

Research report

Neonatal monosodium glutamate alters circadian organization of feeding, food anticipatory activity and photic masking in the rat

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Abstract

In rodents, parenteral administration of monosodium glutamate (MSG) induces marked degeneration of the retina and arcuate nucleus (AN) and disrupts daily rhythms of food intake. We quantified the effects of neonatal MSG (2 mg/g SC, postnatal days 1, 3, 5, 7, 9) on the expression of feeding and activity rhythms in adult rats under schedules of light–dark (LD), constant dark (DD), restricted daily feeding and total food deprivation. AN lesions were confirmed by neuropeptide Y (NPY) immunocytochemistry and Nissl stain. Compared to age-matched control rats, the amplitude (quantified as LD ratios) of daily food intake and food-bin activity rhythms was significantly attenuated in MSG rats in LD 12:12 and on the first day of DD. Control rats, but not MSG rats, showed lower amplitude rhythms in DD compared to LD. The phase angle of feeding and activity rhythms did not differ between groups in either condition. In a short LD cycle (2:2), control rats, but not MSG rats, showed significant inhibition (masking) of activity during the 2 h light periods. When food access was restricted to a 4 h daily meal, MSG rats showed enhanced expression and persistence of food-entrained anticipatory activity rhythms by comparison with control rats. These results indicate that attenuation of daily feeding rhythms in MSG rats is due in part to loss of direct inhibitory effects of light on behavior, and that the AN likely modulates, but does not mediate entrainment of feeding-related rhythms to daily cycles of LD or food access. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Feeding behavior in mammals is organized in the temporal domain by a circadian system consisting of at least two pacemakers that generate endogenous circadian timing signals, associated input pathways that entrain these signals to local time cues and output pathways that confer circadian rhythmicity to effector systems for behavior and physiology. The circadian pacemaker mediating entrainment of feeding rhythms to daily light–dark (LD) cycles is located in the hypothalamic suprachiasmatic nuclei (SCN) [31]. A second circadian pacemaker is located at an unknown site outside of the SCN and is responsible for generating daily rhythms of food anticipatory activity (FAA) that emerge in many species when food access is restricted to a fixed time of day [41,44]. Coupling mechanisms that link these pacemakers to effector systems regu-

lating ingestive behavior and metabolism are not yet well delineated, but likely involve direct and indirect interactions with a distributed network of hypothalamic and extrahypothalamic areas and peripheral glands [29,32]. The present study utilized neonatal monosodium glutamate (MSG) treatment as an ablation tool to examine the role of the arcuate nucleus (AN) in the expression of daily rhythms of food intake and FAA in the rat.

Parenteral administration of MSG during the first week of life induces substantial and irreversible loss of neurons in the retina and AN in rats, hamsters and mice [9,34,46,48]. Destruction of AN neuropeptide cells, including neurons immunoreactive for neuropeptide Y (NPY), is extensive or complete [37]. MSG lesions are associated with a syndrome characterized by stunted growth, increased adiposity, hypophagia and reduced function of several hormone systems [30]. Neonatal MSG treatment is also reported to disrupt daily rhythms of food intake and activity; studies consistently show an attenuation of the day–night rhythm of food intake [10,11,33,56,57]. Similar effects have been

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reported in rats sustaining electrolytic lesions of the AN [38], and complete loss of circadian organization of activity has been reported in voles following AN lesions that spare the SCN [20]. These results suggest that the AN may be a conduit through which the circadian system imposes daily rhythms on ingestive behaviors and metabolism.

Consistent with this idea, the AN has direct and indirect anatomical links with the SCN circadian pacemaker [24,29]. AN–NPY cells project to sites in the hypothalamus that regulate feeding and metabolism [29] and AN–NPY release exhibits a daily rhythm that peaks early in the night, when spontaneous feeding activity is also maximal [1,25]. Exogenous NPY is the most potent orexigen yet discovered, and maximally stimulates food intake early in the night [55]. Attenuation of daily variations in hypothalamic NPY by continuous intraventricular NPY infusion dampens the daily rhythm of food intake [4].

Although these observations converge on a hypothesis that AN–NPY cells may be an integral component of one pathway through which the circadian pacemaker regulates the timing of feeding behavior, studies to date have presented a relatively limited analysis of the nature of disrupted ingestive rhythms following neonatal MSG exposure. Daily rhythms can be characterized by their period, phase and amplitude. In nocturnal animals, environmental light regulates these parameters in two ways, first, by entraining the pacemaker generating the rhythms (i.e., period and phase control), and, second, by directly inhibiting activity, a process known as photic masking [3,44]. Direct inhibition of activity by light (negative masking), and stimulation of activity by dark (positive masking), functions to accentuate daily rest–activity rhythms driven by the circadian pacemaker (direct effects of light and dark on arousal are reversed in diurnal animals) [7,8,19]. These effects can be revealed by a comparison of rhythm amplitude in standard LD cycles (e.g., a 12 h photoperiod) with amplitude in non-standard ‘skeleton’ photoperiods (e.g., 30 min of light twice a day, simulating dawn and dusk) or in constant dark (DD). Rhythm phase and waveform may also be influenced by characteristics of the LD cycle, including the light intensity and waveform of the LD cycle (in laboratory settings, typically a square wave).

Although MSG kills retinal ganglion cells and greatly reduces the size of the optic nerves in rats, hamsters and mice, sufficient retinal circuitry is retained to mediate entrainment of circadian rhythms to standard LD cycles [9,14,45,48,49]. However, it is conceivable that photic pathways necessary for the direct masking effects of light are functionally impaired, and that attenuation of LD-entrained rhythms of food intake in MSG-treated rats is due to reduced photic masking of behavior. Therefore, in the first part of this study, to evaluate the contribution of direct and indirect effects of light to daily feeding rhythms in MSG-treated rats, we measured circadian parameters of food intake and feeding-related activity in LD (standard 12:12 and ultradian 2:2 schedules) and DD.

In the second part of this study, we evaluated the effects of MSG treatment on entrainment of circadian FAA rhythms. Endogenous NPY release is enhanced during food deprivation and increases prior to a scheduled daily meal, and exogenous NPY induces a true motivation to feed (i.e., operant responses to acquire food) [27,28,58]. These functional correlates of AN NPY activation may be taken to suggest that the AN, in addition to interfacing with the light-entrainable SCN pacemaker, may also play a role in regulating daily rhythms of FAA that emerge during restricted feeding schedules. Previous studies have shown that adaptation to a 4 h diurnal feeding schedule, measured in terms of daily meal size, correlates with entrainment of FAA [6] and is impaired in MSG-treated mice [35]. In addition, food-entrained behavioral rhythms (expressed as anticipatory activity directed at a food bin) are significantly enhanced in the Zucker fatty rat [43], a genetic model of obesity in which the AN–NPY system is up-regulated [13]. We have previously observed that some rats sustaining very large medial hypothalamic lesions that destroy the AN, among other structures, fail to exhibit an entrained pattern of FAA to a scheduled daily mealtime [2]. Therefore, in the present study, we also evaluated whether MSG lesions alter entrainment of anticipatory activity rhythms to daily feeding schedules.

