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Several lines of evidence have implicated the hypothalamic ventromedial nucleus (VMH) in the control of caloric homeostasis. For example, the activity of VMH neurons depends on energy availability. We tested the hypothesis that energy balance may involve the remodeling of the dendritic arbor of VMH neurons. We compared two groups of animals: one group had *ad libitum* access to food, and the other experienced 10-d restricted access to food. As expected, the food-deprived group lost body weight and had reduced levels of glucose, insulin, and leptin. VMH neurons were visualized after Golgi impregnation, and dendrite length was measured. Food deprivation had differential effects on VMH neurons. In particular, within the ventrolateral VMH, for neurons with long primary dendrites (LPDs) that extended in the lateral, but not medial, direction, the LPDs were 31% shorter. These same neurons exhibited a 32% reduction in the number of other dendrites without a change in soma size. In contrast, within the dorsomedial VMH, for neurons with medially, but not laterally, extended LPDs, the soma area was reduced by 28%. However, neurons in the dorsomedial VMH did not display a change in the length or number of dendrites, regardless of LPD direction. Thus, although structural changes during calorie depletion occur in both the dorsomedial and ventrolateral VMH, only the latter exhibits a remodeled dendritic arbor. These results also suggest that the direction of the LPD may be an important marker of neuronal function in the VMH. (*Endocrinology* 149: 93–99, 2008)

MANY STUDIES HAVE implicated the hypothalamic ventromedial nucleus (VMH) in the control of caloric homeostasis. This suggestion was initially based on the effects of VMH lesions on body weight, food intake, and insulin secretion (1, 2). Obesity and hyperphagia also were noted in a woman with a discrete neoplasm that bilaterally destroyed the VMH (3), and after unilateral surgical resection of the VMH (4). These lesion studies lead to the view that the VMH inhibits food intake.

More recent studies on the molecular biology, neurochemistry, and neurophysiology of VMH neurons provide further support for its role in energy homeostasis. For example, the specific glucose transporter isoform glucose transporter 4 and the ATP-sensitive potassium channel are expressed in the VMH (5, 6). In addition, VMH neurons exhibit electrophysiological responses to local glucose administration (7–9). The tyrosine kinase receptor, type 2 (TrkB), and its ligand, brain-derived neurotrophic factor (BDNF), also may regulate food intake in the VMH (10, 11). In particular, reduced expression of either the TrkB receptor or BDNF is associated with obesity and altered autonomic activity. Another metabolic signaling system found in the VMH is the receptor for leptin, a hormone critical for the regulation of adiposity (12). Leptin administration activates neurons in the dorsomedial VMH (13), and VMH lesions disrupt the ability of leptin treatment to reduce body weight (14). Obesity has been observed in transgenic mice lacking the gene for steroidogenic factor 1 (SF1), a transcription factor necessary for the normal development of the VMH (15). Furthermore, a recent study demonstrated that leptin directly acts on SF1-expressing neurons in the VMH to mediate body weight regulation (16). This increase in body weight results from not only increased food intake, but also markedly decreased spontaneous motor activity and modestly increased plasma insulin levels. Together, these studies provide strong evidence for the role of VMH neurons in energy homeostasis.

Recently, several laboratories have reported that changes in energy availability or metabolic cues may alter local activity in the hypothalamus (17–19). One locus for a change in synaptic activity was a VMH projection to the proopiomelanocortin neurons in the arcuate nucleus, which are known to inhibit feeding behavior. The altered neurotransmission was consistent with the model that increased activity in the VMH reduces feeding behavior. These studies point to fasting- or leptin-induced changes in VMH neurotransmission but do not address the question of the possible underlying morphological changes. These recent studies prompted our general hypothesis that the central control of energy balance involves structural remodeling within the VMH.

Neural plasticity in the VMH has been studied previously in the context of hormone-induced reproductive behavior in female rats. Estradiol treatment promotes the development of axodendritic synapses in the VMH and increases dendritic spine density (20–23). The effects of estradiol depend on the type of dendrite. VMH neurons have simple dendritic trees, with a single long primary dendrite (LPD) and several short primary dendrites. Therefore, the goal of the present study was to test the specific hypothesis that food deprivation may

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Abbreviations: BDNF, Brain-derived neurotrophic factor; LPD, long primary dendrite; SF1, steroidogenic factor 1; TrkB, tyrosine kinase kinase receptor, type 2; VMH, hypothalamic ventromedial nucleus.

