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Final Report for NASA COOPERATIVE AGREEMENT #NCC2-593

June 3, 1994

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June 3, 1994
San Jose State University

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Final Subproject Report for Cooperative Agreement #NCC2-593

RAT LONG TERM HABITABILITY AND BREEDING UNDER LOW LIGHT INTENSITY (5 LUX)

FINAL REPORT of subproject for COOPERATIVE AGREEMENT #NCC2-593.

January 7, 1994

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NASA 5 lux habitability study
Final Subproject Report 01/07/94
(Cooperative Agreement NCC2-593)
San Jose State University

INTRODUCTION

Previously we published a report which established lighting standards for animal experiments relative to NASA projects on the ground and during space flight (HOLLEY, et al., 1988). In this document we indicated that rats should be maintained under lighting conditions of 40 lux when possible, but that an acceptable range for extended periods was 75-5 lux (sunlight simulating spectrum). This was in contrast to guidelines stipulated in the National Institute of Health's "Guide for the Care and Use of Laboratory Animals". Originally, the GUIDE recommended 800-1100 lux for animal maintenance, but the current edition (1985) has lowered that recommendation to approx. 323 lux. This change resulted primarily from studies showing that albino rats raised under 800-1100 lux could be shown histologically to have damaged retinas. To our knowledge, however, functional impairment has not been demonstrated. In support of lower light intensities for animal maintenance, Kupp et al. (1989) conducted a 2 year study to evaluate the effects of 34-36 foot candles (ftc) on rodent retinas after finding that 55 ftc (592 lux) caused retinal damage in albino rats. At 34-36 ftc (366-387 lux) they found no retinal damage.

Since lower light intensities would result in considerable energy conservation on space vehicles, low light would be an advantage in animal studies in space. It is well established that the estrous cycle of the rodent is synchronized by the light:dark cycle (Elliot and Goldman, 1981). We were concerned that maintaining rodents at the lower end of the "recommended" envelope (e.g., 5 lux) might negatively affect their reproduction. We were particularly interested in chronic exposure effects.

With these considerations and upon consultation with colleagues at NASA, we decided to perform a series of experiments to further validate our recommendation that vivarium rats could be maintained under minimum 5 lux light intensity. Parameters assessed were reproductive activity, growth, and well-being (clinically normal appearance) of test animals reared under 5 lux versus controls reared under 40 lux light intensity.

MATERIALS AND METHODS

The parental generation consisted of 3-4 month old Sprague-Dawley rats (*Rattus norvegicus*, Simonsen Laboratories, Gilroy, CA). We were supplied with two males from the same litter and two females from the same litter (different from the males). The males and females, therefore came from separate lineage. Additionally, we requested animals from the supplier that had been housed on lower vivarium animal racks to reduce the possibility of retinal damage from ceiling mounted lights. These four animals provided our first generation animals which we then bred for a second generation and so forth for a total of three generations (F-1 through F-3 generations).

Animals were fed a lactation/growth type rodent diet (Wayne Laboratory Diet MRH22/5 Rodent Blocks #8640) throughout the study. They were housed on laboratory hardwood bedding in ventilated (fans provided approx. 20 air changes per hour), light-tight cabinets inside a light shielded room. The cabinet dimensions were 26"x30"x26". Each cabinet held two "shoe box" style Nalgene rat cages. A total of four cabinets were used for this study. Lighting within the cabinets was provided by sunlight simulating

fluorescent bulbs (VitaLite, Durotest Corp.). Light intensity was adjusted using a calibrated IL-1700 Research Radiometer (Industrial Light, Inc., Newburyport, MA). The photoperiod throughout was 12L:12D.

Throughout the experiment, all animals were protected from exposure to any stray light. Routine maintenance was performed with the room door closed and with the lights off in the room. Only one cabinet door was opened at a time. All procedures, such as weighing, were accomplished within the room, and animals remained in the room until they were removed from the experiment.

Experimental Protocol

The animals were separated into two groups each originally consisting of one breeding pair. Group A was maintained in strict 5.0 lux lighting conditions; group B was maintained under 40-50 lux lighting conditions. The animals were acclimated at these lighting conditions for 2 weeks before being allowed to mate. For breeding, the female was introduced into the male's cage. When pregnancy was detected by abdominal palpation, the female was removed to her own cage. Litters, at the time of birth, were counted and culled to equal sizes generally consisting of approximately eight pups; except for the third generation which was culled to six pups each due to a small litter size. Pups were weighed weekly and weights were recorded. Additionally, all animals were regularly examined by animal technicians and the consulting veterinarian.

Litters were weaned and separated by sex at approximately 21 days of age; thereafter they were culled so as to comply with housing requirements stipulated in the

GUIDE. At three to four months of age, a male and female were chosen at random from each litter to carry on the line; at that time the remaining animals in the litter were removed. This process was performed for three generations. The original four parents were kept throughout the entire study under experimental lighting conditions; however, after the first generation was weaned, they were switched to a diet of Wayne Rodent Blocks #8604 (regular rat chow). The four were regularly examined as previously mentioned so as to monitor for ill-effects of long term housing at these light intensities. Subsequent parental generations were removed after their progeny were weaned.

RESULTS

In all cases, female rats conceived within one week of being exposed to the males. Additionally, in all cases, the litter sizes and birth weights were within the normal range as listed in Laboratory Animal Medicine (ref.). Moreover, all of the pups' eyes opened at the typical time following birth.

As can be seen in Figure 1, the average birth weights of the 40 lux and 5.0 lux pups were similar in each generation. Birth weight decreased from one generation to the next for all three generations. Figures 2 through 4 and Table 1 show mean rat weights by sex, age, and generation. Table 1 also contains individual rat weights. The rats growth rates paralleled each other in comparing the 5.0 lux to the 40 lux. Sexual dimorphism in weight is apparent after approximately 30 days of age.

On November 13, 1989, Dr. Sig Rich, the consulting veterinarian, examined the first generation of pups, eight born and raised under 5.0 lux lighting conditions and

eight born and raised under 40 lux. He found all to be alert, active, clean, and in apparently good physical condition. In subsequent examinations of all the generations and the four original parents, the veterinarian consistently reported these same observations with only two exceptions late in the experiment.

Throughout this study, only two physiological problems were observed. In the second generation of 40 lux pups, we noticed that three had dried red discharge around their eyes. The veterinarian suspected that this resulted from irritation due to the bedding. On April 2, 1990, at age 4 months, a male rat of this group was sent to the University of Missouri, College of Veterinarian Medicine, Research Animal Diagnostic and Investigative Laboratory. Their summary diagnosis indicated that no conclusive evidence of a serious, naturally occurring infectious disease existed.

Secondly, on August 15, 1990, the original 5.0 lux male (17 months of age) was observed to have discharge around his eyes and muzzle in addition to a hunched posture indicative of illness. He was also sent to the University of Missouri, College of Veterinary Medicine, Research Animal Diagnostic and Investigative Laboratory. He was diagnosed to have had renal failure and a salivary gland carcinoma. We note that this is common in white albino rats 1-2 years of age. Neither the eye discharge problem with the 40 lux pups nor the problems in the original 5.0 lux male could be related to the light intensity conditions.

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Holley, D.C., C.M. Winget, and H. A. Leon. Lighting Requirements in Microgravity.- rodents and nonhuman primates. NASA Technical Memorandum #101077, December, 1988, 273 pp.

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Table 1. Individual and mean weights by sex and age for generation F-1 rats.

Generation F1 weights of male rats raised under 40 lux light conditions.

Age (days)	1.0	8.0	15.0	22.0	29.0	36.0	43.0	50.0	57.0	65.0	71.0	78.0	85.0	92.0	99.0
	7.2	21.2	35.9	53.2	96.3	137.4	182.3	206.8	267.7	308.0	318.0	340.0	340.0	361.0	376.0
	6.8	18.8	33.6	52.2	85.6	127.6	178.9	201.1	262.9	315.0	323.0	365.0	368.0	398.0	411.0
	6.9	21.0	36.1	53.0	92.3	134.7									
	7.0	20.8	35.3	50.6	86.6	122.4									
	7.6	21.7	35.7	53.3	94.6	140.8	180.0	202.3	255.4	296.0	292.0	312.0	317.0	343.0	367.0
	7.6														
	7.1														
	7.2														
Mean	7.2	20.7	35.3	52.5	91.1	132.6	180.4	203.4	262.0	306.3	311.0	339.0	341.7	367.3	384.7
StdDev	0.3	1.1	1.0	1.1	4.8	7.5	1.8	3.0	6.2	9.6	16.6	26.5	25.5	28.0	23.2

Generation F1 weights of male rats raised under 5 lux light conditions.

Age (days)	1.0	8.0	15.0	22.0	29.0	36.0	43.0	50.0	57.0	65.0	71.0	78.0	85.0	92.0	99.0
	6.4	15.4	35.2	44.8	86.7	124.0									
	7.3	18.2	39.8	51.8	91.7	137.6	184.2	214.3	252.5	300.0	301.0	331.0	329.0	355.0	371.0
	7.5	17.0	37.5	48.2	91.3	134.7	182.5	216.7	258.0	305.0	309.0	341.0	338.0	359.0	384.0
	7.3	17.5	38.0	51.2	88.1	131.6	178.2	210.1	256.4	298.0	300.0	338.0	333.0	350.0	373.0
	7.5														
	5.0														
Mean	6.8	17.0	37.6	49.0	89.4	132.0	181.6	213.7	255.6	301.0	303.3	336.7	333.3	354.7	376.0
StdDev	1.0	1.2	1.9	3.2	2.4	5.8	3.1	3.4	2.8	3.6	4.9	5.1	4.5	4.5	7.0

Generation F1 weights of female rats raised under 40 lux light conditions.

Age (days)	1.0	8.0	15.0	22.0	29.0	36.0	43.0	50.0	57.0	65.0	71.0	78.0	85.0	92.0	99.0
	7.3	22.3	37.0	53.6	90.1	131.1	163.2	187.5	213.1	229.0	238.0	242.0	250.0	258.0	267.0
	7.2	20.6	35.6	52.3	84.1	121.6	147.4	168.4	191.8	206.0	206.0	225.0	237.0	237.0	255.0
	7.1	19.4	34.4	50.6	86.2	124.0	145.0	163.3	189.8	194.0	204.0	216.0	213.0	228.0	234.0
	7.1														
Mean	7.2	20.8	35.7	52.2	86.8	125.6	151.8	173.0	198.2	209.7	216.0	227.7	233.3	241.0	252.0
StdDev	0.1	1.4	1.3	1.5	3.0	4.9	9.9	12.8	12.9	17.8	19.1	13.2	18.8	15.4	16.7

Generation F1 weights of female rats raised under 5 lux light conditions.

Age (days)	1.0	8.0	15.0	22.0	29.0	36.0	43.0	50.0	57.0	65.0	71.0	78.0	85.0	92.0	99.0
	7.0	16.9	35.1	45.9	77.8	111.2	135.8	152.3	174.0	189.0	192.0	201.0	205.0	208.0	222.0
	6.3	15.7	35.7	46.2	79.7	112.3	140.3	163.1	181.7	202.0	207.0	222.0	232.0	229.0	239.0
	6.5	15.8	36.4	46.8	82.3	113.6									
	6.2	13.7	32.6	41.7	72.9	104.8	125.1	149.3	163.7	177.0	182.0	193.0	198.0	195.0	211.0
	7.2														
	6.7														
Mean	6.7	15.5	35.0	45.1	78.2	110.5	133.7	154.9	173.1	189.3	193.7	205.3	211.7	210.7	224.0
StdDev	0.4	1.3	1.7	2.3	4.0	3.9	7.8	7.3	9.0	12.5	12.6	15.0	18.0	17.2	14.1

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Table 2. Individual and mean weights by sex and age for generation F-2 rats.

Generation F2 weights of male rats raised under 40 lux light conditions.

Age (days)	0.0	7.0	14.0	22.0	28.0	35.0	42.0	50.0	56.0	63.0	70.0	77.0	84.0
1	5.9	14.8	34.4	48.6	83.8	124.3							
1	6.3	16.5	37.4	51.1	87.6	133.8							
1	6.2	12.9	31.4	50.1	81.7	131.3	159.7	210.3	253.5	298.2	324.0	345.0	357.0
1	5.9	15.8	34.6	51.9	81.9	126.6	153.5	206.8	251.7	296.8	316.4	336.0	345.0
1	6.1	17.0	36.7	56.1	91.0	140.6	172.9	226.3	270.0	315.0	334.0	359.0	365.0
1	6.1	15.9	31.7	44.9	79.0	121.9							
Mean	6.1	15.5	34.4	50.5	84.2	129.8	162.0	214.5	258.4	303.3	324.8	346.7	355.7
StdDev	0.2	1.5	2.5	3.7	4.4	6.9	9.9	10.4	10.1	10.1	8.8	11.6	10.1

Generation F2 weights of male rats raised under 5 lux light conditions.

Age (days)	1.0	7.0	14.0	21.0	28.0	35.0	42.0	49.0	56.0	63.0	70.0	84.0	91.0
1	6.7	18.0	34.9	47.0	82.5	126.3	174.0	224.0	261.2	291.9	315.5	359.0	373.0
1	7.0	18.5	35.7	45.7	82.9	127.0	171.0	220.1	257.5	283.9	309.3	342.0	351.0
Mean	6.8	18.2	35.3	46.4	82.7	126.7	172.5	222.1	259.4	287.9	312.4	350.5	362.0
StdDev	0.2	0.4	0.5	0.9	0.3	0.5	2.1	2.8	2.6	5.7	4.4	12.0	15.6

Generation F2 weights of female rats raised under 40 lux light conditions.

Age (days)	0.0	7.0	14.0	22.0	28.0	35.0	42.0	50.0	56.0	63.0	70.0	77.0	84.0
1	6.1	13.3	34.8	50.9	80.7	122.3	156.4	182.3	205.5	223.0	226.1	250.0	250.0
1	5.9	14.5	34.2	48.0	71.5	109.3	138.7	164.3	181.0	201.5	208.2	208.0	212.0
1	6.0												
1	5.9												
1	6.2												
Mean	6.0	13.9	34.5	49.5	76.1	115.8	147.6	173.3	193.3	212.3	217.2	229.0	231.0
StdDev	0.1	0.8	0.4	2.1	6.5	9.2	12.5	12.7	17.3	15.2	12.7	29.7	26.9

Generation F2 weights of female rats raised under 5 lux light conditions.

Age (days)	1.0	7.0	14.0	21.0	28.0	35.0	42.0	49.0	56.0	63.0	70.0	84.0	91.0
1	6.4	18.5	35.2	45.4	77.1	113.4	142.0	164.8	179.3	190.9	204.5	217.5	223.5
1	7.0	18.7	34.2	45.9	76.4	110.9	143.0	162.8	181.3	184.4	200.0	212.4	220.0
1	6.6	17.2	33.9	44.6	74.5	109.7	138.0						
1	5.8	14.5	31.2	41.1	68.6	95.3	119.0						
1	6.5	17.1	33.9	45.6	76.4	107.3	134.0						
1	6.4	17.7	35.1	42.7	71.3	105.7	132.0	152.9	167.6	176.9	186.5	202.0	207.0
Mean	6.4	17.3	33.9	44.2	74.1	107.1	134.7	160.2	176.1	184.1	197.0	210.6	216.8
StdDev	0.4	1.5	1.4	1.9	3.4	6.4	8.8	6.4	7.4	7.0	9.4	7.9	8.7

Table 3. Individual and mean weights by sex and age for generation F-3 rats.

Generation F3 weights of male rats raised under 40 lux light conditions.

Age (days)	0.0	8.0	14.0	21.0	28.0	35.0	42.0	49.0	56.0	63.0	70.0	77.0	84.0	91.0
	5.9	20.4	35.2	54.0	86.4	136.9	185.8	246.1	278.1	312.4	335.9	351.6	367.2	387.2
	5.8	20.1	35.0	55.6	87.8	137.7	186.7	247.2	284.2	323.0	350.6	371.3	389.5	404.8
	6.0	19.1	33.5	53.5	85.8	127.5	176.5	233.3	264.6	297.3	313.7	338.4	350.9	374.5
	6.1	19.6	37.0	55.3										
	5.7													
	5.6													
	5.3													
	5.5													
Mean	5.7	19.8	35.2	54.6	86.7	134.0	183.0	242.2	275.6	310.9	333.4	353.8	369.2	388.8
StdDev	0.3	0.6	1.4	1.0	1.0	5.7	5.6	7.7	10.0	12.9	18.6	16.6	19.4	15.2

Generation F3 weights of male rats raised under 5 lux light conditions.

Age (days)	0.0	7.0	14.0	21.0	28.0	35.0	42.0	49.0	56.0	63.0	70.0	77.0	84.0	91.0
	6.4	19.5	40.0	59.6	92.4	146.5	197.1	248.2	296.6	337.4	357.5	396.7	411.9	430.7
	5.9													
	6.2													
	5.7													
	6.0	19.5	40.0	59.6	92.4	146.5	197.1	248.2	296.6	337.4	357.5	396.7	411.9	430.7

Generation F3 weights of female rats raised under 40 lux light conditions.

Age (days)	0.0	8.0	14.0	21.0	28.0	35.0	42.0	49.0	56.0	63.0	70.0	77.0	84.0	91.0
	5.7	19.9	35.5	55.5	80.7	118.1	148.3	166.5	178.9	185.2	199.8	209.3	216.9	219.5
	5.8	19.0	33.1	53.2	76.6	111.0	136.0	151.7	166.4	176.5	186.7	197.5	201.3	213.7
	6.2													
	5.7													
	5.8													
	5.8													
Mean	5.8	19.5	34.3	54.4	78.7	114.6	142.2	159.1	172.7	180.9	193.3	203.4	209.1	216.6
StdDev	0.2	0.6	1.7	1.6	2.9	5.0	8.7	10.5	8.8	6.2	9.3	8.3	11.0	4.1

Generation F3 weights of female rats raised under 5 lux light conditions.

Age (days)	0.0	7.0	14.0	21.0	28.0	35.0	42.0	49.0	56.0	63.0	70.0	77.0	84.0	91.0
	6.4	20.5	40.7	58.7	84.2	117.9	143.7	156.2	170.2	177.5	191.4	200.5	203.8	208.6
	5.9	16.9	38.2	55.4	79.1	113.5	136.6	156.1	168.4	180.3	187.9	201.3	206.7	205.3
	6.3	19.1	39.4	55.3										
	5.9	20.5	41.3	60.4										
	5.8	17.0	36.9	54.8										
	6.4													
	6.6													
Mean	6.2	18.8	39.3	56.9	81.7	115.7	140.2	156.2	169.3	178.9	189.7	200.9	205.3	207.0
StdDev	0.3	1.8	1.8	2.5	3.6	3.1	5.0	0.1	1.3	2.0	2.5	0.6	2.1	2.3

Figure 1. Mean ± S.E.M. birth weight listed by generation and light level.

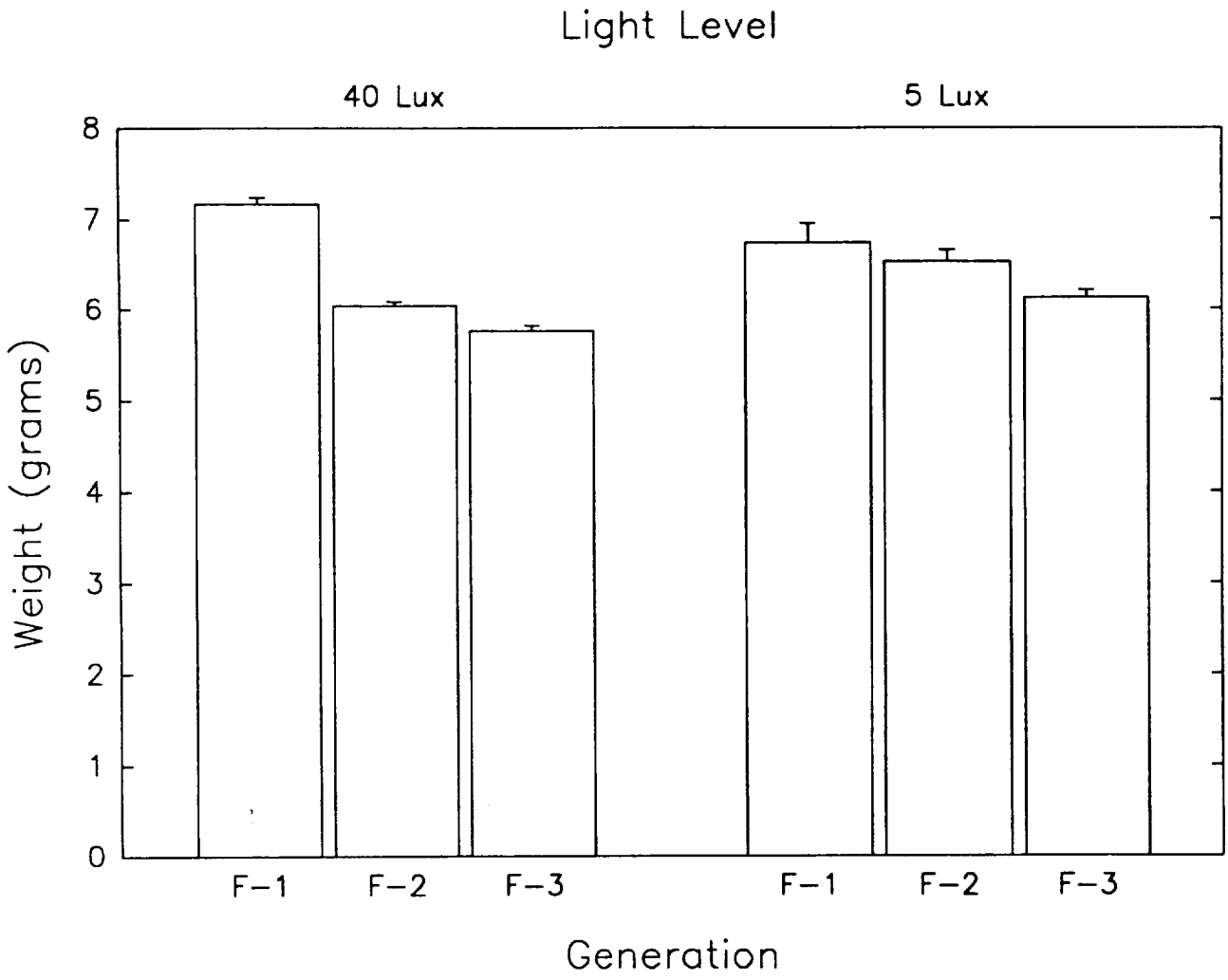


Figure 2. Mean ± S.E.M. weight of rats for generation F-1 by age, sex, and light level.

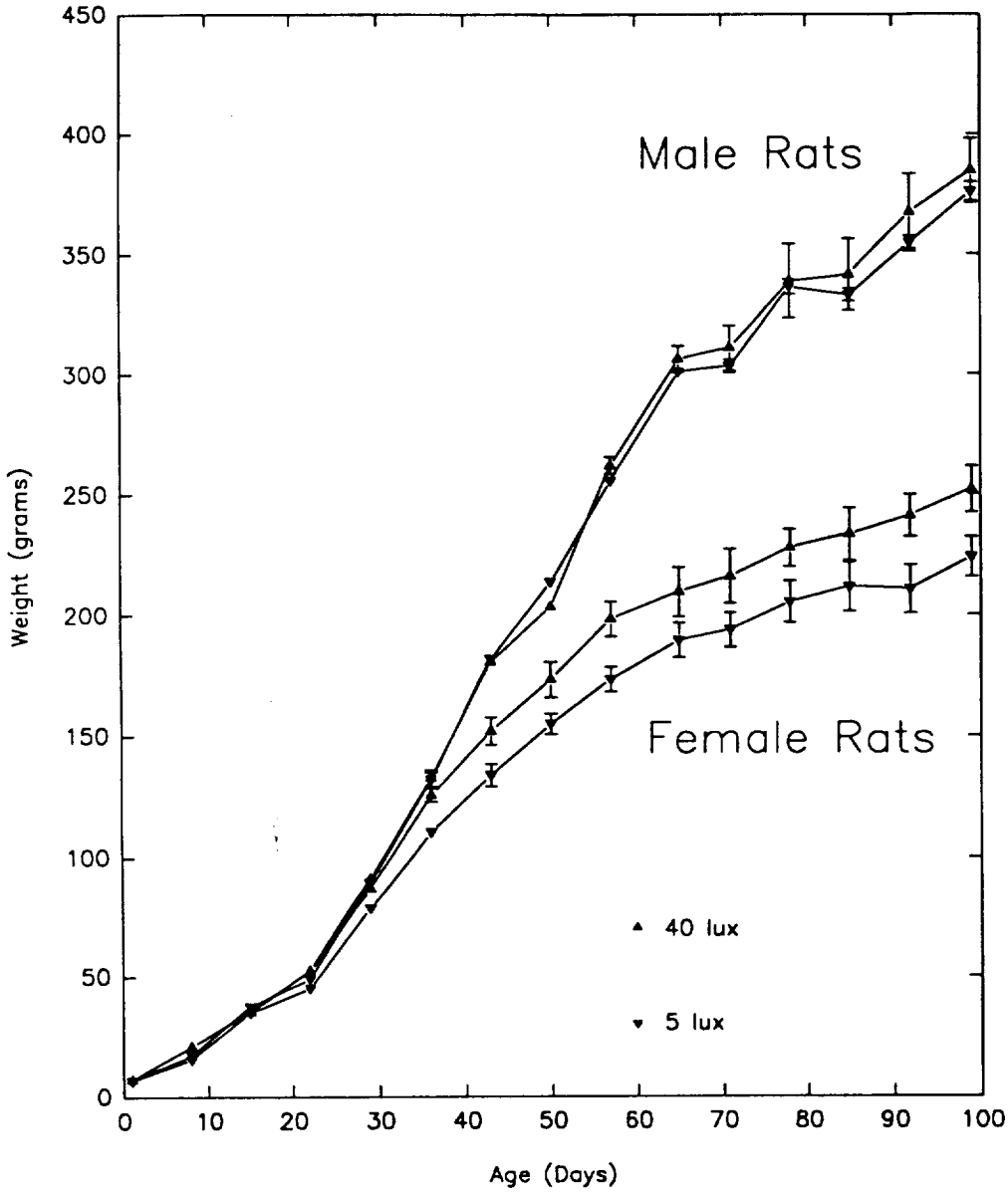


Figure 3. Mean ± S.E.M. weight of rats for generation F-2 by age, sex, and light level.

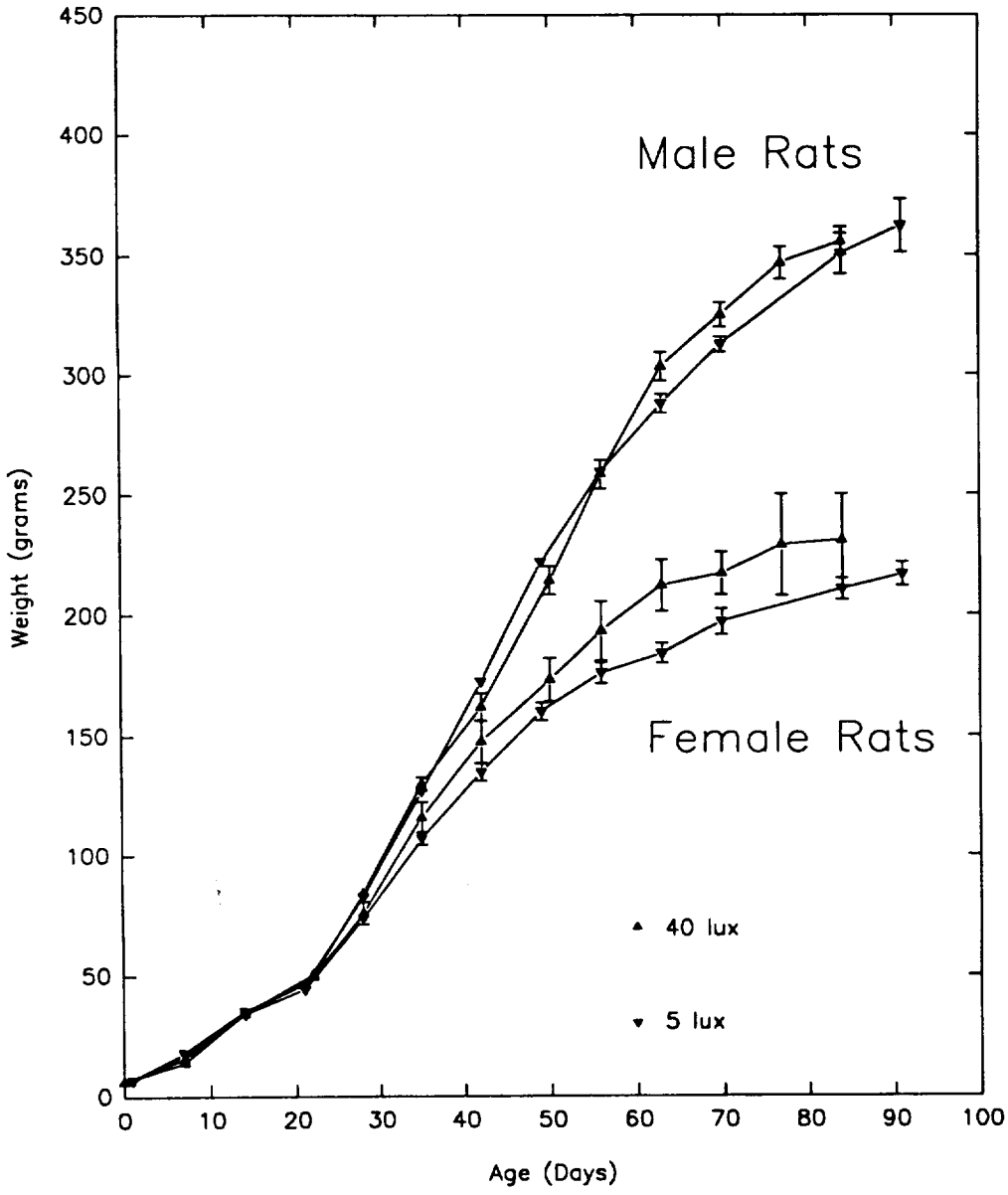
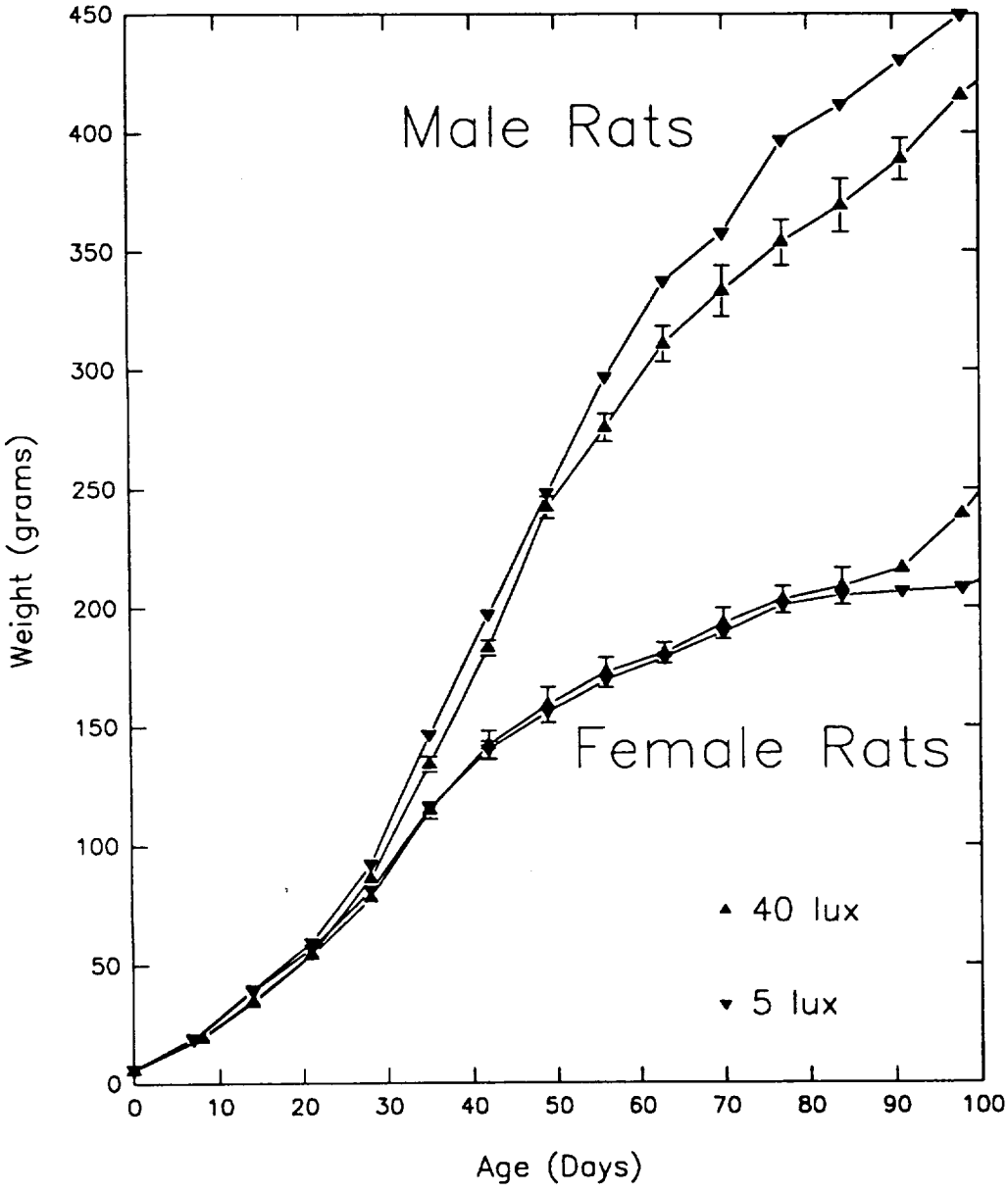


Figure 4. Mean ± S.E.M. weight of rats for generation F-3 by age, sex, and light level.



Final Report for NASA COOPERATIVE AGREEMENT #NCC2-593
June 3, 1994
San Jose State University

B) Effects of Low Light Intensity on the Rat Circadian System

Final Subproject Report for Cooperative Agreement #NCC2-593

Part 1. New Findings Regarding Light Intensity and its Effects as a Zeitgeber in the Sprague-Dawley Rat.

Tischler, A.C., C.M. Winget, D.C. Holley, C.W. DeRoshia, J. Gott, G. Mele, and P.X. Callahan, 1993

The Physiologist 36(1, Suppl.):S-125 - S-126

Part 2. Circadian Entrainment of Male Sprague-Dawley Rats Using Low Light Intensity

Tischler, A.C., D.C. Holley, C.W. DeRoshia, J. Gott, S. Okumura, G. Mele, C.M. Winget, and P.X. Callahan, 1992

The FASEB Journal 6(5):A1832

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Volume 6, Number 5 February 28, 1992

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CIRCADIAN ENTRAINMENT OF MALE SPRAGUE - DAWLEY RATS USING LOW LIGHT INTENSITY.

