The limbic system and food-anticipatory circadian rhythms in the rat: ablation and dopamine blocking studies

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Rats behaviorally anticipate a fixed, daily opportunity to feed by entrainment of circadian oscillators that are physically separate from the light-entrainable circadian pacemaker that has been localized to the suprachiasmatic nucleus. Neural substrates mediating food-entrained rhythms are unknown. A variety of anatomical and functional observations suggest possible involvement of the limbic system and its dopaminergic component in the regulation of these rhythms. To test this hypothesis, the activity rhythms of rats bearing large, combined ablations of the hippocampus and amygdala or nucleus accumbens and medial forebrain anterior to the thalamus were examined under ad-lib feeding, 2-h feeding, and total food deprivation conditions. Some hippocampal-amygdala rats showed alterations of free-running rhythms under ad-lib feeding, but none of the ablations impaired the rats’ ability to anticipate daily feeding, or ‘remember’ the phase of feeding time during subsequent food deprivation. Additional groups of intact rats were treated with the dopamine antagonist haloperidol (0.3 mg/kg or 20 mg/kg) 30 min prior to daily feeding, but this also did not prevent the emergence of food-entrained rhythms. The limbic and dopaminergic systems do not appear to play a necessary role in the generation or entrainment of food-anticipatory circadian rhythms.

INTRODUCTION

Rats normally feed and forage at night, but if food is restricted to a single meal scheduled at a fixed time in the day, they will, within a few days, exhibit a new bout of intense locomotor activity that anticipates mealtime by 1–3 h. This food-anticipatory activity is thought to be regulated by a self-sustaining, food-entrainable pacemaker with circadian limits to entrainment (reviewed in refs. 6, 33), since it does not occur if the feeding schedule differs appreciably from 24 h, it persists at its usual phase position when food is withheld for several days, and it realigns to a new mealtime by gradually shifting in 2–4-h jumps over several days.

The putative food-entrainable pacemaker is physically and functionally separate from light-entrainable circadian pacemakers. If the rat is blinded or maintained in constant dark, the light-dark (LD) entrained rhythm of behavior free-runs with a stable periodicity usually different from 24 h, but the food-entrained rhythm of anticipatory behavior remains coupled to the daily mealtime. The rat thus simultaneously expresses two rhythms of behavior with different periodicities. If the rat is then subjected to complete ablation of the hypothalamic suprachiasmatic nuclei (SCN), the free-running, light-entrained rhythm is abolished but the food-entrained rhythm is unaffected. The neural system regulating daily rhythms in the rat has thus been conceptualized as multi-oscillatory, with separate substrates mediating synchrony to periodic photic and non-photonic events. Other studies indicate that, although separable, these oscillators do exhibit mutual coupling under certain conditions.

Ablation, stimulation and transplant studies have firmly established the SCN as the site of a circadian pacemaker with a primary role in photic entrainment. However, little is known of the physical substrates necessary for food-entrainable rhythms. Ventromedial hypothalamic ablation disrupts food-anticipatory rhythms of behavior, body temperature and corticosterone secretion, but this effect is
transiem\textsuperscript{20,20}. Paraventricular hypothalamic (PVN) ablation eliminates food-anticipatory cage activity in some rats, but not anticipatory food-bin activity\textsuperscript{22}. Cell-specific ablation of the lateral hypothalamus also does not eliminate food-anticipatory rhythms\textsuperscript{32}. These areas were reasonable targets for localization studies, since they have in common a role in the regulation of ingestive behavior and anatomical links to the SCN that could mediate the hypothesized coupling between food- and light-entrainable pacemakers\textsuperscript{59,60}.

Other targets of localization studies have included the adrenal\textsuperscript{23} and pituitary glands (F.K. Stephan, personal communication), neither of which appears to be necessary for food anticipation. Comparatore and Stephan\textsuperscript{9} have recorded food-entrained circadian motility rhythms in isolated rat intestines, suggesting possible involvement of that organ in food anticipation. Parasympathetic denervation of this organ, by vagotomy, has no effect on food-entrained rhythms of corticosterone\textsuperscript{37}, wheel running\textsuperscript{10} or general cage activity (Mistelberger, Houp and Moore-ede, unpublished observations), but a full assessment of the intestine's role in anticipatory behavioral rhythms remains to be done.

