Thyroid Hormone Signalling Genes Are Regulated by Photoperiod in the Hypothalamus of F344 Rats

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Abstract

Seasonal animals adapt their physiology and behaviour in anticipation of climate change to optimise survival of their offspring. Intra-hypothalamic thyroid hormone signalling plays an important role in seasonal responses in mammals and birds. In the F344 rat, photoperiod stimulates profound changes in food intake, body weight and reproductive status. Previous investigations of the F344 rat have suggested a role for thyroid hormone metabolism, but have only considered Dio2 expression, which was elevated in long day photoperiods. Microarray analysis was used to identify time-dependent changes in photoperiodic responsive genes, which may underlie the photoperiod-dependent phenotypes of the juvenile F344 rat. The most significant changes are those related to thyroid hormone metabolism and transport. Using photoperiodic manipulations and melatonin injections into long day photoperiod (LD) rats to mimic short day (SD), we show photoinduction and photosuppression gene expression profiles and melatonin responsiveness of genes by in situ hybridization; TSHβ, CGA, Dio2 and Oatpc1 genes were all elevated in LD whilst in SD, Dio3 and MCT-8 mRNA were increased. NPY was elevated in SD whilst GALP increased in LD. The photoinduction and photosuppression profiles for GALP were compared to that of GHRH with GALP expression following GHRH temporally. We also reveal gene sets involved in photoperiodic responses, including retinoic acid and Wnt/ß-catenin signalling. This study extends our knowledge of hypothalamic regulation by photoperiod, by revealing large temporal changes in expression of thyroid hormone signalling genes following photoperiod switch. Surprisingly, large changes in hypothalamic thyroid hormone levels or TRH expression were not detected. Expression of NPY and GALP, two genes known to regulate GHRH, were also changed by photoperiod. Whether these genes could provide links between thyroid hormone signalling and the regulation of the growth axis remains to be investigated.

Introduction

Many mammals and birds from temperate latitudes anticipate seasonal changes in climate and in response adapt their physiology and behaviour accordingly. These changes require resetting of a number of endocrine systems associated with reproduction, growth and energy balance, and through this strategy the species optimise their chances of survival for their offspring.

Recent work has demonstrated that the hypothalamus combines both role of photoperiodic time measurement as well as neuroendocrine regulator of physiology. [1–3]. Intra-hypothalamic thyroid hormone metabolism has been shown to play an important role in photoperiod-dependent seasonal responses. Pioneering studies by Yoshimura and colleagues have established the importance of hypothalamic thyroid hormone, T3, in the photoperiodic reproductive response of the Japanese quail [3]. More recent work in Siberian hamsters demonstrated that intrahypothalamic Silastic implants of T3 promoted long day-like reproductive and body weight responses in short day housed animals [2]. This suggested that thyroid hormone metabolism within the hypothalamus is important to photoperiodic regulation in mammals as well as birds. Furthermore in mammals, melatonin produced in the pineal gland was recently shown to act on the pars tuberalis (PT) to relay the photoperiod signal into the hypothalamus via the thyroid hormone signalling system. In long day housed sheep, melatonin-responsive cells in the PT increase production of thyrotrophin (TSH) relative to short day levels [1], a process recently shown to be coordinated by the photoperiod-responsive transcription factor Eya3 [4]. Thus TSH acts locally within the hypothalamus, through stimulation of the TSH-receptor expressing cells, a response also seen in the quail [5]. This leads to up-regulation of expression of type II deiodinase (Dio2), a key enzyme controlling thyroid hormone bioactivity. Dio2 converts thyroxine (T4) into the bioactive tri-iodothyronine (T3) in TSH-receptor (TSH-R) expressing cells located in the tanyocytes of the ependymal layer of the hypothalamus. Increased expression of Dio2 should increase T3 availability within this region. In the quail, decreased expression of the T3 catabolizing enzyme, type 3 deiodinase (Dio3) has been reported in long days [6] which should reduce the clearance of active T3 in long days. Following a switch in photoperiod from long day (LD) to short day (SD), Dio2 decreases whereas the expression of Dio3 increases, which should produce a
net decrease in hypothalamic T3 levels in SD. Consistent with this, the hypothalamic levels of T3 were shown to be ten fold lower in SD than LD whilst plasma levels were similar in both photoperiods in the quail [3]. Similar changes in Dio2 gene expression occur in mammalian species such as the Soay sheep, Syrian hamster, the photoperiodically sensitive Fischer 344 (F344) rat, and mice [1,2,7–9]. The Siberian hamster differs in that only Dio2 changes with photoperiod whilst no Dio3 was observed in the ependymal cells in the Syrian hamster [2]. Despite this, it can be anticipated that the net effect of the changes in species including the Siberian hamster, would be high levels of hypothalamic T3 in LD and low levels during SD. Consistent with this, studies in the Siberian hamster have shown that central thyroid hormone metabolism plays a critical role in the seasonal control of body weight and reproduction [2].

The F344 rat also shows profound reductions in food intake and body weight in response to SD [10]. Previous investigations of hypothalamic genes involved in the food intake and body weight response to altered photoperiod in F344 rats demonstrated marked, but opposite changes in neuropeptide Y (NPY) and agouti-related peptide (AgRP) expression in the arcuate nucleus (ARC) [11]. It was postulated that upregulation of AgRP in LD was associated with the higher levels of food intake, whereas upregulation of NPY in the SD was associated with a reduced drive for growth. Study of the thyroid hormone signalling system in response to photoperiod in F344 rats has been limited to consideration of Dio2 expression, which showed lower levels in SD than LD [9]. The relationship between intra-hypothalamic thyroid hormone signalling and body composition has not been investigated.

In this study we examined changes in hypothalamic gene expression in F344 rats in response to altered photoperiod by microarray. Amongst the most robust changes are genes related to pituitary/hypothalamic thyroid hormone responses. These changes were confirmed by in situ hybridization. In addition we investigated the dynamics of both photoperiod induction of gene expression using short to long day transition as well as photoperiod suppression, using transitions from long to short day and melatonin injections to mimic short day responses in long day housed rats.

We also identified changes in response to photoperiod of a number of other gene sets, including retinoic acid signalling genes but these are not the subject of this paper.

Results

TRH and thyroid hormone levels in F344 rats on LD and SD for 28 days

Over a 28 day period F344 rats held in SD (8 h:16 h, light:dark) exhibited a slower rate of body weight gain (17.7% divergence, \( p<0.001 \)) and reduced food intake relative to rats held in LD (16 h:8 h, light:dark) [previously shown in [11]]. In brain sections from these rats, TRH mRNA expression in the paraventricular nucleus of the hypothalamus was modestly, but significantly suppressed in SD relative to LD rats after 28 days (d) in the different photoperiods (SD 12.5% lower, \( p<0.01 \), Figure 1).

Microarray of hypothalamus from LD and SD F344 rats

To explore how signalling through thyroid hormone-related pathways may have been affected at earlier time points, gene expression was compared using Affymetrix microarray of the hypothalamus taken from LD and SD rats after 3, 14 and 28d in the respective photoperiods, with follow up in situ hybridization on candidate genes.