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This study was performed to define the lower limits of light intensity for circadian entrainment in albino rats. Groups of six were maintained individually in isolation cabinets for approx. 60 d (22°C, food and water ad lib.). The following parameters were monitored continuously, reduced and saved in ten minute bins: gross locomotor activity (LMA), drinking time, and feeding time. The photoperiod consisted of control (12L:12D), constant conditions (at test light intensity), both 2-4 weeks; followed by control, 1 week. Lighting was sunlight simulating fluorescent (Vitalite) and varied among trials: 10, 5, 1, and 0.1 Lux (Lx). An additional trial used 40 Lx during L:D, and dark (< 0.01 Lx) during constant conditions. Significant ($p<0.05$) circadian rhythms were detected in all parameters during all L:D control periods (cosinor analysis). During constant conditions all animals exhibited free-running rhythms with period being directly proportional to light intensity. LMA periods (complex demodulation and periodogram analyses; trial mean ± S.D., hrs) during constant conditions were: dark, 24.26±.06; 0.1 Lx, 24.45±.04; 1 Lx, 25.09±.04; 5 Lx, 25.27±.24; 10 Lx, 25.49±.06. These studies indicate that light as low as 0.1 Lx can entrain the circadian system of the white laboratory rat. (Funded by NASA Coop. Agreement #NCA2-593).

NEW FINDINGS REGARDING LIGHT INTENSITY AND ITS EFFECTS AS A ZEITGEBER IN THE SPRAGUE-DAWLEY RAT

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INTRODUCTION

Circadian rhythmicities are oscillations of physiological cycles designed to create temporal organization. Circadian rhythms ensure that physiological mechanisms are expressed in proper relationship to each other and the 24 hour day. Light is the main zeitgeber ("time giver") for biological clocks. The daily variations in light intensity from dawn to dusk, and seasonally due to the rotation of the earth, act upon organisms to give them photoperiodic information. This entrainment allows them to vary biologically to prepare for reproduction, hibernation, migration and the daily adaptations necessary for survival.[1] In most mammals, the suprachiasmatic nucleus of the anterior hypothalamus has been implicated as the central driving mechanism of circadian rhythmicity. The photic input from the retina, via the retino-hypothalamic tract, and modulation from the pineal gland help regulate the clock. In this study we investigated the effects of low light intensity on the circadian system of the Sprague-Dawley rat. A series of light intensity experiments were conducted to determine if a light level of 0.1 Lux will maintain entrained circadian rhythms of feeding, drinking, and locomotor activity.

The intensity of light to which an animal is exposed is one determinant of the length of the free-running period of a circadian clock in a constant environment. In 1960, Jurgen Aschoff showed that more intense light shortens the free-running period in diurnal organisms, but lengthens the period in nocturnal animals. Aschoff further concluded that under more intense light, the time an animal is active, compared with the time it is at rest, increases in diurnal animals, but decreases in nocturnal animals. Also, in diurnal species, the total amount of activity during a free-running period increases with light intensity, while the reverse is true in nocturnal species.[2]

METHODS

Groups of six male Sprague-Dawley albino rats (initial weight 250 g; final weight 350 g) were obtained from Simonsen Laboratories. Rats were selected from the lowest shelves on the racks by the vendor in order to select animals with exposure to low light intensity, thus controlling for possible retinal damage. Typical lighting levels in many animal vivariums exceed 1000 Lux. At this level it is now known that retinal damage can occur in albino rats. It is also important to establish a light history on the animals, because in circadian rhythm research, prior exposure to light can elicit aftereffects lasting up to 100 days. [1]

The rats were housed individually in Nalge metabolism cages made of lexan and polycarbonate. These materials pass all wavelengths of light in the visible spectrum, and a portion of the ultraviolet spectrum . We used a full-spectrum fluorescent light source (Duro-Test Vita-Lite) simulating the spectral qualities of natural sunlight. The metabolism cages were housed in individual light tight cabinets. The fluorescent light sources were located centrally over each metabolism cage. Using a calibrated radiometer, light intensities were adjusted to the appropriate experimental levels and set no higher than each specific intensity at the eye level of each animal.

An initial baseline intensity of 10 Lux was tested because previous studies in laboratory rodents indicated entrainment at this level.and above. An experiment was performed utilizing a light intensity of 5.0 Lux, a level determined by concensus at a NASA Lighting Requirements in Microgravity Workshop. [3] Two additional experiments were performed utilizing 1.0 Lux and 0.1 Lux(a level less than that of full moonlight,approximately 0.4 Lux).

The following protocol was used for each experiment:The rats were acclimated for a period of 1 week to their new environment at each light intensity. The light cycle was adjusted for a 12 hour period of light, starting at 0700, and 12 hours of darkness beginning at 1900. This 12L:12D cycle at each intensity was imposed for 3 weeks.Immediately following the 12L:12D cycle, the rats were exposed to a 2 week period of constant light (LL).At the conclusion of the LL period, the 12L:12D light cycle was reinstated for 1 week to re-entrain the animals. Daily animal care was performed in the dark at time intervals outside the range

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of circadian entrainment (18 hours before or 33 hours after last maintaining the rats).A final experiment was conducted using 40 Lux for the 12L:12D cycle, followed by an 18-day period of constant darkness (<0.01 Lux) and a return to the LD cycle.

The data format represented below is a raster plot. It is a record of activity over a 24 hour period which is double plotted so that the pattern of the circadian rhythm can easily be seen by the unaided eye. This raster plot visually depicts the difference between the entrained and free-running periods at an intensity of 0.1 lux

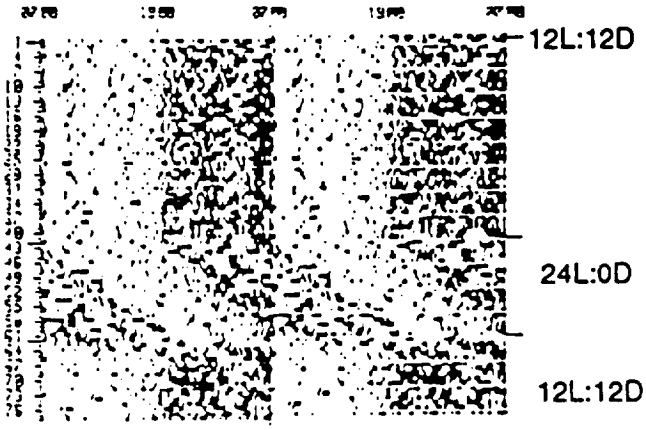

Figure 1

Table 1. Complex Demodulation Mean Period ± S.E.

Lux	Activity	Drinking	Feeding
0.01	24.28±0.01	24.30±0.03	24.29±0.01
0.1	24.30±0.07	24.45±0.26	24.70±0.05
1	24.84±0.14	25.29±0.13	25.53±0.06
5	24.99±0.34	25.50±0.03	25.46±0.13
10	25.56±0.14	25.85±0.10	25.23±0.63

Period lengths were estimated from linear regression of acrophases (peak of harmonic fit to circadian rhythm) obtained from complex demodulation analyses [Table 1].Cosinor analysis was utilized to obtain acrophase, mean amplitude, and significance level of the rhythms monitored.

RESULTS AND DISCUSSION

Visual inspection of the raster plots clearly indicated that circadian rhythmicity was maintained in all parameters monitored during LD. Results of cosinor analysis showed significant circadian rhythms (alpha=0.05) were detected in all parameters during the control periods.

Furthermore,the results demonstrate that the free-running period length is directly proportional to the logarithm of light intensity [figure 2].

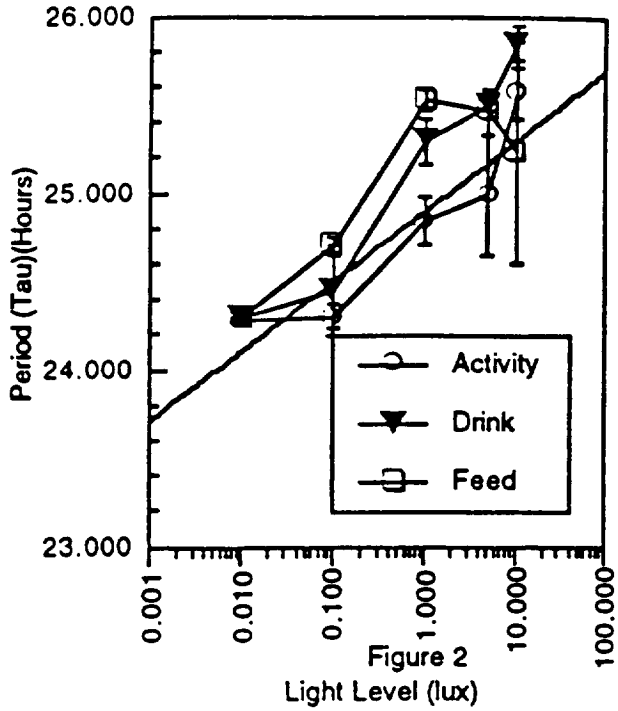

Figure 2

This is consistent with Aschoff's Rule which states that the circadian frequency varies linearly with the logarithm of the intensity.With increasing light intensity, the circadian frequency of dark-active animals decreases(the period lengthens).

CONCLUSION

These experiments show that extremely low light levels (0.1 lux) can entrain the circadian system of the white laboratory rat. Therefore, the power requirement[3] (5.0 lux)specified for Sprague-Dawley rats in microgravity is sufficient to maintain normal circadian rhythmicity.

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[1] Moore-Ede,M.C.,F.M. Sulzman and C.A. Fuller,1982. The Clocks That Time Us: The Circadian Timing System in Mammals. Harvard Univ. Press.[Boston].

[2] Carpenter,Gail A., and Grossberg,S.,1984. A Neural Theory of Circadian Rhythms:Aschoff's Rule in diurnal and nocturnal mammals.Am.J. Physiol. 16:R1067-R1082

[3]NASA Technical Memorandum 101077,1988. editors:Holley,D.C.,Winget,C.M.,and Leon,H.A. Lighting Requirements in Microgravity-Rodents and Non-Human Primates. p.5-7.

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Part 3. Experiment Protocol, Methods, and Data

Effects of Low Light Intensity on the Circadian System of the Rat

FINAL REPORT of sub project for COOPERATIVE AGREEMENT #NCC2-593.

June 2, 1994, 14:14

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Effects of Low Light Intensity on the Rat Circadian System
Final Subproject Report June 2, 1994
(Cooperative Agreement #NCC2-593)
San Jose State University

INTRODUCTION

In 1957, Franz Halberg coined the term "circadian" -- from "circa-" meaning about or around, and "-dian" meaning day. Circadian rhythmicities are oscillations of biological processes designed to create physiological temporal organization. Circadian rhythms ensure that physiological mechanisms are expressed in proper relationship to each other during the 24 hour day. Furthermore, circadian rhythms help optimize the economy of biological systems and better prepare organisms to foresee and cope with predictable alterations in the environment.

Light is the main zeitgeber ("time giver") for biological clocks. The daily variations in light intensity from dawn to dusk, and seasonally from the rotation of the earth, act upon organisms to give them photoperiodic information. This entrainment allows them to vary biologically to prepare for reproduction, hibernation, migration and the daily adaptations necessary for survival. In most mammals, the suprachiasmatic nucleus of the anterior hypothalamus has been implicated as the "Big Ben" mechanism of circadian rhythmicity. The photic input to the retina, retinalhypothalamic tract, and pineal gland are the "pendulum" components of the clock. In this study we investigated the effects of low light intensity on the circadian system of the Sprague-Dawley rat. A series of light intensity experiments were conducted to determine if a source with a sunlight simulating spectrum and a light level of 0.1 Lux will maintain entrained circadian rhythms of feeding, drinking, and locomotor activity.

LIGHT INTENSITY EFFECTS

The intensity of the light to which a mammal is exposed is one determinant of the length of the free-running period of its circadian clock in a constant environment. In 1960, Jurgen Aschoff showed that more intense light shortens the free-running period in diurnal organisms, but lengthens the period in nocturnal animals. Aschoff further concluded that under more intense light, the time an animal is active, compared with the time it is at rest, increases in diurnal animals, but decreases in nocturnal species. Also, in diurnal species, the total amount of activity during a free-running period increases with light intensity, while the reverse is true in nocturnal species.

METHODS

Groups of six male Sprague-Dawley albino rats (initial weight 250 g; final weight 350 g) were obtained from Simonsen Laboratories. They were selected from the lowest shelves on the racks in order to select animals with a low exposure to light, controlling for possible retinal damage. Typical lighting levels in most laboratories are approximately 1000 Lux; at this level it is now known that retinal damage can occur in albino rats. It is also important to establish a light history on the animals, because in circadian rhythm research, prior exposure to light can elicit after-effects lasting up to 100 days. Each rat was examined to ensure that its eyes were tracking properly as a test of visual acuity.

The rats were housed individually in Nalgene metabolism cages made of lexan and polycarbonate. These materials pass all wavelengths of light in the visible spectrum, and a portion of the ultraviolet. Therefore, we used a full-spectrum fluorescent light source (#1032 T12-15, 14 watts, Vita-Lite, Duro-Test, Corp, North Bergen, NJ) simulating the spectral qualities of natural sunlight.

The metabolism cages were housed in individual light tight wooden units (26w x 30h x 26d inches). The fluorescent light sources were located centrally over each metabolism cage. Light intensities were adjusted to the appropriate experimental levels using a Lutron light meter. All tested light intensities were adjusted to be no higher than each specific intensity at the eye level of the animal in an upright position.

EXPERIMENT PROTOCOLS

Control animals were exposed to 10 Lux intensity because previous studies using laboratory rodents indicated entrainment at this level. A second experiment was performed utilizing a light intensity of 5.0 Lux, the light level minimum suggested by the NASA Lighting Requirements in Microgravity Workshop (TM 101077; December 1988). Two additional experiments were performed utilizing 1.0 Lux and 0.1 Lux, a level less than that of full moonlight (approximately 0.4 Lux). A final experiment was conducted using 40 Lux for the 12L:12D cycle, followed by an 18-day period of constant darkness (<0.001 Lux) and a return to the LD cycle.

The protocol for each experiment was as follows:

1. The rats were acclimated for a period of 1 week in order to have them adapted to their new environment at each light intensity.

2. The light cycle was adjusted for a 12 hour period of light, starting at 0700, and 12 hours of darkness beginning at 1900. This 12L:12D cycle with each intensity was imposed for 3 weeks.

3. Immediately following the 12L:12D cycle, the rats were exposed to a 2 week period of constant light, LL.

4. At the conclusion of the LL period, the 12L:12D light cycle was reinstated for 1 week to re-entrain the animals.

5. Daily animal care was performed in the dark at time intervals outside the range of circadian entrainment (18 hours before or 33 hours after the last animal maintanance).

DATA ANALYSIS

The data format represented in this report is called the raster plot or actogram. It is a record of activity over a 24 hour period which is 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. In the light intensity studies, the onset and offset coincide with the 12L:12D light cycle. In the so-called "free-running" condition, the animal is released to a constant environmental condition (i.e., constant light, LL). In LL, each day's activity will drift a little with the "intrinsic" rhythm of the internal biological clock. This is a means of indicating whether or not the

animal was previously entrained to the light cycle (an exogenous stimulus). The raster plot visually depicts the difference between entrainment and the free-running period for each light intensity tested.

RESULTS

Visual analyses of the raster plots clearly show that all parameters monitored maintained circadian rhythmicity at each light intensity tested. Note: in all captions LMA = gross locomotor activity.

PERIOD ANALYSIS

Period lengths were calculated by a linear regression of acrophases obtained from complex demodulation analyses.

Power spectral analysis was performed and periodograms were plotted for all 12L:12D and LL data sets for final period information.

COSINOR ANALYSIS

Cosinor analysis was utilized to obtain phase angle (at the peak of sine wave fit), acrophase (at the peak of sine wave fit), mean amplitude, and significance level of the rhythms monitored. Results for the first three are shown in Table 3, Table 4, and Table 5.

Results of cosinor analysis showed significant circadian rhythms (alpha=0.05) in all parameters during the control period (12L:12D).

The results of the periodograms demonstrate free-running period values with period being directly proportional to logarithm of light intensity. This is consistent with Aschoff's Rules:

1. Circadian frequency varies with the logarithm of light intensity.

2. With increasing light intensity, the circadian frequency of dark-active animals decreases (the period lengthens).

CONCLUSION

These studies indicate that 0.1 Lux (light lower than full moonlight, 0.4 Lux), entrained the circadian system of the white laboratory rat.

Table 1. Summary of all experiment times and applied light.

Experiment	Start	End	Type	Days
8801Lite10	25-Jan-88:1900			
	25-Jan-88:1900	17-Feb-88	LD 10	23
	17-Feb-88	19-Feb-88:1100	DD	2
	19-Feb-88:1100	05-Mar-88:1600	LD 10	15
Total Days				40

Expt	Start	End	Type	Days
8803Lite10	25-Mar-88:1900	10-May-88:1426	LD 10	46
Total Days				46

Expt	Start	End	Type	Days
8806Lite01	6-02-88:1604	7-11-88:0650	LD 1	39
	7-11-88:0700	7-25-88:1000	LL 1	14
	7-25-88	7-28-88:1400		2
Total Days				55

Expt	Start	End	Type	Days
8808Lite0.1	8-01-88:1740			1
	08-02-88	9-15-88	LD 0.1	44
	09-15-88	10-04-88:1500	LL 0.1	19
	10-04-88:1533	10-05-88:1440	LL 5	1
	10-05-88	10-26-88:1600	LD 5	21
Total Days				85

Expt	Start	End	Type	Days
8812Lite05	12-07-89	12-13-89		6
	12-13-89:1610	2-06-89:1510	LD 5	54
	02-06-89	2-24-89:1307	LL 5	18
Total Days				78

Expt	Start	End	Type	Days
8906Lite40	6-12-89:1603	6-14-89:0751		2
	6-14-89:0751	7-03-89	LD 40	19
	7-03-89:1027	7-21-89	DD 0.01	18
	7-21-89	8-02-89:1237	LD 40	12
	8-2-89	8-22-89:1410		
Total Days				51

Table 1. Summary of all experiment times and applied light

Expt	Start	End	Type	Days
9107Lite10	7-13-91			
	7-22-91	8-04-91	LD 10	13
	8-04-91	8-14-91	LL 10	10
	8-14-91	8-18-91	LL 10	4
	8-18-91	8-24-91	LD 10	6
Total Days				33
Total of all experiments				388

Table 2. Dates used in cosinor period analysis

Expt	Start	End	Type	Days
8906Lite40	6-25-89	7-03-89	LD	8
	7-05-89	7-13-89	DD	8
Expt	Start	End	Type	Days
8808Lite0.1	9-07-88	9-15-88	LD	8
	9-17-88	9-25-88	LL0.1	8
Expt	Start	End	Type	Days
8806Lite01	7-03-88	7-11-88	LD	8
	7-13-88	7-21-88	LL	8
Expt	Start	End	Type	Days
8812Lite05	1-29-89	2-06-89	LD	8
	2-08-89	2-16-89	LL	8
Expt	Start	End	Type	Days
9107Lite10	7-27-91	8-04-91	LD	8
	8-06-91	8-14-91	LL	8

Table 3. Periods from cosinor analysis.

LMA Period

Light Level	0.01	0.1	1	5	10
LD 1	24.06	24.03	24.32	23.92	23.98
LD 2	24.21	23.94	23.72	23.92	23.88
LD 3	23.97	24.33	23.81	23.95	24.08
LD 4	23.82	23.94	23.74	23.86	24.21
LD 5	23.92	23.70	23.93	---	23.99
LD 6	24.00	23.50	23.77	23.80	23.61
Mean	24.00	23.91	23.88	23.89	23.96
Std Dev	0.13	0.29	0.23	0.06	0.20
LL 1	24.32	24.55	25.09	25.45	25.15
LL 2	24.47	25.34	24.95	25.88	25.32
LL 3	24.48	24.70	24.68	24.63	25.31
LL 4	24.39	24.57	24.97	25.57	25.46
LL 5	24.26	25.03	25.03	24.57	---
LL 6	23.96	24.94	24.96	25.74	24.98
Mean	24.31	24.85	24.95	25.31	25.24
Std Dev	0.19	0.31	0.14	0.57	0.19

DRINKING Period

Light Level	0.01	0.1	1	5	10
LD 1	24.09	24.18	23.74	24.01	24.02
LD 2	23.92	24.05	24.00	23.68	24.06
LD 3	23.95	23.83	23.84	23.80	23.96
LD 4	23.77	23.87	24.15	24.12	24.21
LD 5	23.46	23.69	23.55	24.00	23.95
LD 6	24.15	24.01	23.64	24.07	23.90
Mean	23.89	23.94	23.82	23.95	24.02
Std Dev	0.25	0.17	0.22	0.17	0.11
LL 1	24.37	25.20	25.52	25.54	25.52
LL 2	24.58	24.95	24.81	25.62	25.30
LL 3	24.66	25.00	25.64	25.49	26.01
LL 4	24.44	24.84	25.41	25.12	26.16
LL 5	24.36	24.66	---	25.65	25.97
LL 6	24.20	24.02	25.05	25.64	25.83
Mean	24.43	24.78	25.28	25.51	25.80
Std Dev	0.16	0.41	0.35	0.20	0.33

Table 3. Periods from cosinor analysis.

Feeding Period

Light Level	0.01	0.1	1	5	10
LD 1	24.04	24.37	23.65	24.06	24.15
LD 2	23.99	24.37	23.69	23.65	24.11
LD 3	23.71	24.21	24.05	24.03	24.21
LD 4	23.93	24.04	24.01	24.22	24.13
LD 5	23.83	23.93	23.82	23.96	24.00
LD 6	23.65	24.34	23.70	23.56	23.68
Mean	23.86	24.21	23.82	23.91	24.05
Std Dev	0.15	0.19	0.17	0.26	0.19
LL 1	24.05	25.20	25.18	25.80	25.94
LL 2	24.64	24.66	24.88	25.54	25.16
LL 3	24.33	24.86	26.14	25.41	25.62
LL 4	23.73	24.47	25.35	25.38	25.58
LL 5	24.57	24.63	25.50	25.99	25.75
LL 6	24.31	24.16	24.66	25.35	25.47
Mean	24.27	24.66	25.29	25.58	25.59
Std Dev	0.34	0.35	0.52	0.26	0.26

Table 4. Acrophase from cosinor analysis.

LMA Acrophase (hours after midnight)

Light Level	0.01	0.1	1	5	10
LD 1	22.27	26.76	23.19	21.21	23.18
LD 2	22.96	23.01	23.82	21.91	23.15
LD 3	23.32	23.22	23.90	22.23	22.30
LD 4	24.09	25.10	24.07	21.92	22.14
LD 5	24.23	24.41	23.07	24.89	24.04
LD 6	23.40	24.95	24.00	21.81	25.23
Mean	23.38	24.58	23.67	22.33	23.34
Std Dev	0.72	1.38	0.43	1.30	1.15
LL 1	23.66	27.42	27.77	24.98	28.63
LL 2	23.99	23.15	25.32	23.38	27.44
LL 3	24.02	26.29	27.76	27.49	28.27
LL 4	25.14	25.55	27.25	25.71	26.34
LL 5	24.54	23.70	26.56	32.96	33.73
LL 6	25.05	25.15	27.04	22.79	30.84
Mean	24.40	25.21	26.95	26.22	29.21
Std Dev	0.61	1.59	0.92	3.71	2.67

DRINKING Acrophase (hours after midnight)

Light Level	0.01	0.1	1	5	10
LD 1	22.64	21.32	24.14	21.81	23.43
LD 2	23.91	23.35	22.57	22.90	23.50
LD 3	23.72	23.43	23.91	22.83	23.85
LD 4	24.11	23.99	22.61	21.21	22.91
LD 5	26.45	23.80		21.09	23.68
LD 6	22.75	21.37	24.55	21.37	24.10
Mean	23.93	22.88	23.56	21.87	23.58
Std Dev	1.38	1.21	0.91	0.81	0.41
LL 1	24.37	23.76	26.76	25.70	24.98
LL 2	24.42	22.44	26.82	24.59	26.32
LL 3	24.05	23.44	23.84	22.95	23.29
LL 4	23.90	23.09	26.52	25.16	23.67
LL 5	24.28	23.65		22.42	26.78
LL 6	23.71	25.83	26.23	23.73	26.85
Mean	24.12	23.70	26.03	24.09	25.32
Std Dev	0.28	1.15	1.25	1.28	1.58

Table 4. Acrophase from cosinor analysis.

FEEDING Acrophase (hours after midnight)

Light Level	0.01	0.1	1	5	10
LD 1	22.64	20.37	25.47	20.78	22.81
LD 2	30.43	20.78	22.87	22.68	21.96
LD 3	23.03	21.10	23.20	22.34	23.07
LD 4	22.25	23.32	23.39	22.36	20.49
LD 5	23.11	21.88	22.22	21.23	23.41
LD 6	23.25	20.77	23.78	23.52	24.40
Mean	24.12	21.37	23.49	22.15	22.69
Std Dev	3.11	1.08	1.10	1.00	1.34
LL 1	24.42	23.86	27.44	23.44	23.72
LL 2	23.87	27.01	24.64	24.32	24.66
LL 3	24.29	23.09	21.72	22.69	25.83
LL 4	25.48	24.56	24.52	23.62	21.93
LL 5	22.19	25.14	25.19	21.28	26.08
LL 6	22.78	25.08	26.00	23.92	28.52
Mean	23.84	24.79	24.92	23.21	25.13
Std Dev	1.19	1.34	1.90	1.09	2.25

Table 5. Rhythm amplitude from cosinor analysis.

LMA Rhythm amplitude

Light Level	0.01	0.1	1	5	10
LD 1	534.50	138.13	236.12	417.86	852.12
LD 2	343.11	650.04	310.48	268.32	879.45
LD 3	407.46	369.85	568.86	323.29	420.56
LD 4	48.26	295.94	446.67	34.92	342.86
LD 5	202.69	504.34	908.54	9.98	821.28
LD 6	298.23	240.62	434.96	273.87	219.44
Mean	305.71	366.49	484.27	221.37	589.28
Std Dev	167.96	185.68	237.82	163.34	294.31
LL 1	508.31	206.99	262.22	271.29	398.90
LL 2	341.15	350.09	290.11	191.37	433.41
LL 3	311.97	394.18	463.56	224.87	320.11
LL 4	91.17	213.46	321.40	28.45	177.76
LL 5	215.15	302.29	610.11	18.52	500.92
LL 6	280.03	147.11	441.02	216.34	118.78
Mean	291.30	269.02	398.07	158.48	324.98
Std Dev	138.55	95.02	131.97	107.76	149.93

DRINKING Rhythm amplitude

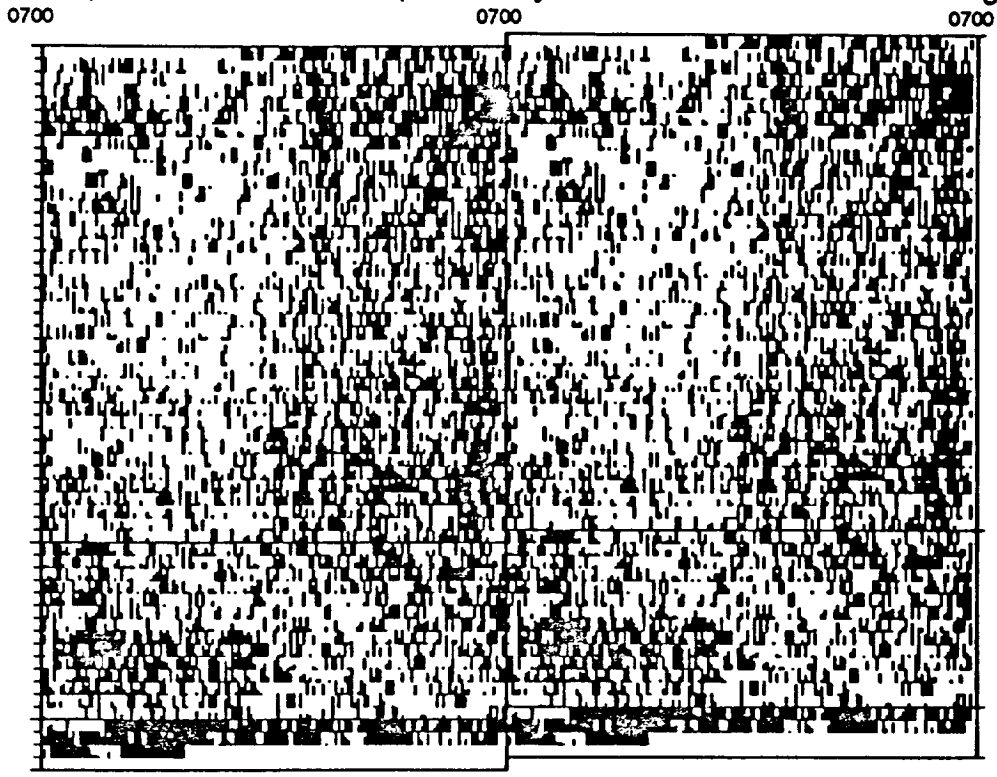
Light Level	0.01	0.1	1	5	10
LD 1	2.89	2.56	3.71	3.69	3.74
LD 2	2.62	3.62	3.28	2.39	3.32
LD 3	3.56	3.59	2.98	3.20	3.06
LD 4	3.47	2.68	3.79	2.80	3.95
LD 5	2.58	1.07		3.45	5.14
LD 6	2.93	2.51	2.59	3.02	2.95
Mean	3.01	2.67	3.27	3.09	3.69
Std Dev	0.42	0.93	0.50	0.46	0.81
LL 1	2.78	1.83	2.94	2.34	1.60
LL 2	2.05	1.81	2.17	2.25	1.20
LL 3	2.14	2.19	1.91	2.26	1.35
LL 4	3.64	1.76	2.94	1.42	2.37
LL 5	3.28	1.04		1.75	1.69
LL 6	2.92	1.27	1.89	2.30	1.71
Mean	2.80	1.65	2.37	2.05	1.65
Std Dev	0.62	0.42	0.53	0.38	0.41

Table 5. Rhythm amplitude from cosinor analysis.