The limbic system has also been suggested as the possible substrate of a food-entrainment mechanism\textsuperscript{1,59}. The hippocampus (Hi), for example, is thought to participate in temporal mapping processes\textsuperscript{12,38,52} that could extend to 'learning' of mealtimes. Consistent with this idea, neurotransmitter levels in the Hi exhibit diurnal rhythms that synchronize with feeding time\textsuperscript{39}. Watts\textsuperscript{9} has suggested that any one of several limbic nuclei, including the Hi, amygdala (Am) or nucleus accumbens (Acb), could participate in food entrainment. These limbic areas receive inputs from the SCN and subparaventricular zone (sPVz; an area strongly linked to the SCN), both directly and secondarily via the lateral (LS) and medial (MS) septal nuclei, thalamic paratemporal (PT) and paraventricular (PVT) nuclei, and possibly the bed nucleus of the stria terminalis (BNST). In turn, these areas may feed back to the SCN area via the LS and ventral subiculum (Sub), and have widespread, direct influence over much of the hypothalamus and descending medial forebrain bundle (MFB). Limbic nuclei thus have the requisite bidirectional coupling with the SCN pacemaker that one might expect of a food-entrainable pacemaker, and are well situated to modulate arousal, foraging behavior and neuroendocrine processes known to be synchronized by scheduled feeding.

There are additional reasons for suspecting that the Acb in particular might regulate food-anticipatory rhythms. (1) Dopamine activity in the Acb correlates with the level of exploration and activity (see ref. 2 and is increased prior to feeding time in food-entrained rats (Ref. 19; C. Blaha and T. Phillips, personal communication). (2) The Acb and its dopaminergic afferents appear to mediate the increases in locomotor activity normally observed in rats during food deprivation or following amphetamine injections\textsuperscript{44}. (3) Chron intake of methamphetamine causes a striking reappearance of circadian rhythms in previously arrhythmic SCN-ablated rats\textsuperscript{21}. These rhythms are apparent food- but not light-entrainable\textsuperscript{22}. (4) DA activity in the Acb is increased by rewarding stimuli, including variety of drugs\textsuperscript{19} and food intake\textsuperscript{5,41}. It is likely that a non-photic stimuli that are capable of eliciting anticipatory rhythms, such as food, water\textsuperscript{35} or salt\textsuperscript{47}, have strong reward properties. One stimulus that does not increase dopamine metabolism in the Acb, namely ingestion of palatable, non-nutritive foods, also does not entrain anticipatory rhythms\textsuperscript{31}. Conceivably, mesolimbic dopamine signal, if not part of the timir mechanism itself, may represent part of the input (entrainment) pathway to a food-entrainable oscillator.

This study used ablation and pharmacological (dopamine) blocking procedures to directly test the hypothesis that limbic nuclei or a dopaminergic subsystem mediate food-entrained rhythms. Although some of these procedures affected various characteristics of light-entrainable activity rhythms, none altered the rat's ability to behaviorally anticipate feeding time.

**MATERIALS AND METHODS**

**Surgery and histology**

Adult male Wistar rats (300–450 g; Charles River, Montreal) were anesthetized for stereotoxic surgeon with sodium pentobarbital. HI ablations were produce in 10 rats using a manually guided Pasteur pipette an aspiration pump. Since these aspirations spared ventricle portions of the dente gyrus (DG) and Sub, 5 additional rats were subjected to aspirations combined with electrolytic lesions. Lesions (7 per side) were made with stereotoxic guided, bipolar stainless steel, teflon-coated electrodes passing 2 mA for 20 s from a 1 mm expose tip. Stereotoxic coordinates relative to bregma were Al = -2.3, -3.3 -4.3, -5.3, -5.3, -5.7, -5.8, -5.8 ML = 4.7, 4.7, 4.7, 4.0, 4.7, 4.0, 4.7; DV = -9.8, -10.0, -10.0, -10.2, -10.2, -10.2, -10.0, -10.2. Following surgery, each rat received a large dose of diazepean (10–15 mg/kg, i.p.) to control convulsions that often follow hippocampal lesions. Smaller supplementar doses were administered periodically over the next 24 h

Radiofrequency ablations of the Acb were made in
rants using stainless steel, verathane-coated electrodes with 0.75 mm exposed tips passing 20 mA for 15 s. Coordinates with respect to bregma were AP 5.1, 4.6, 4.1, 3.6, 3.1; ML ± 1.6; DV - 6.3. The incisor bar was set at 5.0 mm above the interaural line.

Following behavioral testing, all rats were sacrificed by pentobarbital overdose and perfused intracardially with saline and formalin. Brains were paraffin-embedded, sectioned, mounted and stained with cresyl violet for light microscopic inspection.

