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Do the suprachiasmatic nuclei oscillate in old rats as they do in young ones?

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Satinoff, Evelyn, Hua Li, Thomas K. Tcheng, Chen Liu, Angela J. McArthur, Marija Medanic, and Martha U. Gillette. Do the suprachiasmatic nuclei oscillate in old rats as they do in young ones? *Am. J. Physiol.* 265 (*Regulatory Integrative Comp. Physiol.* 34): R1216-R1222, 1993.—The basis of the decline in circadian rhythms with aging was addressed by comparing the patterns of three behavioral rhythms in young and old rats with the in vitro rhythm of neuronal activity in the suprachiasmatic nuclei (SCN), the primary circadian pacemaker. In some old rats, rhythms of body temperature, drinking, and activity retained significant 24-h periodicities in entraining light-dark cycles; in others, one or two of the rhythms became aperiodic. When these rats were 23–27.5 mo old they were killed, and single-unit firing rates in SCN brain slices were recorded continuously for 30 h. There was significant damping of mean peak neuronal firing rates in old rats compared with young. SCN neuronal activities were analyzed with reference to previous entrained behavioral rhythm patterns of individual rats as well. Neuronal activity from rats with prior aperiodic behavioral rhythms was erratic, as expected. Neuronal activity from rats that were still maintaining significant 24-h behavioral rhythmicity at the time they were killed was erratic in most cases but normally rhythmic in others. Thus there was no more congruence between the behavioral rhythms and the brain slice rhythms than there was among the behavioral rhythms alone. These results, the first to demonstrate aberrant SCN firing patterns and a decrease in amplitude in old rats, imply that aging could either disrupt coupling between SCN pacemaker cells or their output, or cause deterioration of the pacemaking properties of SCN cells.

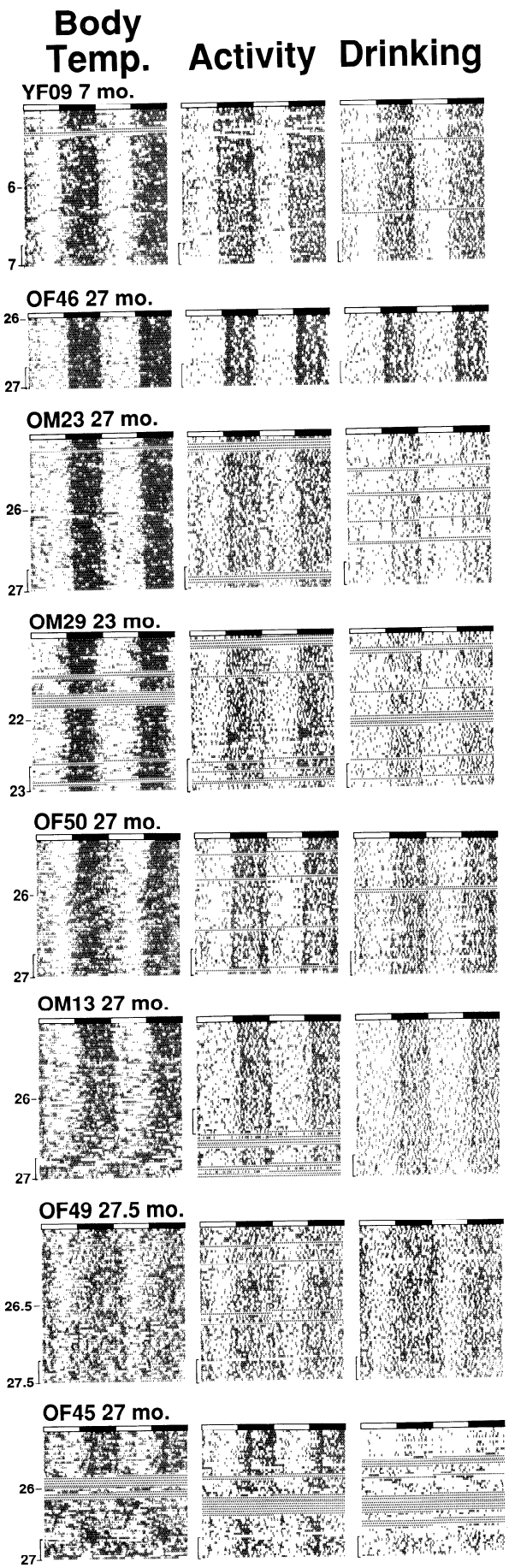
brain slice; circadian rhythms; body temperature; activity; drinking; rat