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alter the morphology of VMH neurons in a dendrite-specific fashion.

Materials and Methods

Animals

Adult male Sprague Dawley rats (n = 14) were housed in wire mesh cages with food (Purina rat chow; Purina Mills, LLC, St. Louis, MO) and tap water continuously available, unless otherwise stated. The temperature in the colony was maintained at 22 C with a 12:12 reverse light-dark cycle. Animals were allowed at least 1 wk to acclimate to the colony before any measurements were commenced. During the second week, animals were weighed daily, just before the beginning of the dark phase, and given preweighed food to monitor baseline food intake. The Institutional Animal Care and Use Committee of the University of Pennsylvania approved all procedures with animals.

Food deprivation regimen

Once the animals were acclimated to the colony and baseline body weight and food intake were established, the animals were assigned to one of two groups, matched for body weight. The control group (n = 7) was provided with continued *ad libitum* access to food. The food-deprived rats (n = 7) were given access to food only on alternate days, such that they had 24-h access, followed by 24-h deprivation. This regimen was followed for 10 d, during which food intake for both groups was monitored.

Tissue and plasma collection

At the end of the food restriction regimen, animals were deeply anesthetized (50 mg/kg ketamine and 20 mg/kg xylazine, ip) just before the lights going out. Blood samples were taken by cardiac puncture. A drop of whole blood was assayed for glucose level. The remaining blood was separated by centrifugation, and the plasma was frozen for future assay. RIAs for leptin, insulin, and testosterone were performed by the Diabetes and Endocrinology Research Center, University of Pennsylvania.

Golgi impregnation and morphological analysis

Animals were killed by decapitation, and the brains were removed. The brains were prepared for Golgi impregnation using the FD Rapid Golgi Stain kit (FD Neurotechnologies, Ellicott City, MD). In brief, brains were incubated in a potassium dichromate, mercuric chloride, and potassium chromate solution for 2 wk. After this incubation, hypothalamic sections, including the VMH, were obtained using a Vibratome (200 μm, Vibratome Series 1000; Vibratome, St. Louis, MO). The sections were mounted onto gelatinized slides and coverslipped. Individual neurons (n = 5–12 per animal) and their processes were visualized at $\times$100 using camera lucida. Soma area and dendrite length were measured in triplicate using National Institutes of Health Image 1.62 (National Institutes of Health, Bethesda, MD).

Several additional features of the dendrites were noted, as described previously (23, 24). First, the type of dendrite was categorized as either primary or secondary, based on whether it emerged from the cell body or another dendrite, respectively. The primary dendrites were further classified according to their length. For each neuron the longest primary dendrite was assigned as the LPD, and all other primary dendrites were referred to as "short primary dendrites" (see Fig. 3). In the present study, the direction of the LPDs was classified as one of two mutually exclusive categories: lateral or medial, based on dorsal-ventral axis bisection of each neuron in the coronal plane. An experimenter blind to the treatment groups conducted all morphological analyses.

Statistical analysis

Statistical comparisons between the two treatment groups were performed by repeated measure ANOVA and the Student's *t* test. The distribution of dendrites extended in each of the four possible quadrants in the coronal plane was assessed with a χ^2 analysis. Averages are expressed as the mean $\pm$ SEM. Significance was set at $P < 0.05$.

Results

Body weight, food intake, and hormones

As expected, the food restriction regimen caused a loss of body weight that was apparent after the first 24-h deprivation (Fig. 1). After 10 d of this regimen, the food-deprived rats lost approximately 35 g body weight, whereas the control animals gained about 40 g (repeated measures ANOVA, F = 48.71; $P < 0.01$). During the alternate days when food was available, the food-restricted rats increased their food intake by approximately 42%, whereas the food intake of the control rats remained stable (repeated measures ANOVA, F = 6.21; $P < 0.01$; Fig. 2). Assays performed at the end of the study revealed that blood glucose levels were reduced in the food-deprived group (*t* test, $P < 0.01$), as were plasma insulin (*t* test, $P < 0.002$) and leptin levels (*t* test, $P < 0.0004$; Table 1). Testosterone levels were equivalent in the two groups.

