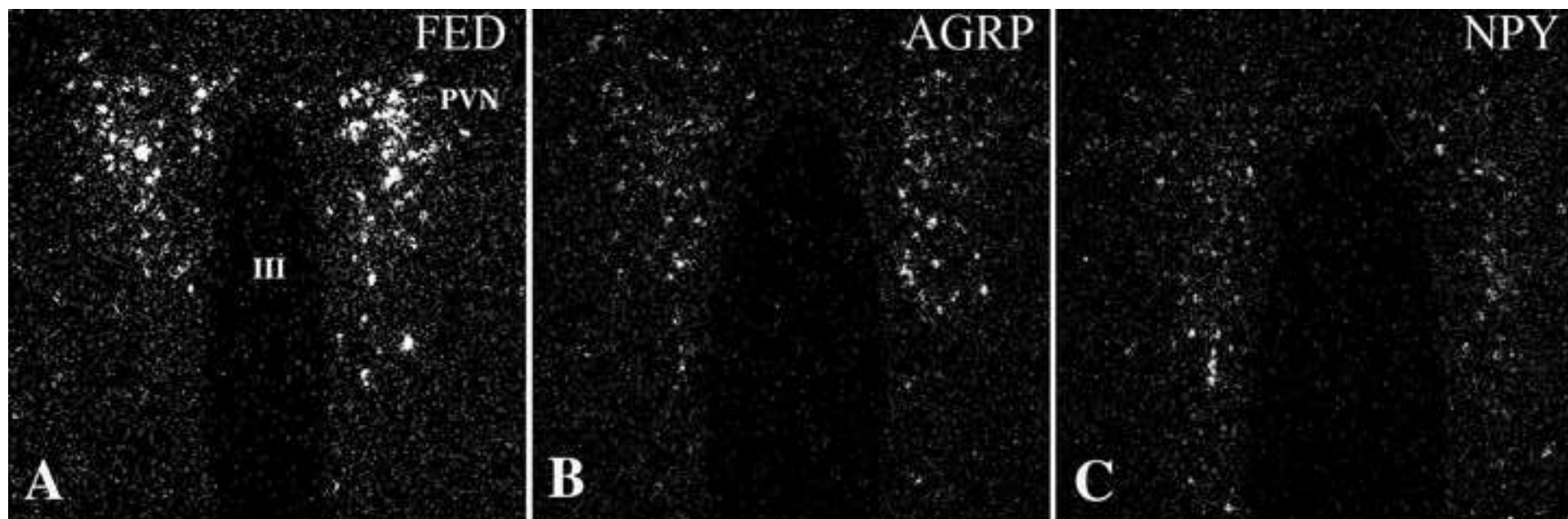




Figure 15  
[Click here to download high resolution image](#)

