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Final Subproject Report for NASA COOPERATIVE AGREEMENT #NCC2-779

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Part II: Circadian Behavioral Study

LED vs. Cool White Fluorescent - 0.1, 1, 10, 40, 80 lux

IN-51

[redacted]

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Abstract

LIGHT-EMITTING DIODES (LED) AND COOL WHITE FLUORESCENT (CWF) LIGHT HAVE SIMILAR EFFECTS ON THE CIRCADIAN SYSTEM OF THE RAT.

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Currently, the light source most commonly used in animal habitat lighting is cool white fluorescent (CWF) light. It was the objective of this study to evaluate a novel LED light source for use in animal habitat lighting by comparing its effectiveness to CWF light in producing and maintaining a normal circadian entrainment. The LED and CWF lights had similar spectral power distributions. Sprague-Dawley rats (175-350 g) were kept individually in metabolic cages, under a strict lighting control: 4 days of acclimation at 12:12 LD, 14 days of 12:12 LD, 14 days of 24:0 LD (free-run), and finally 12:12 LD. Food and water were provided *ad libitum.* Three behavioral parameters were monitored continuously: gross locomotor activity, drinking, and feeding. Combined mean free run periods (tau) were (mean±SEM): 24.6±0.1 and 24.7±0.2 at **0.1 lux**, 25.5±0.1 and 25.7±0.1 at **1.0 lux**, 25.3±0.2 and 25.4±0.2 at **10 lux**, 25.8±0.1 and 25.9 ±0.1 at **40 lux**, and 25.9±0.1 and 25.9±0.1 at **80 lux**, CWF and LED respectively. ANOVA found a significant effect ($p<0.05$) due to light level, but no difference in tau between rats exposed to constant CWF light and rats exposed to constant LED light. This study has shown that LED light can produce the same entrainment pattern as a conventional CWF light at similar intensities (0.1, 1, 10, 40, and 80 lux). LED light sources may be a suitable replacement for conventional light sources used in animal habitat lighting while providing many mechanical and economical advantages.

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Introduction

Needing precise engineering specifications for construction of lighting systems for animal cages to be used in microgravity experiments, in 1988 NASA convened a working group to address this issue and to publish standards (1). At that time, federal guidelines provided little detail on animal habitat lighting requirements (2). Though the latest version of the federal Guide for the Care and Use of Laboratory Animals indicates that intensity and duration of light exposure are important factors for animal holding room lighting, it still does not specify standards for photoperiod or wavelength (3).

Previous space shuttle animal cages (Animal Enclosure Module, Research Animal Holding Facility) have been illuminated by incandescent lights (4) which do not provide optimal spectral characteristics (1) and produce considerable heat. For these reasons, combined with the fact that cool white fluorescent lights (CWF) illuminate most ground animal vivariums, NASA is interested in alternate lighting technologies. Light emitting diodes (LED) have been proposed as a candidate lighting alternative. LED systems are inexpensive, and offer inherent advantages including: spectral control, high efficiency, long operating life, relatively low heat production, ruggedness (solid state), and have certain mechanical/size advantages (5).

The purpose of this study was to evaluate the effectiveness of LED arrays (of similar spectral characteristics) compared to CWF lighting in maintaining photo entrainment of several selected behavioral circadian rhythms in rats: gross locomotor activity, feeding, and drinking. This was evaluated at five light intensities (0.1, 1.0, 10, 40, and 80 lux).

Methods And Materials

We exposed 175-350g male Sprague-Dawley albino rats (Simonsen Laboratories, Gilroy, CA) to light from CWF bulbs or from four color LED arrays at 5 light intensities (0.1, 1.0, 10, 40, 80 lux). Tischler et al. (6,7) found that rat circadian entrainment was maintained using light intensities as low as 0.1 lux. Forty-lux was included since this was the recommendation made to NASA in 1988 by the science working group (1). Recent work done in our laboratory (8) had confirmed that 40 lux is preferable to higher illumination since it produces normal circadian entrainment, normal reproductive activity, and does not cause retinal damage (see part I of this final subproject report).

Cages

Rats were housed individually in Nalgene metabolism cages made of lexan (Nalge Co., Rochester NY). These materials passed all wavelengths of light in the visible spectrum, and a portion of ultraviolet. The metabolism cages were placed inside separate ventilated, radio frequency shielded, light- tight wooden cabinets (I.D. 66cm X 66cm X 76cm). These cabinets were contained in a sound-attenuated, environmental chamber at constant temperature (20 – 22 C°).

Food and water were provided *ad libitum.*

Lights

Each cabinet contained either a CWF lamp (GE Cool White, F14T12 CW, 14 Watt) or a 4-color LED array (Figure 32). Light sources were positioned directly above each cage at approximately 8 inches from the cage floor.

Illuminance levels from the CWF lamps and the LED arrays were set to 0.1, 1, 10, 40, or 80 lux at animal head height using a calibrated radiometer (Model IL-1700, International Light Inc., Newburyport, MA) and a photometer sensor (Model #SED038, Y filter and W diffuser). Total irradiance prodeced by the LED arrays and the CWF lights was measured directly using a calibrated irradiance probe (Model # 038, F filter and W diffuser). The irradiance measures ($\mu W/cm^2$) corresponding to the illuminance settings were: 80 lux: LED 22.4, CWF 25.6; 40 lux: LED 11.2, CWF 13.11; 10 lux: LED 2.8, CWF 3.2; 1.0 lux: LED 0.28, CWF 0.32; 0.1 lux: LED

0.03, CWF 0.04. The LED array spectrum could not be adjusted to exactly match that of the CWF lamps. We, therefore, set the irradiance of each LED color to match that part of the CWF spectrum where most of the energy was contributed by that color LED. Irradiance levels of each LED color were adjusted to be equal to the corresponding color band irradiances of the CWF lamps. The measured spectrum (300nm - 800nm) was divided into three color bands. Each band covered sections of the spectrum where most of the energy was contributed by one or two LED colors. The blue LED band spanned 300 to 535 nm. The green and yellow LED band spanned 540 to 620 nm. The red LED band spanned 625 to 800 nm. Energy distribution for the LED array was: blue 35.8%, green and yellow 47.2%, and red 17%. Energy distribution for the CWF bulb was: blue 34.6%, green and yellow 48.6%, and red 16.8%. For a spectral power distribution curve see Figure 1 in part I of this final project report.

The light schedules for all experiments were similar. An initial 4 day period of acclimation at 12:12 LD was followed by 14 day period of 12:12 LD (LD1), then 14 days of 24:0 LD (free-run) (LL), and finally a 14 day period of 12:12 LD (LD2). Light intensities were set at the beginning of an experiment and remained unchanged for the duration. Lights came on at 0700 and remained on until 1900.

Data Acquisition

The continuously monitored dependent variables were gross locomotor activity (LMA), feeding time, and drinking time. All parameters were recorded automatically every minute. Data are presented in double raster plots (Figures 1-30, and Appendix A). These are continuous records of activity over the course of the experiment, which are double plotted so that the pattern of the circadian rhythm can be easily seen with the unaided eye. In an entrained animal, each day's activity is aligned with a specific onset and offset. During the so-called "free-running" conditions, the animal is exposed to constant environmental conditions (i.e., constant light, LL). In LL, each day's activity will drift a little with the "intrinsic" rhythm of the internal biological clock (9). The appearance of a drift is a means of indicating whether or not the animal was previously entrained to the light cycle (an exogenous stimulus).

Two independent data acquisition systems (DAS) were used to collect and store data from 12 cages. Each system consisted of activity, feeding and drinking sensors connected to a

Keithley data collection and conversion system that was connected to a Sperry microcomputer (MS-DOS). Data were printed (hard copy) and stored on floppy disks.

Activity Sensors

Each metabolism cage was attached to a particle board base which "floated" above the cabinet floor on four steel springs. Rat locomotion caused the cage to move. A three-axis ballistocardiograph accelerometer attached to the top of the metabolism cage frame converted cage movements into a voltage. The ballistocardiograph preamplifier and main amplifier multiplied, filtered, and twice integrated the accelerometer voltage. The amplifier output voltage was approximately proportional to the distance moved by the rat in a few seconds.

Drinking Sensor

Rat drinking water came from a 100 ml plastic bottle equipped with a metal spout with an internal ball bearing to prevent drips. Rats standing on the metal cage floor grid licked the spout to obtain water. A custom circuit sensed the microampere currents that flowed from the metal water spout through the rats body to the metal floor grid. The output of this circuit indicated whether the rat was or was not touching the drinking spout.

Feeding Sensor

A small drawer contained a reservoir of food (Purina 5001 Rodent Chow) which was available to the rat through a small opening off a short tunnel attached to the metabolism cage. An Omnitech sensor (Model #FM8, Omnitech Electronics, Inc., Columbus, OH) utilizing an infrared LED/ photodiode pair surrounded the food drawer opening. A rat reaching into the food reservoir interrupted the infrared beam. The Omnitech monitor converted the beam interruption into a TTL compatible pulse. When the rat interrupted the beam, the monitoring system recorded duration of feeding activity.

Keithley 500 Data Acquisition System (DAS)

Two (one per 6 cages) Series 500 DAS (Keithley Metrabyte, Taunton, MA) converted activity, drinking, and feeding signals into a form which could be passed on to the data acquisition program running in the Sperry microcomputer. Each DAS total sample rate was 100 samples per second, where each channel was sampled at 100/18 samples per second. This produced approximately 300 samples per channel per minute.

Sperry microcomputer

Each Sperry ran the Data Acquisition Program which transferred data from the Keithley, accumulated data for 1 minute, then recorded the data on floppy disks.

Data were recorded at the rate of 1 datum per minute. Each datum is the sum of many samples read from the Keithley data acquisition system. The number of summed samples varied from one datum to the next. This number, N, was recorded with each datum.

Activity data values represented the total amount of physical activity in the preceding minute. This combined intensity and duration information. Feeding and drinking data values represent the relative amount of time used for feeding or drinking.

Circadian Rhythm Analysis

Time series analysis was composed of several filtering steps followed by either frequency or period analysis.

Analysis Methods

Blocked Sensor edit

Occasionally food pellets in the food reservoir stacked up and interrupted the infrared beam. This created invalid data values. The blocked sensor edit removed values which were at least 90% of the number of samples used to create the value.

Missing edit

Data values were not recorded for every time interval, e.g., several were lost during maintenance when the data diskette was changed, and occasionally the computer stopped recording due to software problems, printer paper feed problem, or power failures. The missing edit routine replaced each missing value with an estimated value. Each estimated value was the mean of four adjacent values. Two adjacent values were immediately before and after the missing value. Two adjacent values were 24 hours before and after the missing value.

Decimate

This procedure added successive data values to create a new value. N was the number of summed samples. This reduced the number of samples and provided a smoothing function.

Binary conversion

This counted the number of nonzero data values in a given number of values. N is the number of values checked for nonzero values.

Robust Locally Weighted Regression (RLWR)

This filtering technique used a fixed number of adjacent values to predict what the central value should be in order to create a smooth curve. This routine produced one of two data sets as its output. The first result, smoothed data, allowed RLWR to act as a low pass filter. The second result, the residual (difference between the smoothed data and the original data), allowed RLWR to act as a high pass filter.

Fourier analysis

This applied the discrete Fourier transform to calculate the spectral composition of the last 10 days of data values for the periods LD1, LL, and LD2. The largest spectrum amplitude value occurred at the dominant frequency. This was converted into a period and reported as the free running period, tau.