**Apparatus**

All ablated rats were recorded in Wahmann activity wheels equipped with automated feeders attached externally to the door of the side cage. The feeders employed a motor-driven, computer-controlled carousel to present any one of four food bins at an access window (4 x 5 cm) in the door. Activity at the window was detected by a photosensitive transistor and LED positioned over the food-bin. Wheel running was detected by microswitch closures. Food-bin and wheel-running counts were monitored by an Apple computer and summed and stored to disc at 10-min intervals.

One group of rats used in the dopamine blocking (haloperidol) studies received miniature radiofrequency transmitter implants (Mini-Mitter Co., Sunriver, OR) in the peritoneal cavity for telemetric recording of body temperature and motor activity. Each rat was housed singly in standard plastic rodent cages that rested on individual receiver platforms that converted the radiofrequency signal to digital pulses at a rate proportional to body temperature and amount of movement. Food was available in a food bin attached to the outside of the cage that was accessible via a 4 x 5 cm window. Activity at the food window was detected by photocell. Temperature and both activity measures were monitored continuously by an IBM386 computer using DataquestIII software (Mini-Mitter Co., Sunriver, OR).

A second group of rats tested at a higher haloperidol dose were recorded in the Wahmann wheels used for ablation studies.

**Procedures**

**Hippocampal ablations.** Fourteen rats were blinded by optic nerve severation and recorded in the Wahmann wheels for 2 months with free access to food (Purina rat chow powder in vegetable oil) and water. Seven rats were then subjected to the HI aspiration procedure. After another month of recording, all rats were restricted to a single 2-h meal presented at the same time each day. Free-feeding was resumed after 70-90 days. Four HI rats were subjected to a second 2-h restricted feeding schedule which, after 17-21 days, was altered so that mealtime began 10 min earlier (t = 23.83 h, one rat) or 10 min later (t = 24.16 h, 3 rats) each day, for 16 days. This schedule was designed to separate food-anticipatory rhythms from previously free-running rhythms that overlapped or appeared to entrain to the 24 h feeding cycle.

A second set of 8 visually intact rats were recorded in the Wahmann wheels under a 12:12 LD cycle. These rats received HI aspirations (n = 3) or combined HI-Am lesions (n = 5), followed by at least 25 days of recovery with free access to food and water. Food was then restricted to a 2-h daily meal beginning 5 h after light onset. After 13 days, food was provided ad-libitum for 3 days. Food was then removed for 3 days before a final 10 days of free-feeding.

**Accumbens ablations.** Seven rats subjected to Acb ablations were allowed 2 weeks recovery before recording in Wahmann wheels. After 9 days of ad-lib food access, food was restricted to a 2-h daily meal beginning 3 h after lights on (LD12:12) for 12 days. The rats were then food-deprived for 48 h.

**Haloperidol, 0.3 mg/kg.** Fifteen rats were handled daily for 2 weeks prior to receiving transmitter implants. The rats were then recorded for 2 weeks with free access to food and water. Food was then restricted to a 2-h meal provided each day 5 h after lights-on (LD 12:12). Thirty min prior to each meal, 8 rats received 0.3 mg/kg haloperidol delivered i.p., and 7 rats received physiological saline injections. After 14 days, food was removed for 72 h before ad-lib food access was resumed.

**Haloperidol, 2 mg/kg.** Twelve rats were recorded in Wahmann wheels under LD 12:12 for 2 weeks. For the next 10 days, food was restricted to a single meal of concentrated evaporated milk (12 ml) delivered by intragastric gavage each day 4 h after lights-on. Thirty min prior to intubation, 6 rats received i.p. injections of physiological saline and 6 rats received 2 mg/kg haloperidol. Following the 10th day of restricted feeding, the rats were food deprived for 48 h and then returned to ad-lib food access. The gavage procedure was necessary because high doses of haloperidol impaired feeding behavior.

**Data analysis**

Running-wheel and food-bin activity data monitored by an Apple computer were periodically downloaded to a MacIntosh FX computer for subsequent analyses using Circadia (Behavioral Cybernetics, Cambridge, MA). Temperature and activity data recorded by telemetry were stored and analyzed using DataquestIII and were transferred to the MacIntosh for further analyses and plotting using Circadia. Activity data were plotted in the form of standard actograms and wave-
forms that averaged data for selected blocks of days in individual rats. The periodogram method was used to quantify the free-running period of blinded rats.

