Persistence of a behavioral food-anticipatory circadian rhythm following dorsomedial hypothalamic ablation in rats

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Landry, G. J., M. M. Simon, I. C. Webb, R. E. Mistlberger. Persistence of a behavioral food-anticipatory circadian rhythm following dorsomedial hypothalamic ablation in rats. Am J Physiol Regul Integr Comp Physiol 290: R1527–R1534, 2006. First published January 19, 2006; doi:10.1152/ajpregu.00874.2005.—Circadian rhythms of behavior in rodents are regulated by a system of circadian oscillators, including a master light-entrainable pacemaker in the suprachiasmatic nucleus that mediates synchrony to the day-night cycle, and food-entrainable oscillators located elsewhere that generate rhythms of food-anticipatory activity (FAA) synchronized to daily feeding schedules. Despite progress in elucidating neural and molecular mechanisms of circadian oscillators, localization of food-entrainable oscillators driving FAA remains an enduring problem. Recent evidence suggests that the dorsomedial hypothalamic nucleus (DMH) may function as a final common output for behavioral rhythms and may be critical for the expression of FAA (Gooley JJ, Schomer A, and Saper CB. Nat Neurosci 9: 398–407, 2006). To determine whether the reported loss of FAA by DMH lesions is specific to one behavioral measure or generalizes to other measures, rats received large radio-frequency lesions aimed at the DMH and were recorded in cages with movement sensors. Total and partial DMH ablation was associated with a significant attenuation of light-dark-entrained activity rhythms during ad libitum food access, because of a selective reduction in nocturnal activity. When food was restricted to a single 3-h daily meal in the middle of the lights-on period, all DMH and intact rats exhibited significant FAA. The rhythm of FAA persisted during a 48-h food deprivation test and reappeared during a 72-h deprivation test after ad libitum food access. The DMH is not the site of oscillators or entrainment pathways necessary for all manifestations of FAA, but may participate on the output side of this circadian function.

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accumbens core (38) and the hypothalamic paraventricular nucleus attenuate or eliminate FAA in some measures of behavior (e.g., general locomotor activity) but not in others [e.g., wheel running or activity directed at a food bin (47, 49)]. Ablation of infralimbic neocortex (54) or the hypothysis (25) eliminates the food-anticipatory rhythm of body temperature, but not of behavior. FAA also persists after ablation of the hypothalamic subparaventricular zone (30, 30a), arcuate nucleus (43), ventromedial nucleus (48), mutations affecting the clock genes mCry1/Cry2 (33) and Clock (53), and the leptin receptor gene (45). FAA is also not dependent on endocrine signals from the adrenal gland (15, 60), although adenectomy does eliminate a food-entrainable circadian rhythm of clock gene expression in the oval nucleus of the bed nuclei of the stria terminalis (4, 36). Finally, FAA is not dependent on sensory signals provided by olfactory, optic, trigeminal (gustatory), or visceral autonomic nerves (19, 21, 24, 40), although combined removal of these afferents has not been attempted. Although these studies have failed to identify oscillators and input pathways necessary for entrainment by scheduled feeding, they are instructive in demonstrating that lesions can dissociate behavioral and physiological food-anticipatory responses and may differentially affect behavioral outputs. Non-specific measures of locomotor activity, such as provided by telemetry or tilt cages, may be more susceptible to disruption by lesions, whereas behaviors directed at feeding locations seem to be more resistant (e.g., Ref. 49).

Two other reports merit special attention. Rats with electrolytic or cell-specific lesions of the brain stem parabrachial nucleus (PBN) were shown to express either very little or no food-anticipatory circadian rhythms of core body temperature or activity directed at a food tray, a behavioral measure that in other studies has been resistant to disruption by lesions (22). The PBN has also been shown to exhibit a food-anticipatory rhythm of c-fos expression that, unlike FAA, does not persist if the scheduled daily meal is omitted for one cycle (5). Together, these results suggest that the PBN may be a critical component of the entrainment pathway to food-entrainable oscillators located elsewhere. The PBN is an integrative area for visceral and gustatory sensory information, and projects to a variety of forebrain areas, including the dorsomedial hypothalamic nucleus (DMH) (55, 64). The DMH, referred to as “enigmatic” by Thompson, Swanson and colleagues (64, 65), has recently been conceptualized as an integrative area and final common output for circadian rhythms of sleep-wake, ingestive behavior, and corticosterone (56). The DMH receives direct and indirect input from the SCN, expresses neuropeptides (e.g., neuropeptide Y, orexin) and receptors (e.g., leptin, cholecystokinin, ghrelin) implicated in the control of ingestive behavior and metabolism and is richly interconnected with hypothalamic, preoptic, and some brain stem nuclei involved in regulation of energy input/output or behavioral state (10, 17, 18, 26, 41, 64, 65). Lesions of the DMH disrupt LD-entrained circadian rhythms in these functions (13, 18) and have recently been shown to attenuate or eliminate anticipatory rhythms of general activity and core body temperature measured by telemetry in rats restricted to a 4-h daily meal (30, 30a). DMH damage may explain our own occasional observations of rats or mice that failed to anticipate a daily feeding time following very large, nonspecific radiofrequency lesions of the medial hypothalamus, resulting from presumably faulty electrodes aimed at the SCN or the paraventricular nucleus (6, 37). These results suggest that the DMH may be the site of food-entrainable circadian oscillators or a critical link between such oscillators and circadian outputs.