Time Series Analysis Sequence Used for Activity, Feeding, and Drinking Data

Analysis of activity data used the following steps:

1) Missing edit
2) Decimate with n=5
3) RLWR with n=36 saving the smoothed curve
4) Decimate with n=4
5) RLWR with n=144 saving the residual
6) Fourier analysis

Analysis of feeding data used the following steps:

1) Blocked sensor edit
2) Missing edit
3) Binary conversion (n=20)
4) RLWR with n=9 saving the smoothed curve
5) RLWR with n=144 saving the residual
6) Fourier Analysis

Analysis of drinking data used the following steps:

1) Missing edit
2) Binary conversion (n=20)
3) RLWR with n=9 saving the smoothed curve
4) RLWR with n=144 saving the residual
5) Fourier Analysis

Analysis of Variance

Free running periods from the previous section were tested for effects due to the type of light, the light level, and variation among rats using analysis of variance techniques. The design used was a 2 level nested ANOVA within a 2 way factorial ANOVA (10). The factorial ANOVA portion tested for effects due to light level and light type. The nested analysis portion tested for the effect of variation among rats within the subgroups.

The factorial ANOVA design tested for significant effects due to light type, light level, or the interaction of those two. Several changes in the standard factorial ANOVA design were required to fit the pattern of the time series analysis results. Failure of equipment resulted in an unequal number of taus in the subgroups. These variations of N depended mostly on the random nature of computer failures and appeared to be independent of the type or level of light used. These assumptions allowed the use of unweighted means analysis methods (11) which estimated the partitioning of variation within the ANOVA.

Another addition to the factorial ANOVA design was the nested nature of data within the subgroups. Within each subgroup there was random genetic variation among the rats of the subgroup. For each rat there was random variation among the three estimates of tau. Factorial methods cannot be used here because the three tau values are not independent of one another. Nested methods structure the analysis into levels to account for variation contributed by the random differences between rats and between tau estimates. This structure reduces the error term used for significance testing in the factorial portion of the ANOVA.

Nested analysis tested for significant variation among rats within each subgroup. Variation at this level would mean that individual rats responded differently to the same experimental conditions. Each subgroup contained the feeding, drinking, and activity taus for all rats tested at the same light level using the same light type. Combining the three taus for each rat was allowed by assuming that the taus were <u>not</u> independent of one another and that any differences were due to random variation among parameters. This requires that the same "clock" control feeding, drinking, and activity (9). Our previous findings in this laboratory support this notion (12,13)

Results and Discussion

Circadian Rhythm Analysis

Visual inspection of the raster plots (Figures 1-30, and Appendix A) clearly indicated that circadian rhythmicity was maintained in all parameters monitored. Captions for each plot indicate experiment name, light intensity, and whether the rat was exposed to LED or CWF.

Table 1 lists individual free run periods in chronological order. Table 2 shows the same values listed by light level, parameter measured, and light type. There were 3 measured parameters - activity, drinking, and feeding, and 5 levels of light intensity. Table 3 shows the mean free run periods listed by light level, parameter measured, and light type. Table 4 and Figure 31 show the mean periods and standard errors for combined activity, drinking, and feeding free run periods listed by light level and light type. These were the subgroup values used in the combined ANOVA.

It should be noted that activity was recorded in five of the 12 cages used for this study. All cages in chamber A lacked the activity monitor, as well as cage six in chamber B. The operational accelerometers were of an older model, which could not be repaired, and funds did not suffice to acquire 12 new devices. Other reasons for missing data, or inability to determine tau value (other than random computer failure, as mentioned above) included either: 1) a temporary short circuit or 2) a disconnected cable to a drinking monitor, and 3) an over-filled food drawer which blocked the infrared beam, resulting in the recording of erroneous data.

Combined mean free run periods varied from 24.6 ± 0.1 hours for CWF lights at 0.1 lux to 25.9 ± 0.1 hours for CWF lights at 80 lux and LED lights at 40 and 80 lux (mean ± SEM). Except for both light types at 10 lux, the free run period increased with increasing light level.

Analysis of Variance

Table 5 shows the results of the analysis of variance. The factorial portion of the ANOVA found a significant effect ($p < .05$) due to the level of light. This was expected since the free running period increases with light level (6). The nested analysis found a significant ($p < .05$) amount of variation due to differences among rats within each subgroup.

Conclusions

Time series analysis and ANOVA found a significant effect ($p<0.05$) due to light level, but no difference in the free running period of rats exposed to constant CWF light and rats exposed to constant LED light within a light intensity subgroup.

This study shows that LED light can produce the same entrainment pattern as a conventional CWF light at intensities between 0.1 and 80 lux. LED light sources are a suitable replacement for conventional light sources used in animal habitat lighting while providing many mechanical and economical advantages.

Acknowledgments

I wish to express my deepest appreciation for the following invaluable assistance with this research. Dr. D.C. Holley, who was the main reason I got the project to begin with, for his guidance and sound advice. Mr. G. Mele, for the technical assistance in setting up the light sources, debugging the collection software, and statistical analysis. Mr. D. Heeke for his contribution in running the project. Warm appreciation is extended to the entire staff of Student Research Assistants who helped with the day to day animal maintenance.

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Figure 1. Experiment 9703LED.1B (0.1 Lux) Rat LMA actogram - LED. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

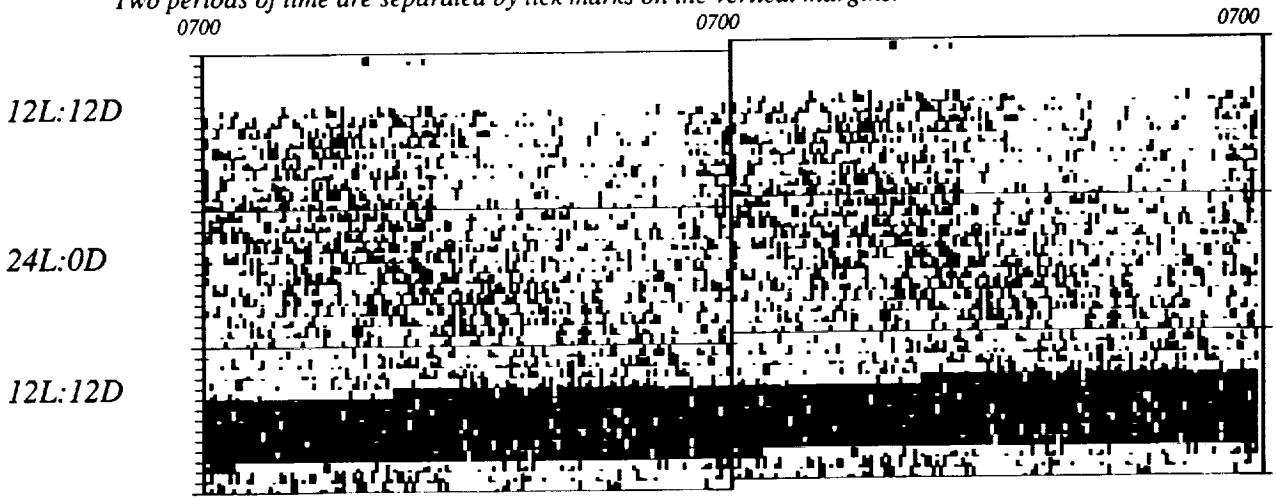

Figure 2. Experiment 9703LED.1B (0.1 Lux) Rat drinking actogram - LED. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.

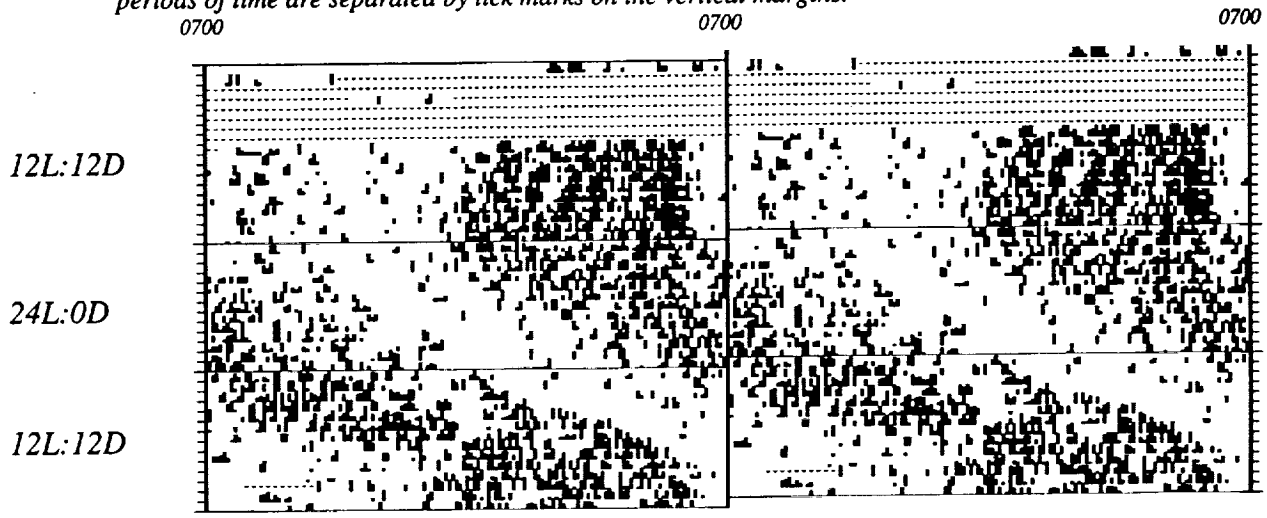

Figure 3. Experiment 9703LED.1B (0.1 Lux) Rat feeding actogram - LED. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins

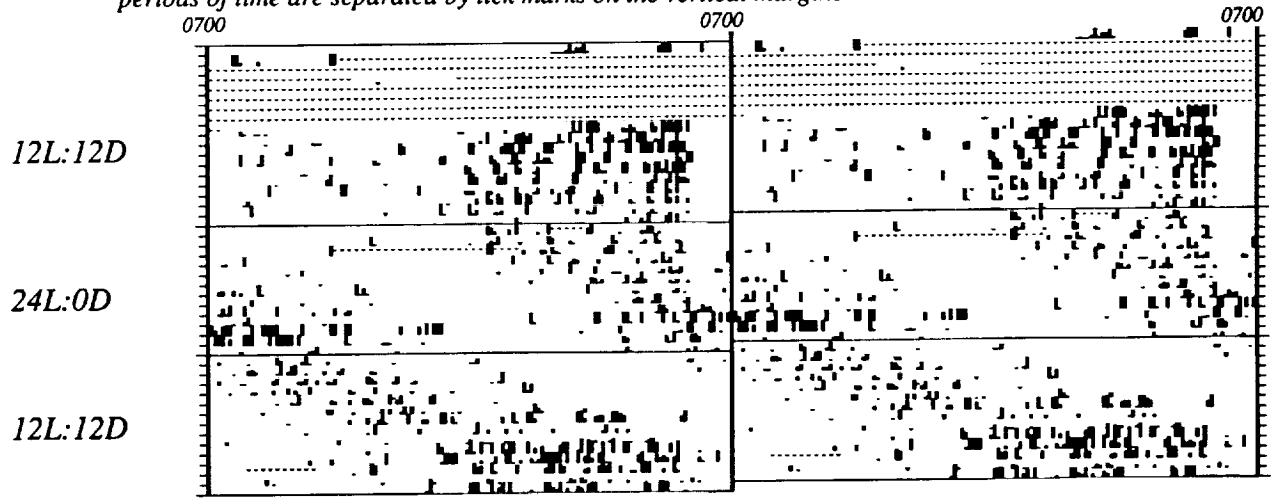

Figure 4. Experiment 9703LED.1B (0.1 Lux) Rat LMA actogram - CWF. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