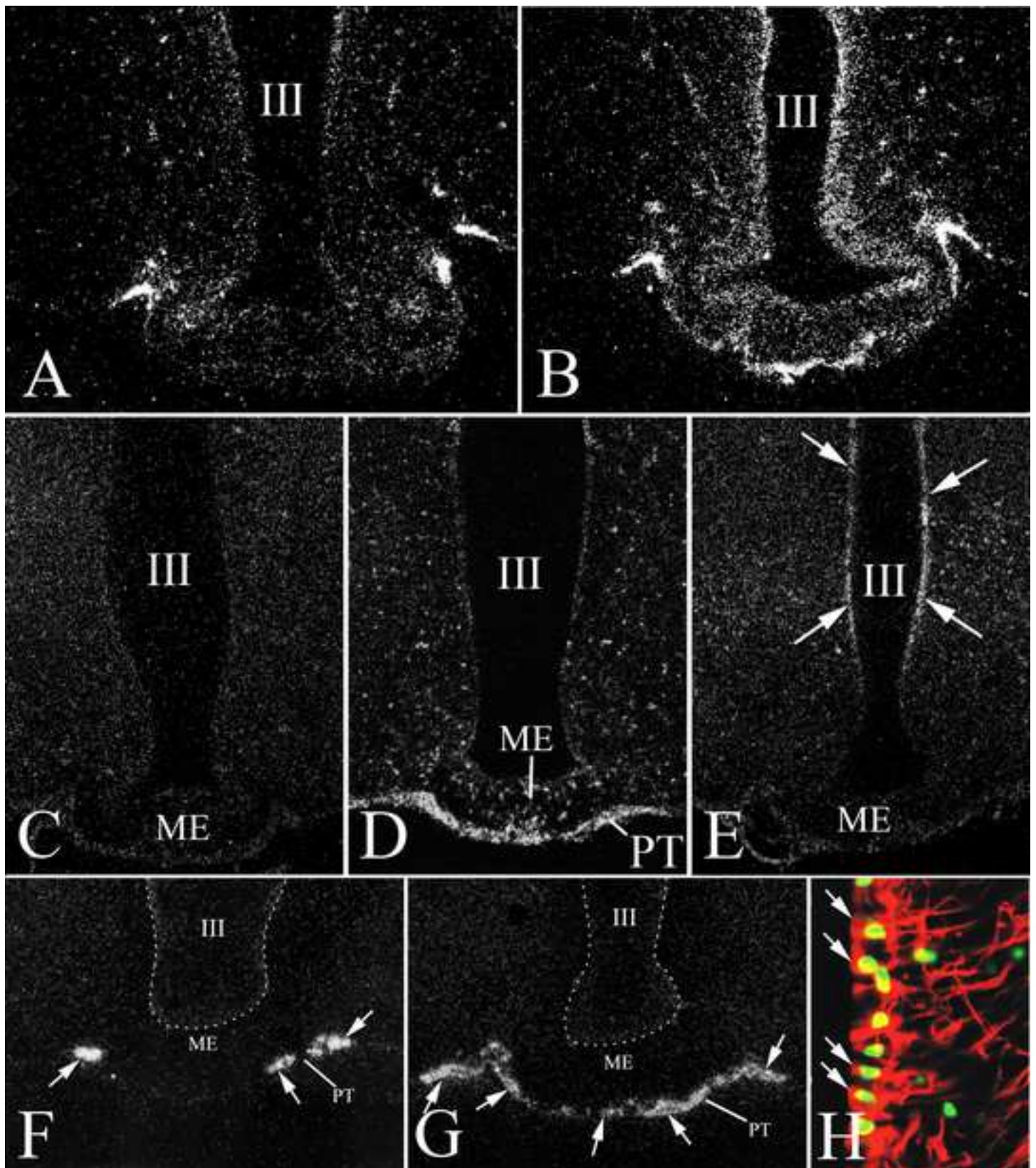
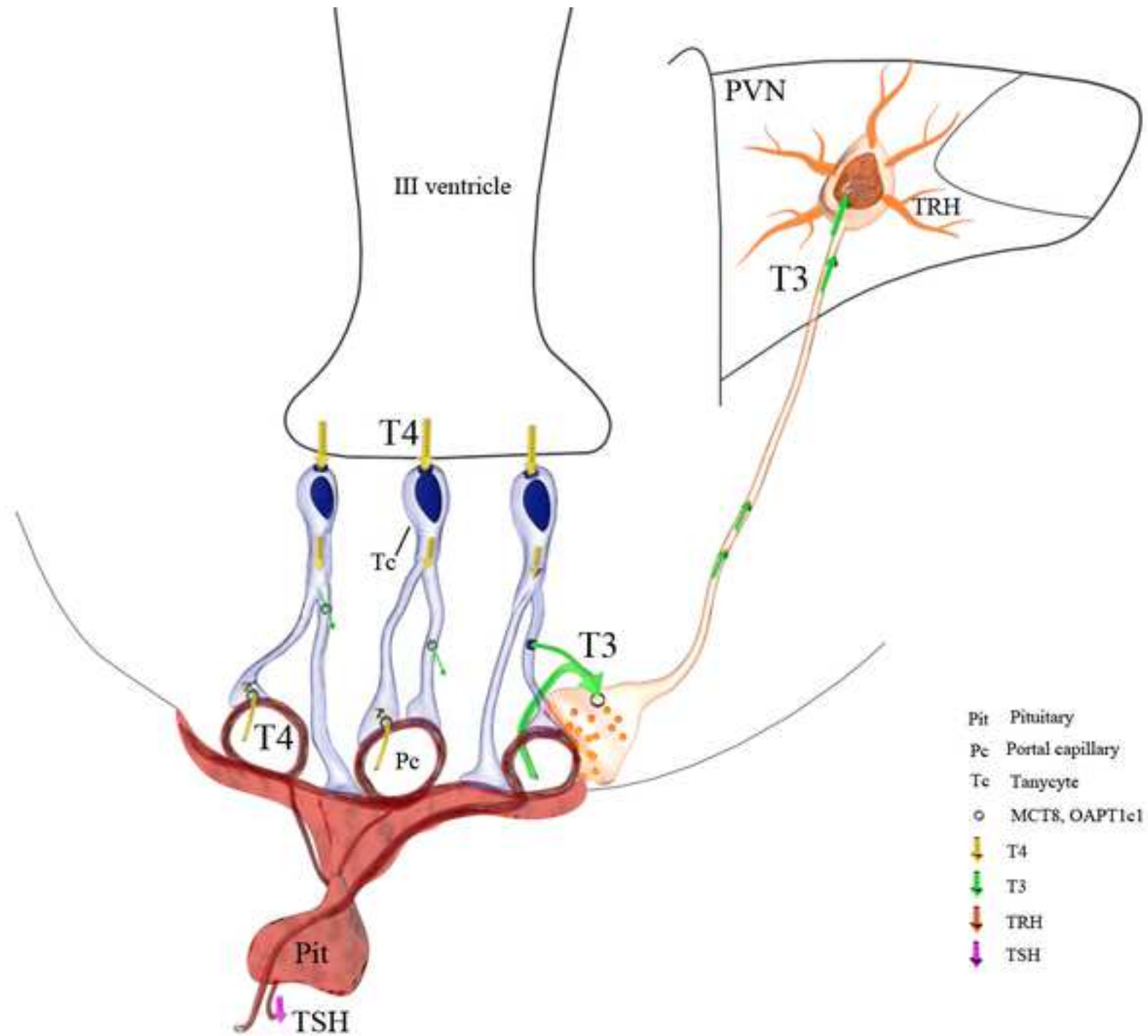




