Regulation of TRH neurons and energy homeostasis-related signals under stress

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Abstract

Energy homeostasis relies on a concerted response of the nervous and endocrine systems to signals evoked by intake, storage, and expenditure of fuels. Glucocorticoids (GCs) and thyroid hormones are involved in meeting immediate energy demands, thus placing the hypothalamo–pituitary–thyroid (HPT) and hypothalamo–pituitary–adrenal axes at a central interface. This review describes the mode of regulation of hypophysiotropic TRHergic neurons and the evidence supporting the concept that they act as metabolic integrators. Emphasis has been placed on i) the effects of GCs on the modulation of transcription of *Trh* *in vivo* and *in vitro*, ii) the physiological and molecular mechanisms by which acute or chronic situations of stress and energy demands affect the activity of TRHergic neurons and the HPT axis, and iii) the less explored role of non-hypophysiotropic hypothalamic TRH neurons. The partial evidence gathered so far is indicative of a contrasting involvement of distinct TRH cell types, manifested through variability in cellular phenotype and physiology, including rapid responses to energy demands for thermogenesis or physical activity and nutritional status that may be modified according to stress history.

Key Words

- ▶ HPA
- ▶ HPT
- ▶ stress
- ▶ metabolism

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Introduction

The tripeptide pglu-his-proNH₂ was isolated from hypothalami and named according to its endocrine function: ‘thyrotrophin-releasing hormone’ (TRH; Boler *et al.* 1969, Burgus *et al.* 1969). TRH is synthesized in the neuronal cell bodies of many brain regions from a precursor protein, prepro-TRH (ppTRH), and processed to yield the biological active peptide (Lechan *et al.* 1986, Lechan & Segerson 1989, Nillni 2010). Several hypothalamic nuclei synthesize TRH (Fig. 1), but the TRH neurons that control thyrotrophin (TSH) release are the hypophysiotropic neurons present in the mid–caudal paraventricular nucleus (PVN) (anterior–medial PVN in mouse), whose axons project to the median eminence

from where TRH is released into the portal vessels of the hypothalamo–pituitary system (Fekete & Lechan 2014; Fig. 1). In humans, hypophysiotropic TRH neurons are present in the medial region of the dorsocaudal portion of the PVN (Fliers *et al.* 2014).

The amount of released TRH that reaches the pituitary is modulated in the median-eminence extracellular space by the activity of the TRH-degrading ectoenzyme (pyroglutamyl peptidase II (PPII)) present on tanycytes, a group of ependymal cells lining the third ventricle whose end-feet are in proximity with TRH terminals and portal vessels (Sánchez *et al.* 2009). In the pituitary and upon binding to its receptor (TRH-R1), TRH controls the release

Figure 1

Schematic localization of TRH endocrine and nonendocrine cell bodies and TRH receptor fields in the hypothalamus and pituitary and of afferents to TRH neurons. TRH-synthesizing neurons in the rat PVN are represented in light purple, the anterior PVN and the mid-caudal PVN in dark purple. Arrows indicate afferents originating from the ARC and the brainstem that regulate the hypothalamic–pituitary–thyroid axis. AGRP co-localizes with NPY in the ARC (orexigenic peptides, blue arrow); NPY innervation to hypophysiotropic TRH neurons originates mainly from the ARC, and some (25%) from the brainstem. Hypophysiotropic TRH neurons receive afferents from ARC neurons synthesizing anorexigenic peptides cocaine–amphetamine regulated transcript (CART)/ α -MSH and CART expressed in adrenergic C1–3 regions in the medulla. ARC neurons expressing the orexigenic peptide galanin innervate the anterior PVN parvocellular subdivision (aPVN), mid and caudal PVN, and GALP only aPVN (pink arrows). Catecholaminergic inputs arising from the brainstem play a key role in regulation of the HPT axis in response to cold. Both noradrenergic

(LC and NTS, orange arrows) and adrenergic (C1–3 regions, brown arrow) axons establish contacts with TRH neurons in the PVN (Fekete & Lechan 2014). AH, anterior hypothalamic area; ARC, arcuate hypothalamic nucleus; AVPO, anteroventral preoptic nucleus; DMH, dorsomedial hypothalamic nucleus; GALP, galanin-like peptide; LA, lateroanterior hypothalamic nucleus; LC, locus coeruleus; LH, lateral hypothalamic area; ME, median eminence; MPA, medial preoptic area; MPO, medial preoptic nucleus; NTS, nucleus solitary tract; OX, optic chiasm; Ppit, posterior lobe of pituitary; PVN, paraventricular hypothalamic nucleus; RCH, retrochiasmatic area; SCh, supraoptic nucleus; SOX, supraoptic decussation; VMH, ventromedial hypothalamic nucleus; 3V, third ventricle. The figure is based on Paxinos & Watson (2005) (The Rat Brain, 5th Edn): Fig. 78 (lateral 0.4 mm) and coronal sections (Figs 24–33; Bregma $-1.4/-3.8$) to delineate approximate location of hypothalamic nuclei; PVN division in Simmons & Swanson (2009)).

of TSH and its transcription and posttranslational modifications, such as glycosylation, that define the bioactivity of TSH (Chiamolera & Wondisford 2009). Under certain physiological conditions, TRH modulates prolactin or growth hormone synthesis and release (Galas *et al.* 2009). The concentration of pituitary TRH-R1 is regulated by TRH and several other hormones (Chiamolera *et al.* 2012, Hinkle *et al.* 2012). Released TSH controls several steps of the synthesis of thyroid hormones (TH) at the thyroid gland, increasing serum levels of thyroxine (T_4) and to a minor degree, 3,5,3'-triiodothyronine (T_3). Deiodinases 1 or 2 (D1, D2) convert T_4 to T_3 in various

tissues and their activity is differentially regulated in a condition- and tissue-specific manner (McAninch & Bianco 2014). TH have crucial roles in basal metabolic rate, thermogenesis, lipid and carbohydrate metabolism, indicating that the hypothalamus–pituitary–thyroid (HPT) axis is an important player in energy homeostasis (Hollenberg 2008, Fliers *et al.* 2014, McAninch & Bianco 2014, Mullur *et al.* 2014).

The activity of the HPT axis is stringently regulated by neuronal stimuli impinging on TRH neurons and by the negative-feedback effects of TH on TRH and TSH synthesis and release (Hollenberg 2008, Fekete & Lechan 2014,

Figure 2

Schematic representation of TRH synthesis. (A) The primary transcript is synthesized in the nucleus as prepro-TRH heterogeneous nuclear RNA (hnRNA) which contains two introns and five exons. (B) After splicing, mature RNA is transported to the cytosol, binds to ribosomes, begins transcription of prepro-TRH mRNA, the leader sequence is cleaved, synthesis of pro-TRH continues with ribosomes linked to rough endoplasmic reticulum (RER) and precursor is transported inside the ER. (C) At the transGolgi pro-TRH may suffer a first cleavage by protein convertase 1 (PC1), proTRH is compartmentalized in secretory granules with the rest of

processing enzymes: PCs cleave at the carboxy end of a pair of basic residues, a carboxypeptidase cleaves the basic residues leaving the immediate precursor of TRH: gln-his-progly (black squares) and cryptic peptides (green); pyroglutaminase converts gln to pyroglu and peptidyl-glycine- α -amidase (PAM) leaves the amino group of the glycine bound to the carboxyl end (forming the amide group) and cleaves the rest of the pro-carbon moieties. Processing occurs as the secretory granule is transported to the nerve terminal (Nillni 2010).

Fliers *et al.* 2014). T_3 downregulation of *Trh* mRNA levels occurs specifically in the hypophysiotropic neurons of the PVN and not in other hypothalamic nuclei expressing *Trh* in rat or mouse (Segerson *et al.* 1987, Sugrue *et al.* 2010). The neuronal concentration of T_3 in the PVN is controlled by D2-conversion of T_4 in tanycytes, the presence of T_3 transporters, and the activity of neuronal D3 that inactivates T_3 , converting it to rT_3 (Fliers *et al.* 2006, Fekete & Lechan 2014). The most commonly used sensor of activation of TRH neurons has been the measurement of *Trh* mRNA levels. Rapid (<2 h) changes in TRH tissue concentration, mostly concentrated at nerve endings have been used as an indirect marker of TRH release because at later times TRH content represents the resultant of synthesis and release but not of degradation because the intracellular peptidases do not modulate TRH levels within secretory granules (Rondeel *et al.* 1991,

van Haasteren *et al.* 1995, Charli *et al.* 1998, Aguilar-Valles *et al.* 2007).

TRH biosynthesis

The *Trh* gene encodes a protein that contains 5–8 (depending on the species, Wallis 2010) repetitions of the gln-his-pro-gly sequence (Lechan *et al.* 1986). Like other neuropeptides, the precursor ppTRH is synthesized in the neuronal soma at the endoplasmic reticulum (Fig. 2B) where it is cleaved to proTRH, compartmentalized at the transGolgi into secretory granules that travel together with processing enzymes to the nerve endings where most of TRH is found (Nillni 2010) (Fig. 2C).

Trh mRNA is transcribed from a unique gene (Fig. 2A); the proximal promoter equivalent to 250 bp upstream of the TATA box contains several response elements (REs) that

Figure 3

Sequences of response elements for transcription factors proven to be involved in regulating *Trh* transcription. (A) T_3 binds to thyroid hormone receptor THR β on site 4 or THRE; corticosterone to GR-cJun on composite GRE (GRE1/2-AP1); leptin to STAT3, and pCREB on CRE; SP1 or KPL4 bind to extended CRE (green dashed line) (Lee *et al.* 1988, Díaz-Gallardo *et al.* 2010a,b, Cote-Vélez *et al.* 2011). (B) Stimulation of transcription has been observed after treatment with noradrenaline, BDNF, and ERK, PKA activators that stimulate phosphorylation of CREB which binds to CRE; PKA activators also induce binding of SP1; pCREB recruits CBP with acetylase

activity that releases the complex of histones and chromatin allowing binding of polymerase II (Pol II) and transcription machinery to proceed with transcription. This mechanism may occur with other transcription factors that present stimulatory activity such as STAT3 or GR-cJun. (Díaz-Gallardo *et al.* 2010b, Cote-Vélez *et al.* 2011). (C) Transcriptional repression of *Trh* transcription may occur by T_3 binding to THR on site 4, recruiting corepressors and deacetylases that compact chromatin avoiding binding of polymerase and basal transcriptional machinery (Hollenberg 2008, Díaz-Gallardo *et al.* 2010a).

provide binding sites for specific transcription factors (TFs) whose activity is modulated by various signals (Lee *et al.* 1988; Fig. 3A). Chromatin remodeling constitutes the first step in transcriptional regulation; recruitment of specific TF, coregulators, and histone acetylases loosens the DNA from histones allowing initiation of transcription by RNA polymerase II (Pol II; Fig. 3B); certain TFs recruit histone deacetylases that compact chromatin repressing transcription (Gadaleta & Magnani 2014; Fig. 3C).

The negative regulation of TRH and TSH synthesis has long been recognized, but the mechanism is still under study (Segerson *et al.* 1987, Guissouma *et al.* 2000, Hollenberg 2008, Chiamolera *et al.* 2012, Vella *et al.* 2014). T_3 binds to TH receptors (THRs) THR α 1, or THR β 1 and THR β 2, that are expressed and regulated in a tissue-specific manner; the same receptor may stimulate or inhibit transcription of multiple proteins in the same tissue (Ramadoss *et al.* 2014). The THR RE in the *Trh*

promoter (site 4, Fig. 3A) binds THRs as homo- or heterodimers with retinoid X receptors (RXRs) *in vitro*; in transiently transfected cells, unliganded receptors activate *Trh* transcription whereas repression requires T_3 to be bound to the receptor (Guissouma *et al.* 2000, Hollenberg 2008, Chiamolera & Wondisford 2009; Fig. 4). Mice with knock-outs of the different THRs were used to demonstrate THR β 2 to be the most important receptor involved in downregulation of *Trh* transcription by T_3 (Abel *et al.* 2001, Chiamolera & Wondisford 2009); however, transfection of *Thr* β -KO mice with either THR β 1 or THR β 2 rescues T_3 -induced TRH expression, but only THR β 1 is able to activate in a ligand-independent manner (Dupré *et al.* 2004). Chromatin immunoprecipitation (ChIP) from stably transfected GH4C1 cells with *Trh* promoter detects acetylated histones 3 and 4, THR β , and bound Pol II; T_3 treatment transiently recruits deacetylases (HDAC2, 3), diminishes Pol II binding and, after 24 h, also THR β

Figure 4

Model of rapid regulation of *Trh* transcription by extracellular signals. The transcription of the pro-*TRH* gene is rapidly increased by activators of CREB phosphorylation and by glucocorticoids. Noradrenaline (NA), BDNF, and dexamethasone or corticosterone, through protein kinase A (PKA), the ERKs MEK/ERK, and serum-glucocorticoid kinase (Sgk), induce the phosphorylation of CREB; pCREB enhances transcription through an interaction with the CRE2 site. Activation of the glucocorticoid receptor (GR) by ligand binding releases it from a multiprotein complex; it is phosphorylated and transported to the nucleus where the GR:Jun heterodimer binds to a composite GRE (cGRE). A cross talk between the two pathways alters the individual transcriptional responses; glucocorticoids interfere with PKA stimulation of *Trh* transcription by a protein–protein interaction that impedes binding of pCREB or GR to their REs (Cote-Vélez *et al.* 2008, 2011, Díaz-Gallardo *et al.* 2010b and references therein).