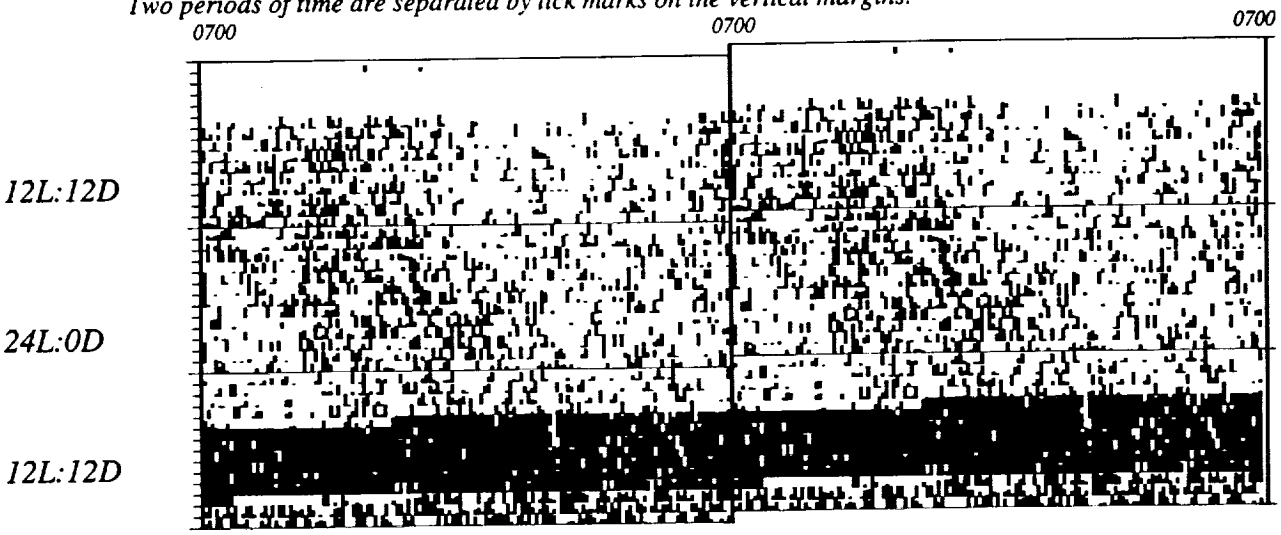

Figure 5. Experiment 9703LED.1B (0.1 Lux) Rat drinking actogram - CWF. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.

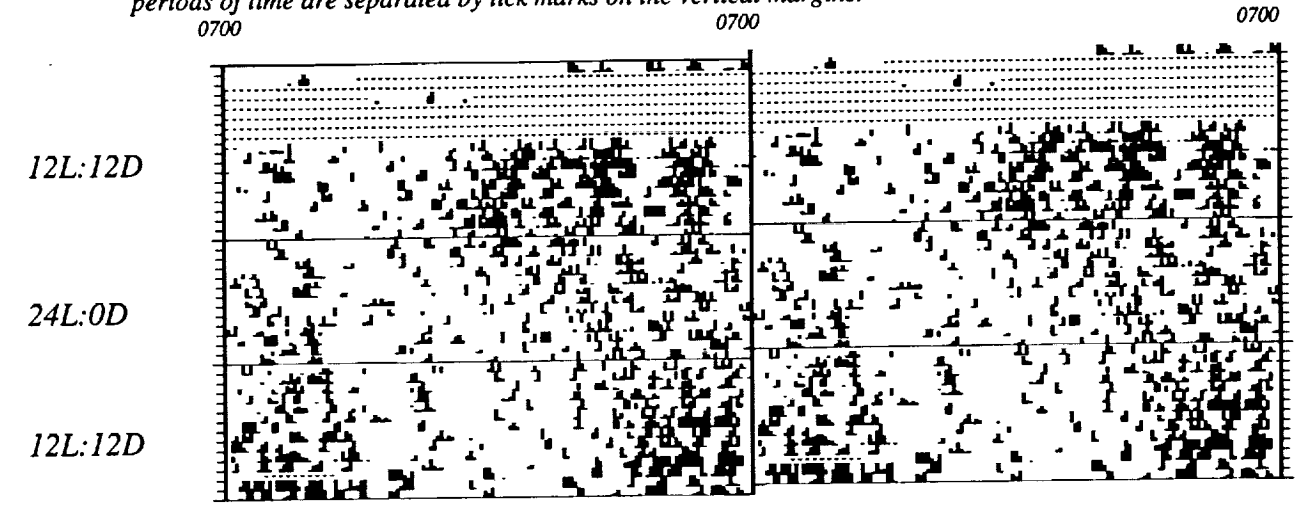

Figure 6. Experiment 9703LED.1B (0.1 Lux) Rat feeding actogram - CWF. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins

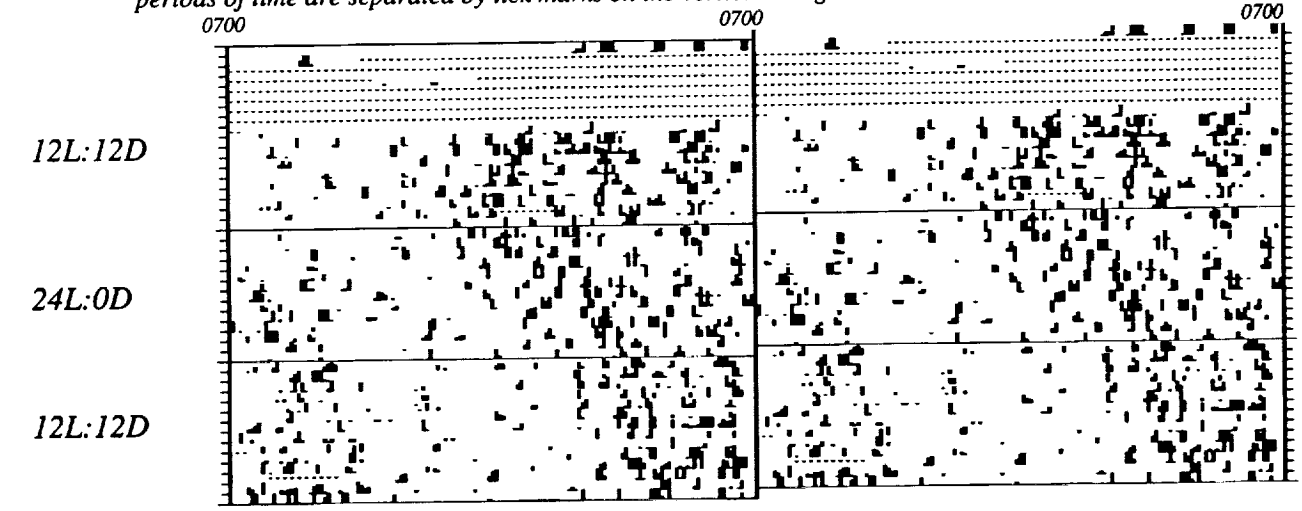

Figure 7. Experiment 9609LED01B (1.0 Lux) Rat LMA actogram - LED. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

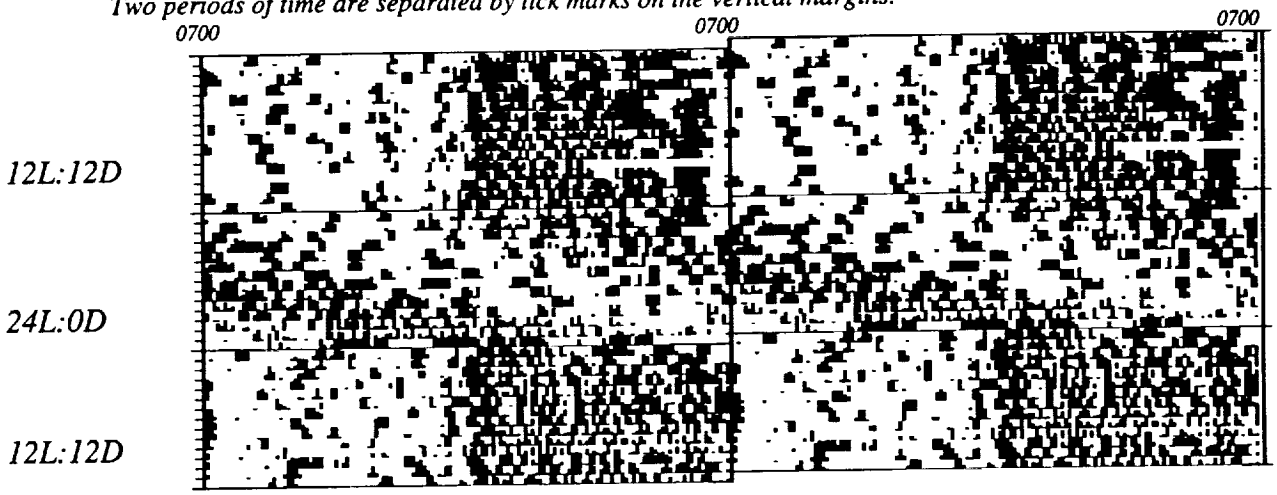

Figure 8. Experiment 9609LED01B (1.0 Lux) Rat drinking actogram - LED. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.

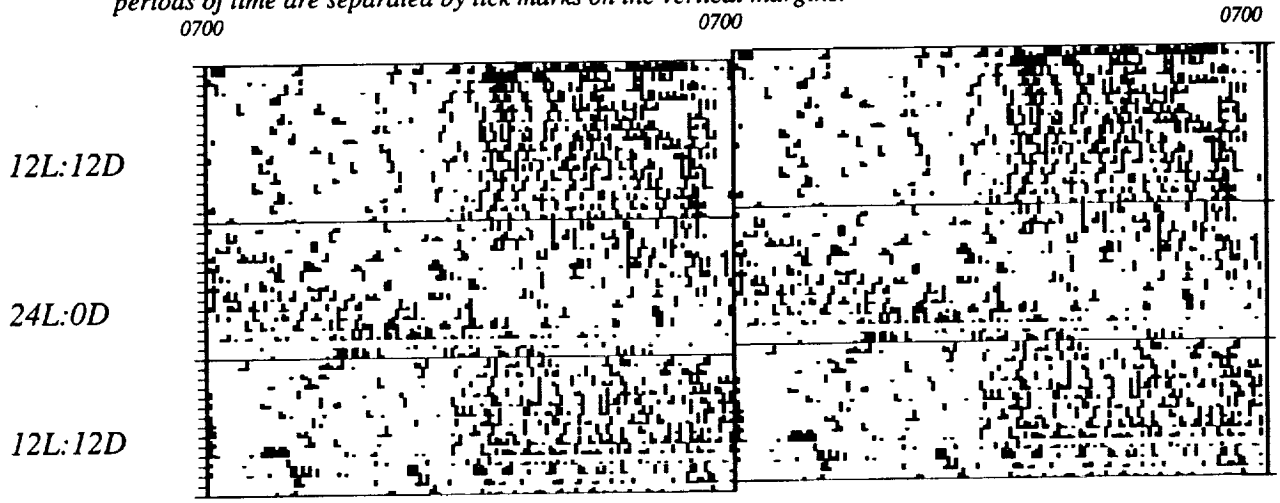

Figure 9. Experiment 9609LED01B (1.0 Lux) Rat feeding actogram - LED. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins

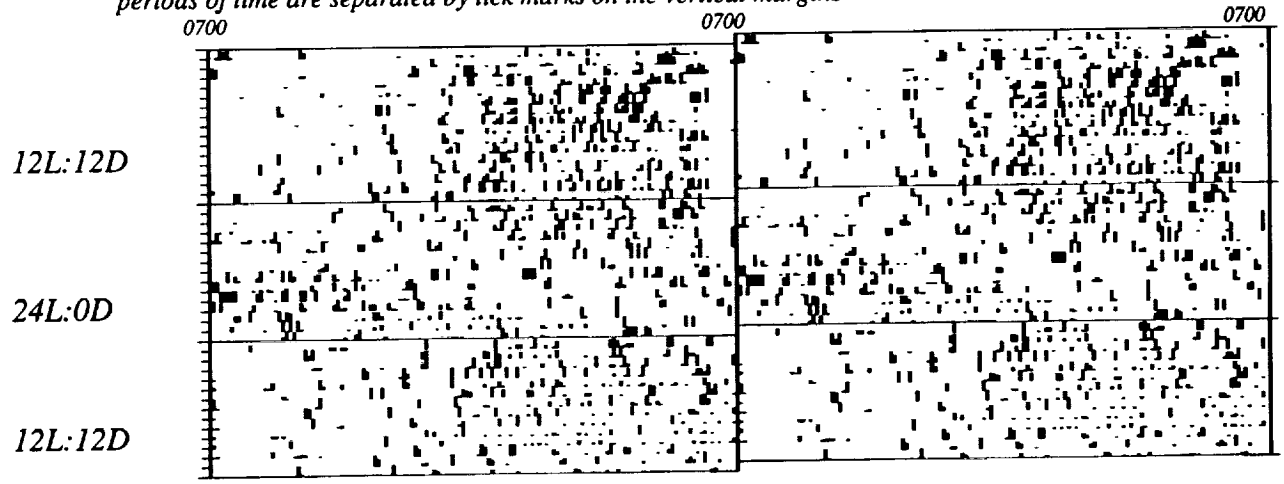

Figure 10. Experiment 9610LED01B (1.0 Lux) Rat LMA actogram - CWF. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

