It seems most likely that these differences in the effect of interferon on the morphology and tumorigenicity can be attributed to inherent differences between the cells of different clones of transformed cells. We stress, however, that interferon-treated cells of both clones fulfilled the criteria for phenotypic reversion. This phenotypic reversion induced in vitro by interferon was obtained in a clone of transformed cells, apparently free of C-type viral particles and reverse transcriptase activity. It seems likely therefore that the effect of interferon in inducing reversion of the transformed phenotype is unrelated to its antiviral activity.

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A computer-based system for collection and analysis of circadian rest-activity data

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Summary. A computer-based system for collection and analysis of circadian rest-activity data was developed. The system has the advantage of a minimal amount of interface hardware and uses standard laboratory computer equipment permitting easy collection and automatic visualization and analysis of the data. The flexibility of the programs allows manipulation of the presentation format as needed and further refinements of the data analyses are possible.

Introduction

Longitudinal recording of motor activity is the principal method that has been used to study rodent circadian rhythms. The rest-activity cycle is usually monitored by means of Esterline-Angus strip chart recordings, the daily plots of activity pasted sequentially below each other, and photographs of double plots used for analysis of period and phase[1]. This method is labor-intensive and does not provide numerical data for quantitative analysis of, for example, the total number of activity wheel revolutions per selected time bin or per 24-h day.

We approached the problem of measuring circadian rest-activity rhythms in animals after several years' experience in applying new electronic and computer technology to the measurement and analysis of human circadian rhythms[2,3]. Since one requirement was to be able to process the animal data in the same way as the human data, it seemed appropriate to apply the more sophisticated data collection and analysis technology to the development of a new computer-based system. Few reports of computer-based rodent motor-activity systems have been found in the literature[4,5]. We present details of a computer-based system that has the advantage of a minimal amount of interface hardware and that uses standard laboratory computer equipment that allows easy implementation in other laboratories. Additionally, we describe the software that was developed to record the circadian rhythm data, and include examples of the computed and plotted results using hamster running-wheel activity data.

Methods

a) Hardware

The system was developed for the continuous monitoring of running-wheel activity of 32 animals in separate cages. The cage and running-wheel (Hazelton Systems, Cincinnati, Ohio) consists of a stainless steel, rectangular cage 22 cm × 11 cm × 11 cm attached to a 36-cm diameter running wheel. Only minimal modifications and additions were necessary to adapt each cage to allow monitoring of each wheel rotation. A 5.5-cm diameter, stainless steel disc was mounted to the hub of each wheel and 2, IR, optical switches (Monsanto MCA8) 180° apart mounted to the support frame of the wheel. As the wheel rotates, the single slot on the disc briefly opens the pathway of the light beam of the optical switch. When both switches have been activated, a single count is generated by the interface logic and recorded by the computer.

Two detectors were used on each cage to prevent false counts which could occur if the animal rocks back and forth, but fails to make a complete revolution. The optical switches and circuitry can be easily removed from the activity wheels for replacement or when the cages are cleaned. A cable joins each cage to a junction box in the animal room, and a single flat cable joins this unit to the interface logic in an outer room.

A block diagram of the data logging system is shown in figure 1. All components, except the interface between the computer and the wheel cages, are commercially available. The PDP-11/03 computer contains 4K words (16 bits) of RAM and 2K words of PROM. The data logging program was written in the high-level programming language PASCAL (OMSI PASCAL-1, Oregon Software, Portland, Oregon), using a routine support system modified for use in read-only memory. Program development and checkout, as well as PROM programming, were done on a larger, disk based, PDP-11/40 system.