Morphological analysis

Golgi impregnation provided excellent visualization of VMH neurons (Fig. 3). The locations of the neurons included in this study are shown in Fig. 4. Five to 12 neurons were analyzed per animal, with a total of 127 neurons. In general, the morphological features of these neurons were similar to those observed in previous studies using Golgi impregnation, Lucifer yellow cell filling, or biolistic cell filling. From these neurons emerged relatively straight, sparsely branched dendrites, and this basic neuronal architecture was similar for the neurons located in the dorsomedial and ventrolateral portions of the VMH.

As in our previous work, we classified dendrites as the LPD, short primary dendrites, or secondary dendrites (23, 24). The number of neurons with LPDs extended in each of the two main directions, lateral and medial, was similar (43 lateral *vs.* 29 medial in the ventrolateral VMH, and 33 lateral *vs.* 28 medial in the dorsomedial VMH; χ^2 tests, $P > 0.05$). Food deprivation selectively reduced the length of LPDs extended in the lateral direction by approximately 110 μm,

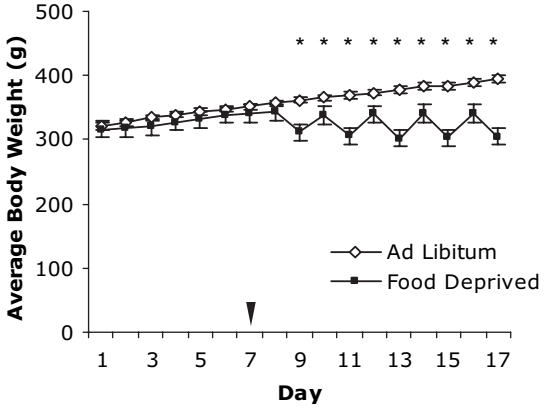

FIG. 1. Line graph illustrating the average body weights for the *ad libitum* control and food-deprived experimental groups. During a week of baseline measurements, all rats had free access to food and water. The food-deprived rats then had access to food only on alternate days for the next 10 d (onset indicated by *arrow*). The body weight of the food-deprived rats significantly decreased as the control group continued to gain weight daily. *, Significant difference between groups (Newman-Keuls, $P = 0.00003$ on d 17).

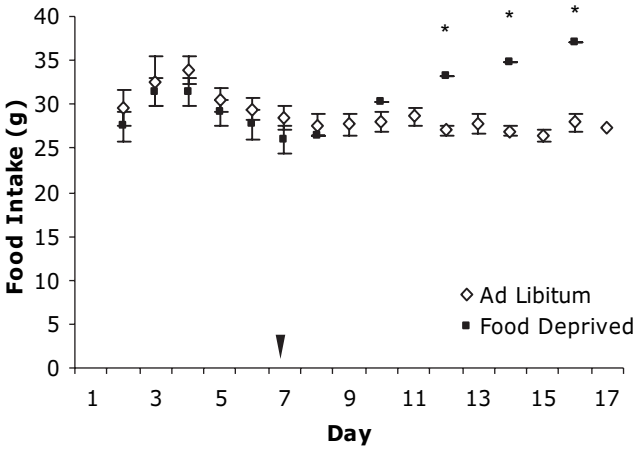

FIG. 2. Scatter plot of the average daily food intake for the control and food-deprived rats. Food intake for the control rats did not change across these 17 d. For the food-deprived rats, food intake increased once the deprivation regimen began (indicated by *arrow*), reaching significance above baseline on d 14 and 16. *, Significant difference between groups (Newman-Keuls, $P < 0.005$ and $P = 0.0001$, respectively).