Given the importance of the behavioral measure in assessing circadian function following brain lesions, we sought to further examine the role of the DMH by using motion detectors with more spatial selectivity than is provided by telemetric movement sensors. To maximize the chances of evaluating rats with unambiguous, complete DMH ablation, we used electrodes, stereotaxic placements, and radiofrequency current parameters designed to produce very large medial hypothalamic lesions. We found that unambiguous DMH destruction produced ingestive deficits and attenuated LD-entrained circadian activity rhythms but did not attenuate behavioral anticipation of a daily meal.

**MATERIALS AND METHODS**

**Subjects and apparatus.** Adult male Sprague-Dawley rats (n = 14, 310 –320 g; Charles River) were housed individually in polypropylene cages (45 × 24 × 20 cm) equipped with wire floors and tops, a water bottle, and a black opaque tube (15 cm long × 8 cm diameter) for sleeping or light avoidance (12:12-h light-dark cycle, ~1,000:0 lux). Food was available in a metal cup mounted on a manually controlled carousel, accessed via a 4 × 4 cm window cut through one end of the cage. The window was covered by a hinged metal gate, which the rats were required to move with their snouts to reach the food cup. Movements of the gate were detected by a microswitch, monitored continuously by an interface and data collection system of our own design. The “sleeping” tube was fixed to the cage floor with the opening at the end of the cage opposite to the feeding window. The rats thus had to cross the length of the cage to move from the tube to the food cup. A motion detector (Quorum RR-150) was positioned above the cage to detect these movements. Activity counts were summed in 10-min intervals and stored for analysis off-line using Circadia (Dr. T. A. Houpt, Florida State University, Tallahassee, FL) operated on a Macintosh computer.

**Surgery and histology.** Seven rats received bilateral radiofrequency lesions of the DMH. The rats were anesthetized for stereotaxic surgery using ketamine (90 mg/kg Ketalean; Bimeda-MTC Animal Health) and xylazine (9 mg/kg Rompun; Bayer) supplemented with isoflurane (0.5–1% Aerrane; Baxter) as needed. The lesion electrodes were stainless steel insect pins (size 0) insulated to within 0.5 mm of the flattened tip. Current was supplied by a Grass LM3 Lesion Maker. Stereotaxic coordinates were ±0.5 mm lateral and 3.5 mm posterior to bregma and 8.5 mm ventral from the dura. Following surgery, body temperature and food and fluid intake were monitored over an 8-day recovery period. All rats survived the procedure.

At the completion of behavioral testing all lesioned rats and two intact control rats were euthanized via pentobarbital sodium overdose and perfused transcardially with saline followed by 10% formalin. The brains were removed, postfixed, cryoprotected in a formalin-sucrose mixture for at least overnight, and sectioned at 50-µm intervals using a cryostat. All sections from the posterior optic chiasm to the medial mammillary nuclei were mounted on slides, stained using cresyl violet, dehydrated, cleared, and coverslipped.

Sections in which lesions or intact DMH were evident were examined under a microscope, photographed with a digital camera, and then carefully inspected on a computer. Lesioned brains were compared with the two intact brains and with the Paxinos and Watson (52) rat brain atlas, supplemented by published work on DMH cell bodies, afferents, and efferents (18, 64, 65). From these comparisons, a percentage of DMH intact was estimated.

