The Musk Shrew (*Suncus murinus*): A Model Species for Studies of Nutritional Regulation of Reproduction

Jennifer L. Temple

Abstract

Reproduction is the most energetically costly process that most female mammals ever undergo. When nutritional resources are scarce, and there is a high probability that females will be unable to cope with this energetic challenge, reproductive processes are inhibited. This process is highly conserved and likely adaptive and reversible when nutritional resources become available. Although the nutritional regulation of reproduction has been described in a number of species, the mechanism and neural sites of action by which this regulation occurs remain elusive. The musk shrew has proven to be a useful model to elucidate the peripheral cues and neuronal mechanisms that underlie nutritional infertility. Current knowledge of the nutritional regulation of reproduction is reviewed, with a focus on mammals. The advantages and disadvantages of using the musk shrew as an animal model for these types of studies are described.

Key Words: calories; fatty acids; glucose; GnRH; LHRH; mating behavior

Introduction

The process of reproductive inhibition in response to insufficient energetic resources is widespread across vertebrate taxa, suggesting that it is a highly adaptive physiological process (Bronson 1989, 2000). In mammals, all of the following functions are inhibited when animals are in a state of negative energy balance, the point at which calorie expenditure exceeds caloric intake: ovulatory cycles (Morin 1986; Schneider et al. 1993, 1997a,b), mating behavior (Dickerman et al.1993; Jones and Lubbers 2001; Temple and Rissman 2000b), steroidogenesis (Schneider and Wade 1990), and gonadotropin secretion (Bronson and Heideman 1990; Helmreich and Cameron 1992). This balance is commonly achieved when nutritional resources are scarce, but it also occurs when excessive energy is expended, as is seen in some athletes (Sanborn et al. 1982). Energetic resources are then allocated toward meeting immediate survival needs, such as maintaining body temperature, metabolism, and foraging (Bronson 2000). Once a positive energy balance is restored, reproductive processes are reinstated. This reinstatement ensures that reproduction and/or lactation coincide with resource abundance, which is favorable to the survival of offspring.

A female must determine when energetic resources are sufficient, not only to support basic life functions but also to reproduce effectively. We know that various environmental cues can be used as predictors. For example, the female must assess nutrient abundance. For some animals such as carnivores, nutrients vary little over the year. Thus in these species, variations in the rate of pregnancy throughout the year are not associated with changes in the nutritional value of prey (Bronson 1989). For other species such as herbivores and insectivores, there are seasonal variations in the availability of nutrients from grasses or insect populations (Bronson 1989). Thus reproductive efficacy is tightly correlated with the nutritional value of their diet.

In addition to energy intake, females must also account for energy expenditure, such as foraging effort and thermoregulation. When nutritional resources become scarce, small mammals such as mice may forage as far as 15 miles in one night in search of food (Bronson 1989), which undoubtedly results in a profound expenditure of energy. In some cases, such as when ambient temperatures are low, it may be more energetically conservative for animals to stay in the nest rather than venture out for food. Somehow, females are able to assess these demands and allocate their energy expenditure accordingly. The process requires an "energy detector," a mechanism to integrate various external cues, and a way to regulate the appropriate behavioral output. The neural substrate that underlies this ability is unknown, but reproduction is believed to be one of the first processes to be inhibited when a female faces an energetic challenge (Bronson 1989).

The term *reproduction* encompasses a myriad of processes. The final common pathway of neuroendocrine inputs in the brain is a group of cells called gonadotropin-releasing hormone (GnRH-I[1]) neurons. These cells are not localized to discrete nuclei but, rather, are distributed in a loose continuum beginning in the accessory olfactory bulb

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[1]Abbreviations used in this article: 2-DG, 2-deoxy-D-glucose; FSH, follicle-stimulating hormone; GnRH-I, gonadotropin-releasing hormone–I; GnRH-II, gonadotropin-releasing hormone–II; HPG, hypothalamic-pituitary-gonadal; LH, luteinizing hormone; MA, mercaptoacetate.

and continuing caudally to the hypothalamus. The majority of GnRH-I neurons send their processes to a region in the ventral forebrain called the median eminence. There the cells secrete GnRH-I in a pulsatile manner into the hypothalamic-pituitary-portal blood supply where it binds to GnRH-I receptors in the anterior pituitary, triggering the release of follicle-stimulating hormone (FSH[1]) and luteinizing hormone (LH[1]). These gonadotropins then travel via the general circulation where they act at the gonads to stimulate steroidogenesis and ovulation. Steroid hormones then feed back on the GnRH-I neurons either to increase or to decrease release of GnRH-I. These processes taken together are termed the *hypothalamic-pituitary-gonadal* (HPG[1]) *axis* (Brown 1994).

The HPG axis is a major target for reproductive inhibition after food restriction, although there are many other aspects of reproduction that are also inhibited when energetic resources are scarce. Mating behavior, pregnancy, and lactation all require tremendous energy expenditure by female mammals. Not surprisingly, these processes rely on sufficient food intake to function properly. For example, if a female becomes pregnant but experiences a shift in energy balance, the litter may be reabsorbed to conserve energy (Zamiri 1978). If a female does not have sufficient energy to lactate, the litter may be lost (Schneider and Wade 1989; Zamiri 1978). For reproduction to be successful, the offspring must survive to reproduce themselves. Therefore even if a female has enough energy to ovulate or mate, these resources must be sustained throughout lactation to maximize offspring survival.