THE BIOLOGICAL CLOCK in the suprachiasmatic nuclei (SCN) produces timing signals that generate circadian rhythms in physiological and behavioral systems. Normal biological rhythm functioning contributes to good health and well-being (1, 2). During aging, many characteristics of rhythmic behaviors deteriorate; the two most commonly seen changes are decreased amplitude and a disruption of normal patterning (13). There are important questions to be asked about these changes, including possible changing relationships among behavioral rhythms and between the SCN and behavioral rhythms, as well as changes within the SCN. Do all circadian rhythms become disrupted at the same time, or does one invariably deteriorate before another, or is there no pattern to the loss of rhythmicity? Does the deterioration reflect changes in the circadian clock itself or in the interaction between the clock and the overt behaviors it regulates?

Changes in rhythmic behaviors could be due to alterations in rhythmic neuronal activity in the SCN of the hypothalamus, a principal pacemaker that organizes circadian rhythms in mammals (18). In rats, SCN lesions disrupt circadian rhythmicity in many behaviors (see Refs. 15 and 19 for review). The SCN generate a near 24-h rhythm of multiunit activity in vivo (8). When isolated in vitro, the SCN continue to produce a circadian rhythm of electrical activity (5, 6) that is stable for multiple cycles and matches the rhythm in vivo (11). The rhythm of 2-deoxyglucose uptake in an SCN slice is proportional to the rate of glucose utilization in light-entrained rats (10). Cultured SCN cells retain the capacity for circadian oscillation (9). Thus the SCN in vitro is an appropriate preparation for assessing SCN rhythms.

Homeostatic regulations also deteriorate in aging organisms (7, 12). This leads to the question of whether the decline in circadian rhythmicity might be a masking effect. Masking usually refers to an environmental cycle exerting a strong exogenous influence on a biological variable without a direct effect on the underlying timing process. We use the term here to refer to a physiological system exerting a strong effect on its overt rhythm. For instance, if renal insufficiency or vasopressin dysfunction caused increased drinking during the day, the drinking rhythm might disappear, but this would not imply loss of control of the rhythm via the pacemaker. Similarly, if a decrease in one rhythm (e.g., activity) caused a reduction in another rhythm to which it is linked (e.g., body temperature), this would also be a masking effect.

Although we did not look for masking effects, we did try to determine whether the deterioration of circadian rhythms in elderly rats was accompanied by changes in rhythmic neuronal activity in the SCN. If changes in behavioral rhythmicity were mirrored by changes in this measure of output of the primary pacemaker, then we could infer that the changes in the behaviors were caused by alterations in neuronal relationships very close to the oscillator as well as possible deteriorations in homeostatic regulations further downstream.