(Umezawa *et al.* 2009). THRβ2 binds to the *Trh* proximal promoter region of chromatin from hypothalamic cells incubated 1 h with T₃ (Díaz-Gallardo *et al.* 2010a), as well as deacetylase (HDAC3), whereas Pol II and histone acetylases diminish compared with controls (Sotelo-Rivera *et al.* 2012). Chromatin from hypothalami of newborns has THRβ or RXR bound to TRH promoter which decreases after 20 h of T₃ treatment (Decherf *et al.* 2013). These results support the hypothesis that T₃–THRβ recruitment of deacetylases that may compact chromatin and inhibit Pol II binding and *Trh* transcription (Fig. 3C). Whether THRβ remains bound to DNA *in vivo* after longer T₃-treatments awaits resolution (Ramadoss *et al.* 2014).

Neuronal stimulation activates kinases such as PKA, PKC, and MAPK/ERK that rapidly enhance *Trh* transcription or mRNA levels in several cell systems (Uribe *et al.* 1995a, Pérez-Martínez *et al.* 1998, Harris *et al.* 2001, Cote-Vélez *et al.* 2005, 2008). The *Trh* proximal promoter contains a

phosphorylated-CREB (pCREB) binding site (CRE2: positions –101/–92 in rat) that is protected, along with adjacent 5' GC box and 3' CACC sequences, by nuclear extracts of cAMP-stimulated hypothalamic cells (Fig. 3A; Díaz-Gallardo *et al.* 2010a,b); these adjacent sites are recognized by SP1/Krüppel-like factors (Kpl; Ren *et al.* 1998, Díaz-Gallardo *et al.* 2010b, Pérez-Monter *et al.* 2011). Deleted or mutated CRE2 or CACC sites resulted in a decrease of 50–80% in basal and 50% in forskolin-induced transcription (the latter also with mutated GC box; Cote-Vélez *et al.* 2011). Multiple-intracellular signals phosphorylate CREB in the nucleus, pCREB binds to its REs and interacts with coactivators (some with histone-acetylase activity) increasing transcription (Fig. 3B; Altarejos & Montminy 2011). SP1 binding may increase the stability of pCREB on CRE2, as has been proposed for a nonconical sequence (Lundblad *et al.* 1998) or, depending on the TF of the SP/KPl family, cause interference. ChIP assays revealed other REs, such as the STAT-binding site, responsive to leptin stimulation (Guo *et al.* 2004). A composite glucocorticoid RE (cGRE), formed from a half-site GRE next to an AP1 RE, binds the glucocorticoid receptor (GR) as a heterodimer with cJun after dexamethasone stimulation (Cote-Vélez *et al.* 2005, Díaz-Gallardo *et al.* 2010b; Fig. 3A).

Caveats regarding biosynthesis studies

Before continuing with this review we would like to stress some problems worthy of consideration when studying the regulation of TRH biosynthesis, given the diversity of experimental paradigms used. Cell lines are homogenous but with a particular set up that differs from the physiological situation, they are usually incubated with drugs for long periods and under steady conditions in contrast to *in vivo* situations where events such as clearance and diffusion play important roles. Transient transfections omit the regulatory steps related to chromatin remodeling (Gadaleta & Magnani 2014). Primary culture of embryonic hypothalamic cells (Uribe *et al.* 1995a, Harris *et al.* 2001), or transfected hypothalami of newborn pups (Guissouma *et al.* 2000), involve a mixture of multiple hypothalamic nuclei whose developmental windows, afferents and receptors, might differ to those exclusive to the PVN; furthermore, they miss the developmental effects of gonadal hormones (McCutcheon & Marinelli 2009). Knockout animals provide important information (Supplementary Table 1, see section on supplementary data given at the end of this article), but early-compensatory responses may lead to altered homeostatic states and/or the animals may have phenotypes that result from the simultaneous

manipulation of many organs that contribute, for example, to altered TH levels (Astapova & Hollenberg 2013); however, they revealed the redundancy of important regulatory molecules (Chiappini *et al.* 2013). Notwithstanding the problems mentioned, these distinct approaches have provided important information for identifying potential modes of *Trh* transcriptional regulation.

The HPT axis in energy balance

Circuits involved in energy balance overlap with those involved in food intake. Afferents from neurons of the brain stem or of the arcuate nucleus (ARC) convey nutritional information to the PVN and other hypothalamic nuclei. ARC neurons detect circulating hormones levels modulated by the nutritional and energy status (ghrelin, leptin, insulin, glucose, and fatty acids) and activate or inhibit neurons that express orexigenic (neuropeptide Y (NPY) and agouti-related peptide (AgRP)) or anorexigenic (pro-opiomelanocortin (POMC) and cocaine amphetamine-regulated transcript (CART) peptides) (Schneeberger *et al.* 2014); *Trh* expression is inhibited by orexigenic and stimulated by anorexigenic peptides (Fekete & Lechan 2014). Table 1 summarizes data on mRNA levels of *Trh* and several ARC peptides, as well as on serum hormones in rodents studied under different paradigms. Caution is required to distinguish measurements in the hypophysiotropic area (approximately 50% of parvicellular PVN neurons (Simmons & Swanson 2009)) normally determined by *in situ* hybridization (ISH) of *Trh* mRNA in mid-caudal PVN, against total PVN by analysis in dissected tissue (Fig. 1; Fekete & Lechan 2014).

TRH-hypophysiotropic neurons are the central integrator of the HPT axis, considered to be metabolic sensors as they decode neuronal and hormonal signals related to the energy status (Lechan & Fekete 2006, Hollenberg 2008). THs have multiple targets responsible for energy distribution and expenditure (Klieverik *et al.* 2009, Mullur *et al.* 2014). Negative energy balance such as fasting, food restriction, or pathological situations, including infection or critical illness, decreases serum levels of TH, and TSH to some extent, as well as of *Trh* expression in hypophysiotropic TRH neurons. In humans, these conditions are recognized as nonthyroidal illness syndrome (NTIS). The principal mechanism of fasting-induced decreased *Trh* expression involves a drop in leptin serum concentrations that lowers α -MSH and CART stimulatory tone, and enhances the inhibitory effect of NPY/AgRP on TRHergic neurons (Fekete & Lechan 2014). Results from animal models of infection or inflammation support the

hypothesis that the mechanism causing NTIS primarily entails increased D2 activity in tanocytes and reduced D1 and D3 in liver and muscle, changes probably produced by cytokines and not by corticosterone (Fekete & Lechan 2014, Fliers *et al.* 2014). The effects are more pronounced in chronic situations where multiple factors intervene, including feeding status. In humans, postmortem analyses of critically ill patients showed low T_4 and T_3 liver concentrations and reduced *TRH* mRNA expression, supporting inhibition of the HPT axis at central level (Fliers *et al.* 2014).

The effects of energy excess on TRHergic neurons are less clear. Body-weight gain (approximately 20%) induced by high-fat diet (HFD), or ovariectomy, is associated with increases in *Trh* expression in the PVN; both conditions augment circulating leptin levels, although only HFD increases TH serum concentrations in rat (Perello *et al.* 2010). Leptin rapidly activates TRHergic neurons, indirectly in mid-PVN through its effects on ARC neurons and directly in caudal-PVN with high concentration of leptin receptors (Huo *et al.* 2004, Perello *et al.* 2006). Leptin's direct effect is further supported by the exclusive increase in *Trh* mRNA in caudal PVN of ovariectomized rats (Uribe *et al.* 2009). The response of TRHergic neurons to feeding conditions differs in obese and lean rodents (Perello *et al.* 2010); under basal conditions *Trh* mRNA levels in the PVN are similar in obese and in lean Zucker rats; however, fasting decreases *Trh* expression more drastically in obese rats than in lean rats, a difference overridden by adrenalectomy (Duclos *et al.* 2005). A different allostatic state in obesity, related to a particular state of receptors causing leptin or insulin resistance, could be the basis of these differences.

Events involved in the regulation of energy balance such as fat metabolism, thermogenesis, body weight, food intake, and fat distribution is sex-dependent. Estrogen modulates the expression of several orexigenic and anorexigenic peptides and hormones (Brown & Clegg 2010). Male rats are more sensitive to insulin and females to leptin; compared with males, females adapt more efficiently to energy deficits, are more susceptible to HPT inhibition by food restriction, have lower pituitary expression of TSH β and serum TSH associated with higher levels of T_3 , and reduced hepatic activity of D1 under basal conditions (Cizza *et al.* 1996, van Haasteren *et al.* 1996, Valle *et al.* 2005, Marassi *et al.* 2007). Estrogen stimulates thyroid function, increasing iodide uptake and thyroid peroxidase activity which would increase TH biosynthesis (Lima *et al.* 2006). The expression of *Trh* in the PVN varies during the estrous cycle with highest levels at diestrous 2

Table 1 Regulation of the HPT axis under chronic or acute stress. The HPT axis is differentially regulated depending on the origin of stress (physical or psychogenic), the duration (acute or chronic), and the animal's energetic and hormonal status

Rodent strain	Acute treatment	Chronic treatment	Food intake	BW or body fat	Hypothalamic TRH peptide	PVN (mRNA)			Arc (mRNA)				TSH	TT ₄	TT ₃	Cort	References	
						Trh	Crh	AgRP	Npy	Cart	Pomc	Bdnf						
Lou/C rat		vs Wistar		↓		+ ↑		* ↑	* ↑	* -	+ ↓	+ ↑		↑	↓	-	↓	Mitchell <i>et al.</i> (2006) and Veyrat-Durebex <i>et al.</i> (2013)
S-D		64 h fast Refeeding				+ * ↓ * 24 h ↑		* ↑ * 4 h ↓	* ↑ * ↓				↓ 24 h ↑	↓	↓	↑	↑	Rondeel <i>et al.</i> (1992) and Sánchez <i>et al.</i> (2008)
S-D		HFD	↑	↑	↑ 3–6 h	+ ↑							↑ 3 h	↑ & FT ₄	↑ & FT ₃			Perello <i>et al.</i> (2006, 2010)
Wistar		Leptin i.c.v. FR 7 days (80%) DIA 7 days DIA/CRH-R2 antagonist	↓	↓	↑	+ ↓	+ ↓		+ ↓ Y1R + - Y1R				-	↓	↓	↑	↑	Jaimes-Hoy <i>et al.</i> (2008) and de Gortari <i>et al.</i> (2009)
C57BL/6J		(-) Leu (7 days)	↓	↓		↑ Regulated by CREB							↑	↓	↓	↑	↑	Xia <i>et al.</i> (2015)
CFY rat		(-) Leu + anti-TRH		-												↓		
Wistar		Protein free diet (12 days) MWM trained 5 days	↓	↓		* ↓							↓	↓	↓			Shi <i>et al.</i> (1993)
Wistar		14 days: exercise vs pair-fed	-	Fat: ↓	-	+ ↑	+ ↑						-	↑	-	↑	↓	Aguilar-Valles <i>et al.</i> (2005, 2007)
Wistar		OVX	↑	↑	-	+ ↑							-			↑		Uribe <i>et al.</i> (2009)
Wistar		OVX + E ₂	↓	↓	-	↓							↑		-	↑		
Wistar	1 dose	Rimonabant	↓	↓		+ ↑	+ ↑		↓	-	↓		↑		-			
C57BL/6J		Daily for 7 days AMIO i.p.: WT	-	-		-	-											Rorato <i>et al.</i> (2013)
Wistar		D2KO	↓			* ↓							↑	-	-			Rosene <i>et al.</i> (2010)
Wistar	L-NAME s.c.				↑ 1 h	# ↑ 2 h							↓ 1 h			-		Uribe <i>et al.</i> (2011)
S-D	Ethanol i.p.				↓ 6 h	* ↑ 6 h PVN							↓ 1 h	↓ & FT ₄	↓ & FT ₃			Zoeller <i>et al.</i> (1995)

Table 1 Continued

Rodent strain	Acute treatment	Chronic treatment	Food intake	BW or body fat	Hypothalamic TRH peptide	PVN (mRNA)			Arc (mRNA)				TSH	TT ₄	TT ₃	Cort	References
						Trh	Crh		AgRP	Npy	Cart	Pomc					
S-D ₁ Wistar	Cold 4 °C 1 h					+ * ↑								↑	↑ 3 h	↑	Uribe et al. (1993),
	Suckling + 30 min cold					* ↑								↑		–	Zoeller et al. (1995), Sánchez et al. (2001) and
	CORT i.p. + 1 h cold vs veh. cold					–								↓		–	↓
Wistar	exposed Open field test				–	+ ↑ 15 min								↑ 30 min	↑ 1 h	↑	Gutiérrez-Mariscal et al. (2012)

Symbols: (*) quantified by ISH (medial PVN); (+) semi-quantified by RT-PCR (total PVN); (#) quantified by northern blot (total PVN). α -MSH, melanocyte-stimulating hormone alpha; AMIO, amiodarone; Arc, arcuate nucleus; Cort, corticosterone; CREB, cAMP response element binding; CRH2, corticotrophin releasing hormone type 2 receptor; DZKO, deiodinase 2 knockout; DIA, dehydration-induced anorexia; E₂, 17 β -estradiol; Food intake, food intake; FR, food restriction; HFD, high-fat diet; ISH, *in situ* hybridization; L-NAME, N^G-nitro-L-arginine methyl ester; Leu, leucine; MWM, Morris water maze; OVX, ovariectomized; POMC, proopiomelanocortin; PVN, paraventricular nucleus; Rimobant, type 1 endocannabinoid receptor blocker; S-D, Sprague-Dawley; Y1r, neuropeptide Y type 1 receptor.

(Uribe *et al.* 1991). However, the effects of estrogen and the consequences of ovariectomy on serum TH and TSH concentrations are controversial and depend on the paradigm studied and age and previous gonadal status (Lima *et al.* 2006, Marassi *et al.* 2007, Uribe *et al.* 2009). Consistent with role of TRH in prolactin secretion, *Trh* mRNA levels increase, parallel to prolactin serum concentration, during the lactation period and in response to suckling (Uribe *et al.* 1993). As lactation progresses, levels of *Trh* mRNA decrease whereas those of prolactin rise; removal of pups normalizes *Trh* mRNA expression proportional to weaning time and inversely proportional to serum corticosterone levels (Uribe *et al.* 1991, 1995b).