Much of the research on nutritional regulation of reproduction has been conducted in rodents. In Syrian hamsters, food deprivation for 48 hr inhibits estrous cycles (Morin 1986; Schneider et al. 1997a,b), steroidogenesis (Schneider and Wade 1990), and mating behavior (Dickerman et al. 1993; Jones and Lubbers 2001). These effects can be overridden if hamsters have significant fat stores before the onset of caloric restriction (Schneider et al. 1997a). In addition, these effects are mimicked by treatment with drugs that inhibit metabolic fuel oxidation in hamsters, mice, and rats (Briski and Sylvester 1998; Bronson 1988; Schneider et al. 1997a,b). These effects suggest that metabolic fuel availability is a cue that is used to assess energetic status. Thus there must be a mechanism that enables the brain to detect the current state of energy balance and either inhibit or permit reproduction. This process potentially involves neuropeptides acting within the hindbrain and the hypothalamus to integrate nutritional cues with reproductive output. This important area of research is beyond the scope of this review, but the literature is replete with recently published information on this topic (Gundlach 2002; Kalra and Kalra 1996; Magni et al. 2000; Schneider et al. 2002).

Several laboratories have used sheep as animal models for investigating this line of research. Among the many advantages of using a large animal model, perhaps the most useful has been the ability to measure pulsatile release of GnRH-I directly. The fact that growth-restricted sheep have a different distribution of GnRH-I neurons than lambs fed ad libitum (I'Anson et al. 1997) suggests that the GnRH-I system is altered in these animals. In addition, food-restricted sheep have a decreased GnRH-I pulse frequency compared with controls fed ad libitum (I'Anson et al. 2000; Prasad et al. 1993). These later studies have contributed the most direct evidence that food restriction inhibits the release of GnRH-I and the activity of GnRH-I neurons.

Although traditional animal models have yielded much useful information about nutritional infertility, there are limitations. Importantly, all of the species mentioned above have hormonal and behavioral estrous cycles (Crews 1984; Xiong et al. 1997). Nutritional manipulation disrupts these cycles, perhaps by altering the GnRH system. The observed effects on reproduction could potentially be secondary to reduced levels of circulating steroid hormones (Schneider and Wade 2000).

To determine whether food restriction directly affects aspects of reproduction aside from steroid hormone levels and/or hormonal cycles, much rodent work is conducted in ovariectomized, steroid hormone-treated females (Dickerman et al. 1993). The use of these treated animals creates two problems: (1) Because the effects of food restriction vary with hormonal state (Morin 1986), the chronic, sometimes supraphysiological, levels of circulating steroid hormones will likely have significant consequences on the severity of the effects of food deprivation. As a result, it is difficult to assess the ecological relevance of the findings of these studies. (2) Because behavioral recovery after refeeding in rodents requires a minimum of 12 hr (Dickerman et al. 1993; Jones and Lubbers 2001), examining primary cues from food intake that reinstate sexual behavior after food restriction is challenging because by the time behavior is restored, secondary and tertiary cues have also been allowed to act. For several years, we have used musk shrews as an alternative model species for the study of nutritional regulation of reproduction. These animals have unique neuroendocrine characteristics that allow us to circumvent some of the issues encountered when using more traditional animal models.

Natural History and Husbandry

Ecology

The musk shrew (*Suncus murinus*) (Figure 1) is an ecologically relevant animal model for studying not only nutritional infertility, but also environmental regulation of reproduction in general. They are indigenous to Southeast Asia and live within 5° latitude of the equator (Harrison 1955; Simpson 1945). They are members of the order *Insectivora,* which is the third largest mammalian order, containing more than 450 species. Musk shrews have a very high metabolism, typical of insectivores, and they store very little fat (Ishii et al. 2002; Temple et al. 2002). In the wild, they breed at all times of the year, and their pregnancy rates vary

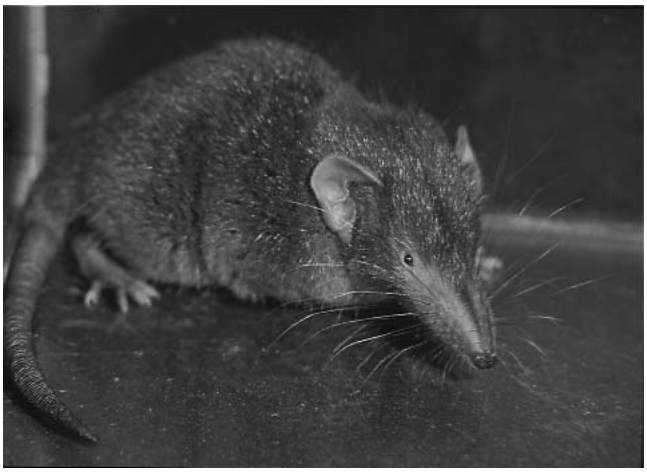

Figure 1 An adult musk shrew (*Suncus murinus).*

with rainfall patterns (Barbehenn 1962; Harrison 1955; Louch et al. 1966). This variation is likely because rainfall and insect populations are tightly correlated, and insects are the musk shrews' primary diet (Advani and Rana 1981).

