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SUPRACHIASMATIC NUCLEUS AND CIRCADIAN CORE TEMPERATURE RHYTHM IN THE RAT

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Abstract—Core temperature was telemetered from 26 singly-housed adult male inbred Fischer rats standardized in an ambient temperature of 24 ± 1°C, in light from 0600–1800 alternating with darkness (L:D 12:12), with food and water freely available. The rats were operated upon first for bilateral electrolytic lesioning of the suprachiasmatic nucleus (SCN) or by a sham-operation, which consisted of an inserted electrode which neither penetrated into the SCN area nor was activated to produce a lesion. Next, a temperature sensor was implanted intraperitoneally. The telemetered data obtained at 10-min intervals from each rat were analyzed by the least-squares fit of certain trial periods (cosinor methods). A circadian population rhythm persisted in the SCN-lesioned rats which sustained destruction of both SCN ($P < 0.01$). The amplitude of the circadian temperature rhythm was attenuated ($P < 0.01$) and the rhythm's acrophase advanced ($P < 0.05$) from mid-dark to a time near the transition from light to darkness. Unilaterial lesions of the suprachiasmatic nuclei altered the circadian amplitude but not the phasing.

INTRODUCTION

THE suprachiasmatic nuclei (SCN) are located adjacent to the midline of the hypothalamus on the dorsal surface of the optic chiasm. Posteriorly, the arcuate nucleus is the immediate neighbor of the SCN (König & Klippel, 1963). The SCN have been described as controlling or generating circadian rhythms, such as those in core temperature, serum corticosterone, locomotor activity, feeding, drinking, and heart rate (Moore, 1978; Menaker *et al.*, 1978; Zucker *et al.*, 1976; Saleh & Winget, 1977). Stephen & Nunez (1977) Saleh *et al.* (1978) and Krieger *et al.* (1977) had previously reported under the same conditions the persistence of a temperature rhythm.

Moore (1978) reported that complete lesions of the SCN abolished all rhythms studied. He defined as complete lesions those in which at least 80% of the cells in the nucleus are destroyed (Moore, 1979). It seemed important to explore whether a small nucleus, like the SCN, constitutes a neural oscillator (Halberg *et al.*, 1959) dispensable yet active as a pace-resetter of one of the important autonomic rhythms.

MATERIALS AND METHODS

A stainless steel electrode, 350 μm in diameter, was used to make electrolytic lesions about 1 mm in diameter (1 mA for 15 s) of the SCN, (coordinates A6.4, L0.0, H3.0; König & Klippel, 1963). Sham-operated controls had electrodes inserted surgically, as was done to experimental animals, but without penetrating the SCN area or activated by the passage of current. Intraperitoneal temperature was telemetered (Halberg *et al.*, 1972) at 10-min intervals from the 26 adult male Fischer rats reported on herein kept at 24°C environmental temperature with food and deionized water available *ad libitum*. The animals were housed singly and kept in L:D 12:12 for 16 weeks, followed by a 6 h delay of the light–dark schedule. After 8 days a continuous light (LL) schedule began and continued for 3 weeks. Except for the data presented in Fig. 5 it is the first 9 days of the L:D data prior to the lighting shift that are pertinent to this paper.

Upon completion of recording, rats were decapitated by a guillotine and the brains preserved in 10% formalin saturated with sucrose. Serial frozen sections were cut at 30 μm, stained with cresyl violet and examined to determine the extent of the lesion and amount of damage to the SCN (Powell, 1964). Brains were classified histologically as sham-(S), bilateral-(B), incomplete unilateral-(U) or incomplete bilateral-(I) suprachiasmatic lesions. The data were analyzed by the so-called cosinor method which is an inferential statistical technique commonly used to analyze time series data (Halberg *et al.*, 1972). It involves in this study the fitting of the data to a chronobiological window from 28 to 20 h with 10-min increments between consecutive trial periods. The program objectively determines the following information: (1) the best period fit; (2) a probability value (P) which indicates the significance of the fit of the best cosine curve to the data; if the P value is 0.05 or less, the fluctuation of the variable studied is presumed to be cyclic and not random and (3) three rhythmic parameters and their dispersions; these are designated as the acrophase (φ), mesor (M) and amplitude (A). The acrophase represents the crest of the best fitted cosine curve in relation to some arbitrarily selected reference time point along the 24 h time scale (in this case, mid-dark or 0000 was used and the symbol φ indicates this was the reference). The acrophase corresponds to the peak in the best fitting curve and hence to the time when the original values are, on the average, highest; it should be noted, however, that the acrophase is not necessarily identical to the time when the "peak" value is recorded. The mesor is the cosinor-determined overall 24 h mean, i.e. the mean of the cosine function best approximating the rhythm; this is equivalent to the 24 h arithmetical mean only if the data points are equidistant and cover an integral number of periods of any relevant rhythm. In the case of data in this paper one can think of the mean and mesor as being similar, if not the same, since the foregoing conditions are largely met. The

