Neural and Hormonal Control of Food Hoarding

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Running Head: Mechanisms Underlying Food Hoarding

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ABSTRACT

Many animals hoard food, including humans, but despite its pervasiveness, little is known about the physiological mechanisms underlying this appetitive behavior. We summarize studies of food hoarding in humans and rodents with an emphasis on mechanistic laboratory studies of species where this behavior importantly impacts their energy balance (hamsters), but include laboratory rat studies even though their wild counterparts do not hoard food. The photoperiod and cold can affect food hoarding, but food availability is the most significant environmental factor affecting food hoarding. Food deprived/restricted hamsters and humans exhibit large increases in food hoarding compared with their fed counterparts, both doing so without overeating. Some of the peripheral and central peptides involved in food intake also affect food hoarding, although many have not been tested. Ad libitum-fed hamsters given systemic injections of ghrelin, the peripheral orexigenic hormone that increases with fasting, mimics food deprivation-induced increases in food hoarding. Neuropeptide Y or agouti-related protein, brain peptides stimulated by ghrelin, given centrally to ad libitum-fed hamsters, duplicates the early and prolonged post-food deprivation increases in food hoarding, whereas central melanocortin receptor agonism tends to inhibit food deprivation- and ghrelin-stimulation of hoarding. Central or peripheral leptin injection, or peripheral cholecystokinin-33, known satiety peptides, inhibits food hoarding. Food hoarding markedly increases with pregnancy and lactation. Because fasted and/or obese humans hoard more food in general, and more high density/high fat foods specifically, than non-fasted and/or non-obese humans, understanding the mechanisms underlying food hoarding could provide another target for behavioral/pharmacological approaches to curb obesity.

Key Words: food deprivation, ghrelin, neuropeptide Y, agouti-related protein, cholecystokinin, leptin, cold exposure, humans, hamsters, rats, Siberian hamsters, lipectomy
OVERVIEW

The vast majority of animals hoard food, including humans [for review see: (172)]. Humans forage (i.e., seek food at stores) and hoard food (i.e., store food in refrigerators, freezers, pantries and cupboards), but we tend to view human food foraging/hoarding trivially as food is abundant in Western societies and an ever expanding list of non-Western civilizations, and is available in ample amounts in a variety of places (e.g., grocery stores and seemingly almost any type of store). Food hoarding by humans tends to be viewed as an excessive and reactive behavior that especially occurs with news of impending inclement weather. These beliefs, however, are superficial and ignore the prominent place that food foraging and hoarding play in the ingestive sequence for nearly all animals, including humans. Not only is foraging and hoarding still important for humans, but based on the data reviewed below, there appears to be separate circuits controlling hoarding relative to food intake.

To understand the regulation of human appetitive ingestive behaviors, it may be useful to briefly engage in an evolutionary perspective. Famine is a threat to survival for many animals, including humans across the millennia (38). Some have posited that famine and the threat of famine have resulted in an energetically ‘thrifty genotype’ contributing to an obese human phenotype because such phenotypes might be ‘selected for’ in famine-prone environments thereby ultimately leading to increases in reproductive success (130). This argument often is used to rationalize the rapid rise in the prevalence of human obesity, but to our knowledge, has not included an additional or alternative mechanism – increases in storage of calories in times of food abundance other than in adipose tissue; that is increases in food hoarding. Thus, in addition to and along with the availability of abundant, inexpensive, calorically dense food that likely contributes to the obesity epidemic, the improvement in the ability to store food due to the
development of long-term storage units (refrigerators, freezers) and preservation processes that extend the shelf lives of foods that otherwise would easily spoil (e.g., dairy, meats, frozen foods) also may be a significant factor in the increase in human obesity. Thus, the necessity to ‘forage’ for food daily or even weekly at the local food store has greatly diminished in many parts of the world – not coincidently, we think, in areas with the fastest increases in obesity/overweight prevalence. Collectively, rather than food hoarding being relegated to the domain of zoologists and field biologists, a deepened understanding of these components of the ingestive behavior sequence may provide insight into behavioral and/or pharmacological treatments for overweight/obesity as we have suggested previously (9; 95).

**WHY DO ANIMALS HOARD FOOD?**

As we have asked previously (95), it seems important to address why food simply is not eaten when found? After all, that would guarantee that the ingested calories would not be available to other animals short of consuming the animal. Food hoarding appears to have evolved to help assure food availability when there are seasonal changes or unpredictable environmental conditions that reduce or restrict access to food [for review see: (172)]. In addition, food hoarding can be more energetically efficient than storing energy as lipid in adipose depots because food hoarding does not require the energetic expense of eating, digesting and converting the food into a long term storage form (largely triacylglyceride and to a considerably lesser extent, glycogen) until it is mobilized for utilization. Another benefit of externally versus internally stored energy is that increases in lipid stores increase body mass and this can reduce the mobility needed to evade predators. So far, food hoarding seems to be only a beneficial evolutionary adaptation. Factors opposing the benefits of food hoarding include
spoilage and theft of hoarded food [for review see: (172)]. Finally, as will be discussed below [see Lipid stores and lipectomy (LIPX)], there are examples of a seemingly integrative process between the external storage of energy as a food hoard and the internal storage of energy as body fat [e.g.; (39; 180)].

**HISTORICAL ROOTS & OUR FOCUS**

The animal behaviorist Wallace Craig in 1918 (34) broadly dichotomized behaviors into ‘appetitive’ -- a set of behavioral responses bringing animals in contact with a goal object, here food, in other cases sex, water and in humans, drugs of abuse, or ‘consummatory’ -- from the word ‘consume’ as in the final act once the objects are acquired/stored, in this case eating/feeding. Note that we are abiding by this dichotomy in our thinking and in this review; thus, although the fate of much of the hoarded food is ingestion, it has not yet met this destiny and therefore it is still in the domain of the appetitive phase of ingestive behavior.

The scope of this review is largely on appetitive ingestive behaviors and is limited, to some extent, on studies attempting to elucidate the mechanisms underlying food hoarding, rarely including food foraging because the possible mechanisms underlying this behavior are even more infrequently studied than those for food hoarding. In addition, we will restrict our discussion of field studies of food hoarding to those that help clarify the mechanistic laboratory studies. We will, however, rely heavily on laboratory models of food hoarding conducted in species known to hoard food naturally in nature – thus, studies of laboratory rats will be infrequently addressed, but discussed, in brief, below.

Laboratory rats are not natural hoarders (29; 164; 175), but instead carry food from the source of the discovered food to a safe place to eat. Moreover, even there, a food cache is not
formed. Instead, when wild rats do hoard, they often bring inedible, frequently shiny objects, to their burrows, but not food (29; 164; 175). ‘Food carrying’ by laboratory rats, however, undoubtedly shares some common underlying mechanisms with those mechanisms underlying natural food hoarding by species where this behavior is a significant part of their ingestive behavioral repertoire – usually animals equipped with cheek pouches that enable substantial amounts of food to be transported home.

Historically, the study of food hoarding in the laboratory was initiated by Wolfe in 1939 (177) who demonstrated that the behavior was quantifiable. Soon after, Morgan et al. (124) proposed the ‘deficit hypothesis’ for triggering food hoarding. This deficit hypothesis for the initiation of food hoarding is somewhat analogous to, but predated, two deficit hypotheses for the initiation of feeding – the ‘glucostatic hypothesis’ of Mayer (113) and the ‘lipostatic hypothesis’ of Kennedy (96). Mayer suggested that food intake is stimulated by glucose utilization (113)], whereas Kennedy suggested it increases with decreases in lipid stores [triacylglycerol in white adipose tissue (WAT) depots]. The major difference between the ‘deficit hypothesis’ for food hoarding and the ‘glucostatic and lipostatic hypotheses’ for food intake is that the former does not explicitly focus on any one metabolic fuel, rather it is based on overall decreases in utilizable metabolic fuels to trigger this behavior (124). The hypothesis was based on the findings of Eliot Stellar who noted that food hoarding in laboratory rats (food carrying, see above) was minimal without previous food deprivation (160). Indeed, the strongest stimuli that triggers food hoarding in hamsters, humans and other species, or food carrying by laboratory rats, is food deprivation with its suite of alterations in peripheral and central processes to be delineated below (see CNS Changes with Food Deprivation; Central Mechanisms; and Peripheral Mechanisms). The energy deficit elicited by food deprivation, as conceptualized by Stellar, was
not believed to be identical to short-term fuel deficits induced by ‘hunger’, but was attributed to a growing energy demand accumulating across days or weeks. Eventually, the energy deficit reached a threshold thereby tipping the scales of energy balance to trigger food hoarding (19).