Stress and the HPT axis

Stress was defined by Selye as the nonspecific response of the body to any demand (Selye 1976). This definition has been revised (Koolhaas *et al.* 2011). The type of stress defines the nature of the response; physiological (systemic) stressors such as cold, pain, and disease involve neuronal circuits that usually decode stimuli at the level of the brain stem, whereas psychogenic stressors are motivated by previous experiences and involve limbic areas such as the amygdala, hippocampus, and frontal cortex; all directly or indirectly converge at the PVN and activate the sympathetic nervous system and the hypothalamus–pituitary–adrenal (HPA) axis (de Kloet 2014, Myers *et al.* 2014). The response of the HPA axis is characterized by increased synthesis and release of corticotrophin-releasing hormone (CRH) from the PVN, adrenocorticotrophin hormone from the pituitary, and glucocorticoids (GCs) from the adrenal cortex. In rodents, paradigms of psychogenic stressors include restraint, chronic defeat stress, or isolation; their responses differ according to the controllability of the stressor, whether the animal senses a previously encountered stressor (homotypic) that causes habituation or a new one (heterotypic) to which a hyper-reaction may occur (Koolhaas *et al.* 2011, Myers *et al.* 2014).

Stress, whether chronic or acute, modifies eating patterns and metabolism, and is considered to be at the base of the metabolic syndrome (Charmandari *et al.* 2005, Maniam & Morris 2012). GC excess or deficiency leads to several metabolic problems (Rose & Herzig 2013). As these hormones act in multiple organs, it is difficult to distinguish direct effects from indirect effects in paradigms such as adrenalectomy or thyroidectomy and hormone replacement. Chronic stress may increase or decrease food intake in humans whereas in animals this usually diminishes unless they are offered a

palatable food, although abdominal adiposity increases (Dallman *et al.* 2007). Chronic GC administration increases *Npy* and *Agrp* expression, the activity of their synthesizing neurons, alters the melanocortin system; and increases food intake; it causes multiple metabolic changes such as insulin resistance, visceral fat accumulation, high serum leptin levels (Dallman *et al.* 2007, Maniam & Morris 2012) and decreased *Trh* expression in the PVN and TSH serum concentrations in rat and in human (Kakucska *et al.* 1995, Alkemade *et al.* 2005). The GC-induced increase in NPY expression could contribute to GC-inhibitory effect on *Trh* mRNA levels in the PVN. Patients with Cushing's disease present hypothyroidism and *Trh* expression in the PVN is reduced in postmortem brains of patients that received GC treatment or suffered major depression (Alkemade *et al.* 2005).

Various forms of long-term psychogenic stress decrease TSH and TH serum levels in rats (Armario & Castellanos 1984, Servatius *et al.* 2000), but reports on *Trh* expression are scarce. Constriction injury of sciatic nerve decreases *Trh* mRNA levels in the whole hypothalamus as well as serum TH levels (Kilburn-Watt *et al.* 2010). Foot shock (14 sessions/day) decreases total and free T_4 and T_3 serum concentrations without affecting *Trh* in the PVN, whereas *Agrp* mRNA levels in the ARC increase (Helmreich *et al.* 2005); a milder form of stress, such as 60 min daily restraint for 2 weeks, does not affect *Trh* mRNA expression in the PVN nor TSH or TH serum levels (Uribe *et al.* 2014). The intensity of the stressors thus seems to determine HPT activity and *Trh* expression; however, despite significant changes not being observed in the latter two paradigms, levels of *Trh* mRNA correlate negatively with those of corticosterone and positively, with body-weight changes.

Situations of increased energy expenditure such as exercise and cold are also considered stressful. Cold exposure rapidly activates the autonomic nervous system and the HPT and the HPA axes. Thermogenesis is achieved primarily by the activity of brown adipose tissue (BAT) and the uncoupling proteins through adrenergic stimulation in concert with TH and lipolysis (Mullur *et al.* 2014). Three-day cold exposure of rats increases BAT D2 and its thermogenic activity, D2 increases in skeletal muscle after 10 days and, total and free T_3 increase whereas total T_4 decreases at both times, indicating increased peripheral TH conversion (Louzada *et al.* 2014). Whether long-term cold exposure is constant or intermittent, the metabolic and behavioral effects (increased food intake or physical activity) vary because adaptive changes occur, although data on the effects of chronic cold exposure on TRH expression are lacking and changes in TSH or TH serum

levels depend on the condition studied (Silva 2011, Yoo *et al.* 2014). Human beings adapt to prolonged exposure to a cold environment by increasing metabolic and physiologic responses; after moving to the Antarctic, energy requirements increase, as well as T_3 production, clearance and distribution, TRH-stimulated TSH release, TSH and thyroglobulin serum levels, while altered total and free TH levels may depend on altitude differences (Palinkas *et al.* 2007). When exposed for 60 h to 50% energy restriction, humans lose considerable body weight but do not decrease TH or TSH serum levels as in temperate conditions, which indicates that cold stimulation overcomes the inhibition produced by food restriction (Case *et al.* 2006).

Exercise is proposed to be a chronic stressor that inhibits HPT and activates HPA axes (Mastorakos & Pavlatou 2005). This depends on the type of exercise, i.e. extenuating or forced versus voluntary (as wheel running; Steinacker *et al.* 2005, Stranahan *et al.* 2008). Compared with naïve sedentary controls, 2-week voluntary running reduces food ingestion (18%); the decreased energy intake in the sedentary pair-fed group causes similar changes to those caused by 40% food restriction for 25 days in rats, decreased levels of serum leptin, TSH and T_3 , BAT-D2, and liver-D1 activities, and increased corticosterone (Araujo *et al.* 2009). Exercise blunted several of the changes produced by the 18% reduced food intake, i.e. *Trh* diminished 30% instead of the 50% seen in the pair-fed rats, and neither T_3 or deiodinases activities were altered; however, white adipose tissue (WAT) mass diminished in exercised rats whose serum leptin concentration decreased much more than that of the pair-fed rats; furthermore, the amount of exercise performed correlated positively with T_3 and *Trh* mRNA levels and negatively with WAT mass (Uribe *et al.* 2014). Exercise thus overrides the signals of energy deficiency, such as low serum leptin concentration; the intermittent activation of PVN–TRH neurons and of TH release (seen after an acute increase in physical activity, see below) may guarantee maintenance of TH levels for adequate fuel supply to oxidizing tissues, such as released fatty acids from WAT (Klieverik *et al.* 2009, Weber 2011). Hypophysiotropic TRHergic neurons may therefore be included in a central homeostatic circuit modulated by exercise, which by maintaining adequate TH-induced lipolysis or serum glucose levels through PVN–sympathetic and parasympathetic hepatic activity guarantee energy supply to metabolic tissues (Fliers *et al.* 2014).

TRH expression in the hypophysiotropic neuron at a given time depends thus on the concentration of T_3 , THR and co-regulators, nutritional status, stress history, and the extent of neuronal and hormonal influences that sense the

'basal' state of the organism and regulate TRH synthesis and release – a process further modified by the environmental influences it must contend with. The HPT axis is inhibited in response to energy deficits or chronic stress. However, animals submitted to energy-demanding situations, such as chronic cold or exercise, have altered HPT axis activity and may present high or normal circulating levels of TH, although *Trh* expression in the PVN and serum TSH levels may be differentially affected (Table 1).

HPT responses to acute stimuli

Several neuromodulators regulate the excitability of TRHergic neurons of the PVN; NPY inhibits neuronal activity whereas α -MSH and leptin stimulates it, effects consistent with the known responses on TSH or T₄ release (Ghamari-Langroudi *et al.* 2010). GCs rapidly suppress glutamatergic excitatory inputs onto various parvocellular neurons of the PVN, including those expressing TRH; the effect is mediated through membrane receptors, whose activation causes the release of endocannabinoids that act at the presynaptic side to inhibit glutamate release (Di *et al.* 2003). TRH neurons in the PVN receive afferents from the suprachiasmatic nucleus that modulate TRH expression in a circadian manner (Kalsbeek *et al.* 2000, Zoeller *et al.* 1990).

Acute stress inhibits the HPT axis at different levels; 1 h restraint or 2 h immobilization decrease *Trh* mRNA expression in the rat PVN together with TRH and TSH release during the following hour; the response to immobilization is higher in female rats (Cizza *et al.* 1996, Gutiérrez-Mariscal *et al.* 2012). However, a stressful condition, such as the defensive burying test that involves increased physical activity (burying), augments the levels of *Trh* mRNA in the PVN but not TSH release (Gutiérrez-Mariscal *et al.* 2008). Situations of energy demand, such as physical activity or cold exposure, cause an immediate response of the HPT and the HPA axes. TH and GCs are rapidly released; the latter mobilize glucose for fast responses and the former activate fuel distribution to oxidizing tissues (Klieverik *et al.* 2009, Rose & Herzig 2013). This is coupled with changes in TRH biosynthesis. For example, physical activity in the open field increases the expression of *Trh* in the PVN accompanied by enhanced TRH and TSH release, but only if animals are tested during the dark period. The stress response is stronger in animals tested during the light hours when the HPT response is blunted (Gutiérrez-Mariscal *et al.* 2012). Another type of exercise, such as swimming in a water maze, activates the HPT, increasing the expression of

Trh mRNA in the PVN and the release of TSH; the response of the HPT is opposite to the degree of stress induced by the test (Aguilar-Valles *et al.* 2005, 2007).

In response to a cold stress, catecholaminergic afferents from the brain stem onto the PVN rapidly contribute to increase *Crh* and *Trh* mRNA levels in the PVN, concomitant with the release of TRH, TSH, and corticosterone (Zoeller *et al.* 1990, 1995, Rondeel *et al.* 1991, Uribe *et al.* 1993). The changes in *Trh* mRNA are transient, with increases at 1 h and normalizing by 2 h, even when animals are kept in the cold for long periods; *Trh* mRNA levels rise again at 6 h but only if animals are exposed during the light period (Uribe *et al.* 1993, Zoeller *et al.* 1995). Acute administration of some drugs alters *Trh* expression and these changes do not always coincide with changes in TSH or TH serum concentrations (Table 1). Although ethanol increases *Trh* mRNA levels in the PVN, it blocks increases induced by cold-exposure (Zoeller *et al.* 1995). Cold increases *Trh* mRNA expression independent of high-TH serum levels (Zoeller *et al.* 1990), but a mild stress 2 h before being submitted to cold suppresses the expected increase in *Trh* mRNA levels in the PVN but not in serum TSH in male rats (Uribe *et al.* 2011).

Cold exposure activates the HPT axis more in non-lactating female rats than in males or lactating females (Sánchez *et al.* 2001). Ovariectomized rats with increased body weight and high leptin serum concentration respond to cold stimulation augmenting *Trh* mRNA levels in the PVN, but the effect is suppressed by high doses of 17 β -estradiol, while serum TSH and corticosterone levels are similar to those found in the ovariectomized animals (Uribe *et al.* 2009). In contrast, food-restricted female rats display greater increases in *Trh* mRNA levels in the PVN than controls after cold exposure, but TSH response is blunted (Jaimes-Hoy *et al.* 2008). These results exemplify situations in which the response to a life-threatening situation such as cold exposure is altered by stress, corticosterone, sex, and other as yet unidentified effectors.

As mentioned, TRH is involved in prolactin release during lactation (Galas *et al.* 2009), and the levels of TRH mRNA increase in mid-PVN 30 min after initiation of suckling (Sánchez *et al.* 2001). TRH hypophysiotropic neurons co-express CART, which is also induced after 1 h of cold exposure (Sánchez *et al.* 2007) but not after suckling. Since CART inhibits prolactin release *in vitro* and cold exposure does not induce the release of prolactin, CART may serve as a co-modulator of TRH in this physiological circumstance, stimulating TRH and TSH release while blocking prolactin release (Sánchez *et al.* 2001, 2007, Raptis *et al.* 2004).

Because GCs affect not only the electrical activity of PVN–TRH neurons, but potentially the expression of *Trh* in rodents and humans, it is relevant to understand how they interact with the stimuli that control the HPT axis. The timing of GR activation is of utmost importance; GR is released from a cytosolic multi-protein complex upon ligand binding, phosphorylated, and translocated to the nucleus within minutes, where it acts as a transcription factor. The dynamics of GR transport and its half life depend on the presence of ligand, DNA binding, and activity of several kinases (Ratman *et al.* 2013, de Kloet 2014). The response of PVN *Crh* expression to restraint is blunted if animals receive corticosterone 1 h but not 3 h previously, supporting the relevance of timing of GR activation; in contrast, the increase in c-FOS due to restraint is not diminished by corticosterone pretreatment (Osterlund & Spencer 2011). *Trh* mRNA levels in the PVN correlate negatively with corticosterone serum concentrations in animals under several circumstances; therefore, we studied the effect of a peripheral corticosterone injection on cold-induced *Trh* expression in the PVN. In male rats kept at room temperature, *Trh* mRNA expression is enhanced (greater than threefold) 2 h after corticosterone injection in the mid- and caudal-PVN, where TRHergic neurons co-express GR (Cintra *et al.* 1990). Rats that received injections of vehicle responded to cold as expected, increasing *Trh* mRNA levels in the three zones of the PVN and TSH serum concentration; however, in corticosterone pre-treated rats, *Trh* expression does not increase further after cold exposure and the response of TSH is suppressed (Sotelo-Rivera *et al.* 2014). GCs may inhibit TRH or TSH release directly at the median eminence or the pituitary respectively (van Haasteren *et al.* 1995, John *et al.* 2003). TRH synthesis and release might be uncoupled in those circumstances when TSH is not increased within an hour of exposure.

