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Persistence of meal-entrained circadian rhythms following area postrema lesions in the rat

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Abstract

Rats anticipate daily meals with increased approaches to a feeder and an increase in core body temperature. Food-anticipatory activity (FAA) is thought to be under the control of a feeding-entrained circadian oscillator (FEO). Ibotenic acid and electrolytic lesions in the region of the parabrachial nuclei (PBN) in the rat severely disrupt FAA (feeder approaches) and temperature rhythms. The PBN receive dense input from the area postrema (AP), which lacks a blood–brain barrier and thus has access to humoral factors in the systemic circulation. The present study assesses development and maintenance of FAA in rats with cautery lesions of the AP. The results demonstrate that AP lesions do not alter FAA. This experiment does not support the hypothesis that the AP in the caudal brainstem detects and relays circulating signals from the periphery that trigger FAA.

Keywords: Food-anticipatory activity; Feeding-entrainable oscillator; Hindbrain

1. Introduction

Circadian rhythms in rodents and other species can be entrained to restricted daily feeding. Wheel running, food-bin approach behavior, unreinforced bar pressing, serum corticosterone, core body temperature and liver enzyme activity all increase prior to a daily timed meal in rats (for reviews, see Refs. [1,10]). Food-anticipatory activity (FAA) is the increase in locomotor activity that precedes a daily meal by about 3 h. The putative feeding-entrained oscillator (FEO) is the proposed mechanism that mediates entrainment by daily meals. Existence of the FEO is supported by the following circadian properties of FAA. FAA free-runs in constant conditions (food deprivation), exhibits limits of entrainment in the circadian range [15], and displays transients in response to phase shifts in mealtime [16]. This circadian clock, although functionally and anatomically distinct from the SCN [18], has not yet been localized. Recent findings regarding entrainment of circadian clock gene expression in peripheral organs to meal time [5,19] point towards a peripheral locus for the FEO.

Communication between the gastrointestinal system and the central nervous system is required for FAA, regardless of clock locus. Because gustatory cues are insufficient as a zeitgeber [11,14], and olfaction is unnecessary for entrainment [2,6], postingestive and/or post-absorptive cues must provide the stimulus for entrainment. Since FAA is a behavioral variable, the brain must be involved as well. Consequently, if the clock is part of the central nervous system, then an input signal regarding meal entrainment must get from the GI system to the brain. If the clock function is attributable to peripheral organs (e.g., small intestine, liver) then a signal triggering a behavioral response at a specific time must be transmitted to the brain.

Excitotoxic and electrolytic lesions directed at the rat parabrachial region severely attenuate, and in some cases abolish anticipatory food bin approaches and the anticipatory increase in core body temperature [7]. Because the parabrachial nuclei (PBN) receives afferents from both the area postrema (AP, which can detect circulating humoral factors) and the nucleus of the solitary tract (NTS, which receives visceral neural input), both the AP and NTS are good candidates for involvement in feeding-entrained rhythms.

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The present study tests the role of AP in feeding entrainment by assessing FAA after AP lesions. Since AP has access to humoral factors in the systemic circulation (lacks a blood–brain barrier), and is heavily connected with the PBN, it is plausible that this site is important in GI system/brain signaling. Therefore, disruption of this pathway prior to exposing the animals to restricted feeding (RF) may interfere with the entrainment and expression of FAA.

2. Methods

2.1. Subjects and surgery

Adult male rats (Sprague Dawley), purchased from Charles River Laboratories, were anesthetized with a mixture of chloral hydrate and sodium pentobarbital. An antibiotic (sulfamethoxazole and trimethoprim, Gensia Laboratories, 0.05 cc im) was administered prior to surgery. The rat's head and neck were shaved and the head was immobilized with a sterotaxic device. The incisor bar was pressed against the top of the nose to make the occipital bone accessible. A midline incision was made from the top of the occipital bone caudally about 1 cm. The skin was then retracted and the muscle bluntly dissected and retracted. When the foramen magnum was exposed, the membranes were cut across the base of the occipital bone and caudally down the midline using small scissors and a dissecting microscope. Small rongeurs were used to slightly enlarge the foramen magnum rostrally. The pial meningeal layer was carefully removed from the brainstem and caudal tip of the cerebellum using blunt forceps. Once visual identification of the AP was achieved, a battery-powered cautery iron was touched to the structure for about 0.5 s. Cautery was skipped for sham-operated subjects. Lesioned rats invariably stopped breathing briefly and were resuscitated by compressing the rib cage. The muscle layer was sutured in two layers and the skin was stapled. Rats were maintained on sweetened condensed milk (diluted 1:2 with water) with vitamin supplements for 1 week after surgery.