Figure 1. Effect of photoperiod on TRH gene expression in the paraventricular nucleus (PVN) of F344 rats. TRH mRNA levels in SD photoperiod are lower than in LD (t-test, \( p = 0.01 \)). mRNA levels were determined by in situ hybridization (LD, n = 10; SD, n = 10). Data are mean ± SE. Autoradiographs of representative regions of coronal brain section images are shown for LD and SD. Arrow indicates PVN. Scale bar = 1.0 mm. doi:10.1371/journal.pone.0021351.g001

Food intake and body weights at each of the time-points (3, 14 and 28d) of rats used for in situ hybridization and microarray are shown in Figure 2A–D. Food intakes were significantly lower in SD rats after 21d while body weights were lower after 17d in the rats used for in situ hybridization (n = 8 per group) but by 21d in the microarray groups (n = 4 per group). Total RNA from the hypothalamus, including the ARC, of F344 rats was analysed using the Affymetrix Rat 230-2 arrays. Genes that were either up- or down-regulated by 1.5 fold or more are shown in Tables 1–3. By microarray no significant change in TRH was seen, and in situ hybridization at the earlier time points 1, 3 and 14d also showed no significant difference in TRH mRNA expression with photoperiod.

Data from the microarray indicated that many genes showed differential expression between LD and SD at each time point but with most occurring at the 28d time point. A number of thyroid hormone signalling-related, retinoid signalling, Wnt/\( \beta \)-catenin signalling and energy metabolism-related genes were amongst those showing the greatest differences in expression in response to altered photoperiod. Several of these, together with some related genes that did not appear in the normalised array results, were analysed by in situ hybridization on coronal brain sections from rats that had been housed in LD or SD photoperiods over the same time course of 3, 14 and 28d, or 1, 3 and 14d.

By microarray, gene transcripts for TSH\( \beta \)and its binding partner glycoprotein hormones, alpha polypeptide (CGA) showed the largest differences in expression levels between LD and SD rats (Tables 2, 3). These results were confirmed by in situ hybridization; furthermore this analysis showed the expression of each gene to be restricted to the PT of the pituitary, with no expression observed in
Figure 2. Photoperiod differentially affects food intakes and body weight gain of F344 rats. (A) Cumulative food intakes of SD rats used for in situ hybridization were significantly lower than LD rats after 21 days. (B) Body weights of rats used for in situ hybridization were significantly lower in SD compared to LD rats after 17d. (C) Cumulative food intakes of SD rats used for microarray were significantly lower than LD rats after 21 days. (D) Body weights of rats used for microarray were significantly lower in SD compared to LD rats after 21d. *, p<0.05; **, p<0.01 and ***, p<0.001.

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Table 1. Genes found by microarray showing the largest responses to photoperiod after 3d.

| Gene Name   | Description                     | Fold Change | p value    |
|-------------|---------------------------------|-------------|------------|
| Genes down-regulated in SD |                    |             |            |
| Hcrt        | Hypocretin                      | –3.3        | 0.0441     |
| CcnA2       | cyclin A2                       | –1.8        | 0.0003     |
| Gpr83       | G protein-coupled receptor B3   | –1.7        | 0.0027     |
| RGD1563347  | similar to RIKEN cDNA 2310015N21 | –1.5        | 0.0117     |
| Ces3        | carboxylesterase 3              | –1.5        | 0.0132     |
| Genes up-regulated in SD |                  |             |            |
| Dao1        | D-amino acid oxidase 1          | 1.5         | 0.0314     |
| Slc6a4      | solute carrier family 6 (5-HT transporter), member 4 | 1.5 | 0.003 |
| LOC362972   | similar to adiponutrin          | 1.5         | 0.0009     |
| TnnC2       | troponin C type 2 (fast)        | 1.5         | 0.0241     |

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the hypothalamus (Figure 3A). Densitometric analysis showed that TSH\(_b\) levels were higher in LD than SD levels by 18.4 fold (\(p<0.001\)) after 3d, by 351 fold (\(p<0.001\)) after 14d and by 732 fold (\(p<0.001\)) after 28d (Figure 3A). Similarly, CGA gene expression was expressed at higher levels in LD compared to SD rats at all time points, although unlike TSH\(_b\), CGA expression levels increased over time in both photoperiods. The LD expression level was greater than the SD level by 2.1 fold (\(p<0.01\)), 4.8 fold (\(p<0.001\)) and 3.5 fold (\(p<0.001\)) at 3, 14 and 28d, respectively (Figure 3B).

Other genes related to TSH signalling that showed photoperiod-responsiveness by microarray included the receptor for TSH (TSH-R) and Dio2. These in addition to Dio3 and a specific thyroid hormone transporter, monocarboxylate transporter 8 (MCT-8) were analysed by in situ hybridization over the time course. All of these genes were expressed in the mediobasal hypothalamic ependymal region around the 3rd ventricle in the F344 rat.

By microarray, there was a small but significant difference in expression of TSH-R between LD and SD at 3d, but at no other time points. Through in situ hybridization TSH-R was strongest in the ependymal region and the ventromedial hypothalamus but was not obvious in the PT, although the hybridization signal was too indistinct to allow densitometric analysis (Figure 3C).

In contrast, levels of Dio2 mRNA, which was expressed in both the ependymal region and the mediobasal hypothalamus, were strikingly different at all time points with the highest expression observed in LD compared to SD after 3d. LD expression levels were greater than SD levels by 9 fold, 11.8 fold, and 3.2 fold (all \(p<0.001\)) at 3, 14 and 28d, respectively (Figure 3D).

Dio3 mRNA levels were also robustly influenced by photoperiod with a significant increase seen as early as 3d in the ependymal layer in SD. The levels in SD increased substantially to peak at 14d before decreasing again by 28d, but remaining higher than LD levels throughout. Dio3 mRNA levels were up-regulated in SD

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**Table 2. Genes found by microarray showing the largest responses to photoperiod after 14d.**

