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Hypothalamic Thyroid Hormone Catabolism Acts as a Gatekeeper for the Seasonal Control of Body Weight and Reproduction

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Seasonal adaptations in physiology exhibited by many animals involve an interface between biological timing and specific neuroendocrine systems, but the molecular basis of this interface is unknown. In this study of Siberian hamsters, we show that the availability of thyroid hormone within the hypothalamus is a key determinant of seasonal transitions. The expression of the gene encoding type III deiodinase (*Dio3*) and Dio3 activity *in vivo* (catabolism of T_4 and T_3) is dynamically and temporally regulated by photoperiod, consistent with the loss of hypothalamic T_3 concentrations under short photoperiods. Chronic replacement of T_3 in the hypothalamus of male hamsters exposed to short photoperiods, thus bypassing synthetic or catabolic deiodinase enzymes located in cells of the ependyma of the third ventricle, prevented the onset of short-day physiology: hamsters maintained a long-day body weight phenotype and failed to undergo testicular and epididymal regression. However, pelage moult to a winter coat was not affected. Type II deiodinase gene expression was not regulated by photoperiod in these hamsters. Collectively, these data point to a pivotal role for hypothalamic DIO3 and T_3 catabolism in seasonal cycles of body weight and reproduction in mammals. (*Endocrinology* 148: 3608–3617, 2007)

BIOLOGICAL RHYTHMS CAN have profound temporal effects on physiology and behavior. Few are more spectacular than the large amplitude changes in food intake, body weight, reproductive behavior, and physiology observed in seasonal mammals and birds (1–3). The molecular basis of the seasonal timing system is poorly understood, although it is clear in mammals that nocturnal melatonin secretion by the pineal gland conveys temporal information to melatonin receptors located at a number of sites within the hypothalamus (4–6). How these sites interface with the neuroendocrine regulation of seasonal physiology and behavior has still to be elucidated.

Recent studies in birds and mammals suggest that central thyroid hormone availability (T_3) may play a significant role in the seasonal regulation of the reproductive axis (7–9). In the Japanese quail, a long-day (LD) breeder, T_3 infusion stimulates gonadal growth in birds housed in short-day (SD) length, consistent with the elevated T_3 synthesis found in the medial basal hypothalamus of LD-housed birds (9). In sheep, a SD breeder, there is an absolute requirement for T_3 to trigger cessation of the breeding season (10–14).

The local availability of T_3 in the hypothalamus is likely to be determined by type II deiodinase (DIO2) that converts T_4 to active T_3 by outer ring deiodination, and type III deiodinase (DIO3), which catalyzes the conversion of T_4 to rT_3, and T_3 to 3′,3′-diiodothyronine (T_2) by inner ring deiodination. The balance of activities of these two enzymes thus determines the availability of active T_3 hormone within the hypothalamus (15). In the Japanese quail under LD, *Dio2* is strongly expressed and *Dio3* is weakly expressed in the basal hypothalamus, consistent with a high level of active T_3 and maintenance of gonadal growth. Conversely, under SD, *Dio2* is weakly and *Dio3* is strongly expressed, consistent with a low level of T_3 and gonadal atrophy (16). In the SD-breeding Saanen goat, *Dio2* is expressed in the hypothalamus at a high level in SD, yet is suppressed by LD (17). This reciprocal pattern of expression of *Dio2* in LD- and SD-breeding animals may provide a common output from divergent temporal input, but the functional significance of the changes in hypothalamic T_3 availability in mammals has not been demonstrated.

The Siberian hamster exhibits gonadal regression as well as profound reductions in food intake and body weight in response to SD. In this study, we have demonstrated a coordinate role for hypothalamic T_3 metabolism in the photoperiodic control of energy balance as well as reproduction.

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Abbreviations: ARC, Arcuate nucleus; DIO2, type II deiodinase; DIOIII, type III deiodinase; dmpARC, dorsal medial posterior arcuate nucleus; LD, long day; SD, short day; VMN, ventromedial nucleus.

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Materials and Methods

Animals and housing

All experiments were carried out on male Siberian hamsters (*Phodopus sungorus*). These were obtained from breeding stocks held at the Rowett Research Institute and the University of Nottingham. All research using animals was licensed under the Animals (Scientific Procedures) Act of 1986 and received ethical approval from appropriate ethical review committees.

Siberian hamsters were individually housed at a constant temperature of 20 C with *ad libitum* access to food and water. Hamsters held in LD photoperiod were exposed to a light/dark cycle of 16 h light/8 h darkness. Hamsters in SD photoperiod were exposed to a light/dark cycle of 8 h light/16 h darkness.

In a basic LD *vs.* SD paradigm, hamsters were housed in LD or SD for 14 wk before they were culled at 3 h after lights on. To look at progression of gene expression change in SD hamsters, adult hamsters were transferred from LD to SD and culled with the corresponding LD controls 2, 4, 8, or 14 wk later. In this experiment, hamsters were culled in the middle of the light phase.

Pinelaectomy or a sham operation was performed on hamsters in LD under general anesthetic (ketamine, 0.4 mg/kg, and xylamine, 0.2 mg/kg; ip). Hamsters were then transferred to SD for 12 wk. Pinealectomy prevented all the SD-induced phenotypic changes, including decrease in body weight, testicular regression, and moult to a winter pelage (data not shown).

Juvenile hamsters, which are very responsive to photoperiod (18, 19), used for DIO3 activity measurements were taken from litters on the day of weaning and transferred to either LD or SD photoperiod and held in these respective photoperiods for 6 wk. These hamsters were culled in the middle of their respective light phase. Adult hamsters used for DIO3 activity measurement were 14 wk in LD or SD and were culled 3 h after lights on.

Deiodinase gene riboprobes

Oligonucleotide primers for type 2 deiodinase were based on mouse type 2 deiodinase sequence (GenBank accession no. AF096875) amplifying a 494-bp DNA fragment between nucleotides 67–560. Forward primer, 5′-CTCTTCCTGGCGCTCTATGACTCG; reverse primer, 5′-TCCTCTTGGTTCCGGTGCTTCTTA. Oligonucleotide primers for DIO3 were based on a consensus sequence for the mouse, rat, and bovine sequence DIO3 (GenBank accession nos. NM_172119, NM_017210, and NM_001010993, respectively) The primers amplify a 390-bp DNA fragment between nucleotides 16–405 of the bovine *Dio3* sequence. Forward primer, 5′-CATSCTGCGCTCYCTGCTGCTTCA; reverse primer, 5′-YGKGCGTAGTCGAGGATGYGCT.

Thyroid hormone receptor riboprobes

Oligonucleotide primers for TRα1 were based on mouse TRα1 (GenBank no. NM_178060), amplifying a 272-bp DNA fragment between bases 1542–1813. Forward primer, 5′-TGACTGACCTCCGCATGATCG; reverse primer, 5′-TGGGCAGTGCAGGGGTGTAT.

Oligonucleotide primers for TRβ1 were based on mouse TRβ1 (GenBank no. S62756), amplifying a DNA fragment of 293 bp between bases 67–359. Forward primer, 5′-CTAACAGTATGACAGAAAATGGCCTTCC; reverse primer, 5′-ATAGCTGGGGATATACCCTTTACATTTC.

Oligonucleotide primers for TRβ2 were based on mouse TRβ2 sequence (GenBank no. NM_009380) amplifying a 246-bp DNA fragment between bases 362–607. Forward primer, 5′-CATGCAGGTCACTGACTACC; reverse primer, 5′-GGCGACTGCACTTGAGGAA.

The conditions for amplification for each of these sequences are given as supplemental data (published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org).