The interference of corticosterone with the cold-induced activation of *Trh* synthesis may be due to a cross talk between activated GR and elements of the PKA pathway, as demonstrated *in vitro*. Hypothalamic cells co-stimulated with dexamethasone and forskolin do not display increased *Trh* mRNA levels as are detected when they are incubated separately with each drug (Pérez-Martínez *et al.* 1998, Cote-Vélez *et al.* 2005), nor the recruitment of pCREB, GR, or Pol II to the *Trh* promoter (Díaz-Gallardo *et al.* 2010b). The effect is probably due to GR–PKA protein–protein interaction with PKA impeding CREB phosphorylation and GR binding to DNA (Sotelo-Rivera *et al.* 2012). Similar cross talk between the PKA and GR intracellular pathways is detected also in cell lines,

supporting the hypothesis that the effect is an intracellular event, and not due to the average of differential responses of TRHergic cells from different hypothalamic nuclei present in primary cultures (Cote-Vélez *et al.* 2005). The interference of GCs with the cAMP-stimulatory effect on *Trh* expression is avoided with inhibitors of the ERK/MAPK pathways (Cote-Vélez *et al.* 2008). These results, although obtained *in vitro*, support the possibility of fast cross talk between signaling pathways that may alter an immediate response (Fig. 2).

As mentioned in the previous section, long-term energy-demanding situations do not seem to keep the HPT axis activated, but the response to acute stimuli may prevail or be modified. One may envisage situations when TRH neurons are activated, TRH synthesis increases and simultaneously processed TRH is released, TSH secretion follows, causing TH release that modulates different metabolic reactions at target organs. The feedback effect includes not only TH at TRH or TSH synthesis, but molecules of the target tissues, GCs if HPA is co-stimulated and TH acting on other brain areas involved in the required circuit. TH affects metabolism acting at various hypothalamic nuclei increasing, for example, glucose production, modulating central autonomic outflow, and eating behavior or stimulating the dorso-medial hypothalamus (DMH) which activates the sympathetic-BAT response involved in thermogenesis (Fliers *et al.* 2014).

TRHergic neurons may thus respond transiently to energy-demanding situations and maintain energy homeostasis. However, stress could impede a fast and efficient response of the HPT axis in which, for example, in cases such as cold exposure or increased physical activity, the lack of opportune lipolysis needed for fuel supply may oblige the organism to display alternative responses, such as increased food intake and decreased activity due to fatigue, thus contributing to the metabolic syndrome.

Hypothalamic TRH neuronal populations

In mammals, various brain circuits contribute to maintenance of energy balance (Schneeberger *et al.* 2014). Central administration of TRH or TRH agonists consistently reduces food intake in normal rodents and hungry rats, and in models of stress-induced feeding increases thermogenesis and arousal (Lechan & Fekete 2006, Akieda-Asai *et al.* 2014). These effects may involve various hypothalamic targets because, for example, local injection of TRH into medial and lateral hypothalamus (LH) reduces feeding in rats (Suzuki *et al.* 1982) whereas administration into the preoptic area, dorsomedial, or ventromedial

hypothalamus (VMH) increases BAT temperature in hamsters (Shintani *et al.* 2005). ISH of *ppTRH* mRNA, its receptors (TRH-R1 and TRH-R2), and inactivating enzyme (PPII) together with immunostaining of *Trh* and of TRH, as well as autoradiography of TRH binding sites, has been used to generate maps of brain TRHergic neurons, receptors, and inactivation sites (Vargas *et al.* 1987, Hökfelt *et al.* 1989, Lechan & Segerson 1989, Heuer *et al.* 2000). However, the circuits in which they are involved are currently poorly understood as few of the projection fields of the TRH neurons have been identified with anterograde and retrograde techniques (Simmons & Swanson 2009, Wittmann *et al.* 2009a,b, Fekete & Lechan 2014). Understanding the role of hypothalamic TRHergic neurons (Fig. 1) requires consideration of their distribution because various regions, such as the LH, PVN, preoptic, suprachiasmatic, and dorsomedial nuclei express *Trh* mRNA (Fig. 1). The physiological state of TRH neurons has been evaluated by few electrophysiological studies on identified neurons and by biochemical markers of activation, such as detection of immediate early genes, or of TRH biosynthesis, but the output of TRH neurons is difficult to obtain *in vivo* because of sampling or sensitivity issues.

Even in a single nucleus, TRH neurons are not homogeneous. In the rat PVN, TRH-expressing neurons are distributed along the rostro-caudal part of the parvocellular and some in the magnocellular subdivisions (Fekete & Lechan 2014). Almost half of the total PVN-TRHergic cells in the parvocellular part of the PVN are non-neuroendocrine or preautonomic, concentrated in the rostral or anterior PVN (aPVN) in the rat, with only few present in the mid-PVN and even fewer in the caudal. As mentioned, the hypophysiotropic or endocrine cells are concentrated in mid- and caudal-PVN (Simmons & Swanson 2009, Fekete & Lechan 2014). In mouse PVN, 69% of TRHergic endocrine cells project to the median eminence, while 17% project to the neurohypophysis, and the rest are non-endocrine cells localized in the anterior zone (Ghamari-Langroudi *et al.* 2010, Kádár *et al.* 2010). PVN-TRHergic neurons differ not only in their afferents, projections, and receptor expression (Fekete & Lechan 2014) but also according to time of birth; rat non-neuroendocrine neurons peak at embryonic days 11–12 and neuroendocrine neurons at days 12–14 (Markakis & Swanson 1997).

The non-neuroendocrine TRHergic neurons of the rat aPVN receive a robust innervation from NPY/AgRP and α -MSH/CART neurons from the ARC (Fekete & Lechan 2014) and from adrenergic and noradrenergic fibers from the brain stem (Füzesi *et al.* 2009). The α -MSH input seems to be functional since central administration of α -MSH

enhances the phosphorylation of CREB in *Trh* neurons (Sarkar *et al.* 2002) and mediates the effect of i.c.v. leptin injection, which rapidly increases pCREB in aPVN-TRH neurons (Perello *et al.* 2006). The adrenergic input may be relevant to thermogenesis because cold induces a fast increase in *Trh* mRNA levels in the aPVN as it does in the hypophysiotropic neurons (Sánchez *et al.* 2001). TRH neurons of the aPVN send projections to the ARC, dorsomedial, ventral-premammillary nuclei, and medial-preoptic region, and to several additional limbic regions such as various amygdaline nuclei, the bed nucleus of the stria terminalis, and lateral septum (Wittmann *et al.* 2009a). TRH neurons of the aPVN do not express GR (Cintra *et al.* 1990), but a corticosterone injection increases *Trh* mRNA levels, probably through direct stimulation of GC membrane receptors, and the cold-stimulatory effect is additive (Myers *et al.* 2014, Sotelo-Rivera *et al.* 2014). *Trh* expression in aPVN is not modulated after suckling or food restriction, whereas it is stimulated after dehydration-induced anorexia (DIA; Sánchez *et al.* 2001, Alvarez-Salas *et al.* 2012). As regulation of biosynthesis is often coupled with changes in peptide release, the results indicate that aPVN-TRH neurons transfer multiple modalities of metabolically relevant information to postsynaptic targets; confirmation of their role will require experimental testing, but it is interesting to note that the intestinal administration of long-chain fatty acids enhances the activity of aPVN neurons (Randich *et al.* 2004), and adiponectin stimulates only non-neuroendocrine PVN-TRH cells (Hoyda *et al.* 2009).

The ARC expresses both *Trh-R1* and *Trh-R2* receptor mRNAs (Heuer *et al.* 2000, Ebling *et al.* 2008), its dorso-medial part receives a dense TRHergic innervation (Lyons *et al.* 2010) arising, at least in part, from the aPVN and some from the perifornical area (Wittmann *et al.* 2009a,b). Cell-specific neuron-mapping techniques in mice demonstrate a strong excitatory drive emanating from subsets of neurons from the PVN expressing TRH and pituitary-adenylate cyclase-activating polypeptide that contact and activate AgRP neurons, inducing intense feeding (Krashes *et al.* 2014). The functional role of TRH in this circuit was not tested, but TRH does not regulate the electrical activity of α -MSH or NPY neurons in slices of rat ARC (Zhang & van den Pol 2012), instead TRH regulates that of tuberoinfundibular dopaminergic neurons present in the dorsal ARC which are surrounded by TRH-ir terminals; TRH causes a transition from phasic to tonic firing which probably decreases dopamine output (Lyons *et al.* 2010).

The DMH has an important role in energy homeostasis (Schneeberger *et al.* 2014). A significant population

of TRH neurons is detected in this nucleus (Hökfelt *et al.* 1989, Horjales-Araujo *et al.* 2014), which receive afferents from the subparaventricular zone, an output region from the suprachiasmatic nucleus (SCN); the DMH sends a glutamate–TRH projection to the LH area (Chou *et al.* 2003) which expresses both TRH receptors, predominantly TRH-R1 (Heuer *et al.* 2000). TRH receptors are probably present in orexin neurons as they respond to TRH with robust increases in their action potential firing rate, an effect that persists under conditions of synaptic isolation (González *et al.* 2009). TRH also increases the firing activity of presynaptic GABAergic interneurons (Hara *et al.* 2009). The DMH–TRHergic neuronal projection onto or near orexin neurons may be part of a circuit required for the circadian activation of behavioral and endocrine functions, including the circadian control of awareness (González *et al.* 2009). In agreement with this possibility, the effect of TRH on locomotor activity is reduced in orexin-ablated mice (Hara *et al.* 2009). TRH also inhibits the activity of melanin-concentrating hormone (MCH) neurons of LH indirectly through the excitation of GABA neurons, a result consistent with the detection of TRH axons terminating on or near GABA neurons (Zhang & van den Pol 2012). Interestingly, *Trh* mRNA levels in the DMH are increased after 2 weeks of moderate exercise compared with the pair-fed group (Uribe *et al.* 2014). The LH contains another large group of TRH cells throughout the rostro-caudal axis of the hypothalamus (Hökfelt *et al.* 1989, Lechan & Segerson 1989, Heuer *et al.* 2000, Horjales-Araujo *et al.* 2014) and in the perifornical region (Wittmann *et al.* 2009a,b). These neurons do not coexpress orexin, MCH, or neurotensin (Horjales-Araujo *et al.* 2014). In the juxtapa-raventricular area, a small group of TRH-immunoreactive cells stains for enkephalin and urocortin 3, and their projections partially overlap those of the aPVN (Wittmann *et al.* 2009b, Horjales-Araujo *et al.* 2014). AgRP and α -MSH populations of terminals form close appositions onto TRH cells in the LH; the TRH-expressing cell population of the LH may link metabolic signals and the generation of arousal (Horjales-Araujo *et al.* 2014). Increased expression of *Trh* is detected in LH of male rats after 5 days of DIA and in the pair-fed group (de Gortari *et al.* 2009) while *Crh* mRNA levels increase only after DIA (Watts *et al.* 1999). DIA and pair-fed rats eat by the fifth day only 20% of the intake of control animals; the increase in TRH expression may be related to increased arousal as increased anxiety is produced by these conditions (Jaimes-Hoy *et al.* 2008).

TRH neurons heavily innervate histaminergic (HA) neurons in all subdivisions of the rat tuberomammillary nucleus (TMN) (Sárvári *et al.* 2012) where TRH-R2 is

present (Gotoh *et al.* 2007). A TRH microinjection above the TMN activates HA TMN neurons and histamine turnover in PVN and VMH projections (Gotoh *et al.* 2007). As hypothalamic neuronal histamine is involved in the regulation of body weight and acts as an anorexic agent (Schneider *et al.* 2014), it is indeed possible that a HA projection contributes to the anorectic effect of TRH.

Are changes in the expression of neuropeptides and TRH in total hypothalamus indicative of a metabolic alteration?

Owing to the crucial involvement of TH thermogenesis and metabolism (Klieverik *et al.* 2009, Silva 2011, Mullur *et al.* 2014), a more active HPT would in principle reflect a higher metabolism. However, a cause–effect relationship between gene expression of ARC peptides and that of TRH in total PVN is difficult to identify (Table 1 and Supplementary Table 1). If, for example, there is an opposite response of *Trh* expression in aPVN versus mid–caudal PVN, the results of the whole PVN may overshadow the endocrine response. Even more if levels of expression are measured in total hypothalamus since TRH in several nuclei may have important roles in energy homeostasis. For example, comparison between lean and fat rats (Lou/C versus Wistar (Veyrat-Durebex *et al.* 2013)) or selectively bred chickens (Byerly *et al.* 2009) show increased *Trh* mRNA but also increased *Npy* and *AgRP* together with decreased *Pomc* in the lean animals which would seem to contradict the effects of these peptides on TRH expression in the PVN (Fekete & Lechan 2014). Although it is not possible to delineate the hypothalamic regions where these reported changes occur, coincident changes in these two species include the enhanced expression of brain-derived neurotrophic factor (BDNF) which *in vitro* increases *Trh* mRNA levels (Ubieta *et al.* 2007). BDNF and its receptor TRKB are expressed in several hypothalamic nuclei including TRHergic cells of the PVN (Ubieta *et al.* 2007). BDNF regulates neurogenesis, neuronal plasticity, and support during development and throughout the animal's life-span (Jeanneteau & Chao 2013). In the hypothalamus, BDNF plays an important part in energy homeostasis (Levin 2007, Rothman *et al.* 2012). Support for this role stems from the effects of transferring the *Bdnf* gene into hypothalami of adult mice, which display decreased body weight and body fat and increased expression of *Mc4r*, *Lepr*, *Trh*, and *Crh* compared with wild type mice despite having strangely greater than tenfold induction of the orexigenic peptides NPY and AgRP. Under HFD, they gained less weight and little fat

compared with normal mice and displayed increased *Mc4r*, *Lepr*, *Trh*, *Crh*, *Cart* (*Cartpt*), and *Pomc* expression, as well as expression of the metabolic genes of target tissues such as WAT and liver (Cao *et al.* 2009). These results, although not defining the particular hypothalamic nuclei involved, reveals the importance of additional participants such as BDNF in re-setting the response of several hypothalamic–peptidergic systems and their relationship with body weight and adipose tissue.