amplitude is defined as one half the total cosine excursion best approximating the rhythm; it represents the distance between the mesor and crest of the cosine function used to approximate the rhythm. It is important to realize that the amplitude value represents only one half the total cosine excursion and it is not equivalent to the difference between the actual peak and trough values of the raw data as plotted on a typical time graph (chronogram). Frequently data are plotted in degrees. If 360° = 24 hr, then 15° = 1 hr. Thus if the reference point was local midnight, −15° would represent 0100 or 0116 would be −19°. The minus sign preceding the degrees is in keeping with mathematical convention. The cosinor permits one to estimate objectively the phase-advance or phase-delay of a rhythm.

Phase is defined as an instantaneous state of a rhythm within a cycle. A phase angle represents a time point in a bioperiodicity considered in relation to another specified time point. An acrophase is the phase angle of the crest, in relation to a specified reference time point, of a single best-fitting cosine. An acrophase-advance or delay indicates the displacement of a bioperiodicity along a time scale to an earlier or a later time. A phase-resetter would imply an organismic entity capable of causing either an advance or a delay in the rhythm, measured, e.g. as an acrophase change in the corresponding direction.

RESULTS

Figures 1 and 2 show the histological picture of the SCN and illustrate how one determines whether a lesion is complete (B), incomplete (I) or unilateral (U). Rats sustaining at least an estimated 80% destruction of the SCN were regarded as B (Moore, 1979).

All 9 B and all 7 S animals studied demonstrated a highly significant ($P < 0.01$) individual 24 h synchronized rhythm in core temperature when the data were analyzed by the cosinor technique for the entire 9 day span (Table 1). For the series from the 5 I and 5 U rats, 1 P value in each group was 0.06, the others were significant at or below 2%. Figure 3 illustrates "folded" (average) data in what is referred to as a plexogram. A curve typical of the response to a B lesion (cf. Fig. 4 for the consistency of this response in 5 out of 5 B rats) is compared with the pattern seen in rats with an I or U lesion. These curves were constructed by first averaging the data of each animal for corresponding clock hours from each of the 9 days of the L:D 12:12 span, by expressing these averages relative to the overall mean (mesor) of the animal's temperature rhythm and by graphing the result for an idealized single 24 h span. The effect of the B lesion on the rhythm of core temperature was to reduce the range over which the temperature rises and falls in a regular manner (to attenuate the amplitude), but it also increased the variability within the range and shifted the time of the acrophase (daily high values) from mid-dark to an earlier time.