**Test procedures.** After recovery from surgery, the rats were returned to their recording cages where they and seven intact rats had...
Results

Histology. Figure 2 illustrates photomicrographs of the hypothalamus from an intact rat (A–C) and rats with partial (E–G) or total (I–K) DMH ablation. The DMH first appears caudal to the paraventricular and anterior hypothalamic nuclei, above the rostral ventromedial hypothalamic nucleus, below the diffuse dorsal hypothalamic area, and extending from the third ventricle laterally to within 100–200 μm of the fornix. The caudal border is considered ambiguous (14); conservatively, it may merge with the arcuate nucleus at the level of the mammillary recess and premammillary nuclei. The rostrocaudal extent of the DMH can be estimated at approximately 1.6 mm based on sagittal sections illustrated in Chou et al. (18). The latter estimate corresponds to the range of sections illustrated for the intact rat in Fig. 2, A–C.

The lesion parameters were intended to produce ablations centered on the DMH and extending 2 mm or more rostrocaudally. Six of seven cases sustained ablations of this size. In three of seven cases, some DMH tissue appeared to be present. In two of these three cases, the lesions were asymmetrical and clearly partial; the smallest lesion (Fig. 2, E–G) spared the lateral third of the DMH on one side and the caudal DMH bilaterally. A second partial lesion spared ~20% of one DMH laterally (Fig. 3A). A third case was also classified as partial; although the cavity extended ~1.6 mm rostral to caudal, some intact DMH cells medial to the fornix on one side could not be ruled out, and the lesion was estimated at ≥90% complete (Fig. 3B).

In four of seven cases, the lesions were very large, producing cavities that extended laterally at least to the fornix on both sides, caudally from the paraventricular nucleus to the premammillary nuclei, and dorsally from the ventromedial nucleus well into the medial thalamus above the roof of the third ventricle (Figs. 2, I–J and 3, C–E). In all of these cases, the lesion cavities subsumed the fornix and mammillothalamic tract on at least one side. The dorsal hypothalamic area and the diffuse and compact regions of the DMH were completely absent. At least partial damage was sustained by the paraventricular (particularly the medial magnocellular portions), subparaventricular, anterior, periventricular, ventromedial (particularly dorsomedially), arcuate, and posterior hypothalamic nuclei, the midline thalamus (reuniens, rhomboid, centromedian nuclei), and the tuberal magnocellular nucleus. Abnormal cells, glia, and apparent debris were evident in parenchyma near the borders of the cavities, indicating that this analysis, based on the cavity size and position relative to key landmarks, is a conservative estimate of the extent of the damage.

Fig. 1. Activity records of representative rats with no lesion (A), partial dorsomedial hypothalamic nucleus (DMH) damage (B), and total DMH ablation (C). Each line represents a day, with time plotted from the left in 10-min bins. Bins in which 3 or more activity counts were registered are represented by a vertical bar. The 12-h dark period is indicated by shading. Mealtime (3 h/day) during food restriction is labeled and indicated by the open bar. Days during which no food was provided are indicated by the black bar to the left of each chart. V, beginning of food deprivation; inverted V, end of deprivation.
Activity levels and nocturnality. During ad libitum food access before restricted feeding, intact rats exhibited a high-amplitude daily rhythm of locomotor activity, averaging 1,828 ± 119 counts/day of which 83 ± 3% were registered at night (Figs. 1A, 2D, and 4A). DMH lesion rats averaged 929 ± 225 counts/day \([I_{12}] = 2.60, P = 0.02\) vs. intact rats] of which 72 ± 6% occurred nocturnally \([I_{12}] = 5.77, P < 0.0001\) vs. intact rats]. The reduction in activity in lesion rats was significant only at night \([I_{12}] = 3.17, P = 0.008\) vs. intact rats; Fig. 4A]. During restricted feeding and food deprivation, nocturnal activity levels increased in the DMH lesion rats, but not in the intact rats (Fig. 4, B and C). Nonetheless, nocturnal activity remained significantly lower in the DMH lesion rats. Activity levels and nocturnality ratios were virtually identical in rats with total and partial DMH lesions.

Food-anticipatory activity. During restricted feeding, all intact and DMH lesion rats exhibited activity in anticipation of the daily meal, as measured by an overhead motion sensor (Figs. 1, A–C; 2, D, H, and I; 3, F–J; and 4B) and by a microswitch at the food bin. The number of counts detected by the microswitch was low throughout the day in intact and lesion rats; therefore only the motion detector activity data were used for quantitative analyses. Total motion detector activity counts during the 3 h before mealtime (FAA) were averaged in 5-day blocks over the 30 days of restricted feeding and compared with activity at the same time of day during the preceding five baseline days when food was available ad libitum (Fig. 5A). Each of the six blocks of restricted feeding days was significantly different from the baseline block in both the intact group \([F_{(11,6)} = 10.24, P < 0.0001]; pairwise comparisons significant at \(P = 0.02\) or better] and DMH lesion group \([F_{(11,6)} = 7.92, P < 0.0001]; pairwise comparisons significant at \(P = 0.003\) or better]. FAA counts remained high during 2 days of food deprivation immediately following the last scheduled meal (e.g., Fig. 1, A–C), declined when food was provided ad libitum, and increased again when food was removed for 3 days (Figs. 1, A–C and 4C), demonstrating persistence of the FAA rhythm in the absence of a daily feeding stimulus in both groups. Between-group comparisons revealed that FAA counts were significantly higher in the DMH lesion group on the last 5-day block of restricted feeding during the 48-h food deprivation test and on subsequent blocks with ad libitum food access.