In situ hybridization

In situ hybridization was carried out as described previously (20). In brief, frozen brain sections mounted on glass slides were fixed in 4% paraformaldehyde in 0.1 M PBS, acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8. Radioactive probes (approximately 10^6 cpm) were applied to the slides in 70 μl hybridization buffer containing 0.3 M NaCl, 10 mM Tris-HCl (pH 8), 1 mM EDTA, 0.05% transfer RNA, 10 mM dithiothreitol, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA, and 10% dextran sulfate. Hybridization was carried out overnight at 55 C. Posthybridzation, slides were washed in 4× standard saline citrate (1× standard saline citrate is 0.15 M NaCl, 15 mM sodium citrate), then treated with ribonuclease A (20 μg/μl) at 37 C and finally washed in 0.1× standard saline citrate at 60 C. Slides were dried and apposed to Biomax MR film for 5–7 d.

Image analysis

Slides containing all brain sections for a complete experiment were apposed to a single sheet of autoradiographic film. Autoradiographic films were scanned at 600 dpi on a Umax scanner linked to a PC running Image-Pro PLUS version 4.1.0.0 analysis software (Media Cybernetics, Wokingham, UK). For each probe, four sections spanning a selected region of the hypothalamus (approximately bregma −2.54 mm to bregma −1.82 mm) were chosen for image analysis.

Integrated optical density for each selected region was obtained by reference to a standard curve generated from the ^{14}C microscale. The integrated optical densities for each section of each animal were added and an average (with SE of the mean) was obtained for a specific treatment. The values of one treatment in an experiment were set to 100% expression value, and other treatment values were calculated accordingly.

DIO3 activity

DIO3 activity was measured in Siberian hamster hypothalamus and cerebellum tissue samples. Frozen tissue samples were homogenized on ice in 10 vol PED1 buffer [0.1 M phosphate and 2 mM EDTA (pH 7.2)] using MagnaLyser Beads and a MagnaLyser apparatus (Roche Molecular Biochemicals, Mannheim, Germany). Homogenates were snap frozen in aliquots and stored at −80 C until further analysis. Protein concentration was measured with the Bio-Rad protein assay (Bio-Rad, Hertfordshire, UK) using BSA as the standard following the manufacturer's instructions. DIO3 activity was measured by duplicate incubations of homogenates (~100 μg protein) for 60 min at 37 C with 1 nM [3′-^{125}I]T_3 (200,000 cpm) in a final volume of 0.1 ml PED50 buffer. Reactions were stopped by addition of 0.1 ml ice-cold methanol. After centrifugation, 0.1 ml of the supernatant was added to 0.1 ml 0.02 M ammonium acetate (pH 4), and 0.1 ml of the mixture was applied to a 4.6 × 250 mm Symmetry C18 column connected to an Alliance HPLC system (Waters, Etten-Leur, The Netherlands). The column was eluted with a linear gradient of acetonitrile (28–42% in 15 min) in 0.02 M ammonium acetate (pH 4.0) at a flow of 1.2 ml/min. The activity in the eluate was measured online using a Radiomatic Z-500 flow scintillation detector (Packard, Meriden, CT).

Intrahypothalamic implantation

Implants were made by thoroughly mixing crystalline T_3 (Sigma-Aldrich, Poole, UK) and medical grade silicone/SILASTIC-brand adhesive (MED-1137; Polymer Systems Technology Ltd., High Wycombe, UK) in a 1:9 ratio. The viscous mixture was tamped into the ends of short (5 mm) pieces of polyethylene tubing (0.5 mm inner diameter, 1.0 mm outer diameter; SIMS Portex Ltd., Hythe, UK) and allowed to cure at room temperature for 48 h. The solid SILASTIC-brand polymer was extruded by means of a wire plunger, then cut under a dissecting microscope into 500-μm lengths. Sham implants were made by omitting the T_3. The implants were washed then incubated in 0.9% sterile saline overnight before insertion.

Microimplants were placed bilaterally in 30 hamsters (n = 15 T_3; n = 15 sham) ranging in age from 19–27 wk. The hamsters were anesthetized with a reversible anesthetic (mixture of 1 mg/kg ketamine and 0.25 mg/kg medetomidine, ip) and injected with an analgesic (0.1 mg/kg carprofen, sc). Animals were then placed in a stereotaxic frame with the height of the incisor bar set at the intraaural level. A small burr hole was drilled in the skull on midline at the point of bregma and a stainless steel 25 g cannula holding the implant in its tip was lowered to 6.5 mm below the surface of the dura at 0.5 mm to the left of the midline as defined by the center of the superior midsagittal sinus. The implant was extruded

by insertion of a 29 g obturator into the cannula, then the process was repeated on the contralateral side. The skin was closed over the wound with Michel clips, and the anesthesia was reversed by the injection of an antisedan (0.2 mg/kg atipamezole, sc). After surgery, all hamsters received daily analgesia and fluid replacement and supplemental highly palatable foodstuffs (wet lab chow and sunflower seeds) to aid recovery from surgery. The wound clips were removed in the second week after surgery under brief isoflurane anesthesia.

Experimental protocol

Hamsters from each experimental group were allocated at 12–16 d post surgery on the basis of matched body weights to remain in the LD photoperiod (n = 6 T_3, n = 6 sham), or to be transferred to a SD photoperiod (n = 8 T_3, n = 7 sham). Body weight and the weight of food pellets consumed were both recorded weekly for 8 wk, then a blood sample was collected by cardiac puncture under terminal sodium pentobarbitone anesthesia (Rhone Merieux, Harlow, UK), and the wet weights of the testes, epididymides, seminal vesicles, and epididymal fat pads were obtained. The brains were rapidly removed and frozen on dry ice, then stored at −80C. Fifteen-micrometer sections of frozen brains were prepared. Every 10th section, spanning a region from the posterior hypothalamus (bregma −2.7 mm) to the preoptic area (bregma −0.22 mm) was stained with toluidine blue. Pelage was scored using a modification of a four-point scale devised by Duncan and Goldman (21).

Carcass lipid determination

Animal carcasses were opened, weighed, frozen to −20 C, then freeze dried in a chamber under a vacuum of 10^{-2} torr and a shelf temperature of 25 C. After drying for 48 h, the carcasses were weighed to give a freeze dried matter then finely ground in an IKA A11basic analysis mill (IKA Werke GMBH & Co., Staufen, Germany).

Homogeneous subsamples were weighed (2.5–5 g) in duplicate for oven dry matter determination and total fat analysis.

The samples were transferred to a conical flask and boiled under reflux in 4 M HCl for 1 h. The hydrolysate was cooled and filtered through approximately 1 g of Celite/Hyflo filter aid contained in a Whatman no. 541 filter paper cone and washed with water until the filtrate was acid free. Each filter was dried overnight at 50 C then placed in an extraction thimble before extraction with 40/60 Petroleum Ether in a Tecator Soxtec System HT 1043 Extraction Unit (Foss, Warrington, Cheshire, UK). The solvent was then evaporated off from the extract and the recovered fat was weighed. The percentage fat extracted from the original sample was then calculated.

Statistical analysis

Values are expressed as mean ± SE. The statistical tests applied in this study were *t* tests or two-way ANOVA with *post hoc* Tukey tests for multiple comparisons as appropriate.