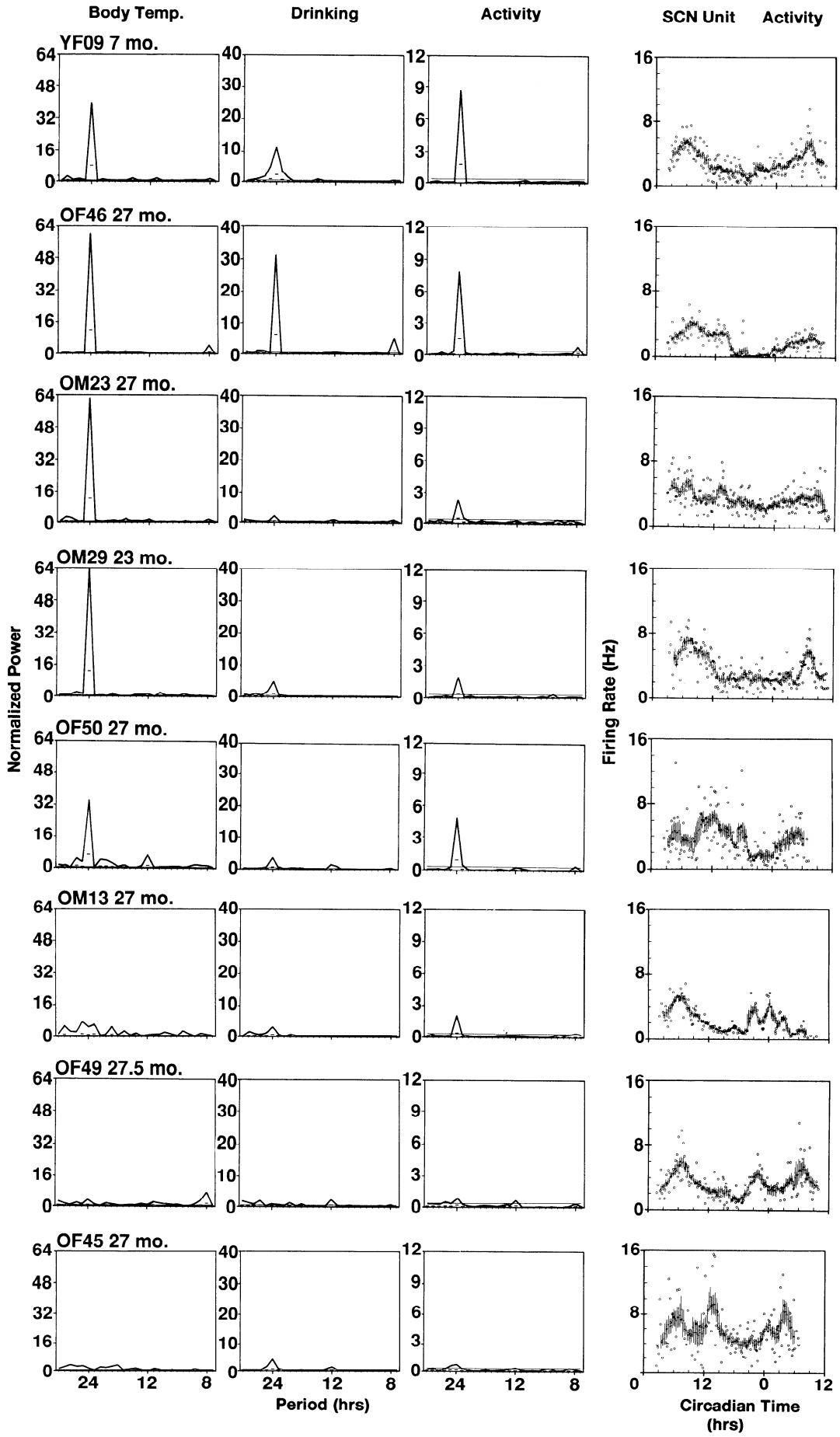


MATERIALS AND METHODS

Behavioral measures. The subjects were male and female Long-Evans rats (Charles River) that were 5–7 mo old ($n = 3$) or 23–28 mo old ($n = 10$) at the time they were killed. Under Ketaset anesthesia (ketamine, 50–75 mg/kg), they were implanted intraperitoneally with temperature telemeters (Mini-Mitter model M) and housed individually in polycarbonate cages (26.7 × 24.1 × 20.3 cm) in a 12:12-h light-dark (LD) cycle at an ambient temperature of 23 ± 1°C. Food and water were always available. Body temperature, locomotor activity, and drinking were monitored every 10 min for 1–6 mo. Licking was measured by a standard drinkometer circuit. The rat had to stand on a metal plate to get access to the drinking tube. When it licked the tube, a circuit was closed. For measuring activity, each cage sat on a platform suspended by four chains. When the rat moved the platform, a movement was recorded. All data were automatically collected by computer. Body temperature was recorded every 10 min and licks and activity were recorded in 20-min bins. Raster plots were generated for all animals. The basic measure of the strength of the circadian rhythms was the periodogram (20), which describes how the variance in a time series may be accounted for by cyclic components at different frequencies. A periodogram is essentially a modified Fourier analysis, which approximates a function by a sum of sine and cosine terms. It is a nonparametric procedure; that is, no specific waveform is assumed a priori. Periodograms reveal not only significant frequencies, but also their relative strength (or power), which is important in the context of determining the properties of oscillators in rats as they age.