A TCU-50 clock (Digital Pathways, Inc., Palo Alto, Cal.) provides the system time base. The program may access the current date and time in the form: month/day/year/hour/minute/second. A tape drive

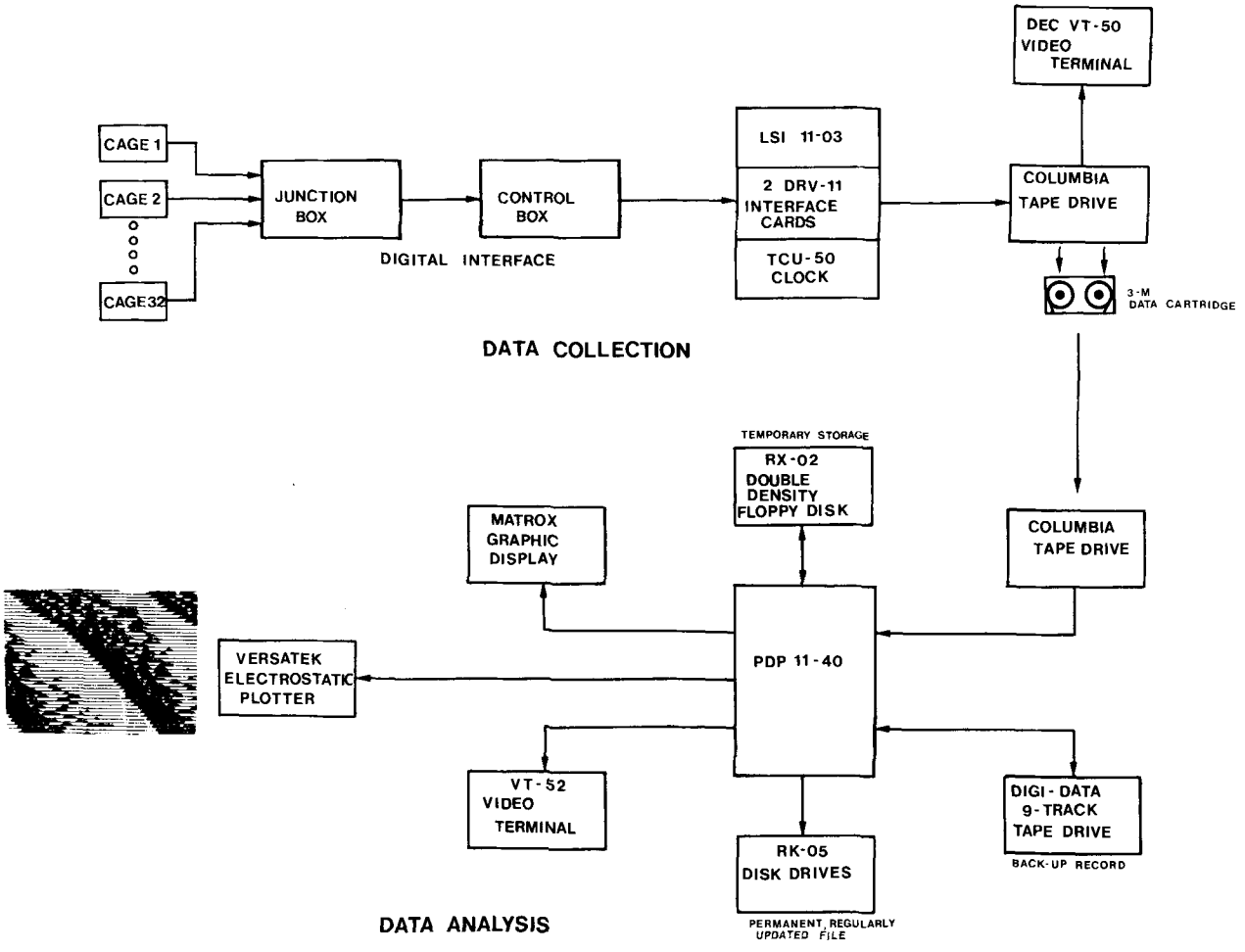

Figure 1. A block diagram of the data collection and analysis system for recording the running wheel activity of 32 animals in separate cages. Two computer-based systems are shown. Data collection was accomplished using a PDP-11/03 minicomputer. The data were stored on a Columbia tape cartridge system and transferred weekly to an identical Columbia unit on the larger PDP-11/40 computer for analysis.