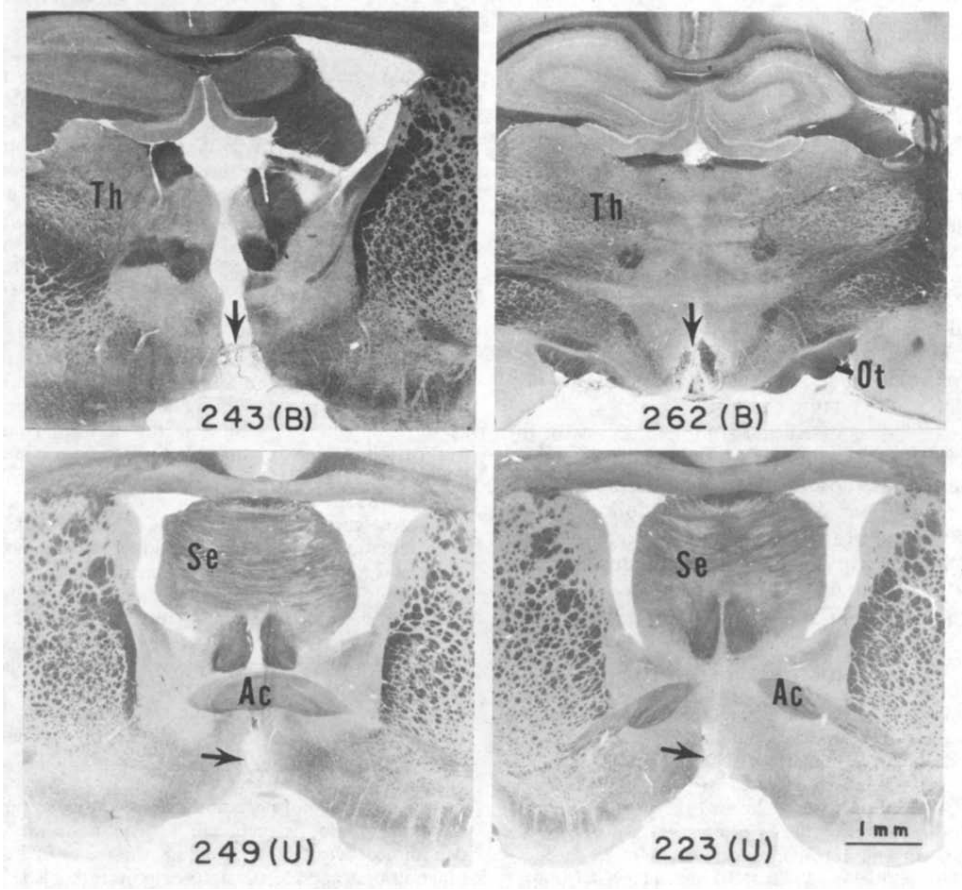

Fig. 1. Photographs of unstained frozen sections (Powell, 1964) show examples of lesions and their placement in the suprachiasmatic nuclear area. Cases 243 and 262 illustrate complete lesions (B). Cases 249 and 223 illustrate anterior unilateral lesions (U). Arrows point to the lesion at the anterior thalamic level, 243 and 262; and at the level of the posterior septum and anterior commissure 249 and 223. Abbreviations: Ac, anterior commissure; Ot, optic tract; Se, septum; Th, thalamus.

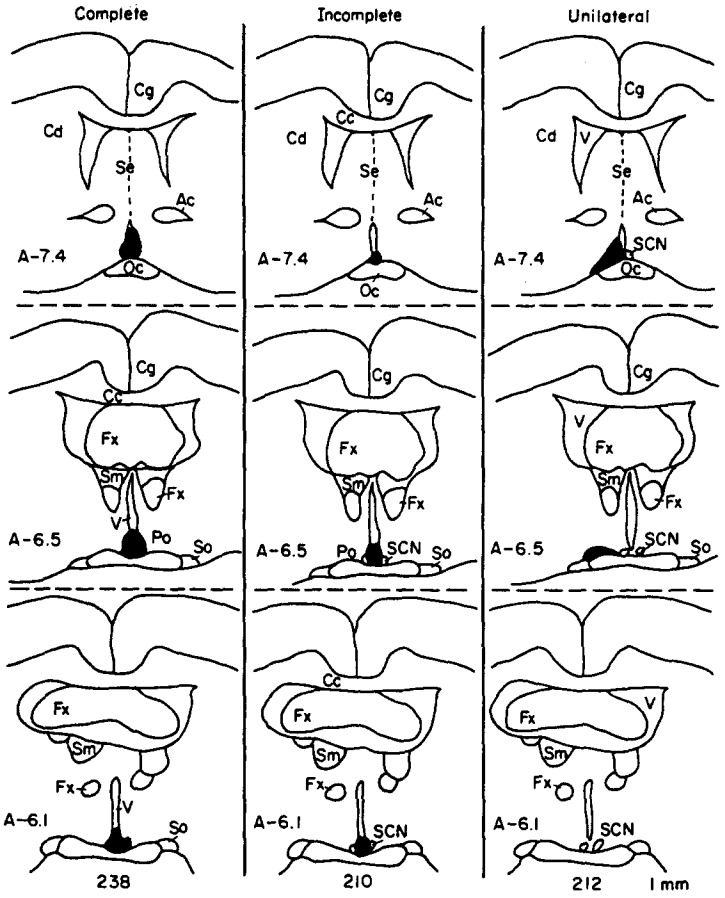

Fig. 2. Lesions of the SCN. Three cases have been chosen to illustrate the types of lesions encountered in this study. Complete, incomplete, and unilateral lesions are represented by cases 238, 210 and 212. The serial diagrams are a composite of sections from fresh tissue tracings of lesions and analyses of cresyl violet stained slides of serial sections through the lesion. Abbreviations: Ac, anterior commissure; Cc, corpus callosum; Cd, caudate-putamen; Cg, cingulate gyrus; Fx, fornix; Oc, optic chiasma; Po, Preoptic region; SCN, suprachiasmatic nucleus; Se, septum; Sm, stria medullaris thalami; So, supraoptic nucleus; V, ventricle.