| Gene Name | Description                                      | Fold Change | \(p\) value |
|-----------|--------------------------------------------------|-------------|-------------|
| **Genes down-regulated in SD** | | | |
| Tsh\(_b\) | thyroid stimulating hormone, beta subunit | −243.96 | 2.52E-12 |
| Gca | glycoprotein hormones, alpha subunit | −27.21 | 1.44E-05 |
| Nmu | neuregulin | −43.27 | 6.86E-08 |
| Crabp1 | cellular retinoic acid binding protein 1 | −23.75 | 7.78E-05 |
| Plunc | palate, lung, and nasal epithelium carcinoma associated | −1.79 | 0.0002 |
| Ces3 | carboxylesterase 3 | −1.75 | 0.0019 |
| Six1 | sine oculis homeobox homolog 1 (Drosophila) | −1.65 | 0.0008 |
| Sfrp2 | secreted frizzled-related protein 2 | −1.59 | 2.41E-05 |
| Tnfsf13 | tumor necrosis factor (ligand) superfamily, 13 | −1.54 | 1.83E-05 |
| Slc10a1 | solute carrier organic anion transporter family, 1c1 | −1.52 | 2.78E-05 |
| Gup1 | Gup1, glycerol uptake/transporter homolog (yeast) | −1.51 | 0.0199 |
| **Genes up-regulated in SD** | | | |
| Ttr | transthyretin | 9.32 | 0.0058 |
| Foxg1 | forkhead box G1 | 4.69 | 0.04 |
| Bhlhb5 | basic helix-loop-helix domain containing, class B5 | 2.34 | 0.0143 |
| Hnrpd | heterogeneous nuclear ribonucleoprotein D-like | 2.14 | 0.0029 |
| Ddn | Dendrin | 2.08 | 0.0099 |
| RGD1565710 | similar to MGC6837 protein | 2.08 | 0.0217 |
| Neurod4 | neurogenic differentiation | 2.04 | 0.0338 |
| Icam5 | intercellular adhesion molecule 5, telenenchalin | 2 | 0.0038 |
| Itpka | inositol 1,4,5-trisphosphate 3-kinase A | 1.92 | 0.009 |
| S100a7 | solute carrier family 17 (Na-dependent inorganic phosphate cotransporter), member 7 | 1.9 | 0.0079 |
| Lhx2 | LIM homeobox protein 2 (predicted) | 1.88 | 0.0263 |
| LOC685826 | similar to reprimir-like | 1.84 | 0.0019 |
| RGD1307524 | similar to Friedreich ataxia region gene X123 | 1.83 | 0.0002 |
| Gda | guanine deaminase | 1.78 | 0.0196 |
| Mlf1 | myeloid leukemia factor 1 | 1.77 | 0.0009 |
| Mx1 | myxovirus (influenza virus) resistance 1 | 1.74 | 0.038 |
| Kcnip2 | Kv channel-interacting protein 2 | 1.7 | 0.0029 |
| Lpl | lipoprotein lipase | 1.65 | 0.002 |
| RGD1566269 | similar to Neuropilin- and tollloid-like protein 1 | 1.64 | 0.004 |
| Myosb | myosin 5B | 1.57 | 0.0156 |

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The table shows the genes found by microarray showing the largest responses to photoperiod after 14d. The fold change and \(p\) values indicate the statistical significance of these changes.
Table 3. Genes found by microarray showing the largest responses to photoperiod after 28d.

| Gene Name        | Description                               | Fold Change | p value   |
|------------------|-------------------------------------------|-------------|-----------|
| **Genes down-regulated in SD** |                           |             |           |
| Tshβ             | thyroid stimulating hormone, beta subunit | −624.09     | 1.07E-13  |
| Cga              | glycoprotein hormones, alpha subunit       | −32.88      | 6.84E-06  |
| Nmu              | neuron U                                  | −5.72       | 7.16E-11  |
| Crabp1           | cellular retinoic acid binding protein 1   | −3.16       | 1.72E-06  |
| Rbp1             | retinol binding protein 1, cellular        | −2.84       | 3.53E-05  |
| Stra6            | stimulated by retinoic acid gene 6 homolog (mouse) | −2.53     | 2.68E-09  |
| Ces3             | carboxylesterase 3                         | −2.24       | 4.08E-05  |
| Serpinf1         | serine (or cysteine) peptidase inhibitor, clade F, member 1 | −2.18     | 4.06E-05  |
| Six1             | sine oculis homebox homolog 1 (Drosophila) | −2.17       | 4.86E-06  |
| Aurkb            | aurora kinase B                           | −2.16       | 1.10E-05  |
| RGD1565710       | similar to MGC68837 protein               | −2.06       | 0.0237    |
| Alas2            | aminolevulinic acid synthase 2            | −2.03       | 0.0122    |
| LOC689064        | beta-globin                               | −2.01       | 0.001     |
| Olfml3           | olfactomedin-like 3                       | −1.95       | 0.0015    |
| S100g            | S100 calcium binding protein G            | −1.83       | 1.34E-05  |
| Tnfsf13          | tumor necrosis factor (ligand) superfamily, member 13 | −1.74   | 4.98E-07  |
| Ifitm1           | interferon induced transmembrane protein 1 | −1.73     | 0.001587  |
| Timp1            | tissue inhibitor of metallopeptidase 1     | −1.73       | 0.000156  |
| Strp2            | secreted frizzled-related protein 2        | −1.73       | 2.66E-06  |
| Col1a2           | procollagen, type I, alpha 2              | −1.72       | 0.0046    |
| **Genes up-regulated in SD** |                           |             |           |
| RGD1307524       | similar to Friedreich ataxia region gene X123 | 1.79     | 0.0002    |
| Tnnc2            | troponin C type 2 (fast)                  | 1.76        | 0.0016    |
| NPY              | neuropeptide Y                            | 1.49        | 0.0236    |

Effect of melatonin, photoinduction and photosuppression on thyroid hormone signalling genes

For the microarray experiments, the effects of either LD or SD were measured after switch from an intermediate photoperiod of 12 h:12 h, light:dark, providing simultaneous photoinduction and suppression. To examine these two effects independently and to establish the role of melatonin, thyroid signalling responses (TSHβ, Dio2, Dio3 and Oatp1c1 gene expression) were examined after switch from either LD14:10 (14 h:10 h, light:dark) or SD10:14 (10 h:14 h, light:dark) photoperiods. These photoperiods were chosen because they have been used previously to demonstrate effectiveness of melatonin injections into LD10:14 to mimic SD physiological responses [12].

To assess the effects of photosuppression and melatonin, F344 rats were divided into three groups. A control group was maintained on LD14:10 whilst a second group was transferred to SD10:14 and a third group was maintained on LD14:10 and given a sub-cutaneous injection of melatonin one hour before lights off to extend the melatonin signal and thereby mimicking a short day response [12]. Food intakes were significantly lower in the LD14:10-melatonin injected and SD10:14 rats compared to LD14:10 control rats after 11d (p<0.001, Figure 5A). The body weights began to diverge but differences did not reach significance (Figure 5B) and paired testes weights also showed no significant differences (Figure S1). Gene expression for TSHβ, Dio2, Dio3 and Oatp1c1 after 3d and 14d of treatment are shown in
Figure 6A–D. Relative to the LD14:10 control group, gene expression of TSHβ, Dio2 and Oatp1c1 was significantly suppressed after 3d of melatonin injections (TSHβ \( p = 0.015 \), Dio2 and Oatp1c1, \( p < 0.001 \)), with the magnitude of the response being greatest after 14d (TSHβ, Dio2 and Oatp1c1, all \( p < 0.001 \)). Melatonin injections gave a comparable response to SD10:14, both in magnitude and temporally. For Dio3, SD10:14 induced a time dependent increase in gene expression only after 14d (\( p < 0.001 \), Figure 6C), although melatonin induced a response of much greater magnitude at 3d, relative to the LD14:10 level of expression (Figure 6C). Dio3 induction was more pronounced under the 8 h:16 h light:dark, SD photoperiod than in the SD14:10 control group (compare Figure 6C with 6G).