Therefore, the majority of the studies discussed below are food deprivation studies or experiments where the consequences of food deprivation/restriction have been mimicked experimentally in ad libitum-fed animals to determine which, of the constellation of peripheral/central changes associated with food deprivation, elicits food hoarding. Finally, in these early studies of food hoarding, the food hoard was removed daily and the animals continued to hoard food despite the ‘disappearing food cache’. This suggested to some (124) that the behavior, in-and-of-itself, was reinforcing. In our laboratory we study food hoarding by Siberian hamsters (*Phodopus sungorus*) and we also remove the food hoard after each hoard measurement [typically 1, 2, and 4 h in many studies – see below; or after 24 h (daily) in longer term studies]. An alternative hypothesis to hoarding being inherently reinforcing is that the animals are hard-wired to seek and store food to survive. Thus, the removal of the only food present in the early studies and our studies requires the animals to seek and eat/store additional food despite the inability to build a sustaining cache, or else starve. It is not surprising, therefore, that they continue to hoard food despite its removal. Thus, this behavior does not imply that hoarding, in-and-of-itself, is reinforcing, any more than daily trips to the grocery store in humans would be self-reinforcing if yesterday’s food purchases always disappeared. A third hypothesis follows from the two above and is rooted in the biological theory of reinforcement; in brief, food hoarding is a fixed action pattern that, when carried out, is thereby reinforced (66).

In studies of food hoarding in our laboratory, we have almost exclusively studied Siberian hamsters because they are prodigious hoarders in nature [K. Wynne-Edwards, personal
communication; (61)] as well as in the laboratory (6; 8; 39; 40; 42-46; 91-94; 110; 168; 169; 178-180). Syrian hamsters also are prodigious food hoarders in nature (127) and in the laboratory (25; 59; 99; 108; 146). We chose Siberian hamsters because of their hoarding prowess and because of the extensive knowledge of their energy balance with regard to the control of their lipid stores, food intake and energy expenditure [for review see: (9; 14; 112; 119)]. Both species are equipped with cheek pouches that extend well beyond their cheeks to their shoulders allowing them to carry substantial amounts of food. Indeed, the word ‘hamster’ is derived from the German ‘hamstern’ which means to hoard and, moreover, Middle Easterners call Syrian hamsters ‘grandfather saddlebags’ because of the appearance of their full cheek pouches (172).

Because of this robust behavior and because of their tendency to control their food intake separately from their food hoarding (see below), the vast majority of the mechanistic and behavioral laboratory studies of food hoarding has been conducted in these two hamster species.

ENVIRONMENTAL INFLUENCES ON FOOD HOARDING

Food availability appears to be the strongest stimulus triggering food hoarding in nature (172) and the stimulus most frequently exploited in the laboratory to study the neural and hormonal mechanisms underlying food hoarding. In addition, we will briefly discuss other environmental influences such as ambient temperature (cold) as well as photoperiod. The underlying mechanisms controlling the effects of photoperiod and/or ambient temperature on food hoarding are not as well studied compared with food availability, however. In nature these three factors – photoperiod, ambient temperature and food availability often covary. For example, in winter, when the days are short and the nights long, ambient temperatures often drop significantly, especially at high latitudes. Because of both the decrease in daylength and ambient
temperature, food availability thereby is often reduced. In the laboratory, of course, these factors can and have been manipulated separately.

**FOOD AVAILABILITY**

Although it might be argued that total food deprivation does not occur naturally for many or most animals, it also seems reasonable to envision food deprivation as on a continuum with naturally-occurring intermeal intervals. When laboratory rats and mice are food deprived and refed, they overeat [e.g., (78; 185)]. By contrast, when various hamster species are food deprived and refed, they do not overeat [Siberian hamsters: (8; 43; 179); Syrian hamsters: (25; 141; 151); Turkish hamsters (*Mesocricetus brandti*; (140); Chinese hamsters (*Cricetulus griseus*; (18)]. The lack of post-food deprivation-induced hyperphagia is not due to a physical inability to overeat because both Siberian and Syrian hamsters do so under several standard conditions that produce overeating by laboratory rats and mice including calorically diluted diets (140; 178) and lesions of the hypothalamic paraventricular nucleus [PVH; (7; 180)]. Silverman and Zucker (151) speculated that the lack of a post-food deprivation-induced increase in food intake by hamsters could have been shaped by environmental selection pressures for building food caches to counteract food shortages.

We previously noted multiple times that the lack of a post-food deprivation-induced hyperphagia by hamsters contrasts with that of humans [e.g., (9; 95; 145)]. We were clearly incorrect and succumbed to the conventional wisdom that humans overeat after a fast. As we now know, the literature does not strongly support post-fast hyperphagia in humans. That is, laboratory studies, the surprisingly few that exist, and studies of the religious prohibitions eating demonstrate a lack of post-fast increases in eating by humans. For example, female non-
restrained eaters fasted for 24 h or food restriction to 1200 kcal do not show a compensatory increase in food intake on subsequent days (105). Similarly, 19 h fasts do not increase subsequent food intake in humans (77), but in a positive report, 36 h fasts modestly increase food intake by ~20% (84). We are unaware of any studies of food intake after the Yom Kippur fast by Jewish individuals and only a preliminary report of a lack of increases in food intake after the one-day fast at the beginning of each month for members of the Church of Jesus Christ of Latter-Day Saints [Mormons; (137)]. Daily fasting occurs during the daylight hours of Ramadan by Muslims (~13 h fast including no water intake). Mixed results are reported for food intake after sunset during Ramadan, with several studies reporting significant increases [(87; 88; 101)], albeit relatively modest ones (~20%, 5%, 6%, respectively), whereas others report no increase [(1; 97)]. Collectively, the food intake following fasting and food restriction in humans and food deprivation in hamsters is more similar than different; there is essentially little or no increase in food intake upon refeeding. Instead, both hungry humans and hamsters ‘overhoard’ with resumption of ad libitum food access. Specifically, hungry people bring home more food after even mild food deprivation compared with their sated counterparts (15; 49; 117). Similarly, Siberian hamsters markedly increase their food hoarding, but not their food intake, when food deprived (6; 8; 43; 179), as do food deprived Syrian hamsters (25; 146). Therefore, hamsters provide a more analogous model to the consummatory ingestive behavior responses to food restriction/deprivation to that of humans than do laboratory rats or mice. Moreover, because hamsters do not overeat after food deprivation, but instead overhoard, they therefore show an uncoupling of the consummatory and appetitive responses to food deprivation/restriction more readily permitting the determination of the underlying mechanisms of each behavior separately. With laboratory rats, however, food intake and food carrying are inextricably intertwined, as
both increase with food deprivation-refeeding thereby not permitting an unraveling of the underlying mechanisms of each ingestive behavior [e.g.,(55; 124)]. More detailed information on post-fast/food deprivation studies from humans, hamsters and food carrying by laboratory rats and mice appears directly below.

Changes in food foraging/hoarding by humans with alterations in food availability:

Most studies of human food hoarding have focused on food purchasing behaviors as an index of food storage because ~85% of the food purchased is consumed at home (138). These studies confirm what your grandmother and mother suggested, and what we all likely have experienced – if you go to the grocery store hungry, you will bring home more food than when sated. Indeed, food deprivation and/or increased hunger leads to increases in the amount of food purchased (49; 117; 170). In addition, obese people purchase more food per person and food with higher fat content and/or caloric density compared with lean people (52; 138).