Results

Deiodinase expression in Siberian hamster brain

Dio2 is expressed in cells of ependymal layer lining the third ventricle and in some cells of the neuropil close to the ependymal wall (Fig. 1A), but expression is not affected by photoperiod (Fig. 1, B and C). *Dio3* expression has a very similar distribution (Fig. 2A), but in contrast, its expression is markedly affected by photoperiod, being present in SD but absent in LD (Fig. 2B). After the switch from LD to SD, there is a strong inverse temporal correspondence between the appearance of DIO3 expression in the ependymal layer and the decrease in body and testes weights over the first 8 wk of SD exposure, at which time both the body and testes weights approach their nadir. Thereafter and up to 14 wk, *Dio3* expression declined to 32% of peak value with no further decline in body or testes weights (Fig. 2C). When hamsters were housed in LD or SD for a period of 25 wk, during

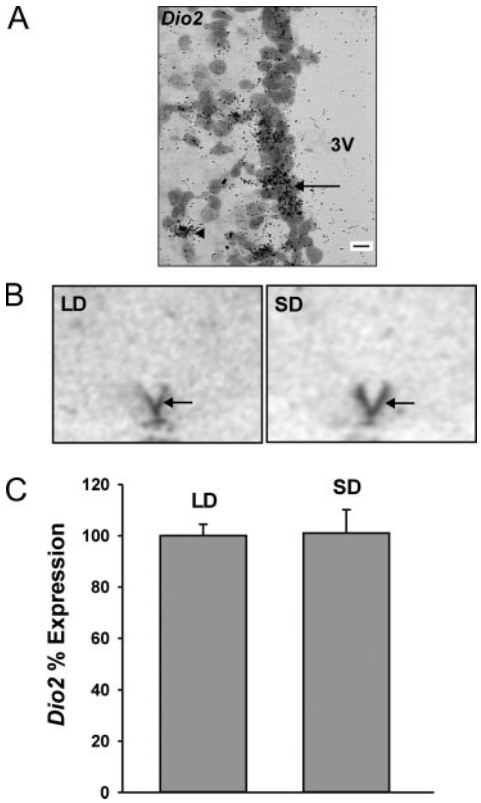

FIG. 1. Localization and effect of photoperiod on *Dio2* gene expression. Siberian hamsters were exposed to photoperiod of 16 h light/8 h dark (LD) or 8 h light/16 h dark (SD) for 14 wk. *In situ* hybridization was performed on 14-μm brain sections with ^{35}S-labeled riboprobes. A, Image from an emulsion-coated slide revealing *Dio2* expression (silver grains indicated by *arrow*) in cells of the ependymal layer. Some labeled cells adjacent to the ependymal layer are also observed (*arrowhead*). *Scale bar*, 10 μM. 3V, Third ventricle. B, Autoradiographic image of the labeled riboprobe hybridized to LD and SD hamster sections in the region of the posterior hypothalamus. The *arrow* indicates hybridization signal in the ependymal layer due to *Dio2* probe hybridization. C, Quantification of the autoradiographic signal by image analysis reveals no difference in expression level of *Dio2* with photoperiod exposure.

which time SD hamsters had reached a nadir of bodyweight by wk 19 and had become refractory to SD, increasing body weight by 16% at wk 25 (wk 19, 25.4 ± 0.5 g *vs.* wk 25, 29.5 ± 1.2 g; n = 14, $P = 0.004$), *Dio3* was not expressed in the ependymal cells of either the LD or SD refractory hamsters (data not shown).

The key role of the changing pattern of nocturnal melatonin secretion in mediating the SD-induced increase in *Dio3* expression was demonstrated by removing the pineal gland of hamsters in LD, and then examining *Dio3* expression after subsequent exposure to SD. Control sham-operated hamsters exposed to SD were found to have a high level of *Dio3* expression, whereas pinealectomized hamsters exposed to SD showed no expression of *Dio3* in the ependymal layer (Fig. 3), similar to a LD phenotype.

Deiodinase activity

Consistent with the increase in *Dio3* gene expression, DIO3 enzyme activity in extracts from dissected blocks of

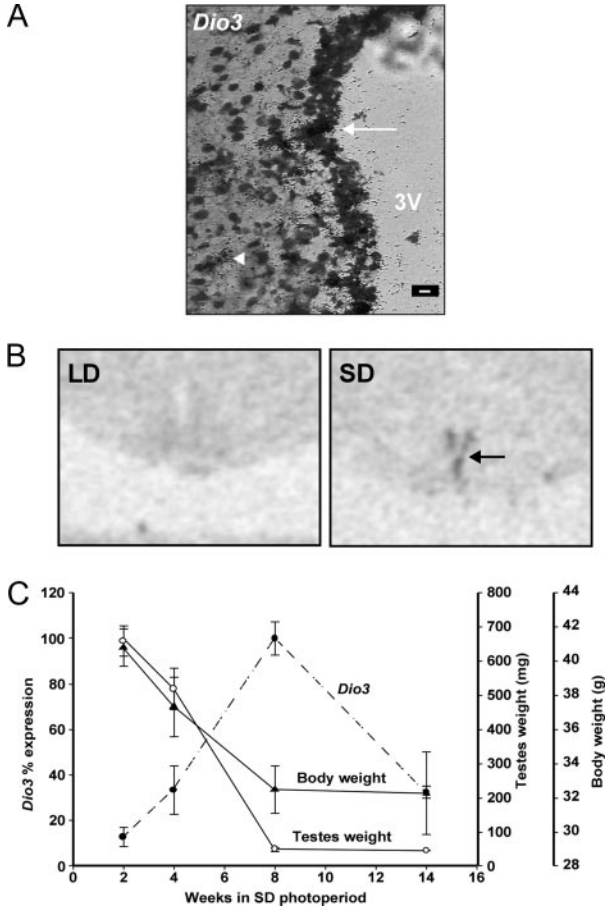

FIG. 2. Localization and effect of photoperiod on *Dio3* gene expression. A, Image from an emulsion-coated slide revealing *Dio3* expression (silver grains indicated by *arrow*) in cells of the ependymal layer. Some labeled cells adjacent to the ependymal layer are also observed (*arrowhead*). *Scale bar*, 10 μM. 3V, Third ventricle. B, Autoradiographic image of the labeled riboprobe hybridized to LD and SD hamster sections in the region of the posterior hypothalamus. In LD, no expression of *Dio3* is found in the ependymal layer, but expression is induced by SD exposure (*arrow*). No hybridization signal is observed with sense probes for either *Dio2* or *3* sequence (not shown). C, Time course of *Dio3* expression and body and testes weight change induced by SD exposure. No expression of *Dio3* was observed in hamsters held in LD for the same duration (not shown).

hamster hypothalamic tissue verified higher enzyme activity in SD animals. Two groups of hamsters were used in this experiment, in juvenile hamsters exposed to SD for 6 wk (body weights, SD 22.1 ± 1.1 g *vs.* LD 29.1 ± 0.8 g; $P = 0.005$), DIO3 activity was higher in SD (SD 5.578 ± 0.231 fmol/mg·min *vs.* LD 2.827 ± 0.249 fmol/mg·min; $P < 0.001$; SD, n = 4, and LD, n = 3). Activity in extracts of the cerebellum of these animals was lower and not altered by photoperiod (SD 0.47 ± 0.195 fmol/mg·min *vs.* LD 0.327 ± 0.142 fmol/mg·min; $P = 0.36$; n = 3 both groups). Hypothalamic tissue of adult hamsters housed in SD for 14 wk had a higher DIO3 activity in SD (SD 1.428 ± 0.085 fmol/mg·min *vs.* LD 0.573 ± 0.022 fmol/mg·min; $P < 0.001$; SD, n = 4, and LD, n = 3). These data are consistent with the relative levels of gene expression found in adult hamsters when exposed to SD for 6 and 14 wk (Fig. 2C).

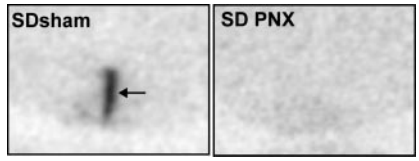

FIG. 3. Effect of pinealectomy on *Dio3* expression assessed by *in situ* hybridization. Hamsters were pinealectomized (SD PNX) or received sham surgery in LD, then exposed to SD for 12 wk. *Arrow* indicates Dio3 expression in the ependymal layer.