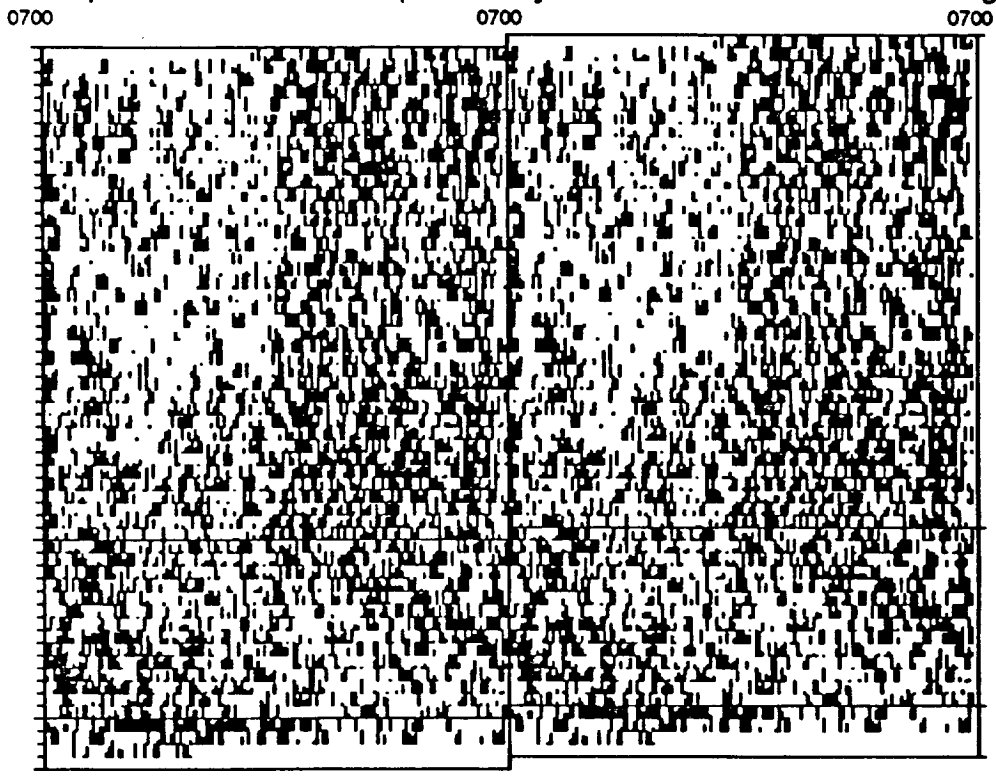
FEEDING Rhythm amplitude

Light Level	0.01	0.1	1	5	10
LD 1	2.68	1.88	1.40	1.57	1.91
LD 2	0.81	1.67	1.73	1.39	2.35
LD 3	1.72	1.32	1.04	1.16	1.49
LD 4	1.83	1.21	1.77	0.87	0.94
LD 5	2.07	1.21	2.07	1.14	1.81
LD 6	1.80	1.95	1.86	1.13	1.66
Mean	1.82	1.54	1.64	1.21	1.69
Std Dev	0.60	0.34	0.37	0.24	0.47
LL 1	2.47	0.64	1.05	0.73	1.34
LL 2	1.58	1.27	1.44	0.98	1.22
LL 3	1.97	0.69	0.54	0.88	1.15
LL 4	1.77	0.58	1.18	1.12	1.06
LL 5	2.21	0.82	1.30	0.97	1.32
LL 6	2.09	1.36	1.21	0.76	0.77
Mean	2.02	0.89	1.12	0.91	1.14
Std Dev	0.32	0.34	0.31	0.15	0.21

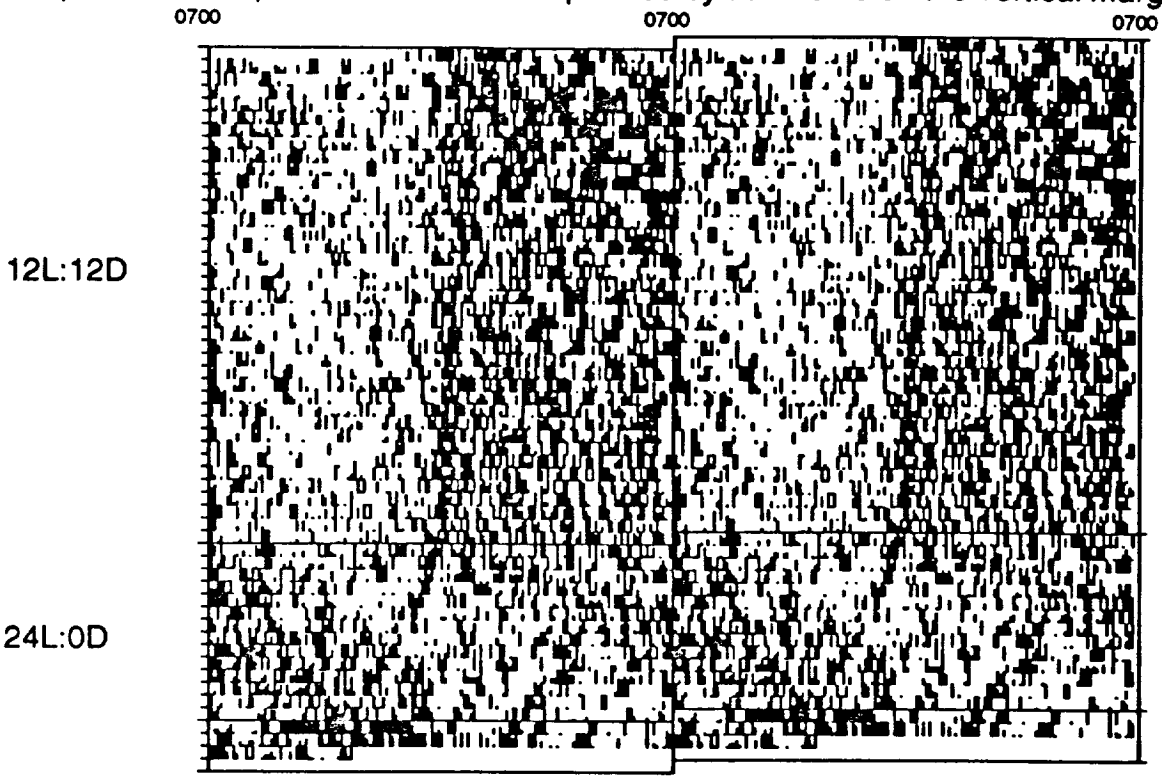
Light experiment 1 (8806Light01) Rat 1 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



Light experiment 1 (8806Light01) Rat 2 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

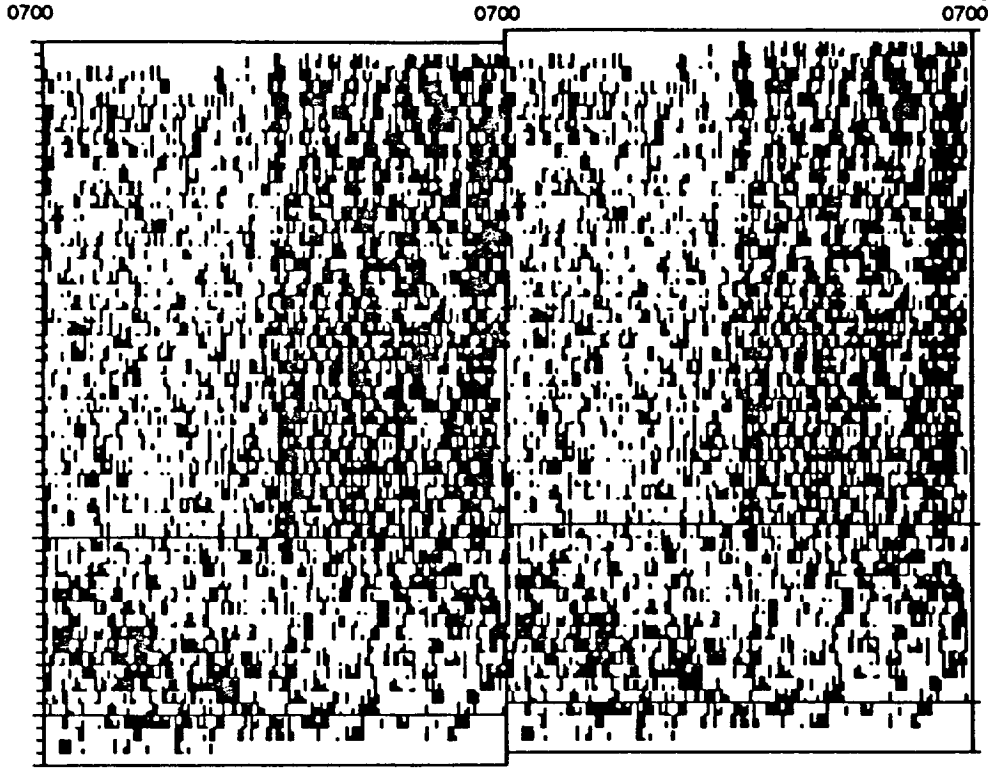


Light experiment 1 (8806Light01) Rat 3 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

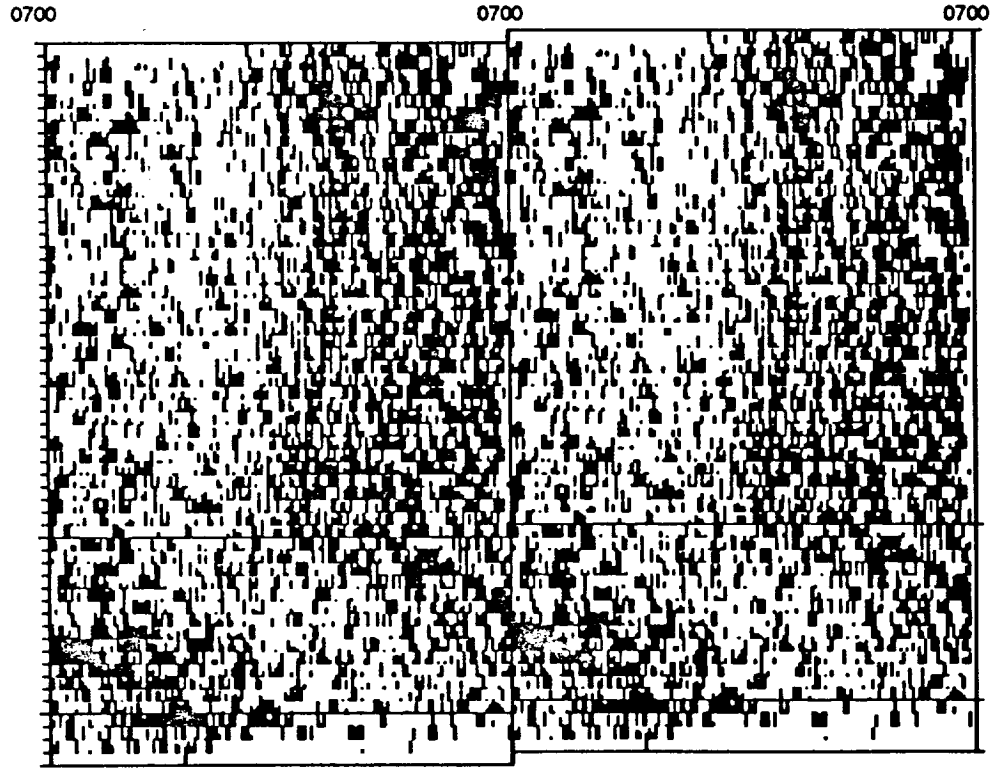


Light experiment 1 (8806Light01) Rat 4 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

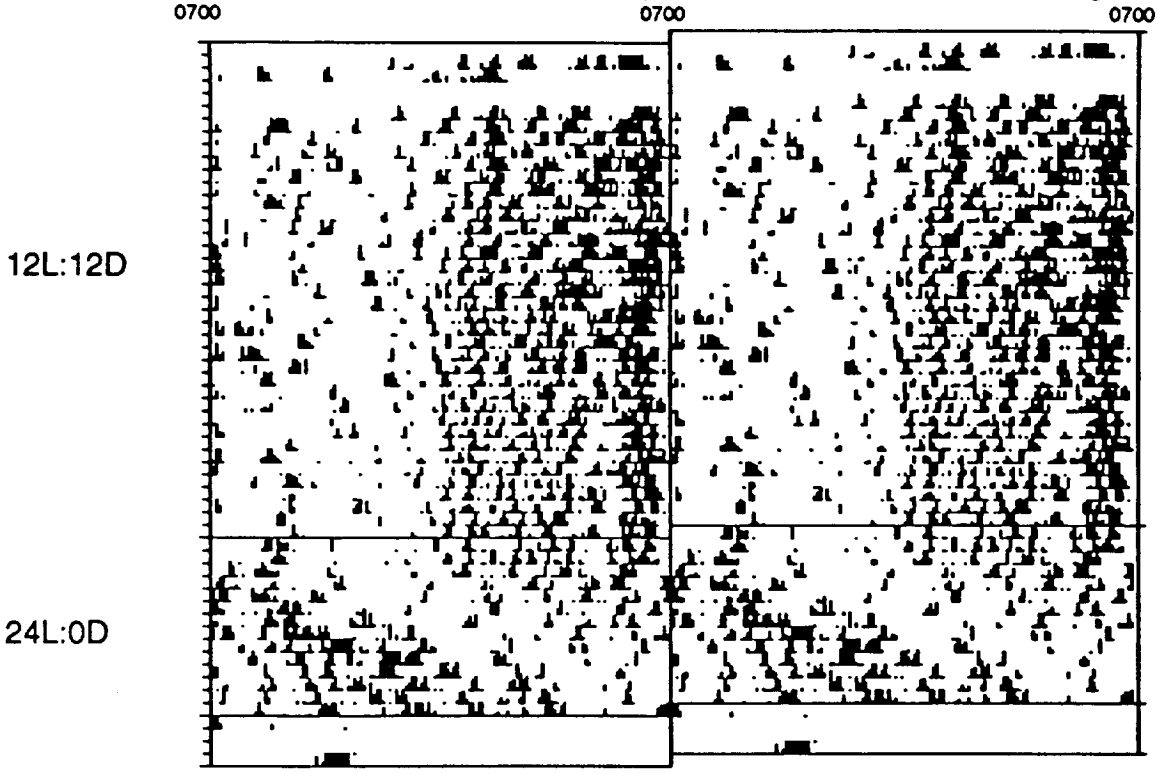
Light experiment 1 (8806Light01) Rat 5 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



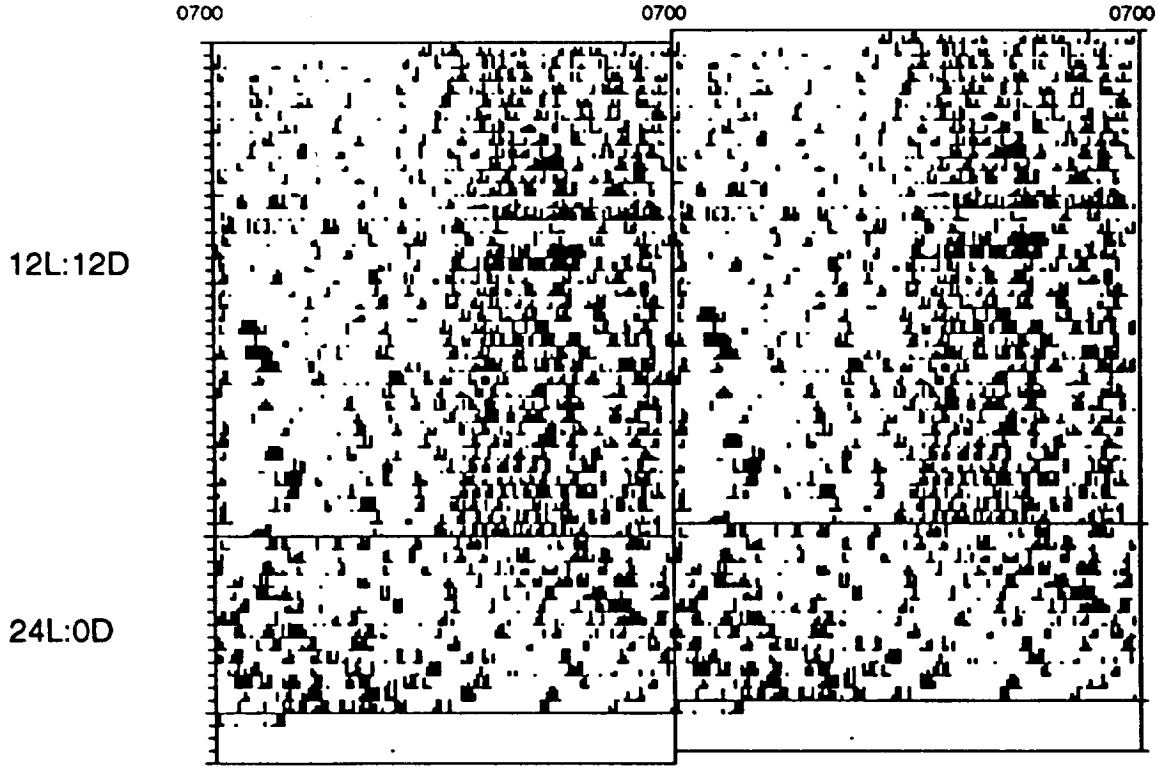
Light experiment 1 (8806Light01) Rat 6 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



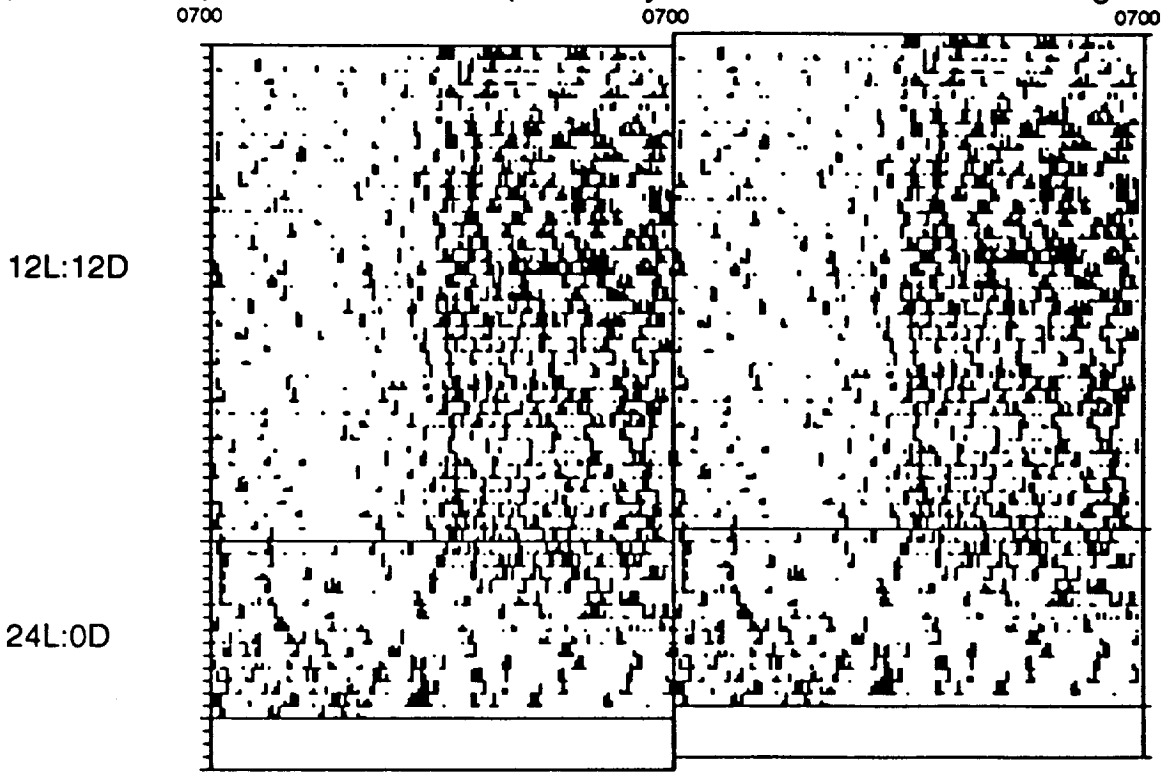
Light experiment 1 (8806Light01) Rat 1 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



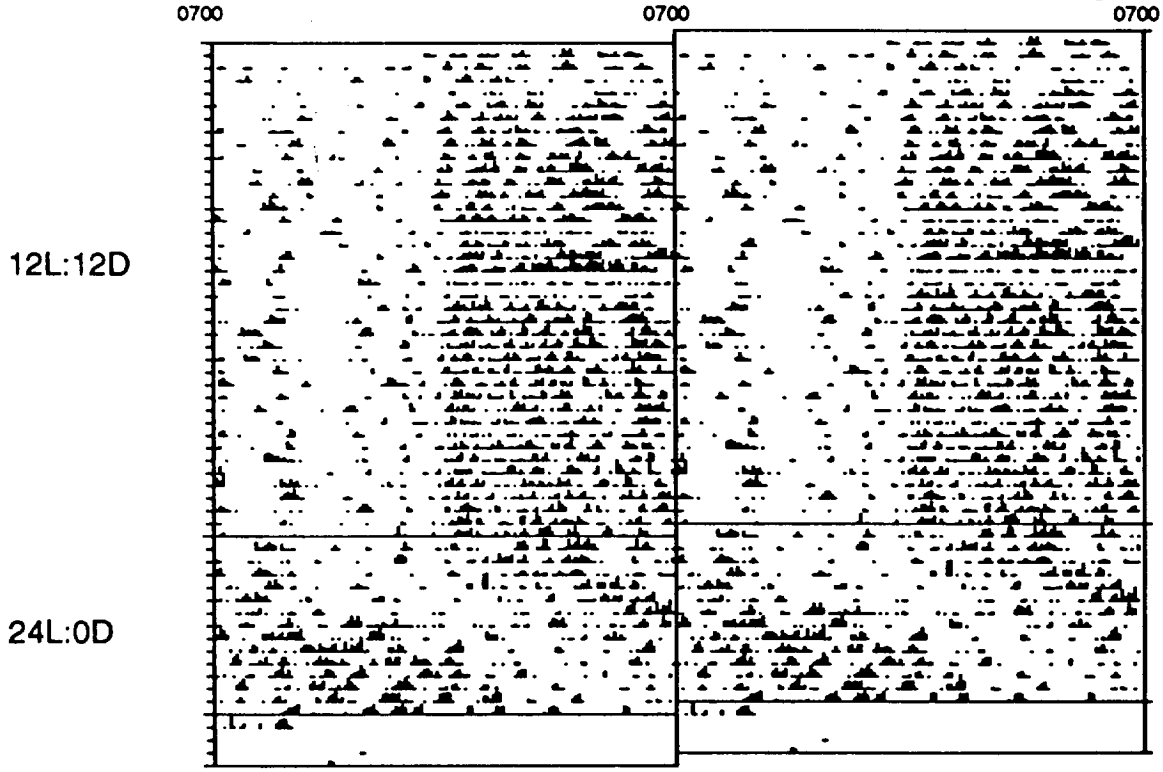
Light experiment 1 (8806Light01) Rat 2 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



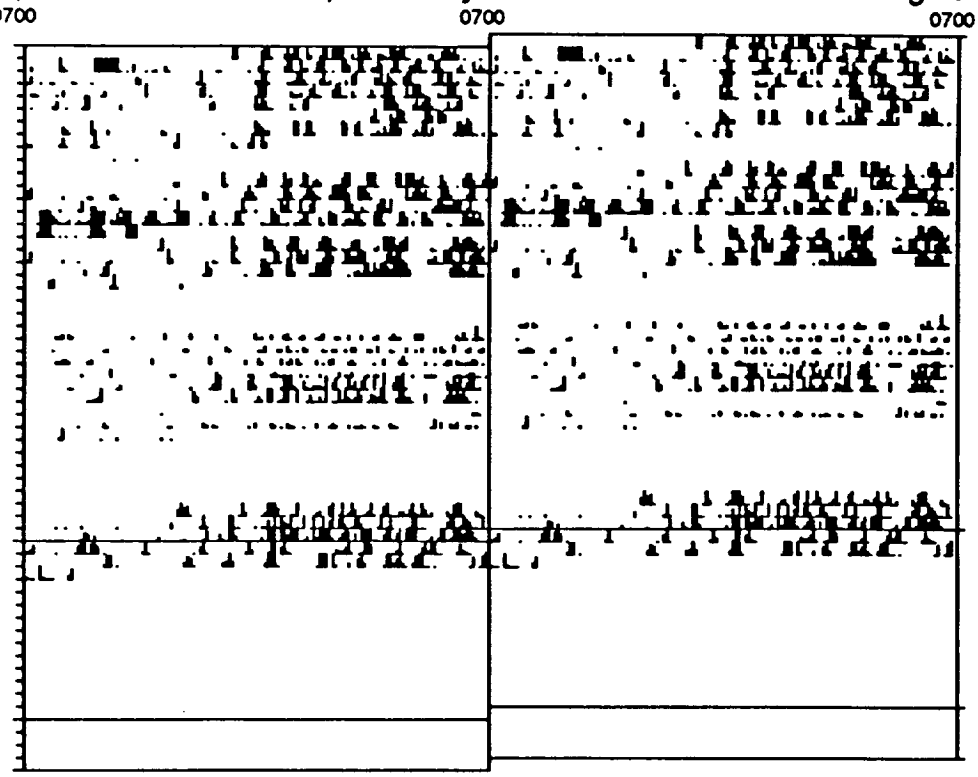
Light experiment 1 (8806Light01) Rat 3 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



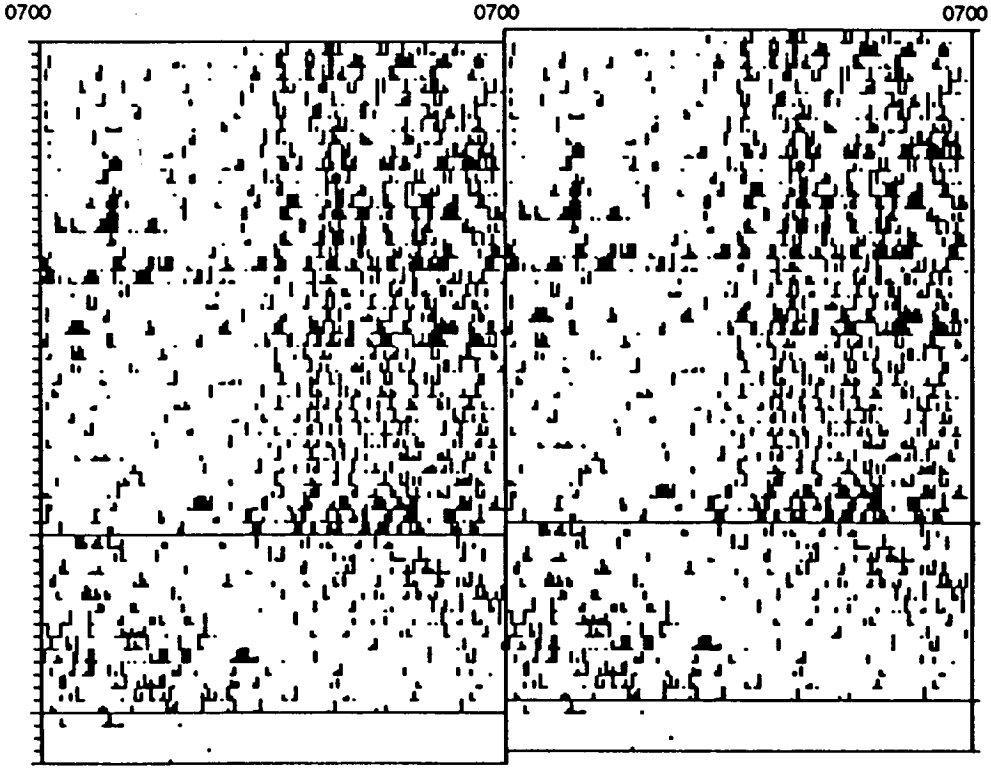
Light experiment 1 (8806Light01) Rat 4 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



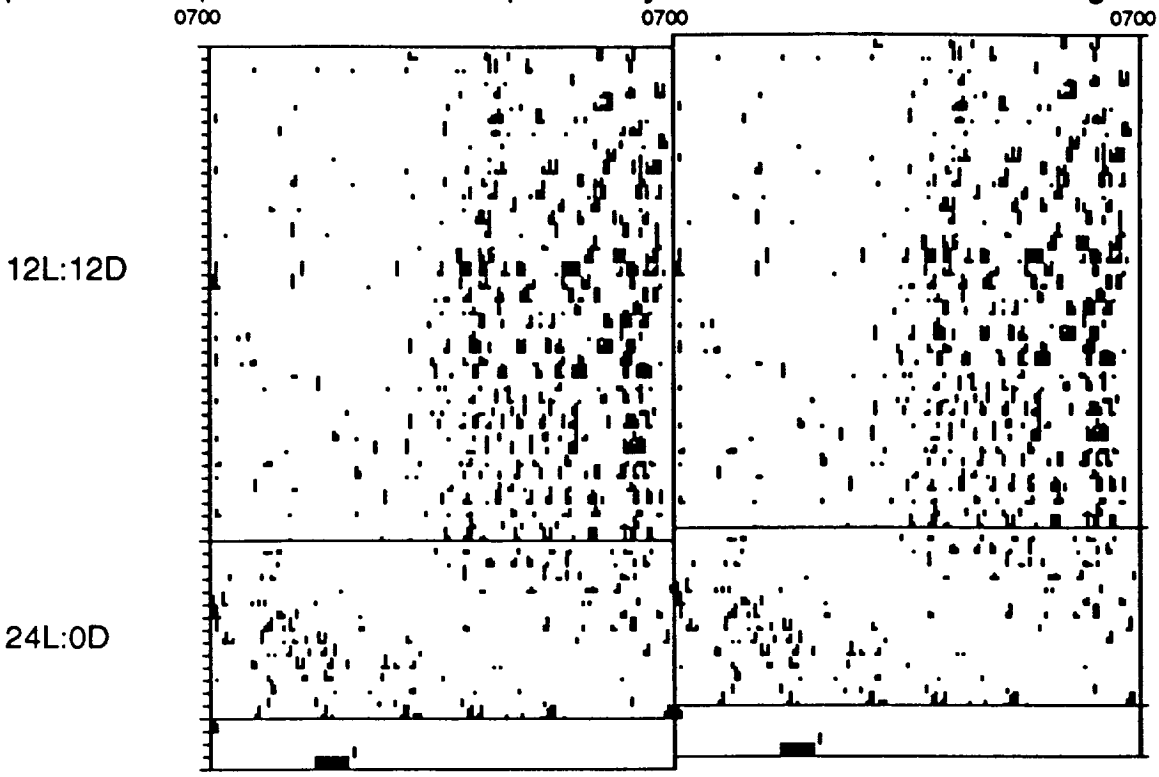
Light experiment 1 (8806Light01) Rat 5 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



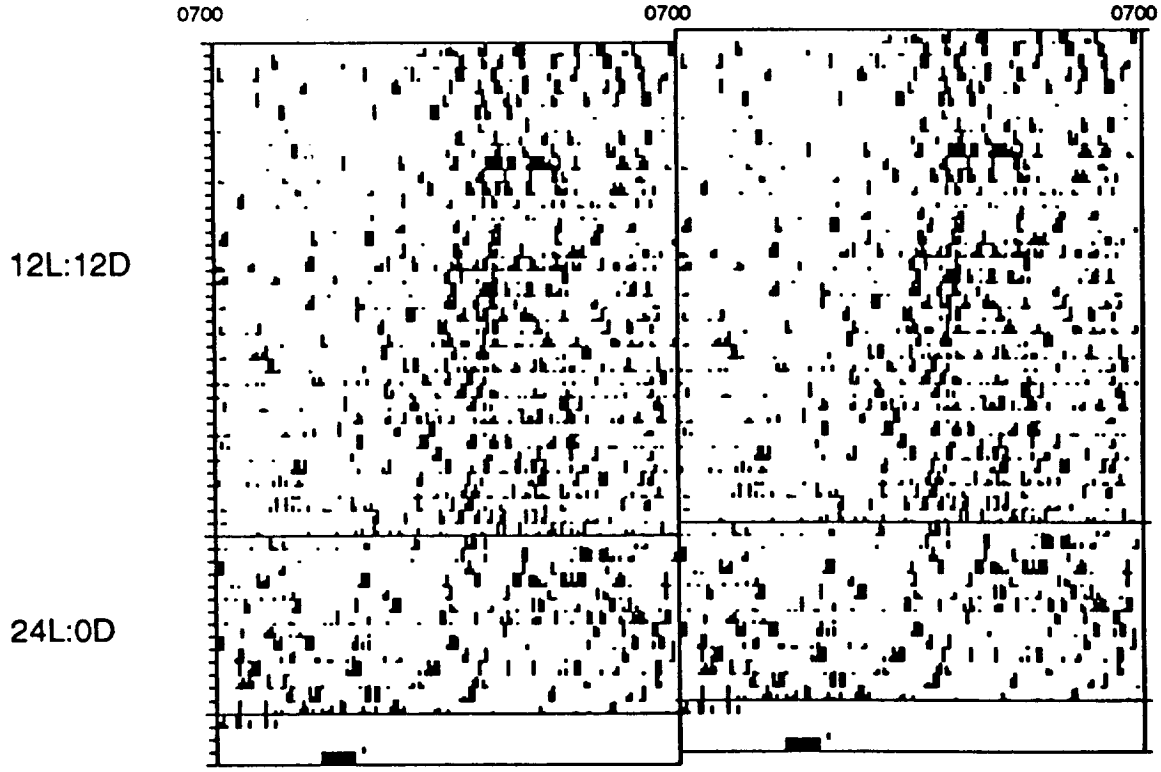
Light experiment 1 (8806Light01) Rat 6 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



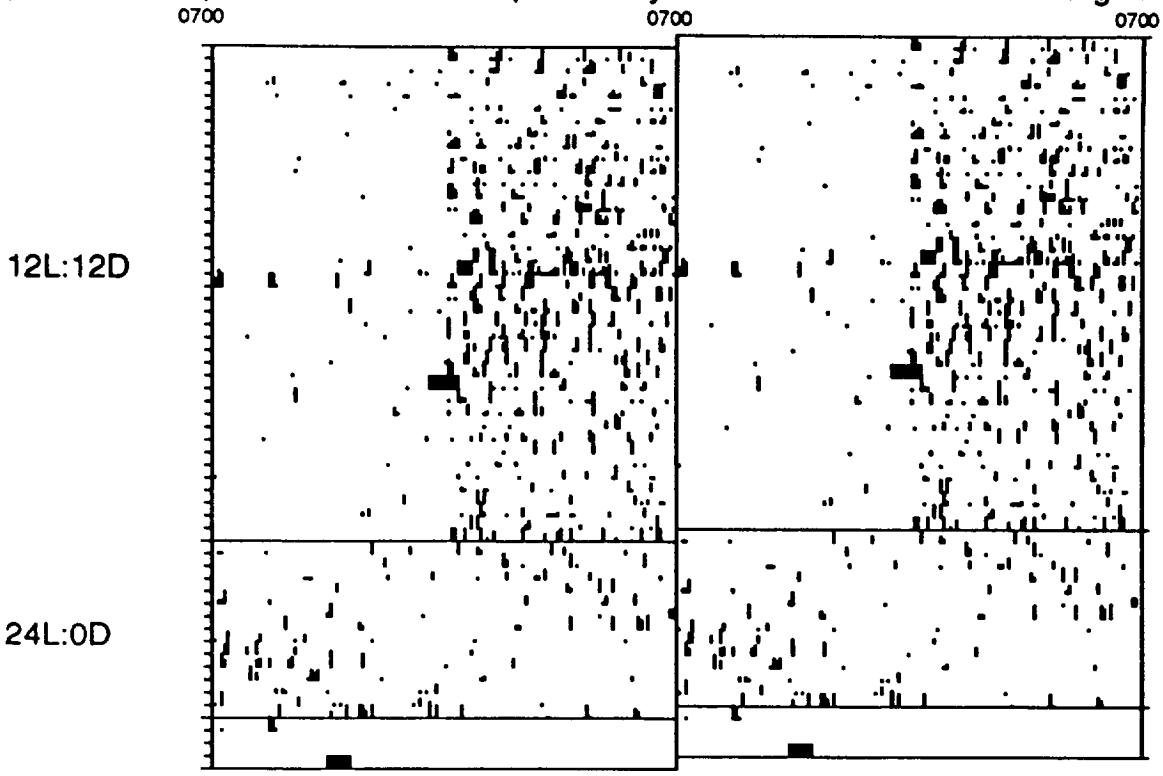
Light experiment 1 (8806Light01) Rat 1 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



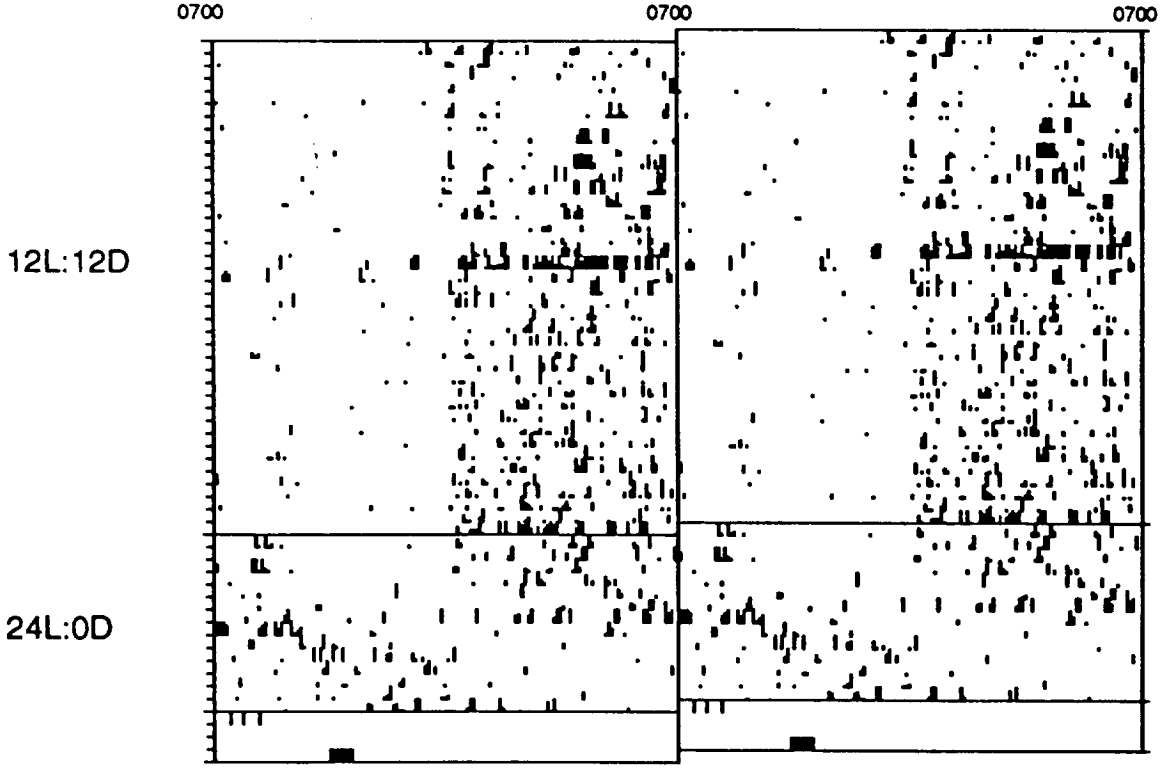
Light experiment 1 (8806Light01) Rat 2 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