SCN slice. After its periodicities were determined, the rat was removed from its cage within 1 h after lights-on and carefully killed by guillotine, and the brain was quickly removed. Coronal slices of the hypothalamus 500 μm thick containing the paired SCN, optic chiasm, third ventricle, and surrounding tissue were prepared and placed in a Hatton-style brain slice chamber (4). The slices were continuously illuminated and perifused with Earle's balanced salt solution (GIBCO), supplemented to 24.6 mM glucose, 26.2 mM sodium bicarbonate, and 0.005% gentamicin and bubbled with 95% O_2-5% CO_2 (pH 7.4) at 37°C. Perifusion rate was 34 ml/h. Extracellular recordings from single cells encountered at random were made using glass microelectrodes as previously described (4, 11). The recording for each slice was maintained continuously for 30–32 h, from circadian time (CT) 2–3 on *day 1* to CT9–10 on *day 2* (lights-on = CT0). Activity within the SCN is represented by a sample of many single-unit firing rates over time. Each raw data point represents the firing rate of a single neuron in the SCN averaged over at least 2 min and assigned the time at the beginning of the averaging bin. Between 120 and 179 cells at random locations within the SCN were counted for each experiment. The firing rates of individual SCN neurons were grouped into 2-h running averages using 15-min lags. The time-of-peak was determined

Fig. 1. Computer-generated double plots of daily body temperature, activity, and drinking of 1 young and 7 old rats for 1–2 mo before they were killed. Data are plotted in 30-min time bins. The mean body temperature and the mean daily water intake and activity were counted. Bins with values above the daily means were plotted as circles. Bins containing measures below the daily means were plotted as blanks. Bin values within ±0.1°C of the daily mean body temperature and within 5% of daily mean licks and activity measures are plotted as an x. Horizontal dotted lines indicate missing data. Black bars at top indicate dark periods. The 10 days from which the periodograms were taken are indicated with a bracket at the side of the graph. Note especially OM23 and OM29. Their behavioral rhythms are almost identical, yet the slice rhythm of OM29 was normal whereas the slice rhythm of OM23 was the flattest seen (Fig. 2).


Body Temp.
Drinking
Activity
SCN Unit Activity
YF09 7 mo.
OF46 27 mo.
OM23 27 mo.
OM29 23 mo.
OF50 27 mo.
OM13 27 mo.
OF49 27.5 mo.
OF45 27 mo.
Normalized Power
Firing Rate (Hz)
Period (hrs)
Circadian Time (hrs)

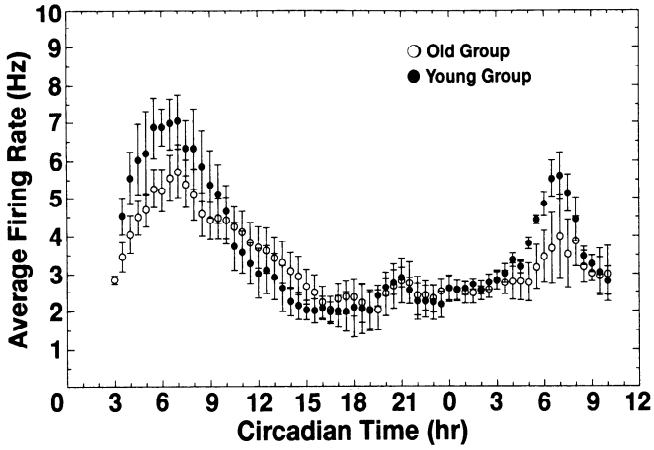


Fig. 3. Circadian variation in the ensemble firing rates within the old (n = 8) and young (n = 4) groups of rats. Two-hour sliding window averages of neuronal firing rates were calculated for individual subjects at 30-min intervals. These mean values were averaged within each group to produce the plotted values. Group averages for time points with fewer than two subjects were not calculated. The amplitude is lower for the old group (P = 0.016, see text).

by visual inspection of a graph of these values for the symmetrically highest point. Recording was performed blind, i.e., without knowledge of the previous behavioral history or age of the rats. In addition to the 13 rats whose behavioral histories had been recorded, slice recordings were also made from two 2-mo-old rats on which prior behavioral rhythms were not recorded.

Curve fitting and statistical analyses. For curve-fitting analysis, single-unit raw data points were grouped into overlapping 2-h bins at 30-min intervals. Such sliding window averaging acts as a low-pass smoothing filter that reveals long-term temporal changes by smoothing their inherent heterogeneity. A periodic parametric curve was fitted to each subject's sliding window averages. The form of the curve was designed to describe the typical shape of a young rat's sliding window average. The equation of the curve is Hz = offset + exp[(amplitude * sin (phase)], with phase = [(CT1)/24] * 2π. Offset and amplitude are parameters that are optimized. Phase fixes the time of peak for the curve at CT7.0, the normal time of peak for young rats (11). Parameter values for the old and young groups were compared using t tests.