Figure 10  
[Click here to download high resolution image](#)



**ELSEVIER LICENSE  
TERMS AND CONDITIONS**

Aug 02, 2013

This is a License Agreement between Csaba Fekete ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

**All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.**

Supplier	Elsevier Limited The Boulevard, Langford Lane Kidlington, Oxford, OX5 1GB, UK
Registered Company Number	1982084
Customer name	Csaba Fekete
Customer address	Szigony u. 43. Budapest, 1083
License number	3200780297608
License date	Aug 02, 2013
Licensed content publisher	Elsevier
Licensed content publication	Brain Research
Licensed content title	Noradrenergic innervation of hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in rats
Licensed content author	Tamás Füzési, Gábor Wittmann, Ronald M. Lechan, Zsolt Liposits, Csaba Fekete
Licensed content date	6 October 2009
Licensed content volume number	1294
Licensed content issue number	
Number of pages	7
Start Page	38
End Page	44
Type of Use	reuse in a journal/magazine
Requestor type	author of new work
Intended publisher of new work	Society for Endocrinology & BioScientifica Ltd
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Order reference number	
Title of the article	Central regulation of the hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions
Publication new article is in	Endocrine Reviews
Publisher of the new article	Society for Endocrinology & BioScientifica Ltd
Author of new article	Csaba Fekete and Ronald M. Lechan
Expected publication date	Jan 2014
Estimated size of new article (number of pages)	45

Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 USD
VAT/Local Sales Tax	0.00 USD / 0.00 GBP
Total	0.00 USD
<a href="#">Terms and Conditions</a>	

## INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at <http://myaccount.copyright.com>).

## GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier at [permissions@elsevier.com](mailto:permissions@elsevier.com))

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and

shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. **Warranties:** Publisher makes no representations or warranties with respect to the licensed material.

10. **Indemnity:** You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. **No Transfer of License:** This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. **No Amendment Except in Writing:** This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. **Objection to Contrary Terms:** Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. **Revocation:** Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

### **LIMITED LICENSE**

The following terms and conditions apply only to specific license types:

15. **Translation:** This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article. If this license is to re-use 1 or 2 figures then permission is granted for non-exclusive world rights in all languages.

16. **Website:** The following terms and conditions apply to electronic reserve and

author websites:

**Electronic reserve:** If licensed material is to be posted to website, the web site is to be password-protected and made available only to bona fide students registered on a relevant course if:

This license was made in connection with a course,

This permission is granted for 1 year only. You may obtain a license for future website posting,

All content posted to the web site must maintain the copyright information line on the bottom of each image,

A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> or the Elsevier homepage for books at <http://www.elsevier.com> , and

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

17. **Author website** for journals with the following additional clauses:

All content posted to the web site must maintain the copyright information line on the bottom of each image, and the permission granted is limited to the personal version of your paper. You are not allowed to download and post the published electronic version of your article (whether PDF or HTML, proof or final version), nor may you scan the printed edition to create an electronic version. A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> . As part of our normal production process, you will receive an e-mail notice when your article appears on Elsevier's online service ScienceDirect ([www.sciencedirect.com](http://www.sciencedirect.com)). That e-mail will include the article's Digital Object Identifier (DOI). This number provides the electronic link to the published article and should be included in the posting of your personal version. We ask that you wait until you receive this e-mail and have the DOI to do any posting.