We know little, if anything, about the social behavior of musk shrews in the wild. One study estimated the population of musk shrews in Guam to be 6.2 shrews per acre (Baker 1946). This finding suggests that shrews likely live in isolation, with occasional contact for the purpose of breeding. We can also speculate that musk shrews do not live in social groups based on laboratory observations that these animals cannot be housed communally.

Bringing Musk Shrews into the Laboratory

In the 1960s, a group of musk shrews was trapped in Guam and brought to the United States by Dr. Gil Dryden. He established a colony and accomplished much of the initial characterization of their physiological and reproductive characteristics. In the 1980s, this colony was transferred to Dr. Emilie Rissman, then at the University of Texas in Austin. The colony was soon brought to the University of Virginia, where it has been maintained since 1987. In general, 40 to 50 animals of each sex are kept for breeding purposes, and their offspring are used for conducting experiments.

Musk shrews are kept on a 14:10 light:dark cycle with an ambient temperature of 71°F. They appear to be crepuscular (most active at dawn and dusk) in their circadian activity levels, thus all behavioral testing is performed within 3 hr of "lights on" or 3 hr before "lights off" (Rissman, unpublished observations). They are housed in hanging Plexiglas cages that contain pine shavings and shredded paper towels for bedding. They are fed ad libitum a mixture of three parts of Purina™ Cat Chow and one part of Mink Chow (Milk Specialities, Wisconsin "Mink Complete Pellets-Grow-Fur" [Growing & Furring Formulation, which has crude protein (not less than 34.0%), crude fat (not less than 20.0%), and crude fiber (not less than 4.0%)]), and the food is provided in small plastic dishes within the cage. Slightly acidic distilled water (pH 5.5) is also provided ad libitum to reduce contamination in the water supply. The cages and water bottles are changed once a week.

Male, breeding female, and sexually naive female musk shrews are housed in separate rooms with airflow from female to male rooms. Although both sexes have a "musky odor," the male odor is much stronger than the female's. Previous studies and unpublished observations have led to the conclusion that male odor may influence female reproduction (Dellovade and Rissman 1994; Rissman 1989; Rissman unpublished observations). Thus in an effort to keep the population of experimental subjects as "sexually naive" as possible, contact with male scent is minimized by separating the food, brooms, dustpans, and gloves for males and females.

To breed musk shrews has not been particularly challenging, perhaps because they become pregnant virtually every time they are paired with a male. Breeder males and females are paired in the male's home cage for no more than 2 hr if the female has a litter of pups, or overnight if she does not. Females appear most likely to become pregnant when they are bred 4 to 5 days after giving birth. Their gestation period is a total of 30 days composed of a 10-day delayed implantation followed by 20 days of gestation once implantation occurs. The average litter size is 2.5 pups per litter (range, 1-6). The pups are housed with their mother until they are 21 days of age. From that time, animals are always housed singly. Male and female musk shrews exhibit high levels of same-sex aggression, thus group housing is not a good option.

Unique Neuroendocrine Characteristics

Musk shrews have the following characteristics that make them suitable for studying reproduction: (1) Females are reflex ovulators, therefore they mate and ovulate virtually every time they are paired with a male (Dryden 1969). (2) Female musk shrews, unlike most other reflex-ovulating species, do not come into estrus before mating. Instead, plasma estradiol levels are undetectable until 10 to 15 hr after mating, when they begin to increase (Fortune et al. 1992; Rissman and Crews 1988). (3) Testosterone, which is converted to estradiol in the brain, is the primary steroid hormone produced by the musk shrew ovary. The plasma levels of testosterone are between 300 and 400 pg/mL at all times before mating and do not increase after mating (Fortune et al. 1992; Temple and Rissman 2000b). Thus musk shrews do not rely on cyclic surges of steroid hormones for the display of mating behavior (Veney and Rissman 1998, 2000). For this reason, most experiments can be performed in gonadally intact females without concern for maintaining high levels of circulating steroid hormones. This aspect of musk shrew neuroendocrinology is similar to humans in which testosterone is known to regulate libido (Davis and Tran 2001). (4) The fact that mating in the musk shrew

induces puberty (Rissman 1992) allows researchers to have complete control over pubertal development simply by regulating whether or not the female is allowed to mate.

Behavioral Regulation of Puberty and GnRH-I

Much of the initial work in musk shrews focused on pubertal development. For example, one of the first studies showed that female musk shrews rarely become pregnant after their first mating bout (Clendenon and Rissman 1990; Rissman 1992). Instead, musk shrews require a second mating separated by at least 24 hr to induce ovulation (Rissman 1992). Once the female gives birth to her first litter, subsequent matings result reliably in pregnancy. This observation led to the conclusion that the first mating bout primes the neuroendocrine system to ovulate in response to subsequent matings. In addition, because the second mating must be separated by at least 24 hr, it was hypothesized that the alterations in the neuroendocrine system were long-term changes involving protein production and perhaps the establishment of novel neural networks. Based on this knowledge, changes in the GnRH-I system as a function of mating became the focus of future investigation.