Effect of thyroid hormone on seasonal phenotype

SILASTIC-brand implants impregnated with T_3 or sham implants were implanted bilaterally into the brain of the Siberian hamsters. After 2 wk of postsurgical recovery, hamsters were placed in either LD or SD and the effect of this manipulation on seasonal physiological responses was monitored for 8 wk. The majority of implants were located in a dorsomedial position in the hypothalamus, close to the ventricular wall and spanning a range from bregma −1.46 to −2.3 mm (Fig. 4A). Only three hamsters were found to have implants out with this range, in one hamster, the site of the implants was centered approximately at −1.34 mm, in the second, the site was centered approximately at −0.7 mm bregma, and the third was centered approximately at bregma

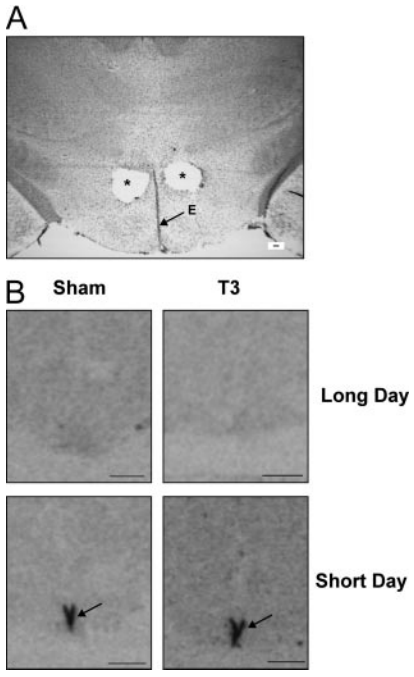

FIG. 4. SILASTIC-brand implant placement. A, A photomicrograph of a brain section showing the approximate location and size of the majority of the SILASTIC-brand implants placed in the brain of the Siberian hamster. *, The site of the implants. Indicated by a labeled *arrow* (E) is the ependymal layer. *Scale bar*, 100 μM. B, Ependymal expression of *Dio3* assessed by *in situ* hybridization after SILASTIC-brand implant placement into the brain of Siberian hamsters. *Upper panels*, LD hamsters; *lower panels*, SD hamsters. *Left column*, Hamsters received sham implants; *right column*, hamsters received T_3-releasing implants. No expression of *Dio3* was observed in LD sham hamsters and four of six LDT3 hamsters. However, *Dio3* expression was observed in the ependymal layer (*arrow*) in all SD sham and SDT3 hamsters. *Scale bar*, 1 mm.

−2.7 mm. No adverse effects of implants (for example, reduced food intake, weight loss, or neurological symptoms) were noted in any of the hamsters. All SD-exposed hamsters, irrespective of implant placement, responded to SD exposure as judged by the induction of *Dio3* in the ependymal layer. No expression of *Dio3* was observed in sham-implanted hamsters maintained in LD (Fig. 4B). Expression was found in two of six LD-exposed hamsters with implants releasing T_3 (data not shown).

Body weight

Body weight was normalized to the weight at the day of transfer to the experimental photoperiod. Vehicle (LD sham)- or T_3-treated (LDT3) hamsters held in LD photoperiod maintained a steady increase in body weight over the course of the experiment (Fig. 5A). Hamsters held in SD with sham implants (SD sham) maintained a steady body weight for 4 wk and declined thereafter, losing 10% (4 g) of body weight at 8 wk in SD photoperiod. However, hamsters with T_3-releasing implants (SDT3) showed a steady body weight increase with an average increase of 7.7% (3.3 g) (Fig. 5A). Epididymal fat pad weights were significantly smaller in SD sham hamsters, compared with other groups ($P < 0.001$). SDT3-treated hamsters had similar epididymal fat pad weights as LD sham or LDT3 hamsters (Fig. 5B). Analysis of total lipid content of these hamsters was consistent with the aforementioned body weight data; LD sham group lipid content 55 ± 4% of body weight, LDT3 group, 45 ± 4%; SD sham, 39 ± 4%; SDT3, 55 ± 4%. A two-way ANOVA showed the SD sham group to be significantly different from the remaining groups ($P < 0.001$).

Food intake

Food intake was monitored weekly after transfer to experimental photoperiod (Fig. 5C). T_3-implanted hamsters tended to have a higher weekly food intake than vehicle implant hamsters, and LD-housed hamsters tended to have a higher weekly food intake than their respective SD groups, but neither comparison reached significance. Over the course of the 8-wk experiment, total food intake was highest and significantly different in T_3-treated animals compared with vehicle treatment ($P = 0.011$), suggesting T_3 increased food intake. Within groups, SDT3 and LDT3 had significantly greater food intake than SD sham ($P < 0.05$).

Reproductive organs

LD sham hamsters had a paired testis weight of 610 ± 80 mg, typical for hamsters housed in this photoperiod. Testes weight in SD sham hamsters was 162 ± 23 mg, demonstrating the imposed SD photoperiod was read and initiated seasonal testicular regression. LDT3 hamsters had a paired testis weight of 798 ± 102, not statistically different from the LD sham group, whereas testes in SDT3 hamsters were 563 ± 106 mg, significantly larger than those in SD sham hamsters ($P < 0.001$), but not statistically different from LD sham hamsters (Fig. 6A). Exposure to SD caused a significant decrease in the weight of both the epididymis and the seminal vesicles (SD sham); implantation of T_3 completely blocked these SD-induced decreases such that these organ weights

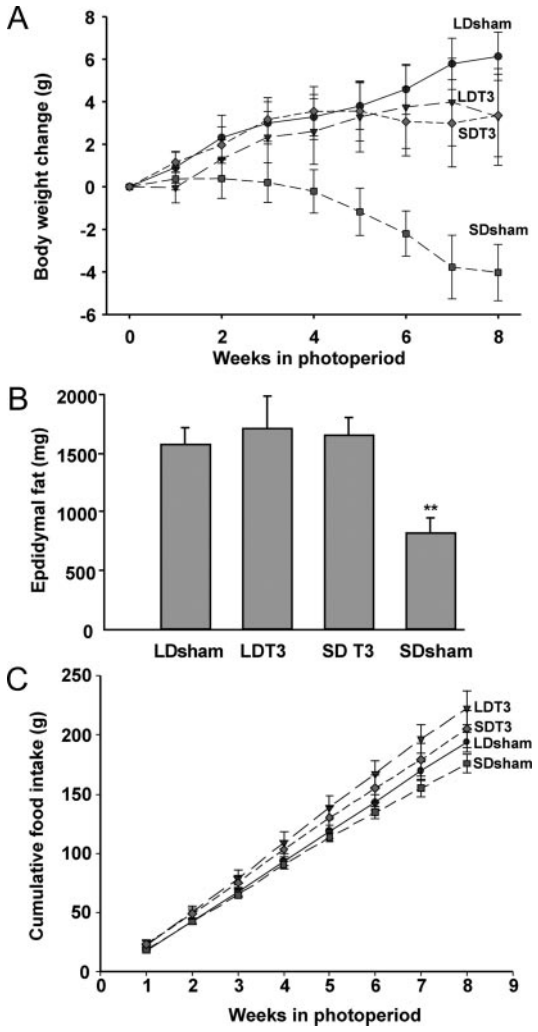

FIG. 5. Body weight and food intake in response to *in vivo* T_3 release. A, SILASTIC-brand implants impregnated with or without T_3 were placed into the brain of Siberian hamsters. Body weight measurement was taken on a weekly basis. Values were normalized for each group to 0 g on the day of transfer to experimental photoperiod after a 2-wk recovery period. B, Average postmortem epididymal fat pads weights. Two-way ANOVA shows significant reduction in fat pad weight in SD sham group only, $P < 0.01$. C, Weekly food intake data recorded after transfer to experimental photoperiod. Two-way ANOVA shows significant effect of T_3 treatment over sham, $P < 0.05$. Food intake in SD sham group is less than all other treatments, $P < 0.05$.