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

18. **Author website** for books with the following additional clauses:

Authors are permitted to place a brief summary of their work online only.

A hyper-text must be included to the Elsevier homepage at

<http://www.elsevier.com> . All content posted to the web site must maintain the copyright information line on the bottom of each image. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version.

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

19. **Website** (regular and for author): A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx>. or for books to the Elsevier homepage at <http://www.elsevier.com>

20. **Thesis/Dissertation:** If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply

single copies, on demand, of the complete thesis and include permission for UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission.

## 21. Other Conditions:

v1.6

**If you would like to pay for this license now, please remit this license along with your payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check or money order referencing your account number and this invoice number RLNK501081814.**

**Once you receive your invoice for this order, you may pay your invoice by credit card. Please follow instructions provided at that time.**

**Make Payment To:  
Copyright Clearance Center  
Dept 001  
P.O. Box 843006  
Boston, MA 02284-3006**

**For suggestions or comments regarding this order, contact RightsLink Customer Support: [customercare@copyright.com](mailto:customercare@copyright.com) or +1-877-622-5543 (toll free in the US) or +1-978-646-2777.**

**Gratis licenses (referencing \$0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.**

---

---

**ELSEVIER LICENSE  
TERMS AND CONDITIONS**

Aug 02, 2013

This is a License Agreement between Csaba Fekete ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

**All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.**

Supplier	Elsevier Limited The Boulevard, Langford Lane Kidlington, Oxford, OX5 1GB, UK
Registered Company Number	1982084
Customer name	Csaba Fekete
Customer address	Szigony u. 43. Budapest, 1083
License number	3200781179430
License date	Aug 02, 2013
Licensed content publisher	Elsevier
Licensed content publication	Brain Research
Licensed content title	DARPP-32 and CREB are present in type 2 iodothyronine deiodinase-producing tanycytes: implications for the regulation of type 2 deiodinase activity
Licensed content author	Csaba Fekete, Emese Mihály, Sorin Herscovici, Jeremy Salas, Helen Tu, P. Reed Larsen, Ronald M Lechan
Licensed content date	17 April 2000
Licensed content volume number	862
Licensed content issue number	1-2
Number of pages	8
Start Page	154
End Page	161
Type of Use	reuse in a journal/magazine
Requestor type	author of new work
Intended publisher of new work	Society for Endocrinology & BioScientifica Ltd
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Order reference number	
Title of the article	Central regulation of the hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions
Publication new article is in	Endocrine Reviews
Publisher of the new	Society for Endocrinology & BioScientifica Ltd



## article

Author of new article	Csaba Fekete and Ronald M. Lechan
Expected publication date	Jan 2014
Estimated size of new article (number of pages)	45
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 USD
VAT/Local Sales Tax	0.00 USD / 0.00 GBP
Total	0.00 USD
Terms and Conditions	

## INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at <http://myaccount.copyright.com>).

## GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier at [permissions@elsevier.com](mailto:permissions@elsevier.com))

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the



transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

### LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation:** This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce

the content word for word preserving the integrity of the article. If this license is to re-use 1 or 2 figures then permission is granted for non-exclusive world rights in all languages.

16. **Website:** The following terms and conditions apply to electronic reserve and author websites:

**Electronic reserve:** If licensed material is to be posted to website, the web site is to be password-protected and made available only to bona fide students registered on a relevant course if:

This license was made in connection with a course,

This permission is granted for 1 year only. You may obtain a license for future website posting,

All content posted to the web site must maintain the copyright information line on the bottom of each image,

A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> or the Elsevier homepage for books at <http://www.elsevier.com> , and

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

17. **Author website** for journals with the following additional clauses:

All content posted to the web site must maintain the copyright information line on the bottom of each image, and the permission granted is limited to the personal version of your paper. You are not allowed to download and post the published electronic version of your article (whether PDF or HTML, proof or final version), nor may you scan the printed edition to create an electronic version. A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> . As part of our normal production process, you will receive an e-mail notice when your article appears on Elsevier's online service ScienceDirect ([www.sciencedirect.com](http://www.sciencedirect.com)). That e-mail will include the article's Digital Object Identifier (DOI). This number provides the electronic link to the published article and should be included in the posting of your personal version. We ask that you wait until you receive this e-mail and have the DOI to do any posting.