2.2. Test of conditioned taste aversion (CTA) acquisition

Since the acquisition of a CTA is dependent on the AP [4], a behavioral test was used to screen the rats for successful and unsuccessful lesions.

Once the rats had surpassed their pre-operative weights and were steadily gaining weight (2–3 weeks following surgery), they were placed on an 18 h/day water-deprivation schedule. After 3 days, rats were given access for 30 min to 5% sucrose and then injected with LiCl (0.15 M, 12 ml/kg ip). Water was then returned for an additional 5.5 h. Following the pairing, the 18-h water deprivation was resumed. Fourty-eight hours after the pairing of sucrose and LiCl, all rats were again given access to 5% sucrose for 30 min. Sucrose intake was measured by weighing the bottles before and after presentation. For each rat, CTA expression was calculated from the sucrose intake 48 h after the LiCl injections as a percentage of the rat's sucrose intake prior to the LiCl injection (percent suppression).

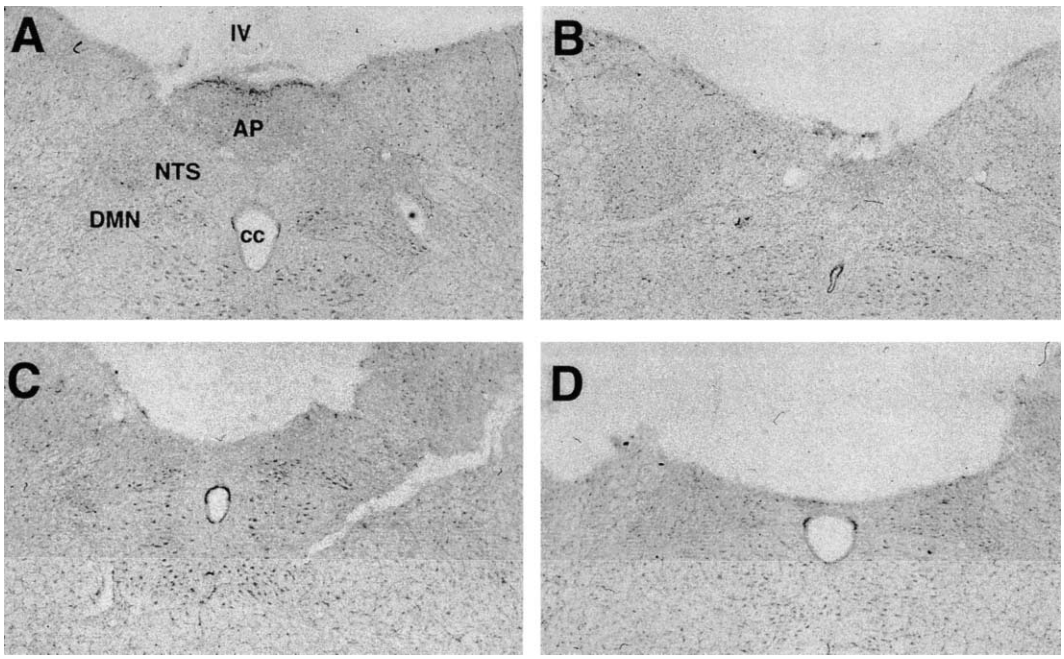

Fig. 1. Four characteristic brain sections from the experiment. (A) A sham subject, with labels indicating relevant brain nuclei. (B) An incomplete lesion. (C) A complete lesion. (D) A lesion that is too large, including damage to the underlying NTS. IV: fourth ventricle, cc: central canal, DMN: dorsal motor nucleus of the vagus.

Following the behavioral phase of the experiment (see below), a second CTA acquisition test was performed to test for the development of possible compensatory mechanisms. For the posttest, a NaCl solution (0.45%) was used and two pairings rather than one were performed. Also, the shams were included for comparison in the posttest, but not the pretest.