In the photoinduction experiment where rats were transferred from SD10:14 to LD14:10, food intakes and body weights were significantly greater in LD14:10 rats from 6d and 11d respectively following the switch, compared to SD10:14 rats (\( p < 0.001 \) for both. Figure 5C,D). Significant induction of TSHβ, Dio2 and Oatp1c1 and inhibition of Dio3 gene expression were observed at 3d (TSHβ, Dio2 and Oatp1c1, \( p < 0.001 \), Dio3, \( p < 0.015 \), Figure 6E–H). For Dio2, Oatp1c1 and Dio3, these responses were sustained at 14d (Dio2 and Oatp1c1, \( p < 0.001 \); Dio3, \( p < 0.01 \), Figure 6F–H) whilst...
the magnitude of the response for TSHβ was further increased by 14d (TSHβ p<0.001, Figure 6E).

GHRH and potential regulatory genes (GALP and NPY)

Two hypothalamic neuropeptides, differentially expressed on the microarray from LD and SD F344 rats, were NPY and GALP. Both genes have been implicated in the regulation of growth hormone releasing hormone (GHRH), and hence growth, and galanin-like peptide (GALP) is a potential target protein for thyroid hormone [13–15]. GALP expression by microarray increased significantly in LD compared to SD after 14d and 28d (−1.32, p=0.004 at 14d; −1.39, p=0.001 at 28d). In contrast, NPY mRNA increased significantly in SD compared to LD after 28d (1.49, p<0.02). By in situ hybridisation it was confirmed that both NPY and GALP genes are differentially expressed in the hypothalamus, and more specifically in the ARC (Figure 7A,B). However the temporal expression profiles were markedly different for the two peptides. NPY showed a rapid decrease in expression after only 3d in LD relative to SD; this differential expression was sustained over 28d (p<0.001 at all time points, Figure 7A). In contrast, the response of GALP to photoperiod change was much slower. A significant increase in GALP expression was seen in LD compared to SD only after 14d (p<0.001) but not at the earlier time points of 1 and 3 days (Figure 7B). A significant increase in GALP expression was also seen in the photoinduction paradigm only after 14d of the photoperiod switch from SD10:14 to LD14:10 (p<0.001) with no change apparent after 3d (Figure 7C). This time-course of change in GALP mRNA levels was also investigated in response to melatonin injections and in the photosuppression paradigm. GALP mRNA decreased significantly only after 14d of melatonin injections into LD14:10 rats compared to control LD14:10 rats (p = 0.038), but not after 3d or 14d in SD10:14 (Figure 7D).

In response to photoinduction, GHRH expression in the ARC changed significantly after 3d (p<0.01) of treatment and was sustained after 14d (p<0.05, Figure 7E). In the suppression paradigm, GHRH expression decreased significantly after 3d in SD10:14 (p<0.01) but the decrease after 14d was not statistically significant (Figure 7F). With melatonin injections, GHRH decreased significantly only after 14d (p<0.01, Figure 7F).

Other genes showing large changes in expression in LD compared to SD by microarray included the retinoic acid-related genes, CRABP1, CRBP1 (Tables 2, 3) and the retinoic acid receptor Stra6 (Table 3). Genes with functions related to appetite and energy balance regulation included NPY which was higher in SD than in LD, and AMN which was the opposite (Tables 2, 3). We recently described the expression of CRBP1 and Stra6 in the rat hypothalamus, and the SD induction of NPY [11,16], and consideration of these and many of the other genes are outside the scope of this paper and will be the subjects of future studies.

Discussion

This is the first study to examine the effects of photoperiod on gene expression in the mammalian hypothalamus, using the F344 rat as a model. A wide range of differentially expressed genes was identified, but with three prominent gene sets being revealed: 1. those involved in thyroid hormone signalling, transport and metabolism; 2. those involved in vitamin A/retinoic acid signalling and 3. those involved in Wnt/β-catenin signalling. In this study we have focussed on the thyroid hormone signalling-related gene set with the other gene sets being the subjects of other studies.

Of the thyroid-related signalling genes, TSHβ and CGA, expressed in the PT, exhibited the biggest difference in expression between long and short photoperiods. These findings accord with those from other species [1,5]. Non-covalent bonding of the TSHβ and CGA subunits is required for biological activity of TSH [17] and hence the presence and changes in the two subunits suggests that there is functional TSH production. The presence of the TSH receptor is important for the central TSH signalling pathway to function as reported for other species, and here we detected expression of the TSH-receptor in the F344 rat ependymal region. In sheep it has been shown that the downstream response to TSH-receptor activation in this region involves an increase in cAMP production, and in quail, sheep and Syrian hamsters includes the LD activation of Dio2 to generate active T3 [1,2,5,8,18]. In our experiments, Dio2 mRNA was induced time-dependently by LD in the F344 rat whereas it was suppressed in SD10:14 and LD14:10 melatonin injected rats. The time-course of Dio2 gene induction or suppression under LD and SD seems to correlate well with the time-frame of TSHβ expression, suggesting a functional linkage. Taken together the data from this study provide further evidence in support of the consensus from quail, sheep and mice for retrograde signalling by PT derived TSH in the hypothalamus.
providing photoperiodic control of signal transduction and gene expression in the ependymal region, specifically Dio2 [1,5,7].

There is one difference of note, which is the contrast between the temporal dynamics of expression of TSHβ in the rat in this study and those results for the Syrian hamster. In the latter, suppression of Dio2 by SD was shown to occur ahead of a change in TSHβ [19]. On this basis it has been hypothesised that for Syrian hamsters, TSHβ is involved in the induction phase of the photoperiod response only, and that suppression of this system may require a different, unknown mechanism [19,20]. In this study, as early as 3d following either melatonin injections into LD14:10 rats or SD10:14 treatments, levels of TSHβ and Dio2 gene expression were similarly inhibited. Likewise during photoinduction from SD10:14 to LD14:10, the time-course of increased gene expression for TSHβ and Dio2 was comparable. Thus there is no evidence of temporal dissociation of TSHβ and Dio2 gene expression for either photoinduction or suppression in the rat. In this study, our data suggest that the SD photosuppression is most likely due to photoperiod-driven melatonin production.

As in other species, Dio2 is not the only thyroid hormone metabolising enzyme under photoperiodic control in the F344 rat. Dio3, a gene encoding an enzyme regulating T3 inactivation, was strongly induced in SD within 3d, peaking at 14d. This response was stimulated by melatonin injections into LD14:10 rats with a similar time-course. Once again similar Dio3 responses to SD have been observed in other species [1,2,6,7]. Acting in concert with Dio2, these changes in Dio3 would be predicted to cause an increase in local ependymal T3 concentrations in LD and a fall in SD as seen in the quail [3]. Surprisingly, in this study only a small rise in T3 after 3d was observed, which failed to reach statistical significance.