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

18. **Author website** for books with the following additional clauses:

Authors are permitted to place a brief summary of their work online only.

A hyper-text must be included to the Elsevier homepage at

<http://www.elsevier.com> . All content posted to the web site must maintain the copyright information line on the bottom of each image. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version.

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

19. **Website** (regular and for author): A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> . or for books to the Elsevier homepage at <http://www.elsevier.com>

**20. Thesis/Dissertation:** If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission.

**21. Other Conditions:**

v1.6

If you would like to pay for this license now, please remit this license along with your payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check or money order referencing your account number and this invoice number RLNK501081822.

Once you receive your invoice for this order, you may pay your invoice by credit card. Please follow instructions provided at that time.

**Make Payment To:**  
Copyright Clearance Center  
Dept 001  
P.O. Box 843006  
Boston, MA 02284-3006

For suggestions or comments regarding this order, contact RightsLink Customer Support: [customer care@copyright.com](mailto:customer care@copyright.com) or +1-877-622-5543 (toll free in the US) or +1-978-646-2777.

Gratis licenses (referencing \$0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.

---

---

## ELSEVIER LICENSE TERMS AND CONDITIONS

Aug 02, 2013

This is a License Agreement between Csaba Fekete ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

**All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.**

Supplier	Elsevier Limited The Boulevard, Langford Lane Kidlington, Oxford, OX5 1GB, UK
Registered Company Number	1982084
Customer name	Csaba Fekete
Customer address	Szigony u. 43. Budapest, 1083
License number	3200771258249
License date	Aug 02, 2013
Licensed content publisher	Elsevier
Licensed content publication	Brain Research
Licensed content title	Hypothalamic dorsomedial nucleus neurons innervate thyrotropin-releasing hormone-synthesizing neurons in the paraventricular nucleus
Licensed content author	Emese Mihály, Csaba Fekete, Gábor Légrádi, Ronald M. Lechan
Licensed content date	9 February 2001
Licensed content volume number	891
Licensed content issue number	1–2
Number of pages	12
Start Page	20
End Page	31
Type of Use	reuse in a journal/magazine
Requestor type	author of new work
Intended publisher of new work	Society for Endocrinology & BioScientifica Ltd
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No

## Order reference number

Title of the article Central regulation of the hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions

Publication new article is in Endocrine Reviews

Publisher of the new article Society for Endocrinology & BioScientifica Ltd

Author of new article Csaba Fekete and Ronald M. Lechan

Expected publication date Jan 2014

Estimated size of new article 45  
(number of pages)

Elsevier VAT number GB 494 6272 12

Permissions price 0.00 USD

VAT/Local Sales Tax 0.00 USD / 0.00 GBP

Total 0.00 USD

Terms and Conditions

## INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at <http://myaccount.copyright.com>).

## GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier at [permissions@elsevier.com](mailto:permissions@elsevier.com))

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. **Reservation of Rights:** Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. **License Contingent Upon Payment:** While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. **Warranties:** Publisher makes no representations or warranties with respect to the licensed material.

10. **Indemnity:** You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. **No Transfer of License:** This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. **No Amendment Except in Writing:** This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. **Objection to Contrary Terms:** Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. **Revocation:** Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

## **LIMITED LICENSE**

The following terms and conditions apply only to specific license types:

15. **Translation:** This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article. If this license is to re-use 1 or 2 figures then permission is granted for non-exclusive world rights in all languages.

16. **Website:** The following terms and conditions apply to electronic reserve and author websites:

**Electronic reserve:** If licensed material is to be posted to website, the web site is to be password-protected and made available only to bona fide students registered on a relevant course if:

This license was made in connection with a course,

This permission is granted for 1 year only. You may obtain a license for future website posting,

All content posted to the web site must maintain the copyright information line on the bottom of each image,

A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> or the Elsevier homepage for books at <http://www.elsevier.com> , and

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

17. **Author website** for journals with the following additional clauses:

All content posted to the web site must maintain the copyright information line on the bottom of each image, and the permission granted is limited to the personal version of your paper. You are not allowed to download and post the published electronic version of your article (whether PDF or HTML, proof or final version), nor may you scan the printed edition to create an electronic version. A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> . As part of our normal production process, you will receive an e-mail notice when your article appears on Elsevier's online service ScienceDirect ([www.sciencedirect.com](http://www.sciencedirect.com)). That e-mail will include the article's Digital Object Identifier (DOI). This number provides the electronic link to the published article and should be included in the posting of your personal version. We ask that you wait until you receive this e-mail and have the DOI to do any posting.

