Published in final edited form as:

Gen Comp Endocrinol. 2011 May 1; 171(3): 269–274. doi:10.1016/j.ygcen.2011.02.024.

Cholecystokinin activation of central satiety centers changes seasonally in a mammalian hibernator

Jessica P. Otis^a, Helen E. Raybould^b, and Hannah V. Carey^{a,*}

^aDepartment of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, 2015 Linden Dr., Madison, WI 53706, USA

^bDepartment of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, University of California-Davis, 1321 Haring Hall, Davis, CA 95616, USA

Abstract

Hibernators that rely on lipids during winter exhibit profound changes in food intake over the annual cycle. The mechanisms that regulate appetite changes in seasonal hibernators remain unclear, but likely consist of complex interactions between gut hormones, adipokines, and central processing centers. We hypothesized that seasonal changes in the sensitivity of neurons in the nucleus tractus solitarius (NTS) to the gut hormone cholecystokinin (CCK) may contribute to appetite regulation in ground squirrels. Spring (SPR), late summer (SUM), and winter euthermic hibernating (HIB) 13-lined ground squirrels (*Ictidomys tridecemlineatus*) were treated with intraperitoneal CCK (100 µg/kg) or vehicle (CON) for 3 h and Fos expression in the NTS was quantified. In CON squirrels, numbers of Fos-positive neurons in HIB were low compared to SPR and SUM. CCK treatment increased Fos-positive neurons in the NTS at the levels of the area postrema (AP) and pre AP during all seasons and at the level of the rostral AP in HIB squirrels. The highest absolute levels of Fos-positive neurons were found in SPR CCK squirrels, but the highest relative increase from CON was found in HIB CCK squirrels. Fold-changes in Fos-positive neurons in SUM were intermediate between SPR and HIB. Thus, CCK sensitivity falls from SPR to SUM suggesting that seasonal changes in sensitivity of NTS neurons to vagally-derived CCK may influence appetite in the active phase of the annual cycle in hibernating squirrels. Enhanced sensitivity to CCK signaling in NTS neurons of hibernators indicates that changes in gut–brain signaling may contribute to seasonal changes in food intake during the annual cycle.

Keywords

Hibernation; Torpor; Appetite; Cholecystokinin; Satiety

1. Introduction

Hormones released from the gut that act on neural inputs to the brain are important regulators of appetite in vertebrates. Prominent among these is cholecystokinin (CCK), a

peptide released by enteroendocrine cells in the proximal small intestine in response to lipid and protein digestive products. CCK activates CCK₁ receptors (CCK₁R) located on vagal afferents on the gut wall that terminate in the nucleus tractus solitarius (NTS), a brainstem region important in satiety signaling [31,33,36]. There is crosstalk between NTS regions that receive satiety information from the periphery and regions of the hypothalamus important for appetite control including the arcuate, paraventricular, and ventromedial nuclei. These hypothalamic sites integrate peripheral information on short term energy intake via the gut and long term energy stores from adipose tissue to ultimately regulate appetite. Exogenous administration of CCK increases satiation, as indicated by a reduction in food intake and activates neurons in the NTS as evidenced by increased expression of the immediate early gene Fos [9,34,38]. In addition to satiety signaling, CCK facilitates lipid and protein digestion by stimulating the release of pancreatic hormones and bile and promoting stomach emptying, thus matching luminal contents to absorptive capacity.

Hibernation is an adaptation that allows animals to survive adverse environmental conditions that limit food availability and/or increase energy demands. Obligate seasonal hibernators, such as ground squirrels and marmots eat and fatten during the active season, reduce food intake in the fall prior to the start of torpor and fast completely during the hibernation season [1,24,30]. The mechanisms that produce annual rhythms of food intake in hibernating mammals are poorly understood, and the potential role of the gut–brain axis in appetite regulation has received little attention. We hypothesized that the sensitivity of NTS neurons to the satiety-inducing effects of CCK may change seasonally in ground squirrels, thus indicating a role for gut–brain signaling in food intake regulation in hibernators. In this study we examined the seasonal sensitivity of NTS neurons to exogenously administered CCK as indicated by changes in expression of Fos in NTS regions known to be involved in appetite control in spring (SPR), summer (SUM) and euthermic hibernating (HIB) 13-lined ground squirrels (*Ictidomys tridecemlineatus*) [38]. We hypothesized that HIB squirrels would be more sensitive to CCK, with more Fos-positive NTS neurons, than fattening SPR and SUM active squirrels. It was also predicted that SPR squirrels would be more sensitive to CCK than more fattened, leptin-resistant SUM squirrels [1,18].