One potential explanation for this disparity between the profound changes in gene expression of the deiodinases and the relatively unchanged hypothalamic thyroid hormone levels may be
Figure 6. Effects of melatonin, photosuppression and photoinduction on thyroid hormone signalling genes. (A–D) Graphs are results of in situ hybridizations that show effects of SD10:14 (SD) and melatonin injection into LD14:10 (LD + mel) on TSHβ, Dio2, Dio3 and Oatp1c1 levels of gene expression respectively, compared to LD14:10 (LD) at 3d and 14d time points. TSHβ, Dio2 and Oatp1c1 mRNA levels are suppressed by melatonin injection after 3d (TSHβ, p = 0.015, Dio2 and Oatp1c1, p < 0.001) and suppressed further by 14d (TSHβ, Dio2 and OATP1c1, all p < 0.001). Dio3 in contrast was induced by melatonin injection after 3d (p < 0.001) and by both melatonin and SD10:14 after 14d (p < 0.001). (E–H) Graphs show the effects of transfer of rats from SD10:14 to LD14:10 on gene expression levels of TSHβ, Dio2, Dio3 and Oatp1c1 respectively, after 3 and 14d. TSHβ, Dio2 and Oatp1c1 gene expression are significantly induced whilst Dio3 is inhibited at 3d (TSHβ, Dio2 and Oatp1c1, p < 0.001; Dio3 p < 0.015). For Dio2, Oatp1c1 and Dio3, these responses were sustained at 14d (Dio2 and OATP1c1, p < 0.001; Dio3 p < 0.01) whilst the magnitude of the response for TSHβ was further increased by 14d (TSHβ p < 0.001). Bars with different letters are significantly different. Data are mean ± SE.
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Figure 7. Comparison of effects of photoperiod on NPY, GALP and GHRH. (A) ARC NPY mRNA levels show decreased expression in LD compared to SD rats as early as 3d post photoperiodic switch, and the response is sustained over 28d (p<0.001 at all time points). (B) A significant increase in ARC GALP expression in LD compared to SD occurs only after 14d (p<0.001) but not at the earlier time points of 1 and 3 days. (C) The photoinduction paradigm shows GALP mRNA increases only after 14d of the photoperiod switch from SD10:14 to LD14:10 (p<0.001) with no change apparent after 3d. (D) GALP mRNA decreases significantly only after 14d of melatonin injections into LD14:10 rats compared to control LD14:10 rats (p=0.038), but not after 3d or 14d in SD10:14. (E) ARC GHRH expression changes significantly 3d (p<0.01) after photoperiod transfer and this was sustained after 14d (p<0.05). (F) In the suppression paradigm GHRH expression decreases significantly after 3d in SD10:14 (p<0.01) but a decrease after 14d does not reach significance. Melatonin injections causes GHRH to decrease significantly only after 14d (p<0.01). Autoradiograph images of coronal brain sections adjacent to graphs show representative hybridization signals in the hypothalamus for the same riboprobe at the time points of maximum difference in expression (scale bar = 1.0 mm for all images). Data are mean ± SE.
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local, dynamic changes in thyroid hormone transporters that can rapidly regulate thyroid hormone levels. Monocarboxylate transporter, MCT-3 was found to be strongly expressed with higher levels in SD than LD in the ependymal region of the hypothalamus. Cells containing this transporter are thought to rapidly equilibrate thyroid hormone levels with the ability to import as well as release thyroid hormones [21]. Elevated MCT-3 mRNA levels were also observed in Siberian hamsters under SD and it has been proposed that these may be involved in the decline in mRNA levels were also observed in Siberian hamsters under SD and it has been proposed that these may be involved in the decline of active hypothalamic T3 mRNA. A second thyroid hormone transporter, Oatp1c1, was found by microarray and confirmed by in situ hybridization to be strongly expressed in the ependymal region, with sustained higher levels in LD compared to SD. Oatp1c1 mRNA was also found to be responsive to melatonin injections in LD14:10 rats, which mimicked the SD response. This transporter has been reported to transport T4 from the CSF into endothelial cells with higher affinity than T3 and also mediates bidirectional transport [23]. In cells where Oatp1c1 and deiodinases are co-expressed, Oatp1c1 increases access to the deiodinases, greatly increasing substrate metabolism, thus indicating that Oatp1c1 expression can be rate limiting for iodothyronine metabolism by the deiodinases [24]. However, the properties and role of Oatp1c1 in the photoperiodic mammal appear to be slightly different from those in chicken due to differences in substrate preference [25]. Also Oatp1c1 was found to be present in abundance in the ependymal cells lining the ventro-lateral walls of the third ventricle, yet there was no detectable change in chicken mRNA expression with photoperiod [25]. Thus, the local rapid equilibration of thyroid hormone levels possible with these transporters, which differ with photoperiod, may account for the difference in the level of detectable thyroid hormone in the rat compared to the quail [3]. These results highlight not only that there may be differences in the regulation of thyroid hormone transport and metabolism between species, but also show how important it is to gain a complete picture of all the players and components regulating thyroid bioavailability in the hypothalamus so as to fully understand their relevance to physiological function. It also remains a possibility that there could be a phase angle difference in daily rhythmicity in the thyroid hormone and TRH responses.

While the lack of large changes in hypothalamic T3 and T4 in this study, in contrast to quail [3], may seem surprising in view of the profound changes in gene expression of thyroid hormone related genes, there are physiological reasons why sustained perturbed changes in T3/T4 may not be expected. Thyroid hormones in the hypothalamus are known to feedback and inhibit TRH to provide a tightly regulated set-point of TRH and the thyroid endocrine axis [26,27]. The unchanged hypothalamic thyroid hormone levels observed in this study and the modest change in TRH mRNA in the paraventricular nucleus are consistent with this model of set-point control. Nonetheless, it should be noted that photoperiod was not without some effect on TRH gene expression, causing a slight decrease in SD, indicating some effect of photoperiod on TRH neurons.

Overall, the players necessary for the transduction of photoperiodic information, from the induction of TSHβ and CGA in the PIT1, through retrograde signalling in to the hypothalamus to adjust deiodinase activities and thereby modulate local thyroid hormone signalling, all appear to be in place in the F344 photoperiodic rat, as described in other species. The lack of detectable thyroid hormone change may simply be because it is a transient event that is rapidly equilibrated after the downstream output is activated, emphasising the need to investigate hormone flux rather than static levels. These thyroid hormone-dependent changes are thought to impact on seasonal reproduction and body weight [1–3]. However, the output from this signalling system remains to be identified.

One of the genes identified on the microarray as differentially expressed between LD and SD rats, and a potential target of triiodothyronine and intermediate between thyroid hormone signalling and GHRH is GALP. GALP was originally discovered as a ligand of galanin receptors in the porcine hypothalamus [28]. GALP distribution in the rat brain is predominantly in neural cell bodies in the hypothalamic ARC, median eminence and infundibular stalk [29,30]. GALP expression may be regulated by thyroid hormone, given that thyroidecomized rats have been reported as having significantly fewer GALP-expressing cells in the ARC than sham operated controls, and replacement of thyroxine partially reverses this effect [14]. GALP is also regulated by leptin and insulin and is thought to participate in the regulation of reproduction and metabolism [31] and may play a role in growth regulation. GALP can stimulate growth hormone secretion in the Rhesus monkey and rat [32,33]. In addition, recently, a role for GALP in stimulating intracellular calcium concentrations in GHRH neurons in the ARC, but not in NPY or POMC neurons, has been reported [13]. GHRH is the main stimulatory neuromodulator involved in regulating the secretion of growth hormone in mammals [34].