RESULTS

Body temperature, activity, and drinking rhythms were recorded for 35–178 days. Figure 1 shows these patterns of body temperature, activity, and drinking of one young and seven old rats for the last 1–2 mo before death. It is readily apparent that there is no consistency between age and rate of decline in circadian rhythms in these outbred rats. One extreme was rat OF46 in which all three rhythms remained strongly entrained to the LD cycle, as was true of the young rats whose SCN activity patterns were analyzed in vitro and 30 other young rats in the laboratory. The other extreme is represented by rat OF45, which had no body temperature or drinking rhythm but maintained a weakly significant activity rhythm. Two male rats, OM23 and OM29, had very similar, strongly entrained patterns of body temperature and weaker rhythms of activity and drinking. The body temperature rhythm in a third male, OM13, appeared to free-run while activity and drinking rhythms were still discernible.

Periodograms for the last 10 days of data for the rats in Fig. 1 are shown in Fig. 2. In general, the behaviors of the old rats were very variable with respect to rhythmicity. One behavior could be rhythmic without any correlation between it and the presence or strength of rhythmicity in the other two behaviors. In four of the old rats body temperature showed a strong 24-h period, while at the same time, the 24-h periodicity of activity and drinking might also be strong (OF46), be considerably weakened (OM23, OM29), or show more power in one rhythm (drinking) than another (activity; OF50). A fifth rat (OM13) had many weakly significant ultradian frequencies in body temperature and weak circadian drinking and activity rhythms. The body temperature rhythm of OF49 had a weak 8-h periodicity. At the same time there was a weak 12-h and 24-h periodicity in the drinking rhythm and a 12-h rhythm in activity. OF45 lost its body temperature and drinking rhythms but maintained a circadian period in activity. In summary, these three behavioral output measures of the pacemaker were not correlated with each other either within an individual rat or between rats.

The right side of Fig. 2 shows the 30-h free-running pattern of the ensemble single-unit activity in the SCN slice; recording began 1 h after the rats were killed. The top record (YF09) is characteristic of SCN activity in young rats (11), with a sinusoidal pattern that has a single peak in SCN unit activity at CT7 on both *day 1* and *day 2* and a peak amplitude of ~6 Hz. This agrees closely with previous work with respect to time and amplitude of the first peak recorded in vitro (11). The brain slice recording of OM29 looks like that of a normal young rat, with a single peak per day at around CT7. Yet the SCN unit activity of OM23, whose behavioral rhythms are essentially identical to those of OM29, was the flattest of any of the old animals, with no clear initial peak. OF46, which had three strong behavioral rhythms, had a slice rhythm that did not have a sharply defined peak. The other four rats shown in Fig. 2 had aberrant patterns of SCN neuronal activity. Most rats had multiple peaks that appeared at unexpected times, and they were not the same time for any rat (e.g., OF50, at CT4, 10, 12, and 18, OM13 at CT21 and 24, OF49 at CT22, and OF45 at CT13–14, 0–1, and 4). Thus there was no more correlation between this fourth measure of pacemaker output, pattern of SCN firing rate, and the three behavioral measures, than there was among the behavioral measures themselves.

Fig. 2. *Three left columns*: periodograms of body temperature, activity, and drinking of the same rats as in Fig. 1 for the 10 days before the slice recording. The periodogram was normalized by making the total variance = 1. The power of each variable can be compared with the power for the same variable in other rats but not with different variables in the same rat. The horizontal line is the white noise level. The dashed line (in some cases only visible in the graphs with high power) is the lower limit of the 99.99% confidence interval for each frequency. *Right column*: SCN unit activity recorded in constant light for at least 30 hr. Solid line is 2-h sliding window average with data grouped in 15-min intervals. Circles are individual cells.