2. Methods

2.1. Animals

The protocol was approved by the University of Wisconsin-Madison Animal Care and Use Committee. Thirteen-lined ground squirrels of both sexes were trapped in the vicinity of Madison, WI in July and August. Squirrels were housed individually in a room maintained at 22 °C with a photoperiod of 12L:12D with free access to food (Teklad rodent chow 7001) supplemented with sunflower seeds and water. SUM squirrels ($n = 10$) were held under these conditions for 5 weeks prior to use (Table 1). In the fall (September–October), HIB and SPR squirrels ($n = 10$ for both) were transferred to a room maintained at 4 °C and 24 h dark. Squirrels were exposed to brief periods of low light (<10 min) every day to monitor torpor–arousal behavior with the sawdust method [27]. Food and water were removed following the start of torpor–arousal bouts. Squirrels in the HIB group hibernated ~35 days (Table 1). After ~85 days in hibernation SPR squirrels were transferred back to the room at

22 °C, 12L:12D, and given free access to food and water for ~30 days prior to use. Table 1 shows the age distribution of squirrels among treatment groups, body masses and hibernation characteristics.

2.2. Experimental protocol

SUM and SPR squirrels were fasted for 20 h and body temperature (measured rectally) was ~37 °C prior to treatment. Euthermia (T_b ~37 °C) was artificially induced in HIB squirrels by moving them to a warm (20 °C), well-lit room for 3 h prior to treatment. In each season squirrels were divided into a CCK treatment group and a vehicle control (CON) group ($n = 5$ in all groups). Squirrels were anesthetized briefly with an isoflurane/O₂ mixture (3%/L; Baxter Healthcare Corporation), given CCK-8 (100 µg/kg; Sigma #C2175) or vehicle (saline) IP, and allowed to recover. This dose of CCK-8 was shown in a preliminary study to induce NTS Fos expression in ground squirrels. Exactly 3 h later squirrels were anesthetized with the isoflurane/O₂ mixture and transcardially perfused with heparin (1000 U/ml; APP Pharmaceuticals, LLC), followed by 300 ml saline, and 500 ml 4% paraformaldehyde (Sigma). After perfusion brains were removed and post-fixed in 4% paraformaldehyde for 1 h followed by a 25% sucrose solution for 2 h at room temperature. Brains were then stored at 4 °C until further use.

2.3. Immunohistochemistry

Transverse sections of the medulla (100 µm) were cut by a vibratome and placed into phosphate buffered saline (PBS) at 4 °C. Sections were washed three times with PBS for 10 min each and then blocked for 1 h in a 2% goat serum (GS)-PBS solution. All antibodies were diluted in a solution of GS-PBS containing 0.2% Triton X-100 and 0.1% BSA (Sigma). The blocking medium was drained, and sections were incubated with anti-Fos antibody (1:1000) (SC-52, rabbit polyclonal; Santa Cruz Biotechnology, Santa Cruz, CA) for 3 h at room temperature. Sections were washed three times and then incubated with biotinylated goat anti-rabbit secondary antibody (1:200) for 2 h at room temperature. After additional washing sections were incubated with avidin–biotin complex solution (Standard Elite Vectastain ABC Kit; Vector) for 3 h at room temperature, followed by incubation with 3,3'-diaminobenzidine (DAB) dissolved in PBS (30 mg/100 ml) for 5 min at room temperature. H₂O₂ (0.3% in ddH₂O) was added to the DAB solution (50 µl/ml), and the peroxidase reaction was allowed to continue until a light brown color emerged. Sections were taken through an ethanol–xylene dehydration procedure and mounted on slides with Depex mounting medium. Slides were examined using conventional light microscopy and images acquired in a rostrocaudal order and analyzed using Scion Image analysis software [12,36]. The NTS was outlined using computer software (Fig. 1), a grey threshold level was uniformly set and nuclei that showed Fos labeling at or above the threshold were counted. About 25 contiguous tissue sections were analyzed for each brain. Although the entire area of the NTS was analyzed, counts are expressed as the number of Fos-labeled nuclei per 10,000 pixels. This provides an index of the density of labeling. Four rostrocaudal levels of the NTS were identified and defined as caudal to the area postrema (AP), at the AP, pre AP, and rostral to the AP. Fos-positive neurons were quantified in each of these areas.