2. Materials and methods

2.1. Subjects, drug treatment and apparatus

Six male Wistar rats received subcutaneous injections of MSG (2 mg/g, 100 g/ml) on postnatal days 1, 3, 5, 7 and 9. The animals were housed in a colony room until 6 weeks of age when they, along with six age-matched, vehicle-injected control rats (one female), were transferred to individual polypropylene cages in ventilated isolation cabinets. Cages were equipped with wire floors and internal food bins designed to monitor feeding-related activity. Contact with the feeder closed a circuit, producing a 5 V pulse counted by a PC-based data collection system that saved counts to disc at 10 min intervals (Activity Counting System, Simon Fraser University). During the restricted feeding schedule, food was available in a bin mounted on the outside of the cage, accessible through a 7 cm square window. A photocell was positioned across the food-bin to detect feeding-related activity.

2.2. Procedures

Food-bin activity was recorded for 10 days in LD (14:10, i.e., 14 h light, 10 h dark) and then 22 days in LD 12:12, with food (Purina lab chow 5001) and water available *ad libitum*. Food intake was then measured every 3 h for 24 h, by weighing the food bins and underlying spillage. Five days later, the lights were turned off for 48 h

of DD, beginning at the usual time of dark onset. Food intake was measured every 3 h for 24 h, beginning at the usual time of lights-on. Following 3 days of re-entrainment to LD, the 48 h DD test was repeated. After another 4 days of re-entrainment to LD, the LD cycle was shortened to 2:2 (2 h light, 2 h dark), with the first 2 h dark period beginning at the usual time of dark-onset. LD 2:2 was maintained for 24 h, after which LD 12:12 was reinstated for the balance of the study. Five days later the rats were food deprived for 42 h beginning at dark-onset. For the next 30 days, food was provided for 4 h each day in the middle of the light period, beginning 7 h before dark onset. The animals were then food deprived for 72 h, provided with food ad libitum for 5 days, and then food deprived for a final 72 h, after which they were sacrificed for histological analysis to confirm MSG neurotoxicity.

2.3. Histology

The rats were anesthetized with sodium pentobarbital (65 mg/kg) and received an intra-lateral ventricular injection of colchicine (75 μ g in 25 μ l phosphate buffered saline, PBS) using stereotaxic procedures. After 24 h, the rats were overdosed with pentobarbital and perfused transcardially with 100 ml cold PBS, followed by 100 ml 4% paraformaldehyde. The brains were removed and soaked overnight in 4% paraformaldehyde, after which they were transferred for 24 h to a 20% sucrose solution for cryoprotection. The brains were then sectioned at 50 μ m intervals using a freezing stage microtome, and alternate sections were saved for NPY immunocytochemistry.

Slices were washed in a 0.3% H_2O_2 bath to inactivate endogenous peroxidase. This was followed by three 10-min baths in PBS and 0.2% Triton X-100 (PBSx). Sections were incubated for 90 min in 10% normal goat serum (Vector Laboratories) and then for 48 h at 4°C in a polyclonal rabbit anti-NPY antibody (1:15,000; Peninsula Laboratories). The tissue was then rinsed in PBSx (3 $\times$ 10 min) and incubated in secondary goat anti-rabbit antibody (Vector Laboratories; Vectastain Elite ABC Kit) for 1 h at room temperature. Following another three 10-min washes in PBSx, the tissue was incubated in avidin–biotin–peroxidase complex and was visualized using 3% H_2O_2 , 0.05 M diaminobenzidine (DAB), and 1% NiCl₂ in Tris buffer (pH = 7.2). The DAB reaction was terminated by washing after 5 min. The tissue was then mounted on gelatin-coated slides, dried and coverslipped. After an initial evaluation of NPY immunoreactivity (IR) by light microscope, the tissue was counterstained with Neutral Red Nissl stain.

2.4. Data analysis

Activity data were transferred to a Macintosh computer for display and analysis. Group mean average waveforms of food-bin activity and food intake were constructed for MSG and saline-treated rats in LD 12:12 and on the first

day of DD. The waveforms revealed that the phase of the daily rhythms of food intake and food-bin activity did not differ between these days. Therefore, rhythm amplitude was quantified by calculating 12:12 ratios in both LD and on the first full day of DD (the first DD test was used for food intake data, and the second DD test for food-bin activity). Ratios were also calculated for total food-bin activity in the 2 h light and 2 h dark periods during the LD 2:2 cycle. Ratios were also expressed as ‘nocturnality’ scores (percentage of total food intake occurring in the dark).

FAA was quantified by dividing the 30 days of restricted feeding into six blocks of 5 days. For each rat, an anticipation ratio (cumulative activity during the 3 h before mealtime divided by total daily activity, excluding the 4 h mealtime) was calculated for each block of days. The duration of anticipation was quantified by defining activity onset as the first of three consecutive 10 min bins within 3

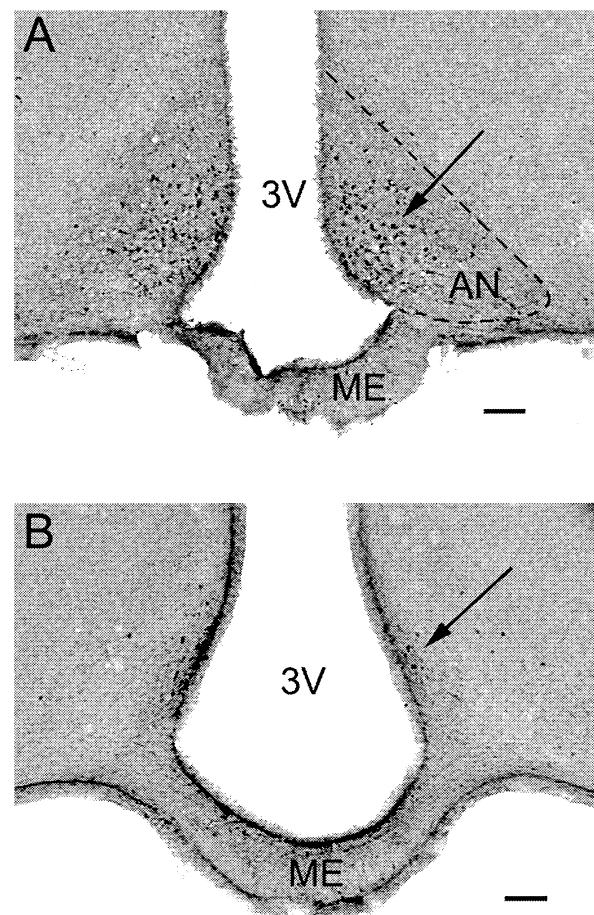


Fig. 1. Photomicrographs of NPY-IR in the AN of a representative control (A) and MSG-treated rat (B). In the control brain, the AN exhibits a uniform NPY-IR fiber plexus, the borders of which are denoted by the dashed line. NPY-IR cell bodies are labeled black, and cluster in the medial region of the AN. In the MSG-treated animal, the NPY-IR fiber plexus is absent, and only a few labeled cells are apparent close to 3rd ventricle (3V), and scattered in the median eminence (ME). The scale bar denotes 100 μ m.