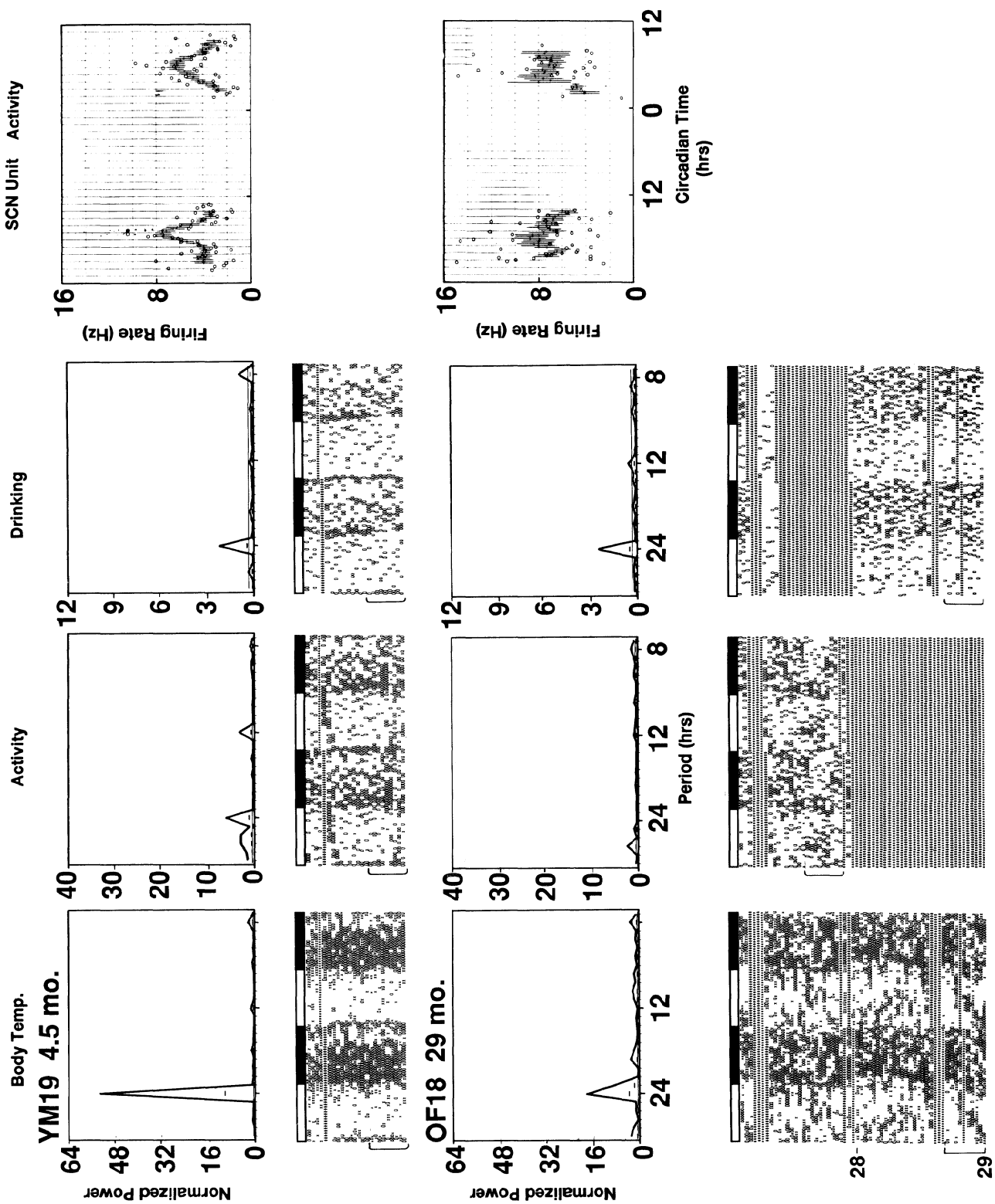


Fig. 4. Three left columns: periodograms of body temperature, activity, and drinking of young male rat YM19, 4.5 mo-old and old female rat OF18, 29-mo-old. Right column: SCN unit activity recorded in constant light for 7 h, from CT3–10 on *day 1*, and at the same time on *day 2*.