Unfortunately, in addition to the paucity of descriptive studies of human food hoarding as affected by food restriction/fasting/hunger, we are unaware of any studies examining the possible neural or hormonal mechanisms underlying this appetitive ingestive behavior in humans. There is one pathological state associated with ‘overhoarding’ – individuals with Prader-Willi-Syndrome (PWS). PWS is a rare genetic disorder due to deletion of genes on chromosome 15 resulting in a plethora of physiological and behavioral disorders including cognitive abnormalities, obesity and obsessions with food.

In PWS, food seeking and food hoarding behaviors far exceed that seen by non-PWS individuals [e.g., (67)]. PWS is characterized by hyperphagia and severe obesity starting around age one (67). The most common abnormal ingestive behaviors observed in PWS include food obsession, food stealing, stealing money to purchase food and food hoarding. Behavioral
intervention in a group home includes rigorous supervision and locking refrigerators, freezers and pantries [(67); for further discussion of PWS and hoarding see Clinical Relevance below].

**Foraging Effort:**

In nature, variation in foraging effort often involves the distance traveled to the food source [for review see: (83)]. Laboratory rats carry less food as the length of the tubing leading to the food source increases (28). In terms of physical effort, but not distance, as the number of lever presses required to obtain food pellets increases, Syrian hamsters hoard less food (104). In reality, both of these models require little effort to obtain food – traveling a few meters (28) or pressing a light-weight lever several times (104). Therefore, we adapted and modified the wheel running-based foraging effort model of Perrigo and Bronson (136) to more closely incorporate two vital components of foraging in the wild – time and distance required to obtain food. Briefly, two cages are connected by convoluted tubing where the top “foraging” cage contains a running wheel, food source and water, and the bottom “burrow” cage is opaque, covered and contains a cotton nest to simulate an underground burrow. Wheel revolutions are monitored by a computer that controls food pellet delivery upon completion of a predetermined number of wheel revolutions (42). We tested the relation between foraging effort and food hoarding in Siberian hamsters in this system by increasing the number of wheel revolutions required to deliver a food pellet [i.e., 10, 50, 100 or 200 revolutions per pellet; (42)]. Whereas, food intake is quite constant across these disparate foraging efforts, food hoarding is more complex in that food hoard size initially increases with the increase in the foraging requirement starting with 0 (‘free, non-contingent’ food) and peaking at 10 revolutions/pellet, followed by a progressive decrease in hoarding as the foraging requirement continues to increase (42). This relation suggests that a moderate increase in effort stimulates food hoarding, but that once the exercise-induced energy
deficit exceeds some level, food is eaten rather than hoarded (42), a finding that makes intuitive
sense as well.

**Photoperiod and Pineal Melatonin**

The photoperiod is faithfully coded into a biological signal via the nocturnal duration of
pineal melatonin secretion [for review see: (12)]. The progression of the daylength (nights
getting longer, or vice versa) determines a suite of seasonal photoperiodic responses that include
changes in pelage, body fat stores, reproductive status, food intake [for review see: (12)] and
food hoarding. The most striking seasonal changes in food hoarding are by small mammals that
overwinter at high latitudes. These animals carry a huge energy burden due to their large surface
area-to-volume ratio resulting in increases in body heat loss while simultaneously experiencing
declining temperatures and food availability. Therefore, winter preparations for some of these
species include increasing cached food to help offset their forthcoming energy burden. Collared
pika, (*Ochotona collaris*) hoard hay in nature, with haying increasing in the middle of the
summer extending into the fall suggesting preparatory accumulation of food for overwintering
(126). A northern subspecies of white-footed mouse (*Peromyscus leucopus noveboracensis*) and
deer mice (*Peromyscus maniculatus bairdii*) increase food hoarding in short days combined with
cold compared with long days and normal vivarium temperatures in the laboratory (5). Testing
these environmental stimuli separately, the photoperiod exerts more control than does the cold on
food hoarding by *P. m. bairdii*, whereas cold exerts more control than over food hoarding than
the photoperiod for *P. l. noveboracensis* (5). Yellow pine chipmunks (*Tamias amoenus*) do not
increase their lipid reserves before their fall-winter hibernation season, as with other chipmunk
species, but instead increase their food hoarding (100).
The role of the photoperiod in food hoarding by hamster species is not clear. We tested the effects of the photoperiod on ad libitum hoarding in Siberian hamsters with the added challenge of food deprivation (the latter topic alone is discussed in depth and comprising the majority of the remainder of this review). Food hoarding does not increase at 11 weeks of short day exposure compared with their long day-housed counterparts (179), a time when body and fat mass of the former are at their natural nadirs and food intake has declined to ~30 % of long day values (173). Because food hoarding was measured after the short day-induced body and lipid masses and food intake reached their nadirs, it was possible we missed short day-induced increases in food hoarding made during the early weeks of short ‘winter-like’ days. Therefore, we recently tested the food hoarding response during the dynamic period of body/lipid mass loss [first 5-6 weeks of short days (173)] using our wheel running-based foraging system. The typical decrease in body mass occurred in short days, but food hoarding did not increase compared to their long day-housed counterparts (168). This suggests that the naturally occurring short day-induced body/lipid losses do not engender similar hoarding responses as seen with food deprivation or lipectomy, both conditions that decrease body fat, discussed below.

By contrast to Siberian hamsters, female Syrian hamsters transferred from long to short days increase their body mass and food intake (13) and hoard significantly more food than their long day-housed counterparts (59). The short day-induced increases in body mass, food intake and food hoarding are dependent on an intact pineal gland (and therefore dependent upon normal short day-released melatonin signals), as pinealectomy blocks these responses (59).

What are the mechanisms underlying the photoperiod-induced adjustments in food hoarding? Quite simply, we do not know. Correlational studies show that changes in the daylength alter peptides in photoperiod-responsive species that also have been implicated in the
peptidergic control of food intake in laboratory rats and mice. These peptides, however, have only cursorily been studied with regard to their role in photoperiod-triggered changes in food hoarding. Even though Siberian hamsters do not exhibit short day-induced increases in food hoarding (168; 179), Syrian hamsters do, as noted above, and this responses is pineal/MEL dependent (59); however, tests of candidate neuropeptides that change with the photoperiod/MEL that might affect food hoarding have not been made. Based on our successful strategy to test neurochemicals involved in food intake in laboratory rats and mice for their potential role in the neurochemical control of food hoarding in Siberian hamsters (40; 41; 44; 45; 91-94; 169), we speculate that neuropeptide Y (NPY) could be involved in the short-day induced increase in food hoarding by Syrian hamsters. Thus, although there are some possible leads in the search for the underlying mechanisms involved in photoperiod/MEL-mediated food intake, determining the underlying neurochemistry/neuroanatomy mediating such changes appears to be an area in need of considerable study.

Collectively, food hoarding can be altered with changes in the photoperiod in some species, but it is not as powerful a stimulator of this behavior as might be expected. Alternatively, or in addition, many species accumulate body fat before winter and hibernate or exhibit shallow daily torpor during winter; thus, the need for accumulating external energy stores may be superseded by the pressures to increase lipid stores.

**Cold Exposure**

As noted above, cold exposure has frequently been tested in tandem with changes in the photoperiod, but also independently. Although our knowledge of the many hormonal and neurotransmitter changes associated with short photoperiods is relatively deep, our knowledge of the hormonal and neurotransmitter changes associated with cold exposure is quite shallow.
Moreover, virtually nothing is known whether cold-induced changes in hormones or neurochemicals are involved with the typically observed cold-induced increases in food hoarding discussed below. Therefore, we will discuss the effects of cold exposure on food hoarding and then describe some of the neurochemical and hormonal changes associated with cold exposure followed by speculation as to their potential to underlie cold-induced increases in food hoarding.