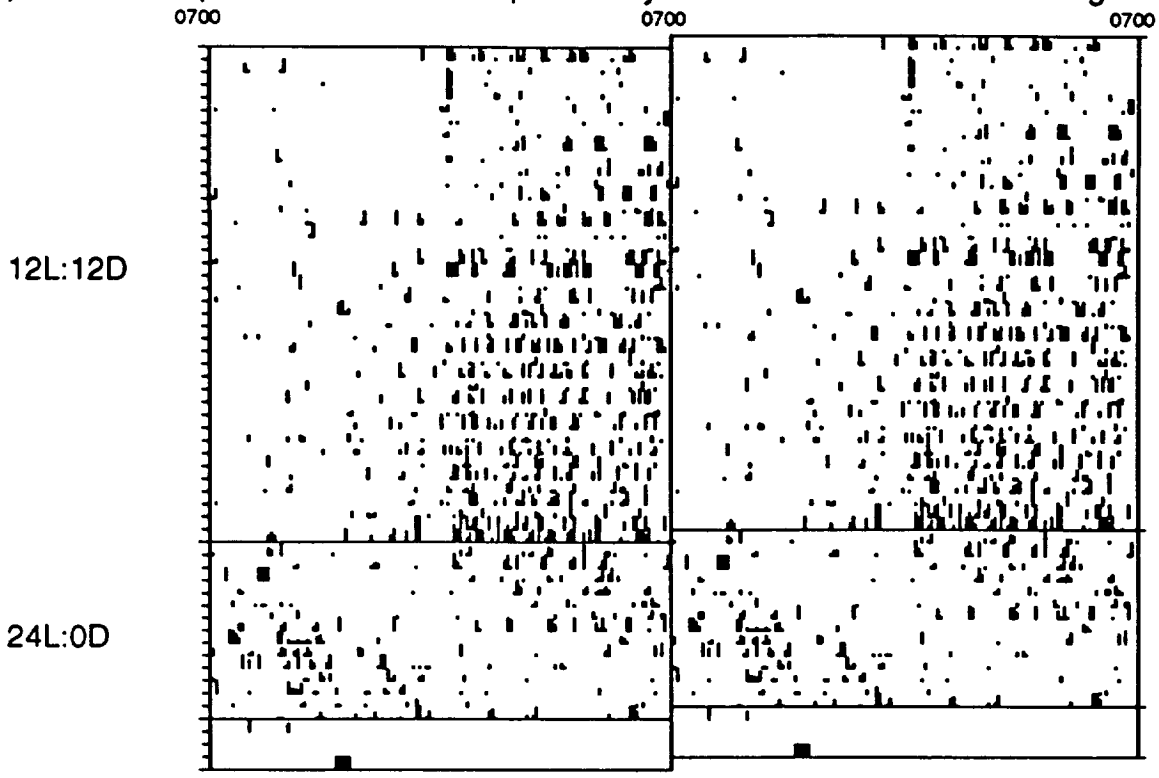
Light experiment 1 (8806Light01) Rat 3 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



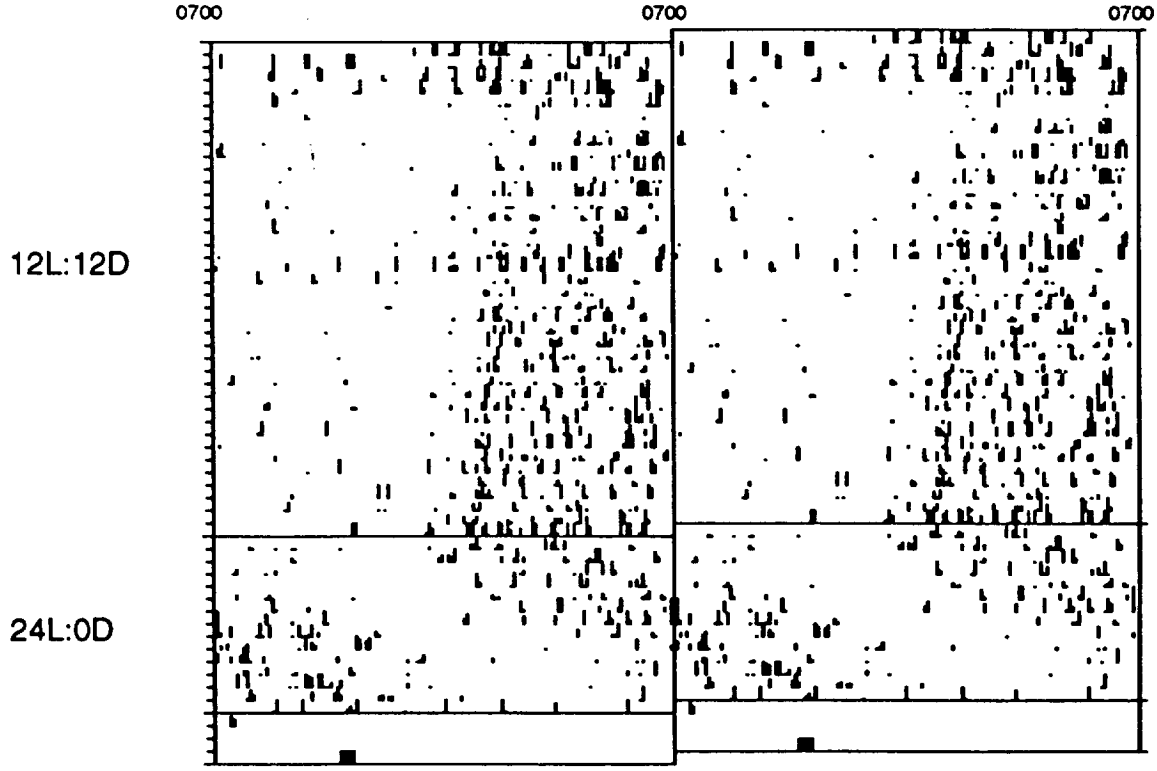
Light experiment 1 (8806Light01) Rat 4 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



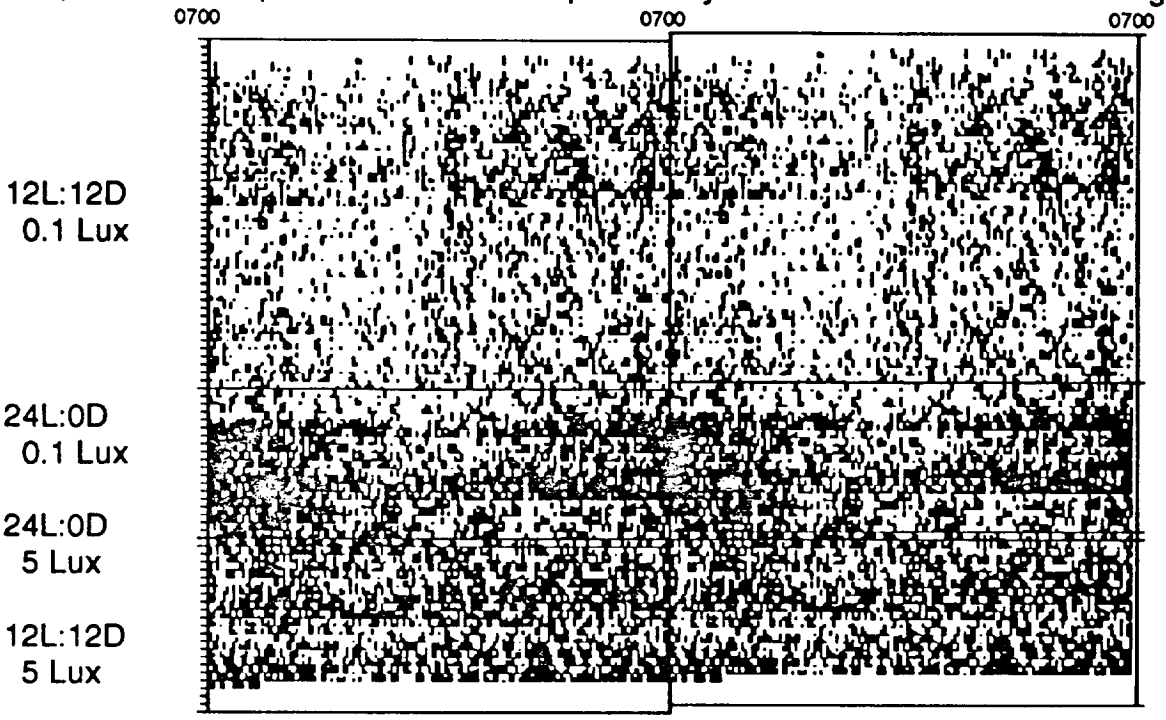
Light experiment 1 (8806Light01) Rat 5 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



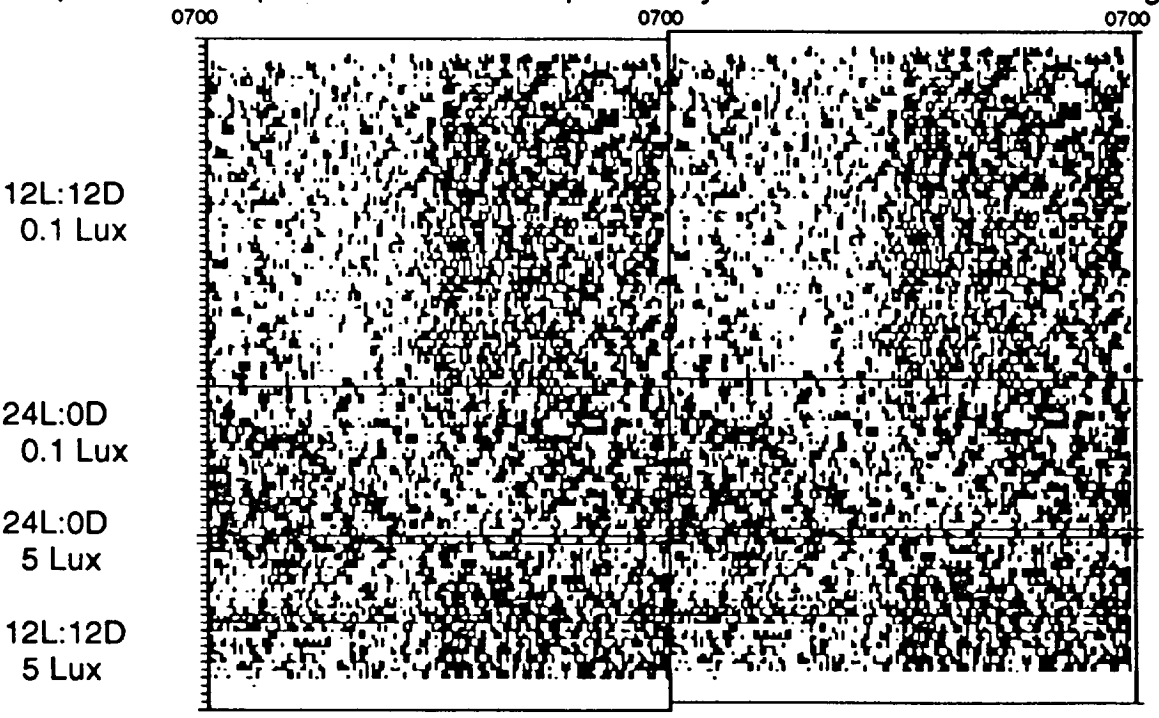
Light experiment 1 (8806Light01) Rat 6 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



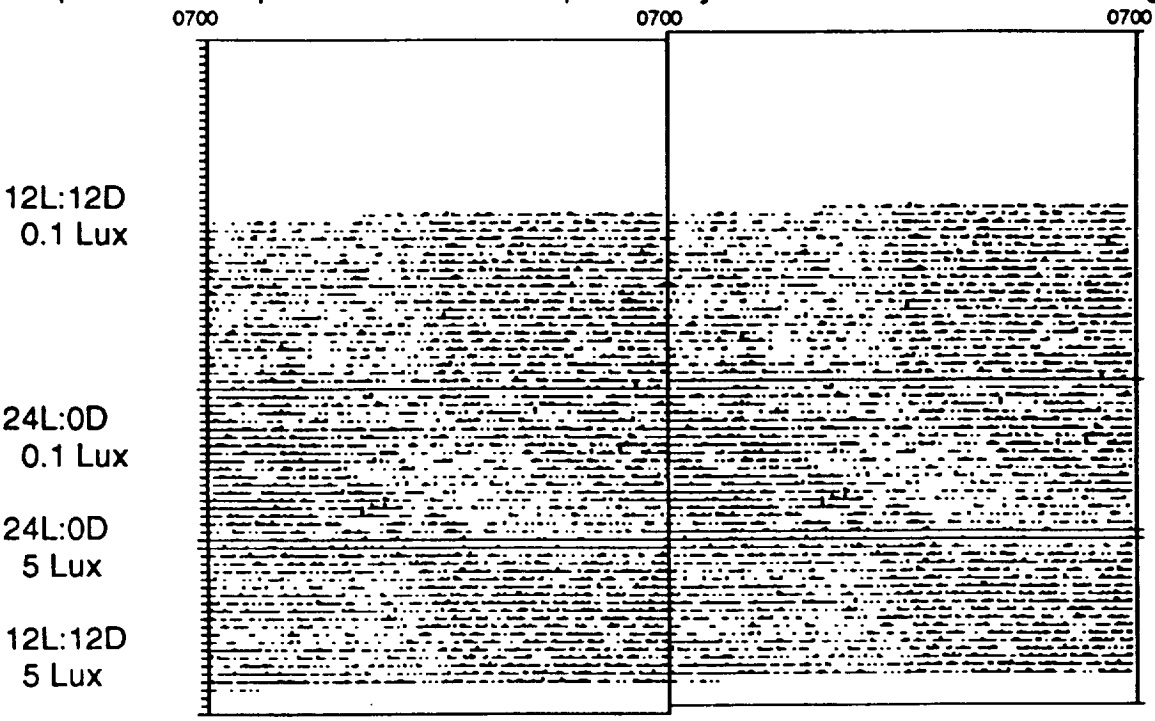
Light experiment 2 (8808Light0.1) Rat 1 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



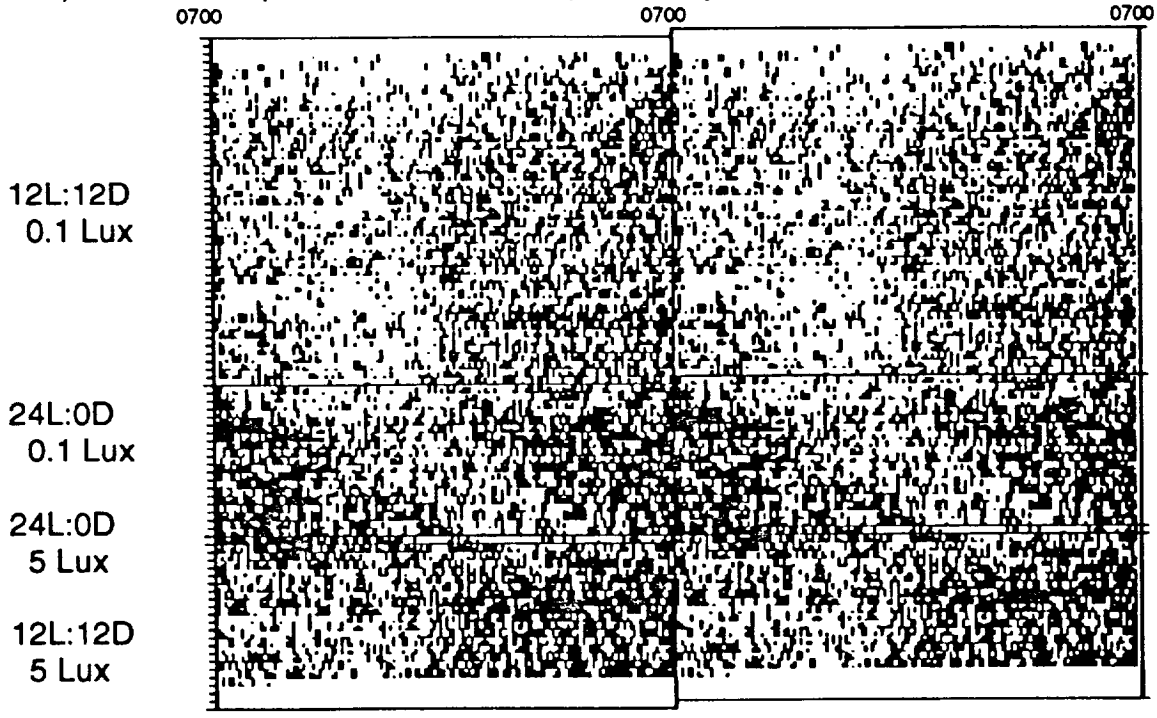
Light experiment 2 (8808Light0.1) Rat 2 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



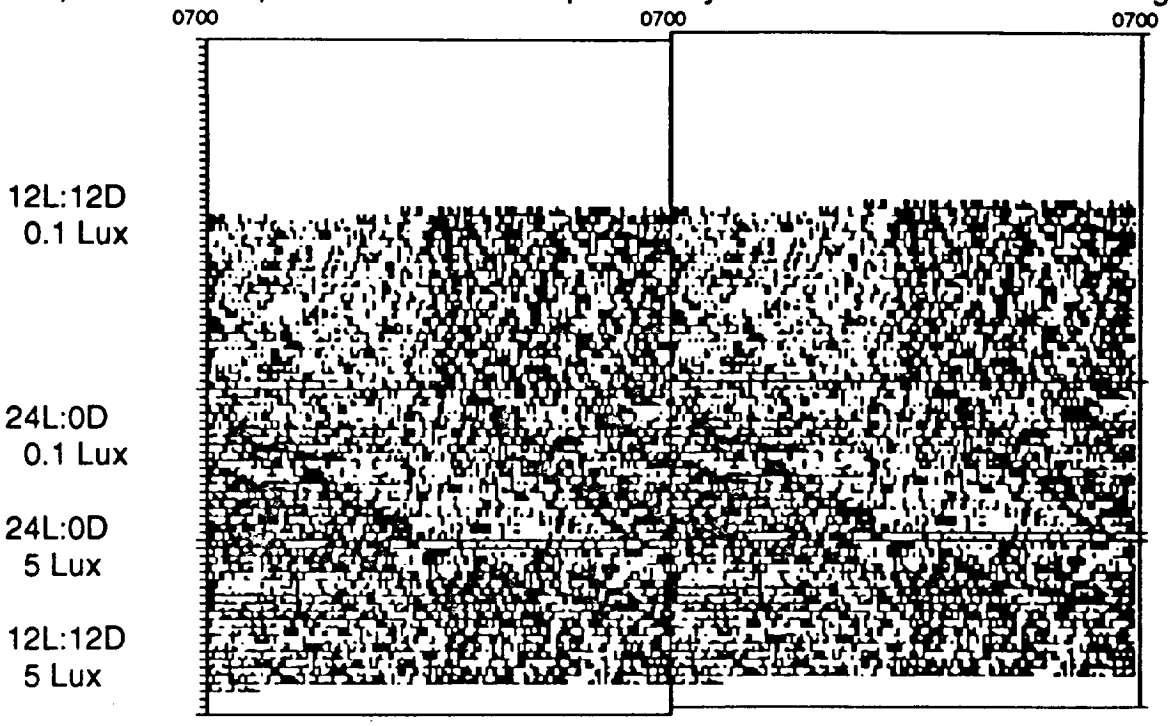
Light experiment 2 (8808Light0.1) Rat 3 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



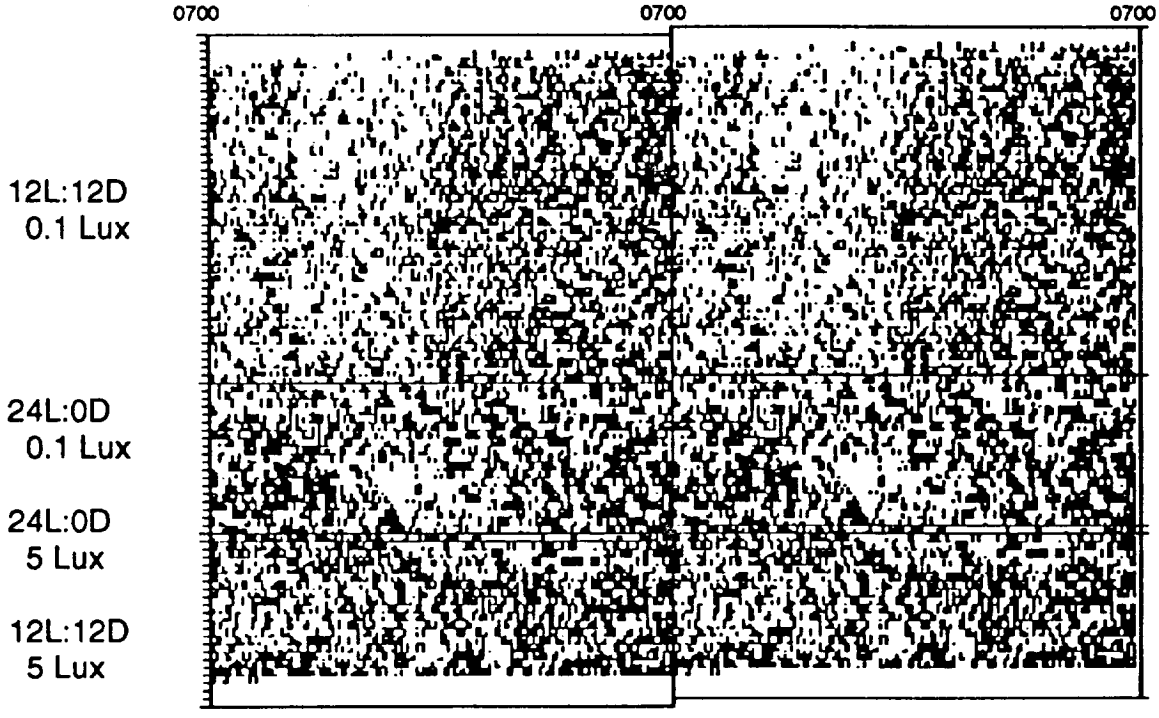
Light experiment 2 (8808Light0.1) Rat 4 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



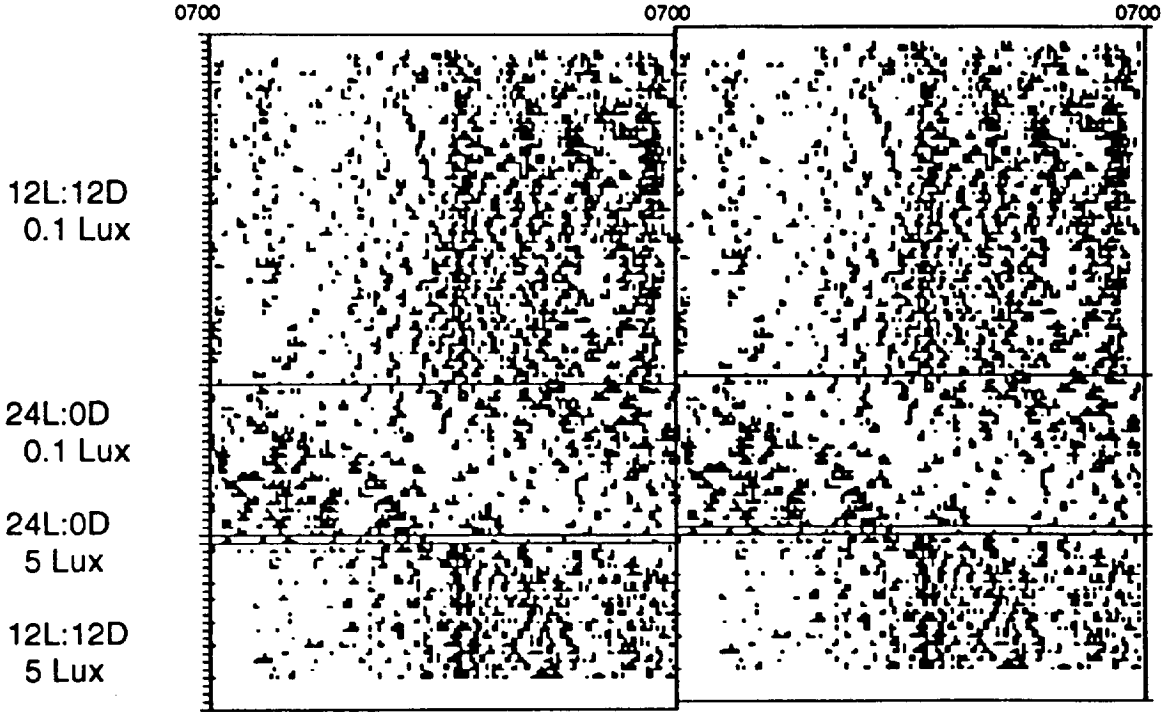
Light experiment 2 (8808Light0.1) Rat 5 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



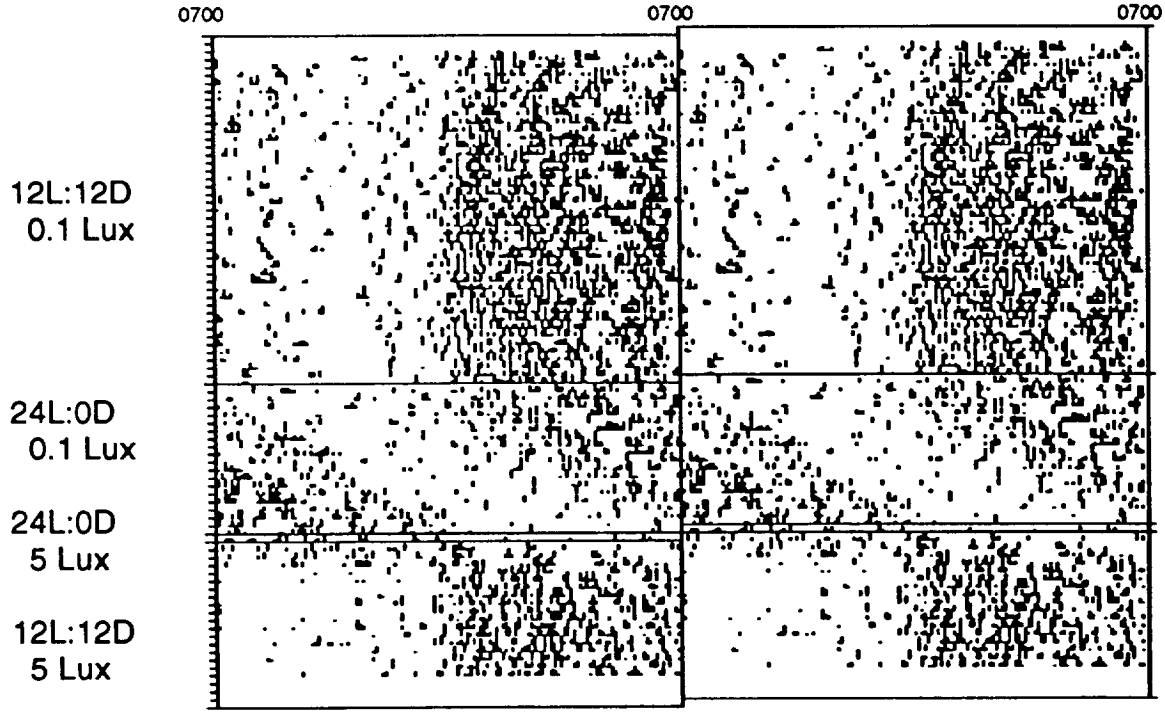
Light experiment 2 (8808Light0.1) Rat 6 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



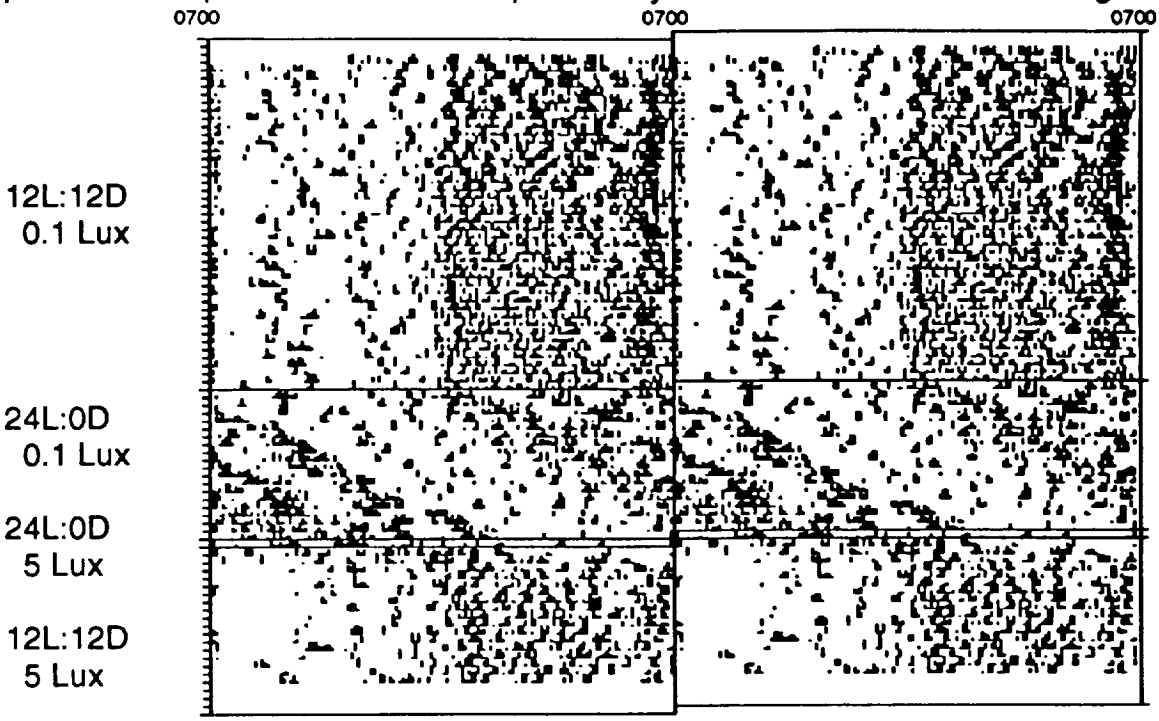
Light experiment 2 (8808Light0.1) Rat 1 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



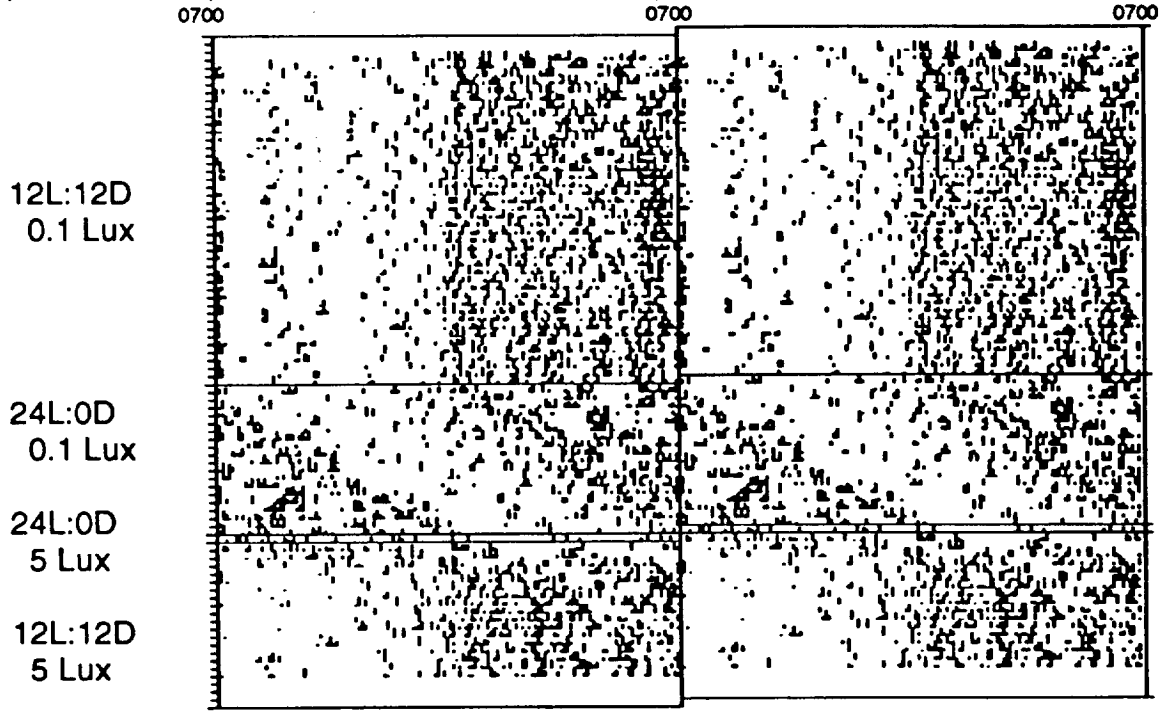
Light experiment 2 (8808Light0.1) Rat 2 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



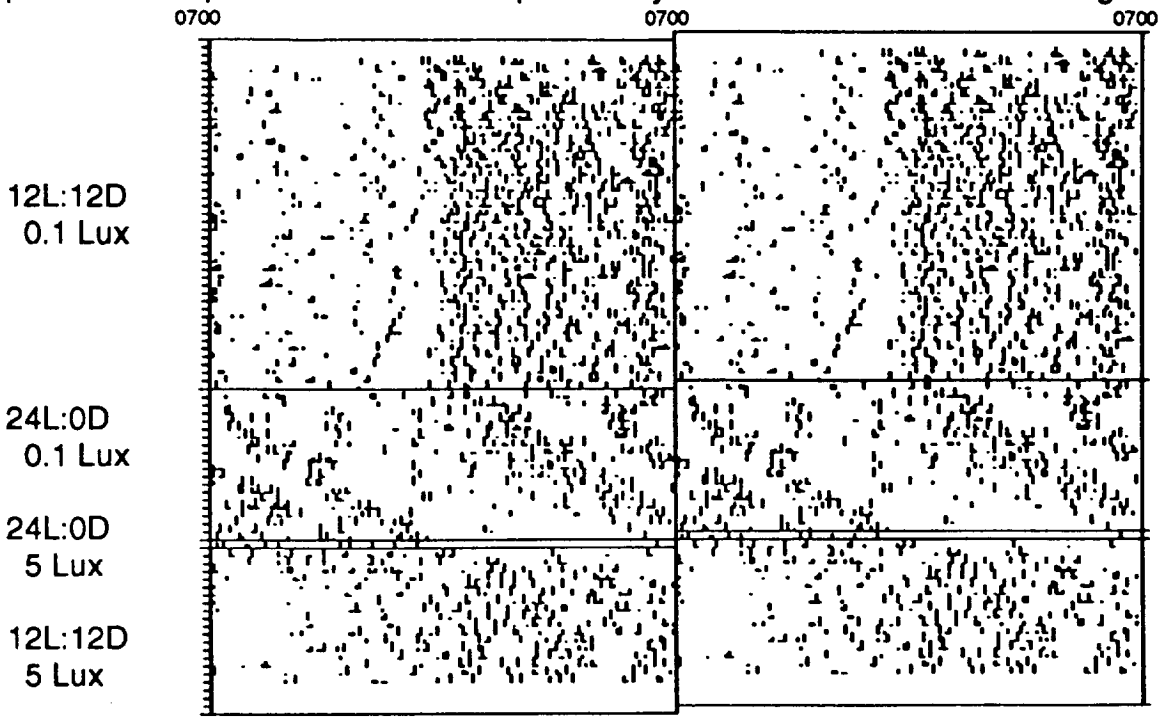
Light experiment 2 (8808Light0.1) Rat 3 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