(Model 300B, Columbia Data Products, Inc., Baltimore, Md.) is attached between the computer and the video terminal (Model VT-50, Digital Equipment Corp., Maynard, Mass.) using an RS-232C serial line. Data is stored on 3M DC-300 data cartridges, which have a storage capacity of 250,000 characters. The Columbia drive can operate in a transparent mode or in a mode where it records on tape all characters transmitted over the line. Data cartridges are normally replaced once per week and the activity data transferred to disk using an identical Columbia data cartridge attached to the larger PDP-11/40 system.

b) Software

Data collection. Whenever the data cartridge is replaced, the operator enters the number of sampling intervals per 15 min TCU-50 clock time. The program then waits until the next quarter hour time before beginning sampling. After the program is initiated, it will continuously poll the 32 input latches while waiting for the previously computed end-of-interval time. Whenever the input latch is set, the corresponding location in a 32-word array is incremented by one. When the current time is equal to the calculated end-of-interval time the program writes out to both the terminal and the tape drive the current time plus the value of the 32 counters. The 32 counters are then reset to zero, a new end-of-interval time is calculated, and the program resumes polling the input latches.

Activity data are displayed on the terminal (and simultaneously stored on the cartridge tape) in ASCII character format. Each data record corresponds to the time interval which was specified by the operator at the beginning of the recording session. Each record contains the date and time of the beginning of that interval plus the number of wheel revolutions within that interval for each of the 32 cages. An example, using an interval of 15 min is:

6/23/80 5:15:0
16, 35, 31, 23, 12, 3, 0, 0, 0, 15, 31, 10, 0, 0, 0, 17
0, 0, 0, 12, 11, 9, 25, 4, 0, 6, 31, 21, 0, 0, 15, 3
6/23/80 5:30:0
21, 13, 9, 22, 40, 0, 61, 31, 21, 12, 27, 15, 3, 0, 0, 0
12, 3, 19, 32, 0, 5, 15, 21, 0, 3, 19, 35, 20, 0, 47, 18

Each 250,000 character cartridge can store about 2000 data records. Using a recording interval of 15 min, this capacity provides up to 500 h of data logging, or about 20 days. Storing the data in ASCII form allows it to be easily edited if it becomes corrupted due to a power outage or tape error, but has the disadvantage of using tape less efficiently than if the activity counts were encoded in binary form.

A program named UPDATE is used to concantenate the newly collected data to each of 32 files containing continuous activity data for up to 1 year. These continuous files store data in a more compact format in which each count interval is assigned a unique location in the file and the time of that interval is computed from its position in the file. Since the continuous files are compatible with those used for long-term human activity monitoring, the same programs used for the human activity data can be used with the animal data.

Data analysis. Once UPDATE has transcribed the activity data into the continuous file format, various programs are available for data analysis, all written in

DATE	SUM	MEAN	SD	MAX	ASUM	AMEAN
18-Jan-80	7193	74.93	132.56	433	6710	291.74
19-Jan-80	11486	119.65	176.40	509	10947	364.90
20-Jan-80	11342	118.15	172.54	559	10057	359.18
21-Jan-80	9501	98.97	181.09	539	8890	423.33
22-Jan-80	7314	76.19	156.90	529	6282	369.53
23-Jan-80	8963	93.36	173.74	571	8302	395.33
24-Jan-80	11960	124.58	202.19	609	11901	396.70
25-Jan-80	8913	92.84	176.07	575	8448	422.40
26-Jan-80	5322	55.44	139.48	561	4229	422.90
27-Jan-80	6972	72.63	174.35	628	6679	477.07
28-Jan-80	11419	118.95	212.82	685	10820	491.82
29-Jan-80	12298	128.10	201.29	637	11689	417.46
30-Jan-80	8916	92.88	191.31	608	8510	472.78