Mating has a significant effect on GnRH-I production in the female musk shrew. Between 24 and 48 hr after the initial mating bout, females had a significant increase in GnRH-I content (assayed by high-performance liquid chromatography) in the forebrain compared with unmated controls and females that had received mating stimulation less than 24 hr before sacrifice (Dellovade et al. 1995b). In addition, when females were given a second mating bout, the levels of GnRH-I decreased dramatically compared with females receiving only a single mating, in which GnRH-I content remained elevated. This decrease in GnRH-I content after a second mating bout suggests that GnRH-I is being released and will lead to ovulation.

The data described above have been strengthened by immunocytochemical studies, which allow for anatomical resolution, showing that the number of GnRH-I immunoreactive cells increases in the specific regions of the forebrain after mating as well as after brief, nonmating interactions with males (Dellovade and Rissman 1994; Dellovade et al. 1995a,b). Together, these data suggest that the first time a female mates with a male, GnRH-I production is increased. This increase "primes" the system for subsequent mating interactions in which GnRH-I is then released and ovulation is induced. This priming is likely necessary only the first time a female mates because ovulation is induced in sexually experienced musk shrews by a single mating bout (Rissman 1992).

A Second Form of Gonadotropin-releasing Hormone: GnRH-II

Most species have at least two forms of GnRH in the brain, and some have three (Millar 2003). In species that have more than one form, the form found in the mesencephalon is always gonadotropin-releasing hormone II (GnRH-II[1]) (King and Millar 1992). GnRH-II is the most evolutionarily conserved form of GnRH, and it is found across all vertebrate taxa, including mammals. In 1993, Dellovade and colleagues reported the presence of a nonmammalian form of GnRH located in the mesencephalon in the musk shrew. Further testing revealed that it was, in fact, GnRH-II (Dellovade et al. 1993; Sealfon et al. 1997; then referred to as chicken GnRH-II because it was the second form isolated in chickens). Musk shrews were the first placental mammal in which GnRH-II was found, although it had been reported in marsupials (King et al. 1990). Since then, this form of GnRH has been identified in a number of mammalian species, including tree shrews (Kasten et al. 1996), rhesus and stumptail monkeys (Lescheid et al. 1997; Urbanski et al. 1999), humans (White et al. 1998), and capybaras (Montaner et al. 1999). In addition, GnRH-II has been identified in the midbrain of mouse using reverse transcription-polymerase chain reaction (RT-PCR) (Chen et al. 1998). Yet these data are controversial because the sequence for GnRH-II is not found in the mouse genome.

Because GnRH-I and GnRH-II differ by only three amino acids, it is believed that functional specificity of these peptides is conferred by the receptor (King and Millar 1992). There are at least two types of GnRH receptors, referred to as type-I and type-II (Millar et al. 2001). These receptors have different distributions within the brain and pituitary as well as different binding affinities for different forms of GnRH (Millar et al. 2001). For example, in the mammalian species that have been examined, the type-I GnRH receptor is localized primarily to the pituitary, where the type-II GnRH receptor is found in a number of brain regions, including those involved in sexual behavior (Millar et al. 2001). There is also a type-III GnRH receptor that has been isolated in fish and amphibians, but it has not been found in mammals (Millar 2003).

Until recently, no physiological function had been ascribed to GnRH-II. There has been some speculation that GnRH-II could be an FSH-releasing hormone. Although GnRH-II appears to release FSH preferentially in ovariectomized, estrogen-progesterone blocked rats, very large doses are required, and these experiments have not been replicated in other animals or in vitro (McCann et al. 2001; Yu et al. 1988). Thus although the function of GnRH-II is unknown, the high degree of conservation in the amino acid sequence across species suggests that it plays a role in some critical process.

Suitability of the Musk Shrew as a Model

Several features of the musk shrew that make it an excellent model for studying nutritional regulation of reproduction include the following: (1) They have a very high metabolic rate and therefore must eat virtually continuously to survive (Ishii et al. 2002). Based on this need, they are likely to be exquisitely sensitive to small reductions in food intake. (2) They have very little body fat (Temple et al. 2002) and thus

cannot rely on stored energy to carry them through periods of low food availability. (3) Mating behavior in musk shrews is not regulated by surges or peaks of steroid hormones (Fortman et al. 1992; Rissman and Bronson 1987). Thus it appears that the musk shrew constantly monitors food intake, and not steroid hormone levels, in an effort to regulate mating behavior.

Mild Food Restriction Inhibits Mating Behavior

The following studies tested the hypothesis that mating behavior would be inhibited by small reductions in food intake. Reducing food intake to 60% of ad libitum for 48 hr was sufficient to reduce the percentage of females that displayed mating behavior significantly (Gill and Rissman 1997). In addition, this effect was not altered by prior sexual experience of the animal, which suggests that the phenomenon is not specific to puberty (Gill and Rissman 1997). Colleagues then hypothesized that female musk shrews would show rapid behavioral recovery after this food restriction paradigm, given that it is not necessary for steroid hormone levels to increase before mating. Studies revealed that females fed 60% of their normal ad libitum intake for 48 hr were significantly less likely to display mating behavior, as has been shown previously. Nevertheless, females subjected to the same food restriction paradigm but allowed ad libitum access to food 90 min before the mating bout were just as likely to mate as ad libitum-fed animals (Temple and Rissman 2000b; Figure 2). These effects were observed in both gonad-intact females and gonadectomized, testosterone-treated females. Additionally, plasma testosterone levels were measured in females from all feeding conditions, and no differences were found. Thus it appears that food restriction does not decrease circulating testosterone levels (Temple and Rissman 2000b).