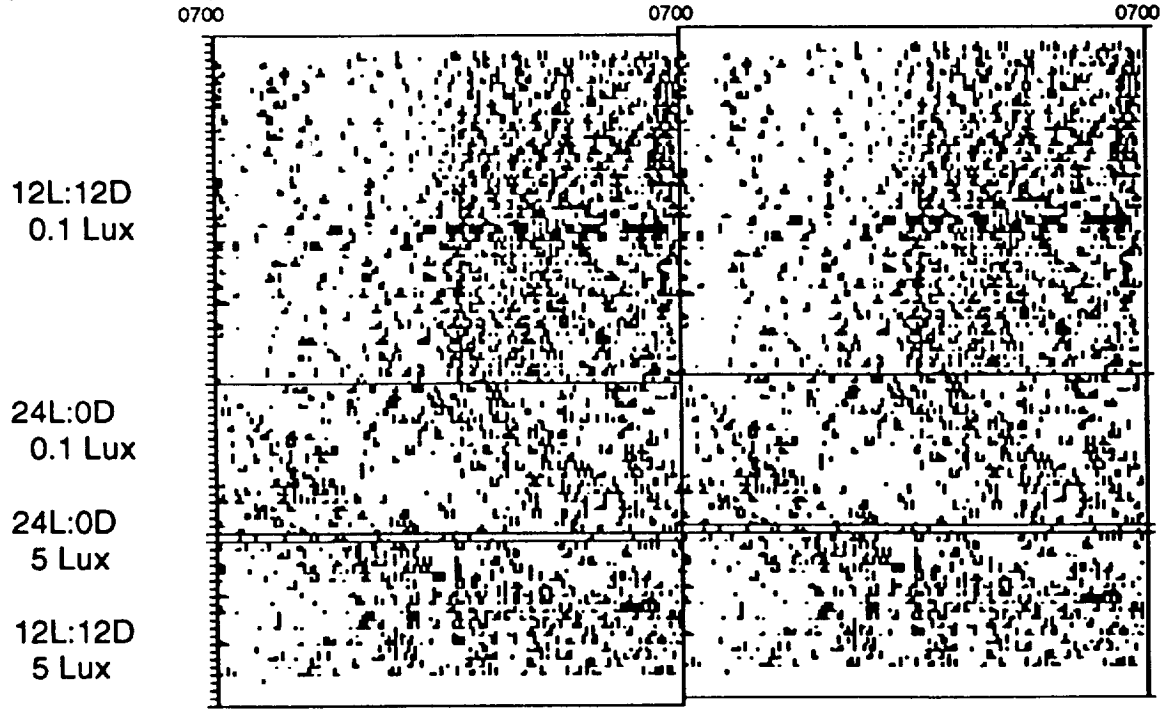
Light experiment 2 (8808Light0.1) Rat 4 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



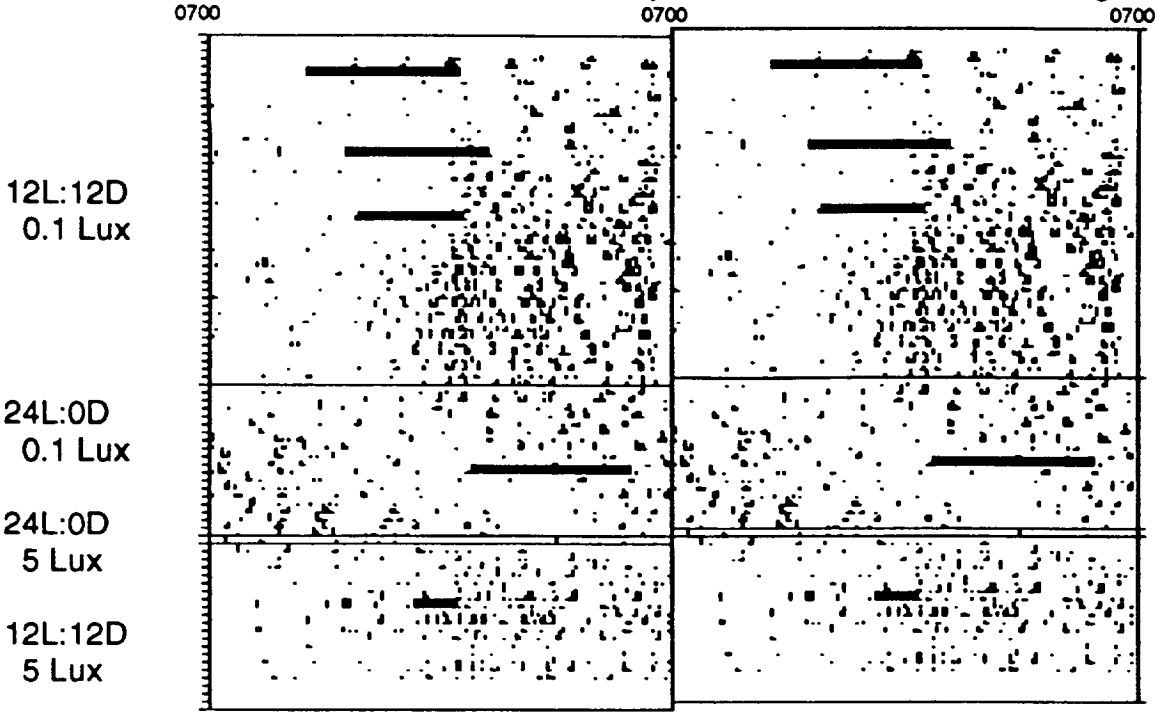
Light experiment 2 (8808Light0.1) Rat 5 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



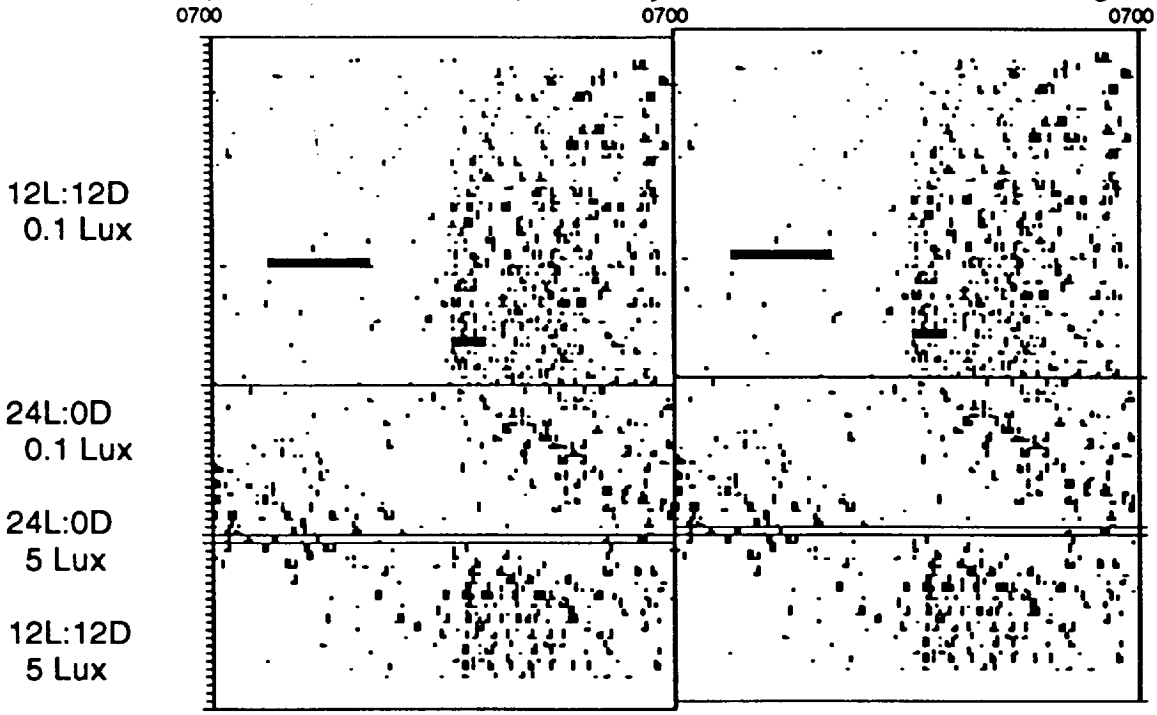
Light experiment 2 (8808Light0.1) Rat 6 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



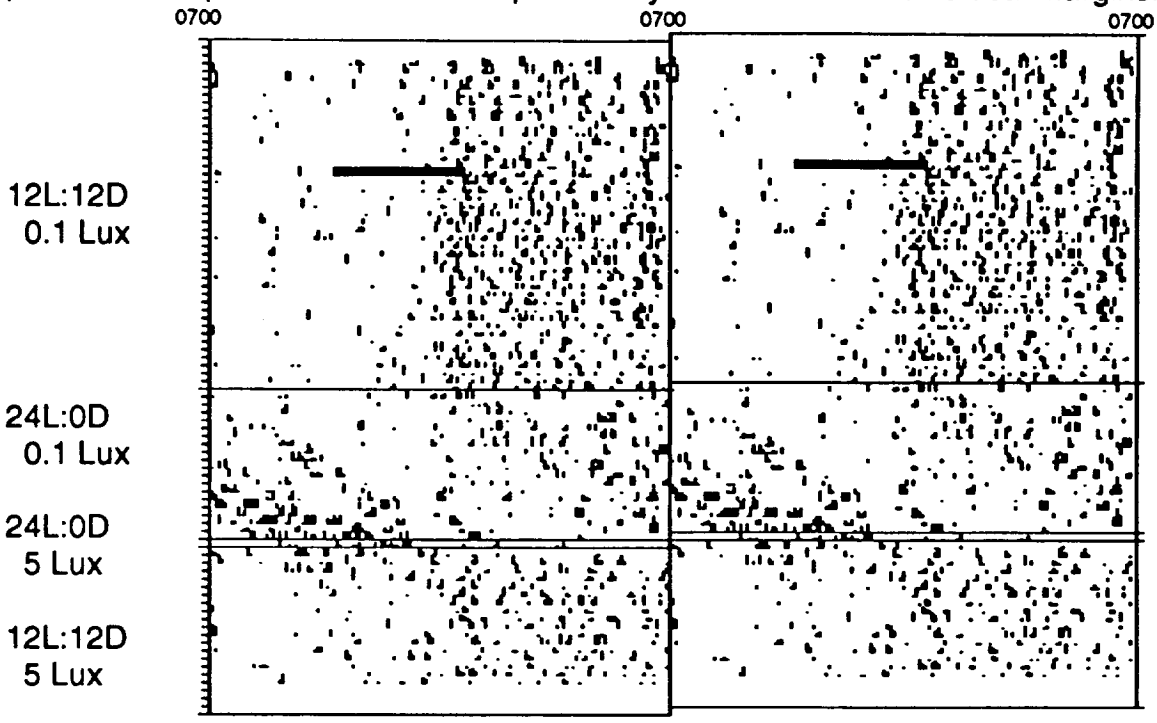
Light experiment 2 (8808Light0.1) Rat 1 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



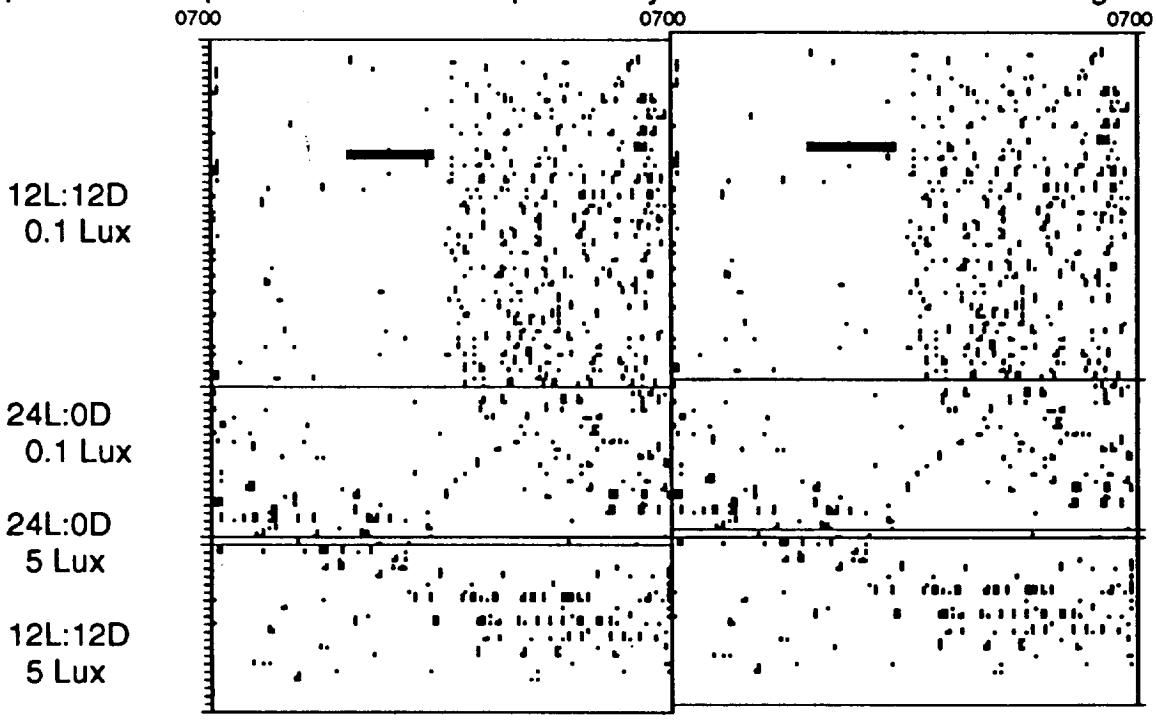
Light experiment 2 (8808Light0.1) Rat 2 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



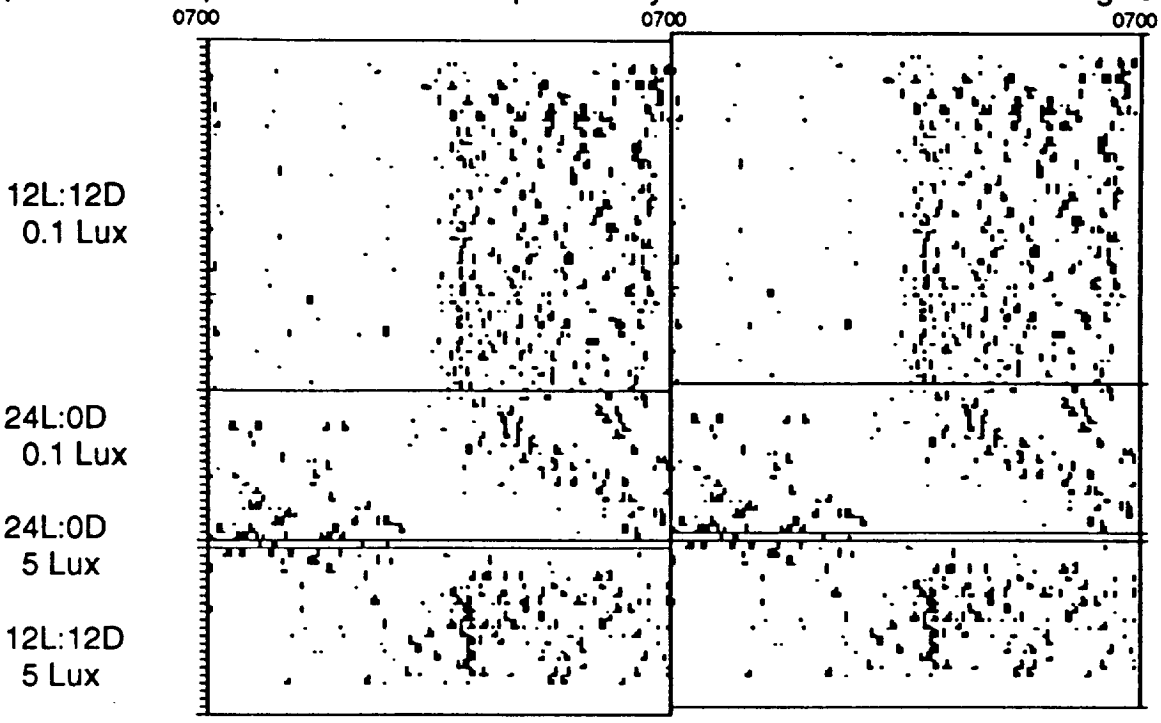
Light experiment 2 (8808Light0.1) Rat 3 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



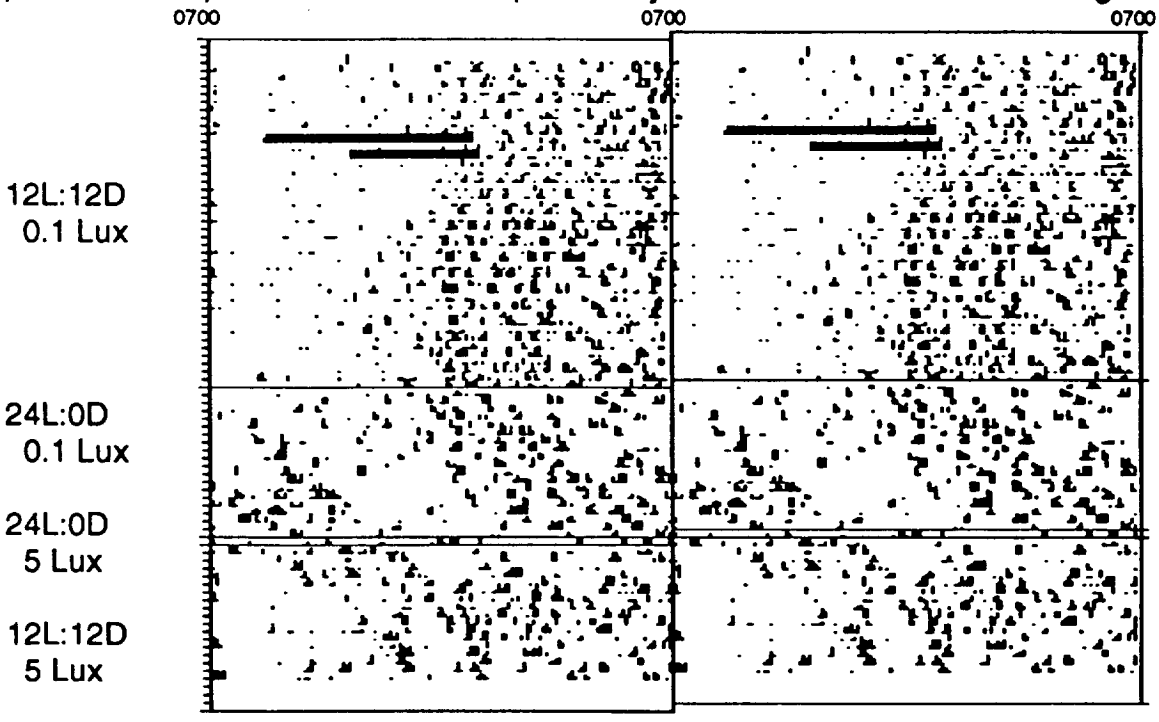
Light experiment 2 (8808Light0.1) Rat 4 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



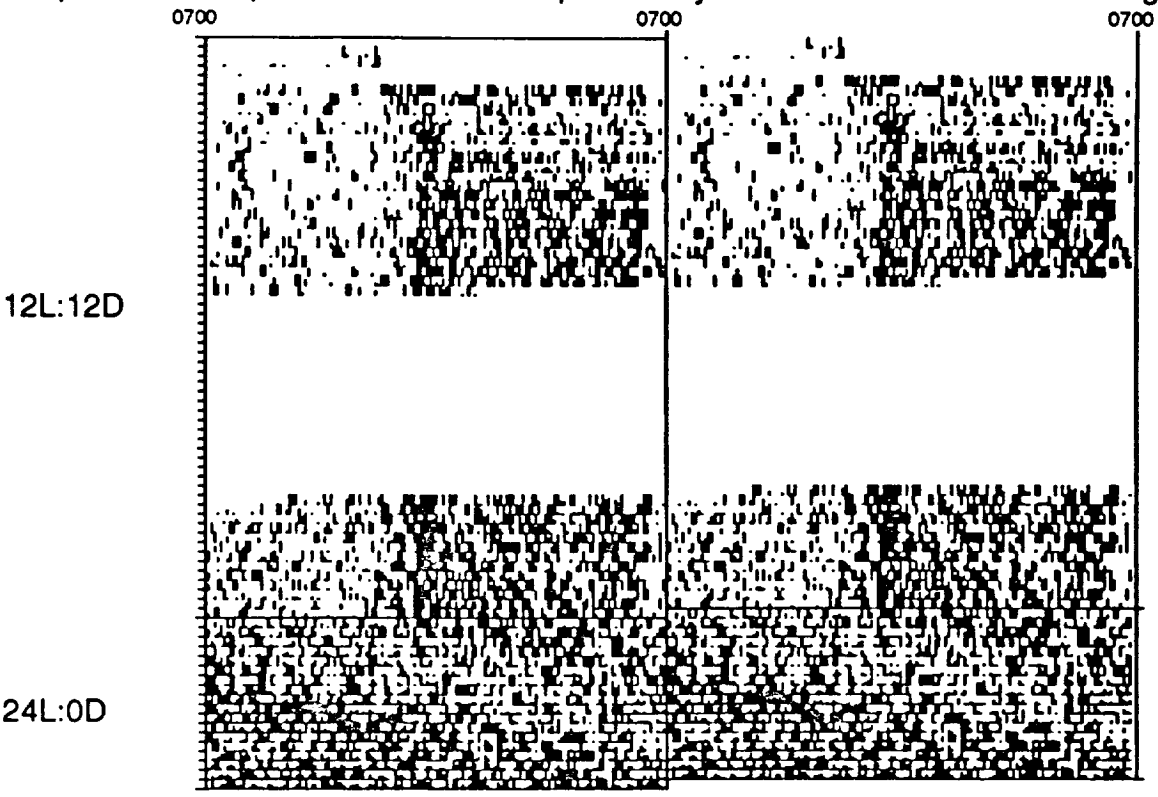
Light experiment 2 (8808Light0.1) Rat 5 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



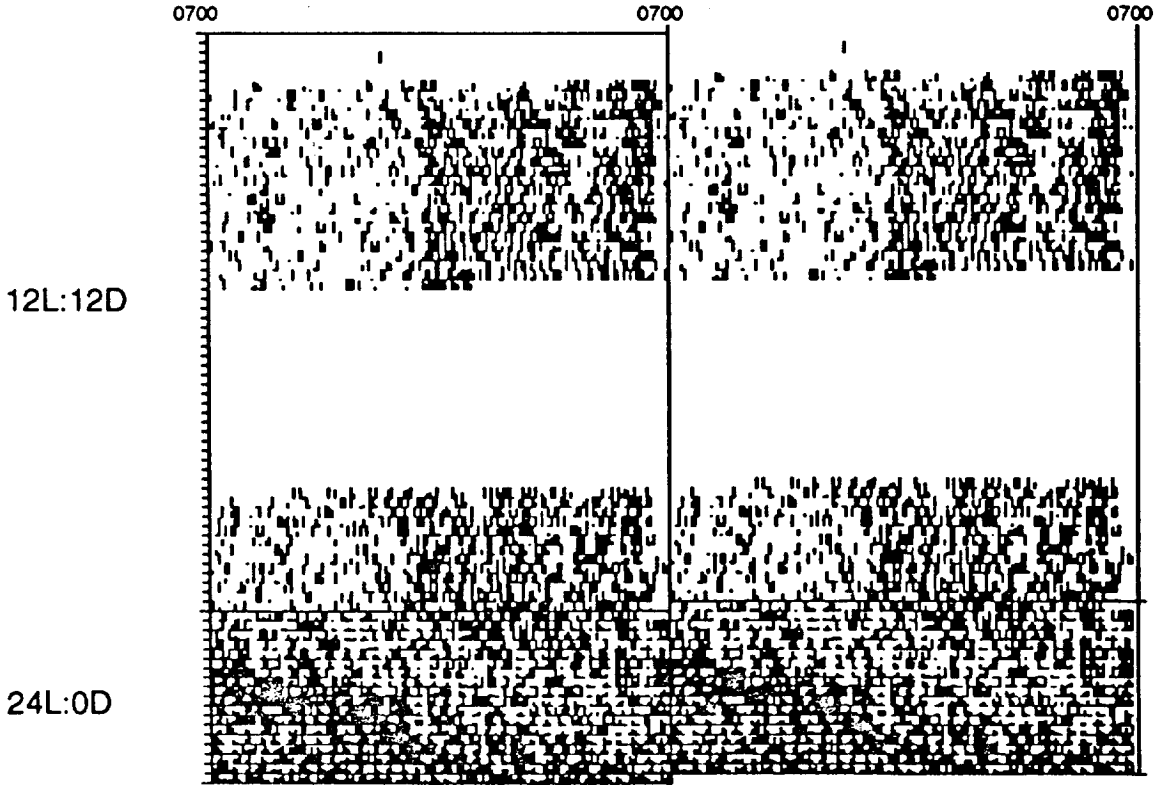
Light experiment 2 (8808Light0.1) Rat 6 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



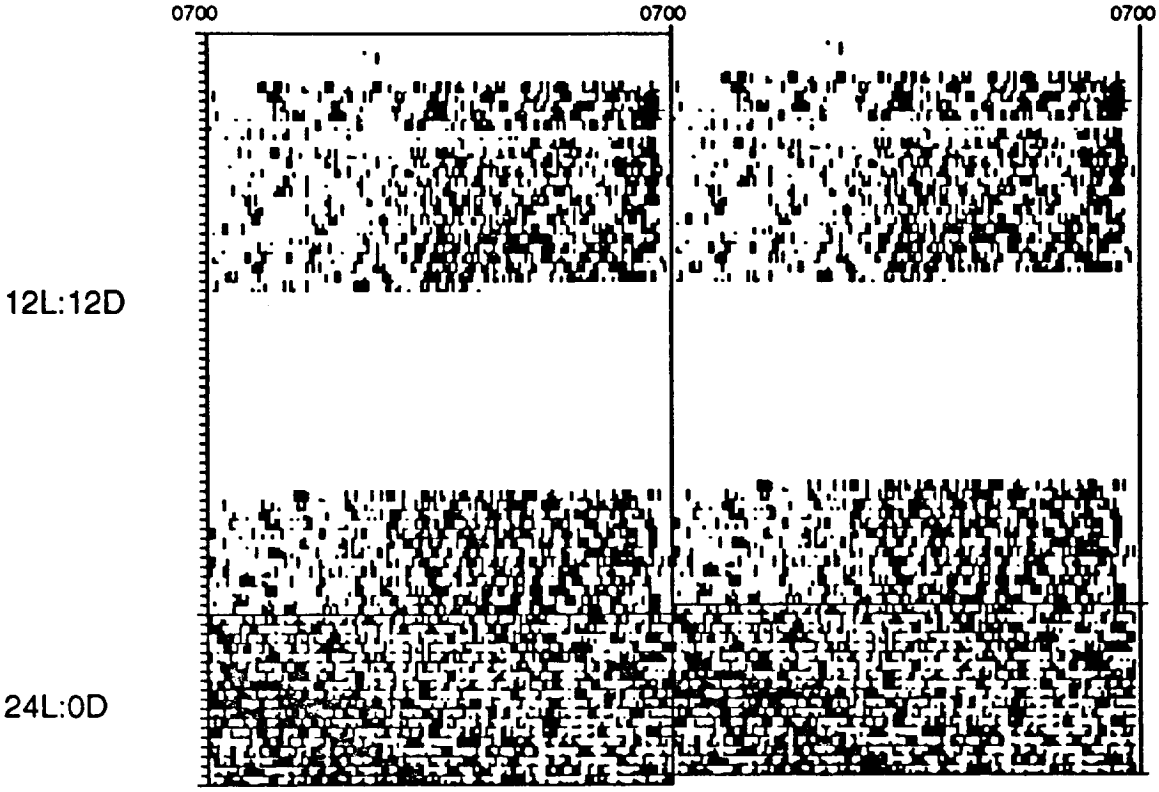
Light experiment 3 (8812Light05) Rat 1 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



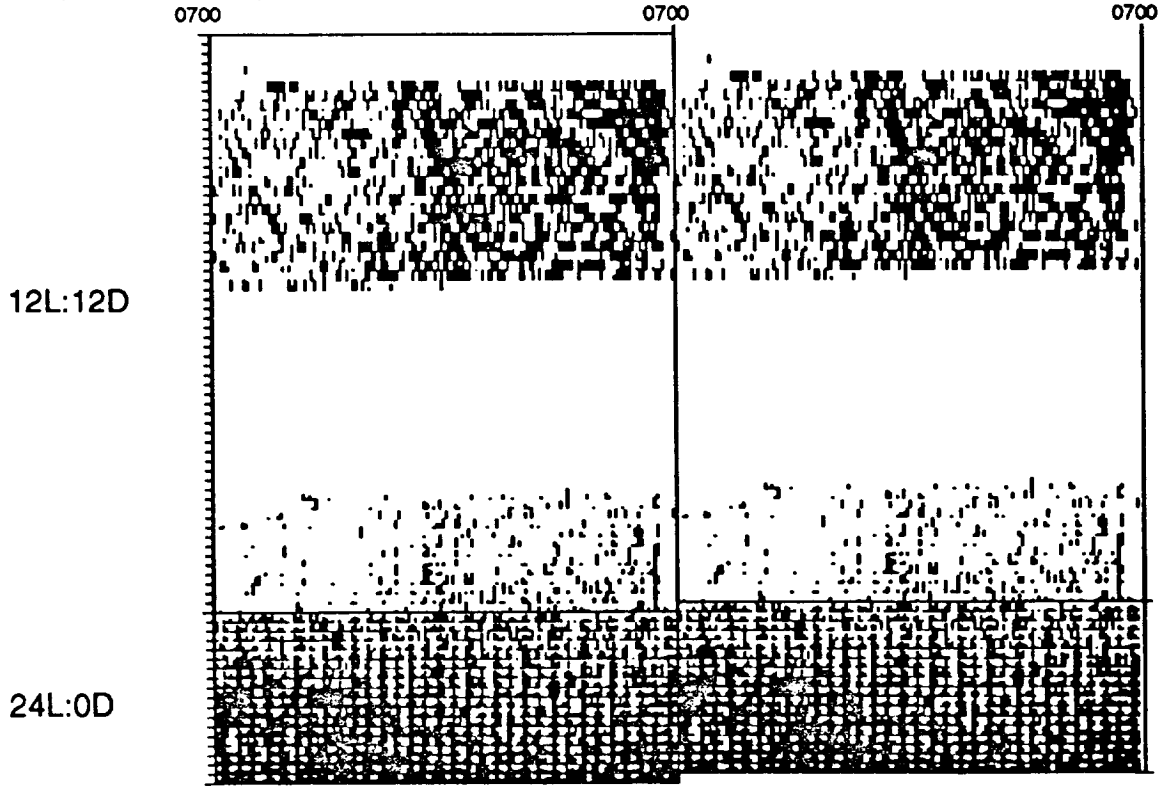
Light experiment 3 (8812Light05) Rat 2 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



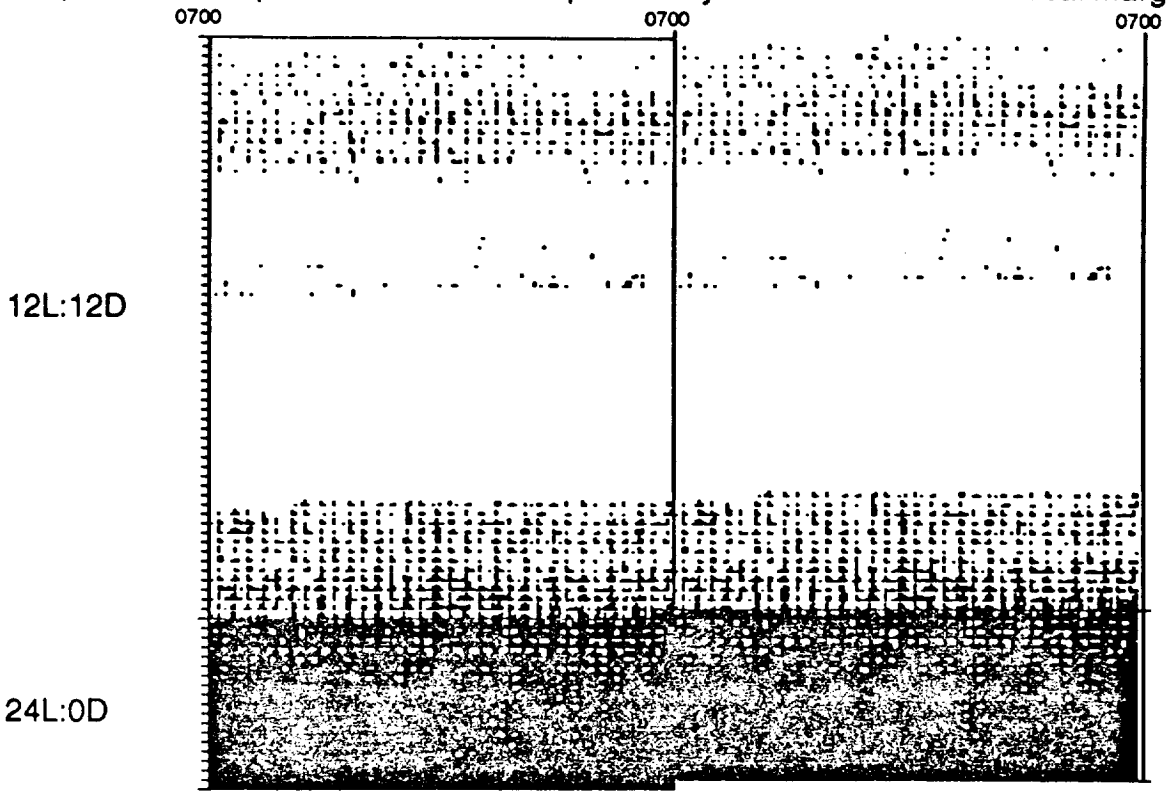
Light experiment 3 (8812Light05) Rat 3 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