Another metabolic relevant brain area, the hindbrain

The brain stem is an important part of the circuitry involved in sensing and conveying information regarding the nutritional and energy conditions of the organism (Schneeberger *et al.* 2014). *Trh* is synthesized in the raphe pallidus (Rpa), raphe obscurus (Rob), and parapyramidal regions; these neurons project to neurons of the dorsal motor nucleus of the vagus, the nucleus tractus solitarius, and the ventrolateral medulla. They contain TRH-R1 and leptin receptors involved in coordinating vagal and sympathetic outflows (Taché *et al.* 2006). As in mid-PVN, *Trh* expression in these nuclei is downregulated in hyperthyroidism (Yuan & Yang 1999) and upregulated by cold exposure (Taché *et al.* 2006). Some TRH neurons in the caudal raphe receive orexin afferents and project to premotor neurons that innervate BAT increasing thermogenesis; others project to the vagal circuit involved in hepatic, pancreatic, and gastrointestinal function (Taché *et al.* 2006). In contrast to the inhibitory effects of fasting on HPT activity, hindbrain *Trh* mRNA levels are elevated in rats fasted for 24 or 48 h, and refeeding restores them to normal levels (Ao *et al.* 2006). Studies of the regulation of TRH expression in these neurons contribute to the understanding of the role of brain TRH in metabolism and thermogenesis.

Neuroendocrinology revisited

Recognition of the effects of environment on metabolism and epigenetic changes has led to questions with regard to what are the adequate controls in experimental research with rodents? Although laboratory rodents have been domesticated for hundreds of generations, their ‘standard’ living conditions are sedentary with no other activity but to eat *ad libitum* and develop obesity (Martin *et al.* 2010). This is evident comparing pair-fed with naïve rats; calorie restriction even as low as 18% of naïve food intake and loss of 8% body weight increased their corticosterone serum

concentrations (Uribe *et al.* 2014). In the case of 300 g male rats, normal cages do not allow standing, reducing even further their possibility of physical activity (RSPCA 2011). Stressful conditions include differences in individual versus group housing depending on the age and sex of the animal, whether animals are transported or isolated within 1–3 h previous to the experiment, etc. (Koolhaas *et al.* 2011, Uribe *et al.* 2011).

Postnatal development, lactation, and adolescence are periods of high sensitivity to stress when epigenetic changes may program the individual to having low stress resilience, changing the expression of key molecules such as BDNF and GR in a region-specific manner; in hippocampus their inhibited expression alters the inhibitory feedback on the activity of the HPA leading to hyperactive stress responses and reduced learning (Meaney & Szyf 2005, Pervanidou & Chrousos 2012, Bath *et al.* 2013). The nutrition or stress experience of mothers during gestation as well as litter size has long-lasting effects on metabolism and stress response (Breton 2013). In the case of the HPT axis and TRH expression in particular, reports are scarce. Dexamethasone injections during the last week of gestation diminish *Trh* mRNA levels in the PVN in female offspring and TRH fibers and T_4 in both sexes during the second week; only *Trh* expression remained low in adult females, accompanied by low body temperature during estrous and diestrous (Carbone *et al.* 2012a). Low levels of immunoreactive TRH are also detected in the fibers of the LH (Carbone *et al.* 2012b). This treatment blunts stress-induced *Bdnf* expression in male rats or HFD-induced increases in females (Carbone & Handa 2013).

Neonatal stress produced by maternal separation causes epigenetic changes in hippocampal *Gr* and *Bdnf* promoters, their reduced expression contributes to the altered HPA-response to stressors in adulthood (Meaney & Szyf 2005), *Bdnf* expression is altered in a strain-, sex-, and region-specific manner (Bath *et al.* 2013). Adequate programming of activity and response of the HPT axis is altered under various conditions. Maternal separation increases *ppTrh* mRNA in the aPVN and circulating T_4 serum concentrations of adult rats, and attenuates the response to exercise or cold exposure but that to fasting is normal (Jaimes-Hoy *et al.* 2013). Offspring of mothers that experienced a 40% food restriction, during gestation and lactation were hypothyroid (low FT_4 and high TSH) and had a blunted response to 24 h cold exposure (Ayala-Moreno *et al.* 2013). In contrast, post-natal overnutrition during lactation, which predisposes the offspring to being overweight, leads to leptin resistance and overexpression

of orexigenic pathways (Breton 2013), alters the HPT axis in rats in contrast to the situation opposite to what is observed after HFD because *Trh* expression in the PVN and T_4 serum concentration are decreased and the response to fasting is suppressed (Rodrigues *et al.* 2009, Aréchiga-Ceballos *et al.* 2014) compared with HFD-induced overweight, which display increased *Trh* mRNA and TH serum levels, but without an altered fasting response (Perello *et al.* 2010).

The opposite scenarios apply to mice grown in enriched environments; their body weight is reduced, BDNF expression is increased in ARC and DMH/VMH, *Trh* in the PVN and in the DMH/VMH, despite decreased expression of *Pomc*; animals with access to wheels for running for several weeks maintain lower body weight and reduced adiposity, but *Trh* mRNA levels tend to decrease and *Crh* and *Crh*-receptors increase in contrast to the effects of the enriched environment (Cao *et al.* 2011). The contradictory results observed between the effects of neonatal stress and those of growing in an enriched environment on the relationship of GR and BDNF (Jeanneteau & Chao 2013) include the responses of the HPA, HPT, and SNA that affect energy balance. Improvements and standardized housing conditions seem required to define adequate controls and obtain reproducible data between different laboratories, together with adequate reports of the animals' age and body weight, as some correspond to the adolescent period (McCutcheon & Marinelli 2009). Another recently discovered but very important problem is the materials used in animal houses such as water bottles made of polycarbonate that release chemicals that act as endocrine disruptors causing deleterious effects, particularly during development (Zoeller 2010), and the differential leakage that occurs depending on the state and treatment of the bottle (Guart *et al.* 2013).

Conclusion

This review summarizes what is known about the regulation of the TRHergic neurons involved in energy homeostasis and the interfering effects of stress. In spite of the limitations of the *in vitro* systems or transgenic mice models, evidence indicates demonstrate an inhibitory role of T_3 and a stimulatory one of pCREB, SP1/KPL, GR-cJun, and STAT3 on *Trh* transcription; interaction of the transduction pathways involved may explain some of the interfering effects of stress on neuronal stimulations observed in various *in vivo* situations, but they have not yet been fully placed in a sub-type of TRH neuron context.

Trh expression levels in the PVN, detected during chronic 'static' situations, are difficult to relate to changes in other known modulators as they represent a 'snap shot' that might not readily correspond to the activity of the target organs involved. TRH neurons can rapidly respond to energy demands for thermogenesis or physical activity; these responses may be modified due to nutritional status, and stress history. We propose that combined approaches of chronic situations with acute responses might lead to a better understanding of TRH physiology in the PVN and in other relevant metabolic hypothalamic nuclei.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/JOE-14-0593>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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References

- Abel ED, Ahima RS, Boers ME, Elmquist JK & Wondisford FE 2001 Critical role for thyroid hormone receptor $\beta 2$ in the regulation of paraventricular thyrotropin-releasing hormone neurons. *Journal of Clinical Investigation* **107** 1017–1023. (doi:10.1172/JCI10858)
- Aguilar-Valles A, Sánchez E, de Gortari P, Ramírez-Amaya V, Balderas I, Bermúdez-Ratóni F & Joseph-Bravo P 2005 Analysis of the stress response in rats trained in the water-maze: differential expression of CRH, CRH-R1, GR and BDNF in limbic regions. *Neuroendocrinology* **82** 306–319. (doi:10.1159/000093129)
- Aguilar-Valles A, Sánchez E, de Gortari P, García-Vázquez I, Ramírez-Amaya V, Bermúdez-Ratóni F & Joseph-Bravo P 2007 The expression of TRH, its receptors and degrading enzyme is differentially modulated in the rat limbic system during training in the Morris Water Maze. *Neurochemistry International* **50** 404–417. (doi:10.1016/j.neuint.2006.09.009)

- Akieda-Asai S, Poleni PE & Date Y 2014 Coinjection of CCK and leptin reduces food intake via increased CART/TRH and reduced AMPK phosphorylation in the hypothalamus. *American Journal of Physiology. Endocrinology and Metabolism* **306** E1284–E1291. (doi:10.1152/ajpendo.00664.2013)
- Alkemade A, Unmehopa UA, Wiersinga WM, Swaab DF & Fliers E 2005 Glucocorticoids decrease thyrotropin-releasing hormone messenger ribonucleic acid expression in the paraventricular nucleus of the human hypothalamus. *Journal of Clinical Endocrinology and Metabolism* **90** 323–327. (doi:10.1210/jc.2004-1430)
- Altarejos JY & Montminy M 2011 CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. *Nature Reviews. Molecular Cell Biology* **12** 141–151. (doi:10.1038/nrm3072)
- Alvarez-Salas E, Aceves C, Anguiano B, Uribe RM, García-Luna C, Sánchez 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. (doi:10.1210/en.2011-2011)
- Ao Y, Go VL, Toy N, Li T, Wang Y, Song MK, Reeve JR Jr, Liu Y & Yang H 2006 Brainstem thyrotropin-releasing hormone regulates food intake through vagal-dependent cholinergic stimulation of ghrelin secretion. *Endocrinology* **147** 6004–6010. (doi:10.1210/en.2006-0820)
- Araujo RL, Andrade BM, da Silva ML, Ferreira AC & Carvalho DP 2009 Tissue-specific deiodinase regulation during food restriction and low replacement dose of leptin in rats. *American Journal of Physiology. Endocrinology and Metabolism* **296** E1157–E1163. (doi:10.1152/ajpendo.90869.2008)
- Aréchiga-Ceballos F, Alvarez-Salas E, Matamoros-Trejo G, Amaya MI, García-Luna C & de Gortari P 2014 Pro-TRH and pro-CRF expression in paraventricular nucleus of small litter-reared fasted adult rats. *Journal of Endocrinology* **221** 77–88. (doi:10.1530/JOE-13-0458)
- Armario A & Castellanos JM 1984 Effect of acute and chronic psychogenic stress on corticoadrenal and pituitary–thyroid hormones in male rats. *Hormone Research* **20** 241–245. (doi:10.1159/000180003)
- Astapova I & Hollenberg AN 2013 The *in vivo* role of nuclear receptor corepressors in thyroid hormone action. *Biochimica et Biophysica Acta* **1830** 3876–3881. (doi:10.1016/j.bbagen.2012.07.001)
- Ayala-Moreno R, Racotta R, Anguiano B, Aceves C & Quevedo L 2013 Perinatal undernutrition programmes thyroid function in the adult rat offspring. *British Journal of Nutrition* **110** 2207–2215. (doi:10.1017/S0007114513001736)
- Bath KG, Schilit A & Lee FS 2013 Stress effects on BDNF expression: effects of age, sex, and form of stress. *Neuroscience* **239** 149–156. (doi:10.1016/j.neuroscience.2013.01.074)
- 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. *Biochemical and Biophysical Research Communications* **37** 705–710. (doi:10.1016/0006-291X(69)90868-7)
- Breton C 2013 The hypothalamus–adipose axis is a key target of developmental programming by maternal nutritional manipulation. *Journal of Endocrinology* **216** R19–R31. (doi:10.1530/JOE-12-0157)
- Brown LM & Clegg DJ 2010 Central effects of estradiol in the regulation of food intake, body weight, and adiposity. *Journal of Steroid Biochemistry and Molecular Biology* **122** 65–73. (doi:10.1016/j.jsmb.2009.12.005)
- 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-NH₂ sequence. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences. Série D: Sciences Naturelles* **269** 1870–1873.
- Byerly MS, Simon J, Lebihan-Duval E, Duclos MJ, Cogburn LA & Porter TE 2009 Effects of BDNF, T₃, and corticosterone on expression of the hypothalamic obesity gene network *in vivo* and *in vitro*. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **296** R1180–R1189. (doi:10.1152/ajpregu.90813.2008)
- Cao L, Lin EJ, Cahill MC, Wang C, Liu X & During MJ 2009 Molecular therapy of obesity and diabetes by a physiological autoregulatory approach. *Nature Medicine* **15** 447–454. (doi:10.1038/nm.1933)
- Cao L, Choi EY, Liu X, Martin A, Wang C, Xu X & During MJ 2011 White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic–adipocyte axis. *Cell Metabolism* **14** 324–338. (doi:10.1016/j.cmet.2011.06.020)
- Carbone DL & Handa RJ 2013 Sex and stress hormone influences on the expression and activity of brain-derived neurotrophic factor. *Neuroscience* **239** 295–303. (doi:10.1016/j.neuroscience.2012.10.073)
- Carbone DL, Zuloaga DG, Lacagnina AF, McGivern RF & Handa RJ 2012a Exposure to dexamethasone during late gestation causes female-specific decreases in core body temperature and prepro-thyrotropin-releasing hormone expression in the paraventricular nucleus of the hypothalamus in rats. *Physiology & Behavior* **108** 6–12. (doi:10.1016/j.physbeh.2012.07.010)
- Carbone DL, Zuloaga DG, Lacagnina AF & Handa RJ 2012b Prepro-thyrotropin releasing hormone expressing neurons in the juxtapaaraventricular region of the lateral hypothalamus are activated by leptin and altered by prenatal glucocorticoid exposure. *Brain Research* **1477** 19–26. (doi:10.1016/j.brainres.2012.08.020)
- Case HS, Reed HL, Palinkas LA, Reedy KR, Van Do N, Finney NS & Seip R 2006 Resting and exercise energy use in Antarctica: effect of 50% restriction in temperate climate energy requirements. *Clinical Endocrinology* **65** 257–264. (doi:10.1111/j.1365-2265.2006.02588.x)
- Charli JL, Vargas MA, Cisneros M, de Gortari P, Baeza MA, Jasso P, Bourdais J, Pérez L, Uribe RM & Joseph-Bravo P 1998 TRH inactivation in the extracellular compartment: role of pyroglutamyl peptidase II. *Neurobiology* **6** 45–57.
- Charmandari E, Tsigos C & Chrousos G 2005 Endocrinology of the stress response. *Annual Review of Physiology* **67** 259–284. (doi:10.1146/annurev.physiol.67.040403.120816)
- Chiamolera MI & Wondisford FE 2009 Minireview: Thyrotropin-releasing hormone and the thyroid hormone feedback mechanism. *Endocrinology* **150** 1091–1096. (doi:10.1210/en.2008-1795)
- Chiamolera MI, Sidhaye AR, Matsumoto S, He Q, Hashimoto K, Ortega-Carvalho TM & Wondisford FE 2012 Fundamentally distinct roles of thyroid hormone receptor isoforms in a thyrotroph cell line are due to differential DNA binding. *Molecular Endocrinology* **26** 926–939. (doi:10.1210/me.2011-1290)
- Chiappini F, Ramadoss P, Vella KR, Cunha LL, Ye FD, Stuart RC, Nillni EA & Hollenberg AN 2013 Family members CREB and CREM control thyrotropin-releasing hormone (TRH) expression in the hypothalamus. *Molecular and Cellular Endocrinology* **365** 84–94. (doi:10.1016/j.mce.2012.09.006)
- 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. *Journal of Neuroscience* **23** 10691–10702.
- Cintra A, Fuxe K, Wikström AC, Visser T & Gustafsson JA 1990 Evidence for thyrotropin-releasing hormone and glucocorticoid receptor-immunoreactive neurons in various preoptic and hypothalamic nuclei of the male rat. *Brain Research* **506** 139–144. (doi:10.1016/0006-8993(90)91210-8)
- Cizza G, Brady LS, Esclapes ME, Blackman MR, Gold PW & Chrousos GP 1996 Age and gender influence basal and stress-modulated hypothalamic–pituitary–thyroidal function in Fischer 344/N rats. *Neuroendocrinology* **64** 440–448. (doi:10.1159/000127150)
- Cote-Vélez A, Pérez-Martínez L, Díaz-Gallardo MY, Pérez-Monter C, Carreón-Rodríguez 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. *Journal of Molecular Endocrinology* **34** 177–197. (doi:10.1677/jme.1.01634)
- Cote-Vélez A, Pérez-Martínez L, Charli JL & Joseph-Bravo P 2008 The PKC and ERK/MAPK pathways regulate glucocorticoid action on TRH transcription. *Neurochemical Research* **33** 1582–1591. (doi:10.1007/s11064-008-9698-5)