(SDT3) were similar to those in sham-implanted hamsters (LD sham) maintained in LD (Fig. 6, B and C).

Pelage

Pelage was scored using a modification of a four-point scale devised by Duncan and Goldman (21). In LD sham and LDT3 groups, pelage score did not change from a LD value of 4 indicating a full summer coat of dark fur (a score of 1 is full winter pelage). In both SD groups, the appearance of the white winter pelage was evident with a greater degree of change in the SD sham group (score 3.0 ± 0.2) compared with the SDT3 group (3.6 ± 0.1), and both groups were significantly different from both groups of LD-housed animals (Fig. 6D; LDT3 *vs.* SDT3, $P = 0.023$; LDT3 and LD sham *vs.* SD

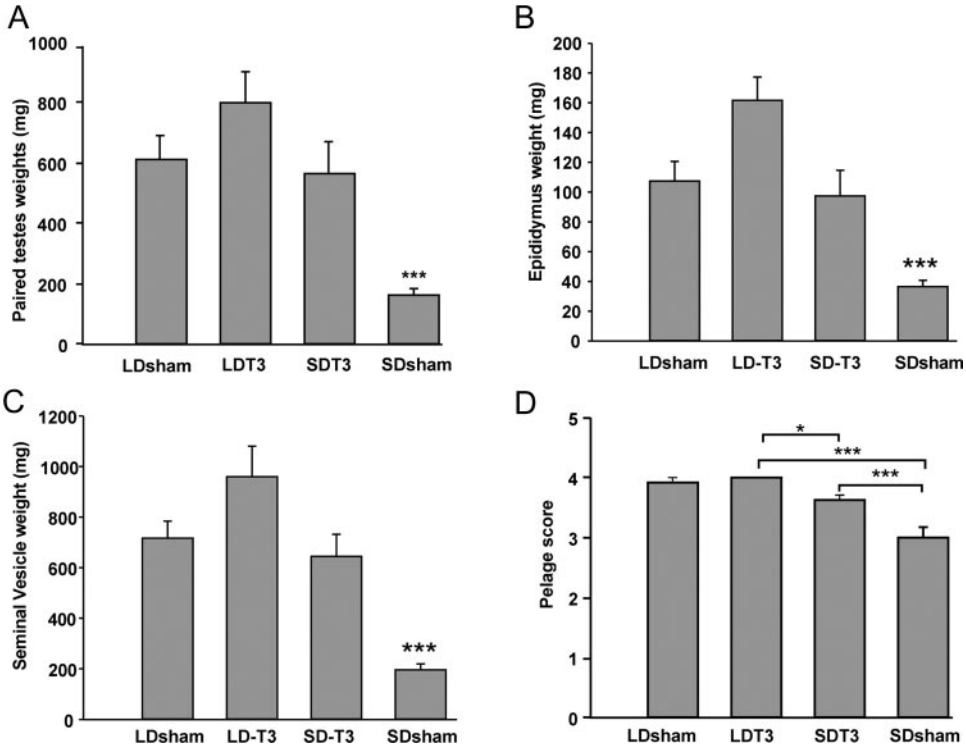

FIG. 6. Reproductive and pelage response to *in vivo* T_3 release. A, Average paired testes weights. Epididymus weight (B) and seminal vesicle weight (C) of hamsters bearing T_3-releasing or sham implants in either LD or SD photoperiod. A two-way ANOVA shows significant reduction in all three measurements in the SD sham group, $P < 0.001$. D, Pelage scores. Two-way ANOVA shows significant effect of SD in the transition of pelage from a dark summer to light winter coloration. *, $P < 0.5$; ***, $P < 0.001$.

sham, $P < 0.001$). However, the moult to winter pelage in the SDT3 group was retarded with respect to the SD sham treatment ($P < 0.001$).

Hypothalamic thyroid hormone receptor expression

Brain tissue of LD- and SD-exposed Siberian hamsters was investigated by *in situ* hybridization for distribution and potential regulation of the three forms of thyroid hormone receptor, α1 (*TRα1*), β1 (*TRβ1*), and β2 (*TRβ2*). *TRα1* showed widespread distribution throughout brain with no apparent effect of photoperiod on expression (data not shown). *TRβ1* had a discrete location in the region of the arcuate (ARC) and ventromedial (VMN) nuclei, and posterior to the premammillary nuclei. Within the ARC, a particularly high level of expression was found in the dorsal medial posterior arcuate nucleus (dmpARC) (Fig. 7A). No effect of photoperiod was found on expression in any region (Fig. 7B). TRβ2 was similarly prominent in the ARC (including the dmpARC) and VMN. The premammillary area and posterior hypothalamus also had high expression of *TRβ2* (Fig. 7C). In SD there was, overall, a 40% increase in *TRβ2* expression throughout the ARC/VMN region (excluding the premammillary area, $P < 0.001$; Fig. 7D). The dmpARC could not be sufficiently discerned to quantify independently. No effect of photoperiod was observed on *TRβ2* expression in the premammillary area.

DIO2 and DIO3 expression in Syrian hamsters

Syrian hamsters are another commonly used photoperiodic model of seasonal reproduction (22). In the ependymal layer of these hamsters, *Dio2* was found to be dramatically decreased in expression, with almost complete cessation in the ependymal layer in SD-exposed animals (Fig. 8). These data concur with recently published observations that *Dio2* expression in the Syrian hamster is under photoperiodic control and dependent upon the melatonin signal (23). However, *Dio3* was not expressed in either LD or SD (Fig. 8).

Discussion

The data presented in this study strengthen the evidence that hypothalamic thyroid hormone metabolism plays a key role in the photoperiod regulation of seasonal physiology. Specifically, we show that thyroid hormones are important to the regulation of both the energy balance and reproductive axes, but are not integral to the control of pelage. Importantly, the *in vivo* data in this study are generated from intact animals, in contrast to many other studies where thyroidectomy has been performed before assessing the effect of T_4 or T_3 replacement (7, 8, 10–14, 24), or a previous study with Siberian hamsters using peripherally administered T_4 or T_3, but not central administered hormone (25). Our study, together with one in quail and one in sheep using centrally administered T_4 or T_3, strongly supports a mechanism of T_3 action on the neuroendocrine axis mediated in the brain (9, 13).