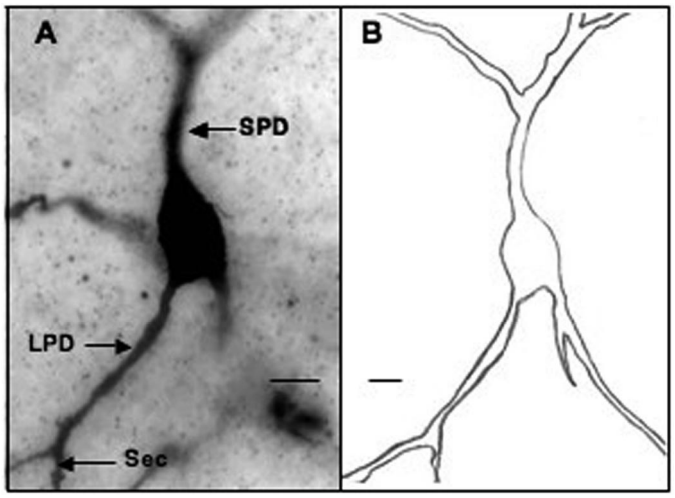

FIG. 3. Digital photomicrograph (*panel A*) and the corresponding camera lucida drawing (*panel B*) of a representative VMH neuron. The proximal region of the LPD, with a secondary dendrite (Sec), and one of the short primary dendrites (SPDs) are indicated with *arrows*. Note that camera lucida drawings are based on multiple z planes, thereby depicting the extension of dendrites that are not fully visible in photomicrographs. Also note that the LPD extends beyond the view of this image. VMH neurons were photographed at ×20. *Scale bar*, 25 μm for both *panels A and B*. *Scale bars* are not identical because the two images are enlarged by slightly different amounts.

or by 31% in the ventrolateral VMH (Student's *t* test, $P < 0.005$; Fig. 5). The lengths of medially extended LPDs in the ventrolateral VMH were not affected by the food restriction, nor were LPDs extended in either direction in the dorsomedial VMH.

The altered length of laterally extended LPDs in the ventrolateral VMH may reflect one of several possible mechanisms, with different implications for the other dendrites, as shown diagrammatically in Fig. 6. First, a laterally extended LPD may be a structural marker for a particular cell type. If so, the retraction of laterally extended LPDs may be based on a neuron-specific response to altered energy availability. In this scenario, other dendrites on these neurons may be affected by food deprivation. Alternatively, laterally arriving afferents may mediate the change in dendrite length. In this case all laterally extended dendrites, not just the LPDs, may be affected by energy depletion. To distinguish between these two possibilities, we classified short primary dendrites according to the direction of the LPD of that neuron and according to their own direction. For neurons with the LPD extended laterally, there was a 32% reduction in the number of non-LPD dendrites (4.22 ± 0.31 in the *ad libitum* group *vs.* 2.86 ± 0.49 in the food deprived group; $P < 0.05$; Fig. 7). In contrast, for neurons with the medially extended LPDs, there was no effect of food deprivation on the length or number of other dendrites. With regard to the alternative mechanism involving all laterally extended dendrites in the ventrolateral VMH, food deprivation did not alter the length of either short primary dendrites or secondary dendrites that extended in the lateral direction, as shown in Fig. 8.

Food deprivation also selectively reduced the soma size of VMH neurons. In particular, for neurons in the dorsomedial VMH with medially extended LPDs, the area of the soma was reduced by approximately 93 μm^2 or by 28% in the dorsomedial VMH (Student's *t* test, $P < 0.0005$; Fig. 9). For neurons in the dorsomedial VMH, there was no effect of food deprivation on the length or number of short primary dendrites or secondary dendrites, regardless of the direction of the LPD. Likewise, for neurons in the ventrolateral VMH, there was no effect of food deprivation on soma size, regardless of the direction of the LPD.

Discussion

The goal of this study was to test the hypothesis that systemic changes in energy status are associated with structural modifications of rat VMH neurons. The food restriction regimen reduced body weight, increased food intake, and prompted changes in metabolic hormones, such as insulin and leptin. There were several striking effects on neuronal morphology in the VMH. First, neurons in the ventrolateral VMH with laterally extended LPDs exhibited a retraction of the LPDs as well as a pruning of other dendrites. Second,

TABLE 1. Plasma values after 10 d on the food deprivation regimen

	Ad libitum	Food deprived	*P* value
Glucose (mg/dl)	79.4 ± 2.1	45.1 ± 8.9	<0.01
Insulin (ng/ml)	0.63 ± 0.17	0.45 ± 0.32	<0.002
Leptin (ng/ml)	5.66 ± 0.31	1.94 ± 0.51	<0.0004
Testosterone (ng/ml)	0.80 ± 0.35	0.86 ± 0.21	0.88 (ns)

Values are expressed as mean ± SEM. ns, Nonsignificant.