When analyzing for the best period fit using a chronobiological window from 28 to 20 h a prominent 24.0 period stood out consistently for the data of all four S animals considered in Fig. 4 ($P < 0.01$). The fit of a 24 h period to the data from B, U and I rats also allowed the rejection of the assumption of no circadian rhythm—by the test of the zero amplitude (Halberg *et al.*, 1972), as can be seen from the P values in Table 1. It is also clear from the results of analyses contained in Table 1 that the amplitude of B rats as compared to S rats was only reduced and not eliminated. A test of the differences in circadian amplitude after mean cosinor computation (Halberg *et al.*, 1977) reveals that on the average the B rats have a lower circadian amplitude and an earlier acrophase, as measured by the fit of a 24 h cosine curve (Powell *et al.*, 1977; Halberg *et al.*, 1977). Figure 4 shows more 9 day series folded into a single idealized day—such data for 5 rats with B lesions are being compared with data from 4 rats with S lesions. It is clear from the data, even by inspection with the unaided eye, that an amplitude reduction and phase shift did occur. High values in B rats most frequently occurred at the transition from light to dark, as is indicated in Fig. 4 by an arrow. The arrow with the data on S rats points to high values occurring at other times.

The unaided eye can also readily see when examining Fig. 4 why an obliteration of the circadian rhythm may have been reported by some workers when values averaged for the entire light span are compared with values averaged for the dark span. Clearly in the rats with B lesions, there is a rise above the rhythm-adjusted mean (mesor) during the second half of the habitual light span when values in the S animals are below the mesor and stay below the mesor until near the end of the daily light span at 1800. Thus comparison of data obtained in the dark span with those obtained in the light span will not detect the rhythm as Table 2 shows. Table 2 further illustrates (although it does not list P values) that a division of the day into 4 spans did, however, under our conditions, indicate a statistically significant within-day difference ($P < 0.01$). It is only when averaging is done for 12 h spans representing the light and dark spans, respect-

Table 1. Rhythmometric summary of telemetered intraperitoneal temperature from several groups of inbred Fischer rats

Group	Rat No.	Rhythm (%)	*P*	Amplitude (°F)	Acrophase* (95% Confidence limits)
B†	238	16	<0.01	0.55	−313° (−294°, −331°)
B†	243	27	<0.01	0.39	−298° (−285°, −311°)
B†	245	27	<0.01	0.38	−253° (−240°, −266°)
B†	247	13	<0.01	0.36	−254° (−234°, −274°)
B†	251	18	<0.01	0.37	−253° (−236°, −269°)
B†	253	18	<0.01	0.35	−237° (−220°, −254°)
B†	258	47	<0.01	0.81	−322° (−313°, −330°)
B†	262	8	<0.01	0.38	−287° (−267°, −322°)
B†	264	5	<0.01	0.67	−298° (−262°, −333°)
U	212	72	<0.01	1.16	−15° (−10°, −19°)
U	223	3	0.06	1.65	−340° (−302°, −36°)
U	249	76	<0.01	1.51	−356° (−352°, −1°)
U	256	72	<0.01	1.05	−7° (−2°, −12°)
U	266	68	<0.01	1.04	−8° (−3°, −32°)
I	210	13	<0.01	0.30	−338° (−317°, −359°)
I	214	4	0.02	0.17	−217° (−178°, −257°)
I	216	30	<0.01	0.86	−9° (−358°, −21°)
I	219	51	0.06	1.59	−351° (−304°, −38°)
I	236	51	<0.01	0.74	−353° (−346°, −1°)
S	246	21	<0.01	0.58	−8° (−335°, −22°)
S	248	66	<0.01	0.82	−1° (−356°, −7°)
S	250	59	<0.01	0.93	−358° (−352°, −4°)
S	252	61	<0.01	1.01	−7° (−1°, −13°)
S	255	75	<0.01	0.87	−6° (−1°, −10°)
S	257	12	<0.01	0.78	−1° (−341°, −23°)
S	263	64	<0.01	1.04	−357° (−351°, −363°)

* Acrophase in degrees, with 24 h ≡ 360° and 15° = 1 h.