2.4. Statistical analysis

Animal characteristics (Table 1) were compared by one-way ANOVA followed by Fisher's post-hoc test. To assess differences in numbers of Fos-positive neurons in the treatment groups we used two-way ANOVA with season and treatment as independent factors and an interaction term between them both. Bonferroni's test was used to compare groups when significance was found for season and treatment by ANOVA. When significance was found for the interaction term, contrast tests were used to determine seasonal differences in increases from baseline with CCK treatment. Levene's test was used to determine variances among groups, and data from the level of the rostral AP were square root transformed to equalize variability. All data sets had normal distributions. Significance level was set at $P < 0.05$.

3. Results

3.1. Animals

SPR squirrels were sampled in April, SUM squirrels were sampled in late summer (August 27 and 28), and HIB squirrels were sampled early in the hibernation season (October 21–23; ~35 days in hibernation) when they are known to be hypophagic (Table 1) [1,11,24,30]. HIB squirrels weighed less than SPR and SUM squirrels ($F = 10.34$, $P < 0.001$). Both HIB groups were in hibernation for the same length of time (~35 days), had the same mean body mass, and lost the same amount of weight on average (~45 g) during hibernation. SPR CON squirrels were in hibernation longer (105 ± 24.6 days) than SPR CCK squirrels (71 ± 14.8 days; $P < 0.05$), but spent the same amount of time (~29 days) out of hibernation prior to CCK treatment.

3.2. CCK activation of NTS neurons

There were fewer Fos-positive neurons in CON HIB squirrels than in CON SPR or CON SUM squirrels in all of the NTS regions studied (~2- to 13-fold) (Figs. 1 and 2). This suggests that despite high body temperatures and metabolic rates, the level of basal activity in NTS neurons is lower in fasted, euthermic hibernators than in fed, non-hibernating squirrels.

We found significant main effects of season and treatment on the number of Fos-positive neurons at the caudal AP ($F = 6.68$, $P = 0.005$ and $F = 4.33$, $P = 0.048$), but the interaction term between the factors was not significant indicating that the effect of CCK relative to basal Fos levels in this region was similar in all seasons (Fig. 3). Post-hoc analysis revealed that CCK treatment in SUM activated a greater absolute number of neurons at the caudal AP than in SPR or HIB (10.4 ± 2.5 vs. 3.7 ± 1.0 and 3.4 ± 1.1 nuclei/10,000 pixels).

In the levels of the AP, both season and treatment, as well as the interaction between season and treatment, had significant effects on the number of Fos-positive neurons ($F = 11.29$, $P < 0.001$; $F = 59.06$, $P < 0.001$; $F = 4.14$, $P = 0.029$) (Fig. 3). CCK induced a ~4-fold increase in Fos-positive neurons in SPR squirrels and a ~2-fold increase in SUM. The increase in Fos-positive neurons with CCK treatment from CON levels was significantly greater in SUM than in SPR squirrels. A nearly 16-fold increase in Fos-positive cells was induced by

CCK at the AP of HIB squirrels, likely due in part to the very low level of Fos-positive neurons in HIB CON squirrels.

Season and treatment also had significant effects on the number of Fos-positive neurons at the pre AP level ($F = 9.36$, $P = 0.001$; $F = 80.83$, $P < 0.001$) (Fig. 3). CCK induced a ~4-fold increase in Fos-positive neurons in SPR, a ~3-fold increase in SUM, and a ~12-fold increase in HIB. There were significantly more Fos-positive neurons in SPR CCK squirrels than in HIB CCK squirrels.