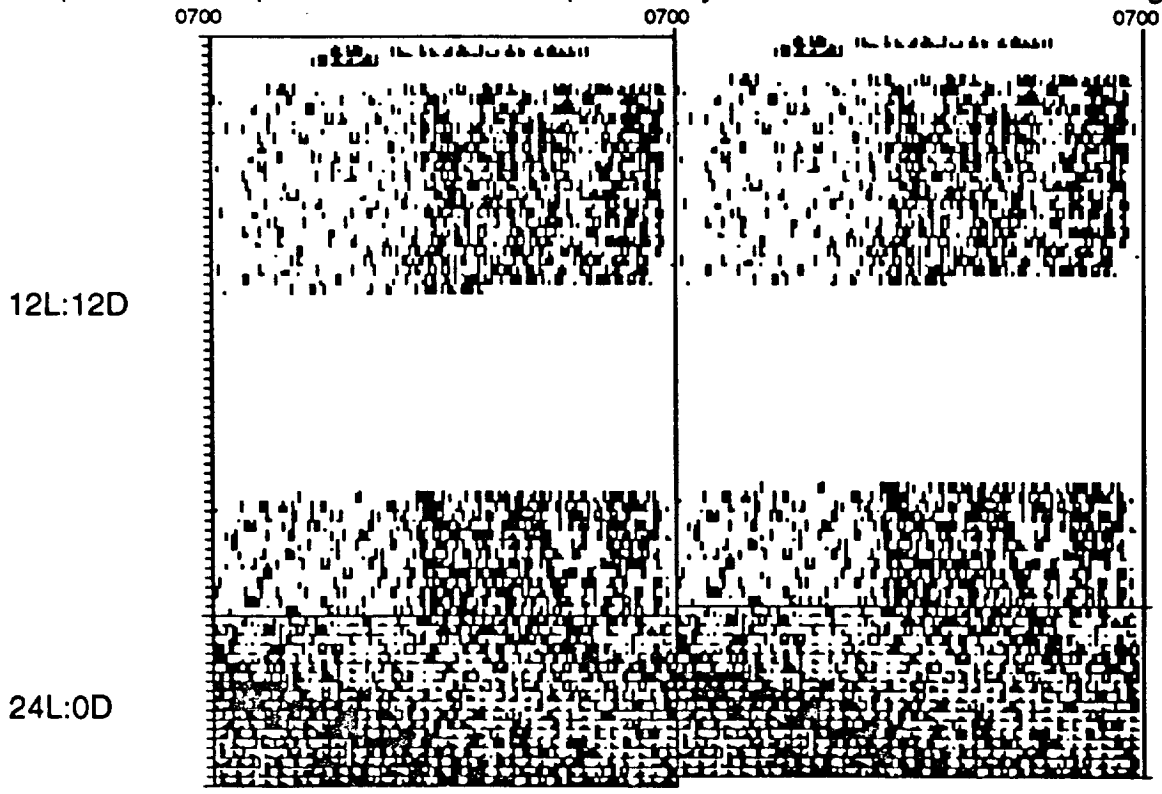
Light experiment 3 (8812Light05) Rat 4 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



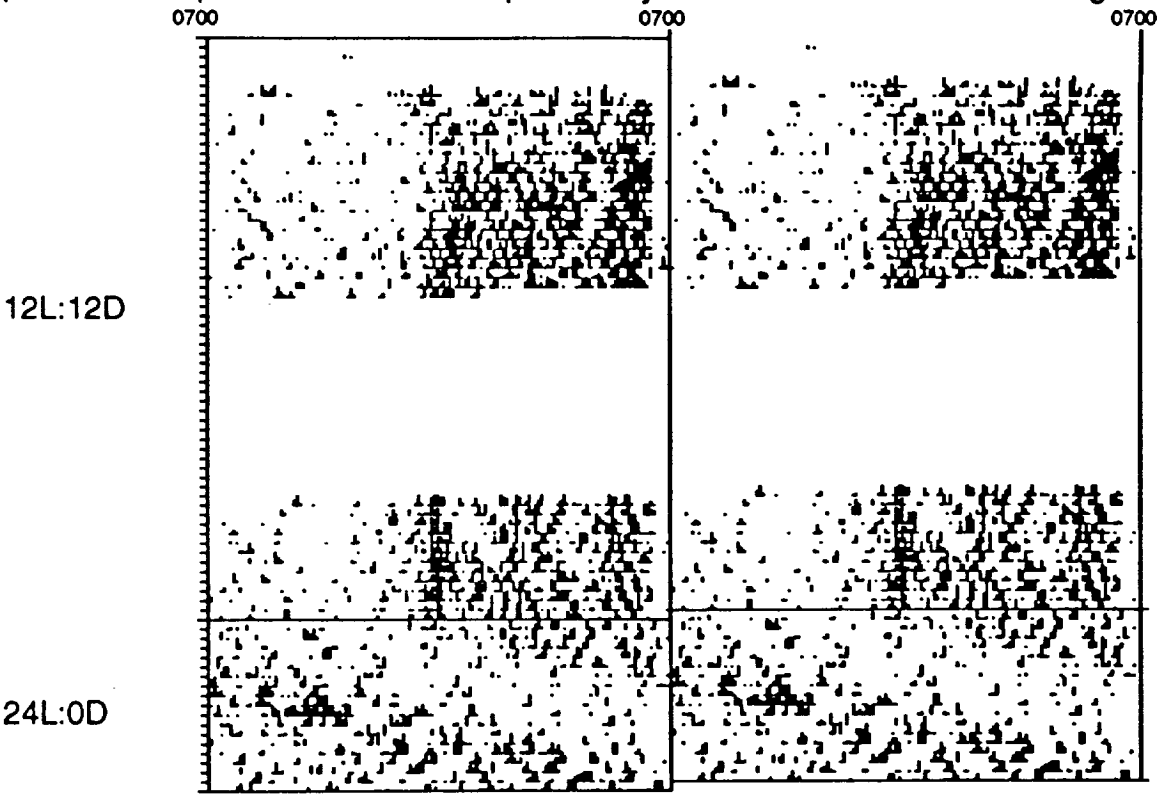
Light experiment 3 (8812Light05) Rat 5 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



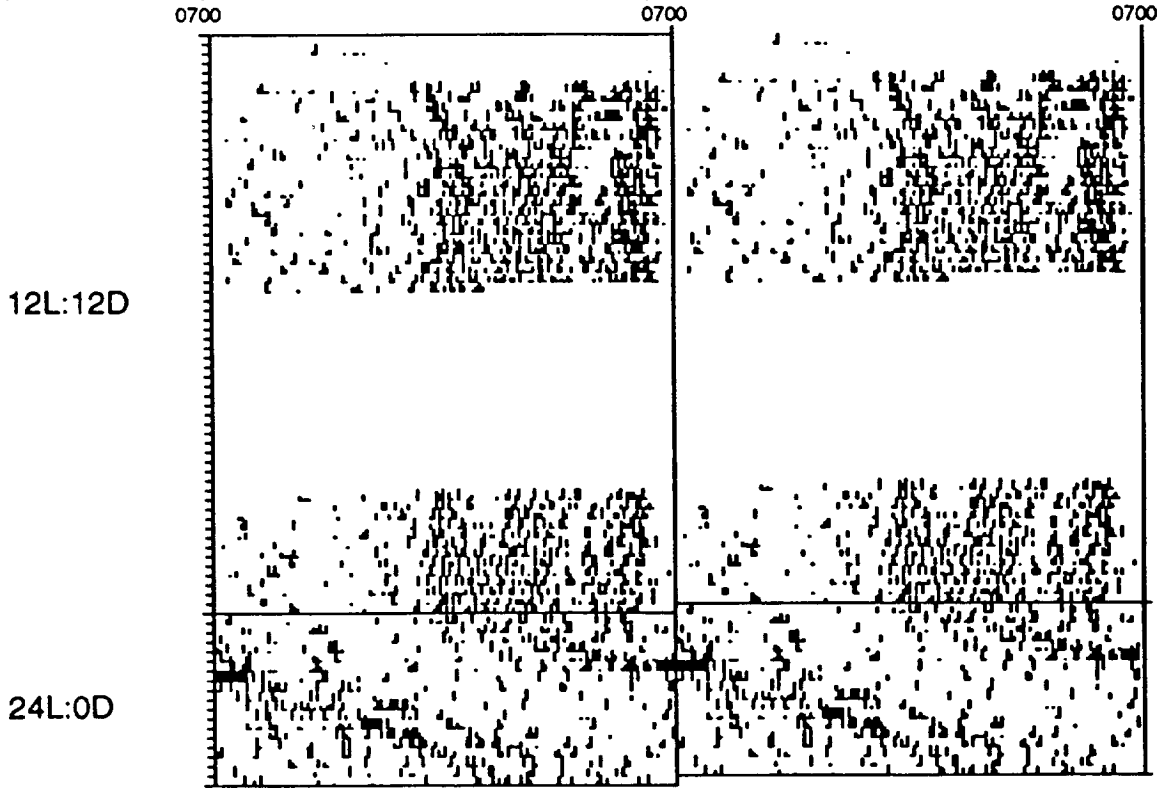
Light experiment 3 (8812Light05) Rat 6 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



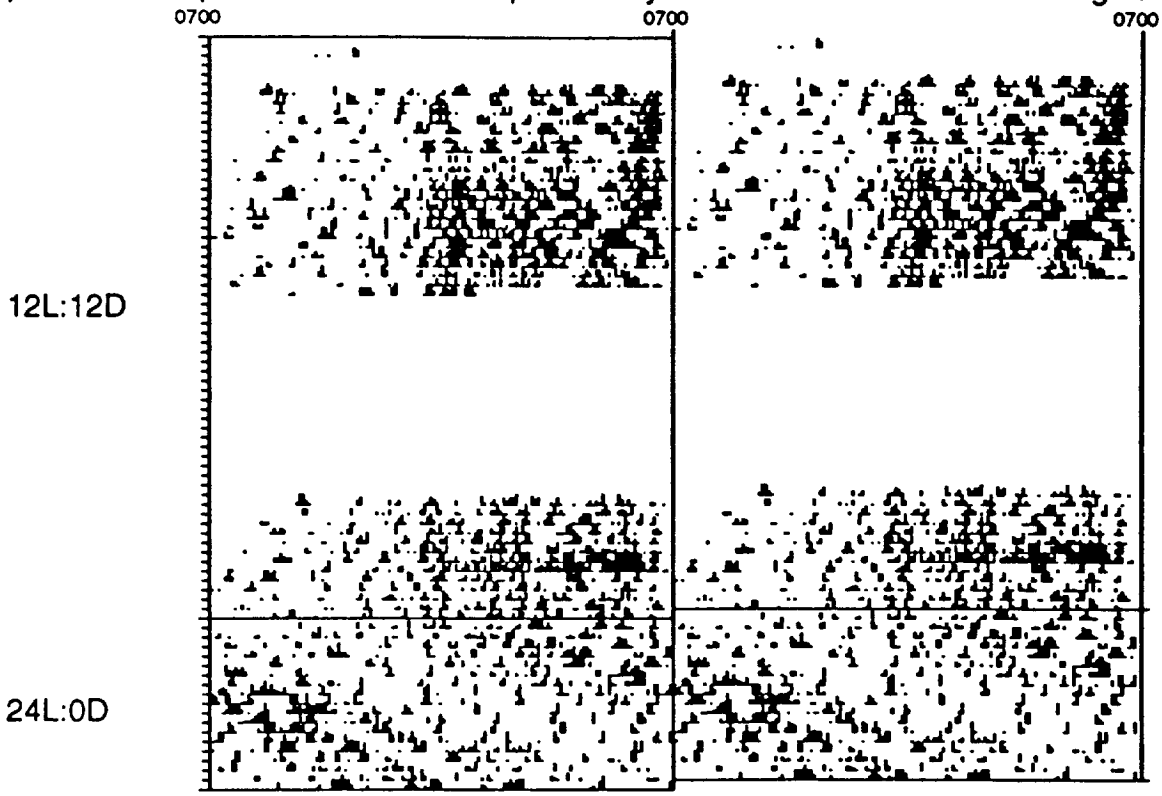
Light experiment 3 (8812Light05) Rat 1 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



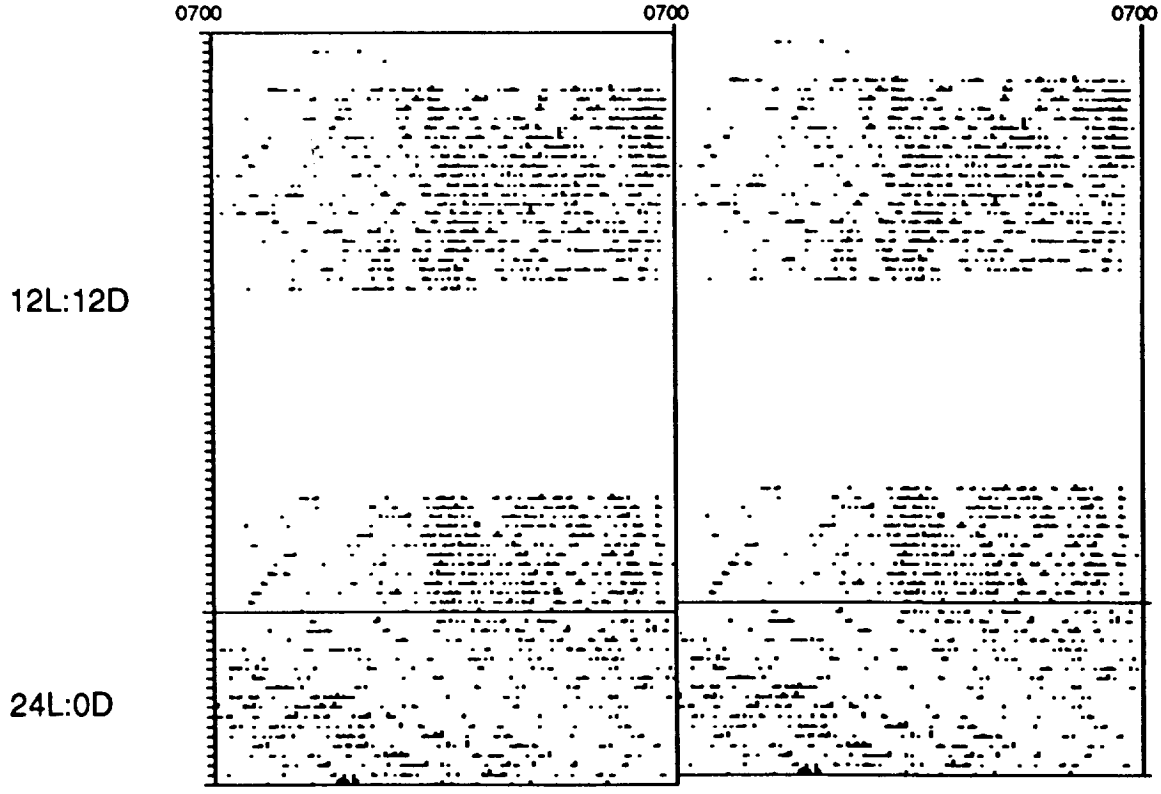
Light experiment 3 (8812Light05) Rat 2 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



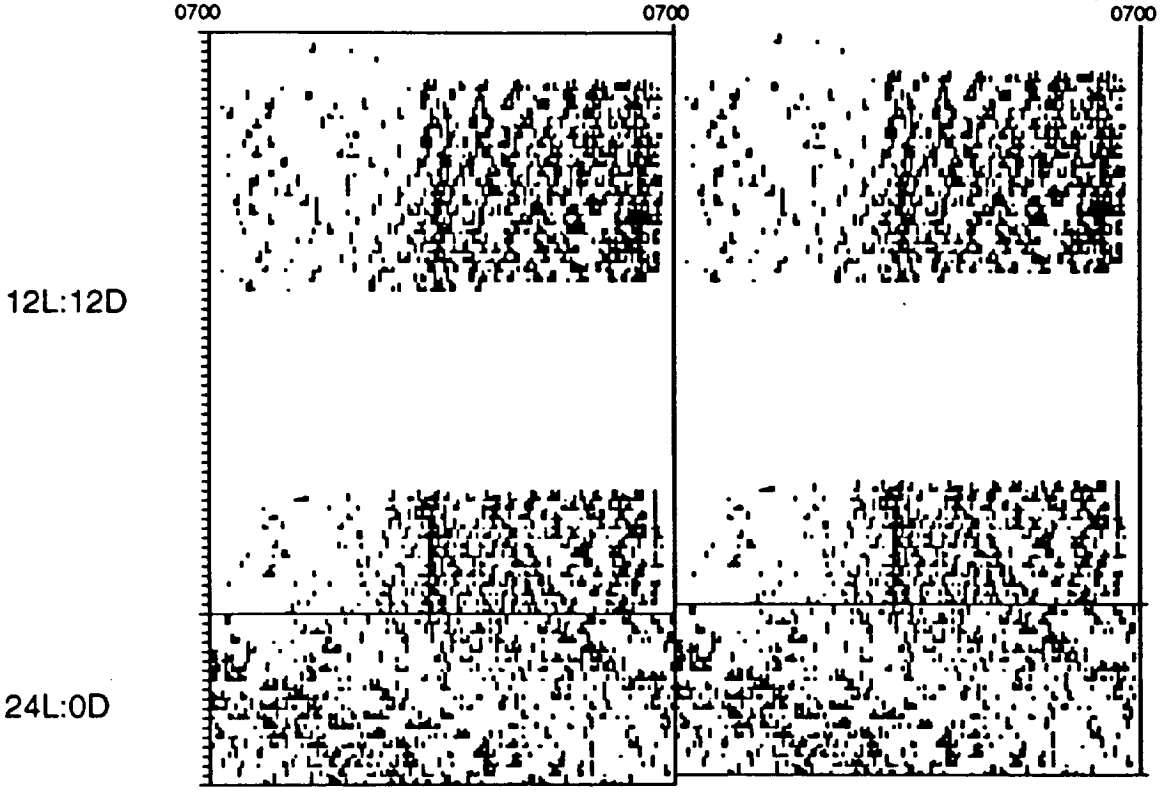
Light experiment 3 (8812Light05) Rat 3 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



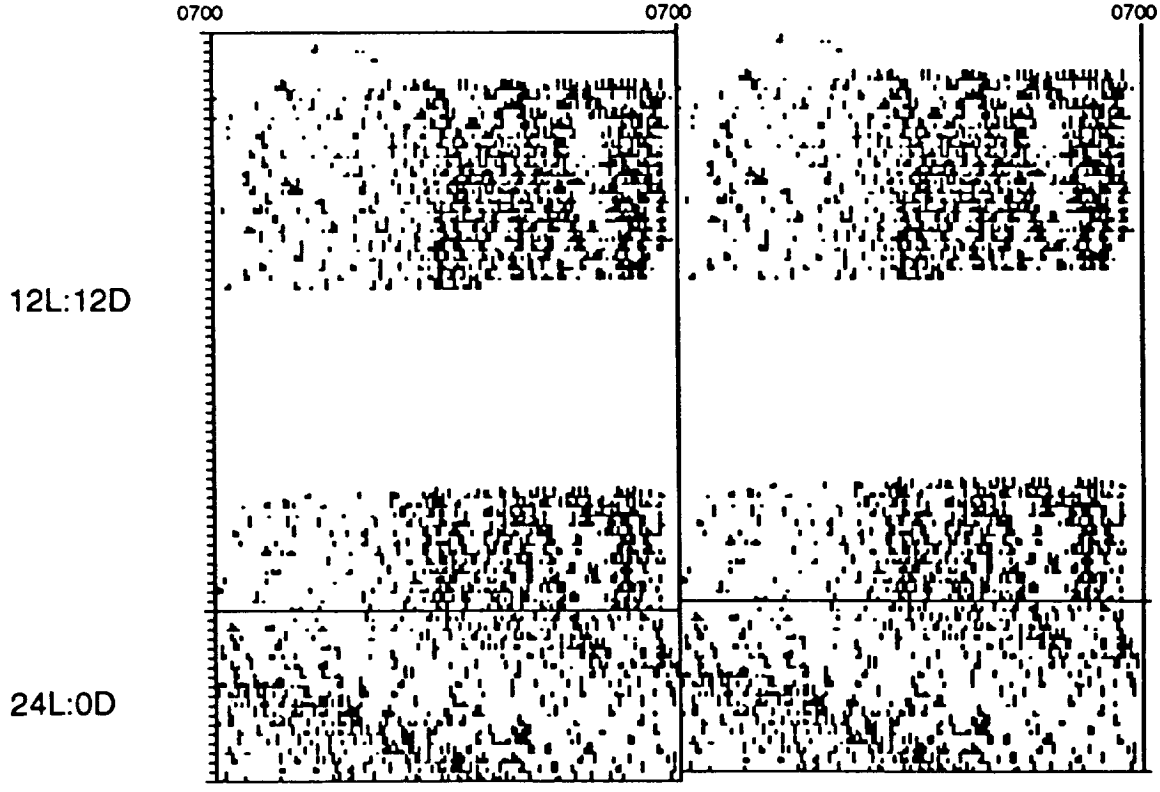
Light experiment 3 (8812Light05) Rat 4 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



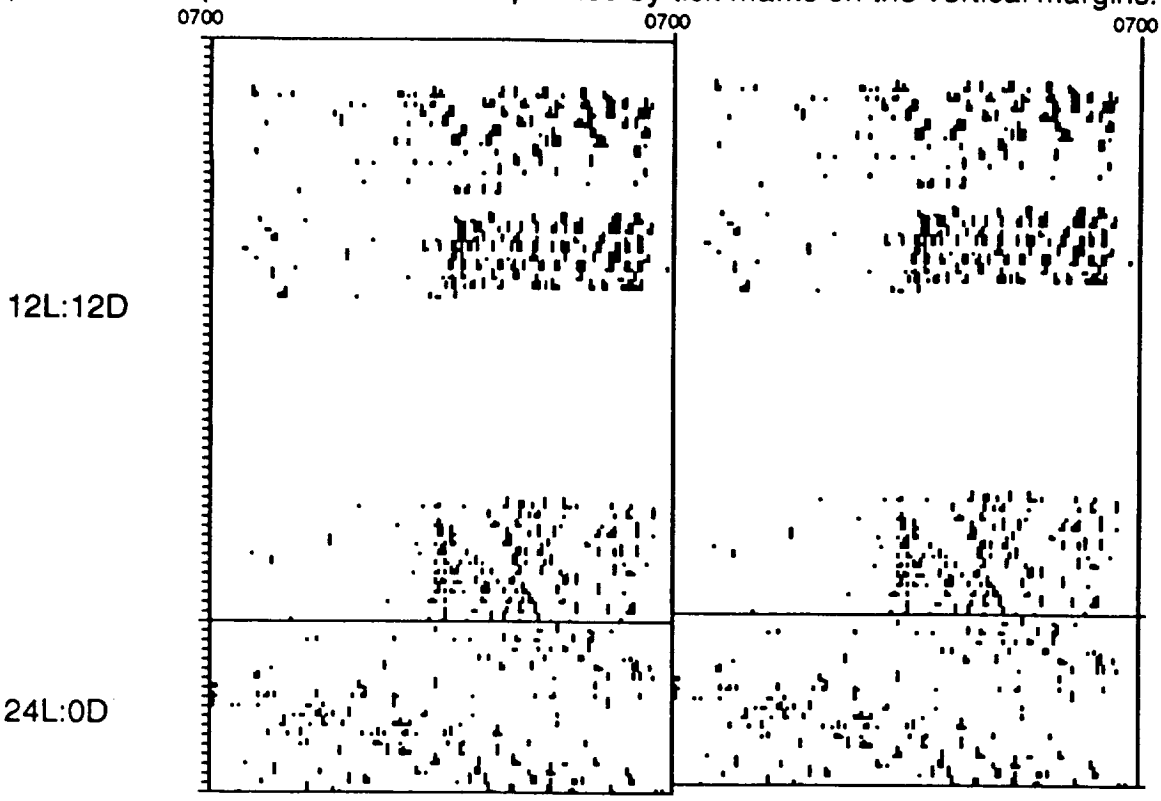
Light experiment 3 (8812Light05) Rat 5 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



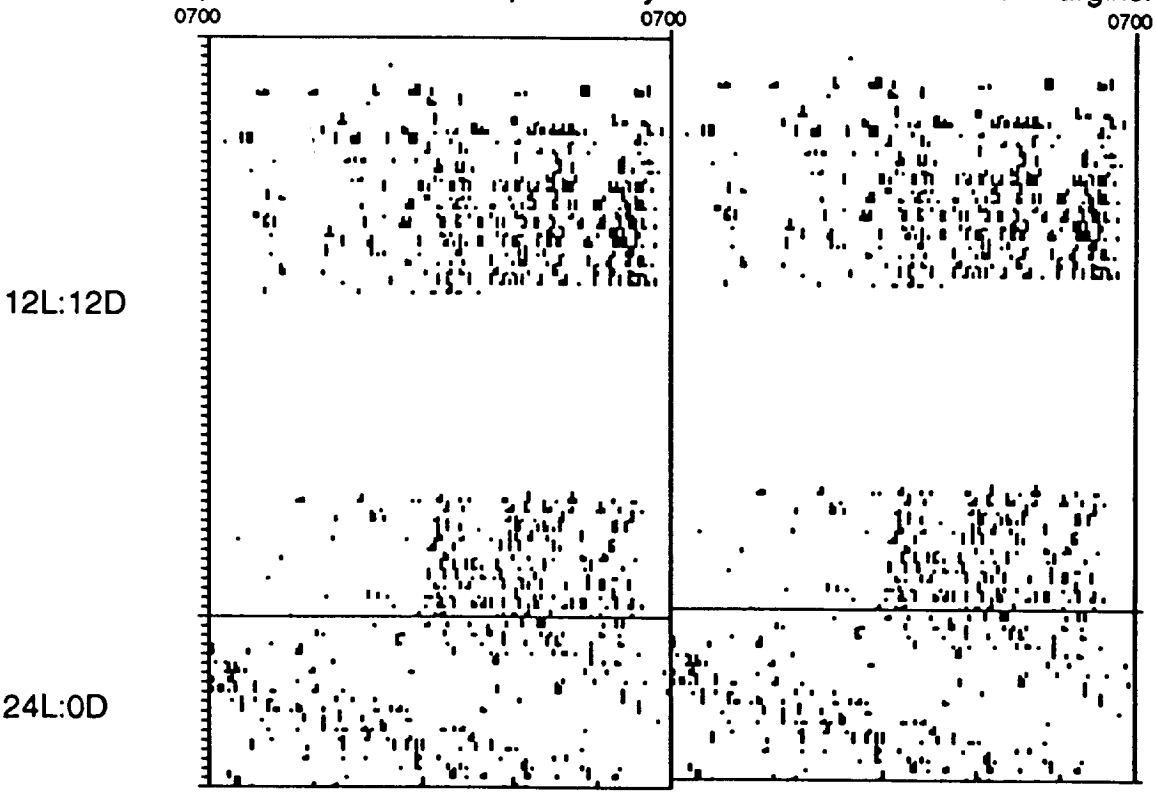
Light experiment 3 (8812Light05) Rat 6 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



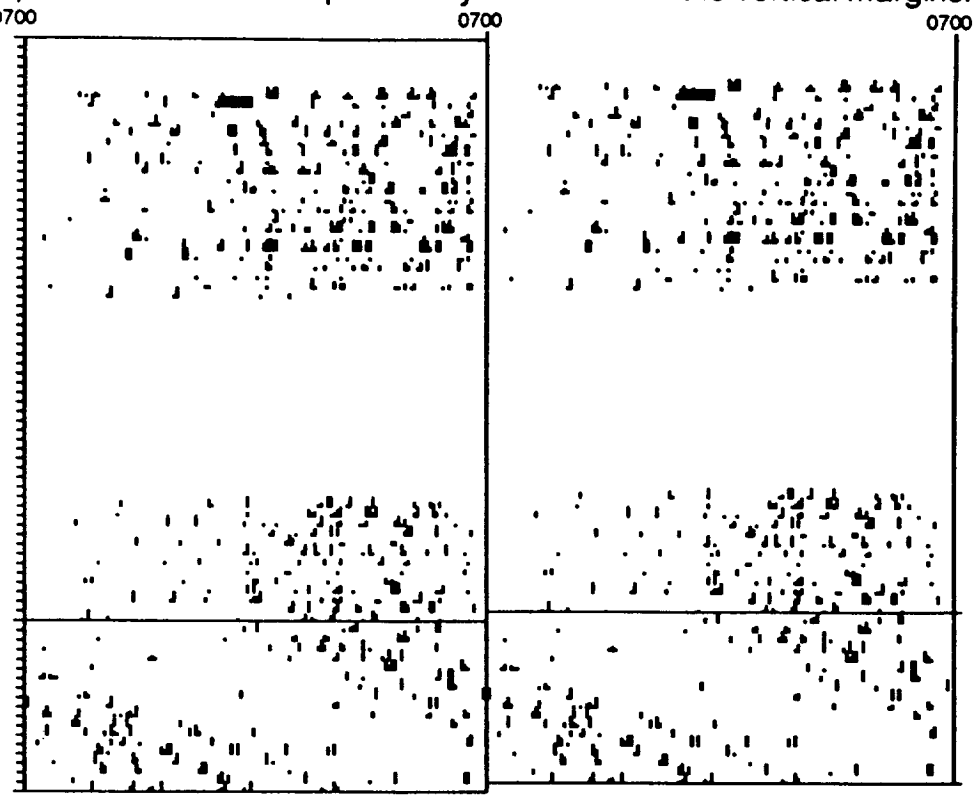
Light experiment 3 (8812Light05) Rat 1 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



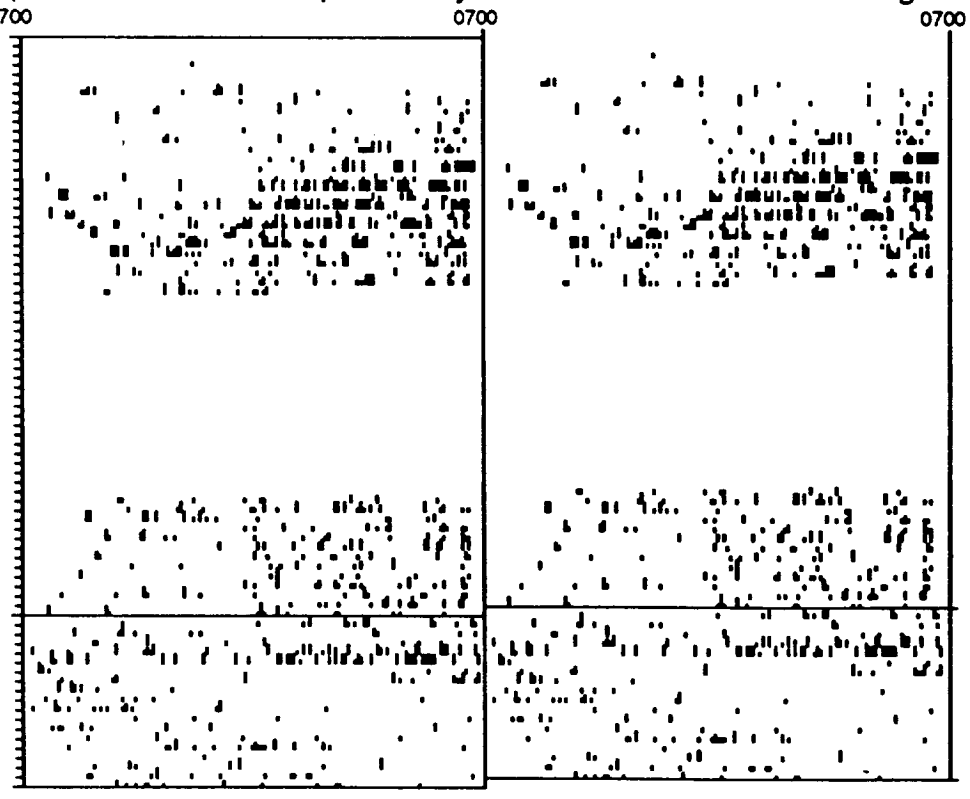
Light experiment 3 (8812Light05) Rat 2 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



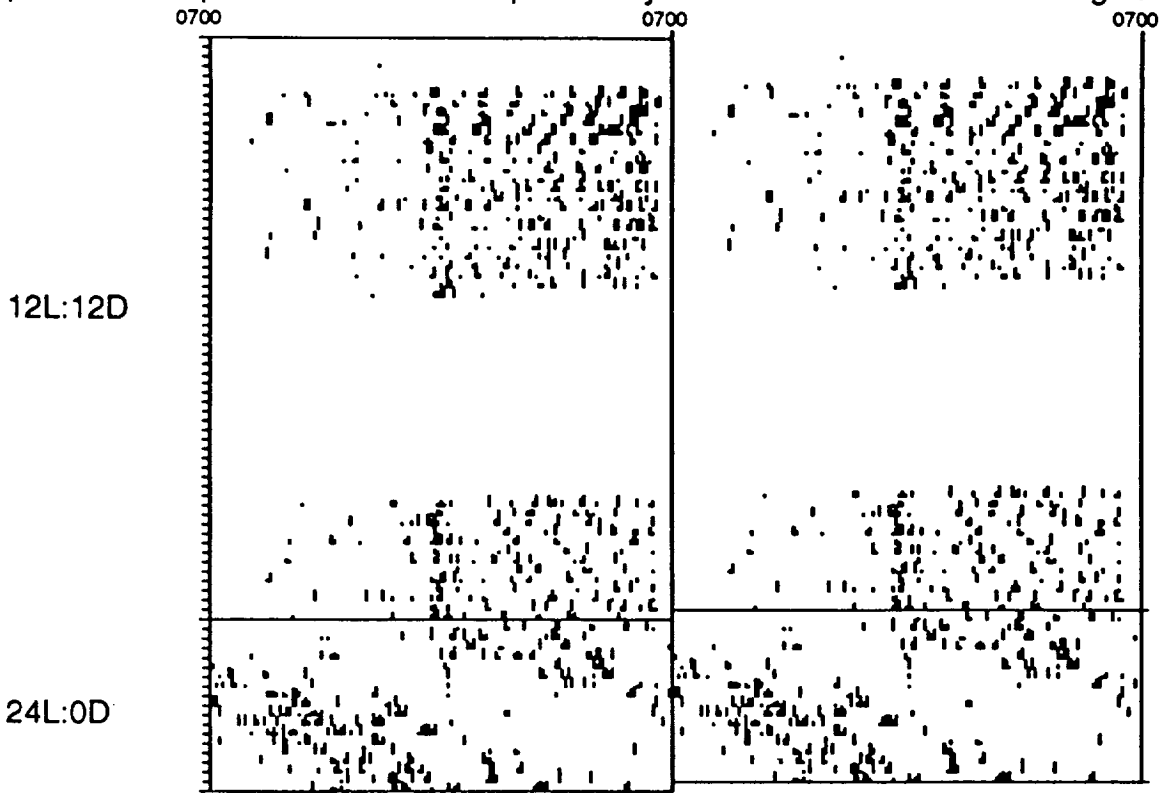
Light experiment 3 (8812Light05) Rat 3 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