In our study on Siberian hamsters, the expression of Dio2, the synthetic enzyme of the T_3 pathway, was unaffected by photoperiod. This contrasts with an initial report of an inhibitory effect of SD on Dio2 expression in Siberian hamsters (26). However, it should be noted that in the study by Watanabe *et al.* (26), the inhibitory effect of SD exposure was observed in hamsters transferred from LD to SD when weaned at three wk of age to prevent testicular maturation (26). Consistent with our observations, Watanabe *et al.* (19) recently reported that the induction of Dio2 did not occur in

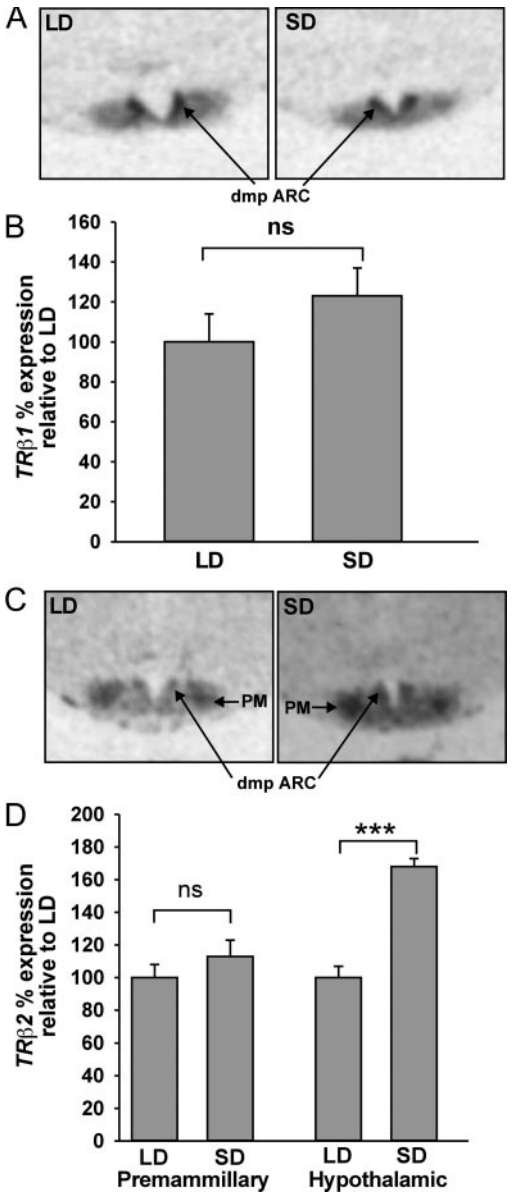

FIG. 7. Effect of photoperiod on thyroid hormone β receptor expression in the hypothalamus. A, *In situ* hybridization for thyroid hormone β1 (*TRβ1*). Expression was observed in dmpARC and adjacent hypothalamic areas. B, Quantification of *TRβ1* expression in the hypothalamus shows no difference in expression between LD- and SD-exposed hamsters. C, *In situ* hybridization for *thyroid hormone β2* (*TRβ2*). Expression was observed in dmpARC and adjacent hypothalamic areas. Expression of *TRβ2* was distinct in the premammillary area (PM). D, Quantification of expression in the premammillary area (PM) did not reveal a LD-SD difference. However, quantification of *TRβ2* expression in the remainder of the hypothalamic region shows *TRβ2* to be significantly increased in SD (***, $P < 0.001$).

Siberian hamsters maintained from birth in LD and then transferred at 7 wk of age to SD.

Dio3 expression in the Siberian hamster, a catabolic enzyme for T_3, was profoundly regulated by photoperiod, consistent with the hypothesis that regulation of T_3 availability to the neuroendocrine axis is an important component of a mechanism gating seasonal physiology.

The gradual increase in *Dio3* expression with time after

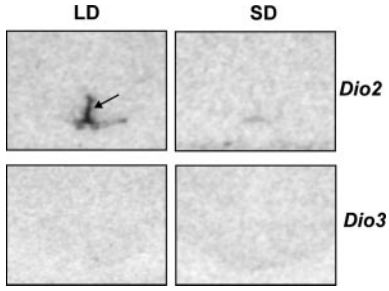

FIG. 8. Deiodinase gene expression in the ependymal layer of Syrian hamsters. *Upper panels*, *Dio2* expression. *Lower panels*, *Dio3* expression. *Left column*, LD hamsters; *right column*, SD hamsters. Expression of *Dio2* was present in the ependymal layer (*arrow*) only in LD. No *Dio3* expression was found in either photoperiod exposure.

transfer of hamsters from LD to SD is consistent with the slow decompression of the melatonin signal (Fig. 2C; Ref. 27) and appears to match the time-course of the decline in body and testes weight. Although we have not ruled out possible circadian regulation at a transcriptional or translation level, a previous study found rat brain DIO3 activity does not vary over the course of a 24-h period (28), and our previous work has found no evidence for circadian regulation of seasonal responsive genes in the ependymal layer or dmpARC (29–31). It is noteworthy that by 8 wk, *Dio3* expression had reached its highest value when body and testis weight of the hamsters were approaching their nadir. Beyond this time, *Dio3* expression declined, indicating that it is refractory to the SD signal. Photorefractoriness, in terms of body weight, is not evident until 20 wk of SD exposure (32, 33). Thus, these data indicate the decline in *Dio3* expression is likely to be an early molecular event in the overall process of photorefractoriness. These data are consistent with a recent demonstration of elevated *Dio3* expression at 10 wk SD exposure and absence in photorefractory Djungarian hamsters (19).

Interestingly a previous study, based on reduced hypothalamic T_4 binding protein and chemical thyroidectomy, has suggested that a reduction in central T_4 may be required to induce reproductive photorefractoriness in Siberian hamsters (34). Furthermore, in Syrian hamsters where *Dio2* is the principal regulator of T_3 levels in the hypothalamus, *Dio2* expression does not increase in the photorefractory state (23). Taken together, these data suggest that the role of thyroid hormone in regulating reproductive physiology and behavior is complex.

The increased expression of *Dio3* during early exposure to SD predicts that the level of T_3 within the hypothalamus is reduced due to increased catabolism, and this permits the transition from a LD (high body weight, reproductively active) to a SD (low body weight, reproductively inactive) phenotype. Consistent with this prediction, chronic release implants of T_3 placed directly into brain tissue, to bypass the ependymal cells and avoid degradation by DIO3, prevented SD body weight and reproductive responses and allowed the LD body weight and reproductive phenotypes to be maintained. The decline in *Dio3* beyond 8 wk in SD might be predicted to permit a reversal of body weight decline. However, *Dio3* mRNA, although in decline, is still present until a time point after 16 wk in SD. This, or low turnover of DIO3 protein, may enable T_3 to be maintained at low levels. Al-

ternatively there may be other factors involved in maintaining body weight at its nadir.

In part, it seems likely that the T_3 effect on body weight in SD hamsters was achieved through overall higher levels of food intake relative to SD sham controls. Indeed, it has been shown in rats that T_3 injected into the VMN increases food intake (35). Although information on the spread of T_3 from our implants could not be determined, a study by Anderson *et al.* (36) using microimplants releasing radiolabeled T_4 placed in the premammillary region of sheep brain, found radioactivity to be spread throughout the hypothalamus, with a peak of concentration extending to 1 mm from the implant site 5 d after implant placement. A similar release in our study would result in T_3 availability to all parts of the hypothalamus.

It is interesting to note that, although statistical significance was not achieved, there is trend toward a higher weekly food intake in LD groups compared with their SD counterparts, consistent with a study demonstrating that Siberian hamsters in SD reduce food intake (37).

The effect of T_3 implants on testes weight in SD-exposed hamsters was also striking. Hamsters bearing T_3 implants did not show testicular regression in the winter photoperiod. Similarly, other measures of reproductive status in these hamsters were as expected from the testes weight with only SD sham-treated hamsters showing a decline in epididymis and seminal vesicle weights typical of seasonal regression (Fig. 6). Therefore, we conclude that the effect of T_3 release into the brain has served to maintain reproductive competence in the Siberian hamster. This mechanism has a parallel with gonadal regression found in Japanese quail brought about by SD photoperiod induced change in the balance of the T_3 synthetic and catabolic enzymes and T_3 availability. In support of this, intracerebroventricular infusion of T_3 stimulated gonadal growth, demonstrating a key role of T_3 in the brain in the seasonal reproductive axis (9). This is in contrast to the findings in sheep, in which it has been demonstrated that T_3 is an absolute requirement to bring about testicular regression in rams or anoestrous in ewes, terminating the breeding season (10–14).