At the rostral AP level, both main effects influenced the number of Fos-positive neurons, and the interaction term was also significant ($F = 0.007$, $P = 0.007$; $F = 28.86$, $P < 0.001$; $F = 0.009$, $P = 0.009$) (Fig. 3). In this region CCK increased the number of Fos-positive neurons in HIB squirrels by ~24-fold, but had no significant effect in SPR and SUM squirrels (Fig. 3). There was a greater increase from basal levels in Fos-positive neurons with CCK treatment in HIB than in SPR or SUM squirrels.

4. Discussion

It has long been appreciated that seasonal hibernators display endogenous rhythms in food intake during the course of the annual hibernation cycle [1]. Hibernators resume food intake after emergence in spring, increase food intake as they fatten during summer, then begin to reduce feeding concomitant with a decline in above-ground activity in late summer [1,11]. This seasonal rhythm in the drive to feed can be reproduced in captivity when ambient conditions remain relatively constant [24,30]. Yet, the mechanisms responsible for the dramatic changes in appetite across the annual hibernation cycle are still poorly understood. There is compelling evidence that signaling between the gut and central appetite centers, including the NTS, plays a crucial role in satiation in rodents and other mammals and can be modulated by changes in adiposity [10]. In this study we asked whether there are seasonal changes in activity of NTS neurons that are known to regulate satiation in other rodent species, and whether NTS neurons respond differently to the gut hormone CCK, therefore implicating the gut-brain axis in feeding behavior of hibernators.

We observed a strong seasonal influence on basal activity of neurons in NTS regions known to be involved in integrating food-derived signals from the gut. The low numbers of Fos-positive neurons in the four rostrocaudal regions of the NTS in CON HIB squirrels suggest that afferent input from stimuli that maintain basal neuronal activity in these regions is greatly reduced during the hibernation season when animals are naturally hypophagic, compared to SPR and SUM when food intake is more regular. This seasonal decrease in basal NTS activity is not due to low body temperature or depressed metabolism, because all HIB squirrels were fully aroused (body temperature ~37 °C) when sampled. Rather, it may reflect the chronic absence of feeding-related signals to these brain regions, which may downregulate basal gene expression and attenuate signaling pathways that originate in the NTS and subsequently affect satiety and/or digestive function. There are few studies that have examined seasonal changes in Fos expression in the brain of hibernating species and none have examined the NTS [4,7,25]. It has been reported that expression of Fos transcripts in the cerebral cortex and hypothalamus of summer golden-mantled ground squirrels

(*Callospermophilus lateralis*) are similar to that of fully aroused hibernators, which is inconsistent with our findings for NTS in summer and HIB 13-lined ground squirrels [25]. Thus, basal levels of transcriptional activity during the seasonal hibernation cycle may vary with specific brain regions.

CCK is released from enteroendocrine cells in response to lipids and proteins in the gut lumen and communicates satiety to the NTS via the vagus nerve and its nodose ganglia. Mice lacking the vagal CCK₁ receptor have diminished satiation in response to food intake which results in larger, less frequent meals when on a high fat diet [13]. To determine whether seasonal changes in food intake might involve the gut-vagal-NTS axis, we administered peripheral CCK, which can activate vagal CCK receptors within the gut wall, to squirrels during different seasons and measured the central neural response as indicated by Fos-positive neurons in the NTS.