2.3. Food-anticipatory activity

Once successful lesions were identified, 16 subjects (nine lesions, seven sham surgical controls) were transferred to feeder-approach boxes that are fully described elsewhere [17]. Briefly, two compartments attached to one side of the plastic boxes contained food and water, respectively. The food tray was mounted on a sliding carrier that was operated by air pressure under computer control, allowing automated delivery and removal of food. The entry to the food compartment was about 5 cm above the cage floor, and was accessible regardless of whether the food tray was in the available or unavailable position. An infrared photobeam was mounted across the entry to the feeding compartment and continuously monitored by computer. The number of seconds per 10 min the photobeam was broken was stored on disk. Food and water were replenished daily after food access ended. A 12:12 light–dark cycle was maintained in the room (lights on at 0800 h). Water was available ad lib throughout the experiment.

After 12 days of ad lib feeding to allow the rats to acclimate to the boxes, a RF schedule was initiated on day 18 (of 2000; see Fig. 2). Food was available for 2 h beginning at 1500 h. One rat died during RF, reducing the group numbers to eight APx and seven controls. In order to assess persistence of FAA during fasting conditions, food was not delivered on days 38 and 39. Therefore, FAA on days 39 and 40 cannot be attributed to an interval mechanism since there was no meal on the days previous to these. On day 56, rats were put back on ad lib feeding.

2.4. Histology

Rats were overdosed with sodium pentobarbital, then perfused first with heparinized 0.9% NaCl containing 0.5% sodium nitrate, then with 4% paraformaldehyde in 0.1 M sodium phosphate buffer. The brains were dissected, post-fixed for 2 h, and then transferred into 30% sucrose for cryoprotection. Forty microns coronal sections were cut on a freezing, sliding microtome through the rostral–caudal extent of the dorsal vagal complex (bregma −12.8 to −14.3 mm; coordinates based on Ref. [13]). Alternate sections were mounted onto gelatin-subbed microscope slides, and stained with methyl green (Vector Laboratories). Damage to the AP and underlying NTS was qualitatively rated by two observers.

3. Results

3.1. Lesions

In order to behaviorally assess AP lesions, two CTA acquisition tests were performed, one before and one after

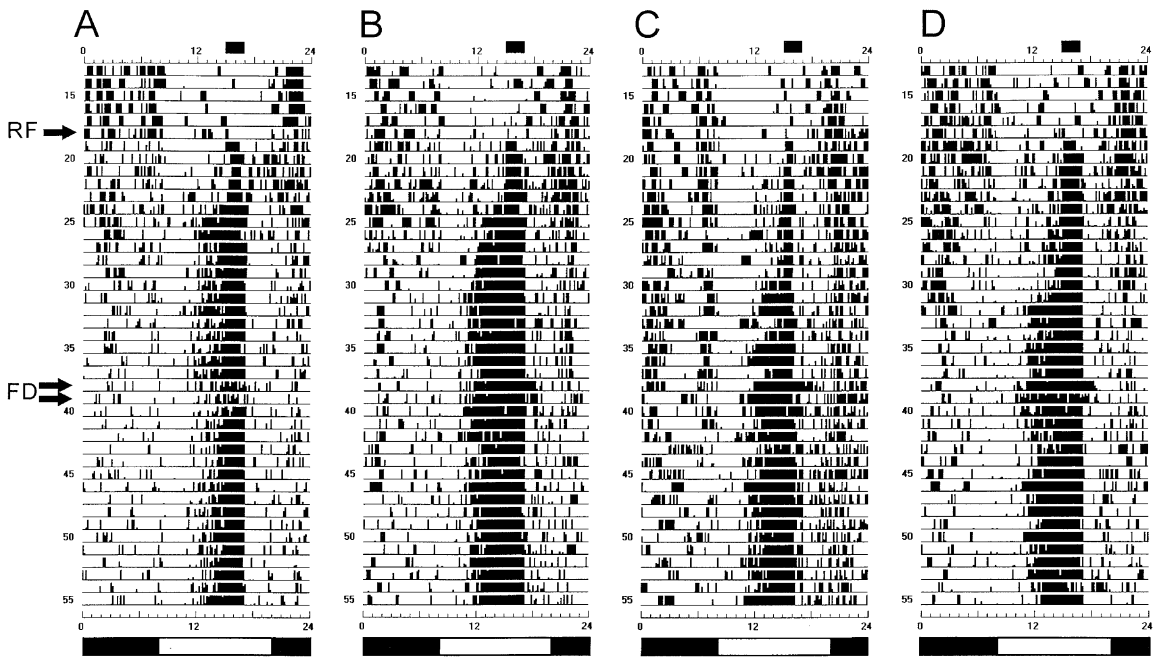

Fig. 2. Event records of feeder-approach behavior. Time of day (h) is shown across the top and bottom of each record and day of the year (2000) along the left side. RF was implemented on day 18. The timing and duration of the meal is represented by the dark bar above the records. The meal was not presented (FD) on days 38–39. The light/dark cycle is depicted by the bar below each record. Records A and B: lesioned rats. Records C and D: sham surgical controls.