† B = bilateral complete suprachiasmatic lesion; U = unilateral suprachiasmatic lesion; I = incomplete suprachiasmatic lesion; S = sham-operated animals.

Table 2. Mean hourly deviation from temperature mesor for groups investigated (Means for consecutive spans of 6 or 12 hours)

Kind of lesion \ Lighting span (Clock-hour)	Light		Dark	
	0600–1200	1200–1800	1800–0000	0000–0600
Sham (S)	−0.70 ± 0.07	−0.54 ± 0.04	+0.51 ± 0.10	+0.76 ± 0.04
Unilateral (U)	−0.80 ± 0.09	−0.78 ± 0.06	+0.66 ± 0.14	+0.99 ± 0.07
Bilateral (B)	−0.32 ± 0.12	+0.18 ± 0.04	+0.26 ± 0.03	+0.14 ± 0.05
	0600–1800		1800–0600	
Sham (S)	−0.62 ± 0.03		+0.64 ± 0.06	
Unilateral (U)	−0.79 ± 0.05		+0.83 ± 0.13	
Bilateral (B)	−0.14 ± 0.13		+0.20 ± 0.13	

Each temperature expressed first as a deviation from the mesor of corresponding series. These deviations were summarized for each consecutive hour of a 24 h span into which temperatures over a 9-day recording span at 10 min intervals were "folded" (averaged). These mean deviations were further summarized as tabulated above for four 6 h and two 12 h spans with indications of light and darkness. The circadian rhythm, well established by analysis of variance and by cosinor methods for all three groups (S, U, B) is readily lost, as the table reveals once data are averaged for the light and dark spans. This loss is simply the consequence of a change in timing as the table also reveals.

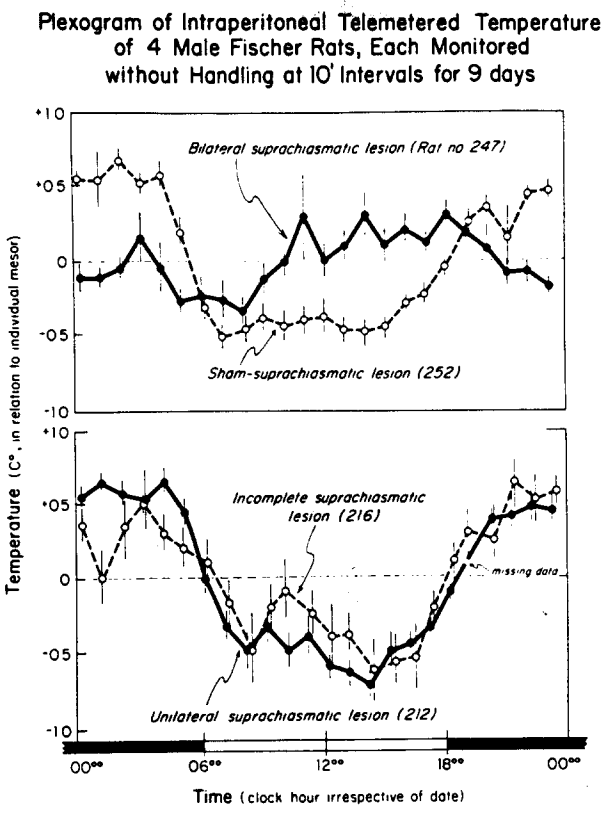

Fig. 3. Individual temperature plexograms from the four animals also summarized in Table 2. The means and standard errors of nine days of hourly data are illustrated. See text for explanation of plexogram.