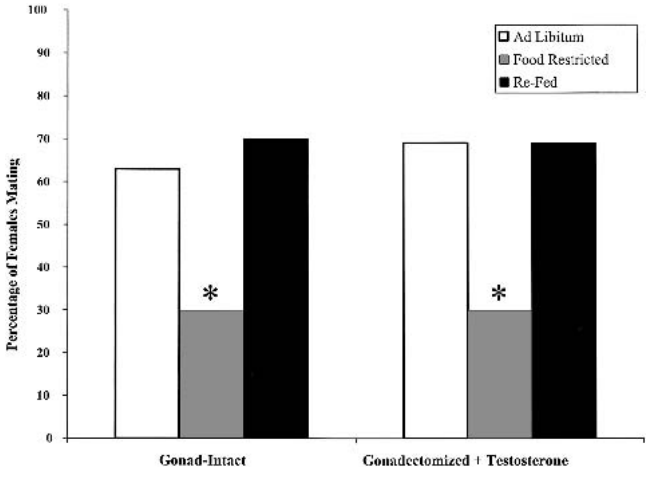

Figure 2 Percentage of females that mated after being fed ad libitum, food restricted to 60% of ad libitum for 48 hr, or food restricted and refed for 90 min before mating. Females in these studies were either gonad intact (left group of bars) or gonadectomized and treated with testosterone (right group of bars). * = significantly different ($p < 0.05$) from other feeding conditions.

The recovery rate in females subjected to a more stringent food restriction paradigm (animals fed 50% of ad libitum intake for 48 hr) was also examined. In these females, behavioral recovery was not observed until 12 hr after refeeding (Temple and Rissman 2000b). These results are important because the rapidity of behavioral recovery after mild food restriction allows us to analyze the primary cues from food intake that initiate mating behavior easily.

Mild Food Restriction Alters the GnRH-I System

The next set of experiments investigated the effects of food restriction and refeeding on the neuroendocrine system. Female musk shrews were subjected to the 60% food restriction/90-min refeeding paradigm for all of the following studies. To assess hypothalamic-pituitary-gonadal axis functioning, the following factors were measured: GnRH-I immunoreactive cell number, GnRH-I content in the preoptic area and median eminence, and ovulation in response to exogenous GnRH-I administration. Food-restricted females had significantly more GnRH-I immunoreactive cells in the preoptic area, and more GnRH-I in the median eminence. In addition, although most of them ovulated after GnRH-I administration, the food-restricted females had significantly fewer corpora lutea compared with ad libitum-fed females. Refeeding for 90 min reversed all of these deficits, such that refed females were similar to ad libitum-fed females for all measurements (Temple and Rissman 2000a; Figure 3). These striking data suggest that once nutrition is restored, multiple aspects of the GnRH-I system are able to recover from food restriction rapidly.

Oxidation of Metabolic Fuels Is Required for Refeeding to Restore Reproduction

Because mating behavior and HPG axis functioning are restored very rapidly in the musk shrew, experiments were designed to identify necessary cues from the food for these effects to take place. Musk shrews are an excellent model for this determination because mating behavior can be used as the reproductive measure, which is completely noninvasive.

In our first study,[2] we examined mating behavior in ad libitum-fed, food-restricted, and refed females given a single injection of either saline or 2-deoxy-D-glucose (2-DG[1]; an inhibitor of glycolysis) 2 hr before mating. 2-DG had no effect on ad libitum-fed or food-restricted females,

[2]In this article, subsequent discussions of the author's studies refer to work with colleagues in their laboratory at the University of Virginia Department of Biology.