- Cote-Vélez A, Pérez-Maldonado A, Osuna J, Barrera B, Charli JL & Joseph-Bravo P 2011 Creb and Sp/Krüppel response elements cooperate to control rat TRH gene transcription in response to cAMP. *Biochimica et Biophysica Acta* **1809** 191–199. (doi:10.1016/j.bbagr.2011.01.005)
- Dallman MF, Akana SF, Pecoraro NC, Warne JP, la Fleur SE & Foster MT 2007 Glucocorticoids, the etiology of obesity and the metabolic syndrome. *Current Alzheimer Research* **4** 199–204. (doi:10.2174/156720507780362236)
- Decherf S, Seugnet I, Becker N, Demeneix BA & Clerget-Froidevaux MS 2013 Retinoic X receptor subtypes exert differential effects on the regulation of *Trh* transcription. *Molecular and Cellular Endocrinology* **381** 115–123. (doi:10.1016/j.mce.2013.07.016)
- Di S, Malcher-Lopes R, Halmos KC & Tasker JG 2003 Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. *Journal of Neuroscience* **23** 4850–4857.
- Díaz-Gallardo MY, Cote-Vélez A, Carreón-Rodríguez A, Charli JL & Joseph-Bravo P 2010a 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. (doi:10.1159/000228833)
- Díaz-Gallardo MY, Cote-Vélez A, Charli JL & Joseph-Bravo P 2010b A rapid interference between glucocorticoids and cAMP-activated signalling in hypothalamic neurones prevents binding of phosphorylated cAMP response element binding protein and glucocorticoid receptor at the CRE-like and composite GRE sites of thyrotrophin-releasing hormone gene promoter. *Journal of Neuroendocrinology* **22** 282–293. (doi:10.1111/j.1365-2826.2010.01966.x)
- Duclos M, Timofeeva E, Michel C & Richard D 2005 Corticosterone-dependent metabolic and neuroendocrine abnormalities in obese Zucker rats in relation to feeding. *American Journal of Physiology. Endocrinology and Metabolism* **288** E254–E266. (doi:10.1152/ajpendo.00087.2004)
- Dupré SM, Guissouma H, Flamant F, Seugnet I, Scanlan TS, Baxter JD, Samarut J, Demeneix BA & Becker N 2004 Both thyroid hormone receptor (TR) β 1 and TR β 2 isoforms contribute to the regulation of hypothalamic thyrotropin-releasing hormone. *Endocrinology* **145** 2337–2345. (doi:10.1210/en.2003-1209)
- Ebling FJ, Wilson D, Wood J, Hughes D, Mercer JG, Morgan PJ & Barrett P 2008 The thyrotropin-releasing hormone secretory system in the hypothalamus of the Siberian hamster in long and short photoperiods. *Journal of Neuroendocrinology* **20** 576–586. (doi:10.1111/j.1365-2826.2008.01702.x)
- Fekete C & Lechan RM 2014 Central regulation of hypothalamic–pituitary–thyroid axis under physiological and pathophysiological conditions. *Endocrine Reviews* **35** 159–194. (doi:10.1210/er.2013-1087)
- Fliers E, Unmehopa UA & Alkemade A 2006 Functional neuroanatomy of thyroid hormone feedback in the human hypothalamus and pituitary gland. *Molecular and Cellular Endocrinology* **251** 1–8. (doi:10.1016/j.mce.2006.03.042)
- Fliers E, Kalsbeek A & Boelen A 2014 Mechanisms in endocrinology: Beyond the fixed set point of the hypothalamus–pituitary–thyroid axis. *European Journal of Endocrinology* **171** R197–R208. (doi:10.1530/EJE-14-0285)
- Füzesi T, Wittmann G, Lechan RM, Liposits Z & Fekete C 2009 Noradrenergic innervation of hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in rats. *Brain Research* **1294** 38–44. (doi:10.1016/j.brainres.2009.07.094)
- Gadaleta RM & Magnani L 2014 Nuclear receptors and chromatin: an inducible couple. *Journal of Molecular Endocrinology* **52** R137–R149. (doi:10.1530/JME-13-0170)
- Galas L, Raoult E, Tonon MC, Okada R, Jenks BG, Castaño JP, Kikuyama S, Malagon M, Roubos EW & Vaudry H 2009 TRH acts as a multifunctional hypophysiotropic factor in vertebrates. *General and Comparative Endocrinology* **164** 40–50. (doi:10.1016/j.ygcen.2009.05.003)
- 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. *Molecular Endocrinology* **24** 2366–2381. (doi:10.1210/me.2010-0203)
- González JA, Horjales-Araujo E, Fugger L, Broberger C & Burdakov D 2009 Stimulation of orexin/hypocretin neurones by thyrotropin-releasing hormone. *Journal of Physiology* **587** 1179–1186. (doi:10.1113/jphysiol.2008.167940)
- de Gortari P, Mancera K, Martínez A, Cote-Vélez A & Joseph-Bravo P 2009 Involvement of CRH-R2 in eating behavior and in the response of the HPT axis in rats subjected to dehydration-induced anorexia. *Psychoneuroendocrinology* **34** 259–272. (doi:10.1016/j.psyneuen.2008.09.010)
- Gotoh K, Fukagawa K, Fukagawa T, Noguchi H, Kakuma T, Sakata T & Yoshimatsu H 2007 Hypothalamic neuronal histamine mediates the thyrotropin-releasing hormone-induced suppression of food intake. *Journal of Neurochemistry* **103** 1102–1110. (doi:10.1111/j.1471-4159.2007.04802.x)
- Guart A, Wagner M, Mezquida A, Lacorte S, Oehlmann J & Borrell A 2013 Migration of plasticisers from Tritan™ and polycarbonate bottles and toxicological evaluation. *Food Chemistry* **141** 373–380. (doi:10.1016/j.foodchem.2013.02.129)
- Guissouma H, Becker N, Seugnet I & Demeneix BA 2000 Transcriptional repression of TRH promoter function by T₃: analysis by *in vivo* gene transfer. *Biochemistry and Cell Biology* **78** 155–163. (doi:10.1139/o00-009)
- Guo F, Bakal K, Minokoshi Y & Hollenberg AN 2004 Leptin signaling targets the thyrotropin-releasing hormone gene promoter *in vivo*. *Endocrinology* **145** 2221–2227. (doi:10.1210/en.2003-1312)
- Gutiérrez-Mariscal M, de Gortari P, López-Ruvalcaba C, Martínez A & Joseph-Bravo P 2008 Analysis of the anxiolytic-like effect of TRH and the response of amygdalar TRHergic neurons in anxiety. *Psychoneuroendocrinology* **33** 198–213. (doi:10.1016/j.psyneuen.2007.11.002)
- Gutiérrez-Mariscal M, Sánchez E, García-Vázquez A, Rebolledo-Solleiro D, Charli JL & Joseph-Bravo P 2012 Acute response of hypophysiotropic thyrotropin releasing hormone neurons and thyrotropin release to behavioral paradigms producing varying intensities of stress and physical activity. *Regulatory Peptides* **179** 61–70. (doi:10.1016/j.regpep.2012.08.010)
- 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. *Journal of Endocrinology* **145** 143–153. (doi:10.1677/joe.0.1450143)
- van Haasteren GA, Linkels E, van Toor H, Klootwijk W, Kaptein E, de Jong FH, Reymond MJ, Visser TJ & de Greef WJ 1996 Effects of long-term food reduction on the hypothalamus–pituitary–thyroid axis in male and female rats. *Journal of Endocrinology* **150** 169–178. (doi:10.1677/joe.0.1500169)
- Hara J, Gerashchenko D, Wisor JP, Sakurai T, Xie X & Kilduff TS 2009 Thyrotropin-releasing hormone increases behavioral arousal through modulation of hypocretin/orexin neurons. *Journal of Neuroscience* **29** 3705–3714. (doi:10.1523/JNEUROSCI.0431-09.2009)
- Harris M, Aschkenasi C, Elias CF, Chandrankunnel A, Nillni EA, Bjørbaek C, Elmquist JK, Flier JS & Hollenberg AN 2001 Transcriptional regulation of the thyrotropin-releasing hormone gene by leptin and melanocortin signaling. *Journal of Clinical Investigation* **107** 111–120. (doi:10.1172/JCI10741)
- Helmreich DL, Parfitt DB, Lu XY, Akil H & Watson SJ 2005 Relation between the hypothalamic–pituitary–thyroid (HPT) axis and the hypothalamic–pituitary–adrenal (HPA) axis during repeated stress. *Neuroendocrinology* **81** 183–192. (doi:10.1159/000087001)
- Heuer H, Schäfer MK, O'Donnell D, Walker P & Bauer K 2000 Expression of thyrotropin-releasing hormone receptor 2 (TRH-R2) in the central nervous system of rats. *Journal of Comparative Neurology* **428** 319–336.