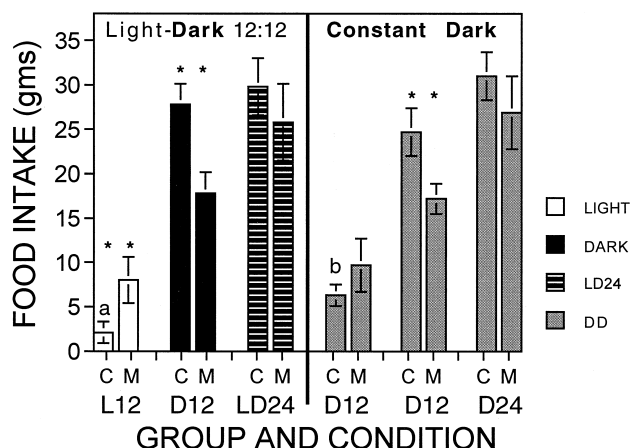


Fig. 2. Average food intake in the 12 h light and 12 h dark periods in LD, and for the same 12 h intervals in DD, in control (C) and MSG-treated (M) rats. ** Denote significant difference between groups ($p < 0.001$). a,b Denote significant difference within groups ($p < 0.05$).

h of feeding time that exceeded the 24 h mean level of activity for that block of days. The peak level of anticipatory activity was defined as the maximal value observed in any 10 min bin following the onset of anticipation as defined. The persistence of the FAA rhythm during the food deprivation tests was evaluated by averaging the deprivation days within each 72 h test and calculating the mean level of activity during the 3 h corresponding to prior mealtime and the 3 h time-blocks that immediately preceded and followed prior mealtime. These three means were then expressed as ratios with respect to total daily activity. For comparative purposes, the same calculations were made for the 5 days of ad libitum feeding between deprivation tests.

Within and between group differences were evaluated by ANOVA and *t*-tests. Results for the single female rat in the control group were not distinguishable from those of

the male rats, thus the data were combined. Means are reported $\pm$ S.D.

3. Results

3.1. Histology

Visual inspection of Nissl stained sections revealed a striking reduction in the size of the optic nerves, optic chiasm and optic tracts in all MSG-treated rats. The AN exhibited a paucity of Nissl-stained neurons by comparison with control rats. Cell counts revealed a marked reduction of NPY-IR cells and fibers in the mediobasal region of the hypothalamus, caudal to the SCN (60% decrease, by comparison with control rats). In control rats, AN-NPY cells were clustered in the ventromedial, periventricular region of the AN, and these cells were virtually absent in MSG treated rats (e.g., Fig. 1a,b). The distribution and density of NPY-IR in the SCN appeared normal in all MSG rats.

3.2. Daily rhythms of food intake

Food intake was measured at 3 h intervals for 1 day in LD and 1 day in DD. There was a trend for lower food intake in MSG rats, compared to control rats, in both LD (-13.4% , $p = 0.06$) and DD (-13.6% , $p = 0.1$; Fig. 2). Both groups exhibited significant daily rhythms of food intake in LD, with food consumption greater in the dark than in the light ($p < 0.001$ for both groups; Fig. 2). The shape of the waveform was similar between groups, and there was no apparent difference in phase (i.e., timing with respect to the LD cycle; Fig. 3a). However, MSG rats ate 280% more than control rats in the light period ($p < 0.001$) and 36% less in the dark period ($p < 0.001$) and consequently had a significantly lower LD ratio (LD ratios = 0.07 ± 0.04 vs. 45 ± 0.13 , in control and MSG groups, respectively, which correspond to nocturnality scores of

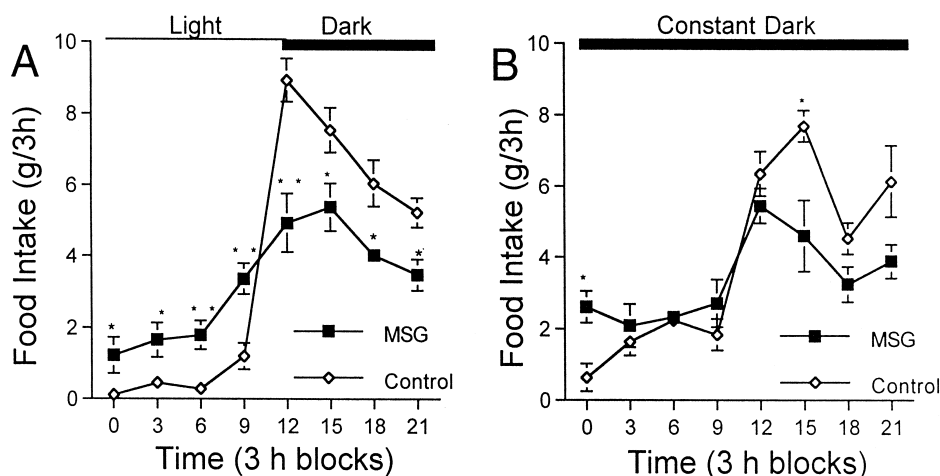


Fig. 3. Group mean waveforms of food intake in MSG (filled squares) and control (open diamonds) rats measured at 3 h intervals in LD 12:12 (A) and DD (B). Differences between groups significant at $p < 0.05$ (*) or $p < 0.005$ (**).

93% and 69%, respectively; $p < 0.001$). In DD, the shape and phase of the circadian waveforms of food intake were again quite similar in the two groups (Fig. 3b). However, control rats showed a significant reduction in the 12:12 ratio of food intake compared to the ratio in LD (DD ratio = 0.26 ± 0.06 , ‘nocturnality’ = 80%; $p < 0.001$), whereas MSG rats did not show a significant change in this ratio (DD ratio = 0.54 ± 0.16 , ‘nocturnality’ = 66%;

$p > 0.05$; Fig. 2). Group differences in food intake were evident at only two time points in DD, rather than at all eight time points as in LD (Fig. 3b).

3.3. Daily rhythms of food-bin activity

Food-bin activity records from representative control and MSG-treated rats are presented in Fig. 4. Activity