Wake onset time	Sleep onset time	Length (h:min)
17:00 18-Jan-80	21:15 18-Jan-80	4:15
0:15 19-Jan-80	1:45 19-Jan-80	1:30
5:45 19-Jan-80	7:00 19-Jan-80	1:15
17:15 19-Jan-80	22:15 19-Jan-80	5:00
0:45 20-Jan-80	3:15 20-Jan-80	2:30
6:15 20-Jan-80	9:15 20-Jan-80	3:00
17:15 20-Jan-80	23:15 20-Jan-80	6:00
3:30 21-Jan-80	4:30 21-Jan-80	1:00
8:15 21-Jan-80	9:30 21-Jan-80	1:15
17:30 21-Jan-80	22:30 21-Jan-80	5:00
7:30 22-Jan-80	9:00 22-Jan-80	1:30
18:00 22-Jan-80	21:00 22-Jan-80	3:00
0:30 23-Jan-80	1:30 23-Jan-80	1:00
8:00 23-Jan-80	10:00 23-Jan-80	2:00
18:30 23-Jan-80	21:45 23-Jan-80	3:15
4:15 24-Jan-80	5:30 24-Jan-80	1:15
9:45 24-Jan-80	10:30 24-Jan-80	0:45
18:30 24-Jan-80	23:45 24-Jan-80	5:15
5:30 25-Jan-80	7:30 25-Jan-80	2:00
19:00 25-Jan-80	0:30 26-Jan-80	5:30
19:30 26-Jan-80	22:30 26-Jan-80	3:00
8:00 27-Jan-80	9:00 27-Jan-80	1:00
19:30 27-Jan-80	22:45 27-Jan-80	3:15
7:45 28-Jan-80	9:45 28-Jan-80	2:00
19:45 28-Jan-80	0:15 29-Jan-80	4:30
8:00 29-Jan-80	10:30 29-Jan-80	2:30
20:15 29-Jan-80	1:45 30-Jan-80	5:30
9:15 30-Jan-80	11:15 30-Jan-80	2:00
20:45 30-Jan-80	24:00 30-Jan-80	3:15

The statistical results of the first 13 days of the experiment shown in figure 2 are tabulated. Each line corresponds to 1 day in the continuous file and includes the total counts of activity (SUM), the mean and standard deviation (MEAN, SD), and maximum value during the day (MAX). The ASUM and AMEAN are defined as activity sums and means and are computed by summing all values in the day which are not zero and not adjacent to any zero values. AMEAN is determined by dividing the ASUM by the number of locations which contributed to it, and gives a good estimate of average activity during active periods. For the same 13-day period, the results of the sleep/wake onset time program are also shown. Comparing the onset times with the actograms in figure 2 shows both the major and minor periods of activity within a 24-h segment.

PASCAL (OMSI PASCAL-2). Four programs which are particularly useful are CPLOT, CDISP, CIRCAD and CPRINT.

CPLOT produces the raster scan plots on a similar format to that produced by the Esterline-Angus strip chart method. The user, however, can select among various options before plotting the continuous file on a Versatek printer/plotter (Xerox Corp., Santa Clara, Cal.). The options include: selection of the height of each raster line; scaling of data and histograms; selecting the specified time window of the data; and whether to display the results in a linear or semi-logarithm form (see fig. 2). CPLOT is excellent for observing an overall activity pattern as a function of time.