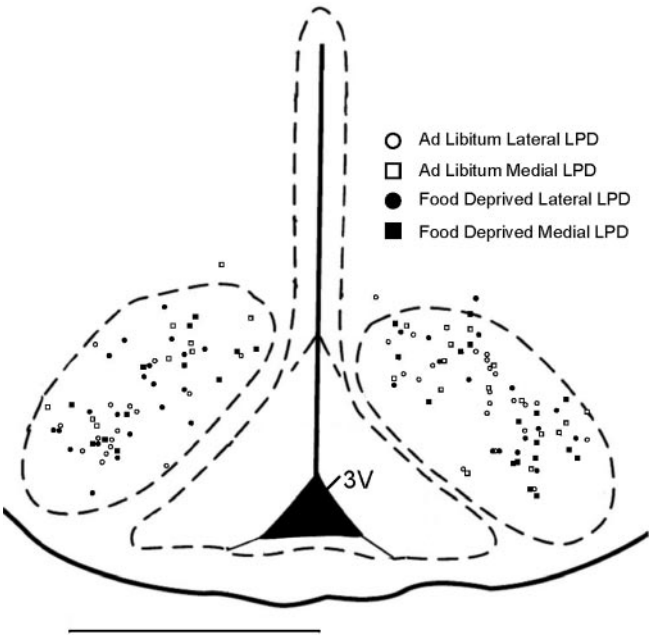

FIG. 4. Drawing of the mediobasal hypothalamus summarizing the location of all VMH neurons in this study, including neurons from both the *ad libitum* (*open symbols*) and food-deprived (FD) (*filled symbols*) animals. *Circles* represent the neurons with LPDs extended in the lateral direction, whereas *squares* represent VMH neurons with LPDs extended in the medial direction. The drawing is based on Paxinos and Watson (34). 3V, Third ventricle.

neurons in the dorsomedial VMH with medially extended LPDs exhibited a reduction in soma size. These results inform our understanding of the dendritic architecture of VMH neurons, the two major subdivisions of the VMH, and the possible relation between neuronal morphology and neuronal activity.

The present data contribute to the novel concept that the direction of the LPD may be a structural marker for the functional network of VMH neurons. Whereas the short pri-

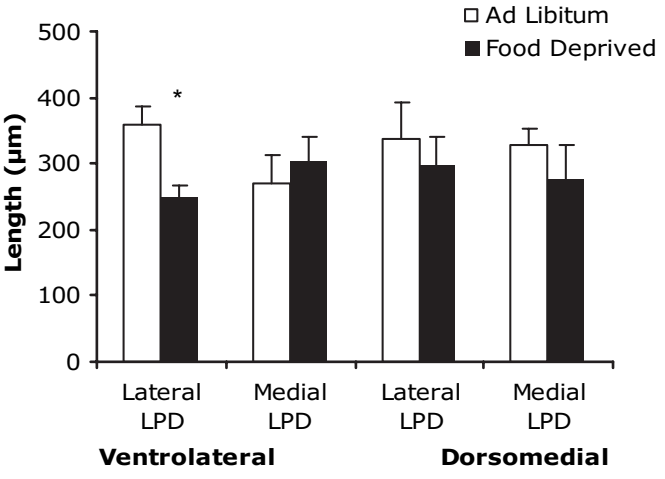

FIG. 5. Bar graph depicting the length of LPDs in *ad libitum* and food-deprived rats according to direction. In the ventrolateral VMH, LPDs extended in the lateral, but not medial, direction were significantly shortened after food deprivation. *, Significant difference between groups ($P = 0.005$). There was no effect of food deprivation on LPD length in the dorsomedial VMH.