Recall from above (Photoperiod and Pineal Melatonin) that in one subspecies of deer mice temperature trumps photoperiod in *P. l. noveboracensis* captured from high latitudes (5). Cold ambient temperature also stimulates other adaptive responses such as shallow daily torpor and it has been suggested that increases in food hoard size may counter the need for torpor by *P. leucopus* perhaps explaining their infrequent torpor bouts (167). Cold exposure increases the percentage of Siberian hamsters hoarding food [individual food hoard size was not quantified (110)]. In laboratory rats, the amount of food carrying is inversely related to ambient temperature (115). The cold-induced increase in food carrying by laboratory rats is negated if insulative nesting material also is supplied (56). This finding demonstrates the dominance of thermal insulation over food storage when there is an opportunity to choose between the two cold coping strategies. Furthermore, the dominance of food carrying over increasing thermal insulation can be reinstated if cold exposed rats are concurrently food deprived (56). These latter data suggest that energetic strategies chosen to cope with cold exposure vary depending upon the choices available to the animal (56). In sum, cold exposure triggers increases in food intake and food hoarding/carrying across a number of species in laboratory tests, but the mechanisms underlying this enhancement of food hoarding by cold exposure is unknown.

Some of the possible underlying mechanisms for cold-induced increases in food hoarding may be found in the neurochemical/neuroendocrine changes associated with cold-induced increases
in food intake and/or thermogenesis from laboratory rats and other species that do not hoard food as part of their ingestive behavioral repertoire, a proven successful strategy noted above and below. Using this strategy, one possible peptidergic mechanism underlying cold-induced increases in food intake (and perhaps, therefore, cold-induced food hoarding in species such as hamsters where these stimuli promote food hoarding over food intake) is the orexigenic peptide, NPY. Centrally administered NPY greatly stimulates food intake in laboratory rats (32; 154) and Siberian hamsters (21) and to our knowledge all species tested to date [for review see: (10)]. Laboratory rats acutely exposed to the cold have increases in NPY protein concentrations, as measured by radioimmunoassy, in several brain areas thought to be involved in food intake [e.g., hypothalamic paraventricular nucleus (PVH), ventromedial hypothalamic nucleus (VMN)], dorsomedial hypothalamic nucleus (DMH) and lateral hypothalamic area (LH) (114)], but when measured by immunohistochemistry, they are decreased (134). Thus, these discordant results do not clearly point to NPY as a potential mediator of cold-induced food intake. In addition, any involvement of cold-induced increases in NPY would have to be reconciled with the ability of NPY to inhibit brown adipose tissue (BAT) thermogenesis (17).

Another possible candidate underlying cold-induced increases in food intake and thus, perhaps cold-induced increases in food hoarding, is agouti-related peptide (AgRP), a powerful and long-lasting orexigen that increases food intake up to a week with a single central injection in laboratory rats (73). Although the data on AgRP with cold exposure are scant, cold exposed (28 d) Brandt's voles (Lasiopodomys brandti) have increases in hypothalamic AgRP gene expression compared with their warm exposed controls accompanied by no changes in several other proven factors involved in food intake in laboratory rats and mice including hypothalamic expression of the long form of leptin receptor, suppressor-of-cytokine-signaling 3, NPY, pro-
opiomelanocortin (POMC) and cocaine-and amphetamine-related transcript (CART) mRNA (166). Melanin concentrating hormone (MCH), an orexigen among its many effects [for review see: (182)] and corticotropin-releasing factor, an anorexigen among its many effects [for review see: (182)], significantly increase and decrease in the hypothalamus, respectively when laboratory rats are exposed to 4 °C (48), suggesting two other possible factors to test for their role in cold-induced increases in food hoarding.

A possible peripheral signal that is permissive of the increased hoarding occurring during cold exposure is the leptin. Chronically cold exposed Siberian hamsters when fed ad libitum do not lose body or fat pad mass (Teubner, Vaughan and Bartness, unpublished observations), overeat (23) and have greatly reduced circulating leptin concentrations (Teubner, Vaughan and Bartness, unpublished observations; see PERIPHERAL CONTROL: Leptin and Ghrelin for specific discussion of leptin).

Collectively, based on the rather scant measures of changes in orexigenic/anorexigenic neuropeptides and associated receptors/intracellular signaling proteins in cold-exposed animals, we can only speculate as to their potential involvement in cold-induced increases in food hoarding and suggest possible roles of AgRP and MCH as stimulators. In addition, it is possible that cold exposure removes an inhibition of hoarding by decreasing the efficacy of satiety factors, but this notion has not been approached.

CHANGES IN REPRODUCTIVE STATUS

Pregnancy, Lactation and Gonadal Steroids

Mammalian species exhibit several strategies to cope with the most energetically challenging stimulus for mammals, pregnancy and/or lactation [for review see:(122)]. These
strategies include increasing food intake during both pregnancy and lactation with/or without decreases in energy expenditure, and fattening during pregnancy followed by tapping into these stored lipid fuels during lactation, among several other strategies [for brief review see within: (6)]. One typically expects fattening during pregnancy, such as accomplished by eastern wood rats [Nemotoma floridana](116), human females (51) and laboratory rats (128)]. By contrast, Siberian hamsters reduce their body fat by ~ 40-50% across pregnancy and lactation (147). Accompanied or perhaps triggered by these decreases in body fat during pregnancy/lactation are marked increases in food hoarding peaking just before parturition and again during early lactation (6). Food intake, by contrast, increases only late in pregnancy in this species, but throughout lactation, the latter likely to fuel milk production (6). Because Siberian hamsters exhibit postpartum estrus, they also often are concurrently pregnant while lactating and, with this incredible energy drain where both fetuses and pups are energetically supported, food hoarding can increase by as much as ~ 2,500% or more (6). These marked increases in the level of food hoarding suggest an important role of cached food that appears to be adaptive because they offer a within-burrow, readily accessible energy supply to subserve these energetically expensive reproductive conditions (6).

Pregnancy- and lactation-induced increases in food hoarding are not specific to Siberian hamsters, as Syrian hamsters hoard more food during both conditions than their virgin controls (120). More specifically, although the size of the food hoard was not measured, the percentage of hamsters hoarding food rises to nearly 100% just before parturition and as lactation progresses toward weaning (60). Similarly, laboratory rats increase food carrying during gestation (20). No one has determined whether it is the energy drain(s), the hormonal milieu, or both that produce central alterations resulting in these marked increases in food storage. Strategies for revealing
the underlying mechanisms triggering these marked rises in food hoarding with pregnancy and/or lactation include induction of pseudopregnancy or exogenous combinations of estrogen, progesterone, prolactin and other hormones altered during pregnancy and/or lactation to mimic the natural hormonal milieu.

Studies of the effects of gonadal steroids on food hoarding or food carrying (laboratory rats) in non-pregnant and/or lactating species have been quite limited. Female laboratory rats (76) and Syrian hamsters (53) decrease food hoarding at a time when naturally-occurring circulating concentrations of the ovarian hormone estradiol are high. This is most apparent when Syrian hamsters are food restricted to ~75% of their ad libitum intake with the rise in food hoarding occurring during the day after ovulation (associated with peaks in progesterone) and its fall with increases in estradiol [and decreases in progesterone; (99)]. The more precise delineation of the effects of estradiol, progesterone or their combination on food hoarding have been achieved using ovariectomized rodents given exogenous hormone treatments to mimic the naturally-occurring endogenous fluctuations of these hormones across the estrous cycle. Specifically, peripheral 17-ß estradiol injection decreases the ovariectomy-induced increases in food carrying by laboratory rats, with no effect of progesterone alone (33), supporting the notion that high circulating estrogen concentrations are associated with an inhibition of food hoarding. The combination of estradiol and progesterone given systemically to ovariectomized Syrian hamsters delays the increases in food hoarding that accompany food restriction compared with vehicle-treated ovariectomized controls and triggers an increase the preference for male hamsters when a choice between food to hoard and a male are offered (99). Finally, little is known about the role of androgens in food hoarding. Castration of Mongolian gerbils increases food hoarding, whereas castration plus testosterone propionate injections reverses this effect (132).
In summary, naturally-occurring changes in ovarian steroids, such as occur across the estrous cycle, and with pregnancy and lactation (the latter two also associated with changes in circulating prolactin among other hormones), can modify food hoarding or carrying and, relative to the ovarian steroids, these effects are modified by the energy status of the animal and the presence of more natural environmental stimuli such as males (99).