One explanation of the action of T_3 in SDT3 hamsters is to affect melatonin output from the pineal gland. However, the annual moulting cycle regulated by a durational melatonin signal at the level of the pars tuberalis (38), is not affected in either SDT3 or SD sham hamsters, albeit the moult is delayed in T_3-implanted hamsters. This, together with the demonstration that *Dio3* expression in the ependymal layer in SD is not affected by the implants (Fig. 4), would indicate that SD melatonin output has not been altered. The delay in the moult to winter pelage of the SDT3 hamsters is likely to be due to the maintenance of LD circulating levels of testosterone due to the presence of nonregressed testes, because the data are consistent with a previous study that showed interference of the winter moult in SD if the circulating level of testosterone is artificially elevated by testosterone implants (21).

The lack of induction of *Dio3* in pinealectomized hamsters exposed to SD would suggest that the photoperiodic control of this gene is mediated by melatonin. Therefore, another explanation for the action of T_3 would be to impede the ability of melatonin receptors in the brain to read the melatonin signal. However, in both SDT3 and SD sham hamsters, SD-induced *Dio3* expression was clearly evident in all SD-exposed hamsters and, therefore, not a realistic explanation.

Dio3 expression was not observed in any of the LD sham hamsters and was observed in two of six LD hamsters bearing a T_3 implant. Examination of the site of the implant in these latter hamsters shows a possible penetration of the ependymal wall, and this may contribute to an increased exposure of T_3 at the ventricular surface of the ependymal layer. Nevertheless, elevated *Dio3* expression would be consistent with previous studies that show elevated T_3 induces *Dio3* expression (15). With elevated *Dio3* typical of SD-exposed hamsters, these hamsters would not be expected to show reduced body weight because the mimicking of the SD reduction of T_3 would be negated by the availability of T_3 to hypothalamic control mechanisms.

Recent data by Freeman *et al.* (39) demonstrates a partial response of Siberian hamsters to exogenous peripherally administered T_3 on testicular recrudescence in SD or regression when placed in SD, and there was no effect on body weight. However, one cannot distinguish between a peripheral and central mediated effect of T_3. Indeed, elevated Dio3 in the ependymal layer would explain why peripherally administered T_3 would not be effective.

Distribution of *TRβ1* and *TRβ2* receptors was found throughout the hypothalamic region. *TRβ1* was not photoperiodically regulated. However, *TRβ2* was up-regulated in SD-exposed hamsters. The increase in *TRβ2* could be a result of the reduced availability of T_3 ligand due to elevation of *Dio3*.

TRβ1 and *2* were expressed more highly in the dmpARC than in the surrounding ARC region. This is noteworthy due to the relative closeness of the dmpARC to tanycytes of the ependymal layer and the photoperiodically regulated expression of *RXRγ* in this structure (29), and RXRγ being a potential partner of thyroid hormone receptors (40). The dmpARC is an area of hamster hypothalamus that shows dramatic changes in expression of key genes that have possible significance for body weight regulation including *histamine H3 receptor*, *RAR*, *RXRγ*, and *VGF* (29, 30). A possible mechanism of regulation by T_3 in the dmpARC may involve the reduced availability of T_3 coupled with reduced *RXRγ* expression. The increased availability of T_3 in our implant experiments may have been able to overcome this restriction.

Consideration must also be given to the distinctive presence of *TRβ2* receptors in the premammillary area, where evidence exists in sheep that melatonin and possibly T_4/T_3 act to regulate seasonal reproduction (36, 41).

Unlike the Siberian hamster, short photoperiod negatively regulates the expression of *Dio2* (23), but has no effect on *Dio3* expression, in the ependymal layer of the Syrian hamster. This suggests that, although T3 availability may be a universal component of seasonal neurondocrine regulation, different species have evolved different mechanisms to alter the seasonal T_3 levels.

It should be noted that no melatonin receptor expression has been reported in the ependymal cells (42, 43), whereas this mRNA has been detected in other hypothalamic regions

(4–6). Therefore the photoresponsiveness of *Dio3* up to and after 8 wk in SD is unlikely to be a consequence of a direct melatonin action on these cells.

Together with our previous findings showing the down-regulation of mRNA for *CRBP1*, *GPR50*, and *nestin* in the ependymal layer of SD hamsters (31), the current study strongly supports the view that cells of the ependymal layer are a key component involved in relaying photoperiodic time to neuroendocrine axes controlling seasonal energy balance and reproduction in Siberian hamsters.

It remains to be determined how T_3 may regulate the key genes involved in driving the physiological and behavioral changes. One strong candidate for the mediation of the seasonal reproductive response is kisspeptin, an effector of GnRH expression and release, which has been shown to be regulated in the ARC in both Syrian and Siberian hamsters by SD photoperiods (22, 44). Therefore, one hypothesis with respect to the regulation of kisspeptin is T_3 could act as a transcriptional regulator of this gene and thus maintain reproductive competence of SD hamsters receiving the T_3 implant. This hypothesis would also propose a link between the tanycytes expressing the deiodinase genes to the neurons expressing kisspeptin and neurons involved in body weight and energy expenditure.