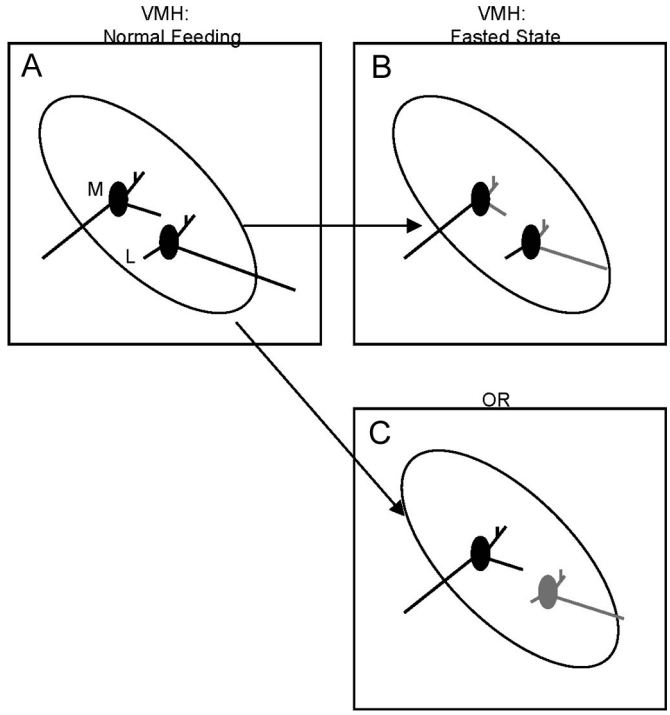

FIG. 6. Illustration of two possible mechanisms that may underlie the observed reduction in the length of laterally extended LPDs in the ventrolateral VMH. A, Two representative neurons in the VMH in the *ad libitum* condition. The neuron on the *right* has a laterally (L) extended LPD, whereas the neuron on the *left* has a medially (M) extended LPD. B, Depiction of how these neurons may change morphology during food restriction, with all laterally extended dendrites becoming shorter (note that both neurons are affected). This scenario implicates a role for laterally arriving afferents. C, An alternative pattern of changed morphology, with a loss of dendrites on neurons with laterally extended LPDs dendrites (note that the neuron on the *left* is not affected). This scenario suggests that neurons with laterally extended LPDs are specifically involved in energy balance compared with other ventrolateral VMH neurons.

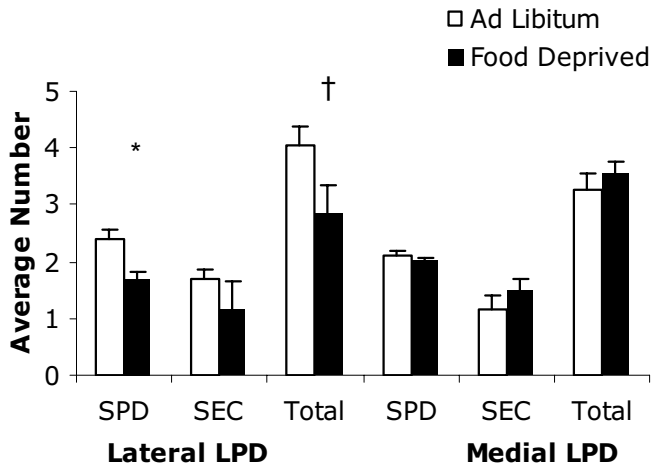

FIG. 7. Bar graph depicting the number of short primary and secondary dendrites (SPDs and SECs, respectively) in *ad libitum* and food-deprived rats, according to the direction of the LPD. Within the ventrolateral VMH, neurons with laterally, but not medially, extended LPDs exhibited a reduction in the number of other dendrites (*, $P < 0.005$; †, $P < 0.05$). There was no effect of food deprivation on dendrite number in the dorsomedial VMH (data not shown).

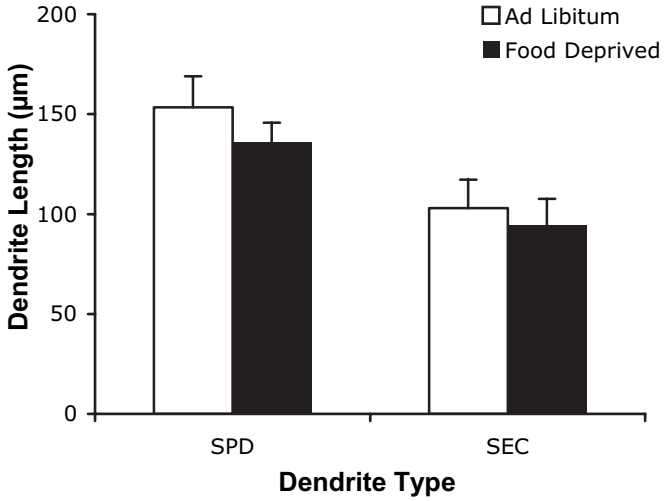

FIG. 8. Bar graph depicting the length of laterally extended short primary dendrites (SPDs) and secondary dendrites (SECs) in *ad libitum* and food-deprived rats in the ventrolateral VMH. In either case there was no effect of food deprivation on the length of these dendrites.