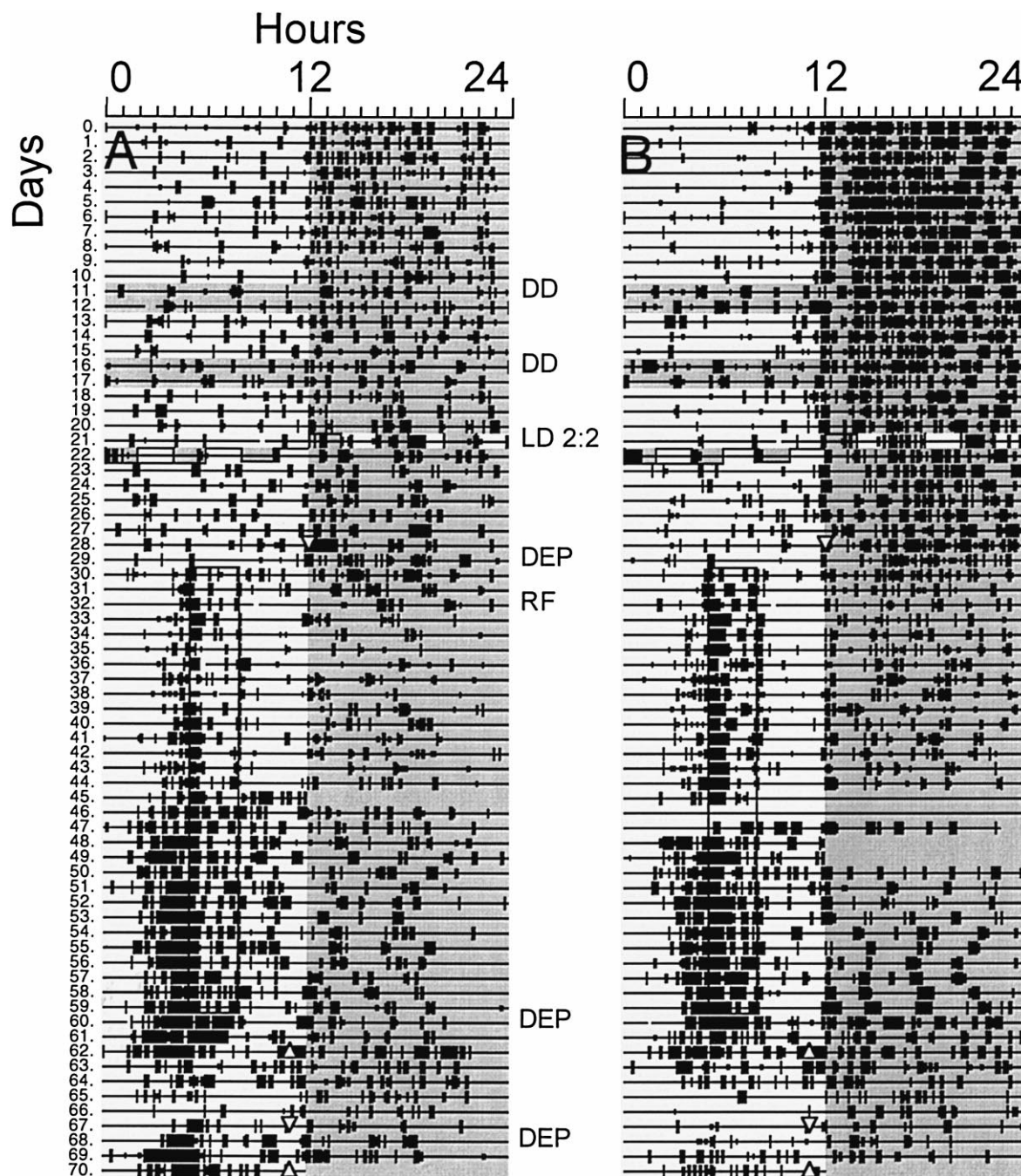


Fig. 4. Actograms depicting food-bin activity in representative MSG-treated (A) and control (B) rats. Each line represents 1 day, with time in 10 min bins plotted from left to right and consecutive days aligned vertically. Vertical deflections on the lines indicate time bins when food-bin counts were recorded. Shading represents lights-off. The vertical column beginning on day 31 represents the 4 h mealtime during restricted feeding. Inverted triangles represent the beginning of food deprivation tests, and upright triangles the end of these tests. Abbreviations: DD (constant dark), LD 2:2 (2 h light–2 h dark cycle), DEP (no food available), RF (restricted feeding schedule).

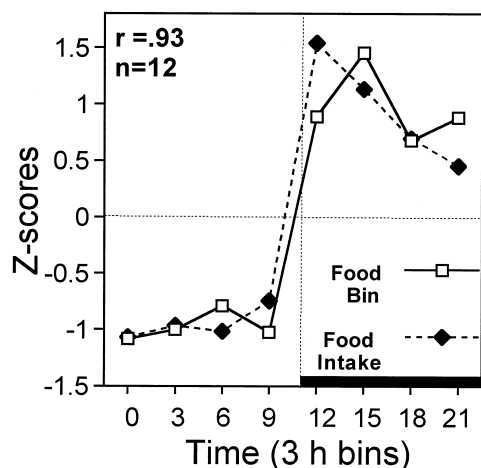


Fig. 5. Normalized scores for food intake (dotted line) and food-bin activity (solid line) accumulated in 3 h intervals in LD 12:12 ($N = 12$ rats, both groups combined).

counts during ad libitum food access were registered whenever rats touched the feeder and thus all counts do not necessarily represent feeding bouts. To evaluate the extent to which food-bin activity mirrored feeding activity, food-bin and food intake data were converted to Z-scores and plotted in 3 h bins. The two curves are highly correlated (Pearson bivariate correlation $r = 0.93$ for both

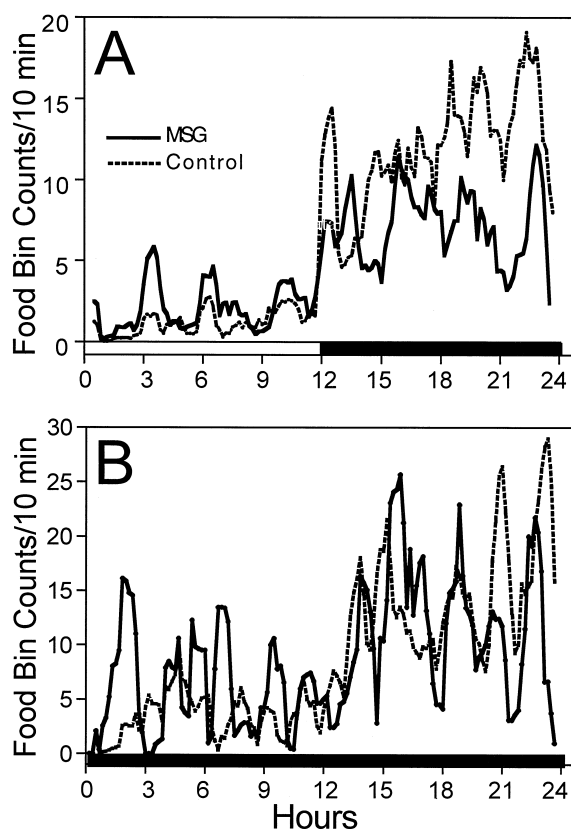


Fig. 6. Group mean average waveforms of food-bin activity in control (dotted lines) and MSG-treated (solid lines) rats in LD (A) and DD (B). Lights-off is denoted by the heavy bar along the abscissa.

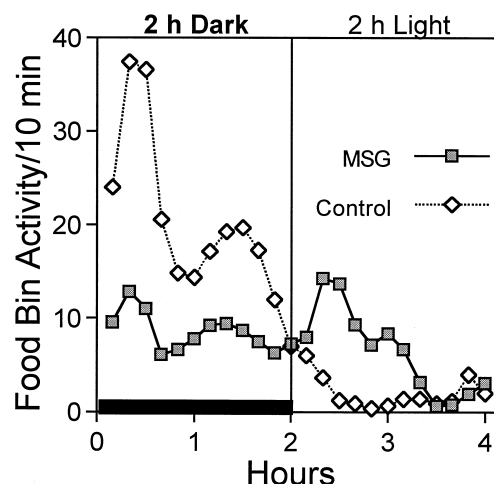


Fig. 7. Group mean average waveforms of food-bin activity in control and MSG-treated rats in LD 2:2. Activity data were averaged at a folding period of 4 h (2 h light, 2 h dark).

groups combined, $p < 0.001$; Fig. 5), indicating that daily rhythms of food-bin counts do accurately reflect variations in feeding activity.

Food-bin activity in each rat was averaged over 5 days of LD prior to the DD tests. The MSG rats had a significantly lower LD ratio of activity (0.50 ± 0.19 vs. 20 ± 0.12 , respectively, $p < 0.05$), whereas in DD, the ratios did not differ significantly between groups (0.48 ± 0.34 vs. 37 ± 0.15 , respectively, $p > 0.05$; Fig. 6a,b). The 12:12 ratios did not differ between LD and DD in the MSG group, but were significantly lower in DD than in LD in the control group ($p < 0.05$).