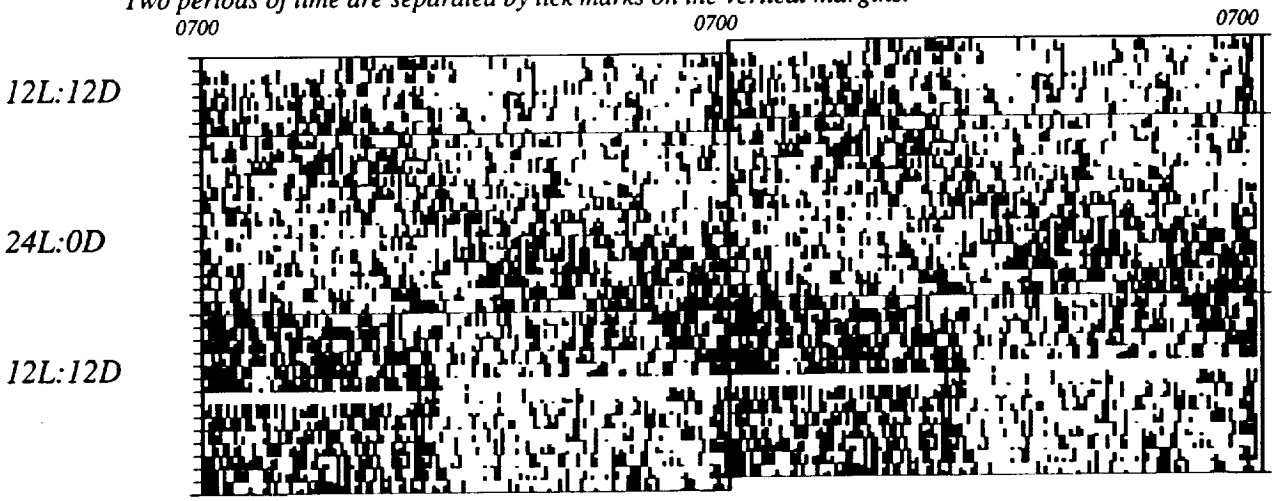

Figure 11. Experiment 9610LED01B (1.0 Lux) Rat drinking actogram - CWF. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.

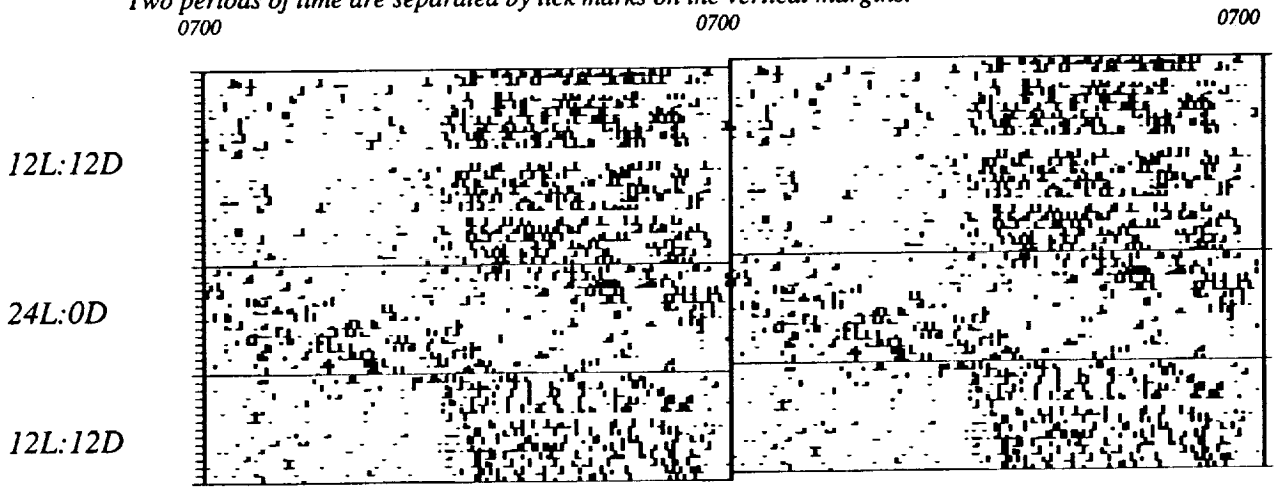

Figure 12. Experiment 9610LED01B (1.0 Lux) Rat feeding actogram - CWF. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins

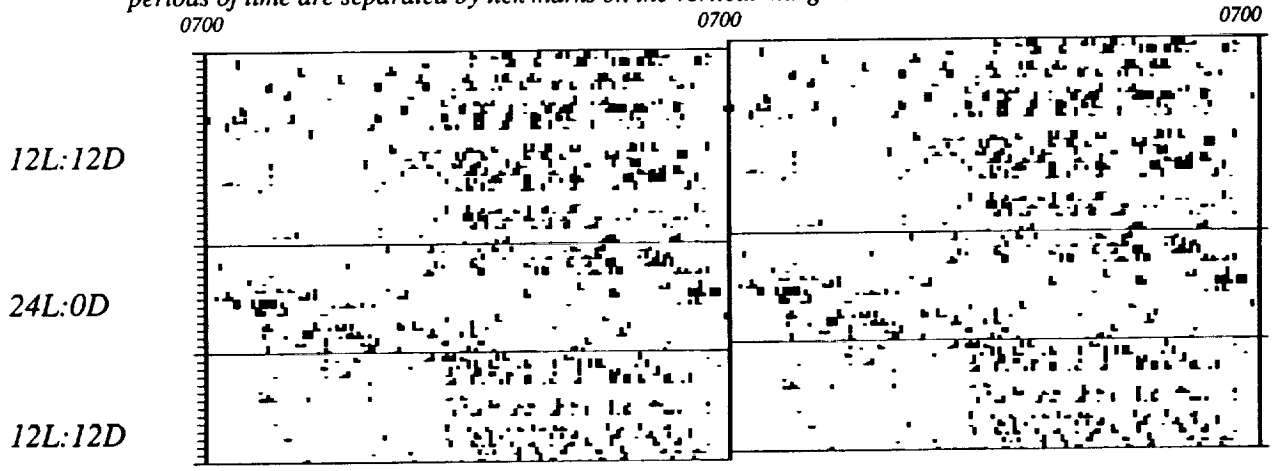

Figure 13. Experiment 9510LED10 (10 Lux) Rat LMA actogram - LED. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

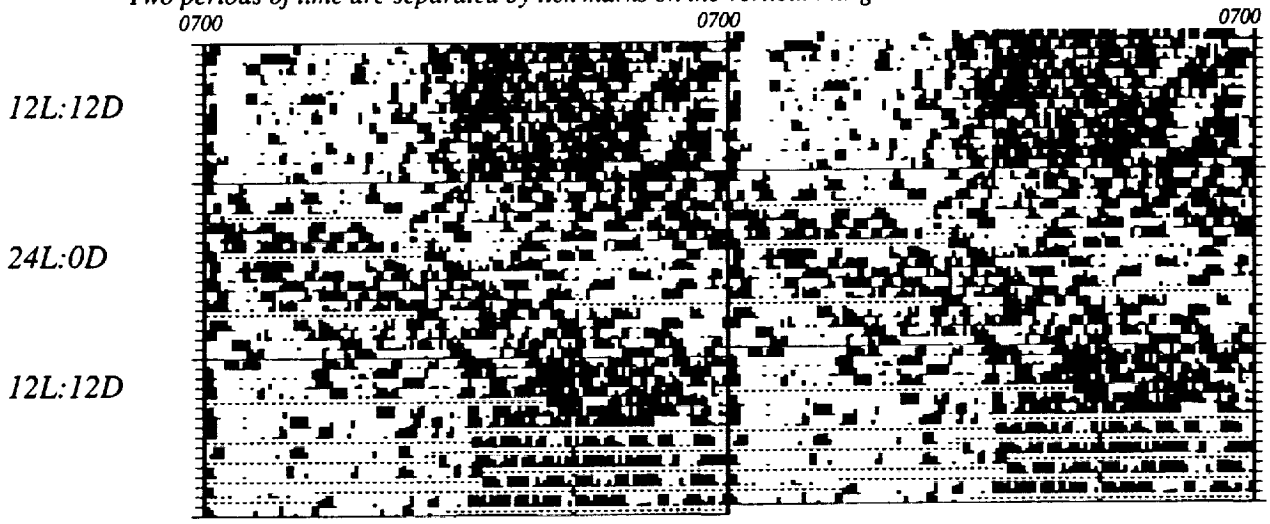

Figure 14. Experiment 9510LED10 (10 Lux) Rat drinking actogram - LED. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.

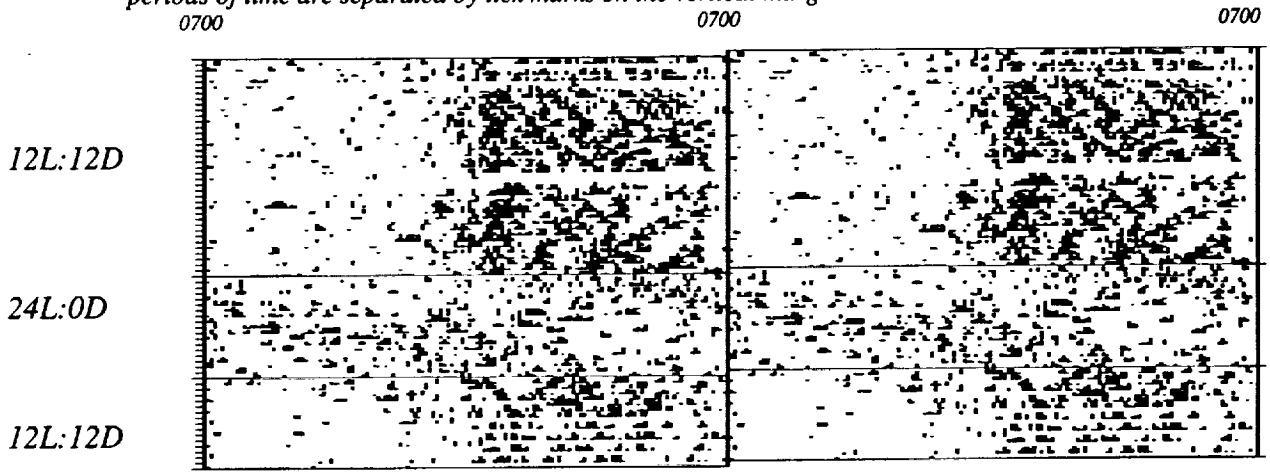

Figure 15. Experiment 9510LED10 (10 Lux) Rat feeding actogram - LED. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins

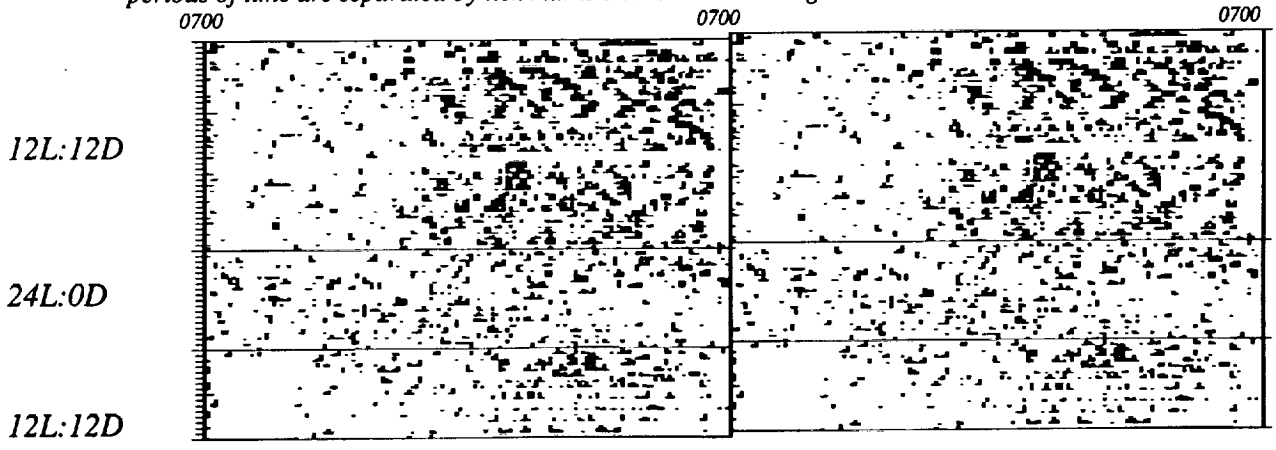

Figure 16. Experiment 9601LED10 (10 Lux) Rat LMA actogram - CWF. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

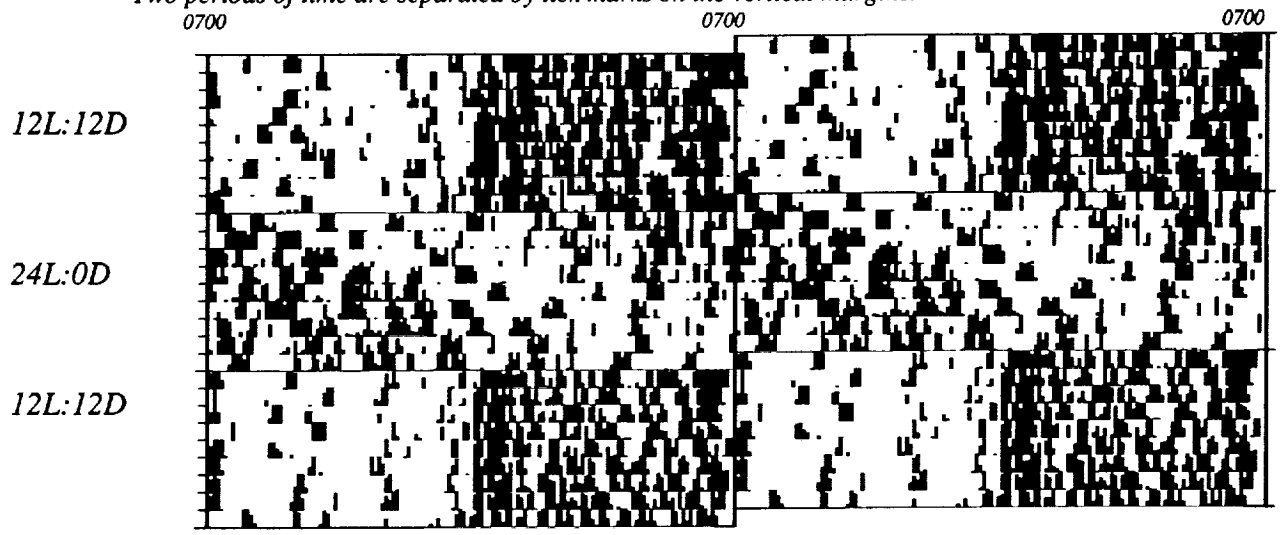

Figure 17. Experiment 9601LED10 (10 Lux) Rat drinking actogram - CWF. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.

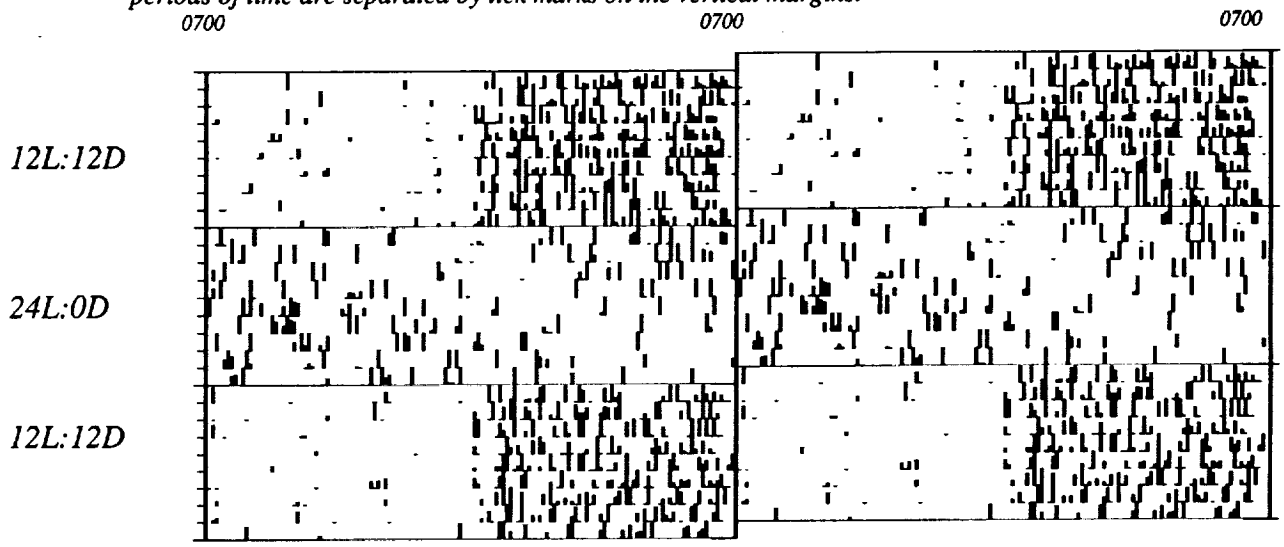

Figure 18. Experiment 9601LED10 (10 Lux) Rat feeding actogram - CWF. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins

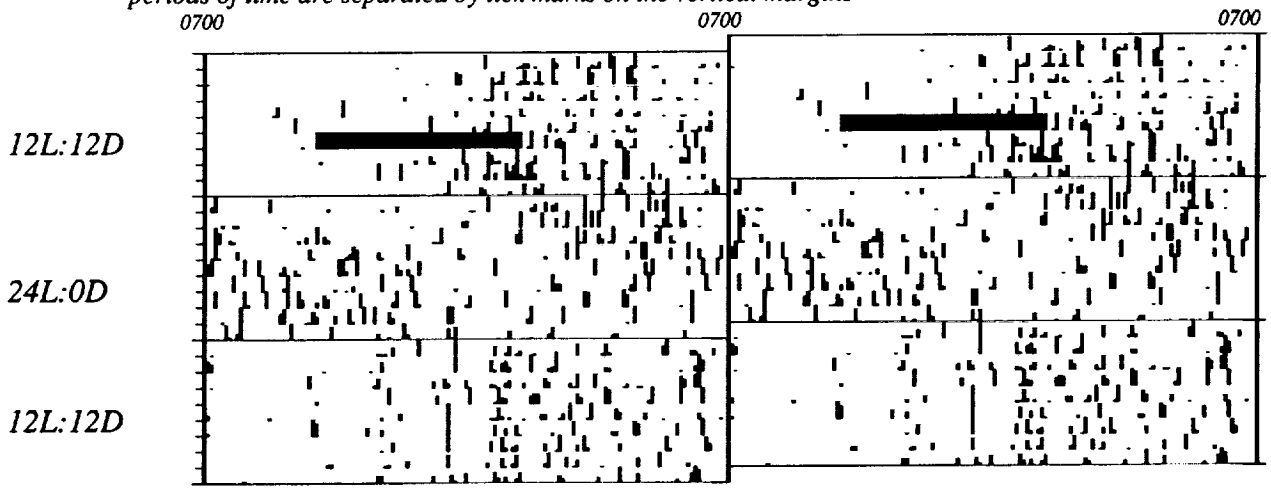

Figure 19. Experiment 9605LED40 (40 Lux) Rat LMA actogram - LED. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

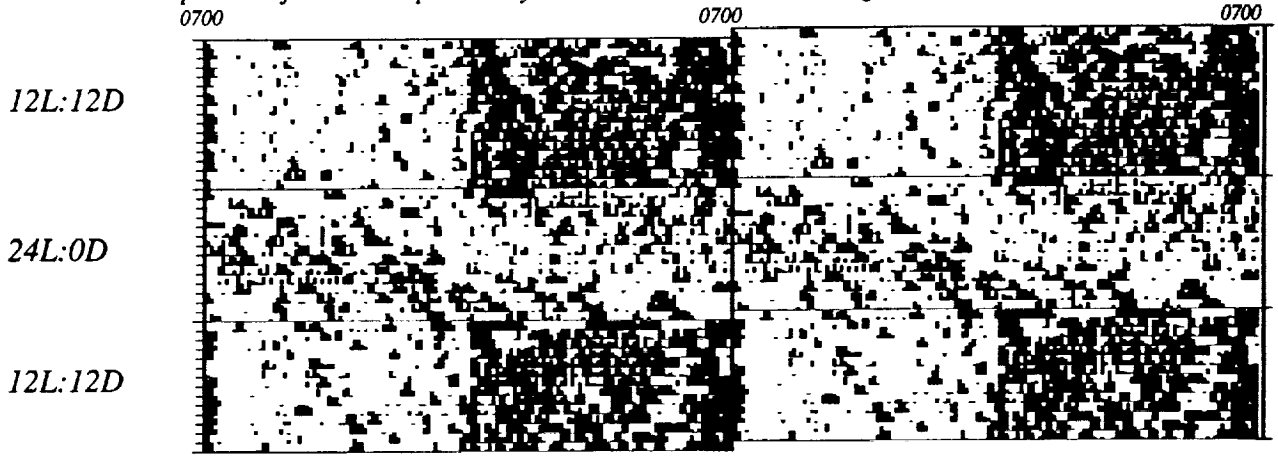

Figure 20. Experiment 9605LED40 (40 Lux) Rat drinking actogram - LED. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.