mary dendrites may be available to inputs from local interneurons, the LPD may be positioned to receive inputs from extranuclear regions. Thus, the lateral *vs*. medial direction of the LPD may dictate integration in very different functional networks. Our results suggest that neurons residing within the ventrolateral VMH may respond to energy balance cues as long as their LPDs extend laterally. An extensive fiber plexus is situated laterally to the VMH, with afferents arriving from numerous other brain regions (25). Likewise, neurons residing in the dorsomedial VMH respond to energy balance cues if their LPDs extend medially, but not laterally. Such medially extended LPDs may be contacted by afferents occurring from the medially located arcuate nucleus, a region

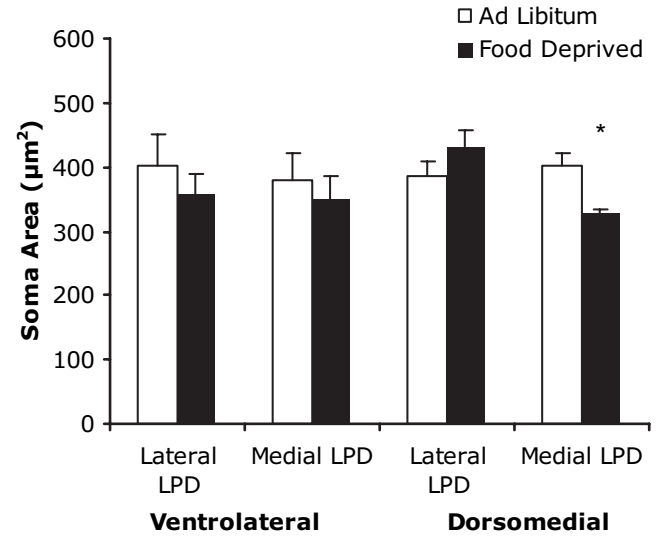

FIG. 9. Bar graph depicting the soma area of VMH neurons in *ad libitum* and food-deprived rats according to the direction of the LPD. Within the dorsomedial VMH, neurons with medially, but not laterally, extended LPDs exhibited a reduction in soma area (*, $P < 0.0002$). There was no effect of food deprivation on soma size in the ventrolateral VMH.

uniquely critical for energy balance. Previously, the function of VMH neurons had been inferred based on the presence of hormone receptors or axonal projection to extranuclear targets (24, 26), although recent work has begun to identify molecular markers of VMH neurons (27). Thus far, SF1 is the clearest marker for neurons involved in calorie regulation, and it is mainly expressed in the dorsomedial, rather than ventrolateral, VMH (16). Our results suggest that in addition to neurochemical markers, the topographically organized innervation of the LPDs may define the function of VMH neurons. It remains unknown whether or not the direction of the LPD is indicative of the axonal projection target of these neurons.

Previously, we observed differential effects of estrogen treatment on the LPD *vs*. short primary dendrites in the VMH (23, 24), and differential effects of mating experience on the secondary dendrites (28). In those studies we measured dendritic spine density, which was not quantified here. Future studies will be needed to determine whether there are concomitant changes in dendritic spines and synaptic inputs in the VMH after challenges to energy balance.

A surprising aspect of these results was that changes were produced on neurons in both the dorsomedial and ventrolateral VMH. Evidence has suggested that the dorsomedial and ventrolateral VMH have distinct functions based on neurochemical markers and Fos activation. In particular, the dorsomedial region has been implicated in energy balance and the ventrolateral in reproductive behavior (as reviewed in Ref. 29). The observed changes in soma size in the dorsomedial VMH were consistent with this framework. However, in the ventrolateral VMH, neurons with laterally extended LPDs also exhibited deprivation-induced changes. A possible implication of this result is that the affected neurons in the ventrolateral VMH play a role in reproductive function rather than energy balance, given that food deprivation disrupts male reproductive function (30, 31). Testosterone levels were not reduced by the food deprivation regimen in the present study, which suggests that testosterone is unlikely to have mediated the changes in the dendritic arbor of neurons in the ventrolateral VMH. However, it is possible that the food deprivation schedule had central effects on brain circuits involved in sexual behavior, including those within the ventrolateral VMH. Regardless of the possible differences in the function of these two subregions, these results suggest that there are fundamental differences in the responses of dorsomedial *vs*. ventrolateral neurons to changes in energy status.