- (doi:10.1002/1096-9861(20001211)428:2<319::AID-CNE10>3.0.CO;2-9)
- Hinkle PM, Gehret AU & Jones BW 2012 Desensitization, trafficking, and resensitization of the pituitary thyrotropin-releasing hormone receptor. *Frontiers in Neuroscience* **13** 180. (doi:10.3389/fnins.2012.00180)
- Hökfelt T, Tsuruo Y, Ulfhake B, Cullheim S, Arvidsson U, Foster GA, Schultzberg M, Schalling M, Arborelius L, Freedman J *et al.* 1989 Distribution of TRH-like immunoreactivity with special reference to coexistence with other neuroactive compounds. *Annals of the New York Academy of Sciences* **553** 76–105. (doi:10.1111/j.1749-6632.1989.tb54479.x)
- Hollenberg AN 2008 The role of the thyrotropin-releasing hormone (TRH) neuron as a metabolic sensor. *Thyroid* **18** 131–139. (doi:10.1089/thy.2007.0251)
- Horjales-Araujo E, Hellysaz A & Broberger C 2014 Lateral hypothalamic thyrotropin-releasing hormone neurons: distribution and relationship to histochemically defined cell populations in the rat. *Neuroscience* **277** 87–102. (doi:10.1016/j.neuroscience.2014.06.043)
- Hoyda TD, Samson WK & Ferguson AV 2009 Adiponectin depolarizes parvocellular paraventricular nucleus neurons controlling neuroendocrine and autonomic function. *Endocrinology* **150** 832–840. (doi:10.1210/en.2008-1179)
- Huo L, Münzberg H, Nillni EA & Bjørbaek C 2004 Role of signal transducer and activator of transcription 3 in regulation of hypothalamic *trh* gene expression by leptin. *Endocrinology* **145** 2516–2523. (doi:10.1210/en.2003-1242)
- Jaimes-Hoy L, Joseph-Bravo P & de Gortari P 2008 Differential response of TRHergic neurons of the PVN in animals submitted to food restriction or dehydration induced anorexia and cold exposure. *Hormones and Behavior* **53** 366–377. (doi:10.1016/j.yhbeh.2007.11.003)
- Jaimes-Hoy L, Espinosa-Ayala C, Charli JL & Joseph-Bravo PI 2013 Maternal separation persistently alters the expression of key elements of the neuroendocrine TRH system. Endo 2013, The Endocrine Society's 95th Annual Meeting & Expo, San Francisco, CA, USA. Abstract MON-428.
- Jeanneteau F & Chao MV 2013 Are BDNF and glucocorticoid activities calibrated? *Neuroscience* **239** 173–195. (doi:10.1016/j.neuroscience.2012.09.017)
- John CD, Christian HC, Morris JF, Flower RJ, Solito E & Buckingham JC 2003 Kinase-dependent regulation of the secretion of thyrotrophin and luteinizing hormone by glucocorticoids and annexin 1 peptides. *Journal of Neuroendocrinology* **15** 946–957. (doi:10.1046/j.1365-2826.2003.01081.x)
- Kádár A, Sánchez E, Wittmann G, Singru PS, Füzesi 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. *Journal of Comparative Neurology* **518** 3948–3961. (doi:10.1002/cne.22432)
- Kakucska I, Qi Y & Lechan RM 1995 Changes in adrenal status affect hypothalamic thyrotropin-releasing hormone gene expression in parallel with corticotropin-releasing hormone. *Endocrinology* **136** 2795–2802. (doi:10.1210/endo.136.7.7789304)
- 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.
- Kilburn-Watt E, Banati RB & Keay KA 2010 Altered thyroid hormones and behavioural change in a sub-population of rats following chronic constriction injury. *Journal of Neuroendocrinology* **22** 960–970. (doi:10.1111/j.1365-2826.2010.02038.x)
- Klieverik LP, Coomans CP, Enderit E, Sauerwein HP, Havekes LM, Voshol PJ, Rensen PC, Romijn JA, Kalsbeek A & Fliers E 2009 Thyroid hormone effects on whole-body energy homeostasis and tissue-specific fatty acid uptake *in vivo*. *Endocrinology* **150** 5639–5648. (doi:10.1210/en.2009-0297)
- de Kloet ER 2014 From receptor balance to rational glucocorticoid therapy. *Endocrinology* **155** 2754–2769. (doi:10.1210/en.2014-1048)
- Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Flügge G, Korte SM, Meerlo P, Murison R, Olivier B, Palanza P *et al.* 2011 Stress revisited: a critical evaluation of the stress concept. *Neuroscience and Biobehavioral Reviews* **35** 1291–1301. (doi:10.1016/j.neubiorev.2011.02.003)
- Krashes MJ, Shah BP, Madara JC, Olson DP, Strohlic DE, Garfield AS, Vong L, Pei H, Watabe-Uchida M, Uchida N *et al.* 2014 An excitatory paraventricular nucleus to AgRP neuron circuit that drives hunger. *Nature* **507** 238–242. (doi:10.1038/nature12956)
- Lechan RM & Fekete C 2006 The TRH neuron: a hypothalamic integrator of energy metabolism. *Progress in Brain Research* **153** 209–235. (doi:10.1016/S0079-6123(06)53012-2)
- Lechan RM & Segerson TP 1989 Pro-TRH gene expression and precursor peptides in rat brain. Observations by hybridization analysis and immunocytochemistry. *Annals of the New York Academy of Sciences* **553** 29–59. (doi:10.1111/j.1749-6632.1989.tb54476.x)
- 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. (doi:10.1126/science.3079917)
- Lee SL, Stewart K & Goodman RH 1988 Structure of the gene encoding rat thyrotropin releasing hormone. *Journal of Biological Chemistry* **263** 16604–16609.
- Levin BE 2007 Neurotrophism and energy homeostasis: perfect together. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **293** R988–R991. (doi:10.1152/ajpregu.00434.2007)
- Lima LP, Barros IA, Lisbôa PC, Araújo RL, Silva AC, Rosenthal D, Ferreira AC & Carvalho DP 2006 Estrogen effects on thyroid iodide uptake and thyroperoxidase activity in normal and ovariectomized rats. *Steroids* **71** 653–659. (doi:10.1016/j.steroids.2006.03.007)
- Louzada RA, Santos MC, Cavalcanti-de-Albuquerque JP, Rangel I, Ferreira AC, Galina A, Werneck de Castro JP & de Carvalho DP 2014 Type 2 iodothyronine deiodinase is up-regulated in rat slow- and fast-twitch skeletal muscle during cold exposure. *American Journal of Physiology. Endocrinology and Metabolism* **307** E1020–E1029. (doi:10.1152/ajpendo.00637.2013)
- Lundblad JR, Kwok RP, Laurance ME, Huang MS, Richards JP, Brennan RG & Goodman RH 1998 The human T-cell leukemia virus-1 transcriptional activator Tax enhances cAMP-responsive element-binding protein (CREB) binding activity through interactions with the DNA minor groove. *Journal of Biological Chemistry* **273** 19251–19259. (doi:10.1074/jbc.273.30.19251)
- Lyons DJ, Horjales-Araujo E & Broberger C 2010 Synchronized network oscillations in rat tuberoinfundibular dopamine neurons: switch to tonic discharge by thyrotropin-releasing hormone. *Neuron* **65** 217–229. (doi:10.1016/j.neuron.2009.12.024)
- Maniam J & Morris MJ 2012 The link between stress and feeding behaviour. *Neuropharmacology* **63** 97–110. (doi:10.1016/j.neuropharm.2012.04.017)
- Marassi MP, Fortunato RS, da Silva AC, Pereira VS, Carvalho DP, Rosenthal D & da Costa VM 2007 Sexual dimorphism in thyroid function and type 1 iodothyronine deiodinase activity in pre-pubertal and adult rats. *Journal of Endocrinology* **192** 121–130. (doi:10.1677/joe.1.06901)
- Markakis EA & Swanson LW 1997 Spatiotemporal patterns of secretomotor neuron generation in the parvocellular neuroendocrine system. *Brain Research. Brain Research Reviews* **24** 255–291. (doi:10.1016/S0165-0173(97)00006-4)
- Martin B, Ji S, Maudsley S & Mattson MP 2010 “Control” laboratory rodents are metabolically morbid: why it matters. *PNAS* **107** 6127–6133. (doi:10.1073/pnas.0912955107)
- Mastorakos G & Pavlatou M 2005 Exercise as a stress model and the interplay between the hypothalamus–pituitary–adrenal and the hypothalamus–pituitary–thyroid axes. *Hormone and Metabolic Research* **37** 577–584. (doi:10.1055/s-2005-870426)
- McAninch EA & Bianco AC 2014 Thyroid hormone signaling in energy homeostasis and energy metabolism. *Annals of the New York Academy of Sciences* **1311** 77–87. (doi:10.1111/nyas.12374)

- McCutcheon JE & Marinelli M 2009 Age matters. *European Journal of Neuroscience* **29** 997–1014. (doi:10.1111/j.1460-9568.2009.06648.x)
- Meaney MJ & Szyf M 2005 Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. *Dialogues in Clinical Neuroscience* **7** 103–123.
- Mitchell SE, Nogueiras R, Rance K, Rayner DV, Wood S, Dieguez C & Williams LM 2006 Circulating hormones and hypothalamic energy balance: regulatory gene expression in the Lou/C and Wistar rats. *Journal of Endocrinology* **190** 571–579. (doi:10.1677/joe.1.06576)
- Mullur R, Liu YY & Brent GA 2014 Thyroid hormone regulation of metabolism. *Physiological Reviews* **94** 355–382. (doi:10.1152/physrev.00030.2013)
- Myers B, McKlveen JM & Herman JP 2014 Glucocorticoid actions on synapses, circuits, and behavior: implications for the energetics of stress. *Frontiers in Neuroendocrinology* **35** 180–196. (doi:10.1016/j.yfrne.2013.12.003)
- Nillni EA 2010 Regulation of the hypothalamic thyrotropin releasing hormone (TRH) neuron by neuronal and peripheral inputs. *Frontiers in Neuroendocrinology* **3** 134–156. (doi:10.1016/j.yfrne.2010.01.001)
- Osterlund C & Spencer R 2011 Corticosterone pretreatment suppresses stress-induced hypothalamic–pituitary–adrenal axis activity via multiple actions that vary with time, site of action, and *de novo* protein synthesis. *Journal of Endocrinology* **208** 311–322. (doi:10.1530/JOE-10-0413)
- Palinkas LA, Reedy KR, Shepanek M, Smith M, Anghel M, Steel GD, Reeves D, Case HS, Do NV & Reed HL 2007 Environmental influences on hypothalamic–pituitary–thyroid function and behavior in Antarctica. *Physiology & Behavior* **92** 790–799. (doi:10.1016/j.physbeh.2007.06.008)
- Paxinos G & Watson C 2005 *In The Rat Brain in Stereotaxic Coordinates*, 5th ed. San Diego, CA, USA: Elsevier.
- 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. (doi:10.1210/en.2005-1533)
- 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. *American Journal of Physiology. Endocrinology and Metabolism* **299** E976–E989. (doi:10.1152/ajpendo.00448.2010)
- Pérez-Martínez L, Carreón-Rodríguez A, González-Alzati ME, Morales C, Charli JL & Joseph-Bravo P 1998 Dexamethasone rapidly regulates TRH mRNA levels in hypothalamic cell cultures: interaction with the cAMP pathway. *Neuroendocrinology* **68** 345–354. (doi:10.1159/000054383)
- Pérez-Monter C, Martínez-Armenta M, Miquelajauregui A, Furlan-Magaril M, Varela-Echavarría A, Recillas-Targa F, May V, Charli JL & Pérez-Martínez L 2011 The Krüppel-like factor 4 controls biosynthesis of thyrotropin-releasing hormone during hypothalamus development. *Molecular and Cellular Endocrinology* **333** 127–133. (doi:10.1016/j.mce.2010.12.020)
- Pervanidou P & Chrousos GP 2012 Metabolic consequences of stress during childhood and adolescence. *Metabolism* **61** 611–619. (doi:10.1016/j.metabol.2011.10.005)
- Ramadoss P, Abraham BJ, Tsai L, Zhou Y, Costa-e-Sousa RH, Ye F, Bilban M, Zhao K & Hollenberg AN 2014 Novel mechanism of positive versus negative regulation by thyroid hormone receptor $\beta 1$ (TR $\beta 1$) identified by genome wide profiling of binding sites in mouse liver. *Journal of Biological Chemistry* **289** 313–328. (doi:10.1074/jbc.M113.521450)
- Randich A, Chandler PC, Mebane HC, Turnbach ME, Meller ST, Kelm GR & Cox JE 2004 Jejunal administration of linoleic acid increases activity of neurons in the paraventricular nucleus of the hypothalamus. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **286** R166–R173. (doi:10.1152/ajpregu.00431.2003)
- Raptis S, Fekete C, Sarkar S, Rand WM, Emerson CH, Nagy GM & Lechan RM 2004 Cocaine- and amphetamineregulated transcript co-contained in thyrotropin-releasing hormone (TRH) neurons of the hypothalamic paraventricular nucleus modulates TRH-induced prolactin secretion. *Endocrinology* **145** 1695–1699. (doi:10.1210/en.2003-1576)
- Ratman D, Berghé WV, Dejager L, Libert C, Tavernier J, Beck IM & De Bosscher K 2013 How glucocorticoid receptors modulate the activity of other transcription factors: a scope beyond tethering. *Molecular and Cellular Endocrinology* **380** 41–54. (doi:10.1016/j.mce.2012.12.014)
- Ren Y, Satoh T, Yamada M, Hashimoto K, Konaka S, Iwasaki T & Mori M 1998 Stimulation of the preprothyrotropin-releasing hormone gene by epidermal growth factor. *Endocrinology* **139** 195–203. (doi:10.1210/endo.139.1.5703)
- Rodrigues AL, de Moura EG, Passos MC, Dutra SC & Lisboa PC 2009 Postnatal early overnutrition changes the leptin signalling pathway in the hypothalamic–pituitary–thyroid axis of young and adult rats. *Journal of Physiology* **587** 2647–2661. (doi:10.1113/jphysiol.2009.169045)
- Rondeel JM, de Greef WJ, Hop WC, Rowland DL & Visser TJ 1991 Effect of cold exposure on the hypothalamic release of thyrotropin-releasing hormone and catecholamines. *Neuroendocrinology* **54** 477–481. (doi:10.1159/000125940)
- 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. (doi:10.1159/000126248)
- Rorato R, Miyahara C, Antunes-Rodrigues J & Elias LL 2013 Tolerance to hypophagia induced by prolonged treatment with a CB1 antagonist is related to the reversion of anorexigenic neuropeptide gene expression in the hypothalamus. *Regulatory Peptides* **182** 12–18. (doi:10.1016/j.regpep.2012.12.004)
- Rose AJ & Herzog S 2013 Metabolic control through glucocorticoid hormones: an update. *Molecular and Cellular Endocrinology* **380** 65–78. (doi:10.1016/j.mce.2013.03.007)
- Rosene ML, Wittmann G, Arrojo e Drigo R, Singru PS, Lechan RM & Bianco AC 2010 Inhibition of the type 2 iodothyronine deiodinase underlies the elevated plasma TSH associated with amiodarone treatment. *Endocrinology* **151** 5961–5970. (doi:10.1210/en.2010-0553)
- Rothman SM, Griffioen KJ, Wan R & Mattson MP 2012 Brain-derived neurotrophic factor as a regulator of systemic and brain energy metabolism and cardiovascular health. *Annals of the New York Academy of Sciences* **1264** 49–63. (doi:10.1111/j.1749-6632.2012.06525.x)
- RSPCA 2011 Rats: good practice for housing and care. Research Animals Department, RSPCA, 3rd Edition. <http://science.rspca.org.uk/science-group/researchanimals/ethicalreview/functiontasks/housingandcare>
- Sánchez E, Uribe RM, Corkidi G, Zoeller RT, Cisneros M, Zacarias M, Morales-Chapa C, Charli JL & Joseph-Bravo P 2001 Differential responses of thyrotropin-releasing hormone (TRH) neurons to cold exposure or suckling indicate functional heterogeneity of the TRH system in the paraventricular nucleus of the rat hypothalamus. *Neuroendocrinology* **74** 407–422. (doi:10.1159/000054707)
- Sánchez 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 Research* **1132** 120–128. (doi:10.1016/j.brainres.2006.11.020)
- Sánchez E, Singru PS, Acharya R, Bodria M, Fekete C, Zavacki AM, Bianco AC & Lechan RM 2008 Differential effects of refeeding on melanocortin-responsive neurons in the hypothalamic paraventricular nucleus. *Endocrinology* **149** 4329–4335. (doi:10.1210/en.2008-0411)
- Sánchez E, Vargas MA, Singru PS, Pascual I, Romero F, Fekete C, Charli JL & Lechan RM 2009 Tanycyte pyroglutamate II contributes to regulation of the hypothalamic–pituitary–thyroid axis through glial–axonal associations in the median eminence. *Endocrinology* **150** 2283–2291. (doi:10.1210/en.2008-1643)
- Sarkar S, Légrádi G & Lechan RM 2002 Intracerebroventricular administration of α -melanocyte stimulating hormone increases phosphorylation of CREB in TRH- and CRH-producing neurons of the hypothalamic paraventricular nucleus. *Brain Research* **945** 50–59. (doi:10.1016/S0006-8993(02)02619-7)