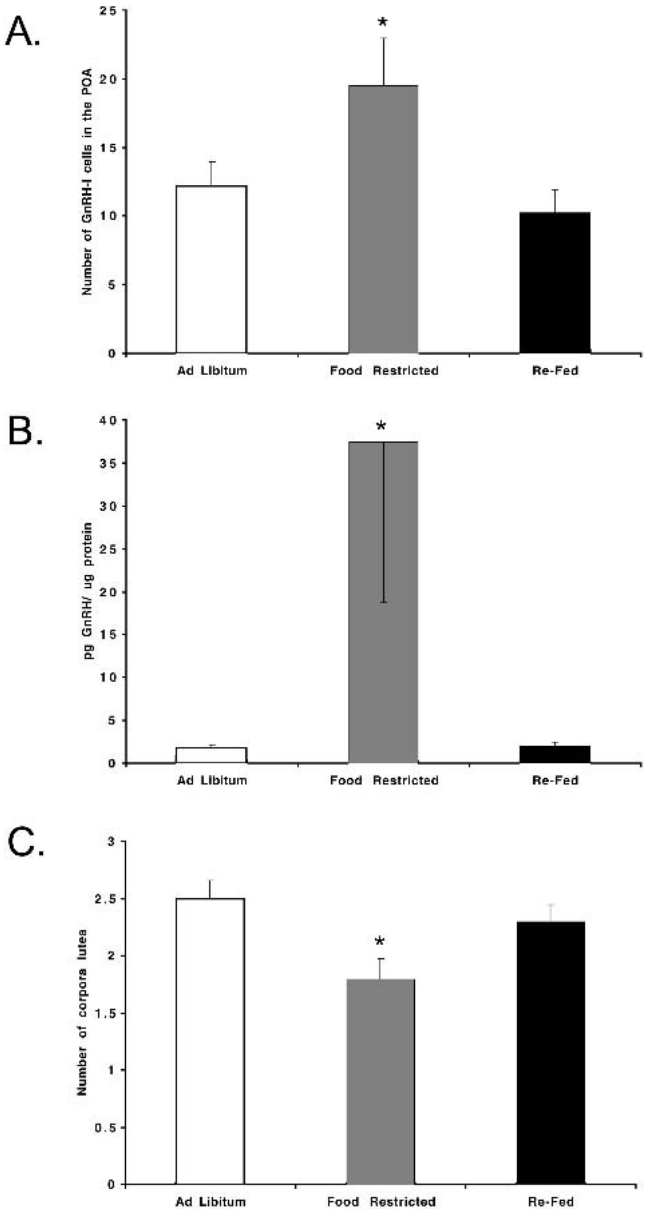

Figure 3 (A) Number of GnRH I-immunoreactive cells in the preoptic area (POA). (B) Amount of gonadotropin-releasing hormone I (GnRH-I) peptide per μg of protein in the median eminence. (C) Number of corpora lutea after an injection of GnRH-I in females that were either fed ad libitum, food restricted to 60% of ad libitum for 48 hr, or food restricted and refed 90 min before sacrifice. * = significantly different ($p < 0.05$) from other feeding conditions.

but the refed females treated with 2-DG before mating were significantly less likely to display mating behavior than ad libitum-fed females or refed females treated with saline (Temple et al. 2002; Figure 4A). In our next study, we used the same experimental design, except that we used mercaptoacetate (MA[1]), an inhibitor of fatty acid oxidation. These results are similar to the results with 2-DG in that the MA treatment prevented the refeeding-induced restoration of

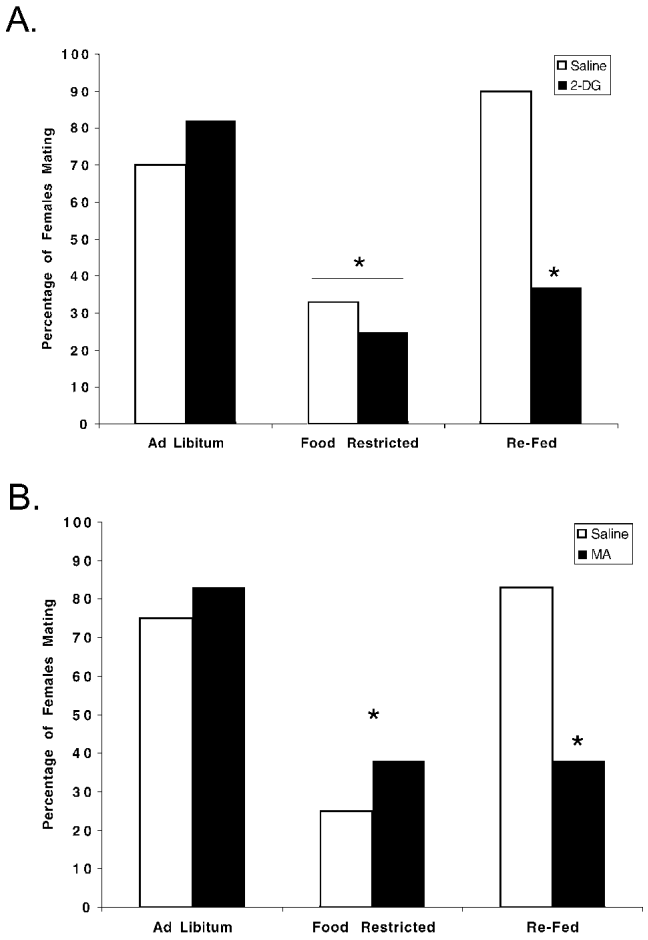

Figure 4 Percentage of females mating that were fed ad libitum, food restricted to 60% of ad libitum for 48 hr, or food restricted and refed 90 min before mating that were treated (A) with saline or 2-deoxy-glucose (2-DG), or (B) with saline or mercaptoactetate (MA). * = significantly different ($p < 0.05$) from other treatments.

mating behavior (Figure 4B). Treatment with the doses of metabolic inhibitors used (350 mg.kg of 2-DG and 20 mg/kg of MA) had no effect on locomotor behavior or aggressive behavior during mating bouts (Temple et al. 2002). In addition, neither glucose nor vegetable shortening could substitute for food and restore mating behavior. These combined data show that musk shrews rely on the simultaneous oxidation of both glucose and fatty acids to have enough energy to mate after food restriction. When either pathway is inhibited, refeeding is no longer able to restore mating behavior. These data are extremely exciting because future studies could lead to the identification of a single, downstream signal that is created by the sum of all types of metabolic fuel oxidation, such as ATP.