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

18. **Author website** for books with the following additional clauses:

Authors are permitted to place a brief summary of their work online only.

A hyper-text must be included to the Elsevier homepage at

<http://www.elsevier.com> . All content posted to the web site must maintain the copyright information line on the bottom of each image. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version.

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by

Heron/XanEdu.

19. **Website** (regular and for author): A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx>. or for books to the Elsevier homepage at <http://www.elsevier.com>

20. **Thesis/Dissertation**: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission.

21. **Other Conditions**:

v1.6

**If you would like to pay for this license now, please remit this license along with your payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check or money order referencing your account number and this invoice number RLNK501081806.**

**Once you receive your invoice for this order, you may pay your invoice by credit card. Please follow instructions provided at that time.**

**Make Payment To:**  
Copyright Clearance Center  
Dept 001  
P.O. Box 843006  
Boston, MA 02284-3006

**For suggestions or comments regarding this order, contact RightsLink Customer Support: [customercare@copyright.com](mailto:customercare@copyright.com) or +1-877-622-5543 (toll free in the US) or +1-978-646-2777.**

**Gratis licenses (referencing \$0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.**

---

---



**ELSEVIER LICENSE  
TERMS AND CONDITIONS**

Aug 02, 2013

This is a License Agreement between Csaba Fekete ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

**All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.**

Supplier	Elsevier Limited The Boulevard, Langford Lane Kidlington, Oxford, OX5 1GB, UK
Registered Company Number	1982084
Customer name	Csaba Fekete
Customer address	Szigony u. 43. Budapest, 1083
License number	3200760265168
License date	Aug 02, 2013
Licensed content publisher	Elsevier
Licensed content publication	Frontiers in Neuroendocrinology
Licensed content title	Negative feedback regulation of hypophysiotropic thyrotropin-releasing hormone (TRH) synthesizing neurons: Role of neuronal afferents and type 2 deiodinase
Licensed content author	Csaba Fekete, Ronald M. Lechan
Licensed content date	August–September 2007
Licensed content volume number	28
Licensed content issue number	2–3
Number of pages	18
Start Page	97
End Page	114
Type of Use	reuse in a journal/magazine
Requestor type	author of new work
Intended publisher of new work	Society for Endocrinology & BioScientifica Ltd
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	6
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Order reference number	
Title of the article	Central regulation of the hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions
Publication new article is in	Endocrine Reviews
Publisher of the new article	Society for Endocrinology & BioScientifica Ltd

Author of new article	Csaba Fekete and Ronald M. Lechan
Expected publication date	Jan 2014
Estimated size of new article (number of pages)	45
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 USD
VAT/Local Sales Tax	0.00 USD / 0.00 GBP
Total	0.00 USD
Terms and Conditions	

## INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at <http://myaccount.copyright.com>).

## GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier at [permissions@elsevier.com](mailto:permissions@elsevier.com))

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of

your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

### **LIMITED LICENSE**

The following terms and conditions apply only to specific license types:

15. **Translation:** This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article. If this license is to

re-use 1 or 2 figures then permission is granted for non-exclusive world rights in all languages.

16. **Website:** The following terms and conditions apply to electronic reserve and author websites:

**Electronic reserve:** If licensed material is to be posted to website, the web site is to be password-protected and made available only to bona fide students registered on a relevant course if:

This license was made in connection with a course,

This permission is granted for 1 year only. You may obtain a license for future website posting,

All content posted to the web site must maintain the copyright information line on the bottom of each image,

A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> or the Elsevier homepage for books at <http://www.elsevier.com> , and

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

17. **Author website** for journals with the following additional clauses:

All content posted to the web site must maintain the copyright information line on the bottom of each image, and the permission granted is limited to the personal version of your paper. You are not allowed to download and post the published electronic version of your article (whether PDF or HTML, proof or final version), nor may you scan the printed edition to create an electronic version. A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> . As part of our normal production process, you will receive an e-mail notice when your article appears on Elsevier's online service ScienceDirect ([www.sciencedirect.com](http://www.sciencedirect.com)). That e-mail will include the article's Digital Object Identifier (DOI). This number provides the electronic link to the published article and should be included in the posting of your personal version. We ask that you wait until you receive this e-mail and have the DOI to do any posting.