PERIPHERAL CONTROL

The distinction between peripheral and central control of any response is one of convenience in presentation and thought, as is the case here. Thus, our presentation of peripheral versus central control of food hoarding is an artificial dichotomy that we are employing to facilitate discussion of the plethora of factors affecting food hoarding. We are clearly cognizant, for example, that metabolic fuels affect central processes, that peripherally-released hormones affect the brain directly and/or through sensory afferents that project to the brain, and that alterations in central neuropeptides can affect peripheral metabolism, hormone release, adiposity and behavior. Therefore, for simplicity and convenience alone, we make this distinction below, but will attempt to integrate central and peripheral factors when possible.

Metabolic fuels

Eliot Stellar in the 1940’s first tested whether metabolic fuel availability could alter food hoarding (food carrying) in laboratory rats by injecting epinephrine peripherally to mobilize lipid and carbohydrate fuels, injecting glucose to supply exogenous carbohydrate fuels and injecting insulin to facilitate the uptake of lipid and carbohydrate fuels into peripheral tissues (158). The results of these manipulations were unremarkable; there were small decreases in food carrying with the highest epinephrine dose, likely secondary to a non-specific debilitating effect (158). We tested the effects of metabolic fuel utilization on food hoarding in Siberian hamsters,
injecting long-lasting insulin to clear metabolic fuels from the circulation thereby creating an energetic challenge that we hypothesized might stimulate food hoarding much in the same way it does food intake in laboratory rats [e.g., (62)] and mice (142). We also created a glucoprivic state by injecting the non-metabolizable glucose analog, 2-deoxy-D-glucose. Neither energetic deficit created by these injections affected food hoarding, nor did lipoprivation created by administering the fatty acid utilization blocker, methyl palmoxirate (8). These treatments were given at dark onset and food hoarding was measured at the beginning of the light period 8 h later (8). Thus, we may have missed acute increases in food hoarding that subsequently could have been compensated by decreases in hoarding resulting in no net change in hoard size. We postulated previously that if the lack of an effect of short term energetic challenges such as these on food hoarding is real and not due to our inability to measure hoarding at the proper time post treatment, then perhaps food hoarding is engaged in response to more prolonged energetic challenges, such as food deprivation/restriction (9), as suggested originally in the ‘deficit hypothesis’ of Morgan (124). Finally, thyroxine injection-induced increases in metabolic rate, or decreases in metabolic rate produced by thyroidectomy or thiouracil, do not affect food carrying by laboratory rats (159). Collectively, although not thoroughly investigated, at present there are no compelling or suggestive data indicating that short-term alterations in energy utilization affect food hoarding.

Lipid stores and lipectomy (LIPX):

Food deprivation triggers marked and prolonged increases in food hoarding and also is associated with decreases in lipid energy stores in addition to a collection of peripheral and central responses (discussed in detail below). At a simple level, food hoards might be viewed as externally stored energy that could functionally replace, or be somewhat interchangeable with,
internally stored energy as triacylglycerol in adipocytes of WAT depots (9). We initially held the view that food hoard size is inversely correlated to total lipid mass in Siberian hamsters (8), as have others for laboratory rats (121). Such a view is admittedly naïve in retrospect because we now know that food hoarding can increase without a detectable decrease in body fat; this belies the proposed inverse relation between body fat and food hoard size. For example, with calorically diluted food, food hoarding increases before body mass/fat decreases (178). Moreover, short photoperiods trigger substantial decreases in body and WAT mass (~30%) in Siberian hamsters (173), but food hoarding is not increased. Furthermore, central administration of the orexigenic peptides NPY and AgRP markedly increase food hoarding, doing so rapidly [(within the first hour or so post injection; (44; 45)] with no decrease in body mass (and therefore likely body fat) either.

The above examples should not, in and of themselves, invalidate the possible relation between lipid metabolic fuels and food hoarding. First, measurements of WAT pad mass are crude and represent an integration of lipid metabolism – lipogenesis, lipid uptake and lipolysis; thus, the process of lipolysis, for example, may be well underway without detectable changes in WAT pad mass. Indeed, we recently reported that central melanocortin receptor agonism increases the sympathetic nervous system drive to WAT (22) and greatly increases phosphorylation of hormone sensitive lipase and perilipin A, two white adipocyte proteins required for sympathetic nervous system/norepinephrine-induced lipolysis (102), one hour post injection showing lipolysis is underway -- yet fat pad mass is unchanged (150). As we previously have noted (39; 42; 95), just as it appears that ‘metabolic flux’ (whether energy is being stored or mobilized) controls reproductive status [for review see: (174)], metabolic flux (in this case lipid flux) may dictate changes in food hoarding. That flux of increased lipid
mobilization could, in turn, alter central and/or peripheral factors that might serve as signals of the food deprived state thereby triggering compensatory responses such as increasing food hoarding. For example, food deprivation, in addition to increasing lipid mobilization, increases the synthesis of ARC AgRP and NPY (139). Thus, ad libitum-fed animals given AgRP/NPY centrally might stimulate the same circuits that are naturally stimulated by endogenous release of AgRP/NPY associated with food deprivation-induced lipid mobilization and in this way trigger food hoarding/intake. Indeed, centrally applied AgRP or NPY increases primarily food hoarding, and to a lesser extent food intake (40; 44; 45; 91-93) despite no discernable or nor actual increase in lipid mobilization (unpublished observations).

To more directly test the effects of decreases in lipid stores, partial LIPX can be used without many of the complicating factors associated with food deprivation [for review see: (112)]. Therefore, we have exploited the ability to produce, at least initially, a marked plunge in total body fat levels by partial LIPX [for review see: (112)]. Removal of both the epididymal and inguinal WAT pads [EWAT and IWAT, respectively; (180) ] triggers increases in food hoarding that wane as the animals marshal the normal compensation for the surgically-induced lipid deficit by increasing the mass of the non-excised fat pads [for review see: (112)]. We reasoned, therefore, that the greater the amount of WAT removed, and thus the larger lipid energy deficit, the greater the increase in food hoarding; however, this was not found (39). Specifically, the smallest lipid deficit, achieved by removal of both EWAT pads, triggered the largest increase in food hoarding (39). It appears, therefore, that the ruling factor was not the size of the lipid deficit per se, but rather the specific fat pad removed. This notion of a fat pad-specific effect of lipectomy is not without precedent, as EWAT lipectomy stimulates compensatory reparation of the surgically-produced lipid loss in the remaining fat depots better
than does IWAT lipectomy, despite the latter producing twice the lipid deficit (111). In addition, we recently reported that EWAT lipectomy of Syrian hamsters completely inhibits spermatogenesis (31), as it does in laboratory rats (153), without other global changes in testicular function (serum testosterone was normal). It is notable that twice the lipid deficit produced by inguinal WAT removal has no effect on spermatogenesis (31). Therefore, it may be that lost lipid from WAT depots that support important functions, such as the evolutionarily-shaped drive to reproduce, are most critical to the animal (EWAT in males) and thereby affect physiology (spermatogenesis, compensatory lipid responses) and behavior (food hoarding) more so than the absolute degree of lipid deficit.

**Leptin and Ghrelin:**

Food deprivation engenders a multitude of central and peripheral changes in physiology that include adjustments in hormones that affect metabolism. For example, food deprivation decreases circulating leptin, a primarily adipocyte-secreted peptide. Leptin is released in rough approximation to the amount of body fat and tends to decrease food intake and increase energy expenditure [for review see: (131)]. By contrast to these catabolic effects of leptin, food deprivation increases circulating ghrelin, a primarily stomach-secreted peptide. Ghrelin tends to increase food intake and decrease energy expenditure and thus has anabolic effects [for review see: (131)]. Considerable effort has focused on the effects of these ‘metabolic hormones’ on consummatory ingestive behavior, but only relatively recently have these hormones been investigated for their role in the control of appetitive ingestive behaviors such as food hoarding.