3.4. Food-bin activity in LD 2:2

During LD 2:2, control rats showed significantly less food-bin activity during the 2 h light periods compared to the 2 h dark periods (LD ratio = 0.21 ± 0.27 , $p < 0.001$).

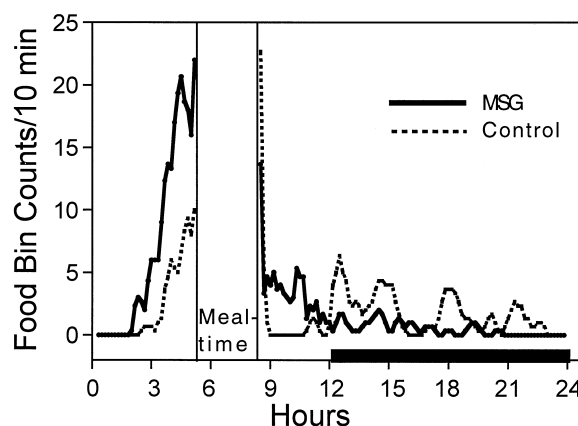


Fig. 8. Group mean average waveforms of food-bin activity during the last 6 days of restricted feeding in control (dashed line) and MSG-treated (solid line) rats. Mealtime is denoted by the opaque box and lights-off by the heavy bar along the abscissa.

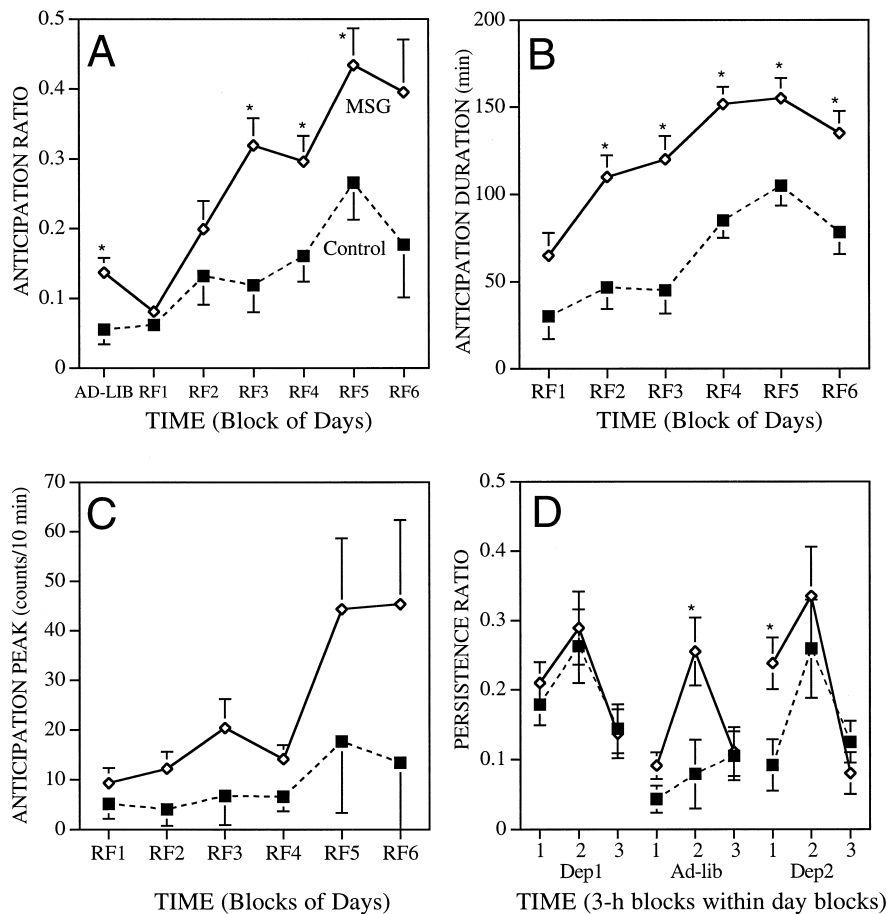


Fig. 9. Parameters of FAA and its persistence during food deprivation in control (dashed lines) and MSG-treated (solid lines) rats. (A) Group mean anticipation ratios during ad libitum feeding (AD-LIB) and six consecutive 5-day blocks of restricted feeding (RF1–RF6). (B) Mean duration of anticipatory activity. (C) Peak level of anticipatory activity per 10 min bin. (D) Persistence ratios during the first (Dep1) and second (Dep2) 3 day blocks of food deprivation after restricted feeding, and the intervening 5 day block of ad libitum feeding (Ad-lib). Within each of these blocks of days, the ratios of activity as a percentage of total daily activity were calculated for hours 2–4 (1), 5–8 (2) and 9–11 (3) of the light period. Mealtime during restricted feeding corresponded to hours 5–8. Significance of group mean differences: * $p < 0.03$, ** $p < 0.01$.

MSG rats did not show a significant difference in mean activity levels in the light and dark periods (LD ratio = 0.99 ± 0.24 ; Fig. 7).

3.5. Food-bin activity during 42 h food deprivation

During the 42 h food deprivation that preceded the daily feeding schedule, food-bin activity was decreased, on average, by 45% in control rats ($p < 0.05$) and by 23% in MSG rats ($p > 0.05$), by comparison with average activity levels during the preceding 3 days of free food access. Activity levels did not differ significantly between groups during deprivation.

3.6. FAA during restricted daily feeding

Inspection of actograms of food-bin activity revealed that all MSG and control rats exhibited FAA by the third week of restricted daytime feeding and that this rhythm persisted for the three circadian cycles (72 h) of food deprivation (Fig. 4a,b and 8). FAA was typically evident within the first 7–10 days of restricted feeding and ap-

peared to stabilize in duration by about the third week. Quantitative comparisons between groups revealed that both the ratio of FAA to total daily activity (anticipation ratio, excluding mealtime; Fig. 9a) and the duration of FAA (b) were significantly greater in the MSG rats during most of the time points. Peak levels of activity/10 min during FAA were greater in MSG rats, but this was statistically only a trend (Fig. 9c). Persistence of the mealtime associated activity in the two groups was equivalent during the 72 h bouts of food deprivation, but was more robust in the MSG rats during the 5 days of ad libitum food access separating the deprivation tests (Fig. 9d).

4. Discussion

4.1. MSG eliminates photic masking of feeding activity

Neonatal treatment with MSG has been shown to reduce the amplitude of LD-entrained rhythms of food intake in rats and mice [10,11,33,56,57]. However, the contribu-

tion of direct and indirect effects of light on feeding rhythms in MSG-treated rats has not previously been assessed. In the present study, we calculated 12:12 ratios of food intake in LD and during the first full day of DD, and found that about 14% of the amplitude of LD-entrained feeding rhythms in intact Wistar rats was attributable to a direct ‘masking’ effect of light on behavior (food intake was 93% nocturnal in LD and 80% ‘nocturnal’ in DD). All of the MSG rats entrained to LD 12:12, but the direct effects of light and dark on food intake and activity were absent, as revealed by a comparison of 12:12 ratios of food intake in LD and DD (69% vs. 66% ‘nocturnality’, respectively). In addition, feeding related activity was significantly attenuated during the light portion of a 4 h LD cycle in the control rats but not in the MSG rats, which showed LD 2:2 ratios not significantly different from 1.0. These analyses indicate that much of the effect of MSG on feeding rhythms is a result of reduced photic masking of behavior.