Previously we showed that GHRH mRNA levels in the ARC were higher after 28d in LD than in SD in the F344 rat [11] and we show here that the SD suppression could be achieved within 3 days. The reciprocal up-regulation of GHRH was observed within 3 days of transfer from SD10:14 to LD14:10. GALP, however, was found to be expressed in the arcuate nucleus of the F344 rat but did not show higher levels of mRNA expression in LD compared to SD until 14d. Although the GALP mRNA expression seen in LD14:10 rats was also attenuated in melatonin injected LD14:10 rats after 14d, there was no significant difference between LD14:10 and SD10:14 rats at 3 or 14d.

At present there is insufficient evidence to invoke GALP as an intermediate between photoperiodically controlled thyroid hormone signalling and GHRH, due to the temporal asynchrony between the changes in GALP and GHRH gene expression, such that GHRH mRNA responses occurred ahead of any changes in GALP mRNA level with either photoperiod or melatonin. This may suggest that GALP is not the primary photoperiod-driven regulator of GHRH, or that it may be involved in sustaining the longer term GHRH changes. Also at present we cannot exclude whether other mechanisms, such as translational or secretory control of GALP may provide routes to the regulation of GHRH release. Previously we have suggested that NPY may provide a mechanism through which GHRH expression may be regulated [11]. The rapid increase in NPY gene expression in SD versus LD rats, 3d post photoperiod switch, without any associated change in food intake, suggests that, in this context, NPY serves a different function to the regulation of appetite. As the time-course of gene expression changes in NPY are comparable to GHRH, the potential for NPY to act as a regulator of GHRH remains a possibility [15].

Methods

Ethics Statement

All animal experiments were performed under strict adherence to UK home office regulations according to the Animals (Scientific Procedures) Act, 1986, and were licensed by the UK home office under Project License PPL60/3615 and approved by the local ethics committee at the University of Aberdeen Rowett Institute of...
Nutrition and Health (Approval numbers: SA07/14E and SA08/17E).

Animals
Rats used in the study were male F344/N, purchased from Harlan Sprague Dawley Inc. USA at approx. 4 weeks old and were acclimatised for at least one week in a 12 h:12 h light dark photoperiod. In all rat experiments, apart from room lighting changes, all other environmental conditions were the same; temperature was 21°C +/-2°C, food (CRM [P] Rat and Mouse Breeder and Grower, standard pelleted diet (Special Diet Services, Witham, Essex, UK)) and water were provided ad libitum. In all experiments where rats were moved into new photoschedules, they were transferred to rooms with the original lights-on time, thus for the SD or SD10:14 rats, the photoperiods were shortened by advancing the lights off time.

Rats for Microarray, gene expression and thyroid hormone assay
Rats were randomly divided into weight matched groups, housed singly and transferred from 12 h:12 h light:dark into LD (16 h:8 h, light:dark) or SD (8 h:16 h, light:dark) photoperiod rooms. For time course analysis, rats were divided into six weight-matched groups (n=8-10 for in situ hybridization, n=6 for thyroid hormone assay and n=4 for microarray) with 3 groups assigned to LD and 3 to SD. Food intakes and body weights were measured and recorded weekly and bi-weekly respectively where possible. After the appropriate number of days, one group of rats from each photoperiod was killed at ZT3 using isoflurane inhalation and decapitation. Trunk blood was collected into 15 ml tubes for serum preparation. Brains were immediately removed and frozen on dry-ice and then serum and brains were stored at -80°C.

Photoperiod suppression by SD and daily melatonin injections
After acclimatization rats were housed initially in a LD photoperiod of 14 h:10 h light:dark (LD14:10) for 13d. Food intakes and body weights were recorded daily and 3x per week, respectively. Melatonin was administered according to Heideman et al [12]. For both the 3 and 14d duration experiments, rats were divided into 3 weight-matched groups (n=8). One LD14:10 group was injected daily with melatonin (100 µg of melatonin dissolved in 0.1 ml of 10% ethanol and 90% physiological saline; delivered subcutaneously) whilst LD14:10 and short day photoperiod (10 h:14 h light:dark, SD10:14) control groups were injected daily with vehicle (0.1 ml of 10% ethanolic saline). Injections were carried out 1 hour before lights off and continued for 3d or 14d at which time rats were anaesthetised using isoflurane inhalation and decapitated during the mid-light phase (3 hrs after lights on for LD and 4 hrs after lights on for SD). Brains were immediately removed and frozen on dry-ice and then stored at -80°C. Testes were dissected and weighed.

Photoperiod induction experiment
Rats were initially exposed to SD10:14 for 13d. Rats were divided into weight matched groups (n=8). Two groups remained in SD10:14, while 2 groups were transferred to LD14:10. Food intakes and body weights were recorded daily and 3x per week, respectively. After 3d, one SD and one LD group was killed and the remaining rats were killed after 14d during the mid-light phase and tissues collected as described above.

Microarray analysis
Hypothalamic blocks encompassing the ARC were dissected whilst frozen and total RNA was extracted for microarray analysis and gene cloning. For microarray, 4 hypothalamic blocks were used for each time point in both photoperiods. The total RNA extracts from individual blocks were applied to separate arrays, thus 24 arrays were used for comparisons. Microarray analysis was done on Affymetrix rat 230-2.0 Genechips by ServiceXS. Data from Service XS, in the CEL file format (containing the raw signal intensities) were transferred to the MadMax website (https://madmax.bioinformatics.nl) where data was analysed using the statistical programming language R [35] and R-libraries offered by the Bioconductor project [36]. The data were normalized using Bioconductor and a GCRI method, a Robust Multiarray Analysis with correction for the GC content of the oligos, and then statistically analysed using the Limma package, which allowed identification of the most differentially expressed genes between different conditions, i.e. photoperiods and time using a nominal p-value <0.05 to represent statistical significance. The MIAME compliant data for experimental conditions and data sets were submitted to the Gene Expression Omnibus and are accessible with the accession number GSE27926 (http://www.ncbi.nlm.nih.gov/geo/).