Among the four NTS regions we examined, induction of Fos activity by CCK was greatest at the AP and pre AP levels compared with the caudal to or rostral to the AP levels. A similar regional activation pattern by CCK is observed in rats [37], which suggests our approach in ground squirrels targets the major appetite-regulatory centers in the brainstem. We found the highest absolute levels of Fos-positive neurons in SPR squirrels, with intermediate levels in SUM, and lowest levels in HIB. However, due to their low basal neural activity, the largest relative increase in Fos-positive neurons in response to CCK was in HIB squirrels, followed by SPR and then SUM animals. In model organisms such as the rat, which shows minimal variations in basal levels of Fos staining in the NTS, differences in absolute levels of Fos-positive neurons among experimental groups are used as indicators of higher or lower responsiveness to a stimulus. Under this criterion, and in agreement with our hypothesis, NTS neurons in SPR squirrels are more sensitive to CCK than SUM squirrels, but in contrast to our hypothesis, both SPR and SUM squirrels are more sensitive to CCK than HIB squirrels. However, there are dramatic differences among the three groups in fold-changes induced by CCK. If fold-change from baseline is used as the main criterion, NTS sensitivity to CCK is greatest in HIB, followed by SPR and lowest in SUM. Thus, satiety in response to a meal is most easily induced in HIB (should food be ingested), intermediate in SPR and least in SUM. This effect would tend to promote continued food intake in SUM despite their already sizable adipose stores. CCK increased the numbers of Fos-positive neurons in the rostral AP but had no effect in that region in SPR and SUM squirrels, which further suggests the HIB squirrels' enhanced sensitivity to feeding-derived cues that reach the brain-stem and induce a satiation effect. However, full support for this conclusion will require gavage of a lipid meal and subsequent assessment of NTS neural activity (via Fos staining) in the three seasonal groups to insure that intraperitoneal administration of CCK in our studies reflects the normal response to digestion of a meal [38]. The idea that food intake in some rodents can be seasonally regulated in part via CCK signaling is supported by the finding that the ability of exogenous CCK to inhibit food intake in Siberian hamsters (*Phodopus sungorus*) differs in long vs. short photoperiods [3].

There is evidence for crosstalk between CCK satiety signaling and the adipokine leptin, as many vagal afferents and NTS neurons are sensitive to both leptin and CCK [14,28], and CCK and leptin have a synergistic satiation effect when acting together [2,15,28,29,35].

Leptin and CCK cross-stimulate their own secretion, thus creating a feed-forward loop extending their action [21,29]. Although hibernators are not generally considered obese prior to hibernation as their body masses are normal for that time of year, obesity can alter normal signaling pathways that mediate CCK and leptin's effects on satiety. Obese Zucker rats release less CCK into the plasma following a meal than do lean animals, and NTS neural activation in response to CCK is reduced in rats fed a high fat diet [5,10,12,21,26,32]. Leptin resistance and altered leptin receptor expression also alter CCK satiety signaling, and satiety signaling by CCK and leptin is disrupted in the obese and leptin-resistant states [22]. These effects may be relevant to food intake regulation in hibernating species. During the late summer and fall as hibernators fatten and their plasma leptin levels rise, they appear to enter a state of leptin resistance which gradually reverses as body fat decreases during the hibernation season [18]. Thus, the decline in the number of Fos-positive neurons over the active season may result from increased fattening and leptin resistance. Appetite regulation in mammals is a highly complex process, making it likely that other mechanisms contribute to satiation in hibernating species including action of the gut hormones ghrelin and peptide YY, insulin, neuropeptide Y, and hypothalamic AMP kinase [6,8,16,17,19,20,23].

In conclusion, we report here two novel findings that may contribute to seasonal changes in food intake characteristic of hibernating mammals. First, NTS regions known to be involved in regulating food-derived signals from the gut show substantially lower levels of basal neuronal activity in euthermic hibernators compared with active season ground squirrels. Second, NTS neural activity in response to exogenous administration of CCK is altered seasonally, with greatest fold changes in hibernators but highest absolute numbers of activated neurons in spring squirrels. The reduced NTS neural activity during hibernation may result from decreased neural inputs from the gut via the vagus nerve during the long-term winter fast. It is likely that appetite changes in seasonal hibernators result from complex interactions between gut hormones, adipokines, insulin, and regulatory centers in the brain.

Acknowledgments

The authors thank Dr. Cecile Áne for statistical consultation, Nathan Nelson and Jim Sharp for expert technical assistance and Michael Grahn for animal care assistance. This work was funded by the University of Wisconsin School of Veterinary Medicine (HVC) and NIHDDK 41004 (HER).