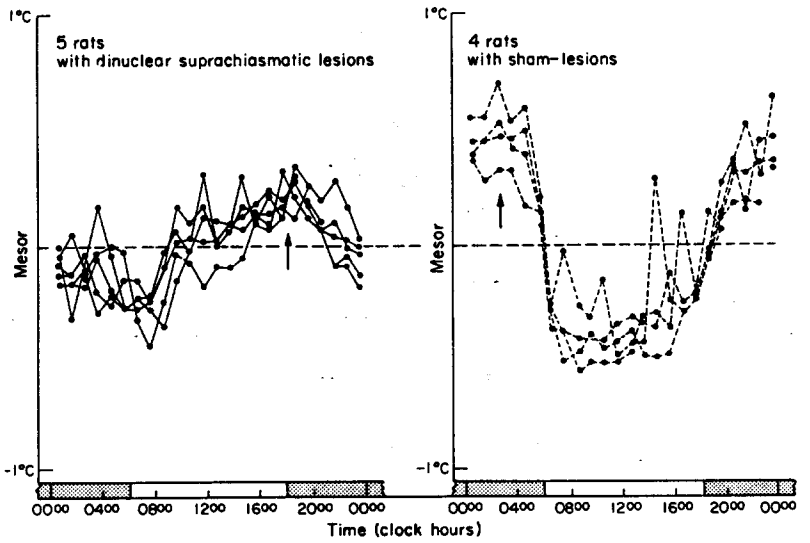

Fig. 4. Individual temperature plexogram of data of individual circadian temperature variations in bilateral suprachiasmatic lesions (B; $n = 5$), on left compared with that in controls (Sham; $n = 4$). All sham-operated animals show a high amplitude rhythm with high values occurring at about 0200 (arrow). All lesioned animals (B) show a lesser extent of circadian change, with a peak occurring by about 1800. All animals were housed in the same telemetry chamber.