This study did not identify the signal of energy availability that led to the change in dendritic arbor and soma size, and there are numerous possible mediators. In fact, the signal may differ for the dorsomedial *vs*. ventrolateral VMH, given the likely differences in innervation and the different nature of the structural changes. One logical candidate is leptin, given that our food deprivation regimen reduced leptin levels and that the VMH possesses leptin receptors (12). Another contending signal of energy status is blood glucose level, considering that glucose levels also were reduced in our food-deprived animals, and the VMH expresses several proteins involved in transducing glucose availability into a membrane potential (5, 6). Possibly downstream of either

leptin or glucose, may be the regulation of either BDNF or its receptor, TrkB, given that the disruption of BDNF-TrkB signaling promotes obesity (10, 11, 32). Interestingly, BDNF expression in the VMH is predominantly found in the ventrolateral region (11). Future studies will be aimed at elucidating these possible mechanisms.

Once the primary metabolic cue has been identified, it will be important to determine whether the VMH neurons respond either directly to the detection of interoceptive signals, as part of a more complex metabolic integration, or as the effector for an adaptive response. Again, this may differ for the dorsomedial *vs*. ventrolateral VMH. To address this question, one may examine VMH neuronal morphology as a function of various energy cues. Alternatively, VMH neuronal morphology may encode the animal's history of food availability, setting the tone for reactivity to changes in energy needs. Therefore, an important goal of future studies will be to determine whether or not the deprivation-induced change in VMH neuronal morphology is reversible. As mentioned previously, changes in the ventrolateral VMH may reflect adaptations in the reproductive system, rather than an integration or effector system for energy balance.

From a cellular perspective, both the reduced soma size and dendrite retraction suggest a reduction in neuronal activity. It remains unclear whether the observed decrease in soma size reflects a reduction in biosynthetic capacity or structural proteins. In either case the reduced soma size may indicate a global reduction in cellular activity, including action potentials. The pruning and retraction of dendrites would seem likely to eliminate certain synaptic inputs, markedly limiting the responsiveness to certain afferents. These general interpretations fit with several lines of evidence that activation of VMH neurons normally promotes a negative energy balance, possibly indirectly via the regulation of the autonomic nervous system (2, 33). Recent experiments suggested that during fasting, there is less activity in a VMH projection to the arcuate nucleus, in particular innervating the anorexigenic proopiomelanocortin neurons (18). Thus, during food deprivation, as in the present experiment, one would expect the VMH to experience reduced activity. Given that we have observed a deprivation-induced atrophy of neurons in the dorsomedial VMH, we propose that neurons with medially extended LPDs are less metabolically active, become atrophied, and provide less drive on downstream neurons. Although the role of the VMH in male sexual behavior has not been well studied, the retraction of dendrites in the ventrolateral VMH may reflect the expected inhibition of mating during times of food scarcity. This model is consistent with the historical view of the VMH as being inhibited during negative energy balance, with the novel suggestion that concomitant changes in reproduction may also be executed within the VMH.

In conclusion, food restriction reduced the length of laterally extended LPDs in the ventrolateral VMH, with a reduction in the number of other dendrites on these same neurons. In addition, food restriction reduced the soma size of neurons in the dorsomedial VMH for neurons with medially extended LPDs. Thus, the direction of the LPDs may be a structural marker defining the functional networks of VMH neurons. These changes in neuronal morphology may reflect reduced neural activity within a hypothalamic network that normally inhibits feeding and promotes energy expenditure. In addition, the observed dendritic plasticity may allow for the integration of energy balance with reproductive function. These results support the general hypothesis that adaptations to energy balance challenges may be regulated in part by structural neural plasticity. Future studies will aim to identify the relevant VMH afferents and metabolic cues that regulate the length of VMH dendrites.

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