References

1. Armitage KB, Shulenberger E. Evidence for a circannual metabolic cycle in *Citellus tridecemlineatus*, a hibernator. *Comp Biochem Physiol.* 1972; 42A:667–688.
2. Barrachina MD, Martinez V, Wang L, Wei JY, Tache Y. Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. *Proc Natl Acad Sci USA.* 1997; 94:10455–10460. [PubMed: 9294232]
3. Bartness TJ, Morley JE, Levine AS. Photoperiod–peptide interactions in the energy intake of Siberian hamsters. *Peptides.* 1986; 7:1079–1085. [PubMed: 3562318]
4. Bitting L, Sutin EL, Watson FL, Leard LE, O'Hara BF, Heller HC, Kilduff TS. C-fos mRNA increases in the ground squirrel suprachiasmatic nucleus during arousal from hibernation. *Neurosci Lett.* 1994; 165:117–121. [PubMed: 8015710]

5. Blevins JE, Morton GJ, Williams DL, Caldwell DW, Bastian LS, Wisse BE, Schwartz MW, Baskin DG. Forebrain melanocortin signaling enhances the hindbrain satiety response to CCK-8. *Am J Physiol Regul Integr Comp Physiol*. 2009; 296:R476–R484. [PubMed: 19109369]
6. Boswell T, Richardson RD, Schwartz MW, D'Alessio DA, Woods SC, Sipols AJ, Baskin DG, Kenagy GJ. NPY and galanin in a hibernator: hypothalamic gene expression and effects on feeding. *Brain Res Bull*. 1993; 32:379–384. [PubMed: 7693306]
7. Bratincsak A, McMullen D, Miyake S, Toth ZE, Hallenbeck JM, Palkovits M. Spatial and temporal activation of brain regions in hibernation: c-fos expression during the hibernation bout in thirteen-lined ground squirrel. *J Comp Neurol*. 2007; 505:443–458. [PubMed: 17912746]
8. Buck MJ, Squire TL, Andrews MT. Coordinate expression of the PDK4 gene: a means of regulating fuel selection in a hibernating mammal. *Physiol Genomics*. 2002; 8:5–13. [PubMed: 11842126]
9. Chen DY, Deutsch JA, Gonzalez MF, Gu Y. The induction and suppression of c-fos expression in the rat brain by cholecystokinin and its antagonist L364,718. *Neurosci Lett*. 1993; 149:91–94. [PubMed: 7682304]
10. Covasa M, Grahm J, Ritter RC. High fat maintenance diet attenuates hindbrain neuronal response to CCK. *Regul Pept*. 2000; 86:83–88. [PubMed: 10672906]
11. Dark J. Annual lipid cycles in hibernators: integration of physiology and behavior. *Annu Rev Nutr*. 2005; 25:469–497. [PubMed: 16011475]
12. Donovan MJ, Paulino G, Raybould HE. Activation of hindbrain neurons in response to gastrointestinal lipid is attenuated by high fat, high energy diets in mice prone to diet-induced obesity. *Brain Res*. 2009; 1248:136–140. [PubMed: 19007755]
13. Donovan MJ, Paulino G, Raybould HE. CCK(1) receptor is essential for normal meal patterning in mice fed high fat diet. *Physiol Behav*. 2007; 92:969–974. [PubMed: 18023701]
14. Ellacott KL, Halatchev IG, Cone RD. Characterization of leptin-responsive neurons in the caudal brainstem. *Endocrinology*. 2006; 147:3190–3195. [PubMed: 16601142]
15. Emond M, Schwartz GJ, Ladenheim EE, Moran TH. Central leptin modulates behavioral and neural responsivity to CCK. *Am J Physiol*. 1999; 276:R1545–R1549. [PubMed: 10233050]
16. Florant G, Lawtence AK, Williams K, Bauman W. Seasonal changes in pancreatic B-cell function in euthermic yellow-bellied marmots. *Am J Physiol*. 1985; 249:R159–R165. [PubMed: 3895984]
17. Florant GL, Fenn AM, Healy JE, Wilkerson GK, Handa RJ. To eat or not to eat: the effect of AICAR on food intake regulation in yellow-bellied marmots (*Marmota flaviventris*). *J Exp Biol*. 2010; 213:2031–2037. [PubMed: 20511516]
18. Florant GL, Porst H, Peiffer A, Hudachek SF, Pittman C, Summers SA, Rajala MW, Scherer PE. Fat-cell mass, serum leptin and adiponectin changes during weight gain and loss in yellow-bellied marmots (*Marmota flaviventris*). *J Comp Physiol B*. 2004; 174:633–639. [PubMed: 15517283]
19. Florant GL, Richardson RD, Mahan S, Singer L, Woods SC. Seasonal changes in CSF insulin levels in marmots: insulin may not be a satiety signal for fasting in winter. *Am J Physiol*. 1991; 260:R712–R716. [PubMed: 2012243]
20. Giroud S, Perret M, Le Maho Y, Momken I, Gilbert C, Blanc S. Gut hormones in relation to body mass and torpor pattern changes during food restriction and re-feeding in the gray mouse lemur. *J Comp Physiol B*. 2009; 179:99–111. [PubMed: 18726602]
21. Guilmeau S, Buyse M, Tsocas A, Laigneau JP, Bado A. Duodenal leptin stimulates cholecystokinin secretion: evidence of a positive leptin–cholecystokinin feedback loop. *Diabetes*. 2003; 52:1664–1672. [PubMed: 12829630]
22. Hayes MR, Skibicka KP, Lechner TM, Guarnieri DJ, DiLeone RJ, Bence KK, Grill HJ. Endogenous leptin signaling in the caudal nucleus tractus solitarius and area postrema is required for energy balance regulation. *Cell Metab*. 2010; 11:77–83. [PubMed: 20074530]
23. Healy JE, Ostrom CE, Wilkerson GK, Florant GL. Plasma ghrelin concentrations change with physiological state in a sciurid hibernator (*Spermophilus lateralis*). *Gen Comp Endocrinol*. 2010; 166:372–378. [PubMed: 20005230]
24. Hill VL, Florant GL. The effect of a linseed oil diet on hibernation in yellow-bellied marmots (*Marmota flaviventris*). *Physiol Behav*. 2000; 68:431–437. [PubMed: 10713281]