In situ hybridization
The expression distribution and mRNA levels of a selection of thyroid-signalling related genes were analysed in forebrain coronal sections. These were cut and analysed by in situ hybridization techniques previously described in detail [11]. Riboprobe templates were prepared as described earlier for the genes TRH [37], TSH[R] [38], Dio2 and Dio3 [2], MCT-8 [22] and GHRH [11]. Riboprobe templates were prepared for GALP, CGA and Oatplc1 by amplifying cDNAs from F344/N rat hypothalamus and then cloning Oatplc1 and GALP into pSC-B-amp/kan (Agilent Technologies UK Ltd, Edinburgh, UK) and CGA into pCR-Blunt 4 TOPO (Invitrogen, Paisley, UK). A TSH-receptor (TSH-R) riboprobe was amplified from mouse cDNA and cloned into pGEM-T-easy (Promega, Southampton, UK). Oligonucleotide primers for GALP were based on rat GALP (GenBank accession no. NM_033237), amplifying a 331 bp DNA fragment between bases 99–429. For GALP, forward primer was 5’-TGTATGCCCCGTTTTTCGAGTTC and reverse primer was 5’-CTATGCGCAGATCTCAGTCTTC. Oligonucleotide primers for CGA were based on rat CGA (GenBank accession no. NM_053918), amplifying a 455 bp DNA fragment between bases 1–455. For CGA, forward primer was 5’-CTGCCCAACACACATCTTCC and reverse primer was 5’-CGCGACGGGTCAGGAGTGC. Oligonucleotide primers for Oatplc1 were based on rat Oatplc1 (GenBank accession no. NM_053441), amplifying a 409 bp DNA fragment between bases 125–533. For Oatplc1, forward primer was 5’-GAGGCCACGTCGGAAGCCGAGA and reverse primer was 5’-AAGACAGGCGACCCCAAGAAC. Oligonucleotide primers for TSH-R were based on mouse TSH-R (GenBank accession no. NM_011648), amplifying a 312 bp DNA fragment between bases 607–918. For TSH-R, forward primer was 5’-TCCAGGGCTGTAGCAAAACTG and reverse primer was 5’-CAGCCCCAGTGAGGTGGGAGA. PCR amplifications of GALP, Oatplc1 and CGA were performed using KOD hotstart (Novagen, Merck Chemicals Ltd. Nottingham, U.K.) and for TSH-R using Go Taq Hotstart (Promega, Southampton, UK). PCR amplification conditions were denaturation for 1 cycle at 95°C for 2 minutes and then 35 cycles (TSH-R), 30 cycles (GALP and Oatplc1) or 25 cycles (CGA) at 95°C for 20 sec (30 sec for CGA and TSH-R), annealing at 58°C for GALP, 55°C for Oatplc1 and TSH-R, and 56°C for CGA for 30 sec and
extension at 70°C for 20 sec for GαLP and Oatplc1 and 72°C for 30 sec for CGα and 45 sec for TSH-R with a 10 minutes extension at 72°C for CGα and TSH-R.

Sense riboprobes were synthesised from the complementary DNA template strands and generated no signals in the hypothalamus or PT (Figure S2). Autoradiographic images were quantified using Image-Pro Plus software version 7.0 (Media Cybernetics UK, Marlow, Buckinghamshire, UK), which computes the integrated optical density of the signal relative to a standard curve generated by 14C autoradiographic microscales (Amersham Pharmacia Biotech UK Ltd, Little Chalfont, Buckinghamshire, UK).

T3/4 analysis

Intracellular hypothalamic T4 and T3 concentrations were measured by radio-immuno assay [39] following extraction of the tissues as previously described in detail [40]. In short, hypothalamic tissue blocks of an average of 49.1 mg each, pooled two by two, were homogenized in a methanol volume 3 times the tissue’s weight. As individual internal recovery tracers, 1500–2000 cpm of the T3 RIA cross-reactivity with T4 was 0.1–0.5%, whereas for T4 the detection limit of 5 fmol and an intra-assay variability of 2.8%. For T3, extracted thyroid hormones ranged from 55 to 75% for T3 and from 40 to 60% for T4. The T3 RIA had a detection limit of 2 fmol and an intra-assay variability of 2.2%. The T4 RIA had a detection limit of 5 fmol and an intra-assay variability of 2.8%. For the T3 RIA cross-reactivity with T4 was 0.1–0.5%, whereas for the T4 RIA cross-reactivity with T3 was 3.5%. All samples were measured within a single assay.

Statistics

Data were analysed by t tests and one or two-way ANOVA and repeated measures ANOVA, followed by Holm-Sidak method for pairwise comparisons as appropriate, using SigmaStat statistical software (Systat Software UK Ltd, Hounslow, London, UK). Results are presented as means ± SEM, and differences were considered significant at p<0.05.

Supporting Information

Figure S1 Paired testes weights of melatonin injected rats. The paired testes weights did not show any significant difference with photoperiod or melatonin injections. Data are mean ± SE.

Figure S2 Autoradiograph images using sense riboprobes. Sense riboprobes showed no detectable hybridization signals in the rat hypothalamic and PT regions. Sense riboprobes used were; (A) TRH, (B) TSHβ, (C) CGα, (D) Dio2, (E) Dio3, (F) MCT-8, (G) Oatplc1, (H) NPY, (I) GαLP, and (J) GHRH. Scale bar = 1.0mm for all images.

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Author Contributions

Conceived and designed the experiments: AWR PJM GH LR. Performed the experiments: GH LR VMD. Analyzed the data: AWR GH LR. Conordinated the reagents/materials/analysis tools: GH LR VMD. Wrote the paper: AWR GH PJM.