Reduced photic masking likely results, at least in part, from MSG-induced loss of retinal ganglion cells and a consequent reduction in total photic efference to effector systems regulating feeding activity. This may be compounded by MSG-induced loss of cells downstream from the retina that mediate the direct masking effects of light on behavior. Two components of the circadian system, the SCN and the intergeniculate leaflet, are retinorecipient and essential for photic entrainment, but neither are necessary for photic masking of behavior [e.g., Refs. [15,26,40]]. Recent work has implicated two other retinorecipient areas, the superior colliculus and pretectum (SC–PT), as mediators of the direct masking effects of light on behavioral state in rats [39]. MSG treatment greatly reduces retinal input to the SC and has been reported to damage its superficial layers [48,53]. Our finding that neonatal MSG eliminates photic masking of feeding activity is consistent with a primary role for the SC–PT in this behavioral effect of light.

Our results differ from one previous study that evaluated the contribution of masking to daily rhythms of feeding in rats [51]. In that study, the amplitude of the daily feeding rhythm, assessed by spectral analysis and visual inspection of waveforms, did not differ across three lighting conditions, including a full photoperiod (12 h light), a skeleton photoperiod (two daily 30 min light pulses simulating dawn and dusk), and DD. The conclusion, therefore, was that direct masking effects of light do not contribute to the amplitude of daily feeding rhythms in rats. However, other studies, including the present, have shown that direct effects of light can modulate the amplitude of some rhythmic variables, including feeding, body temperature, sleep and activity [e.g., Refs. [7,50]]. Differences across rat studies may be related to the light intensities used (e.g., 10 lx of light in Ref. [51], 40 lx in our study), the measure of food intake (e.g., lever pressing in Ref. [51], food weights and food-bin activity in our study)

and the strain of rat (pigmented Long–Evans in Ref. [51], albino Wistars in our study). Additional work will be necessary to evaluate the significance of these methodological factors within and between species.

4.2. *MSG attenuates feeding rhythms independent of its effects on photic masking*

Although elimination of masking effects of light by MSG treatment can explain much of the difference between feeding rhythms in MSG-treated and control rats in LD, it cannot explain the persisting, albeit attenuated, group difference in the amplitude of the feeding rhythm in DD. By comparison with control rats, MSG-treated rats exhibited a significantly lower 12:12 activity ratio in DD, indicating that MSG treatment can compromise the expression of feeding rhythms independent of its effect on photic inhibition of behavior. This could be due to damage to SCN clock cells driving these rhythms or to cells downstream from the clock that regulate food intake and are modulated by circadian pacemaker output. SCN volume in rodents is reported to be either apparently normal [9,49] or slightly reduced [45] following neonatal MSG treatment. However, degenerating cells have not been detected and volume changes may largely reflect the reported 30% reduction of retinohypothalamic neuropil in the SCN [9]. Moreover, partial SCN lesions generally have little effect on overt rhythms unless most of the nucleus is destroyed [e.g., Ref. [22]]. These results suggest that the attenuated amplitude of the feeding rhythm in DD is caused by damage to structures downstream from the SCN.

MSG toxicity is far more severe in the AN, where the entire cell population is greatly reduced. NPY neurons in particular are heavily depleted or eliminated [Ref. [37], present study]. Given the wide range of evidence that AN–NPY cells regulate feeding and exhibit daily rhythms of neural activity, it seems reasonable to infer that damage to these cells is responsible for attenuated feeding rhythms in MSG-treated rats in DD. Taken together, these data support a hypothesis that the AN may normally contribute to the circadian organization of ingestive behavior. However, given that significant daily rhythms of feeding and food-bin activity do persist in MSG-treated rats in both LD and for at least 2 days in DD, the role of the AN is likely one of modulation and not mediation. The circadian system may well employ redundant pathways to regulate the timing of vital behavioral functions such as feeding and foraging.

4.3. *The AN may modulate but does not mediate FAA rhythms*

We have previously noted that very large medial hypothalamic lesions can eliminate food-entrained anticipatory activity rhythms in rats [2]. The survival rate from lesions this large is low, but rats that did survive exhibited extensive damage to the AN. Other structures damaged, including the SCN, PVN, POA, AH and VMH, have

previously been shown to be unnecessary for the expression of food-entrained rhythms, suggesting that the AN might be the critical locus for the effect of these lesions on FAA [41]. However, not only was FAA present in all MSG-treated rats, it was in some respects more robust by comparison with control rats. MSG rats displayed higher amplitude FAA (anticipation ratios) during restricted feeding and a stronger persistence of FAA rhythms during the 5 days of ad libitum food access that separated the two 72 h food deprivation tests (persistence ratios). The duration of FAA was also significantly greater in MSG rats, although that may be more reasonably conceptualized as a measure of the phase angle of FAA entrainment rather than of FAA ‘robustness’.

In a previous study, we found a very similar accentuation of FAA parameters in obese Zucker rats compared to their lean littermates [43]. AN–NPY activity is up-regulated in obese Zucker rats, due to a mutation in the receptor for the adipocyte protein hormone leptin, which is secreted in proportion to body fat levels, and which regulates feeding and metabolism at least in part by actions on AN–NPY cells [12,13,28]. It appears from our two studies that rats with either genetically up-regulated NPY or neurotoxin down-regulated NPY exhibit the same accentuated FAA phenotype. In both models leptin signaling is disrupted, and FAA is enhanced. In dietary-induced obesity, leptin signaling is intact, and FAA is strongly attenuated [47]. These results together suggest that the generation and entrainment of FAA rhythms is not dependent on AN–NPY cells, but that the amplitude of these rhythms is modulated by leptin feedback to the AN, i.e., leptin signaling at the AN likely mediates the inhibitory effect of body fat on FAA.

Food seeking behavior is enhanced by both food deprivation and by hypothalamic NPY microinjections, and food deprivation is associated with an increase in AN–NPY release [27,28]. Conceivably, AN–NPY cells may mediate the hyperactivity induced by food deprivation. MSG-treated rodents have typically been reported to be hypoactive, and may fail to increase food intake following a bout of food deprivation [10,35]. However, by our measure of food-bin activity, hypoactivity in MSG rats was evident only as a statistical trend. In addition, we saw a general decrease of activity, rather than an increase, in both groups during food deprivation. The effects of deprivation on activity in rodents may depend on the behavior measured; wheel running activity typically increases substantially during deprivation, but general cage activity is less affected [6,41]. The lack of group difference in this measure of activity during food deprivation may therefore be considered inconclusive regarding a role for AN–NPY in the induction of hyperactivity by food deprivation.

4.4. MSG-induced obesity and circadian rhythms

In rodents, neonatal exposure to MSG produces obese adult animals. This is due in large part to loss of AN cells

that mediate negative feedback regulation of body weight provided by leptin. [12,28]. Another factor that may underlie obesity in MSG-treated rodents is disrupted circadian rhythmicity of food intake. Attenuation of daily rhythms of food intake appears to be a common feature in animal models of obesity [e.g., Refs. [16,17,21,23,42]], while in humans obesity has been associated with attenuated diurnal rhythms of plasma leptin and increased food consumption late in the day [5,18,36,52,54]. One study of obese Zucker rats showed that if excessive daytime feeding is prevented without significantly reducing total daily food intake, weight gain is attenuated by about 20% [42]. Similar effects were not obtained in rats made obese by VMH lesions [21], and it remains to be determined whether altered circadian timing plays a role in the development of obesity following neonatal MSG exposure.