the behavioral phase of the experiment. Of 14 surgical subjects, the nine rats with the lowest percent suppression score were used for the experiment. Eight of these nine had a negative percent suppression, meaning these rats increased their intake of the sucrose solution on the test day relative to their intake on the day of pairing. This indicates a complete inability to form a CTA in these subjects, and therefore a complete loss of function in the AP. The ninth rat had 33% suppression, indicating the acquisition of a relatively small aversion to the solution. The mean percent suppression for the subjects included in the experiment was $-68.9 \pm 26.7\%$. The sham surgical subjects were not tested prior to the experiment.

The posttest indicated some recovery of the capacity to form a taste-aversion in these rats. Mean suppression after the first pairing of the NaCl solution and the LiCl injection was $14.5 \pm 7.8\%$ for the APx rats, compared with $28.6 \pm 6.3\%$ for the sham rats. This indicates some aversion acquisition since only two of the seven rats tested showed an increase in intake relative to the day of pairing (negative suppression value). The data for one lesioned rat were lost for the first posttest when the bottle containing the solution was spilled. After the second pairing of the posttest, this rat had the 2nd lowest suppression score of the lesioned group. Therefore, the means stated above for the first pairing of the posttest probably indicate a level of aversion that is artificially higher than was really the case.

Mean suppression of NaCl intake after the second pairing was $89.7 \pm 1.5\%$ for the shams, and $37.1 \pm 7.3\%$ for the AP lesioned rats. All of the lesioned subjects reduced their intake by at least 9% with one rat exhibiting a 73% suppression, demonstrating at least some ability to form a CTA. However, the minimum suppression in the sham group was 83%. Therefore all the shams were better at acquiring an aversion to the NaCl than all the lesioned rats used in the experiment after two pairings with LiCl. Consequently, lesioned rats were clearly impaired in their ability to form a CTA.

Histological examination supported the findings with the CTA tests. All subjects in the lesioned group had significant damage to the AP. Fig. 1 shows characteristic brain sections of a sham lesion, an incomplete lesion, a complete lesion