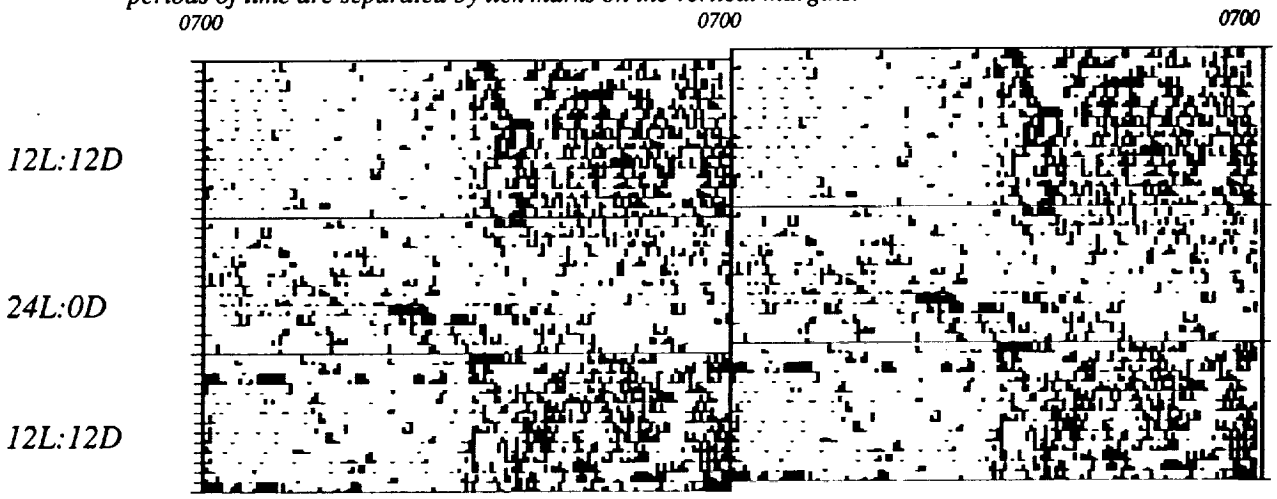

Figure 21. Experiment 9605LED40 (40 Lux) Rat feeding actogram - LED. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins

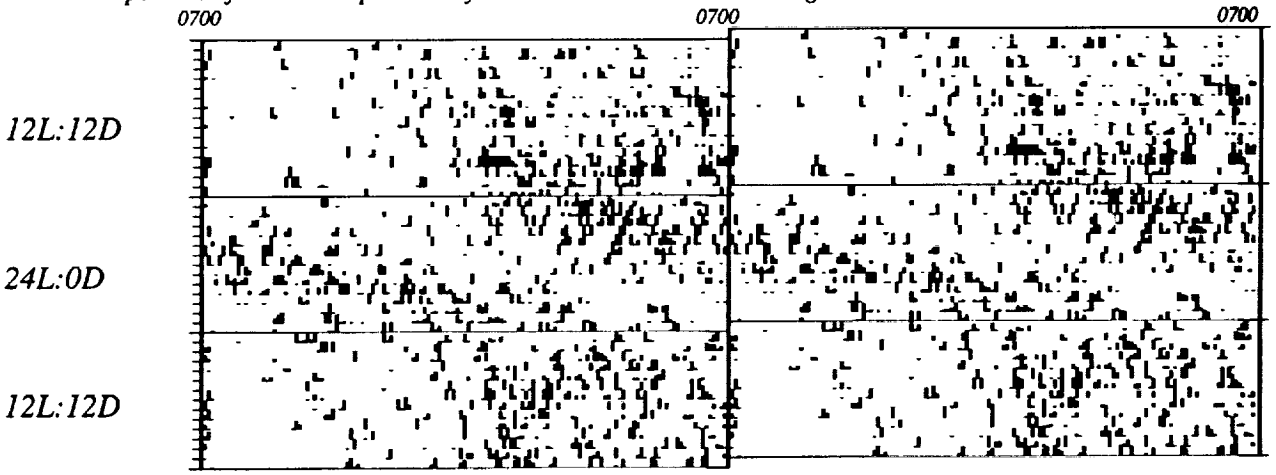

Figure 22. Experiment 9605LED40 (40 Lux) Rat LMA actogram - CWF. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

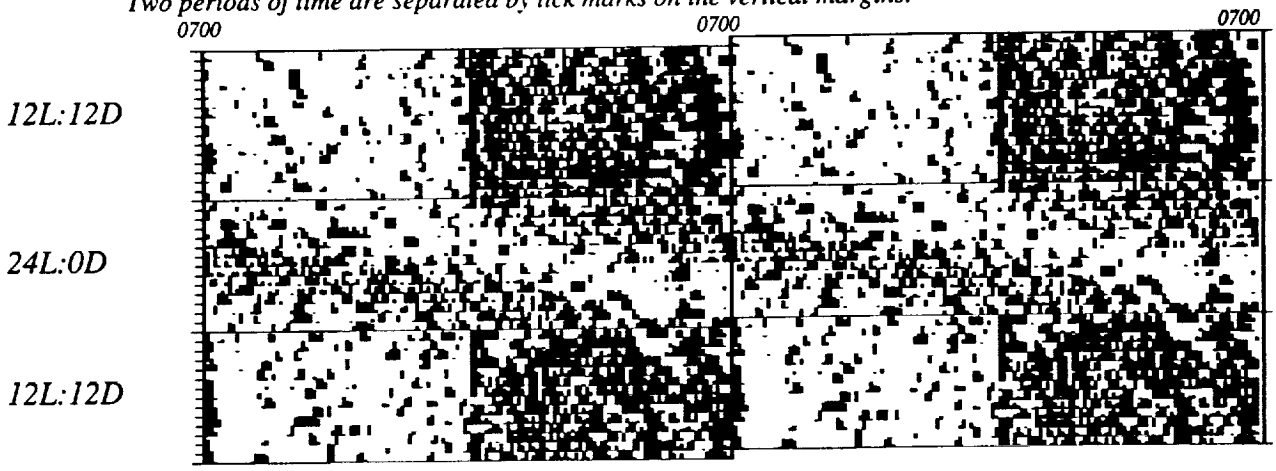

Figure 23. Experiment 9605LED40 (40 Lux) Rat drinking actogram - CWF. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.

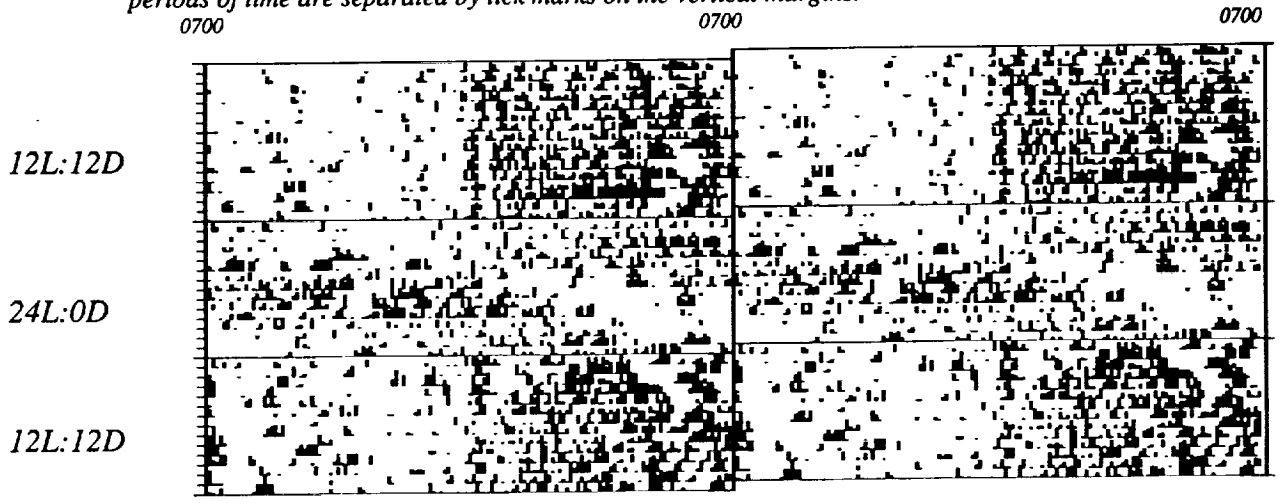

Figure 24. Experiment 9605LED40 (40 Lux) Rat feeding actogram - CWF. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins

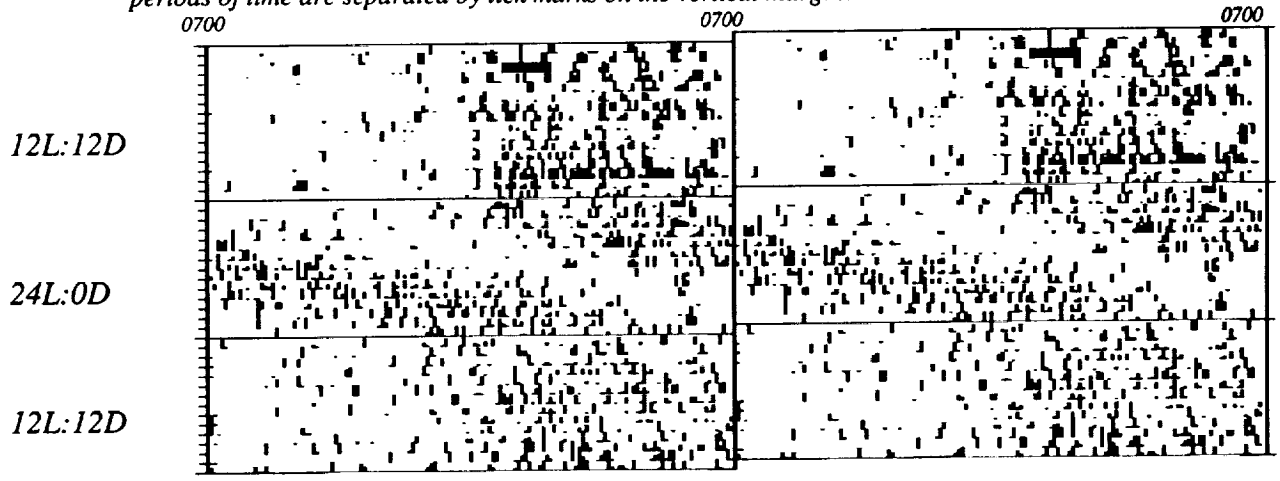

Figure 25. Experiment 9705LED100B (80 Lux) Rat LMA actogram - LED. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

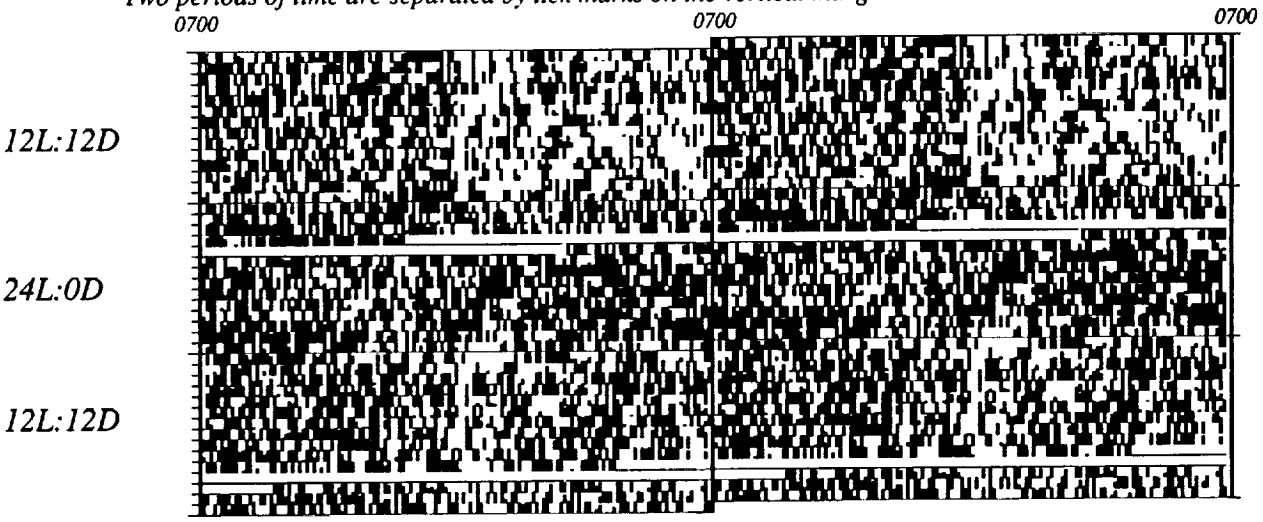

Figure 26. Experiment 9705LED100B (80 Lux) Rat drinking actogram - LED. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.