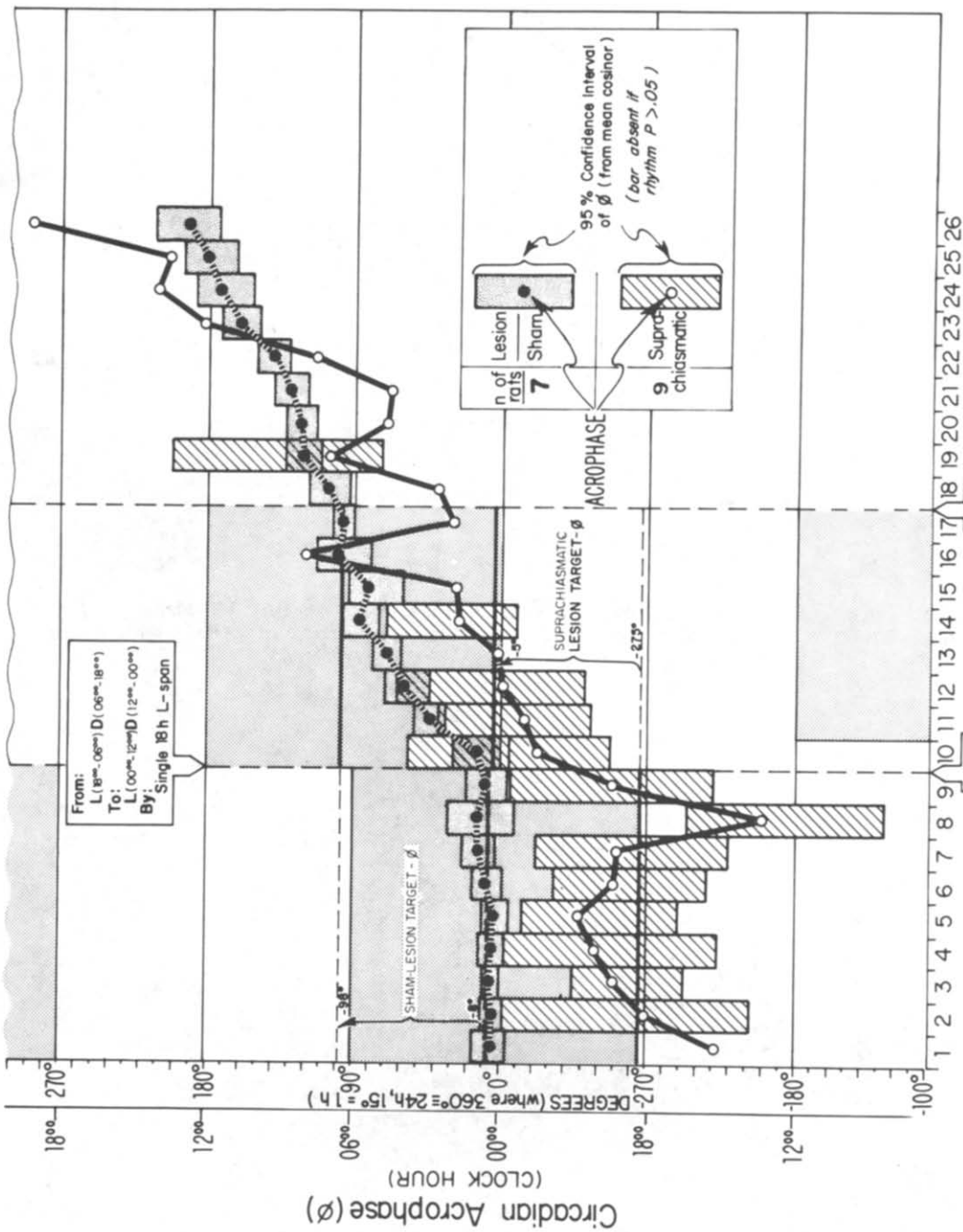

Fig. 5. Mean acrophases and their 95% confidence limits on 26 successive days in sham-operated animals and rats subjected to bilateral suprachiasmatic lesions in 12-h light alternating with 12-h darkness.

ively, that the difference in deviation from the overall rhythm-adjusted mean is lost for the B group ($P > 0.10$), while it remains significant for the U and the S groups ($P < 0.01$). A conclusion from examination of our own data is that dense sampling over more than one or two days may be desirable to describe the lower amplitude rhythm which characterizes B rats. The 9 day span and 10 min interval used in this study were sufficient for this purpose. By contrast, hourly or less dense sampling over one or two 24 h spans, notably when such sampling involves the handling of rats for measurement, may not be sufficient.

A day-by-day population mean cosinor summary of the results on S and B rats is illustrated in Fig. 5 for 26 days. In this case 7 S and 9 B rats were compared. The 95% confidence intervals for the acrophase of the light-dark synchronized B rats are much larger than those for the S rats, thus indicating a noisier rhythm. When the temporal placement along the 24 h scale of the light-dark schedule was abruptly changed by 6 h there was a corresponding gradual shift in the circadian acrophase of both groups. Moreover, when the adjustment of the B rats was compared with the adjustment of the S rats, the group adjustment was much noisier in B rats. As discussed elsewhere (Halberg *et al.*, 1977) the circadian rhythm apparently persisted in individual B rats, even when they were subjected to continuous light (LL) of about 30 lux. In Fig. 5 it is apparent, however, that in LL the group rhythm became desynchronized. Actually an inter-individual desynchronization, as apparent in Fig. 5, starts toward the end of the change in the L:D 12:12 regimen and it is perhaps more pronounced during most of the span when the animals were kept in LL. While in LL the synchronized group rhythm was lost for the B rats, it persisted for the 9 days in LL, in the S rats. The fit of a 24 h cosine curve to each individual series in LL showed a very large decrease in amplitude, as compared to that of S rats, but the zero-amplitude assumption was rejected. A chronobiologic window constructed between 20 and 28 h with 0.1 h intervals between successive trial periods (Halberg *et al.*, 1977) yielded in LL an average period for S rats of 24.77 ± 0.04 h, as compared to a best-fitting circadian period for U rats of 24.74 ± 0.02 h. The average best-fitting period of the B rats in the same chronobiologic window was 23.85 ± 0.093. The difference in variance between the best fitting periods of S and B rats in LL (of 30 lux) was significant ($F = 361.1$; $P < 0.01$).