Administration of GnRH-II Restores Mating Behavior to Food-restricted Females

One of the advantages of using musk shrews is that they have a relatively well-characterized GnRH-II system

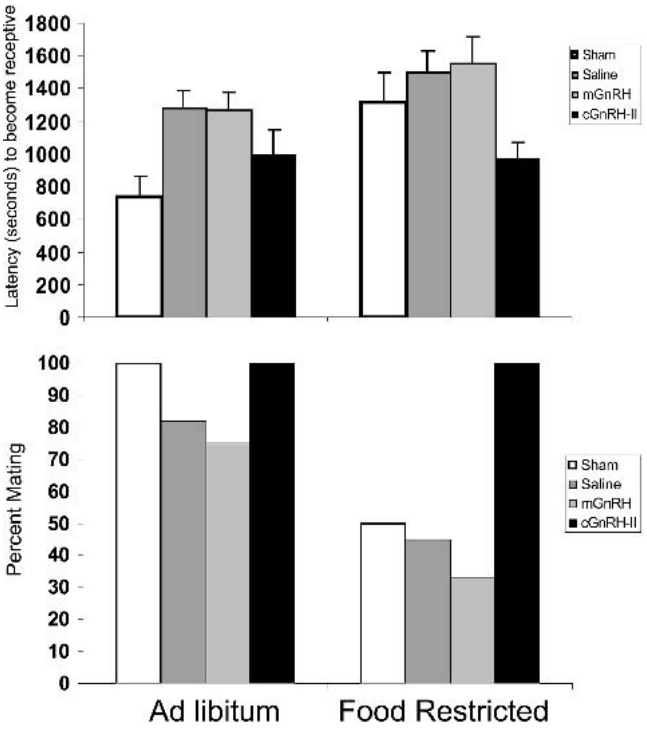

Figure 5 (Top) Latency in seconds for females who were either fed ad libitum or food restricted to 60% of ad libitum for 48 hr to display sexual receptivity after a sham surgery or intracerebroventricular injection of saline, gonadotropin-releasing hormone I (GnRH-I), or gonadotropin-releasing hormone II (GnRH-II). (Bottom) Percentage of ad libitum-fed and food-restricted females that displayed mating behavior after a sham surgery or intracerebroventricular injection of saline, GnRH-I, or GnRH-II. * = $p < 0.05$ compared with other drug treatments within each feeding condition.

(Dellovade et al 1993; King et al. 1994; Rissman et al. 1995). One of the goals of this research has been to determine the function of this highly conserved form of GnRH. We hypothesized that administration of either GnRH-I or GnRH-II would restore mating behavior to food-restricted females. GnRH-I has been shown to support mating behavior in gonadectomized female rats (Moss and McCann 1975; Pfaff 1973). In addition, there is some suggestion from bird studies that GnRH-II may be involved in the regulation of mating behavior (King and Millar 1997; Maney et al. 1997).

To test the hypothesis described above, we administered GnRH-I, GnRH-II, or saline intracerebroventricularly to either ad libitum-fed or food-restricted females 15 min before a mating behavior test. GnRH-II, but not GnRH-I, restored sexual receptivity to food-restricted females (Temple et al. 2003; Figure 5). In addition, food restriction increased the number of GnRH-II immunoreactive cells in the anterior portion of the midbrain and the GnRH-II fiber density in the median eminence (Temple et al. 2003; Figure 6). We also showed that musk shrews have the type-II GnRH receptor and that it is localized to areas of the brain associated with mating behavior, such as the preoptic area and the ventromedial nucleus of the hypothalamus (Temple et al. 2003). These data were the first in mammals showing that GnRH-II plays a role in the regulation of reproductive behavior.

Disadvantages of Using Musk Shrews

Although there are many advantages of using musk shrews as an animal model for behavioral neuroendocrinology research, some disadvantages do exist and include the following: (1) Maintaining a colony can be challenging because musk shrews have unique housing requirements. For example, it is necessary to house males and females in separate rooms and to house all animals individually after weaning. (2) It may be difficult to generalize data from musk shrews to other species that have these endocrine characteristics (e.g., rodents, nonhuman primates, and humans) because musk shrews do not have behavioral or hormonal estrous cycles. This issue is particularly problematic in studying nutritional effects on reproduction because in most species, hormonal cycles are highly affected by caloric manipulation. Often the cycle is one of the initial reproductive processes to be affected. (3) The energetic regulation of musk shrews is likely to be extremely different from species in which energetic resources can be stored because musk shrews store very little body fat.