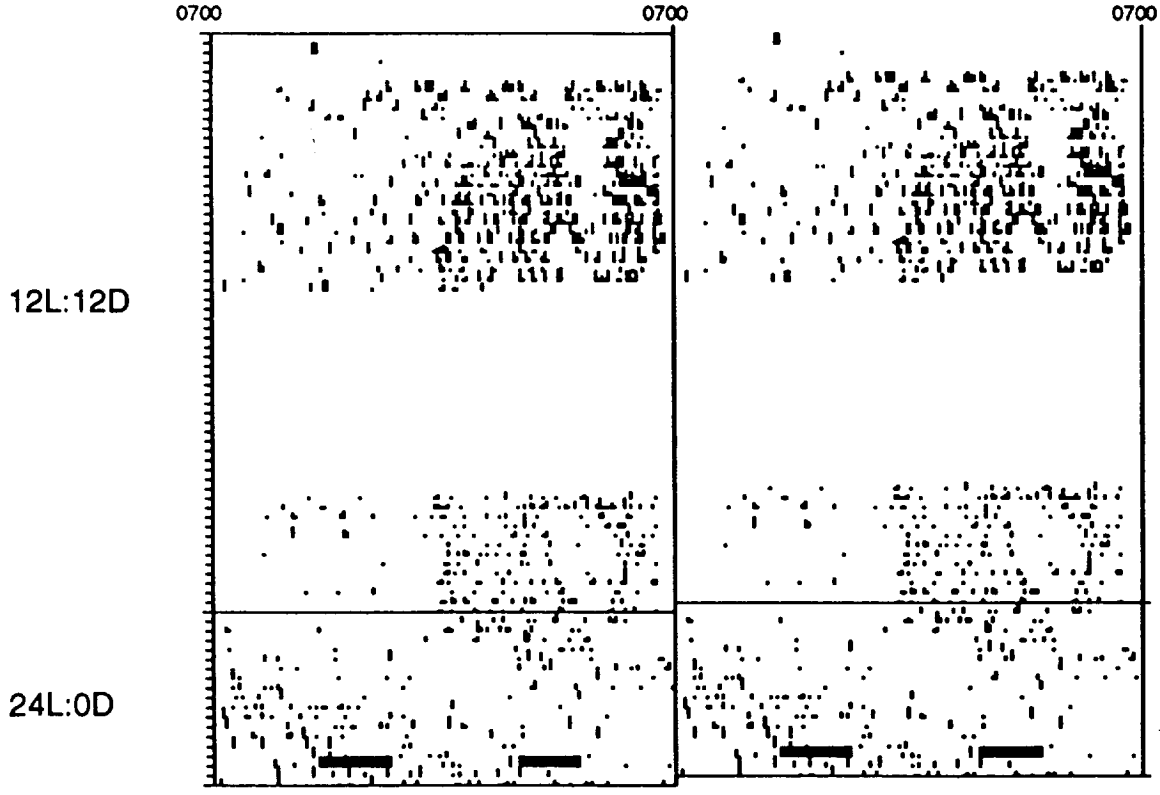
Light experiment 3 (8812Light05) Rat 4 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



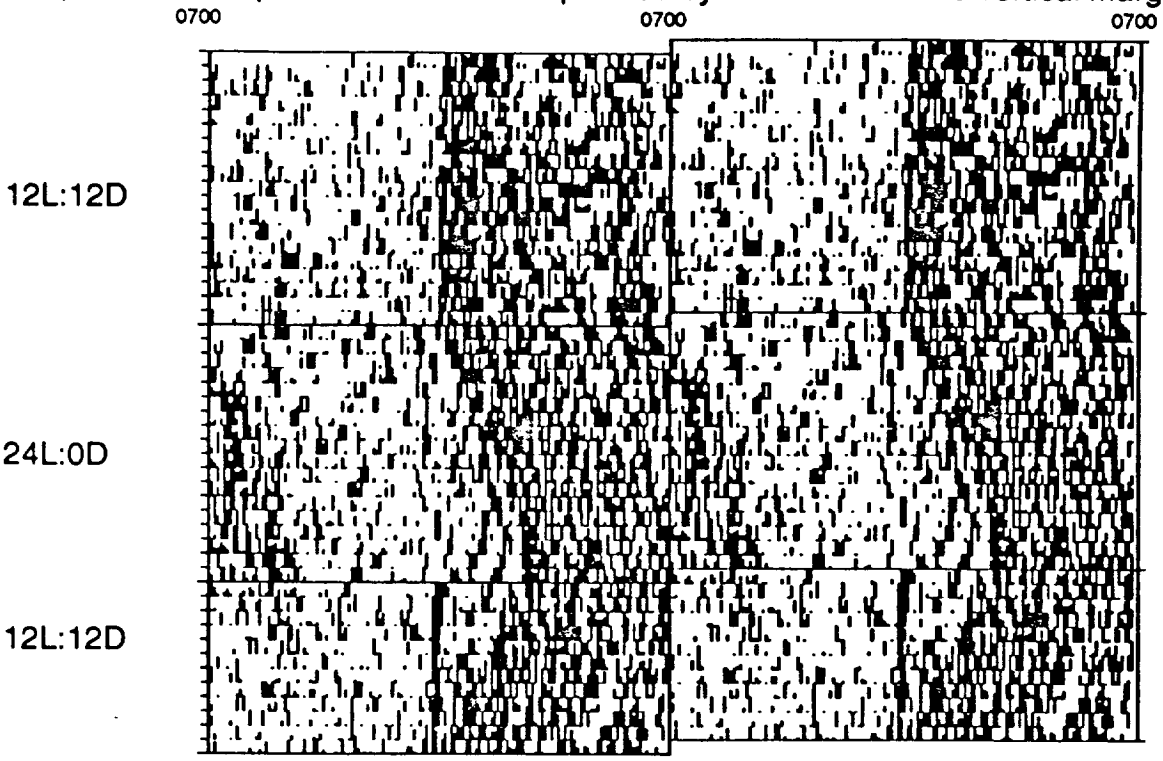
Light experiment 3 (8812Light05) Rat 5 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



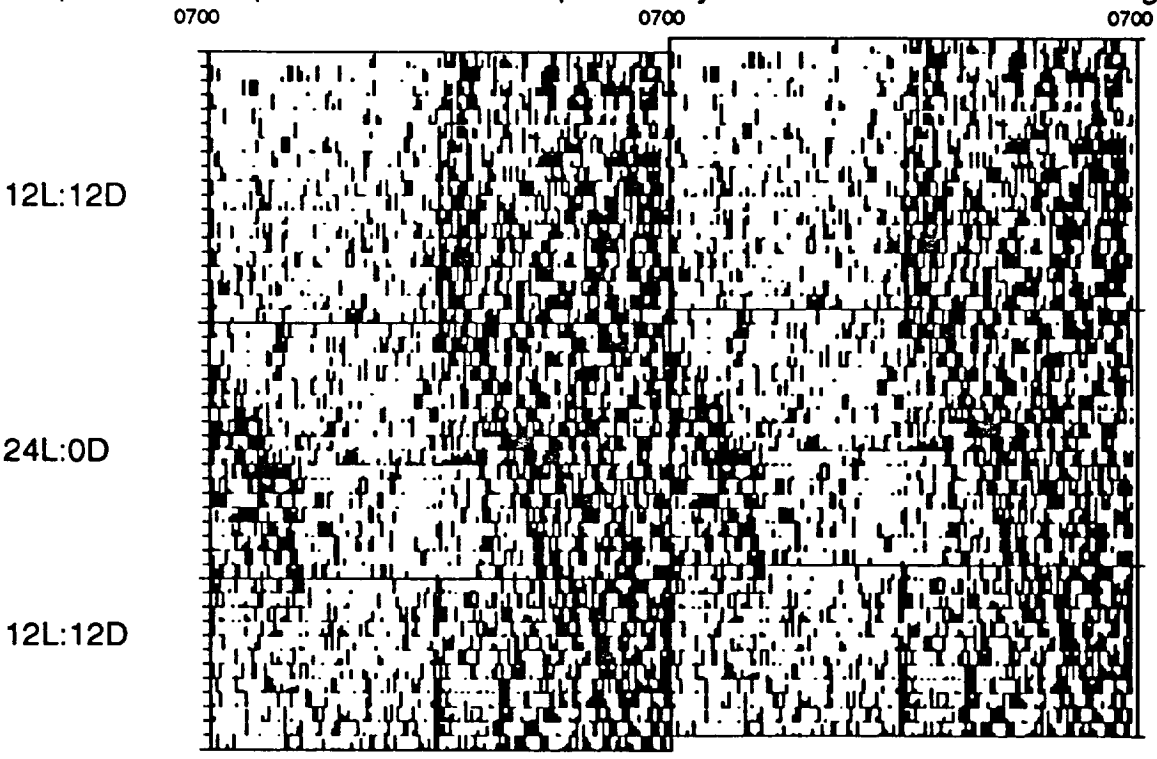
Light experiment 3 (8812Light05) Rat 6 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



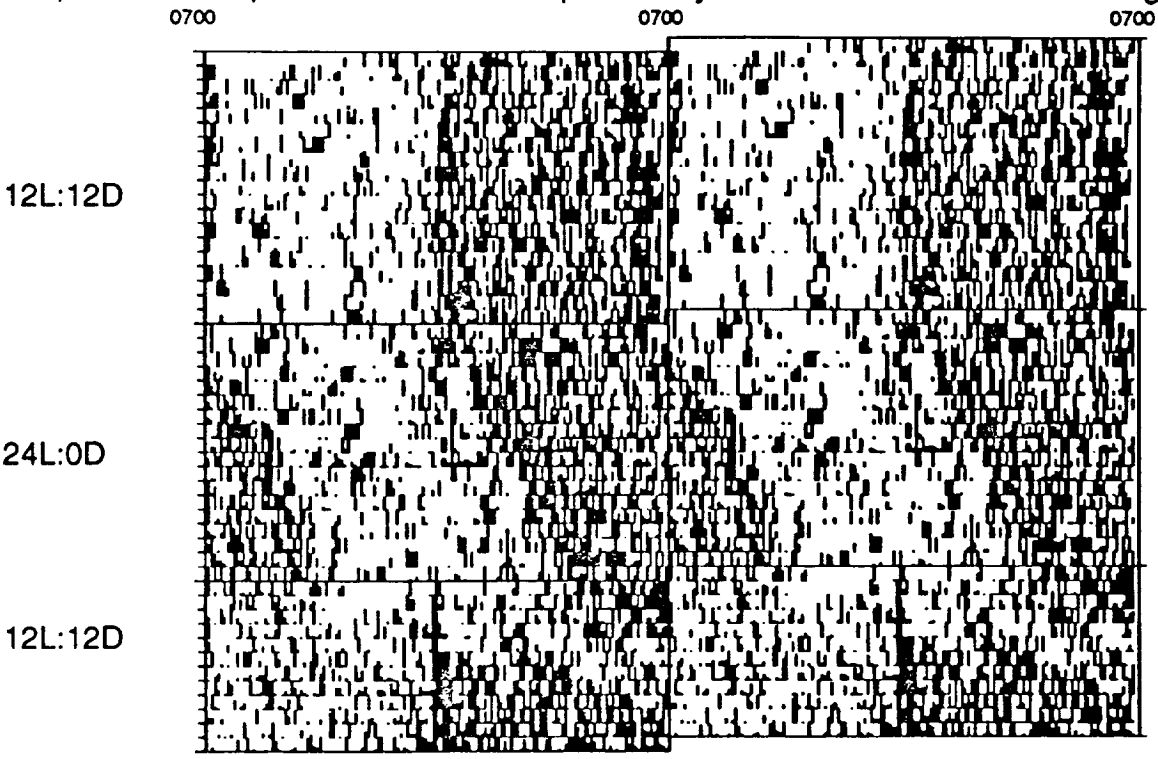
Light experiment 4 (8906Light0.01) Rat 1 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



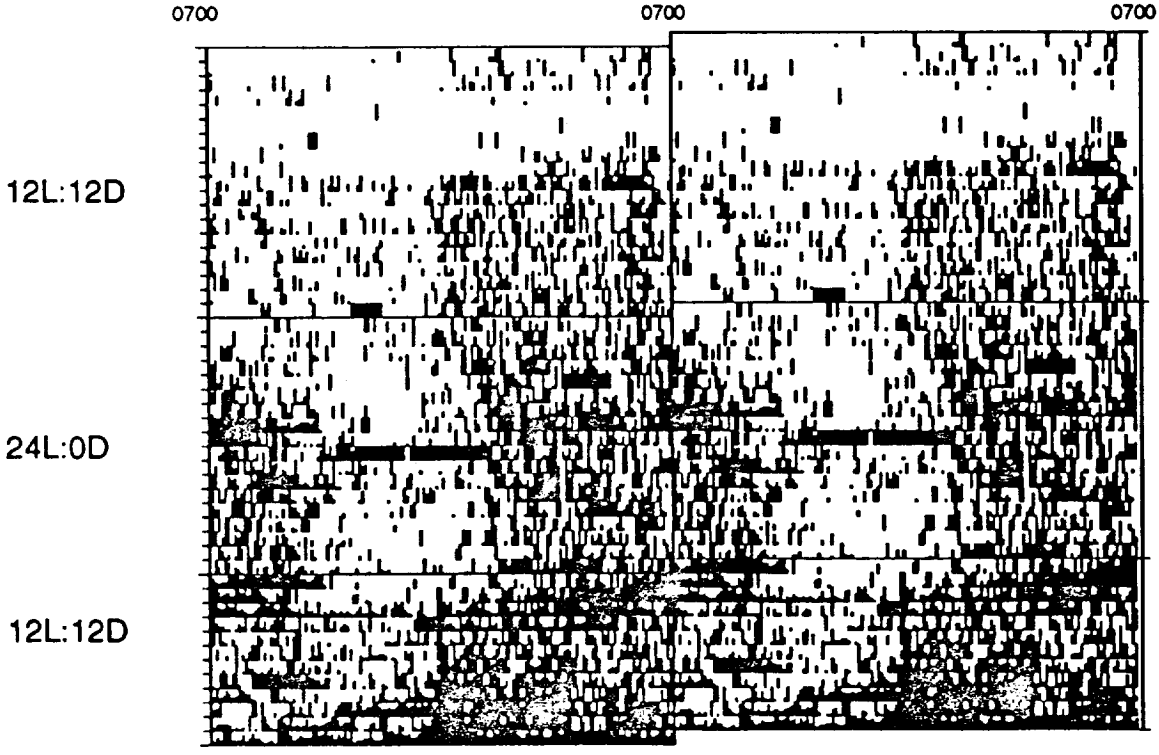
Light experiment 4 (8906Light0.01) Rat 2 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



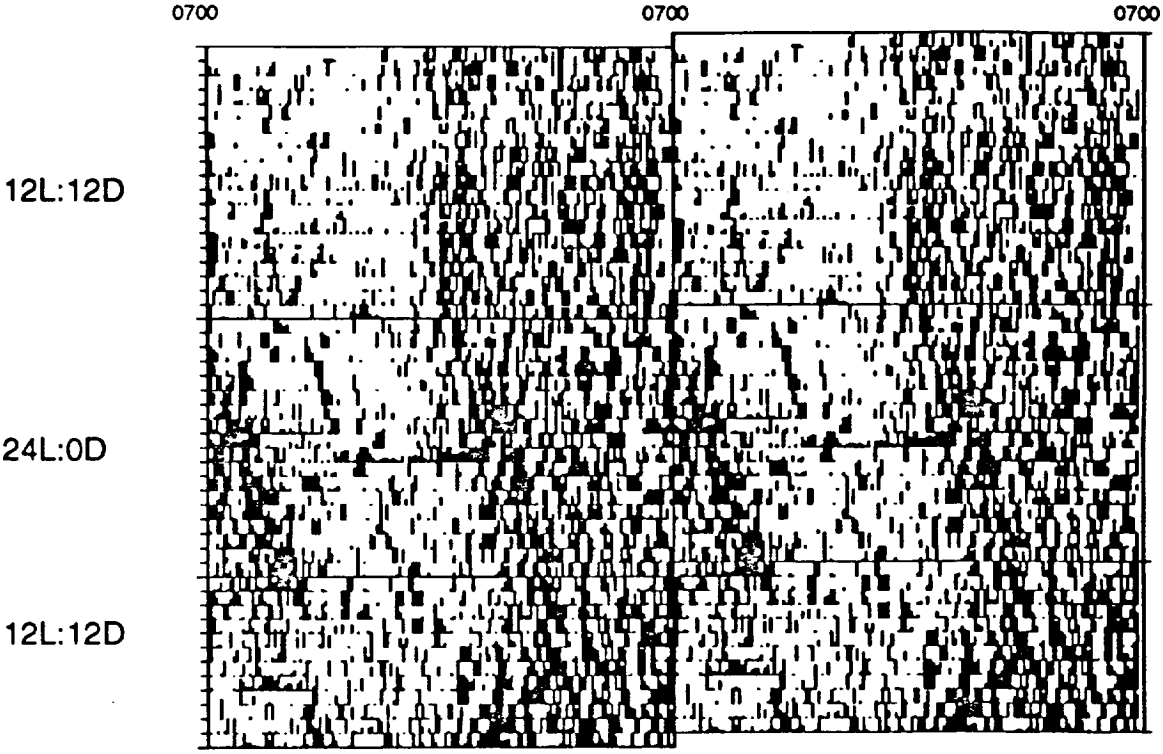
Light experiment 4 (8906Light0.01) Rat 3 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



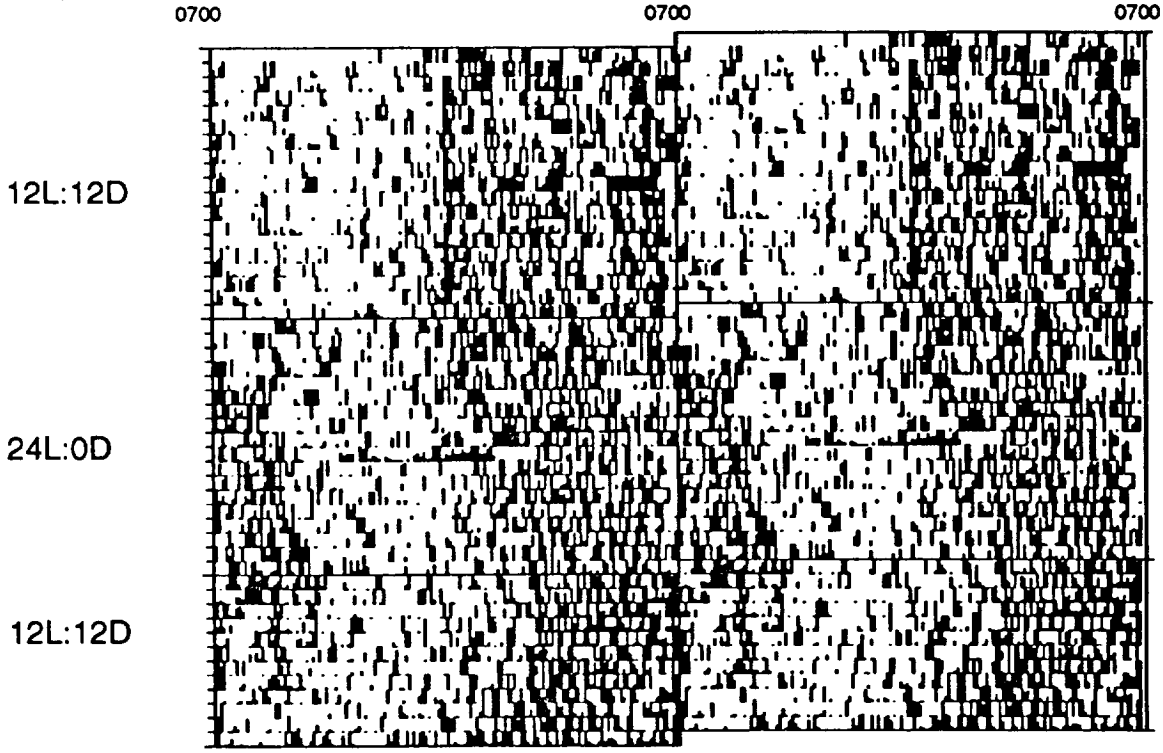
Light experiment 4 (8906Light0.01) Rat 4 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



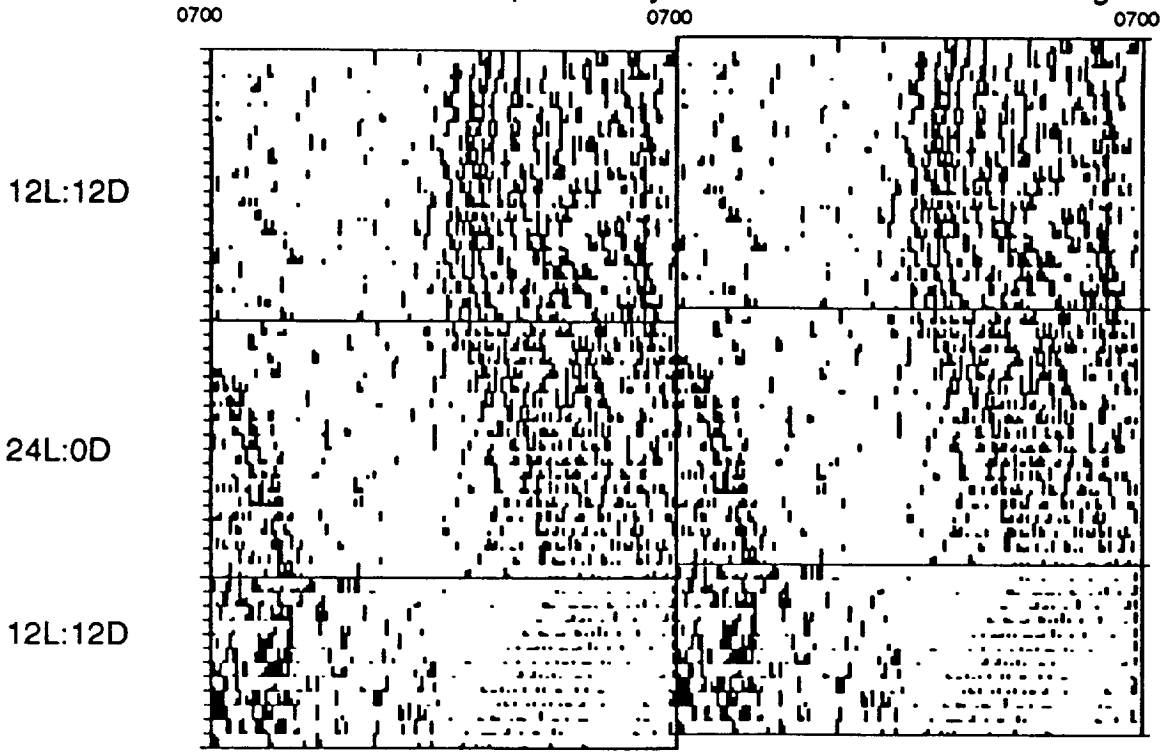
Light experiment 4 (8906Light0.01) Rat 5 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



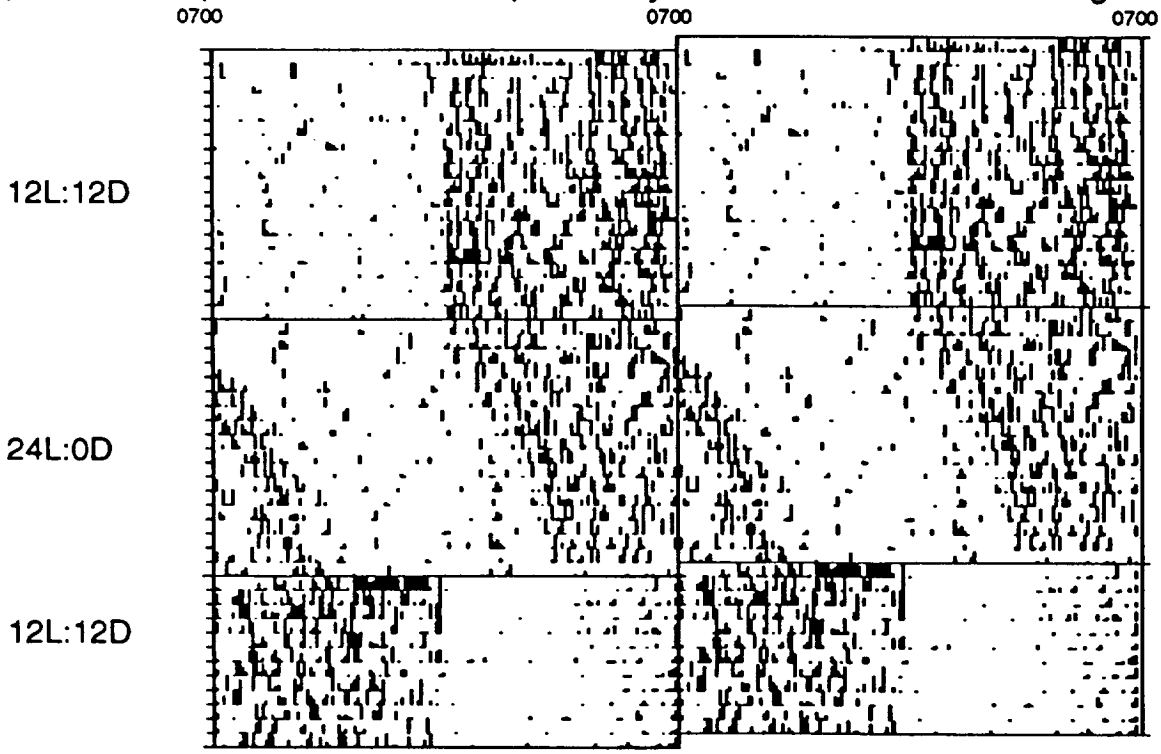
Light experiment 4 (8906Light0.01) Rat 6 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



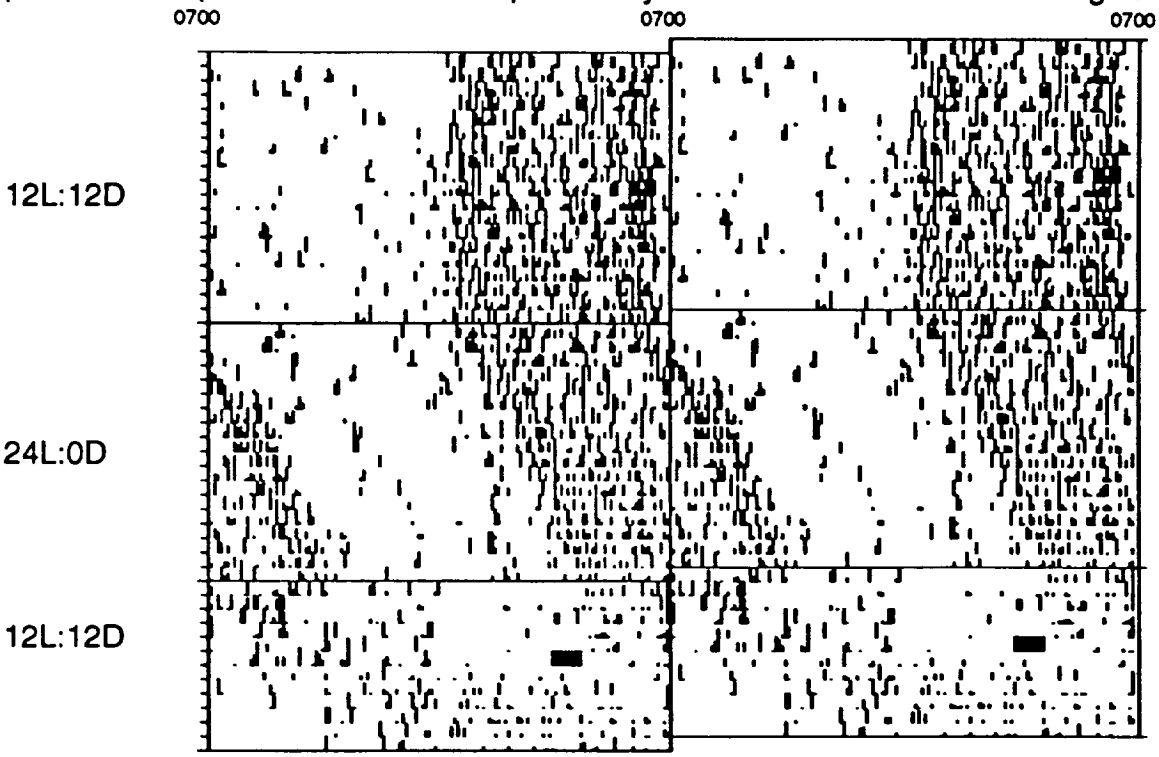
Light experiment 4 (8906Light0.01) Rat 1 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



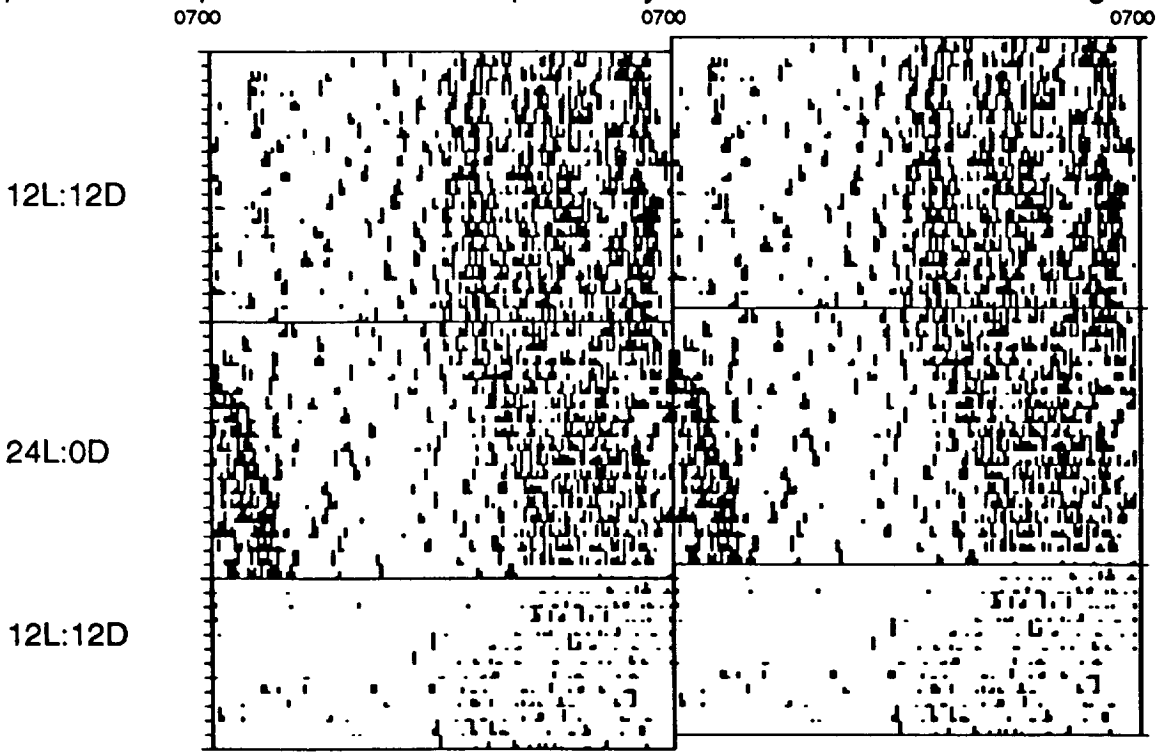
Light experiment 4 (8906Light0.01) Rat 2 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



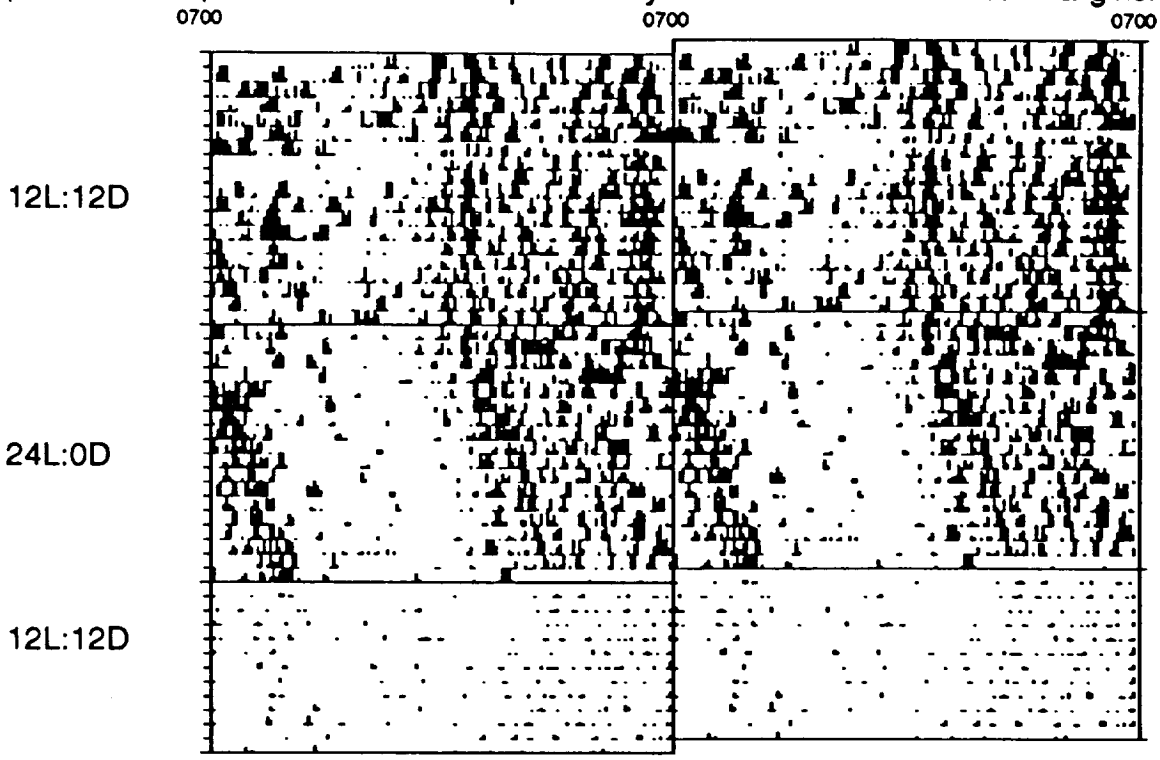
Light experiment 4 (8906Light0.01) Rat 3 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



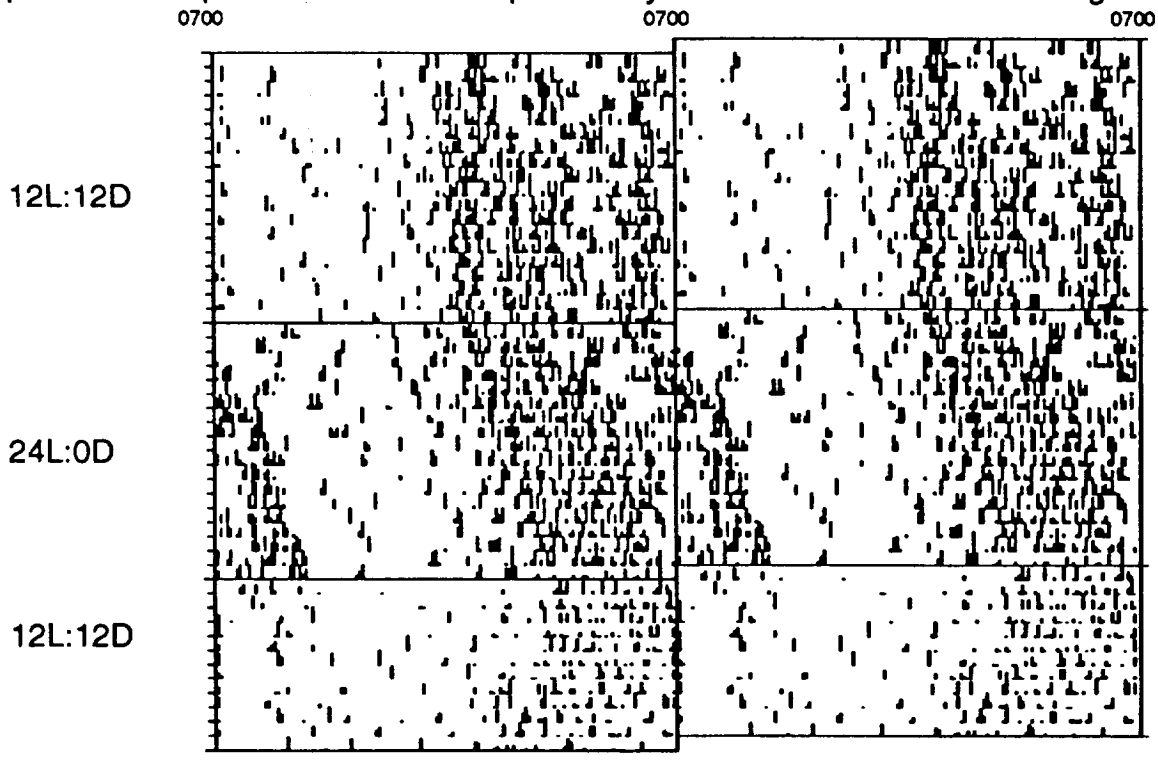
Light experiment 4 (8906Light0.01) Rat 4 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