Circulating ghrelin stimulates ingestive behaviors via its central effects [for review see: (123)]. Peripheral injections of ghrelin or ghrelin receptor agonists increase food intake in laboratory rats (171), as do intracerebroventricular injections [e.g., (3)]. More specifically, the
central sites of action for ghrelin appear to be distributed across the neuroaxis. Thus, parenchymal injections of ghrelin or ghrelin receptor agonists increase food intake if microinjected into brainstem [dorsal vagal complex;(57; 103)], midbrain [ventral tegmental area;(129)] as well as forebrain [ARC (183), PVH (103), DMH (103), LH (103; 163), medial preoptic area (163) and nucleus accumbens (129)] sites. Given that food deprivation increases ghrelin secretion by the stomach in humans, laboratory rats [e.g., (2)] and Siberian hamsters (91) and that food deprivation increases food hoarding in Siberian hamsters (6; 8; 41; 43; 92-94; 169; 179), we reasoned that ghrelin might stimulate food hoarding. Indeed, peripheral ghrelin injection in ad libitum-fed Siberian hamsters at doses that generate circulating active ghrelin concentrations similar to those generated naturally after 24-48 h of food deprivation triggers food deprivation-like increases in food hoarding (91). These increases in food hoarding after a single peripheral ghrelin injection last up to 5 days, similar to food deprivation itself [e.g., (8; 43; 179)], even though there is no evidence of increases in circulating ghrelin 24 h or beyond post-injection (91). Although promoting increases in food intake, ghrelin affects food hoarding to a greater degree than intake in these hamsters (91). Therefore, ghrelin may be the signal by which the periphery is able to communicate feeding state with the brain and increase appetitive ingestive behaviors.

Leptin is another ‘metabolic hormone’ that has a multitude of functions in physiology and pathology. Important here are the effects of leptin on ingestive behavior, as it is conceptually the counterpart to ghrelin, as noted above [for review see:(70)]. Opposite to ghrelin, peripheral exogenous leptin injection/infusion decreases food intake [e.g., (75)] as well as when administered intracerebroventricularly [e.g., (37)]. As with ghrelin, the central sites of action for leptin appear distributed as well and include the ARC (143), VMH (143), and LH
(143) in the forebrain, VTA in the midbrain (81) and dorsal vagal complex in the brainstem (72). Leptin could serve as a signal to decrease food hoarding via action in any of these or other brain sites shown to decrease food intake after microinjection of leptin. Indeed, systemic leptin injections in food deprived Syrian hamsters blocks food deprivation-induced increases in food hoarding (25; 146). We tested the ability of a single peripheral or central leptin injection to block food deprivation-induced increases in food hoarding in Siberian hamsters housed in our foraging/hoarding system (94). Peripherally administered leptin prevented food deprivation-induced decreases in circulating leptin and attenuated food deprivation-induced increases in food hoarding as did a 3rd ventricular leptin injection (94). The inhibition of food hoarding with a 3rd ventricular leptin injection was greater than a peripheral injection, although comparison of peripheral and central injections of any substance is fraught with interpretational difficulties due to access to the sites of action and catabolism differences among other factors. Therefore, it is not possible to ascribe the sites of action for the inhibition of food hoarding by leptin as central or peripheral. In addition, given the prevalence of leptin receptors on vagal afferents (27) that, in turn, potentially could trigger central effects, leptin may have its effects both peripherally and centrally. As with other substances (e.g., ghrelin discussed above, AgRP and NPY discussed below), food hoarding is affected to a greater degree than food intake by leptin in Siberian hamsters (94).

Collectively, leptin and ghrelin appear to provide conceptually opposing peripherally-generated signals that influence a plethora of responses including those involved in energy intake/expenditure, but as described here, also participate in the appetitive ingestive behavior of hoarding. Therefore, the actions of leptin and ghrelin should be expanded to include appetitive
ingestive behaviors as well as the more widely studied and accepted effects on consummatory ingestive behaviors.

**Cholecystokinin**

Cholecystokinin is another peripherally-released peptide that is well-established as an inhibitor of food intake (‘satiety peptide’) that could act to terminate food hoarding. CCK is chiefly produced by the small intestine mucosal epithelium I-cells and released primarily in response to fat and protein intake \([e.g., (106)]\). Exogenously administered CCK [the octapeptide form (CCK-8)] given peripherally inhibits food intake in laboratory rats \([e.g.; (64)]\). Of the circulating CCK forms, CCK-58 is the most common form in humans and dogs (54) with either CCK-33 or CCK-58 most common in rodents (107). The role of CCK in food hoarding only has been tested in Siberian hamsters \([(169); Dailey, Vaughan, and Bartness, unpublished observations]\). CCK-33 is a powerful inhibitor of food foraging and hoarding in fed and food-deprived Siberian hamsters having its major effects early during the first hour post administration during refeeding, as also occurs for its inhibition of food intake (169). By contrast, CCK-8 appears somewhat ineffective in inhibiting food hoarding in this species \(\text{(Dailey, Vaughan, and Bartness, unpublished observations)}\), although it does inhibit food intake (11). CCK has not been tested in other species for its effects on food hoarding, but has been tested for other appetitive ingestive behaviors. For example, CCK-8 decreases exploratory behavior and increases anxiety-like behaviors in laboratory mice at doses capable of inhibiting food intake, but without reducing locomotor activity (35), as well as reducing operant responding for food in pigs (4) and laboratory rats (82). Together these data suggest CCK may play an important role in terminating food hoarding in Siberian hamsters and additional appetitive ingestive behaviors in other species. Other peripheral factors that affect food intake in laboratory
rats, mice and in humans such as amylin, glucagon-like peptide 1, obestatin, and adiponectin have not been tested for their effects on food hoarding.

**CENTRAL MECHANISMS**

Central changes in neurochemicals associated with food deprivation and consequent changes in ingestive behaviors could be due to peripherally-and/or centrally generated signals that trigger subsequent alterations in ingestive behaviors once food access is restored. Instead or in addition, these changes in central neurochemicals could be associated with the peripheral and/or central metabolic consequences of decreases in energy intake. Woods *et al* (181) suggested that some neurochemicals that stimulate food intake in home-cage tests of laboratory rats may instead bring animals into contact with food. In this appetitive phase of the ingestive sequence, if there is little or no effort in obtaining food, as in the typical home-cage testing condition, then the consummatory phase would proceed virtually automatically (181). This notion was based on the inability of central administration of the potent orexigen NPY to stimulate food intake in laboratory rats when no appetitive response was required because food (sucrose solution) was infused intraorally via a chronically implanted catheter (181). If, however, these same animals are given the same dose of NPY centrally, but are required to drink from a sipper spout instead of being passively infused orally with sucrose, NPY produces the expected increase in sucrose intake (181). These data suggest that at least some of the effects of NPY, and perhaps other orexigenic neuropeptides as well, may be due to their stimulation of appetitive ingestive behaviors that brings the animals in contact with the food triggering eating in a reflexive manner (181). The effects of intraoral feeding (and non-intraoral feeding) have been challenged because of adaptations occurring due to the repeated within-animal exposure to the sucrose solution and/or to the peptide (16). Nevertheless, based on these findings,
we hypothesized that neurochemical regulators of food intake also, or instead, may control appetitive responses such as food hoarding and the results of these studies are described below for NPY and AgRP.