- Sárvári A, Farkas E, Kádár A, Zséli G, Füzesi T, Lechan RM & Fekete C 2012 Thyrotropin-releasing hormone-containing axons innervate histaminergic neurons in the tuberomammillary nucleus. *Brain Research* **1488** 72–80. (doi:10.1016/j.brainres.2012.10.010)
- Schneeberger M, Gomis R & Claret M 2014 Hypothalamic and brainstem neuronal circuits controlling homeostatic energy balance. *Journal of Endocrinology* **220** T25–T46. (doi:10.1530/JOE-13-0398)
- Schneider E, Neumann D & Seifert R 2014 Modulation of behavior by the histaminergic system: lessons from HDC-, H₃R- and H₄R-deficient mice. *Neuroscience and Biobehavioral Reviews* **47C** 101–121. (doi:10.1016/j.neubiorev.2014.07.020)
- Segeron 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. (doi:10.1126/science.3116669)
- Selye H 1976 Forty years of stress research: principal remaining problems and misconceptions. *Canadian Medical Association Journal* **115** 53–56.
- Servatius RJ, Natelson BH, Moldow R, Pogach L, Brennan FX & Ottenweller JE 2000 Persistent neuroendocrine changes in multiple hormonal axes after a single or repeated stressor exposures. *Stress* **3** 263–274. (doi:10.3109/10253890009001132)
- Shi ZX, Levy A & Lightman SL 1993 The effect of dietary protein on thyrotropin-releasing hormone and thyrotropin gene expression. *Brain Research* **606** 1–4. (doi:10.1016/0006-8993(93)91561-6)
- Shintani M, Tamura Y, Monden M & Shiomi H 2005 Thyrotropin-releasing hormone induced thermogenesis in Syrian hamsters: site of action and receptor subtype. *Brain Research* **1039** 22–29. (doi:10.1016/j.brainres.2005.01.040)
- Silva JE 2011 Physiological importance and control of non-shivering facultative thermogenesis. *Frontiers in Bioscience* **3** 352–371. (doi:10.2741/s156)
- Simmons DM & Swanson LW 2009 Comparison of the spatial distribution of seven types of neuroendocrine neurons in the rat paraventricular nucleus: toward a global 3D model. *Journal of Comparative Neurology* **516** 423–441. (doi:10.1002/cne.22126)
- Sotelo-Rivera I, Cote-Vélez A, Pérez-Maldonado A, Charli J & Joseph-Bravo P 2012 Corticosterone interferes with the stimulatory effect of cAMP on proTRH transcription by promoting a GR–PKAc interaction and not through chromatin remodelling *15th International & 14th European Congress of Endocrinology*, Florence, Italy. *Endocrine Abstracts* **29** P1115
- Sotelo-Rivera I, Jaimes-Hoy L, Cote-Vélez A, Espinoza-Ayala C, Charli JL & Joseph-Bravo P 2014 An acute injection of corticosterone increases thyrotrophin releasing hormone expression in the paraventricular nucleus of the hypothalamus but interferes with the rapid hypothalamus pituitary thyroid axis response to cold in male rats. *Journal of Neuroendocrinology* **26** 861–869. (doi:10.1111/jne.12224)
- Steinacker JM, Brkic M, Simsch C, Nething K, Kresz A, Prokopchuk O & Liu Y 2005 Thyroid hormones, cytokines, physical training and metabolic control. *Hormone and Metabolic Research* **37** 538–544. (doi:10.1055/s-2005-870419)
- Stranahan AM, Lee K & Mattson MP 2008 Central mechanisms of HPA axis regulation by voluntary exercise. *Neuromolecular Medicine* **10** 118–127. (doi:10.1007/s12017-008-8027-0)
- 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. (doi:10.1210/en.2009-0976)
- Suzuki T, Kohno H, Sakurada T, Tadano T & Kisara K 1982 Intracranial injection of thyrotropin releasing hormone (TRH) suppresses starvation-induced feeding and drinking in rats. *Pharmacology, Biochemistry, and Behavior* **17** 249–253. (doi:10.1016/0091-3057(82)90078-8)
- Taché Y, Yang H, Miampamba M, Martinez V & Yuan PQ 2006 Role of brainstem TRH/TRH-R1 receptors in the vagal gastric cholinergic response to various stimuli including sham-feeding. *Autonomic Neuroscience* **125** 42–52. (doi:10.1016/j.autneu.2006.01.014)
- Ubieta R, Uribe RM, González JA, García-Vázquez A, Pérez-Monter C, Pérez-Martínez L, Joseph-Bravo P & Charli JL 2007 BDNF up-regulates pre-pro-TRH mRNA expression in the fetal/neonatal paraventricular nucleus of the hypothalamus. Properties of the transduction pathway. *Brain Research* **1174** 28–38. (doi:10.1016/j.brainres.2007.08.026)
- Umezawa R, Yamada M, Horiguchi K, Ishii S, Hashimoto K, Okada S, Satoh T & Mori M 2009 Aberrant histone modifications at the thyrotropin-releasing hormone gene in resistance to thyroid hormone: analysis of F455S mutant thyroid hormone receptor. *Endocrinology* **150** 3425–3432. (doi:10.1210/en.2008-1738)
- Uribe RM, Joseph-Bravo P, Pasten J, Ponce G, Méndez M, Covarrubias L & Charli JL 1991 Some events of thyrotropin-releasing hormone metabolism are regulated in lactating and cycling rats. *Neuroendocrinology* **54** 493–498. (doi:10.1159/000125943)
- 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. (doi:10.1159/000126523)
- Uribe RM, Pérez-Martínez L, de Lourdes Covarrubias M, Gómez O, Covarrubias L, Charli JL & Joseph-Bravo P 1995a Phorbol ester or cAMP enhance thyrotropin-releasing hormone mRNA in primary cultures of hypothalamic cells. *Neuroscience Letters* **201** 41–44. (doi:10.1016/0304-3940(95)12125-N)
- Uribe RM, Joseph-Bravo P & Charli JL 1995b Pups removal enhances thyrotropin-releasing hormone mRNA in the hypothalamic paraventricular nucleus. *European Journal of Endocrinology* **133** 354–360. (doi:10.1530/eje.0.1330354)
- Uribe RM, Zacarias M, Corkidi G, Cisneros M, Charli JL & Joseph-Bravo P 2009 17 β -Oestradiol indirectly inhibits thyrotropin-releasing hormone expression in the hypothalamic paraventricular nucleus of female rats and blunts thyroid axis response to cold exposure. *Journal of Neuroendocrinology* **21** 439–448. (doi:10.1111/j.1365-2826.2009.01861.x)
- Uribe RM, Cisneros M, Vargas MA, Lezama L, Cote-Vélez A, Joseph-Bravo P & Charli JL 2011 The systemic inhibition of nitric oxide production rapidly regulates TRH mRNA concentration in the paraventricular nucleus of the hypothalamus and serum TSH concentration. Studies in control and cold-stressed rats. *Brain Research* **1367** 188–197. (doi:10.1016/j.brainres.2010.10.011)
- Uribe RM, Jaimes-Hoy L, Ramírez-Martínez C, García-Vázquez A, Romero F, Cisneros M, Cote-Vélez A, Charli JL & Joseph-Bravo P 2014 Voluntary exercise adapts the hypothalamus–pituitary–thyroid axis in male rats. *Endocrinology* **155** 2020–2030. (doi:10.1210/en.2013-1724)
- Valle A, Català-Niell A, Colom B, García-Palmer FJ, Oliver J & Roca P 2005 Sex-related differences in energy balance in response to caloric restriction. *American Journal of Physiology. Endocrinology and Metabolism* **289** E15–E22. (doi:10.1152/ajpendo.00553.2004)
- Vargas M, Mendez M, Cisneros M, Joseph-Bravo P & Charli JL 1987 Regional distribution of the membrane-bound pyroglutamate amino peptidase-degrading thyrotropin-releasing hormone in rat brain. *Neuroscience Letters* **79** 311–314. (doi:10.1016/0304-3940(87)90450-2)
- Vella KR, Ramadoss P, Costa-E-Sousa RH, Astapova I, Ye FD, Holtz KA, Harris JC & Hollenberg AN 2014 Thyroid hormone signaling *in vivo* requires a balance between coactivators and corepressors. *Molecular and Cellular Biology* **34** 1564–1575. (doi:10.1128/MCB.00129-14)
- Veyrat-Durebex C, Poher AL, Caillon A, Somm E, Vallet P, Charnay Y & Rohner-Jeanrenaud F 2013 Improved leptin sensitivity as a potential candidate responsible for the spontaneous food restriction of the Lou/C rat. *PLoS ONE* **8** e73452. (doi:10.1371/journal.pone.0073452)
- Wallis M 2010 Molecular evolution of the thyrotrophin-releasing hormone precursor in vertebrates: insights from comparative genomics. *Journal of Neuroendocrinology* **22** 608–619. (doi:10.1111/j.1365-2826.2010.01996.x)
- 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. *Journal of Neuroscience* **19** 6111–6121.
- Weber JM 2011 Metabolic fuels: regulating fluxes to select mix. *Journal of Experimental Biology* **214** 286–294. (doi:10.1242/jeb.047050)

- Wittmann G, Füzesi T, Singru PS, Liposits Z, Lechan RM & Fekete C 2009a Efferent projections of thyrotropin-releasing hormone-synthesizing neurons residing in the anterior parvocellular subdivision of the hypothalamic paraventricular nucleus. *Journal of Comparative Neurology* **515** 313–330. (doi:10.1002/cne.22017)
- Wittmann G, Füzesi T, Liposits Z, Lechan RM & Fekete C 2009b Distribution and axonal projections of neurons coexpressing thyrotropin-releasing hormone and urocortin 3 in the rat brain. *Journal of Comparative Neurology* **517** 825–840. (doi:10.1002/cne.22180)
- Xia T, Zhang Q, Xiao Y, Wang C, Yu J, Liu H, Liu B, Zhang Y, Chen S, Liu Y *et al.* 2015 CREB/TRH pathway in the central nervous system regulates energy expenditure in response to deprivation of an essential amino acid. *International Journal of Obesity* **39** 105–113. (doi:10.1038/ijo.2014.65)
- Yoo HS, Qiao L, Bosco C, Leong LH, Lytle N, Feng GS, Chi NW & Shao J 2014 Intermittent cold exposure enhances fat accumulation in mice. *PLoS ONE* **9** e96432. (doi:10.1371/journal.pone.0096432)
- Yuan PQ & Yang H 1999 Hyperthyroidism decreases thyrotropin-releasing hormone gene expression in the caudal raphe nuclei and the parapyramidal regions in rats. *Neuroscience Letters* **276** 189–192. (doi:10.1016/S0304-3940(99)00830-7)
- Zhang X & van den Pol AN 2012 Thyrotropin-releasing hormone (TRH) inhibits melanin-concentrating hormone neurons: implications for TRH-mediated anorexic and arousal actions. *Journal of Neuroscience* **32** 3032–3043. (doi:10.1523/JNEUROSCI.5966-11.2012)
- Zoeller TR 2010 Environmental chemicals targeting thyroid. *Hormones* **9** 28–40. (doi:10.14310/horm.2002.1250)
- Zoeller RT, Kaber 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. (doi:10.1210/endo-127-6-2955)
- Zoeller RT, Simonyi A, Butnariu O, Fletcher DL, Rudeen PK, McCrone S & Petersen SL 1995 Effects of acute ethanol administration and cold exposure on the hypothalamic–pituitary–thyroid axis. *Endocrine* **3** 39–47. (doi:10.1007/BF02917447)

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