25. O'Hara BF, Watson FL, Srere HK, Kumar H, Wiler SW, Welch SK, Bitting L, Heller HC, Kilduff TS. Gene expression in the brain across the hibernation cycle. *J Neurosci.* 1999; 19:3781–3790. [PubMed: 10234010]
26. Paulino G, Barbier de la Serre C, Knotts TA, Oort PJ, Newman JW, Adams SH, Raybould HE. Increased expression of receptors for orexigenic factors in nodose ganglion of diet-induced obese rats. *Am J Physiol Endocrinol Metab.* 2009; 296:E898–E903. [PubMed: 19190260]
27. Pengelley ET, Fisher KC. Rhythmical arousal from hibernation in the golden-mantled ground squirrel, *Citellus lateralis tescorum*. *Can J Zool.* 1961; 39:105–120.
28. Peters JH, Karpel AB, Ritter RC, Simasko SM. Cooperative activation of cultured vagal afferent neurons by leptin and cholecystokinin. *Endocrinology.* 2004; 145:3652–3657. [PubMed: 15105382]
29. Peters JH, Simasko SM, Ritter RC. Modulation of vagal afferent excitation and reduction of food intake by leptin and cholecystokinin. *Physiol Behav.* 2006; 89:477–485. [PubMed: 16872644]
30. Pulawa LK, Florant GL. The effects of caloric restriction on the body composition and hibernation of the golden-mantled ground squirrel (*Spermophilus lateralis*). *Physiol Zool.* 2000; 73:538–546.
31. Raybould HE. Mechanisms of CCK signaling from gut to brain. *Curr Opin Pharmacol.* 2007; 7:570–574. [PubMed: 17954038]
32. Savastano DM, Covasa M. Adaptation to a high-fat diet leads to hyperphagia and diminished sensitivity to cholecystokinin in rats. *J Nutr.* 2005; 135:1953–1959. [PubMed: 16046722]
33. Smith GP, Jerome C, Norgren R. Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. *Am J Physiol.* 1985; 249:R638–R641. [PubMed: 4061684]
34. Strader AD, Woods SC. Gastrointestinal hormones and food intake. *Gastroenterology.* 2005; 128:175–191. [PubMed: 15633135]
35. Wang L, Martinez V, Barrachina MD, Tache Y. Fos expression in the brain induced by peripheral injection of CCK or leptin plus CCK in fasted lean mice. *Brain Res.* 1998; 791:157–166. [PubMed: 9593872]
36. Whited KL, Thao D, Lloyd KC, Kopin AS, Raybould HE. Targeted disruption of the murine CCK1 receptor gene reduces intestinal lipid-induced feedback inhibition of gastric function. *Am J Physiol Gastrointest Liver Physiol.* 2006; 291:G156–G162. [PubMed: 16574983]
37. Zittel TT, De Giorgio R, Sternini C, Raybould HE. Fos protein expression in the nucleus of the solitary tract in response to intestinal nutrients in awake rats. *Brain Res.* 1994; 663:266–270. [PubMed: 7874510]
38. Zittel TT, Glatzle J, Kreis ME, Starlinger M, Eichner M, Raybould HE, Becker HD, Jehle EC. C-fos protein expression in the nucleus of the solitary tract correlates with cholecystokinin dose injected and food intake in rats. *Brain Res.* 1999; 846:1–11. [PubMed: 10536208]