**CNS changes with food deprivation**

Food deprivation alters a host of central and peripheral signals that include alterations in CNS neuropeptides involved with energy balance. The neuropeptide changes occurring with food deprivation that have garnered the most attention involve two sets of neurons in the ARC: 1) neurons that largely co-express the orexigenic neuropeptides NPY and AgRP and 2) neurons that largely co-express the anorexigenic neuropeptide POMC (the post translationally cleaved product is $\alpha$-MSH) and CART. In Siberian hamsters, as in laboratory rats and mice [e.g., (148)], food deprivation increases NPY and AgRP gene expression and inhibits POMC (139) and CART (98) gene expression. Changes in gene expression do not necessarily translate into neuropeptide protein synthesis or its release, but often they appear consistent with such changes [e.g., food deprivation-induced release of NPY in the PVH (85)]. Thus, these well-documented changes in gene expression support the notion that food deprivation ultimately increases the release of the orexigenic peptides NPY and AgRP and inhibits the release of the anorexigenic peptide $\alpha$-MSH at the terminal fields of these ARC neurons [e.g., (PVH) and the perifornical area (PFA) of the hypothalamus]. Therefore, we tested the effects of centrally administered NPY, AgRP and some of their receptor agonists/antagonists to help determine the mechanisms underlying food deprivation-induced food hoarding.

**NPY and its receptor subtypes:**

We tested central administration of NPY on food hoarding because: a) it is the most potent exogenous orexigenic factor discovered (32; 154), b) ARC NPY gene expression is
markedly increased with food deprivation (see above) and c) central injection of NPY mimics the pattern of food intake after food deprivation-refeeding [early onset, dose/duration relation with amount of consumed food, delay to next meal (109)]. Using our foraging/hoarding system, 3rd ventricular NPY injections greatly increase food hoarding (~300 to 1100%) dwarfing the smaller, but still marked increases in food intake [~200 to 300%; (45)].

Of the five NPY receptor subtypes (Y1-Y5), the Y1 and Y5 receptor subtypes appear to be paramount in the control of ingestive behavior (50). Therefore, we tested the role of these NPY receptor subtypes in food hoarding by Siberian hamsters. Injection of the NPY Y1 receptor agonist [Pro34]NPY into the 3rd ventricle increases food hoarding more than food intake, whereas injection of the NPY Y5 receptor agonist [D-Trp34]NPY increases food intake more than food hoarding (45). In neither case, however, were the increases as large as those elicited by equimolar doses of NPY itself (45). Thus, as we noted previously (95), Smith insightfully stated that an essential datum necessary to make a compelling case for the physiological functions of a peptide or its receptor is whether a receptor antagonist produces the opposite effects of exogenous administration of the peptide and/or blocks a physiological response thought to involve the peptide (152). Therefore, further tests for the natural role of these receptors in ingestive behaviors were undertaken using available NPY-R subtype antagonists.

NPY Y1-R antagonist (1229U91) injection into the 3rd ventricular completely blocks fasting- and ghrelin-induced increases in foraging and food intake, but they do not always completely block fasting- and ghrelin-induced increases in food hoarding. Together, these data suggest that the NPY Y1 receptor is important in the modulation of ghrelin- and fasting-induced increases in food foraging and intake, but other NPY receptors and/or other neurochemical
systems appear to be involved in the increases in food hoarding triggered by these two stimuli (93).

Collectively, the above data suggest hypothalamic and/or brainstem NPY-ergic mechanisms are involved in the control of food hoarding in Siberian hamsters, but do not help define the specific sites of action for NPY in this behavior. The brain sites of action for the orexigenic effects of NPY in laboratory rats has been determined by making small volume, parenchymal injections of NPY or agonists of the various NPY receptor subtypes. Several sites of NPY action on food intake include the PVH, VMH, LH (155) and PFA (157). Microinjections of NPY into the PVH or PFA trigger the largest increases in food intake in laboratory rats (155; 157). Both the PVH and PFA possess abundant NPY Y receptors (135) and contain numerous NPY-immunoreactive fibers (47) adding neuroanatomical support for the increases in food intake triggered by parenchymal NPY injections into these sites. More specifically, microinjection of a NPY Y1-R agonist ([Pro34]NPY) into the PVH or PFA triggers dose-dependent increases in food intake in laboratory rats (156) whereas, prior or co- PVH injection of a NPY Y1-R antagonist BIBO 3304 (176) or 1229U91 (184) blocks increases in food intake elicited by PVH injections of NPY. Therefore, we tested whether food deprivation-induced increases in food hoarding could be blocked by NPY Y1-R antagonist (BIBO 3304) microinjection into the PVH or the PFA (40). BIBO3304 injected into the PFA, but not the PVH, nearly completely blocked food deprivation-induced increases in food hoarding (40) further supporting the role of NPY in food deprivation-induced food hoarding, and more specifically, of the PFA and NPY Y1-Rs located there (40). To further test the necessity of NPY in controlling food hoarding, we attempted to destroy the ARC because it supplies some of the NPY projections to the PVH and PFA [e.g., (30)]. This was accomplished by using two
techniques in an attempt to overcome neuroanatomical/neurochemical specificity problems associated with each method thereby providing converging evidence for the role of the ARC and NPY producing neurons in food hoarding. In the first experiment, we either microinjected NPY conjugated to saporin (NPY-SAP) bilaterally into the ARC to kill NPY receptor-bearing neurons or unconjugated saporin (26), however, the NPY-R containing neurons almost invariably also produce AgRP which also appears important in food hoarding control (see directly below). Thus, in the second experiment, we gave neonatal monosodium glutamate (MSG) treatment, which indiscriminately kills most ARC neurons [e.g., (80)], and tested the animals in adulthood. For both methods, we achieved significant ARC cellular destruction (indicated by a significant decrease in cresyl violet cell staining) as well as NPY fiber and NPY Y1 receptor immunoreactivity (ir). Baseline food hoarding was not affected by either treatment, however, food deprivation-induced increases in hoarding were exaggerated ~100 %, rather than being blocked (41). There were significant remaining NPY-immunoreactive fibers in the PVH and PFA, likely emanating from brainstem projections (144), as well as a significant upregulation of Y1 receptors (NPY Y1-Rs) in both areas (41). These findings suggest a ‘denervation supersensitivity’ of the NPY Y1-Rs with the brainstem (144) or non-destroyed ARC NPY-producing neurons as the source of the remaining endogenous NPY likely released by food deprivation in hamsters bearing these lesions. Indeed, up-regulation of NPY Y1-Rs occurs when laboratory rats are treated as neonates with monosodium glutamate and tested as adults and, moreover, analogous to the results of our study (41), 3rd ventricular NPY injections trigger exaggerated increases in food intake in these animals compared with animals possessing an intact ARC (161). Together, the results of the above studies expand the role of NPY in ingestive
behavior to include not only consummatory ingestive behavior (feeding), but also the appetitive ingestive behavior of food hoarding (40; 95).

**Melanocortins:**

α-MSH and AgRP are the natural agonist and *inverse* agonist (antagonist-like effects but with the inhibition of constitutive activity), respectively, for the MC3/4-Rs. As noted above, AgRP and NPY are co-expressed by ARC neurons in laboratory rats (74) and Siberian hamsters (118). Thus, it is not surprising that food deprivation also increases ARC AgRP gene expression in laboratory rats (74) and in Siberian hamsters (118). Single 3rd ventricular or PVH AgRP injection stimulates food intake for several days in laboratory rats [*e.g.*, (73)]. Single 3rd ventricular injection of AgRP in Siberian hamsters markedly increase foraging (~75-400%), food intake (~100-150%) and most notably, food hoarding [200-1200%; (44)], suggesting a greater importance of the melanocortins in food hoarding than intake in this species. We therefore tested whether injection of melanotan II (MTII), a synthetic version of α-MSH that competes with endogenous AgRP release to bind to the melanocortin 3- and 4-receptors (MC3/MC4-Rs), would inhibit food deprivation-refeeding increases in food hoarding. Third ventricular injection of MTII attenuates, but does not completely abolish, food hoarding stimulated by food deprivation (92). Similarly, given that: a) food deprivation increases circulating active ghrelin in Siberian hamsters (91), b) exogenous peripheral ghrelin injection stimulates food hoarding (91) and c) ghrelin stimulates ARC producing AgRP neurons [*e.g.*, (86)], MTII was injected into the 3rd ventricle in an attempt to block ghrelin-induced increases in food hoarding. Central MTII injection attenuates, but does not block, ghrelin-induced increases in food hoarding, similar to its effects on food deprivation-induced hoarding (92), but it does prevent ghrelin-induced increases in food intake (92). Collectively, given that the NPY Y1-R subtype antagonism did not
completely block food deprivation- nor ghrelin-induced increases in food hoarding (93) and that melanocortin receptor agonism did not completely block food deprivation- nor ghrelin-induced increases in food hoarding (92), it may be that a cocktail of central neurochemicals (e.g., increases in AgRP, NPY, decreases in α-MSH) and/or peripherally acting circulating factors (e.g., decreases in leptin and CCK, increases in ghrelin) work together to promote food deprivation-induced increases in food hoarding.