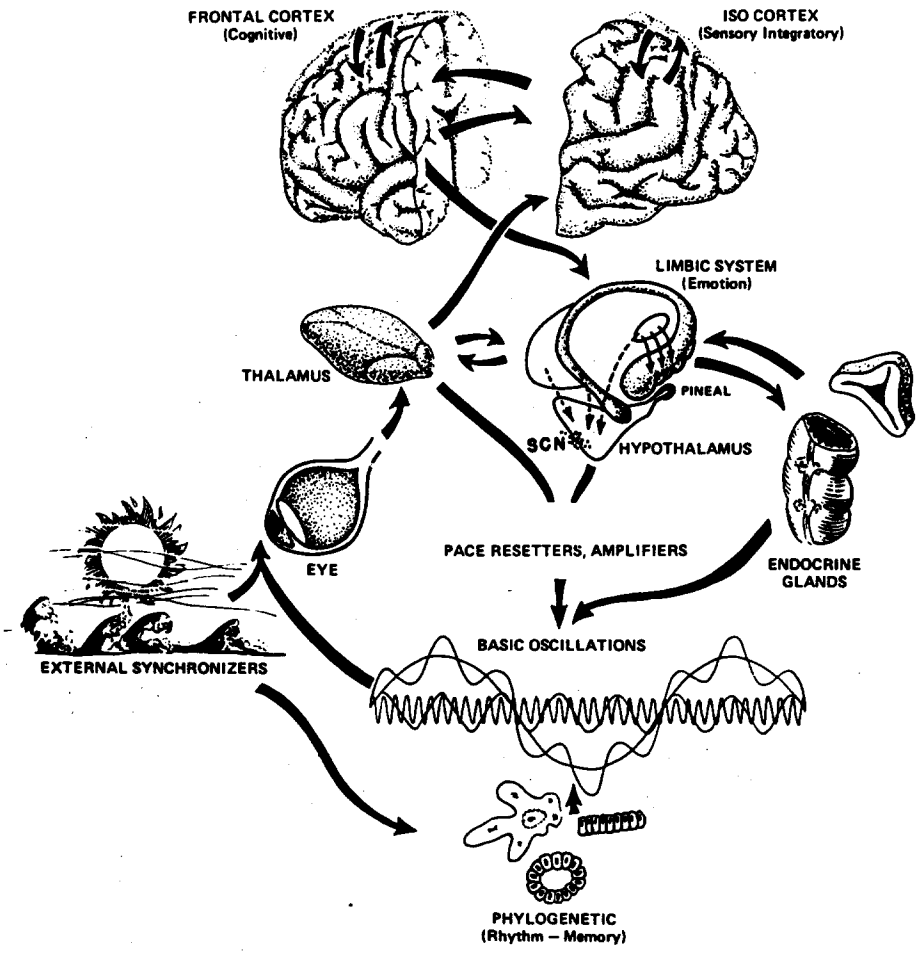

Fig. 6. Schema to illustrate the concept of a collateral hierarchy as an aspect of organismic time structure.

DISCUSSION

The data herein presented on inbred male Fischer rats do not substantiate the frequent claim that the core temperature rhythm is generated by the SCN—that is, it is not a pacemaker of the core temperature rhythm. The SCN does, however, clearly influence the extent and the timing of the temperature rhythm. Because the temperature rhythm changes its characteristics following SCN lesioning under conditions of L:D 12:12 the SCN, is a pace-resetter and amplifier and thus an integrator of information from the complex external and internal information systems reaching this nucleus. The discrepancies between our results and the findings of others who have reported that the SCN is a generator of the core temperature rhythm may arise, at least in part, from differences in density and span of sampling time as well as statistical analysis of the data. From our data it seems essential that the lower amplitude temperature rhythm is monitored for more than one or two 24 h spans.

Moore (1978) suggests that 80% damage of the SCN equates with complete destruction. We use the same criterion. It is in keeping with our results however, that the symmetry of the areas lesioned, as well as the number of cells damaged, may be important. For this consideration we cite the change in the amplitude of the core temperature rhythm in rats subjected to unilateral lesions, Table 1, that need not be a decrease but rather an increase.

The new data support a complex mechanism of spatial and temporal intermodulation in a rhythmic time structure. The scheme in Fig. 6 constitutes an abstract attempt to illustrate some spatial aspects involved. In the case of human beings, socio–ecological factors rather than selected environmental geophysical periodicities such as light–dark seem to be a stronger synchronizer (Halberg *et al.*, 1959; Wever, 1975). A socio–ecological synchronizer may require a structure with cognitive functions such as the frontal lobe of the brain, to process environmental stimuli by means of sensory integration. The integration of several kinds of informational inputs reflected against a memory bank would appear to be essential to an estimate of timing by the CNS. Various brain structures known to be anatomically connected may generate their own rhythms which are in turn interlocked and phased by pace-resetters (and perhaps, pacemakers) which are primarily responsible for the system's oscillating function via neurotransmitter release. As the brain developed, information from environmental synchronizers was transmitted to specific areas of the brain which in turn responded and became pace-resetters for basic tissue rhythms (Fig. 6). The SCN is such a pace-resetter and amplifier. The SCN, however, represents but a cog in the wheel of the overall mechanism, which when damaged changes but does not destroy all basic circadian rhythms (Galicich *et al.*, 1965).

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Key Word Index—Suprachiasmatic nucleus; brain; circadian rhythm; chronobiology; temperature; rat; lesion.