Fig. 1. Photomicrographs of NTS sections at the level of the AP showing immunoreactivity for Fos-protein (dark-stained cells). Sections are from squirrels treated with vehicle (CON, panels A, C, and F) or cholecystokinin (CCK, panels B, D, and F) in spring, summer or hibernation.

Fig. 2.

Fos-positive neurons in NTS of CON squirrels. Numbers of Fos-positive neurons per 10,000 pixels in four regions of the NTS (relative to the area postrema (AP)) in response to vehicle (saline) in spring (SPR), summer (SUM), and euthermic hibernating (HIB) squirrels. Results are means $\pm$ SE, $n = 5$ for all, * indicates significant difference ($P < 0.05$) from SPR and SUM of same NTS region.

Fig. 3. Fos-positive neurons in NTS of CON and CCK squirrels. Numbers of Fos-positive neurons per 10,000 pixels in four NTS regions (relative to the area postrema (AP)) in response to vehicle (CON) or cholecystikinin (CCK) in (A) spring (SPR), (B) summer (SUM), and (C) euthermic hibernating (HIB) squirrels. Results are means $\pm$ SE, $n = 5$ for all, * indicates significant difference ($P < 0.05$) from CON of same NTS region.

Table 1

Squirrel characteristics.

	SPR CON	SPR CCK	SUM CON	SUM CCK	HIB CON	HIB CCK
Date euthanized	April 13 and 14	April 13 and 14	August 27 and 28	August 27 and 28	October 21–23	October 21–23
Adults	1	0	5	5	0	0
Juveniles	4	5	0	0	5	5
Males	2	2	1	2	0	0
Females	3	3	4	3	5	5
Mass at euthanasia (g)	169.6 ± 32.7	148.8 ± 24.8	146.4 ± 6.8	167.4 ± 10.7	109 ± 26.8 [‡]	123 ± 24.6 [‡]
Mass lost in hibernation (g)	49.4 ± 6.9	32.6 ± 7.1 [*]	na	na	49.8 ± 10.0	41.2 ± 13.7
Days since end of hibernation	29 ± 0.6	30 ± 0.6	na	na	na	na
Days in captivity	236 ± 20.4	228 ± 3.5	42.4 ± 0.5	42.6 ± 0.5	72 ± 1.1	72 ± 0.8
Days in hibernation	105 ± 24.6	71 ± 14.8 [*]			34.6 ± 3.0	35.4 ± 2.1

Values are means ± SE; *n* = 5 for all groups. SPR, spring; SUM, late summer; HIB, euthermic hibernator; CON, vehicle treatment; CCK, CCK treatment. na, not applicable. Adults are 1 year at capture, Juveniles are <1 year at capture.

^{*} indicates CCK means differ significantly (*P* < 0.05) from CON in same season;

[‡] indicates HIB means differ significantly (*P* < 0.05) from SPR and SUM means.