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

18. **Author website** for books with the following additional clauses:

Authors are permitted to place a brief summary of their work online only.

A hyper-text must be included to the Elsevier homepage at

<http://www.elsevier.com> . All content posted to the web site must maintain the copyright information line on the bottom of each image. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version.

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

19. **Website** (regular and for author): A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> . or for books to the Elsevier homepage at <http://www.elsevier.com>

**20. Thesis/Dissertation:** If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission.

**21. Other Conditions:**

v1.6

**If you would like to pay for this license now, please remit this license along with your payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check or money order referencing your account number and this invoice number RLNK501081744.**

**Once you receive your invoice for this order, you may pay your invoice by credit card. Please follow instructions provided at that time.**

**Make Payment To:  
Copyright Clearance Center  
Dept 001  
P.O. Box 843006  
Boston, MA 02284-3006**

**For suggestions or comments regarding this order, contact RightsLink Customer Support: [customercare@copyright.com](mailto:customercare@copyright.com) or +1-877-622-5543 (toll free in the US) or +1-978-646-2777.**

**Gratis licenses (referencing \$0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.**

---

---

## JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

Aug 02, 2013

This is a License Agreement between Csaba Fekete ("You") and John Wiley and Sons ("John Wiley and Sons") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by John Wiley and Sons, and the payment terms and conditions.

**All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.**

License Number	3200761172391
License date	Aug 02, 2013
Licensed content publisher	John Wiley and Sons
Licensed content publication	Journal of Comparative Neurology
Licensed content title	Distribution of hypophysiotropic thyrotropin-releasing hormone (TRH)-synthesizing neurons in the hypothalamic paraventricular nucleus of the mouse
Licensed copyright line	Copyright © 2010 Wiley-Liss, Inc.
Licensed content author	Andrea Kádár, Edith Sánchez, Gábor Wittmann, Praful S. Singru, Tamás Füzesi, Alessandro Marsili, P. Reed Larsen, Zsolt Liposits, Ronald M. Lechan, Csaba Fekete
Licensed content date	May 20, 2010
Start page	3948
End page	3961
Type of use	Journal
Requestor type	Author of this Wiley article
Is the reuse sponsored by or associated with a pharmaceutical or medical products company?	no
Format	Print and electronic
Portion	Figure/table
Number of figures/tables	1
Original Wiley figure/table number(s)	Figure 10
Will you be translating?	No
Title of new article	Central regulation of the hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions
Publication the new article is in	Endocrine Reviews
Publisher of new article	Society for Endocrinology & BioScientifica Ltd
Author of new article	Csaba Fekete and Ronald M. Lechan
Expected publication date of new article	Jan 2014
Estimated size of new article (pages)	45

Total 0.00 USD

[Terms and Conditions](#)

## TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a "Wiley Company") or a society for whom a Wiley Company has exclusive publishing rights in relation to a particular journal (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at <http://myaccount.copyright.com>).

### Terms and Conditions

1. The materials you have requested permission to reproduce (the "Materials") are protected by copyright.

2. You are hereby granted a personal, non-exclusive, non-sublicensable, non-transferable, worldwide, limited license to reproduce the Materials for the purpose specified in the licensing process. This license is for a one-time use only with a maximum distribution equal to the number that you identified in the licensing process. Any form of republication granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before may be distributed thereafter). The Materials shall not be used in any other manner or for any other purpose. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Material. Any third party material is expressly excluded from this permission.

3. With respect to the Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Materials without the prior permission of the respective copyright owner. You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Materials, or any of the rights granted to you hereunder to any other person.

4. The Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc or one of its related companies (WILEY) or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is

granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto.

5. NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.

6. WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

7. You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

8. IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

9. Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

10. The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

11. This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

12. Any fee required for this permission shall be non-refundable after thirty (30) days from receipt

13. These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud)



supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.

14. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.

15. WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

16. This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

17. This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

## **Wiley Open Access Terms and Conditions**

Wiley publishes Open Access articles in both its Wiley Open Access Journals program [<http://www.wileyopenaccess.com/view/index.html>] and as Online Open articles in its subscription journals. The majority of Wiley Open Access Journals have adopted the [Creative Commons Attribution License](#) (CC BY) which permits the unrestricted use, distribution, reproduction, adaptation and commercial exploitation of the article in any medium. No permission is required to use the article in this way provided that the article is properly cited and other license terms are observed. A small number of Wiley Open Access journals have retained the [Creative Commons Attribution Non Commercial License](#) (CC BY-NC), which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Online Open articles - Authors selecting Online Open are, unless particular exceptions apply, offered a choice of Creative Commons licenses. They may therefore select from the CC BY, the CC BY-NC and the [Attribution-NoDerivatives](#) (CC BY-NC-ND). The CC BY-NC-ND is more restrictive than the CC BY-NC as it does not permit adaptations or modifications without rights holder consent.