RESULTS

Hippocampal-amygdalectomy ablations

Histology. Histological results from representative rats with small and large Hi lesions are illustrated in Fig. 1. Rats receiving aspirations alone sustained complete removal of the dorsal Hi, most of the lateral Hi, overlying posterior parietal cortex and corpus callosum, and the fimbria fornix (FF). Some rats sustained unilateral damage to the LS and/or caudate putamen (CPu). Small portions of the DG, presubiculum (PrSub), Sub and parasubiculum (PaSub) were spared caudally and ventrally.

Rats receiving Hi aspirations combined with electrolytic lesions sustained complete destruction of the Hi formation including the DG, PrSub, Sub and PaSub. These rats also sustained complete removal of the posterior two-thirds of the entire Am complex and virtually complete destruction of the medial amygdaloid nuclei and intraamygdaloid division of the BNST. Anterior portions of the anterior amygdaloid area, anterodorsal and ventral medial nuclei, medial central nuclei, anterior and ventral basolateral nuclei, dorsolateral lateral nuclei and anterior cortical nuclei were spared. Small infarcts were also present in the dorsal thalamus, including the habenular nucleus, lateral dorsal nucleus, lateral pulvinar, medial and lateral geniculate nuclei and superior colliculi.

Activity rhythms. Despite the amount of tissue removed in these animals, including all of the Hi formation and most of the Am, all of the rats showed essentially normal food-anticipatory activity rhythms. Since the wheel-running and food–bin activity data were virtually identical in these rats, representative activity charts and discussion are limited to the wheel-running data.

Prior to aspiration surgery, the first group of blinded rats exhibited free-running activity rhythms with stable period lengths (τ) ranging from 23.78 to 24.47 h. Following Hi aspirations, all 7 rats showed a shortening of τ by an average of 0.19 ± 0.12 h (e.g. Fig. 2A–E). When food was restricted to a 2-h daily meal, food anticipatory wheel-running and bin activity was apparent in all animals. In four of the lesioned rats (e.g. Fig. 2D,E) and 5 of 7 control rats, a non-24-h free-running activity rhythm coexisted with a 24-h food anticipation activity bout. However, in the remaining animals, the free-running activity rhythm appeared to become synchonized to the feeding schedule. This occurs in about 20% of intact rats tested under similar conditions and is thought to reflect coupling between food- and light-entrainable pacemakers.

To determine whether an independent, food-entrainable pacemaker functioned in these latter rats, a second feeding schedule was implemented, first with a 24-h feeding interval and then with a 23.83- or 24.16 h interval. The rats showed anticipation of these meals at each interval, although this was weaker and sporadic under the 23.83-h schedule. When ad-lib feeding was resumed, a free-running rhythm emerged that had a phase position similar to that apparent prior to the onset of the non-24-h feeding cycle, i.e. this rhythm did not follow the non-24-h feeding cycle, suggesting that food anticipation was generated by a separate substrate.

The second group of rats with intact vision exhibited typical nocturnal activity rhythms under LD 12:12 prior to surgery. Following combined Hi-Am ablation, some rats were hyperactive for a few days (i.e. Fig. 3A) whereas others were hypoactive (i.e. Fig. 3B–D). Nocturnal rhythms eventually returned, although the waveforms were usually altered. When food was restricted to a 2-h daily meal, consistent food anticipation emerged within 2–4 days (e.g. Fig. 3A–D). The amplitude and precision of the food anticipation rhythm in these rats was in most cases as good or better than typical intact rats. When ad-lib feeding was resumed, activity at the former scheduled feeding time was greatly attenuated. However, when the rats were food deprived for 72 h, this activity bout was strongly enhanced, i.e. the rats appeared to ‘remember’ the previous phase of feeding time, in a state-dependent manner. This deprivation test has been used in intact rats to demonstrate that the food anticipation mechanism is self-sustaining and does not require daily resetting (as per an hourglass timer) by food intake. Again, the ablated animals’ performance was as good or better than typical intact rats (Fig. 3A–D).