FAA ratios showed a similar pattern of results (Fig. 5B); ratios during restricted feeding were significantly different from baseline by the first 5-day block in the DMH lesion rats \([F_{(11,6)} = 11.58, P < 0.0001]; P < 0.05\) for each comparison with baseline) and by the second 5-day block in the intact rats \([F_{(11,6)} = 4.92, P < 0.0001]; P < 0.05\) for each comparison with baseline]. FAA ratios were significantly higher in the DMH group at all time points, including the baseline ad libitum food access days [two-way ANOVA, \(F_{(11,1)} = 3.27, P < 0.0001\]; \(P < 0.05\) for each pairwise comparison]. This reflects, in part, the reduced amount of nocturnal activity in rats with DMH lesions. There was no apparent relation between the FAA statistics and the size or completeness of the lesions.

Food and water. Daily food intake increased over the first 7–10 days of restricted feeding (Fig. 6). This was more appa-
Mean intake during restricted feeding was 13.6 ± 0.4 g in intact rats and 13.9 ± 0.4 g in the lesion rats, excluding one hyperphagic outlier. The outlier sustained the smallest partial lesion (Fig. 2, E–G), averaged 22 ± 3 g/day, and exhibited apparent weight gain over the course of behavioral testing.

Daily water intake during restricted feeding averaged 20.1 ± 1.2 ml/day in rats with DMH lesions, and 25.9 ± 1.2 ml/day in intact rats (P < 0.001). There was no difference between rats with partial and total lesions.

DISCUSSION

The DMH has been conceptualized as a site where neural and endocrine signals conveying information about circadian phase, energy states, and other factors are integrated to determine the daily circadian program for sleep-wake, activity, body temperature, and at least some endocrine rhythms (56). Consistent with this hypothesis, daily rhythms of immediate-early gene expression in DMH neurons are regulated by scheduled mealtimes, and ablation of DMH neurons by ibotenic acid eliminates food-anticipatory temperature rhythms and attenuates a rhythm of FAA in proportion to the severity of cell depletion (5, 30, 30a). The DMH has thus been described as critical for entrainment of circadian rhythms by scheduled feeding (30, 30a, 56); conceivably, it could be the site of food-entrainable circadian oscillators or of a final common output pathway for such oscillators. If so, then complete lesions of the DMH should eliminate FAA in all measures of behavior. Alternatively, the DMH could be critical for the expression of FAA in some but not other measures of behavior.
The results of the present study support the latter of these hypotheses; very large radiofrequency lesions that unambiguously destroyed the DMH produced ingestive deficits and attenuated LD-entrained circadian rhythms but did not eliminate FAA detected by a motion detector or a microswitch at the food-bin window. The difference between this result and results reported previously (30, 30a) is presumably related to the measure of behavior and possibly the configuration of the recording apparatus. In the previous study, activity was measured by a radiofrequency transmitter implanted in the abdominal cavity, which detects movement nonspecifically. Other work has shown that anticipatory activity in nonspecific cage activity can be eliminated by hypothalamic lesions that do not affect anticipatory activity directed specifically at a food-access window (49). In the present study, activity was measured by motion detectors situated overhead and at a food-access window. The cage was configured such that the rats could sleep in a dark tube that opened at the end of the cage opposite from the food-bin window. This may have minimized detection of nonspecific daytime activity and increased the amount of activity that was specifically food anticipatory.

Surprisingly, not only was FAA present in all of the DMH-ablated rats, but the magnitude of the rhythms was enhanced by comparison with intact rats. We have previously observed enhanced FAA in rats with other neural ablations or genetic defects (43, 45), but the interpretation of such effects is unclear. In the present case, the DMH lesions were associated with significant reductions in nocturnal activity. Reduced activity at night must have contributed substantially to the increased FAA ratios, given that nocturnal activity was the main part of the denominator in this ratio. The absolute amount of activity during the 3 h before mealtime was also increased in the DMH-ablated rats, and this might be because of an effect of the lesions on one or both of two factors that normally constrain the level of daytime activity: 1) an inhibitory influence of the SCN pacemaker on locomotor activity during the rest phase of the circadian cycle (41) and 2) an inhibitory influence of environmental light on locomotor activity [so-called “negative masking” (8, 51)]. The same factors may explain why there was a tendency for the DMH-ablated rats to eat larger meals during the first week of restricted feeding. Cage lights were relatively bright in this study, and this may have served to amplify differences between intact and ablated rats.