4.5. Conclusions

The present study demonstrates that daily rhythms of food intake and feeding-related activity reflect direct behavioral responses to light and dark added to drive from light- and food-entrainable circadian pacemakers. These influences are presumably integrated with homeostatic and other non-homeostatic factors to regulate the expression of daily cycles of hunger and satiety. Our study illustrates that some interventions that attenuate circadian organization of feeding may do so at least in part by interfering with direct photic regulation of behavior, a factor that has often been overlooked in research on neuroendocrine mechanisms of feeding rhythmicity. The present study also provides evidence that the AN may be one pathway through which light-entrainable and food-entrainable pacemakers modulate feeding-related activities. The AN may have an essential role in mediating the effects of body fat on the expression of food anticipatory rhythms, but in other aspects of circadian regulation of feeding the AN likely plays a modulating rather than mediating role.

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References

- [1] A. Akabayashi, N. Levin, X. Paez, J.T. Alexander, S.F. Leibowitz, Hypothalamic neuropeptide Y and its gene expression: relation to light/dark cycle and circulating corticosterone, *Mol. Cell. Neurosci.* 5 (1994) 210–218.
- [2] M.C. Antle, E.G. Marchant, R.E. Mistlberger, Hypothalamic lesions and absence of food anticipation: role of the arcuate nucleus, *Soc. Res. Biol. Rhythms Abstr.* 4 (1996) 111.

- [3] J. Aschoff, S. Daan, K.I. Honma, Zeitgebers, entrainment and masking: some unsettled questions, in: J. Aschoff, S. Daan, G.A. Groos (Eds.), *Vertebrate Circadian Systems*, Springer-Verlag, New York, 1982, pp. 13–24.
- [4] B. Beck, A. Stricker-Krongrad, J.P. Nicolas, C. Burlet, Chronic and continuous ICV infusion of neuropeptide Y disrupts the nycthemeral feeding patterns in rats, *Ann. N.Y. Acad. Sci.* (1990) 491–494.
- [5] F. Belisle, M.F. Rolland-Cachera, M. Deheeger, M. Guillaud-Bataille, Obesity and food intake in children: evidence for a role of metabolic and/or behavioral daily rhythms, *Appetite* 11 (1988) 111–118.
- [6] R.A. Boakes, Wheels, clocks, and anorexia in the rat, in: M.E. Bouton, M.S. Fanselow (Eds.), *Learning, Motivation, and Cognition. The Functional Behaviorism of Robert C. Bolles*, American Psychological Association, Washington DC, 1997, pp. 163–176.
- [7] A.A. Borbely, Effects of light on sleep and activity rhythms, *Prog. Neurobiol.* 10 (1978) 1–31.
- [8] S.S. Campbell, D.J. Dijk, Z. Boulos, C.I. Eastman, A.J. Lewy, M. Terman, Light treatment for sleep disorders: consensus report: III. alerting and activating effects, *J. Biol. Rhythms* 10 (1995) 129–132.
- [9] I. Chambille, J. Serviere, Neurotoxic effects of neonatal injections of monosodium L-glutamate (L-MSG) on the retinal ganglion cell layer of the golden hamster: anatomical and functional consequences on the circadian system, *J. Comp. Neurol.* 338 (1993) 67–82.
- [10] R. Dawson, J.F. Lorden, Behavioral and neurochemical effects of neonatal administration of monosodium L-glutamate in mice, *J. Comp. Physiol. Psychol.* 95 (1981) 71–84.
- [11] R. Dawson, D.R. Wallace, A pharmacological analysis of food intake regulation in rats treated neonatally with monosodium L-glutamate (MSG), *Pharmacol. Biochem.* 32 (1989) 391–398.
- [12] R. Dawson, M.A. Pellemounter, W.J. Millard, S. Liu, B. Eppler, Attenuation of leptin mediated effects by monosodium glutamate-induced arcuate nucleus damage, *Am. J. Physiol.* 273 (1997) E202–E206.
- [13] S. Dryden, L. Pickavance, H.M. Frankish, G. Williams, Increased neuropeptide Y secretion in the hypothalamic paraventricular nucleus of obese (fa/fa) Zucker rats, *Brain Res.* 690 (1995) 185–188.
- [14] K. Edelstein, J.G. Pfaus, B. Rusak, S. Amir, Neonatal monosodium glutamate treatment prevents effects of constant light on circadian temperature rhythms of adult rats, *Brain Res.* 675 (1995) 135–142.
- [15] K. Edelstein, S. Amir, The role of the intergeniculate leaflet in entrainment of circadian rhythms to a skeleton photoperiod, *J. Neurosci.* 19 (1999) 372–380.
- [16] K. Fukagawa, T. Sakata, H. Yoshimatsu, K. Fujimoto, T. Shiraishi, Disruption of light–dark cycle of feeding and drinking behavior, ambulatory activity induced by development of obesity in the Zucker rat, *Int. J. Obes.* 12 (1988) 481–490.
- [17] K. Fukagawa, T. Sakata, H. Yoshimatsu, K. Fujimoto, K. Uchimura, C. Asana, Advance shift of feeding circadian rhythm induced by obesity progression in Zucker rats, *Am. J. Physiol.* 263 (1995) R1169–1175.
- [18] J. Fricker, S. Giroux, F. Fumeron, M. Apfelbaum, Circadian rhythm of energy intake and corpulence status in adults, *Int. J. Obes.* 14 (1990) 387–393.
- [19] P.H. Gander, M.C. Moore-Ede, Light–dark masking of circadian temperature and activity rhythms in squirrel monkeys, *Am. J. Physiol.* (1983) R927–R934.
- [20] M.P. Gerkema, G.A. Groos, S. Daan, Differential elimination of circadian and ultradian rhythmicity by hypothalamic lesions in the common vole, *Microtus arvalis*, *J. Biol. Rhythms* 5 (1990) 81–95.
- [21] R.M. Gold, G. Sumpster, H. Ueberacher, G. Kapatos, Hypothalamic hyperphagia despite imposed diurnal or nocturnal feeding and drinking rhythms, *Physiol. Behav.* 14 (1974) 861–865.
- [22] M.E. Harrington, T. Rahmani, C.A. Lee, The effects of damage to suprachiasmatic nucleus neurons and efferent pathways on circadian activity rhythms of hamsters, *Brain Res. Bull.* 30 (1993) 655–669.
- [23] A. Ho, A. Chin, Circadian feeding and drinking patterns of genetically obese mice fed solid chow diet, *Physiol. Behav.* 43 (1988) 651–656.
- [24] T.L. Horvath, Suprachiasmatic efferents avoid phenestrated capillaries but innervate neuroendocrine cells, including those producing dopamine, *Endocrin.* 138 (1997) 1312–1320.
- [25] M. Jhanwar-Uniyal, B. Beck, C. Burlet, S.F. Leibowitz, Diurnal rhythm of neuropeptide Y-like immunoreactivity in the suprachiasmatic, arcuate and paraventricular nuclei and other hypothalamic sites, *Brain Res.* 536 (1990) 331–334.
- [26] R.F. Johnson, R.Y. Moore, L.P. Morin, Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract, *Brain Res.* 460 (1988) 297–313.
- [27] D.C. Jewett, J. Cleary, A.S. Levine, D.W. Schaal, T. Thompson, Effects of neuropeptide Y, insulin, 2-deoxyglucose, and food deprivation on food-motivated behavior, *Psychopharmacologia* 120 (1995) 267–271.
- [28] S.P. Kalra, M.G. Dube, A. Sahu, C.P. Phelps, P.S. Kalra, Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food, *Proc. Natl. Acad. Sci. U.S.A.* 88 (1991) 10931–10935.
- [29] S.P. Kalra, M.G. Dube, S. Pu, B. Xu, T.L. Horvath, P.S. Kalra, Interacting appetite-regulating pathways in the hypothalamic regulation of bodyweight, *Endocr. Rev.* 20 (1999) 68–100.
- [30] J.S. Kizer, C.B. Nemeroff, W.W. Youngblood, Neurotoxic amino acids and structurally related analogs, *Pharmacol. Rev.* 29 (1978) 301–318.
- [31] D.C. Klein, R.Y. Moore, S.M. Reppert (Eds.), *Suprachiasmatic Nucleus: The Mind's Clock*, Oxford Univ. Press, New York, 1991.
- [32] S.F. Leibowitz, Neurochemical–neuroendocrine systems in the brain controlling macronutrient intake and metabolism, *Trends Neurosci.* 15 (1992) 491–497.
- [33] J.F. Lorden, A. Caudle, Behavioral and endocrinological effects of single injections of monosodium glutamate in the mouse, *Neurobehav. Toxicol. Teratol.* 8 (1986) 509–519.
- [34] D.R. Lucas, J.P. Newhouse, The toxic effect of sodium L-glutamate on the inner layers of the retina, *Arch. Ophthalmol.* 58 (1957) 193–201.
- [35] V. Ma, A. Ma, D.P. Cameron, Regulation of feeding behavior in monosodium glutamate (MSG) treated mice, *Life Sci.* 42 (1988) 1419–1424.
- [36] V. Matkovic, J.Z. Ilich, N.E. Badenhop, M. Skugor, A. Clairmont, D. Klisovic, J.D. Landoll, Gain in body fat is inversely related to the nocturnal rise in serum leptin level in young females, *J. Clin. Endocrinol. Metab.* 82 (1997) 1368–1372.
- [37] P. Meister, S. Ceccatelli, T. Hokfelt, M. Anden, E. Theodorsson, Neurotransmitters, neuropeptides and binding sites in the rat mediobasal hypothalamus: effects of monosodium glutamate (MSG) lesions, *Exp. Brain Res.* 76 (1989) 343–368.
- [38] M.J. Millan, M.H. Millan, L.D. Reid, A. Herz, The role of the mediobasal arcuate hypothalamus in relation to opioid systems in the control of ingestive behaviour in the rat, *Brain Res.* 381 (1986) 29–42.
- [39] A.M. Miller, W.H. Obermeyer, M. Behan, R.M. Benca, The superior colliculus–pretectum mediates the direct effects of light on sleep, *Proc. Natl. Acad. Sci. U.S.A.* 95 (1998) 8957–8962.
- [40] R.E. Mistlberger, Non-photic entrainment of circadian activity rhythms in suprachiasmatic nuclei ablated hamsters, *Behav. Neurosci.* 102 (1992) 192–202.
- [41] R.E. Mistlberger, Circadian food anticipatory activity: formal models and physiological mechanisms, *Neurosci. Biobehav. Rev.* 418 (1994) 171–195.
- [42] R.E. Mistlberger, H. Lukman, B.G. Nadeau, Circadian rhythms in the Zucker obese rat: assessment and intervention, *Appetite* 30 (1998) 255–267.
- [43] R.E. Mistlberger, E.G. Marchant, Enhanced food anticipatory circadian rhythms in the genetically obese Zucker rat, *Physiol. Behav.* 66 (1999) 329–335.