References

1. Hanon EA, Lincoln GA, Fustin JM, Dardente H, Masson-Pevel M, et al. (2000) Ancestral TSH mechanism signals summer in a photoperiodic mammal. Curr Biol 10: 1147-1152.
2. Barrett P, Ehling FJ, Schuhler S, Wilson D, Ross AW, et al. (2007) Hypothalamic thyroid hormone catabolism acts as a gatekeeper for the seasonal control of body weight and reproduction. Endocrinology 148: 3609-3617.
3. Yoshimura T, Yasuo S, Watanabe M, Igo M, Yamamura T, et al. (2003) Light-induced conversion of T4 to T3 regulates photoperiodic response of gonads in birds. Nature 426: 178-181.
4. Dardente H, Wyse CA, Binnie MJ, Dupre SM, London AS, et al. (2010) A molecular switch for photoperiod responsiveness in mammals. Curr Biol 20: 2193-2198.
5. Nakao N, Oho H, Yamamura T, Anraku T, Takagi T, et al. (2008) Thyrotropin in the pars tuberalis triggers photoperiodic response. Nature 452: 317-323.
6. Yasuo S, Watanabe M, Nakao N, Takagi T, Follett BK, et al. (2005) The reciprocal switching of two thyroid hormone-activating and -inactivating enzyme genes is involved in the photoperiodic gonadal response of Japanese quail. Endocrinology 146: 2551-2554.
7. Oho H, Hoshino Y, Yasuo S, Watanabe M, Nakane Y, et al. (2008) Involvement of thyrotropin in photoperiodic signal transduction in mice. P Natl Acad Sci USA 105: 18238-18242.
8. Revel FG, Sabourau M, Pevet P, Mkilekone JD, Simonneau V (2006) Melatonin regulates type 2 deiodinase gene expression in the Syrian hamster. Endocrinology 147: 4690-4697.
9. Yasuo S, Watanabe M, Igo M, Nakamura TJ, Watanabe T, et al. (2007) Differential response of type 2 deiodinase gene expression to photoperiod between photoperiodic Fischer 344 and nonphotoperiodic Wistar rats. Am J Physiol Regul Integr Comp Physiol 292: R1315-R1319.
10. Heideman PD, Sylvester CJ (1997) Reproductive photopsoniveness in unmanipulated male Fischer 344 laboratory rats. Biol Reprod 57: 134-138.
11. Ross AW, Johnson CE, Bell LM, Reilly L, Duncan JS, et al. (2009) Divergent regulation of hypothalamic neuropeptide Y and agouti-related protein by photoperiod in F344 rats with differential food intake and growth. J Neuroendocrinol 21: 610-619.
12. Heideman PD, Bierl CK, Sylvester CJ (2001) Photoresponsive Fischer 344 Rats are reproductively inhibited by melatonin and differ in 2-[125I] lodomelatonin binding from nonphotopermissive Sprague-Dawley rats. J Neuroendocrinol 13: 225-232.
13. Kuramochi M, Kohno D, Onaka T, Kato S, Yada T (2005) Galanin-like peptide and ghrelin increase cytosolic Ca2+ in neurons containing growth hormone-releasing hormone in the arcuate nucleus. Regul Pep 126: 85-89.
14. Cunningham MJ, Krasnow SM, Gevers EF, Chen P, Thompson CK, et al. (2004) Regulation of galanin-like peptide gene expression by pituitary hormones and their downstream targets. J Neuroendocrinol 16: 10-18.
15. Park S, Peng XD, Frohman IA, Kaeneman RD (2005) Expression analysis of hypothalamic and pituitary components of the growth hormone axis in fasted and streptozotocin-treated neuropeptide Y (NPY)-intact (NPY+/+) and NPY-knockout (NPY-/−) mice. Neuroendocrinology 81: 360-371.
16. Shearer KD, Goodman TH, Ross AW, Reilly L, Morgan PJ, et al. (2010) Photoripple regulation of retinoic acid signaling in the hypothalamus. J Neurochem 112: 246-257.
17. Matzuk MM, Kormiermeier CM, Whitfield GK, Kourides IA, Boime I (1986) The glycoprotein alpha-subunit is critical for secretion and stability of the human thyrotropin beta-subunit. Mol Endocrinol 2: 95-100.
18. Yasuo S, Nakao N, Okaura S, Igo M, Hagisawa S, et al. (2006) Long-day suppressed expression of type 2 deiodinase gene in the mediobasal hypothalamus of the Saanen goat, a short-day breeder: implication for seasonal window of thyroid hormone action on reproductive neuroendocrine axis. Endocrinology 147: 432-440.
19. Yasuo S, Yoshimura T, Ebihara S, Kohno D, Onaka T, et al. (2007) Temporal dynamics of type 2 deiodinase expression after melatonin injections in Syrian hamsters. Endocrinology 148: 4385-4392.
20. Yasuo S, Yoshimura T, Ebihara S, Korf HW (2010) Photoperiodic control of TSH-beta expression in the mammalian pars tuberalis has different impacts on the induction and suppression of the hypothalamo-hypophysial gonadal axis. J Neuroendocrinol 22: 43–50.

21. Friesema EC, Jansen J, Jachtzabel JW, Visser WE, Kester MH, et al. (2008) Effective cellular uptake and efflux of thyroid hormone by human monocarboxylate transporter 10. Mol Endocrinol 22: 1357–1369.

22. Herwig A, Wilson D, Logie TJ, Boelen A, Morgan PJ, et al. (2009) Photoperiod and acute energy deficits interact on components of the thyroid hormone system in hypothalamic tanycytes of the Siberian hamster. Am J Physiol Regul Integr Comp Physiol 296: R1307–R1315.

23. Sugiyama D, Kusuhara H, Taniguchi H, Ishikawa S, Nozaki Y, et al. (2003) Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood-brain barrier: high affinity transporter for thyroxine. J Biol Chem 278: 43489–43495.

24. van der Deure WM, Hansen PS, Peeters RP, Kyvik KO, Friesema EC, et al. (2008) Thyroid hormone transport and metabolism by organic anion transporter 1C1 and consequences of genetic variation. Endocrinology 149: 5307–5314.

25. Nakao N, Takagi T, Iigo M, Tsukamoto T, Yasuo S, et al. (2006) Possible involvement of organic anion transporting polypeptide 1c1 in the photoperiodic response of gonads in birds. Endocrinology 147: 1067–1073.

26. Segerson TP, Kauer J, Wolfe HC, Moltbaker H, Wu P, et al. (1987) Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. Science 230: 78–80.

27. Chiamolera MI, Woodford FE. (2009) Minireview: Thyrotropin-releasing hormone and the thyroid hormone feedback mechanism. Endocrinology 150: 1091–1096.

28. Ohtaki T, Kuman S, Ishibashi Y, Ogi K, Matsui H, et al. (1999) Isolation and cDNA cloning of a novel galanin-like peptide (GALP) from porcine hypothalamus. J Biol Chem 274: 37041–37045.

29. Takasu Y, Matsumoto H, Ohtaki T, Kuman S, Kinada C, et al. (2001) Distribution of galanin-like peptide in the rat brain. Endocrinology 142: 1626–1634.

30. Larm JA, Gundlach AL. (2000) Galanin-like peptide (GALP) mRNA expression is restricted to arcuate nucleus of hypothalamus in adult male rat brain. Neuroendocrinology 72: 67–71.

31. Lawrence C, Fraley GS (2011) Galanin-like peptide (GALP) is a hypothalamic regulator of energy homeostasis and reproduction. Front Neuroendocrinol 32: 1–9.

32. Shahab M, Cunningham MJ, Steiner RA, Plant TM (2005) Galanin-Like peptide elicits a robust discharge of growth hormone in the rhesus monkey (Macaca mulatta). Neuroendocrinology 81: 254–258.

33. Rich N, Reyes P, Reap L, Goswami R, Fraley GS (2007) Sex differences in the effect of prepubertal GALP infusion on growth, metabolism and LH secretion. Physiol Behav 92: 814–823.

34. Gabate MD, Duran-Prado M, Luque RM, Martinez-Fuentes AJ, Quintero A, et al. (2009) Understanding the multifactorial control of growth hormone release by somatotropes: lessons from comparative endocrinology. Ann N Y Acad Sci 1163: 137–153.

35. Boka R, Gentleman R (1996) R: A language for data analysis and graphics. J Comput Graph Stat 5: 299–314.

36. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, et al. (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 5: R80.

37. Ebling FJ, Wilson D, Wood J, Hughes D, Mercer JG, et al. (2008) The thyrotropin-releasing hormone secretory system in the hypothalamus of the Siberian hamster in long and short photoperiods. J Neuroendocrinol 20: 576–586.

38. Sanchez E, Singru PS, Wittmann G, Nouriel SS, Barrett P, et al. (2010) Contribution of TNF-alpha and nuclear factor-kappaB signaling to type 2 iodothyronine deiodinase activation in the mediobasal hypothalamus after lipopolysaccharide administration. Endocrinology 151: 3027–3035.

39. Darras VM, Huybrechts LM, Beighman L, Kuhn ER, Decuyper E (1990) Ontogeny of the effect of purified chicken growth hormone on the liver 5'-monodeiodination activity in the chicken: reversal of the activity after hatching. Gen Comp Endocrinol 77: 212–220.

40. Reynolds GE, Janssens KA, Boyce J, Kuhn ER, Darras VM (2002) Changes in thyroid hormone levels in chicken liver during fasting and refeeding. Comp Biochem Physiol B Biochem Mol Biol 132: 239–245.