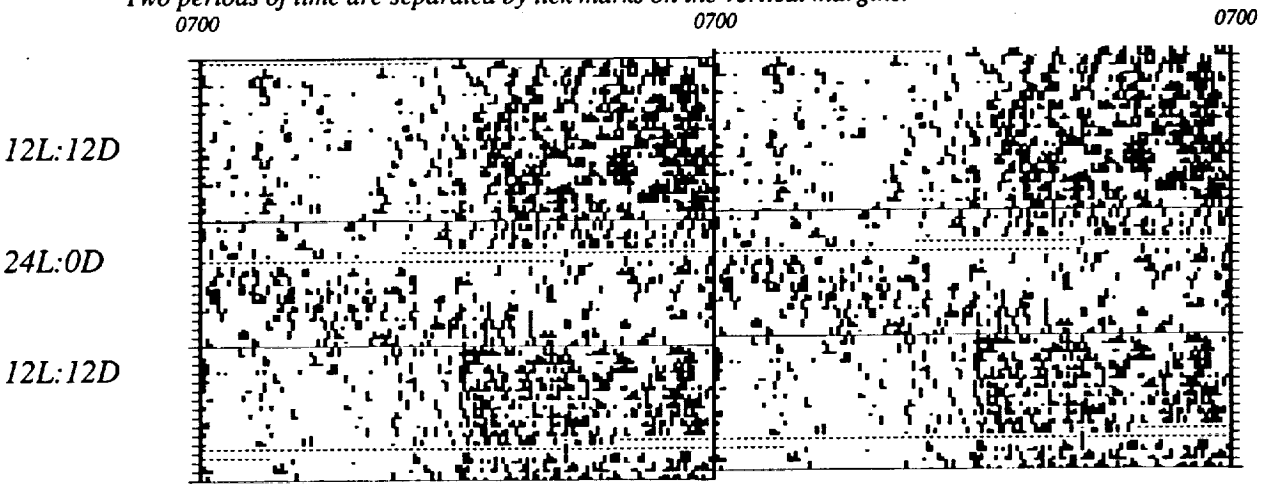

Figure 27. Experiment 9705LED100B (80 Lux) Rat feeding actogram - LED. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins

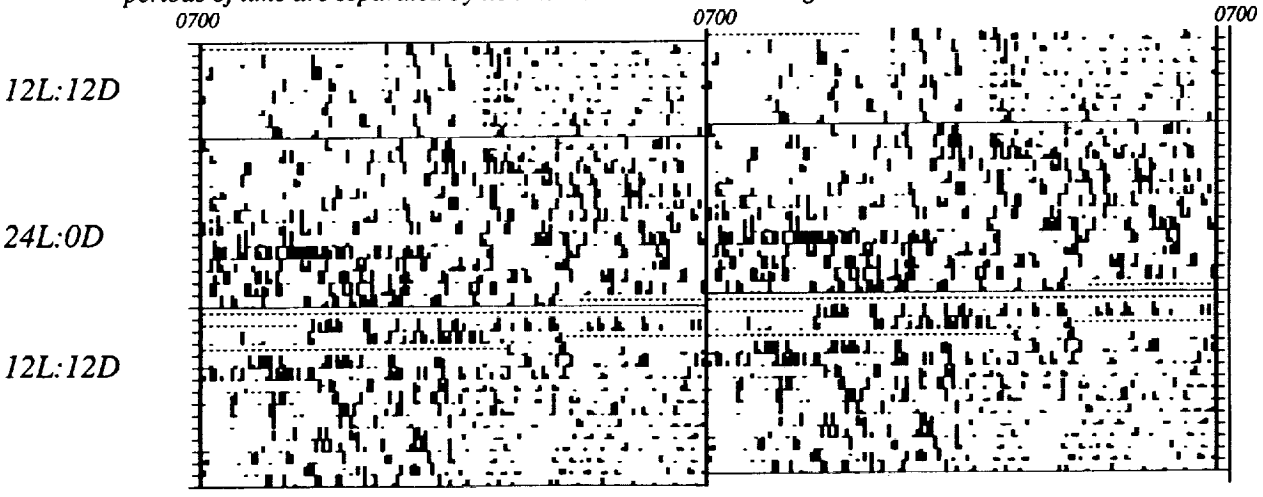

Figure 28. Experiment 9707LED100B (80 Lux) Rat LMA actogram - CWF. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

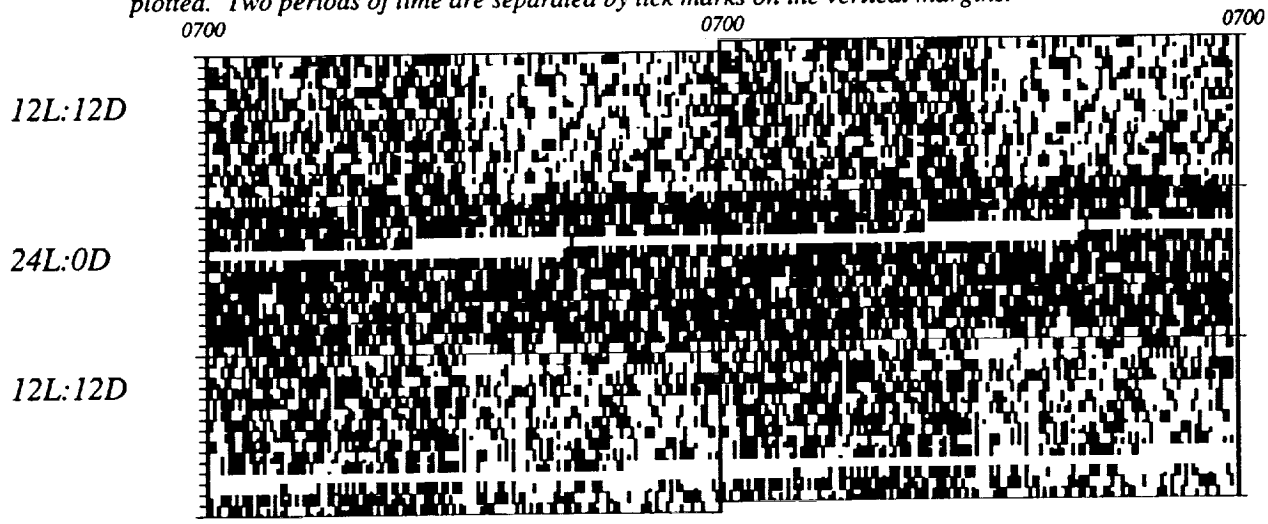

Figure 29. Experiment 9707LED100B (80 Lux) Rat drinking actogram - CWF. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.

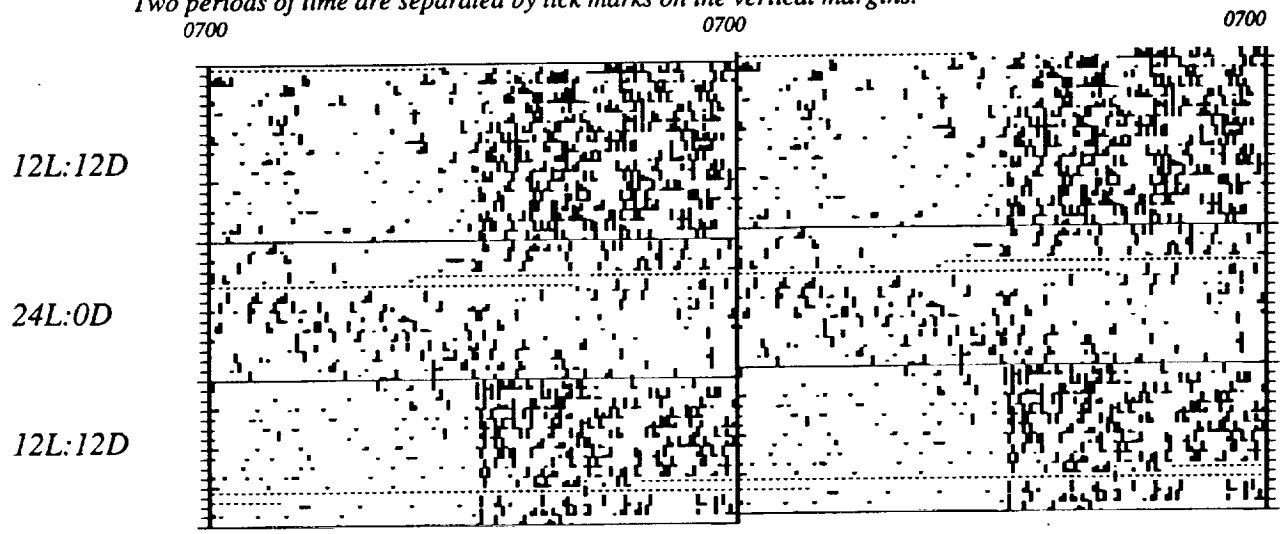

Figure 30. Experiment 9707LED100B (80 Lux) Rat feeding actogram - CWF. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins

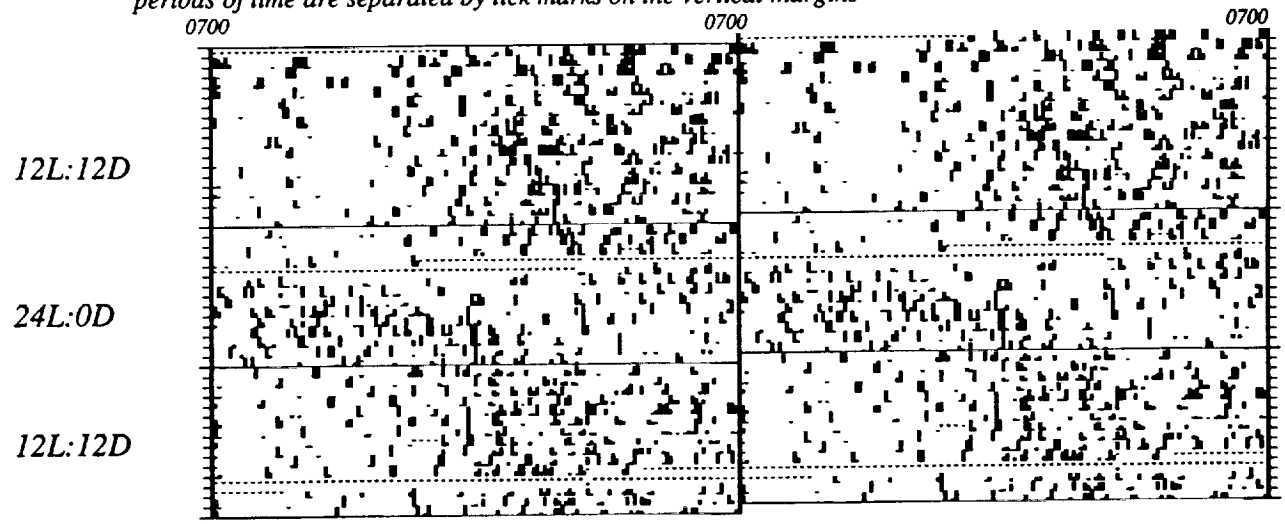

Table 1. Free run periods for rats kept under constant illumination from cool white fluorescent or RYGB LED panels listed in chronological order.