- [44] R.E. Mistlberger, B. Rusak, Circadian rhythms in mammals: formal properties and environmental influences, in: M.H. Kryger, T. Roth, W.C. Dement (Eds.), *Principles and Practice of Sleep Medicine*, 2nd edn., Saunders, Philadelphia, PA, 1994, pp. 277–285.
- [45] S. Miyabo, I. Yamamura, E. Ooya, N. Aoyagi, Y. Horikawa, S. Hayashi, Effects of neonatal treatment with monosodium glutamate on circadian locomotor rhythm in the rat, *Brain Res.* 339 (1985) 201–208.
- [46] J.W. Olney, Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate, *Science* (1969) 719–721.
- [47] J.E. Persons, F.K. Stephan, M.E. Bays, Diet-induced obesity attenuates anticipation of food access in rats, *Physiol. Behav.* 54 (1993) 55–64.
- [48] G.E. Pickard, F.W. Turek, A.A. Lamperti, A.J. Silverman, The effect of neonatally administered monosodium glutamate (MSG) on the development of retinofugal projections and the entrainment of circadian locomotor activity, *Behav. Neural Biol.* 34 (1982) 433–444.
- [49] W.J. Rietveld, J.H. Meijer, J. Ruis, P. Buys, The effect of neonatal administration of monosodium glutamate on the circadian control of food intake in the rat, *J. Interdiscip. Cycle Res.* 17 (1986) 327–334.
- [50] E.L. Robinson, C.A. Fuller, Light masking of circadian rhythms of heat production, heat loss, and body temperature in squirrel monkeys, *Am. J. Physiol.* 276 (1999) R298–R307.
- [51] A.M. Rosenwasser, Z. Boulous, M. Terman, Circadian organization of food intake and meal patterns in the rat, *Physiol. Behav.* 27 (1981) 33–39.
- [52] M.F. Saad, M.G. Riad-Gabriel, A. Khan, A. Sharma, R. Michael, S.D. Jinagouda, R. Boyadjian, G.M. Steil, Diurnal and ultradian rhythmicity of plasma leptin: effects of gender and adiposity, *Clin. Endocrinol. Metab.* 83 (1998) 453–459.
- [53] L. Seress, G. Lazar, B. Kosaras, R.T. Robertson, Regional effect of monosodium-L-glutamate on the superficial layers of superior colliculus in rat, *Cell Tissue Res.* 235 (1984) 453–457.
- [54] M.K. Sinha, J.P. Ohannesian, M.L. Heiman, A. Kriauciunas, T.W. Stephans, S. Magosin, C. Marco, J.F. Caro, Nocturnal rise of leptin in lean, obese and non-insulin-dependent diabetes mellitus subjects, *J. Clin. Invest.* 97 (1996) 1344–1347.
- [55] B.G. Stanley, W.J. Thomas, Feeding responses to perifornical hypothalamic injection of neuropeptide Y in relation to circadian rhythms of eating behavior, *Peptides* 14 (1993) 475–481.
- [56] A. Stricker-Krongrad, C. Burlet, B. Beck, Behavioral deficits in monosodium glutamate rats: specific changes in the structure of feeding behaviors, *Life Sci.* 62 (1998) 2127–2132.
- [57] Y.M. Sun, H.K. Hsu, S.I. Lue, M.T. Peng, Sex-specific impairment in sexual and ingestive behaviors of monosodium glutamate-treated rats, *Physiol. Behav.* 50 (1991) 873–880.
- [58] T. Yoshihara, S. Honma, K. Honma, Effects of restricted daily feeding on neuropeptide Y release in the rat paraventricular nucleus, *Am. J. Physiol.* 270 (1996) E589–E595.