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References

1. **Gerlach T, Aurich JE** 2000 Regulation of seasonal reproductive activity in the stallion, ram and hamster. Anim Reprod Sci 58:197–213
2. **Kortner G, Geiser F** 2000 The temporal organization of daily torpor and hibernation: circadian and circannual rhythms. Chronobiol Int 17:103–128
3. **Morgan PJ, Ross AW, Mercer JG, Barrett P** 2006 What can we learn from seasonal animals about the regulation of energy balance? Prog Brain Res 153:325–337
4. **Watson-Whitmyre M, Stetson MH** 1988 Reproductive refractoriness in hamsters. In: Stetson MH, ed. Processing of environmental information in vertebrates. New York: Springer; 203–218
5. **Turek FW, Losee-Olson S, Swann JM, Horwath K, Van Cauter E, Milette JJ** 1987 Circadian and seasonal control of neuroendocrine-gonadal activity. J Steroid Biochem 27:573–579
6. **Morgan PJ, Barrett P, Howell HE, Helliwell R** 1994 Melatonin receptors: localization, molecular pharmacology and physiological significance. Neurochem Int 24:101–146
7. **Reinert BD, Wilson FE** 1997 Effects of thyroxine (T4) or triiodothyronine (T3) replacement therapy on the programming of seasonal reproduction and post-nuptial molt in thyroidectomized male American tree sparrows (*Spizella arborea*) exposed to long days. J Exp Zool 279:367–376
8. **Dawson A** 1993 Thyroidectomy progressively renders the reproductive system of starlings (*Sturnus vulgaris*) unresponsive to changes in daylength. J Endocrinol 139:51–55
9. **Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K, Ebihara S** 2003 Light-induced hormone conversion of T4 to T3 regulates photoperiodic response of gonads in birds. Nature 426:178–181
10. **Webster JR, Moenter SM, Woodfill CJI, Karsch FJ** 1991 Role of the thyroid gland in seasonal reproduction. II. Thyroxine allows a season-specific suppression of gonadotropin secretion in sheep. Endocrinology 129:176–183
11. **Parkinson TJ, Follett BK** 1994 Effect of thyroidectomy upon seasonality in rams. J Reprod Fertil 101:51–58
12. **Dahl GE, Evans NP, Thrun LA, Karsch FJ** 1995 Thyroxine is permissive to seasonal transitions in reproductive neuroendocrine activity in the ewe. Biol Reprod 52:690–696
13. **Viguie C, Battaglia DF, Krasa HB, Thrun LA, Karsch FJ** 1999 Thyroid hormones act primarily within the brain to promote the seasonal inhibition of luteinizing hormone secretion in the ewe. Endocrinology 140:1111–1117
14. **Thrun LA, Dahl GE, Evans NP, Karsch FJ** 1997 A critical period for thyroid hormone action on seasonal changes in reproductive neuroendocrine function in the ewe. Endocrinology 138:3402–3409
15. **Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR** 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev 23:38–89
16. **Yasuo S, Watanabe M, Nakao N, Takagi T, Follett BK, Ebihara S, Yoshimura T** 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
17. **Yasuo S, Nakao N, Ohkura S, Iigo M, Hagiwara S, Goto A, Ando H, Yamamura T, Watanabe M, Watanabe T, Oda S, Maeda K, Lincoln GA, Okamura H, Ebihara S, Yoshimura T** 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
18. **Timonin ME, Place NJ, Wanderi E, Wynne-Edwards KE** 2006 *Phodopus campbelli* detect reduced photoperiod during development but, unlike *Phodopus sungorus*, retain functional reproductive physiology. Reproduction 132:661–670
19. **Watanabe T, Yamamura T, Watanabe M, Yasuo S, Nakao N, Dawson A, Ebihara S, Yoshimura T** 2007 Hypothalamic expression of thyroid hormone-activating and -inactivating enzyme genes in relation to photorefractoriness in birds and mammals. Am J Physiol Regul Integr Comp Physiol 292:R568–R572
20. **Morgan PJ, Webster CA, Mercer JG, Ross AW, Hazlerigg DG, MacLean A, Barrett P** 1996 The ovine pars tuberalis secretes a factor (s) that regulates gene expression in both lactotropic and nonlactotropic pituitary cells. Endocrinology 137:4018–4026
21. **Duncan MJ, Goldman BD** 1984 Hormonal regulation of the annual pelage color cycle in the Djungarian hamster, *Phodopus sungorus*. I. Role of the gonads and pituitary. J Exp Zool 230:89–95
22. **Revel FG, Saboureau M, Masson-Pevet M, Pevet P, Mikkelsen JD, Simonneaux V** 2006 Kisspeptin mediates the photoperiodic control of reproduction in hamsters. Curr Biol 16:1730–1735
23. **Revel FG, Saboureau M, Pevet P, Mikkelsen JD, Simonneaux V** 2006 Melatonin regulates type 2 deiodinase gene expression in the Syrian hamster. Endocrinology 147:4680–4687
24. **Follett BK, Nicholls TJ** 1985 Influences of thyroidectomy and thyroxine replacement on photoperiodically controlled reproduction in quail. J Endocrinol 107:211–221
25. **O'Jile JR, Bartness TJ** 1992 Effects of thyroxine on the photoperiodic control of energy balance and reproductive status in Siberian hamsters. Physiol Behav 52:267–270
26. **Watanabe M, Yasuo S, Watanabe T, Yamamura T, Nakao N, Ebihara S, Yoshimura T** 2004 Photoperiodic regulation of type 2 deiodinase gene in Djungarian hamster: possible homologies between avian and mammalian photoperiodic regulation of reproduction. Endocrinology 145:1546–1549
27. **Illnerova H, Hoffmann K, Vanecek J** 1984 Adjustment of pineal melatonin and *N*-acetyltransferase rhythms to change from long to short photoperiod in the Djungarian hamster *Phodopus sungorus*. Neuroendocrinol 38:226–231
28. **Campos-Barros A, Musa A, Flechner A, Hessenius C, Gaio U, Meinhold H, Baumgartner A** 1997 Evidence for circadian variations of thyroid hormone concentrations and type II 5′-iodothyronine deiodinase activity in the rat central nervous system. J Neurochem 68:795–803
29. **Ross AW, Webster CA, Mercer JG, Moar KM, Ebling FJ, Schuhler S, Barrett P, Morgan PJ** 2004 Photoperiodic regulation of hypothalamic retinoid signaling: association of retinoid X receptor γ with body weight. Endocrinology 145:13–20
30. **Barrett P, Ross AW, Balik A, Littlewood PA, Mercer JG, Moar KM, Sallmen T, Kaslin J, Panula P, Schuhler S, Ebling FJ, Ubeaud C, Morgan PJ** 2005 Photoperiodic regulation of histamine H3 receptor and VGF messenger ribonucleic acid in the arcuate nucleus of the Siberian hamster. Endocrinology 146:1930–1939
31. **Barrett, P, Ivanova E, Graham ES, Ross AW, Wilson D, Ple H, Mercer JG, Ebling FJ, Schuhler S, Dupré SM, Loudon A, Morgan PJ** 2006 Photoperiodic regulation of cellular retinoic acid-binding protein 1, GPR50 and nestin in tanycytes of the third ventricle ependymal layer of the Siberian hamster. J Endocrinol 191:687–698
32. **Prendergast BJ, Nelson RJ, Zucker I** 2002 Mammalian seasonal rhythms: behaviour and neuroendocrine substrates. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Rubin R, eds. Hormones, brain and behaviour. Vol II. Boston Academic Press; 93–156

33. **Hoffmann K** 1979 Photoperiod, pineal, melatonin and reproduction in hamsters. Prog Brain Res 52:397–415
34. **Prendergast BJ, Mosinger Jr B, Kolattukudy PE, Nelson RJ** 2002 Hypothalamic gene expression in reproductively photoresponsive and photorefractory Siberian hamsters. Proc Natl Acad Sci USA 99:16291–16296
35. **Kong WM, Martin NM, Smith KL, Gardiner JV, Connoley IP, Stephens DA, Dhillo WS, Ghatei MA, Small CJ, Bloom SR** 2004 Triiodothyronine stimulates food intake via the hypothalamic ventromedial nucleus independent of changes in energy expenditure. Endocrinology 145:5252–5258
36. **Anderson GM, Hardy SL, Valent M, Billings HJ, Connors JM, Goodman RL** 2003 Evidence that thyroid hormones act in the ventromedial preoptic area and the premammillary region of the brain to allow the termination of the breeding season in the ewe. Endocrinology 144:2892–2901
37. **Knopper LD, Boily P** 2000 The energy budget of captive Siberian hamsters, *Phodopus sungorus*, exposed to photoperiod changes: mass loss is caused by a voluntary decrease in food intake. Physiol Biochem Zool 73:517–522
38. **Lincoln GA Andersson H, Hazlerigg D** 2003 Clock genes and the long-term regulation of prolactin secretion: evidence for a photoperiod/circannual timer in the pars tuberalis. J Neuroendocrinol 15:390–397
39. **Freeman DA, Teubner BJW, Smith CD, Prendergast BJ** 1 February 2007 Exogenous T3 mimics long day lengths in Siberian hamsters. Am J Physiol Integr Comp Physiol 10.1152/ajpregu.00713.2006
40. **Kliewer SA, Umesono K, Mangelsdorf DJ, Evans RM** 1992 Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. Nature 355:446–449
41. **Malpaux B, Daveau A, Maurice-Mandon F, Duarte G, Chemineau P** 1998 Evidence that melatonin acts in the premammillary hypothalamic area to control reproduction in the ewe: presence of binding sites and stimulation of luteinizing hormone secretion by in situ microimplant delivery. Endocrinology 139:1508–1516
42. **Schuster C, Gauer F, Guerrero H, Lakhdar-Ghazal N, Pevet P, Masson-Pevet M** 2000 Photic regulation of mt1 melatonin receptors in the Siberian hamster pars tuberalis and suprachiasmatic nuclei: involvement of the circadian clock and intergeniculate leaflet. J Neuroendocrinol 12:207–216
43. **Song CK, Bartness TJ** 2001 CNS sympathetic outflow neurons to white fat that express MEL receptors may mediate seasonal adiposity. Am J Physiol Reg Int Comp Physiol 281:R666–R672
44. **Greives TJ, Mason AO, Scotti MA, Levine J, Ketterson ED, Kriegsfeld LJ, Demas GE** 2007 Environmental control of kisspeptin: implications for seasonal reproduction. Endocrinology 148:1158–1166

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