Despite these disadvantages, musk shrews are an extremely useful animal model. Furthermore, collecting data

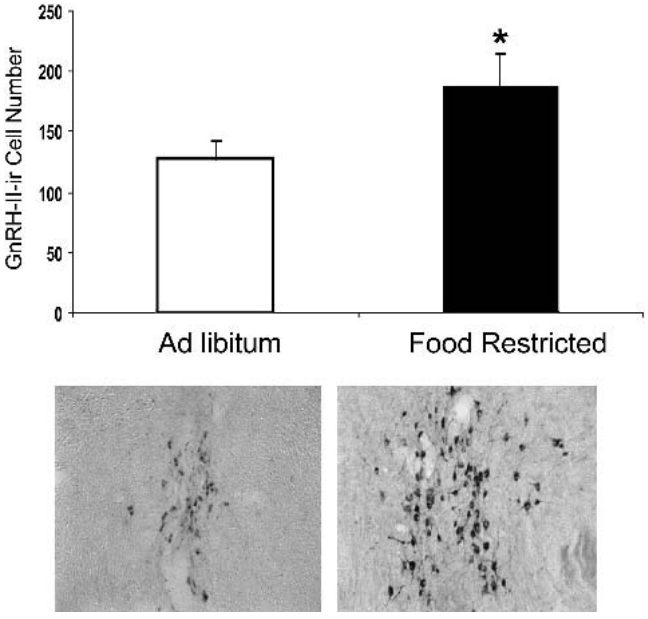

Figure 6 (Top) Mean (± SEM) number of gonadotropin-releasing hormone II immunoreactive (GnRH-II-ir) cells in the anterior portion of the midbrain in females either fed ad libitum or food restricted to 60% of ad libitum for 48 hr. * = $p < 0.05$. (Bottom) Representative photomicrographs of GnRH-II staining from (left) ad libitum-fed, and (right) food-restricted females.

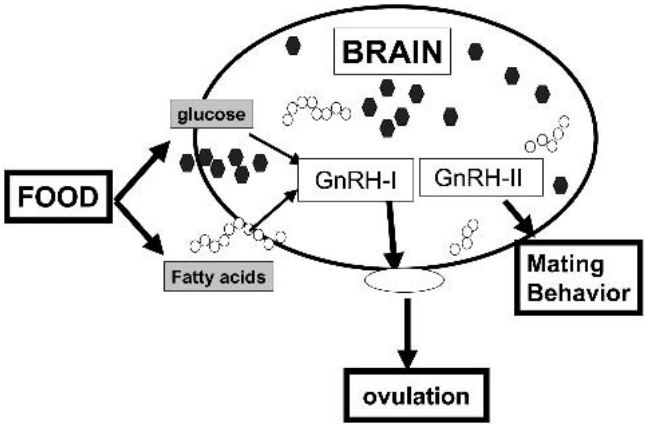

Figure 7 Model of how food breaks down and feeds back on neuronal systems, which then act on downstream behaviors. GnRH-I, gonadotropin-releasing hormone I; GnRH-II, gonadotropin-releasing hormone II.

from multiple species allows us to identify the most fundamental and most highly conserved mechanisms as well as to reconsider current models in light of novel mechanisms discovered in alternative species.

Future Musk Shrew Research

One major unanswered question relates to the location of the neural site of action for food or signals resulting from food intake (Figure 7). Studies are ongoing in the musk shrew and in other species to examine changes in neuropeptide and neurotransmitter systems after food intake. In addition, immediate early gene expression is also being used to examine sites of enhanced neuronal activity after food intake. Once we pinpoint specific brain areas, more precise experiments can be designed to determine how these signals are integrated at the cellular level.

One of the most exciting findings from the musk shrew is that GnRH-II administration restores mating behavior after food restriction (Temple et al. 2003). Colleagues and I have proposed that GnRH-II may serve as a neuropeptide that integrates information about nutritional status with the neuroendocrine system. We are currently exploring these findings further in an effort to determine where GnRH-II is acting and how these neurons are sensitive to nutritional status.

Summary

The musk shrew has proved to be an excellent model for the study of nutritional infertility. Small reductions in food intake affect every aspect of HPG axis functioning, including sexual behavior. Perhaps the most striking effect is that all of the reproductive deficits are reversed within 90 min of refeeding. In addition, because GnRH-II appears to be involved in this regulation, we have uncovered a novel and potentially important role for this highly conserved neuropeptide.

Some of the findings from musk shrews are similar to data that have been reported in other species. For example, in Syrian hamsters, food deprivation for 48 hr inhibits lordosis behavior even when exogenous steroid hormones are provided (Dickerman et al. 1993). Studies in sheep and nonhuman primates have also revealed that food restriction decreases GnRH and LH pulse frequency (Cameron and Nosbisch 1991; Helmrich and Cameron 1992; I'Anson et al. 2000). Interestingly, these deficits can be reversed after a single meal, along a time-course similar to our observations in musk shrews (Cameron 1996).

We believe that the musk shrew has unique neuroendocrine and behavioral characteristics that make it an excellent model to study reproduction and nutritional regulation of reproduction. Our findings complement the findings from other species while adding novel data on the involvement of GnRH-II in the regulation of reproductive behaviors by nutritional cues. Future studies both will increase our knowledge of the neuronal mechanisms that underlie suppression of reproduction during times when nutritional resources are suboptimal and will help uncover cues used by the brain to assess energetic status.

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