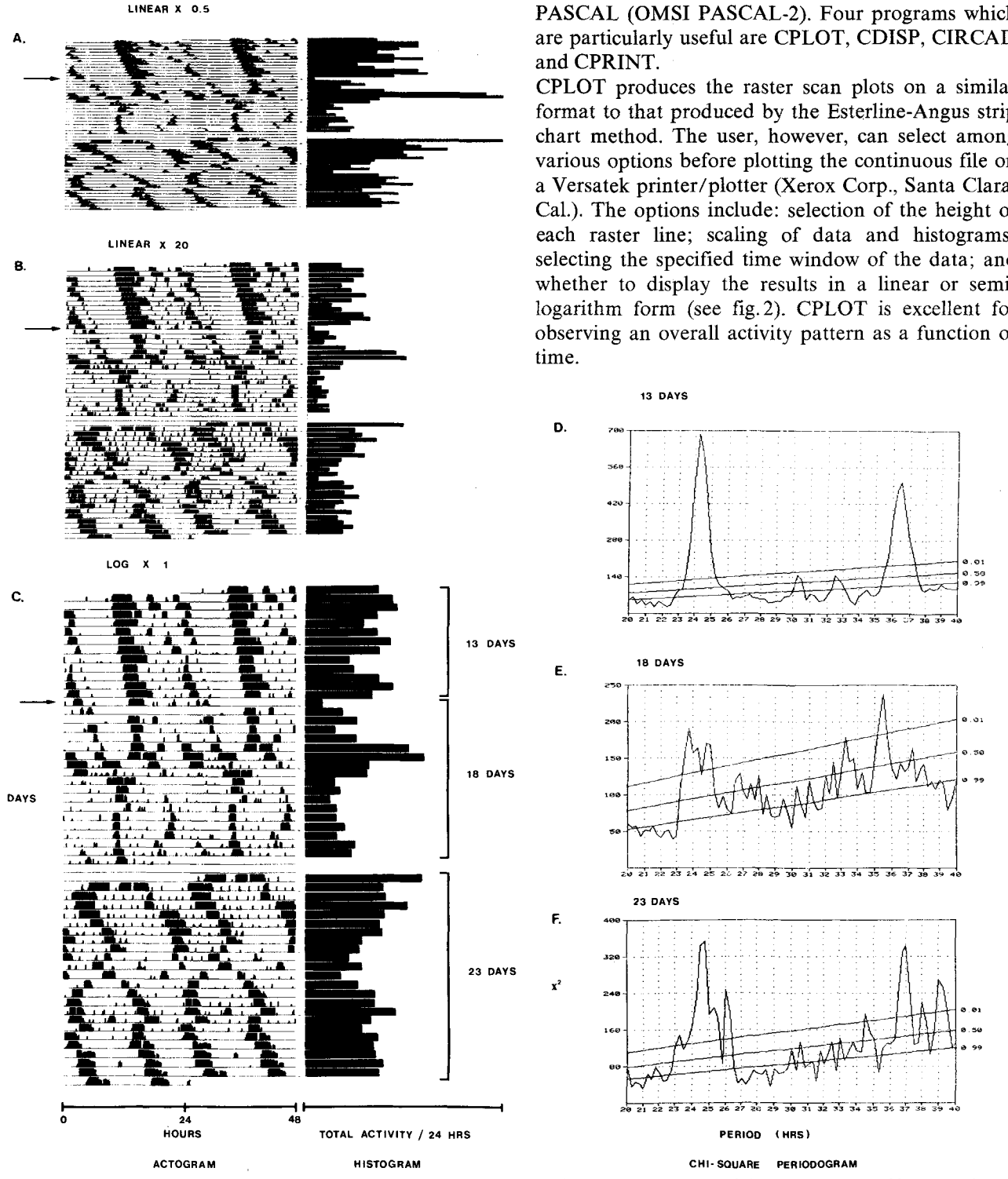

Figure 2. Several analyses of an example of female hamster running-wheel activity taken over a 56-day period (18-Jan-80–14-Mar-80) are shown. 3 different scales for plotting actograms of the same data are displayed: plot *A* shows the actogram when the data (revolutions/min) is multiplied by 0.5; in plot *B* the data are multiplied by a factor of 20; and in plot *C* the log value of the data is used to plot the actogram. The actograms are double plotted in the conventional manner to ease visual inspection of the onset and offset of motor activity, and each day's data plotted beneath the previous day. Immediately to the right of the actograms are plotted the histograms of total motor activity summed over each 24-h period (with different scaling factors shown for each example). The animal was free-running under conditions of constant dim red illumination at the beginning of the experiment. At the day marked with the arrow, the hamsters were implanted with an osmotic minipump that released a constant infusion of the tricyclic anti-depressant drug imipramine. The linear plot *A* shows not only onset and offset of motor activity but also differentiates between high and low (during drug administration) motor activity. The linear plot *B* shows the motor activity saturating the plot as an all or none phenomenon. The logarithmic scale *C* shows a similar pattern; however, fine details of very minor bouts of motor activity (such as the striking regularity in the lower part of actogram) are seen that cannot be picked up in the usual linear scale actogram. Plots *D*, *E*, and *F* show the periodograms obtained by the Chi-square analysis. The rest-activity cycle length period is plotted vs the χ^2 statistic for randomness. Activity levels above the 0.01 (99% level) are statistically non-random. The baseline free run period of 24.25 h can be seen in plot *D*. After the drug is administered, a dissociation of activity components in the circadian range is evident in plot *E* and further in plot *F*.