**Opioids:**

Opioids have a well-known and well-studied role in the central control of food intake with widespread species generality [for review see: (69)]. We are aware of only two published studies where the effects of opioids on food hoarding have been tested (89; 90). Morphine, a mu opiate receptor subtype agonist, increases food hoarding, but not food intake, in deer mice (*Peromyscus maniculatus*), whereas a kappa receptor agonist (U-50488) only increases food intake (89; 90). If the mixed mu and kappa receptor agonist (ketocyclazocine) or a combination of mu and kappa agonists (morphine and U-50488) is given, both behaviors increase (89; 90). Because of the clear separation of appetitive from consummatory ingestive behaviors by stimulation of these two opiate receptor subtypes in deer mice, we tested them in Siberian hamsters in a preliminary study. Food intake and hoarding are unaffected by U-50,488, the kappa receptor agonist, and morphine, the mu receptor agonist, increases food intake one hour post injection, but stimulates food hoarding 1-24 hours post injection. These effects, however, are trivially small [~10%; (9)] relative to the increases described above for NPY or AgRP (as much as ~1200%; see above). The opiate receptor blocker, naltrexone, did not affect food hoarding suggesting there is no tonic opiate stimulation of hoarding in Siberian hamsters (9). Together, although the effects of opiates on food hoarding has been little studied, the consistent,
albeit relatively small, increase in hoarding by mu receptor agonism suggests further study may be warranted.

**CLINICAL RELEVANCE**

Food hoarding is prevalent across animal taxa including humans [for review see: (172)] and food hoarding is increased after food deprivation with obese people hoarding more calorically dense and high fat foods than their lean counterparts (15; 49; 117; 138). Clinically-associated excessive hoarding also occurs with PWS individuals (58; 79) as discussed above. At present there are no effective treatments for PWS, but determining the neuroendocrine factors responsible for excessive food-driven behavior could lead to the discovery of potential drug targets that also may pertain to increases in food hoarding by non-PWS obese individuals.

Several endocrine abnormalities are present in PWS. Ghrelin is extremely high in PWS [1542 + 271 pg/ml; (36)] and this is not due to their obesity _per se_ because non-PWS obese humans have ~ 1/5th the circulating ghrelin concentrations [344 + 45 pg/ml; (36; 149)]. In normal weight people, circulating ghrelin concentrations increase preprandially and decrease postprandially (149). By contrast, in PWS individuals, the postprandial decrease in ghrelin is blunted, perhaps allowing the drive for food to be reinstated much quicker than in non-PWS lean patients (65).

Because ghrelin is elevated in PWS and because ghrelin stimulates food intake, it seems logical that treatments that decrease circulating ghrelin should reduce food-driven behaviors in PWS patients. Somatostatin is one such treatment, as it reduces circulating ghrelin in normal weight subjects (24). Both somatostatin and octreotide (a somatostatin analog) normalize circulating ghrelin concentrations in PWS patients (165), but this is not accompanied by reductions in their exaggerated ingestive behaviors or body weight (165). Post mortem brain examination of PWS
patients finds ghrelin receptor (GHSR1a and GHSR1b) mRNA expression similar to that of normal weight controls (65), as well as normal gene expression and staining for the orexigenic peptides NPY and AgRP (68) and hypocretin (orexin) (63). Thus, it appears that the excesses in ingestive behaviors by PWS individuals are not blatantly associated with alterations in the chemical neuroanatomy of orexigenic peptides; however, this genetic disorder could be associated with dysfunctional anorexigenic signals. That is, PWS individuals may have impaired satiety mechanisms. CCK, the demonstrated satiety factor (see above in Peripheral Mechanisms), is similar in PWS compared with normal weight subjects (67) suggesting a deficit in CCK production does not exist (although its effectiveness may be different, but to our knowledge has not been tested). Peptide YY (PYY), another anorexigenic intestinal peptide normally released postprandially, decreases hunger and food intake in normal weight and non-PWS obese people (65). By contrast, PWS patients have normal fasting levels of PYY, but a blunted PYY response to food intake, perhaps providing a potential mechanism for reduced satiety with PWS (65). Another possible abnormal satiety factor in PWS is oxytocin, an often overlooked satiety peptide [for review see: (133)]. In post mortem brains of PWS individuals, the PVH contains significantly lower total cell number as well as number and volume of PVH oxytocinergic neurons (162). Thus, although some possible orexigenic factors seem relatively normal in PWS patients, except the astronomical increases in fasting circulating ghrelin concentrations [~1500 vs 300 pg/ml(36)], potentially, circulating PYY and hypothalamic oxytocin neuron shortfalls could contribute to the apparent satiety defects in PWS individuals that might promote their increases in food-associated behaviors including their food hoarding.

CONCLUSIONS AND FUTURE DIRECTIONS
Unfortunately, it is difficult to precisely characterize the central/peripheral factors that underlie the control of food hoarding at this time. The reality is that the study of this pervasive behavior that includes humans (172) is in its infancy. Fortunately, progress has been made by applying several strategies including: a) studying species that have food hoarding as a significant component of their ingestive behavioral repertoire [e.g., animals with cheek pouches (or bags, cars etc. for humans)] that permit substantial amounts of foraged food to be brought home for storage, b) studying species that do not necessarily exhibit appetitive and consummatory behaviors in concurrently (hamster species and humans), and c) using the wealth of data on peripheral and central mechanisms in the control of food intake for laboratory rats and mice and testing these substances for their role in food hoarding in natural food hoarding species (e.g., hamster species). Although one could spend several careers repeating the hormonal and neurochemical feeding experiments performed previously in laboratory rats and mice in natural food hoarding species for their effects on food hoarding, this does not seem to be the most prudent use of time and finances; therefore, targeted approaches seem necessary. Thus, we and others (25; 146) have chosen to study the mechanisms underlying food deprivation-induced increases in food hoarding because it is a robust stimulus for increasing this behavior. The studies conducted to date and needed for the future include identification of peripheral signals that impart information to the CNS of the existing energy deficit, the subsequent stimulation of central circuits that ultimately trigger food foraging and especially hoarding and the underlying mechanisms that terminate these appetitive behaviors. Other conditions producing substantial increases in food hoarding such as pregnancy and/or lactation, or cold exposure remain to be studied for their underlying hormonal/neural mechanism using these approaches.
We have made an arbitrary dichotomy of the mechanisms underlying the appetitive ingestive behavior of food hoarding – peripheral factors versus central factors and, as noted, we recognize the important interactions between the brain and periphery. As these studies progress and become more integrative, they will require tests of interactions of peripheral and central factors because individual substances do not appear to account for this complicated behavioral sequence of foraging, hoarding and eating/feeding. Because a complex ‘distributed system’ of multiple redundant sites of action have been identified for other regulatory behaviors/responses such as food intake (71) and thermogenesis (125), it would not be surprising if such a system underlies food hoarding. Therefore, there is plenty of work to be done to understand this pervasive behavior (172).

Finally, as we noted previously (9; 95), current approaches to the obesity crisis have focused on mechanisms controlling consummatory ingestive behavior (eating) and have been largely unsuccessful. Therefore, understanding the mechanisms controlling human foraging and food storing (hoarding) could provide additional targets for pharmaceutical or behavioral manipulations in the treatment or prevention of obesity.
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