Wiley Open Access articles are protected by copyright and are posted to repositories and websites in accordance with the terms of the applicable Creative Commons license referenced on the article. At the time of deposit, Wiley Open Access articles include all changes made during peer review, copyediting, and publishing. Repositories and websites that host the article are responsible for incorporating any publisher-supplied amendments or retractions issued subsequently.

Wiley Open Access articles are also available without charge on Wiley's publishing platform, **Wiley Online Library** or any successor sites.

## Conditions applicable to all Wiley Open Access articles:

- The authors' moral rights must not be compromised. These rights include the right of "paternity" (also known as "attribution" - the right for the author to be identified as such) and "integrity" (the right for the author not to have the work altered in such a way that the author's reputation or integrity may be damaged).
- Where content in the article is identified as belonging to a third party, it is the obligation of the user to ensure that any reuse complies with the copyright policies of the owner of that content.
- If article content is copied, downloaded or otherwise reused for research and other purposes as permitted, a link to the appropriate bibliographic citation (authors, journal, article title, volume, issue, page numbers, DOI and the link to the definitive published version on Wiley Online Library) should be maintained. Copyright notices and disclaimers must not be deleted.
  - Creative Commons licenses are copyright licenses and do not confer any other rights, including but not limited to trademark or patent rights.
- Any translations, for which a prior translation agreement with Wiley has not been agreed, must prominently display the statement: "This is an unofficial translation of an article that appeared in a Wiley publication. The publisher has not endorsed this translation."

## Conditions applicable to non-commercial licenses (CC BY-NC and CC BY-NC-ND)

For non-commercial and non-promotional purposes individual non-commercial users may access, download, copy, display and redistribute to colleagues Wiley Open Access articles. In addition, articles adopting the CC BY-NC may be adapted, translated, and text- and data-mined subject to the conditions above.

## Use by commercial "for-profit" organizations

Use of non-commercial Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee. Commercial purposes include:

- Copying or downloading of articles, or linking to such articles for further redistribution, sale or licensing;
- Copying, downloading or posting by a site or service that incorporates advertising with such content;
- The inclusion or incorporation of article content in other works or services (other than normal quotations with an appropriate citation) that is then available for sale or licensing, for a fee (for example, a compilation produced for marketing purposes, inclusion in a sales pack)
- Use of article content (other than normal quotations with appropriate citation) by for-profit organizations for promotional purposes
- Linking to article content in e-mails redistributed for promotional,

marketing or educational purposes;

- Use for the purposes of monetary reward by means of sale, resale, license, loan, transfer or other form of commercial exploitation such as marketing products
- Print reprints of Wiley Open Access articles can be purchased from: [corporatesales@wiley.com](mailto:corporatesales@wiley.com)

The modification or adaptation for any purpose of an article referencing the CC BY-NC-ND License requires consent which can be requested from [RightsLink@wiley.com](mailto:RightsLink@wiley.com) .

Other Terms and Conditions:

BY CLICKING ON THE "I AGREE..." BOX, YOU ACKNOWLEDGE THAT YOU HAVE READ AND FULLY UNDERSTAND EACH OF THE SECTIONS OF AND PROVISIONS SET FORTH IN THIS AGREEMENT AND THAT YOU ARE IN AGREEMENT WITH AND ARE WILLING TO ACCEPT ALL OF YOUR OBLIGATIONS AS SET FORTH IN THIS AGREEMENT.

## v1.8

**If you would like to pay for this license now, please remit this license along with your payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check or money order referencing your account number and this invoice number RLNK501081785.**

**Once you receive your invoice for this order, you may pay your invoice by credit card. Please follow instructions provided at that time.**

**Make Payment To:**  
Copyright Clearance Center  
Dept 001  
P.O. Box 843006  
Boston, MA 02284-3006

**For suggestions or comments regarding this order, contact RightsLink Customer Support: [customer@copyright.com](mailto:customer@copyright.com) or +1-877-622-5543 (toll free in the US) or +1-978-646-2777.**

**Gratis licenses (referencing \$0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.**

---

---