CDISP allows a more detailed look at the data and can perform some statistical tasks on selected portions of data. In its normal mode, CDISP can display 1 day of activity on a Matrox-driven video monitor (Matrox Electronic Systems, Montreal, Canada). Additionally, however, it can average days, compute amplitude histograms over selected days, and print or plot these results. The program also includes the option of estimating the sleep and wake onset time and the resulting total daily activity time (table). The onset times are determined using a heuristic algorithm based upon threshold filtering. The algorithm searches for these events alternately using a 3-point window for wake onset detection and a 6-point window for sleep onset detection. A wake onset is defined to have occurred whenever each value in the interval is greater than 15 and the sum is greater than 60. A sleep onset is defined to have occurred whenever each value in the interval is less than 15. The time associated with each interval is set to be the first point of that interval. This algorithm has been quite successful using human data and seems to apply to the hamster data well, except that some false detections will occur. The algorithm in effect gives a list of wake-sleep onset times which can then be screened manually.

CIRCAD is a program which implements the Chi-square (χ^2) periodogram analysis of Sokolove[6] to test hypotheses about periodicities in the data. Briefly, the technique entails selecting an epoch for analysis (usually 2 weeks or more), selecting a period of analysis (i.e., 25 h), and then averaging the adjacent 25-h blocks of data which are entirely within the epoch selected. The averages are then subjected to a χ^2 statistical test for randomness. An example of the type of analysis of hamster activity data is shown in figure 2. The plots are shown with 3 statistical levels. Any values above the 0.01 level (99%) are by definition statistically non-random. This same analysis is done for a range of periods usually with increments equal to the sampling interval of the data (15 min for the hamster data). The χ^2 display is very useful in showing periods within the data that are not apparent on a 24-h raster scan display and to quantify intervention effects which may alter the period(s) of an animal's normal daily rhythm.

An auxiliary program called CPRINT prints a daily statistical analysis of the continuous data file (see table). Each line of the table corresponds to 1 day in the file and includes the total sum of data (SUM), the mean and standard deviation (MEAN, SD), and the maximum value during the day (MAX). The ASUM and AMEAN are defined as sums and means and are computed by summing all values in the day which are not zero and are not adjacent to any zero value. This summed value, therefore, indicates no involvement of data during sleep periods. The AMEAN is determined by dividing the ASUM by the number of locations which contributed to it and therefore gives a good estimate of average activity during active periods.

Results

An example of a complex circadian rest-activity rhythm has been taken from experiments described in detail elsewhere[7] to demonstrate how the computer programs were used to analyze hamster activity data. A female hamster originally kept on a light:dark (12:12 h) cycle was studied under conditions of isolation from time cues (constant dim red light), for many weeks. After establishing baseline conditions of free-running rest-activity cycles, the animal was implanted with an osmotic minipump[8] containing the tricyclic anti-depressant drug imipramine (fig. 2) at the arrow mark. Double plots of the rest-activity cycles are shown in 3 versions: plotted on a linear scale; a linear scale saturated plot (showing minor activity bouts not usually seen); and a logarithmic scale plot (emphasizing the activity period). At the right of the actograms are the histograms of total wheel revolutions/24 h. Also shown are the statistical analyses by the χ^2 periodogram method showing 3 different time periods of the experiment: the first 13 days of free running activity; the second 18 days after implantation of the imipramine minipump; and the last 23 days showing continuing effects of the drug. From the statistical analysis of the data, the rest-activity cycle length (period τ) of the baseline free run can be estimated (24.25 h).