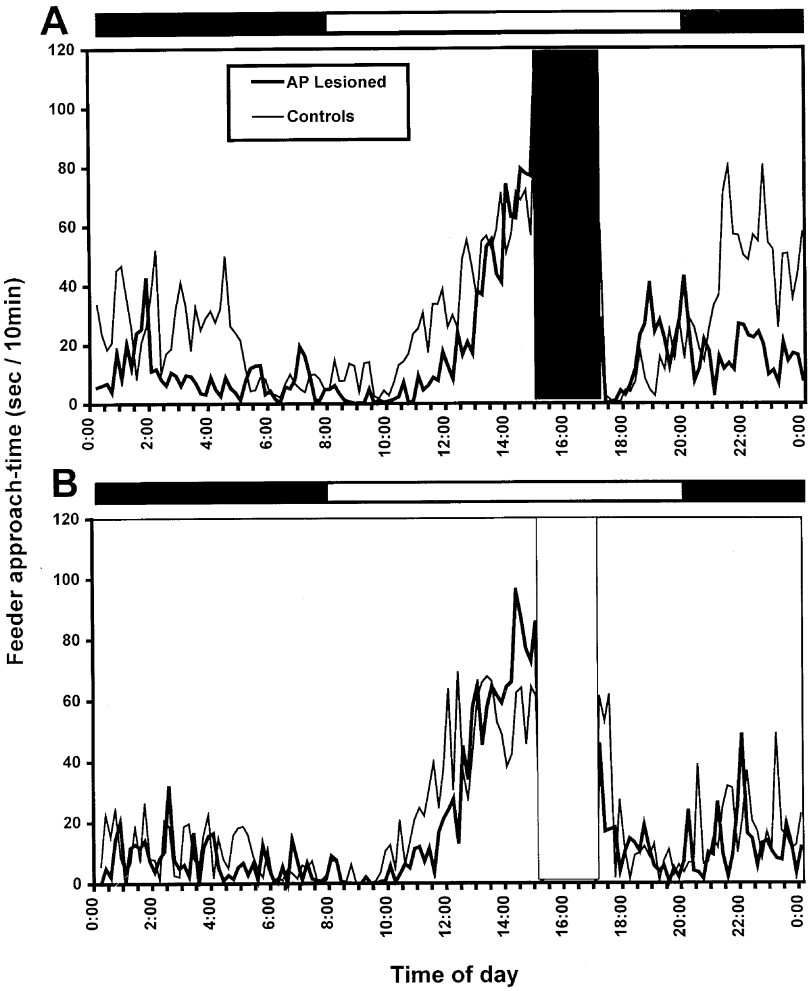

Fig. 3. Waveforms of mean feeder approach-time for 5 days during restricted feeding (A) and for 2 days during food deprivation (B). The black box in A and the white box in B depict the mealtime. Because feeder approach-time is meaningless during the meal, these data are not included for the waveforms.

and a lesion that included NTS damage. Two of the eight surviving rats had lesions that were scored as incomplete, and five of eight had moderate collateral damage to the NTS. Since no differences were apparent in the behavioral records, no rats were excluded from the analysis.

3.2. Body weights

Weight loss during the RF portion of the experiment was within expectations and similar between the groups. For the lesioned subjects, group mean body weight was reduced during RF by 43.8 g (460.3–416.5 g). The sham rats lost an average 63.4 g (516.2–452.8 g).

3.3. Food-anticipatory activity

Fig. 2 depicts four representative event records of feeder approach behavior from the experiment. Records A and B are APx rats, while C and D are sham controls. All lesioned and control rats developed robust FAA within 2 weeks of the initiation of food restriction. Typical variability was noted in the onset time for FAA both between subjects (e.g., Records A and B) and across days in individuals (e.g., Record C).

Fig. 3A shows the distribution of feeder approach behavior averaged for 5 days (days 41–46). These days were chosen because FAA was very stable for most subjects during this time period. It is apparent from the distributions that both groups anticipated the arrival of the meal successfully. The controls seemed to spend more time in front of the feeder during the nighttime than did the APx rats.

Fig. 3B shows the average feeder approach-time for days 49–50, during food deprivation. Although rats were refed on day 50, the anticipatory period occurs before this meal. Therefore, both these days show data that cannot be explained by a 20h interval timing mechanism, since neither bout of FAA was preceded by a meal the day before. The distributions indicate that both groups anticipated the normal mealtime during food deprivation with a similar amplitude to that shown in Fig. 3A. The apparent increase in dark phase feeder approach-time that is present during RF in the control rats (Fig. 3A) is not present during food deprivation.

4. Discussion

This study found that AP lesions fail to attenuate FAA in rats fed a single daily meal. The experiment sought to test the hypothesis that the PBN are part of an ascending pathway whereby the CNS receives input from the G.I. system regarding either clock outputs or the presence of food in the gut. It has been argued that gut/brain communication is necessary for FAA, regardless of the anatomical location of the clock (see Section 1). Since visceral deafferentation with capsaicin [8] and vagotomy [3] both leave FAA unaffected, endocrine signaling is implicated. Since the AP is a circumventricular organ that lacks the blood–brain barrier, the AP rather than other hindbrain structures were targeted in this study.

The role played by the PBN in meal-entrained circadian rhythms is still poorly understood. Two separate experiments utilizing different lesion techniques showed a marked attenuation of the behavioral component of FAA [7]. In one experiment, the anticipatory rise in body temperature was also significantly reduced. The PBN receive dense input from many sites both rostral and caudal in the brain [9,12]. Consequently other circumventricular organs may be involved in FAA by relaying a signal to the PBN.

The NTS also innervates PBN and has access to information regarding the viscera from the vagus nerve and spinal afferents. However, partial bilateral lesions of NTS had no affect on behavioral meal anticipation (unpublished observations). Although these results were not conclusive, the observation that no subjects showed an attenuation of FAA indicates that this structure is not likely to be involved. However, it is possible that large spinal afferents that are not affected by capsaicin treatment are involved. If this were the case, there are a number of other ascending pathways in the spinal cord that bypass the lesion site used in this experiment. Consequently, the signaling pathways between the periphery and the central nervous system that FAA requires are still unknown.

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