Because preparing the brain slice releases each SCN into constant environmental conditions in vitro, an analysis comparing mean SCN firing rates between old and young rats addresses the endogenous rhythmic properties of the old vs. young SCN (Fig. 3). Parameter values for the old and young groups were compared by t test. The t value for the offset term was not significant ($P = 0.737$), suggesting that overall firing rates were not significantly different from each other. This implies that the high firing cells are not clustered around a particular peak but are more spread out. However, the offset parameter only measures overall firing rates indirectly by adjusting the fitted curve up or down on the Hz axis. A stronger comparison of overall firing rats is a t test comparing cell firing rates themselves. Therefore we calculated a mean for each slice and compared old vs. young. There was no significant difference between the groups ($P = 0.322$). This supports the conclusion that the peaks are more scattered in the old group rather than that the high firing cells have disappeared.

The t value for the amplitude term was significant ($P = 0.016$). The mean amplitude values were 1.375 for the young rats and 0.876 for the old rats. This difference indicates that the amplitude of the circadian oscillation in SCN neuronal firing rates is attenuated in old rats. This change in amplitude reflects underlying changes in the behavior of individual neuronal activities such that there is apparent loss of coherence of the timing of appearance of fast units, producing multiple peaks (Fig. 2, *right*), as well as a general increase in variance of the activity, both fast and slow, of the neuronal population at any one time. The nature of these differences can be seen by comparing two behavioral rhythms and slice firing rate from a 5 and a 29-mo-old rat (Fig. 4). In these cases the units were recorded for 7 h, the slice was left untouched for 14 h, and then recordings were made again for 7 h to sample only the peak activities. In the young rat, the firing rates on the second day were as high as on the first day. This is in contrast to the old rat: there was an initial drop in firing rate on the first day, which is never seen in slices from young animals (11), and there were no clear midday peaks. Even when firing rate is damped in slices from young rats, a single major peak at CT6–7 is still clearly seen. In any case the firing rates on the second day in some old rats, such as OF45 and OF49, showed no such damping (Fig. 2).

DISCUSSION

There are two major points in this study. The first is that there is a loss of coherence of behavioral rhythms in aged rats. Circadian rhythmicity in body temperature, drinking, and locomotor activity did not deteriorate in the same order in individual elderly rats. This may result from several causes. Although masking effects due to peripheral factors may have contributed to some disruptions in the behavior, they cannot wholly explain the aggregate data. The latter can be accounted for by assuming that pacemaker or output cells in the SCN consist of coupled oscillators. Whenever sufficient numbers of cells become damaged or die, or the cells lose their coupling with each other, the behavioral rhythm they drive will be lost or weakened while others remain. In this sense, aging can be considered similar to partial SCN lesions in young rats, reducing either the number of oscillators or the synchrony between them. Such lesions have been found to cause differential disruption of the three behavioral rhythms measured in the present experiments (16). Although there are no experiments characterizing coupling among individual SCN cells, there is evidence that there are cellular changes with aging. For instance, a loss of peptidergic neurons in the SCN of old rats has been reported (3, 14). Alternatively, in individual aging animals, the target brain areas for the three behavioral rhythms may be differentially sensitive to signals from the pacemaker.

The second point concerns changes in the activity pattern of neurons within the SCN itself. Examination of individual slice activity records shows that there are peaks in firing rate at times never seen in young rats. The curve of the average firing rate for the old rats (Fig. 3) shows that an underlying circadian rhythmicity remains in the SCN; peaks occur at the expected times on both *day 1* and *day 2*. The aberrant peaks and troughs are superimposed on this basic rhythm. However, the amplitude of the rhythm in old rats is significantly damped at the normal peak times.

The functional identity of SCN neurons based on firing pattern is presently unknown (4). The neuronal activity rhythms measured here are most likely outputs from the SCN clock and not necessarily integral constituents of the clock mechanism (17). Nevertheless, it may seem surprising that deterioration of circadian rhythmicity in this output from the pacemaker, or the coupling between its cellular elements, does not prevent entrainment in the overt behavioral rhythms. This may indicate that different outputs from the SCN maintain entrainment of different rhythms. This hypothesis assumes that the entraining signal (LD) is capable of generating rhythmic SCN output of some kind even if the population of neurons within the SCN does not sustain an autonomous rhythm. In any case, the present results demonstrate that there is no more correlation between the output of the SCN and the rhythmicity of the behavioral rhythms than there is among the behavioral rhythms themselves. Of course, behavioral rhythms were measured under entraining signals that were not present in vitro. We do not know whether an arrhythmic SCN in vitro would have been arrhythmic under the entraining conditions in vivo.