The table shows some additional examples of individual daily measures of motor activity parameters taken from the same data shown in figure 2. Of particular interest are the activity sum (total number of wheel revolutions/24 h), and activity mean (activity per 15-min bin during the active phase). Also, as described earlier, the sleep/wake onset can be estimated and the resulting length of activity time calculated.

Comment

This computer-based system for monitoring animal rest-activity data permits easy collection and automatic visualization and analysis. The flexibility of the programs allows manipulation of the presentation format as needed as was shown with the different linear and log scales displayed in figure 2. The system can also be expanded and modified. For example, after 3 years' experience of data collection and analysis, the periodogram program for statistical analysis and numerical determination of multiple periodicities was developed.

Results of a long-term series of experiments are presented in detail in the accompanying article[7]. Technical details are available on request from the authors.

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Antidepressant drugs can slow or dissociate circadian rhythms[1]

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Summary. The circadian rest-activity cycle of female hamsters was lengthened by chronic administration of the monoamineoxidase inhibitor antidepressant drug clorgyline. Chronic treatment with clorgyline or the tricyclic antidepressant drug imipramine also induced dissociation of circadian activity rhythm components. Thus these drugs may be added to the very small group of substances (including the prophylactic antidepressant and antimanic drug lithium) that modify circadian frequency and/or coupling between circadian rhythms.

Introduction

The temporal stability of circadian oscillations is extremely important for their time-keeping and time-measuring functions[4,5]. Homeostasis of circadian oscillations is reflected in their resistance to chemical fluctuations in the internal milieu. However some endocrinological manipulations have been found to change the free-running circadian period (τ = the duration of 1 complete cycle in an environment free of time cues): ovariectomy, and replacement oestrogen and progesterone in hamsters[6,7]; castration and replacement testosterone in mice[8]; pinealectomy and replacement melatonin in birds[9]; and perhaps also thyroidectomy and replacement thyroid in hamsters[10] and man[11].

Although the number of drugs known to modify circadian rhythms in mammals is small, it is expanding[12]. The first substance to be extensively investigated was deuterium oxide[13]. Carbachol, a cholinergic agonist, and α-bungarotoxin, a nicotinic cholinergic receptor blocker, have been shown to exhibit phase-shifting properties mimicking and blocking, respectively, the effects of light[14,15]. Lithium, used as a prophylactic antidepressant and antimanic drug, slows circadian rhythms and delays phase-position in many species from hamsters to man (reviewed in Wirz-Justice[16]).

There are reasons to suspect that not only lithium, but that antidepressants as a class may alter circadian time-keeping. The timing of many circadian rhythms in physiological, behavioral, and endocrinological parameters appears to be abnormal in depression and mania (for reviews, see Wehr and Goodwin[17] and Wehr and Wirz-Justice[18]). It is therefore of interest for circadian hypotheses of the pathophysiology of affective disorders to investigate whether drugs used in the treatment of this illness act on the circadian system.

The free-running period and the phase-response curve for some standard perturbation, are the only parameters of an overt rhythm that can be measured and said to reflect the corresponding parameter of the underlying oscillator(s)[19]. Studies in both rodents and man on the properties of circadian pacemakers have measured the period (τ) of the rest-activity (sleep-wake) cycle under constant conditions of isolation from external time cues. We report here that drugs selected from the 2 major classes of antidepressants, a monoamine-oxidase inhibitor (MAOI) - clorgyline - and a tricyclic - imipramine - can slow or dissociate circadian rest-activity rhythms; we propose that these effects may be an important mechanism in their antidepressant and mania-inducing actions.

Methods

Data collection and reduction. Hamsters were housed in steel cages attached to running wheels (⌀ 36 cm), with free access to food and water, in an airconditioned room maintained at 22 °C. The room was