Accumbens ablations

Histology. Four of 7 ablated rats showed complete destruction of the Acb. All of the lesions were very large and damaged many structures in addition to the Acb. Histological results of two cases are provided in Fig. 1B,C. The lesion illustrated in Fig. 1B was bilaterally symmetrical. It began at the most rostral pole of the anterior olfactory nucleus (AO) and extended caudally approximately 8 mm into the dorsal thalamus. The Acb and AO were unambiguously absent in this animal. Structures that were heavily damaged or virtual-
s occurs in about 23.83- or 24.16 h conditions and food- and light-dependent, food-enforced latter rats, a extended, first with a 23.83-or 24.16 h of these meals at one and sporadic 1-lib feeding was suggested that had a vent prior to the the rhythm did not, suggesting that a separate subvision exhibited under LD 12:12 Hi-Am ablation, ands (i.e. Fig. 3A), e. Fig. 3B,C,D) ed, although the when food was not food anticipated 3A D). The anticipation rhythm somehow better than when was resumed, time was greatly more food deprived enhanced, i.e. previous phase of manner. This depicts to demonstrate is self-sustaining for an hourglass ablated animals ran typical intact showed complete as were very large lation to the Acb are provided in 1B was bilateral rostral pole of 1 and extended dorsal thalamus. Ily absent in this lamaged or virtu

Fig. 1. Schematics of brain sections from the atlas of Paxinos and Watson (1988). A: hippocampal/amygdala lesions. Ablation sites from representative rats with small and large lesions are indicated by black and dotted areas, respectively. B: nucleus accumbens lesion. C: a second nucleus accumbens lesion, with greater medial forebrain damage. The numbers indicate the plates from the Paxinos and Watson atlas, in mm with respect to bregma.
Fig. 2. Computer-generated, double-plotted actograms of wheel-running activity in 5 blind rats (A–E). Each line represents 48 consecutive h plotted in 10-min time bins. Bins during which wheel-running occurred are represented by vertical deflections from the zero activity line. More intense running is indicated by greater deflections. Consecutive days are also aligned top to bottom. Hippocampal ablations occurred on the day marked by the circle. Vertical hollow bars indicate feeding time during food restriction days. Blanks represent missing data. During the first restriction schedule, food was available for 2 h beginning every 24 h. During the second schedule, the interval was switched after 3–5 weeks to 23.83 h (A) or 24.26 h (B, C). Food anticipation is indicated by the concentrations of wheel activity preceding the mealtimes.

ally removed included (from rostral to caudal) the orbital, infralimbic and dorsal peduncular cortices, cingulate gyrus (CG), anterior MFB, LS and MS, vertical limb of the diagonal band (DB), septohippocampal nucleus (SH), ventral pallidum, and BNST. Structures partially damaged include the anterior and ventral CPu, internal capsule, lateral preoptic area (LPOA), substantia innominata (SI), anterior claustrum and anterior third of the PT and PVT.

The lesion illustrated in Fig. 1C unambiguously destroyed the Acb on one side, but may have spared a very small portion of Acb lateral and ventral to the anterior commissure at its most rostral levels. This lesion virtually removed the entire medial forebrain anterior to the thalamus. The MS and LS, SH, DB, parastrial nucleus, and BNST were absent or at least 90% destroyed. The anterior MFB was destroyed and the dorsal third of the LPOA and medial preoptic areas were absent. The bed nucleus of the stria medullaris was largely destroyed. Other areas damaged include the frontal and piriform cortices, CG, anterior CPu, thalamic PT, PVT, rhomboid and reunion nuclei, and hypothalamic PVN and periventricular nucleus.

Activity rhythms. Although several rats were hyperactive in the wheel-running activity measure, these lesions had no apparent effect on LD-entrained rhythms or food anticipation. Average waveforms of food-bin activity from the rat corresponding to histology (Fig. 1B) are presented in Fig. 4. Prior to food restriction, activity was nocturnal (Fig. 4G). Food
Fig 3. Single-plotted actograms (24 h timescale) of representative sighted rats recorded in LD (A–D). Hippocampal/amygdala lesions occurred at the time indicated by the open circles. Feeding time during food restriction days is indicated by the vertical hollow bar. Down- and up-pointing triangles represent the beginning and ending, respectively, of 3 days of total food deprivation that began 3 days after the end of the restricted feeding schedule. See Fig. 2 for other conventions.

Food anticipation emerged within 3–5 days of food restriction (Fig. 4H), and was apparent at its normal phase position during both days of food deprivation (Fig. 4I).

**Haloperidol treatment**

Rats receiving 0.3 mg/kg haloperidol or saline injections 30 min prior to feeding time showed normal anticipation of feeding time in cage activity (Fig. 4B), body temperature and food-bin activity. Mealtime associated activity was apparent during both days of total food deprivation (Fig. 4C).

Rats receiving 2 mg/kg haloperidol prior to intragastric feeding showed a marked hypoactivity in wheel running throughout the treatment period. Nonetheless, a small amount of activity was usually apparent preceding mealtime in most rats (e.g. Fig. 4E) and was evident at its usual phase position during 2 days of food deprivation (Fig. 4I). Similarly marginal anticipation of intragastric feeding time was also apparent in the saline injected rats.