Previously, declines in rhythmicity in various behaviors could have been interpreted as declines in homeostatic abilities taking place downstream from the pacemaker. The present results demonstrate that aging affects endogenous rhythmicity far more upstream, indeed, within the SCN itself.

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REFERENCES

1. **Aschoff, J.** Survival value of diurnal rhythms. *Symp. Zool. Soc. Lond.* 13: 79-98, 1964.
2. **Brock, M. A.** Chronobiology and aging. *J. Am. Geriatr. Soc.* 39: 74-91, 1991.
3. **Chee, C. A., B. Roozendaal, D. F. Swaab, E. Goudsmit, and M. Mirmiran.** Vasoactive intestinal polypeptide neuron changes in the senile rat suprachiasmatic nucleus. *Neurobiol. Aging* 9: 307-312, 1988.
4. **Gillette, M. U.** Intrinsic SCN rhythms. In: *SCN: The Mind's Clock,* edited by D. C. Klein, R. Y. Moore, and S. M. Reppert. New York: Oxford Univ. Press, 1991, p. 125-143.
5. **Green, D. J., and R. Gillette.** Circadian rhythms of firing rate recorded from single cells in the rat suprachiasmatic brain slice. *Brain Res.* 245: 198-200, 1982.
6. **Groos, G., and J. Hendricks.** Circadian rhythms in electrical discharge of rat suprachiasmatic neurones recorded in vitro. *Neurosci. Lett.* 34: 283-288, 1982.
7. **Hazzard, D. G.** Relevance of the rodent model to human aging studies. *Neurobiol. Aging* 12: 645-649, 1991.
8. **Inouye, S.-I., and H. Kawamura.** Persistence of circadian rhythmicity in a mammalian hypothalamic "island" containing the suprachiasmatic nucleus. *Proc. Natl. Acad. Sci. USA* 79: 5962-5966, 1979.
9. **Murakami, N., M. Takamure, K. Takahashi, K. Utunomiya, H. Kuroda, and T. Etoh.** Long-term cultured neurons from rat suprachiasmatic nucleus retain the capacity for circadian oscillation of vasopressin release. *Brain Res.* 545: 347-350. 1991.
10. **Newman, G. C., and F. E. Hospod.** Rhythm of suprachiasmatic nucleus 2-deoxygluose uptake in vitro. *Brain Res.* 381: 345-350, 1986.
11. **Prosser, R. A., and M. Gillette.** The mammalian circadian clock in the suprachiasmatic nuclei is reset in vitro by cAMP. *J. Neurosci.* 9: 1073-1081, 1989.
12. **Refinetti, R., H. Ma, and E. Satinoff.** Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Exp. Gerontol.* 25: 533-543, 1990.
13. **Richardson, G.** Circadian rhythms and aging. In: *Handbook of the Biology of Aging,* edited by E. Schneider and J. Rowe. New York: Academic, 1990, p. 275-305.
14. **Roozendaal, B., W. A. van Gool, D. F. Swaab, J. E. Hoogendijk, and M. Mirmiran.** Changes in vasopressin cells of the rat suprachiasmatic nucleus with aging. *Brain Res.* 409: 259-264, 1987.
15. **Rusak, B., and I. Zucker.** Neural regulation of circadian rhythms. *Physiol. Rev.* 59: 449-526, 1979.
16. **Satinoff, E., and R. A. Prosser.** Suprachiasmatic lesions eliminate circadian rhythms of drinking and activity, but not of body temperature, in male rats. *J. Biol. Rhythms* 3: 1-22, 1988.
17. **Schwartz, W. A., R. A. Gross, and M. T. Morton.** The suprachiasmatic nuclei contain a tetrodotoxin-resistant circadian pacemaker. *Proc. Natl. Acad. Sci. USA* 84: 1694-1698, 1987.
18. **Takahashi, J. S., and M. Zatz.** Regulation of circadian rhythmicity. *Science Wash. DC* 217: 1104-1111, 1982.
19. **Turek, F.** Circadian neural rhythms in mammals. *Annu. Rev. Physiol.* 47: 49-64, 1985.
20. **Williams, E., and J. Gottman.** *A User's Guide to the Gottman-Williams Time Series Analysis Computer Programs for Social Scientists.* Cambridge, UK: Cambridge Univ. Press, 1982.