DMH lesions have previously been shown to reduce both food and water intake when both resources are freely available (11, 12). In the present study, DMH-ablated rats drank significantly less (~22%) but did not eat less than intact rats during scheduled feeding. This may be because food was limited [DMH-ablated rats overeat relative to intact rats during the first hour following food deprivation (11)] and/or because the powdered chow was mixed with oil, which enhances palatability. The amount of food eaten per scheduled meal increased over the first 7–10 days of restricted feeding in the ablated and intact rats (Fig. 6), likely because of homeostatic factors [i.e., loss of body weight during the first few days of limited access to food (e.g., Ref. 7)] and circadian factors [i.e., gradual shifting of gastrointestinal circadian rhythms from a nocturnal phase to a diurnal phase, thereby permitting larger meals (e.g., Ref. 23)]. Although meal size is only an indirect (and putative) measure of the phase of gastrointestinal rhythms, the gradual increase of meal size during the first week of restricted feeding in the DMH-ablated rats suggests that DMH lesions also do not affect entrainment of peripheral oscillators to the scheduled daytime meal.

The results of the present study support the latter of these hypotheses; very large radiofrequency lesions that unambiguously destroyed the DMH produced ingestive deficits and attenuated LD-entrained circadian rhythms but did not eliminate FAA detected by a motion detector or a microswitch at the food-bin window. The difference between this result and results reported previously (30, 30a) is presumably related to the measure of behavior and possibly the configuration of the recording apparatus. In the previous study, activity was measured by a radiofrequency transmitter implanted in the abdominal cavity, which detects movement nonspecifically. Other work has shown that anticipatory activity in nonspecific cage activity can be eliminated by hypothalamic lesions that do not affect anticipatory activity directed specifically at a food-access window (49). In the present study, activity was measured by motion detectors situated overhead and at a food-access window. The cage was configured such that the rats could sleep in a dark tube that opened at the end of the cage opposite from the food-bin window. This may have minimized detection of nonspecific daytime activity and increased the amount of activity that was specifically food anticipatory.

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![Fig. 5. A: group mean ± SE activity counts registered during hours 4–6 after lights on, averaged in 5-day blocks with food available ad libitum or restricted to 3-h/day or in 1-day or 3-day blocks of total food deprivation (number of days/block is indicated above the x-axis). B: same plotting convention, with food-anticipatory activity expressed as a ratio relative to activity at night and during the first 3 h of the day. For some data points, standard error bars are too small to see. FAA, food-anticipatory activity; DMHx, DMH lesions; ad lib, ad libitum; dep, deprivation.](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00024.2006)

![Fig. 6. Mean ± SE daily food intake of intact rats (dotted line, diamond symbols) and rats with DMH lesions (solid lines, square symbols) during 30 days of food restriction (3 h/day).](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00024.2006)
The results of the present study rule out a role for the DMH as the exclusive site of oscillators mediating entrainment of behavioral rhythms by circadian feeding schedules. However, the results are not inconsistent with a role for the DMH as an integrative area that adjusts the daily timing of at least some physiological and behavioral variables under the influence of circadian, metabolic, and other factors. The DMH is most likely situated downstream, i.e., on the output side of the food-entrainable oscillators critical for anticipatory activity rhythms. Alternatively, a number of brain regions, including the DMH, may be capable of food-entrainable oscillations driven by local oscillating clock cells, and these may be more or less directly coupled to specific outputs. Such a “distributed” organization could explain why lesions in several areas (e.g., nucleus accumbens, infralimbic cortex, paraventricular nucleus, lateral hypothalamic orexin cells, DMH, PBN, hypophysis) have been shown to attenuate at least one food-entrainable circadian rhythm (e.g., temperature, general locomotor activity), whereas no lesion has yet been shown to eliminate all manifestations of food entrainment in all animals tested. A quarter-century on, Stephan’s (60) warning remains pertinent: “If many oscillators exist which are entrainable by food restriction schedules, it may not be possible to abolish anticipatory activity by selective removal, or interference with, specific organ systems,” to which we might add “specific brain regions.”

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