Experiment	Cage #	Light type	Light level	Activity tau	Drinking tau	Feeding tau
9510B	1	Cool White	10 lux	24.5	24.2	24.4
	2	LED	10 lux	25.2	24.8	24.2
	3	LED	10 lux	25.1	24.3	24.3
	4	LED	10 lux	a	25.7	24.1
	5	Cool White	10 lux	24.9	25.7	24.3
	6	Cool White	10 lux	b	24.9	24.9
9601B	1	Cool White	10 lux	25.6	26.1	26.4
	2	LED	10 lux	26.0	25.9	26.7
	3	LED	10 lux	25.8	25.6	25.3
	4	LED	10 lux	25.6	25.9	26.4
	5	Cool White	10 lux	25.3	25.4	25.5
	6	Cool White	10 lux	b	26.0	26.4
9603B	1	Cool White	40 lux	25.9	25.9	25.8
	2	LED	10 lux	25.9	25.9	25.8
	3	LED	40 lux	25.5	25.2	25.6
	4	LED	10 lux	25.5	25.2	25.2
	5	Cool White	10 lux	25.1	25.3	25.2
	6	Cool White	10 lux	b	25.3	25.4
9605B	1	Cool White	40 lux	25.9	25.8	25.9
	2	LED	40 lux	26.1	26.2	26.1
	3	LED	40 lux	26.1	26.0	25.9
	4	LED	40 lux	26.1	26.1	26.2
	5	Cool White	40 lux	26.1	26.3	26.3
	6	Cool White	40 lux	b	25.6	25.9
9607B	1	Cool White	40 lux	25.7	25.7	25.8
	2	LED	40 lux	26.0	a	25.9
	3	LED	40 lux	25.8	25.8	25.5
	4	LED	40 lux	25.6	25.6	25.6
	5	Cool White	40 lux	25.8	26.0	25.6
	6	Cool White	40 lux	b	25.6	25.6
9609B,1	1	Cool White	1 lux	25.8	25.8	25.8
	2	LED	1 lux	25.9	a	25.8
	3	LED	1 lux	26.2	a	25.8
	4	LED	1 lux	25.7	25.8	26.4
	5	Cool White	1 lux	25.6	25.6	25.9
	6	Cool White	1 lux	b	25.6	25.9
9610B,1	1	Cool White	1 lux	25.3	25.3	24.9
	2	LED	1 lux	25.9	a	25.1
	3	LED	1 lux	25.4	25.2	25.6
	4	LED	1 lux	25 3	25.7	25.4
	5	Cool White	1 lux	25.2	25.1	25.0
	6	Cool White	1 lux	b	25.8	25.3
9612A,1	1	LED	1 lux	c	25.8	26.0
	2	Cool White	1 lux	c	25.5	25.6
	3	Cool White	1 lux	c	25.3	25.5
	4	LED	1 lux	c	25.5	25.4
	5	LED	1 lux	c	25.8	26.0
	6	Cool White	1 lux	c	25.6	25.5

Table 1 (Cont.). Free run periods for rats kept under constant illumination from cool white fluorescent or RYGB LED panels listed in chronological order.

Experiment	Cage #	Light type	Light level	Activity tau	Drinking tau	Feeding tau
9701B	1	Cool White	1 lux	25.2	25.4	25.3
	2	LED	1 lux	25.6	25.8	25.8
	3	LED	1 lux	25.1	25.7	25.8
	4	LED	1 lux	25.4	25.9	26.1
	5	Cool White	1 lux	25.3	25.6	25.6
	6	Cool White	1 lux	b	a	25.7
9702A	1	LED	0.1 lux	c	24.4	24.0
	2	Cool White	0.1 lux	c	24.7	24.4
	3	Cool White	0.1 lux	c	24.6	24.6
	4	LED	0.1 lux	c	24.5	24.5
	5	LED	0.1 lux	c	24.1	24.1
	6	Cool White	0.1 lux	c	24.2	24.8
9703B	1	Cool White	0.1 lux	24.4	24.4	24.5
	2	LED	0.1 lux	24.4	24.4	24.6
	3	LED	0.1 lux	24.7	24.7	24.7
	4	LED	0.1 lux	24.7	24.8	25.2
	5	Cool White	0.1 lux	24.2	24.4	24.4
	6	Cool White	0.1 lux	b	24.6	24.7
9704A	1	LED	0.1 lux	c	25.1	25.3
	2	Cool White	0.1 lux	c	24.4	25.3
	3	Cool White	0.1 lux	c	24.8	24.3
	4	LED	0.1 lux	c	a	25.8
	5	LED	0.1 lux	c	25.1	24.7
	6	Cool White	0.1 lux	c	a	24.9
9705B	1	Cool White	80 lux	26.6	26.2	26.5
	2	LED	80 lux	25.9	26.3	25.8
	3	LED	80 lux	26.5	26.3	a
	4	LED	80 lux	a	26.2	26.4
	5	Cool White	80 lux	26.4	26.6	26.5
	6	Cool White	80 lux	b	25.3	26.6
9707A	1	LED	80 lux	c	25.9	25.8
	2	Cool White	80 lux	c	24.8	25.5
	3	Cool White	80 lux	c	25.7	25.9
	4	LED	80 lux	c	25.7	25.6
	5	LED	80 lux	c	a	a
	6	Cool White	80 lux	c	25.3	25.5
9707B	1	Cool White	80 lux	25.9	25.5	26.2
	2	LED	80 lux	26.3	25.7	a
	3	LED	80 lux	25.5	25.3	a
	4	LED	80 lux	a	25.9	a
	5	Cool White	80 lux	a	25.5	25.8
	6	Cool White	80 lux	b	25.6	26.3

a Tau could not be determined.
b No activity data collected in this cage due to a broken accelerometer.
c Since cages were not equipped with activity sensors, data for this parameter were not collected.

Table 2. Individual and free run periods for rats kept under constant illumination from cool white fluorescent or RYGB LED panels listed by light level. Each row represents data from one animal.

	Activity		Drinking		Feeding	
Light Level	Cool White	LED	Cool White	LED	Cool White	LED
0.1 lux			24.7	24.4	24.4	24.0
			24.6	24.5	24.6	24.5
			24.2	24.1	24.8	24.1
	24.4	24.4	24.4	24.4	24.5	24.6
	24.2	24.7	24.4	24.7	24.4	24.7
		24.7	24.6	24.8	24.7	25.2
			24.4	25.1	25.3	25.3
			24.8		24.3	25.8
				25.1	24.9	24.7
1 lux	25.8	25.9	25.8		25.8	25.8
	25.6	26.2	25.6		25.9	25.8
		25.7	25.6	25.8	25.9	26.4
	25.3	25.9	25.3		24.9	25.1
	25.2	25.4	25.1	25.2	25.0	25.6
		25.3	25.8	25.7	25.3	25.4
			25.5	25.8	25.6	26.0
			25.3	25.5	25.5	25.4
			25.6	25.8	25.5	26.0
	25.2	25.6	25.4	25.8	25.3	25.8
	25.3	25.1	25.6	25.7	25.6	25.8
		25.4		25.9	25.7	26.1
10 lux	24.5	25.2	24.2	24.8	24.4	24.2
	24.9	25.1	25.7	24.3	24.3	24.3
			24.9	25.7	24.9	24.1
	25.6	26.0	26.1	25.9	26.4	26.7
	25.3	25.8	25.4	25.6		
		25.6	26.0	25.9	26.4	26.4
	25.1	25.9	25.3	25.9	25.2	25.8
		25.5	25.3	25.2	25.4	25.2
40 lux	25.9	25.5	25.9	25.2	25.8	25.6
	25.9	26.1	25.8	26.2	25.9	26.1
	26.1	26.1	26.3	26.0	26.3	25.9
		26.1	25.6	26.1	25.9	26.2
	25.7	26.0	25.7		25.8	25.9
	25.8	25.8	26.0	25.8	25.6	25.5
		25.6	25.6	25.6	25.6	25.6
80 lux	26.6	25.9	26.2	26.3	26.5	25.8
	26.4	26.5	26.6	26.3	26.5	
			25.3	26.2	26.6	26.4
			24.8	25.9	25.5	25.8
			25.7	25.7	25.9	25.6
			25.3		25.5	
	25.9	26.3	25.5	25.7	26.2	
		25.5	25.5	25.3	25.8	
			25.6	25.9	26.3	

Table 3. Mean ± SEM free run period of individual variables.

	Activity		Drinking		Feeding	
	Cool White	LED	Cool White	LED	Cool White	LED
0.1 Lux	24.3 ± 0.1 (2)	24.6 ± 0.1 (3)	24.5 ± 0.1 (8)	24.6 ± 0.1 (8)	24.6 ± 0.1 (9)	24.8 ± 0.2 (9)
1 Lux	25.4 ± 0.1 (6)	25.6 ± 0.1 (9)	25.5 ± 0.1 (11)	25.7 ± 0.1 (9)	25.5 ± 0.1 (12)	25.8 ± 0.1 (12)
10 Lux	25.1 ± 0.2 (5)	25.6 ± 0.1 (7)	25.4 ± 0.2 (8)	25.4 ± 0.2 (8)	25.3 ± 0.3 (7)	25.2 ± 0.4 (7)
40 Lux	25.9 ± 0.1 (5)	25.9 ± 0.1 (7)	25.8 ± 0.1 (7)	25.8 ± 0.1 (6)	25.8 ± 0.1 (7)	25.9 ± 0.1 (7)
80 Lux	26.3 ± 0.2 (3)	26.1 ± 0.2 (4)	25.6 ± 0.2 (9)	25.9 ± 0.1 (8)	26.1 ± 0.1 (9)	25.9 ± 0.2 (4)

Table 4. Mean ± SEM free run period of combined variables.

	mean of Activity, Drinking, and Feeding	
	Cool White	LED
0.1 Lux	24.6 ± 0.1	24.7 ± 0.2
1 Lux	25.5 ± 0.1	25.7 ± 0.1
10 Lux	25.3 ± 0.2	25.4 ± 0.2
40 Lux	25.8 ± 0.1	25.9 ± 0.1
80 Lux	25.9 ± 0.1	25.9 ± 0.1

Table 5. ANOVA table for the analysis of combined free run periods.

	df	SS	MS	F
light type	1	0.407	0.407	1.284
light level	4	46.863	11.716	36.924**
type vs. level interaction	4	0.342	0.086	0.270
rats within type vs. level	79	25.066	0.317	4.061**
within animals	129	10.079	0.078	
total	217	82.759		

Table 6. Planned comparisons among light levels.

	df	SS	MS	F
0.1 vs. 1 lux	1	22.462	22.462	70.791
1 vs. 10 lux	1	1.664	1.664	5.246
10 vs. 80 lux	1	6.929	6.929	21.838
40 vs. 80 lux	1	0.111	0.111	0.349

Figure 31. Combined mean ± SEM free run period for rats kept under constant illumination from cool white fluorescent or LED panels. There was no difference between LED and Cool White Fluorescent means at each light intensity, ANOVA ($p<0.05$).

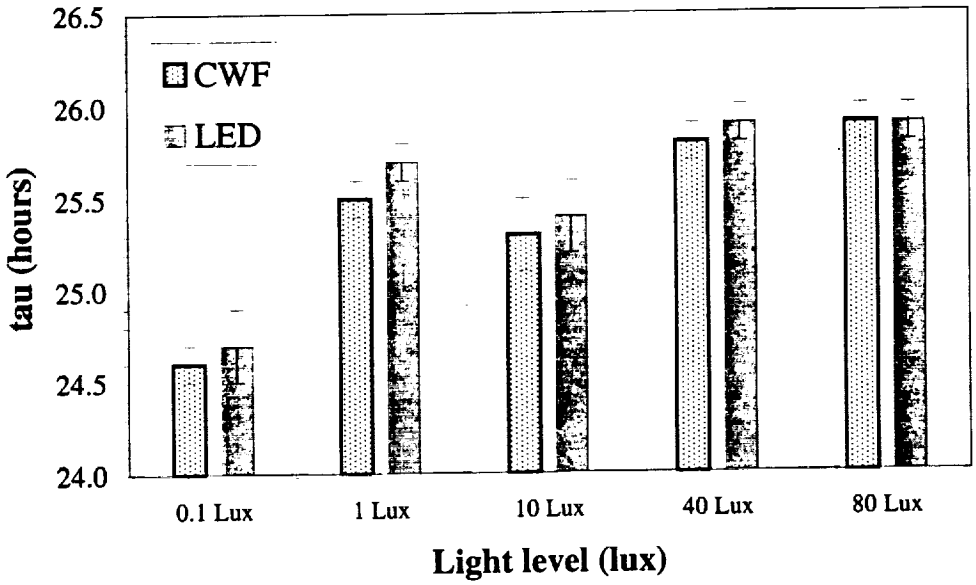

Figure 32. 4-color LED panel schematic.