**DISCUSSION**

We can conclude from this study that neither the Hi formation, including the FF, DG and Sub, nor the Acb are the exclusive site of a timekeeping mechanism for food-anticipatory activity rhythms in the rat. We suspect that the Am complex is also not a critical site for non-photic entrainment since lesions in various animals destroyed either much of the Am proper or eliminated its major efferent pathways (the stria terminalis) or target sites within the subcortical forebrain (the BNST, septum, Acb, and SI). If the Am was a necessary component of a food-entrainment mechanism, we would predict that physical disruption of this magnitude would have some consequences for the expression of food anticipation. Instead, these animals exhibited normal and even, it seemed, supernormal food-entrained rhythms.

Conclusions based on the pharmacological blocking studies are at best equivocal. If dopamine transmission is a part of the food-entrainable clock mechanism or the input pathway necessary for entraining this clock, then one would predict that a complete dopamine blockade would prevent entrainment to a daily mealtime. The difficulty is with achieving a sufficient dopamine block while controlling non-specific performance deficits. At the lower dose (0.3 mg/kg), haloperidol induced transient hypoactivity and reduced, but did not prevent, food intake during the scheduled mealtime. Food anticipation was normal in these rats. At the higher
dose, hypoactivity extended throughout the day. This obviously impaired normal feeding, and necessitated the gavage procedure. Food anticipation was weak in these rats, but was also weak in saline injected, gavage-fed control rats. Thus, reduced levels of food anticipation are confounded with general hypoactivity and the method of food administration. We can state with confidence only that a fully functioning dopamine system, and certainly its mesolimbic (Acb) component, is not required for entrainment to feeding time.

The ablation approach, guided by reasonable assumptions about likely anatomical and functional features of putative food-entrainable oscillators, has to date failed to find evidence for critical input, output or timekeeping components of a food-entrainable circadian system. As suggested previously, it may be that this system consists of a distributed population of neural oscillators represented at one or more levels of the neuroaxis. Only a fraction of this system may be required for normal non-photic entrainment, in the same way that only a fraction of the SCN is needed to generate phasically-entrainable circadian rhythms. If so, it may not be possible to localize the food-entrainable system via a lesion approach. A method of identifying the relevant substrate by metabolic or molecular characteristics of circadian oscillators may instead be required.

Several blind rats in the Hi ablation and control groups showed apparent entrainment of their previously free-running activity rhythms to the restricted feeding schedule. This may reflect coupling between food- and light-entrainable oscillators, or it may reflect entrainment of the light-entrainable oscillator by feedback from the daily bout of food anticipatory activity. This latter interpretation follows from previous observations that free-running rhythms in rats can be...
entrailed, within narrow limits, by a scheduled daily bout of treadmill activity. Whether oscillator coupling or activity entrainment, the present data indicate that the HI formation does not play a necessary role in this process.

All seven of the blinded rats exhibited a shortened free-running \( \tau \) following HI aspiration. Most of these rats also showed lower levels of wheel-running activity, although at least one showed the same or a higher level of activity. Access to running wheels can alter \( \tau \) in blind rats, so conceivably a change in the level of wheel running could be responsible for the \( \tau \) change that followed hippocampocotomy. However, the \( \tau \) change was in the direction opposite to what would be predicted by this explanation; blind rats show a shortening of \( \tau \) during wheel access (i.e. when wheel running is increased), whereas the HI-ablated rats showed a shortening of \( \tau \) in association with decreased running. The \( \tau \) change is thus probably not due to altered feedback of activity levels to the SCN pacemaker.

The HI lesions did not directly damage the SCN, but did partially damage the lateral geniculate, and, in destroying large parts of the HI formation, removed cell populations that are only 2 or 3 synapses removed from the SCN. Thus, \( \tau \) changes may be related to tonic alterations in the afferent stream to the SCN.

Alternatively, \( \tau \) changes may reflect non-specific or phasic effects associated with surgery, such as post-operative edema or the heavy doses of pentobarbital and diazepam used for anesthesia. Single injections of barbiturates and benzodiazepines can alter the phase or period of free-running rhythms in mice and hamsters. Intact rats apparently do not exhibit such responses to benzodiazepines (D. Edgar, personal communication), but similar high dosages have not previously been used. Further studies will thus be necessary to substantiate a role for the HI in the regulation of circadian period.

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