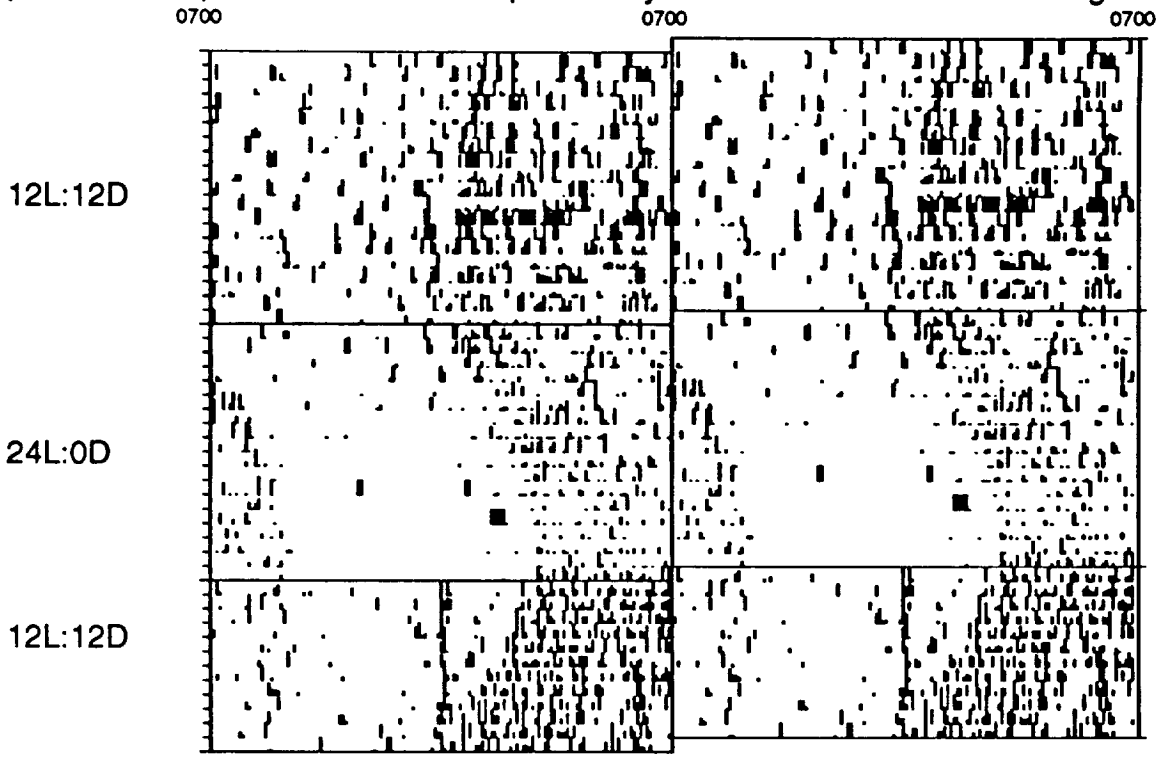
Light experiment 4 (8906Light0.01) Rat 5 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



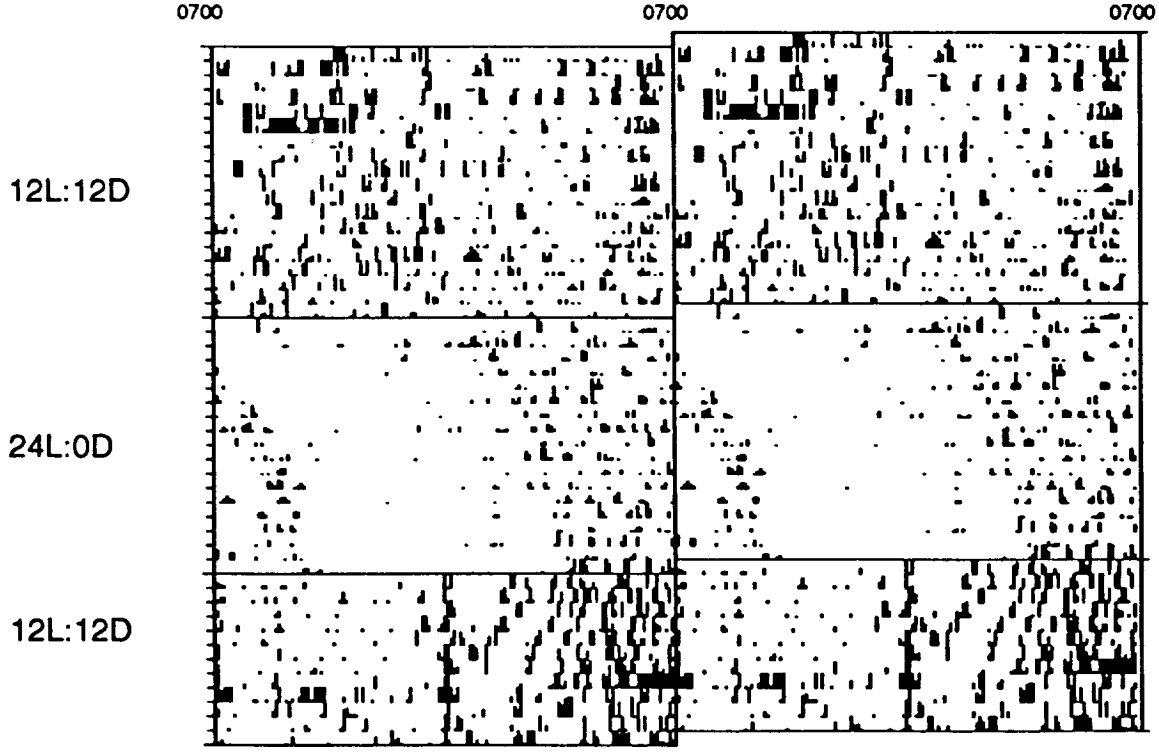
Light experiment 4 (8906Light0.01) Rat 6 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



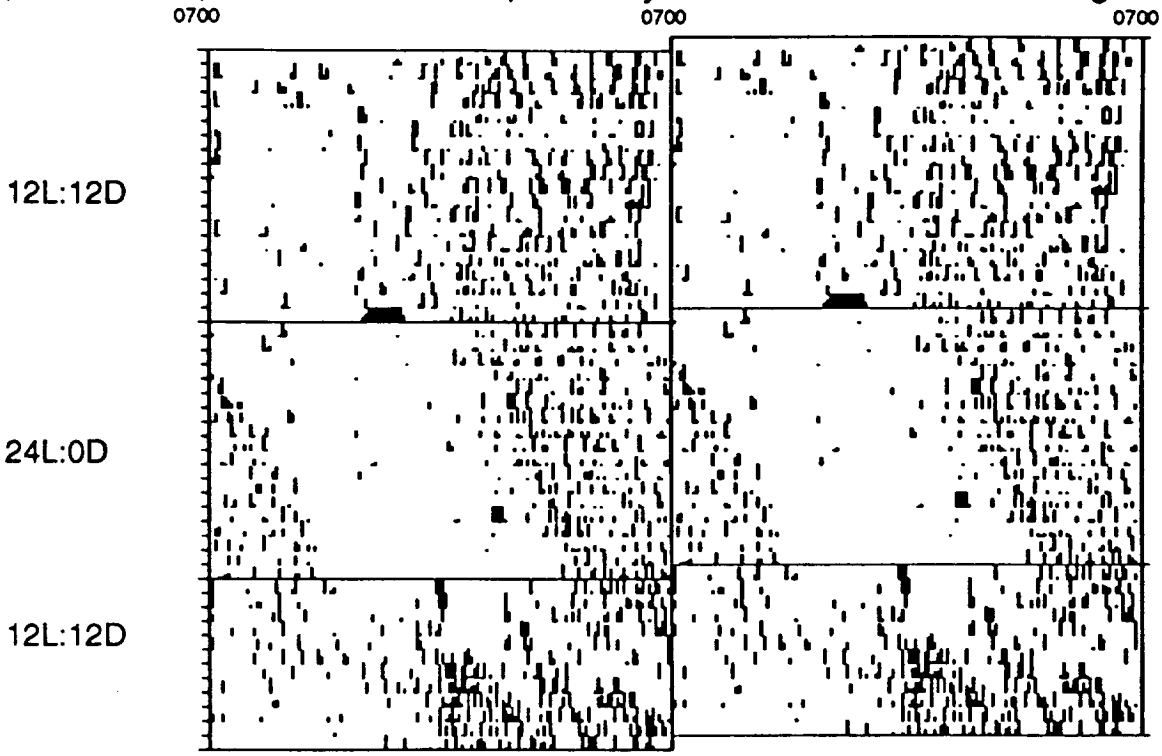
Light experiment 4 (8906Light0.01) Rat 1 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



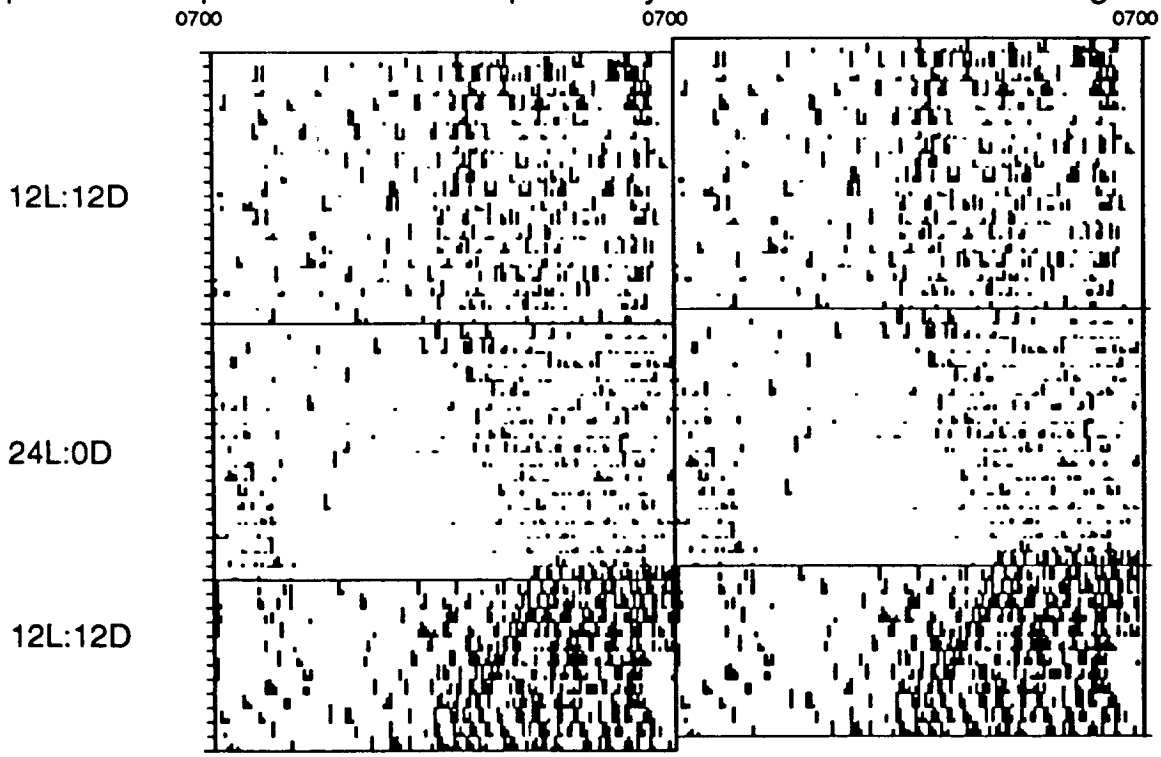
Light experiment 4 (8906Light0.01) Rat 2 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



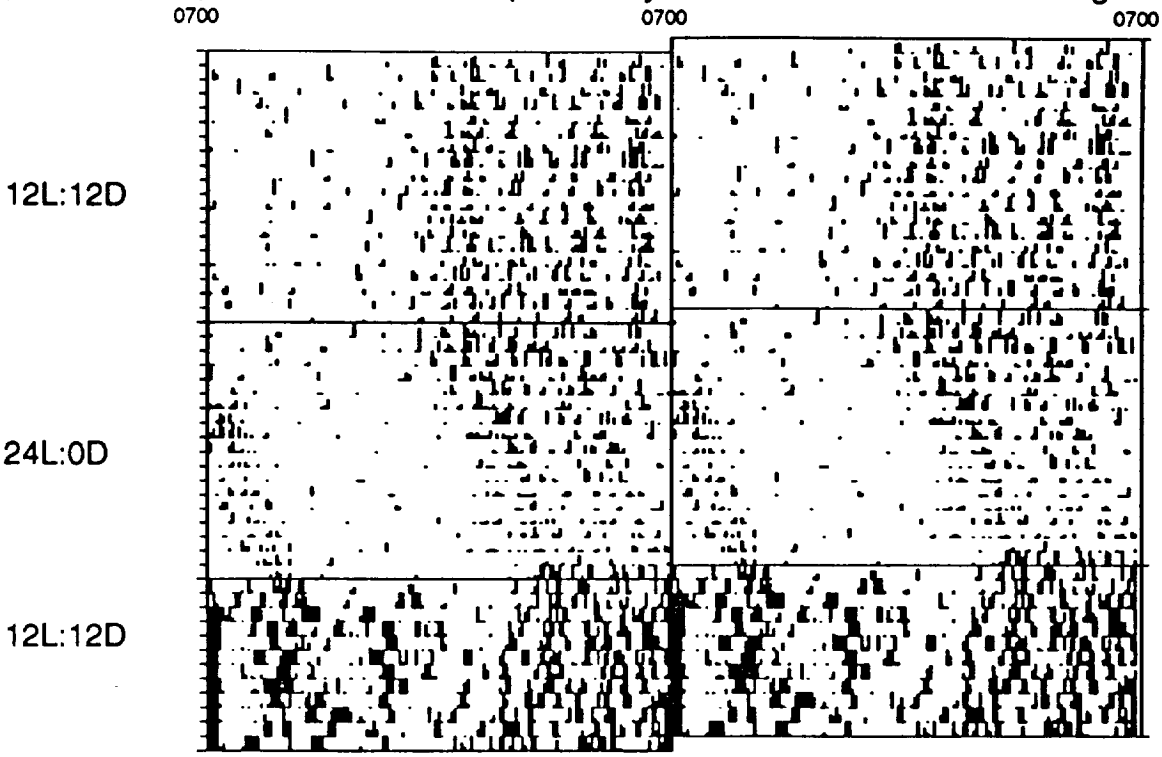
Light experiment 4 (8906Light0.01) Rat 3 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



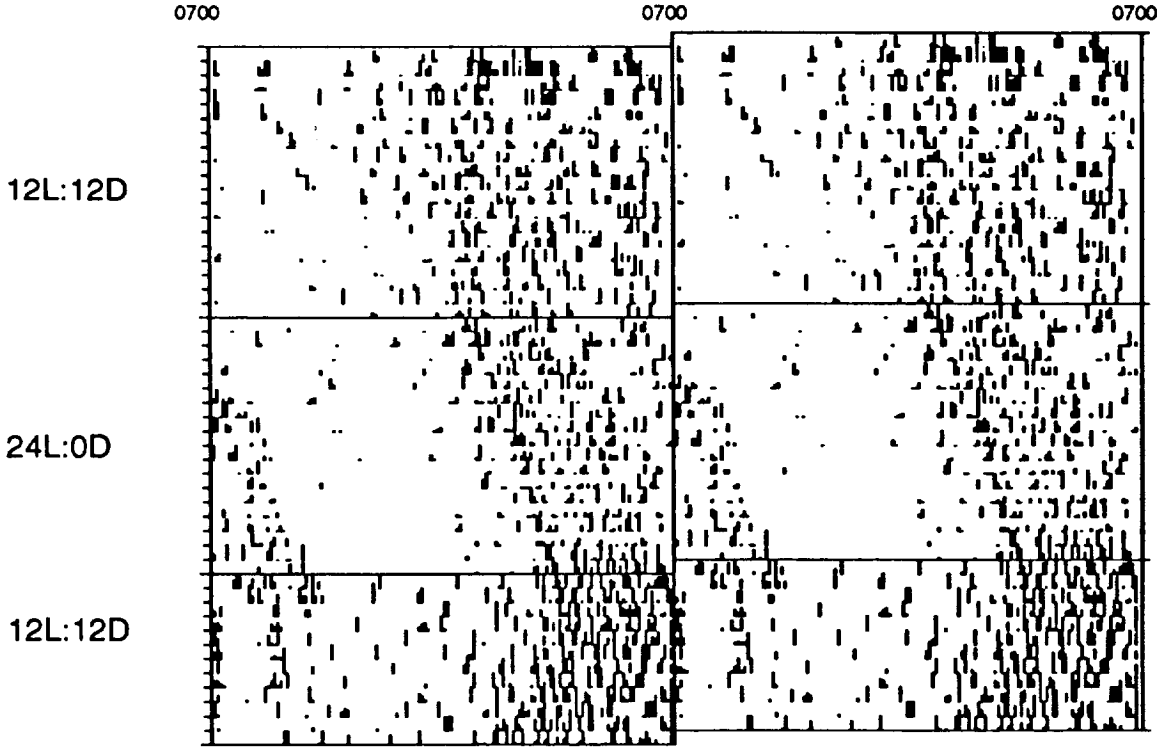
Light experiment 4 (8906Light0.01) Rat 4 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



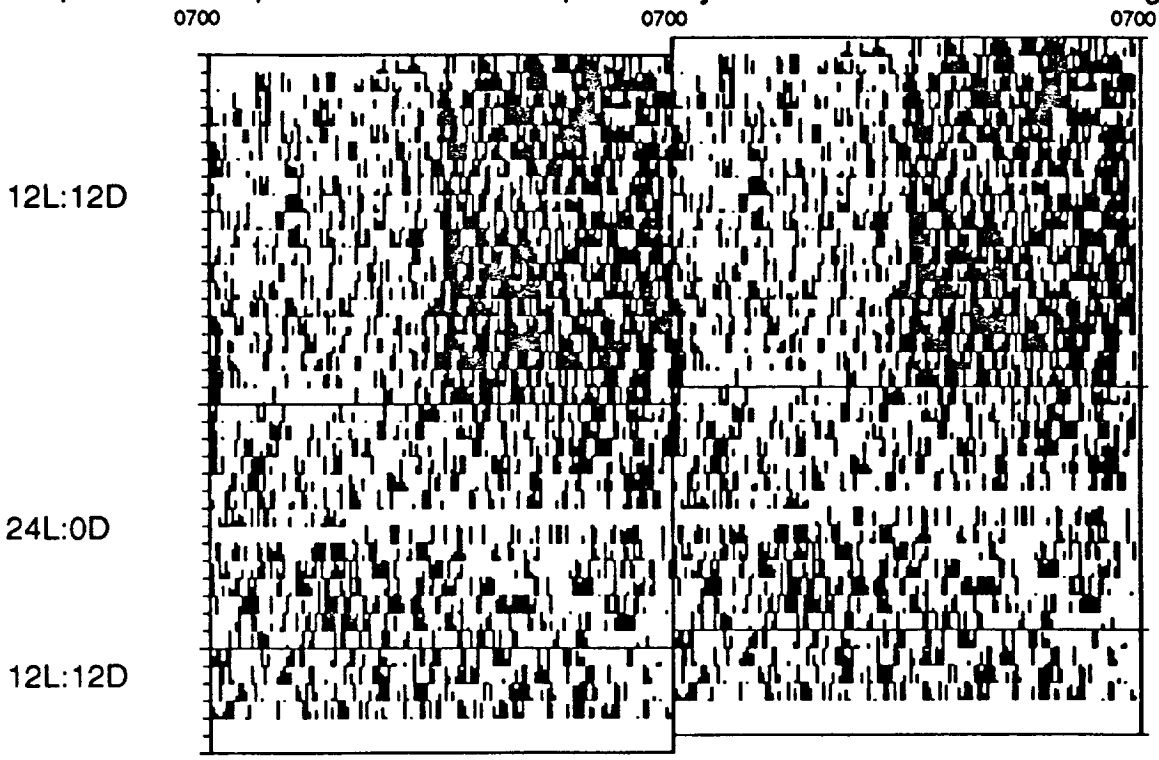
Light experiment 4 (8906Light0.01) Rat 5 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



Light experiment 4 (8906Light0.01) Rat 6 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.

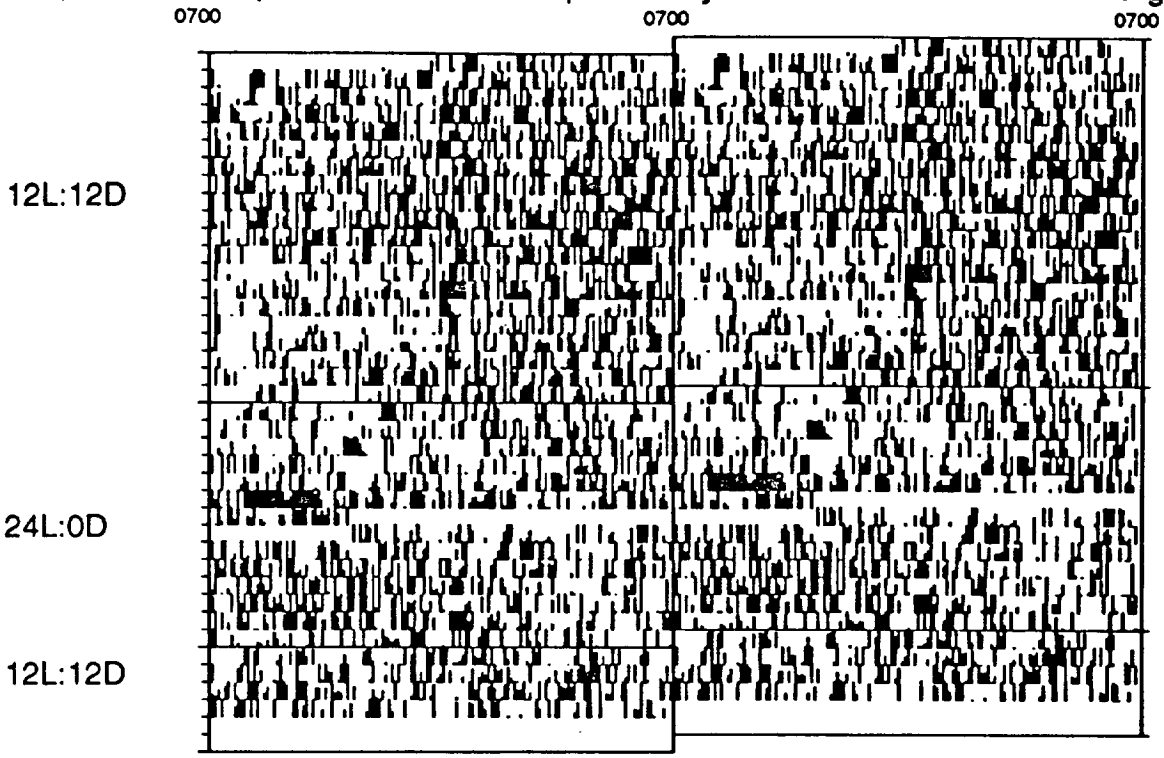


Light experiment 5 (9107Light10) Rat 1 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

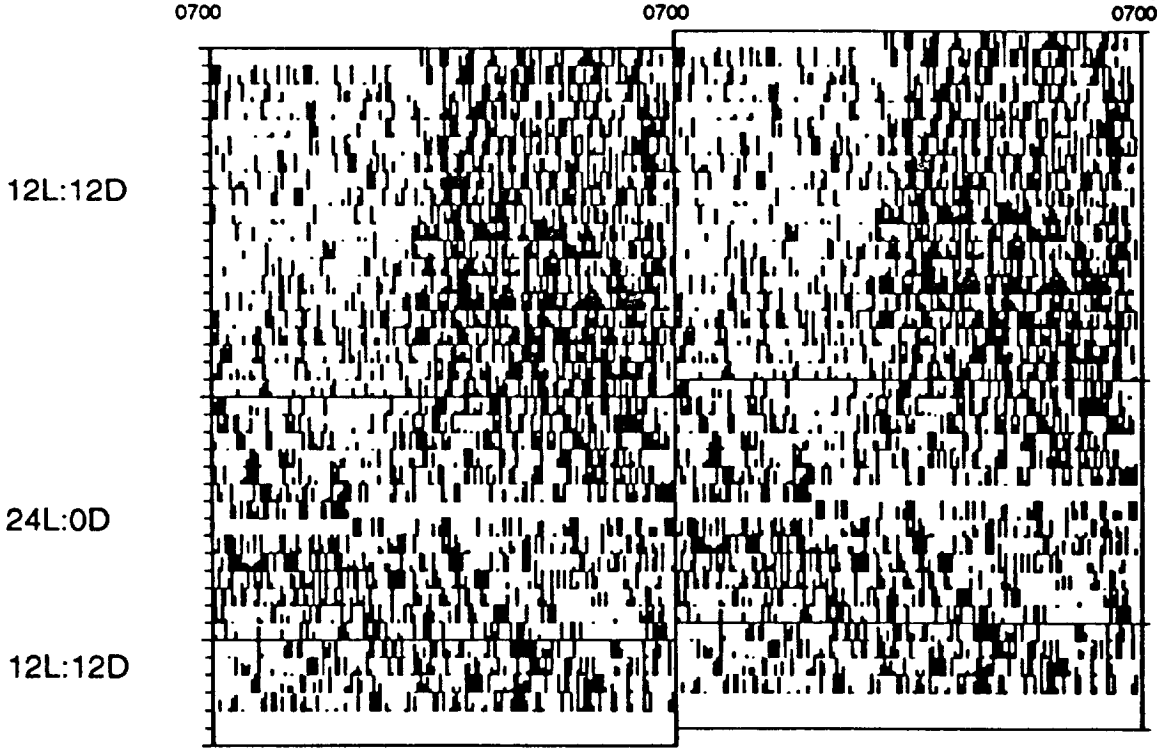


Light experiment 5 (9107Light10) Rat 2 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.

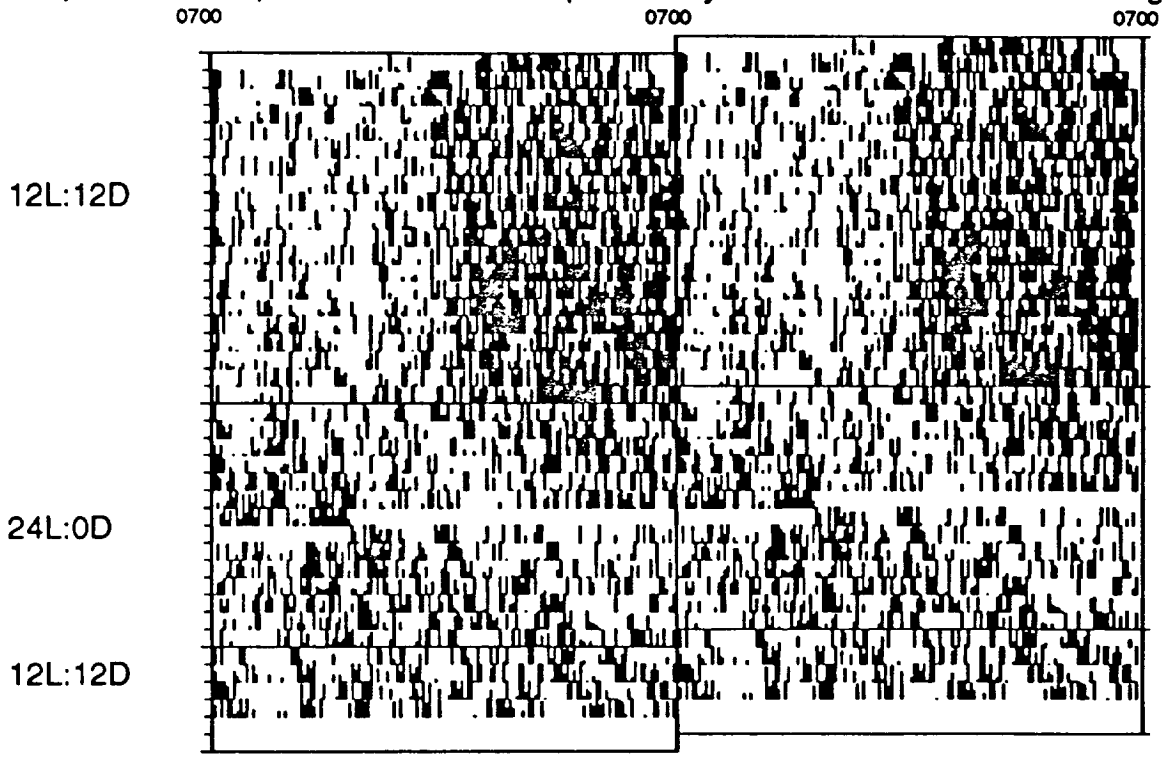
Light experiment 5 (9107Light10) Rat 3 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



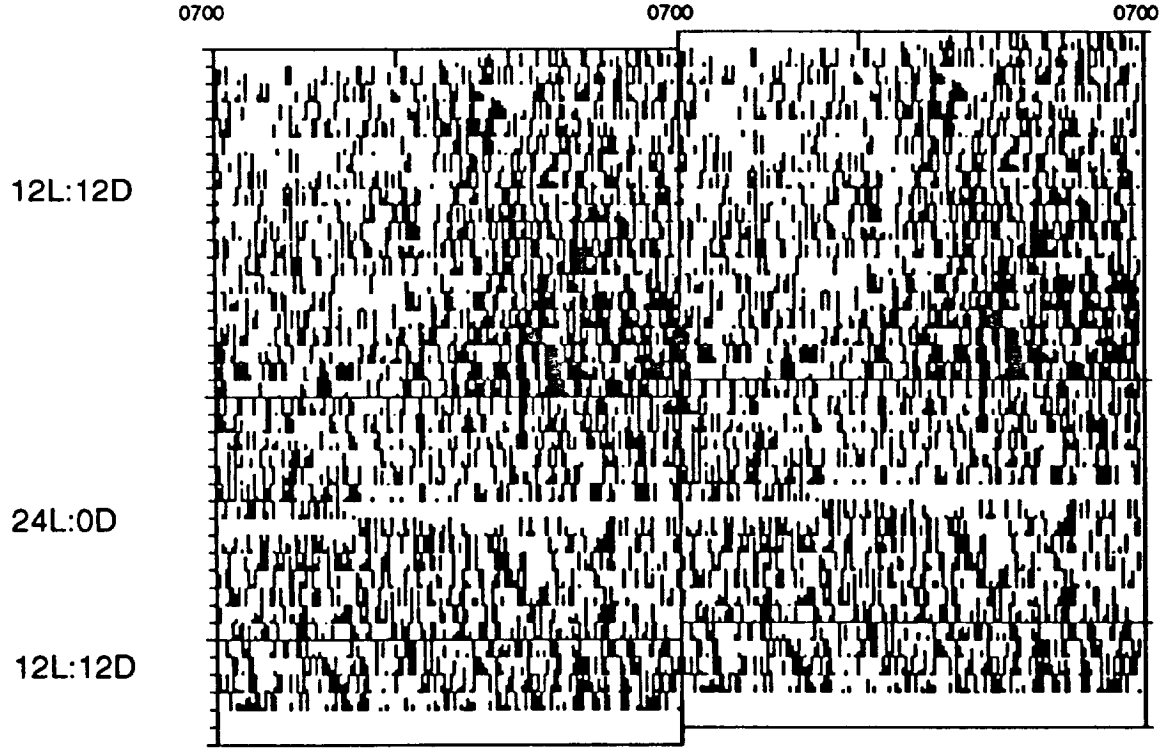
Light experiment 5 (9107Light10) Rat 4 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



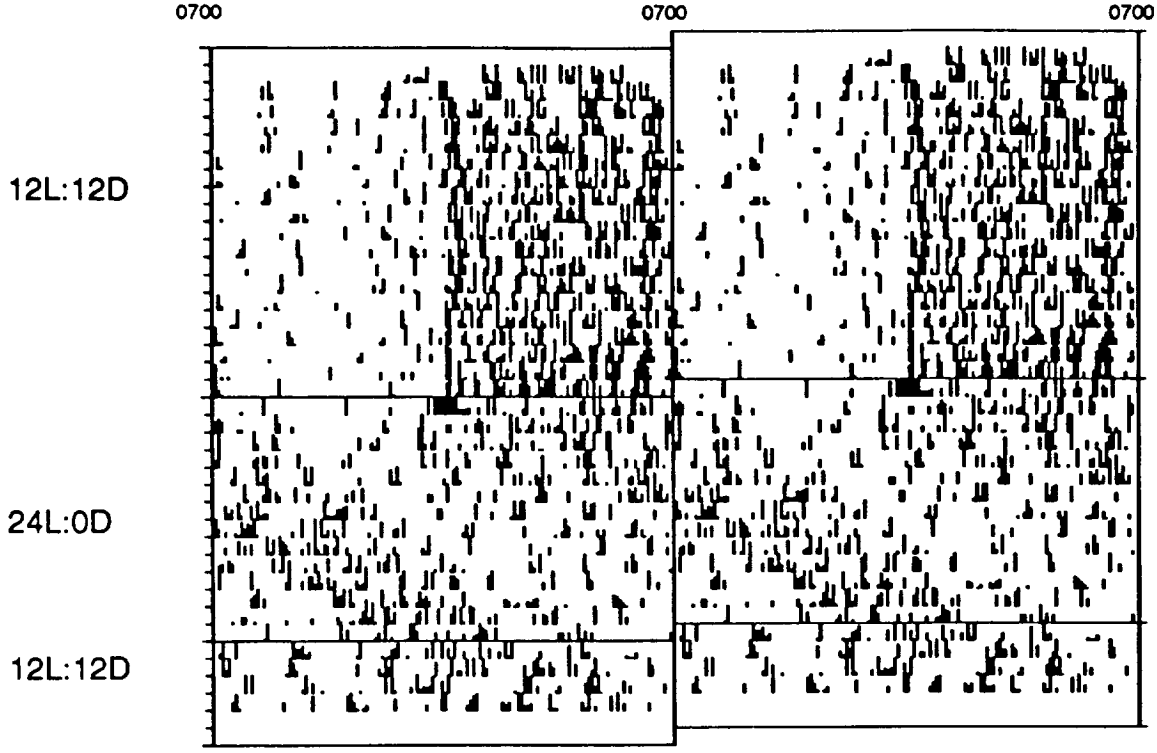
Light experiment 5 (9107Light10) Rat 5 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



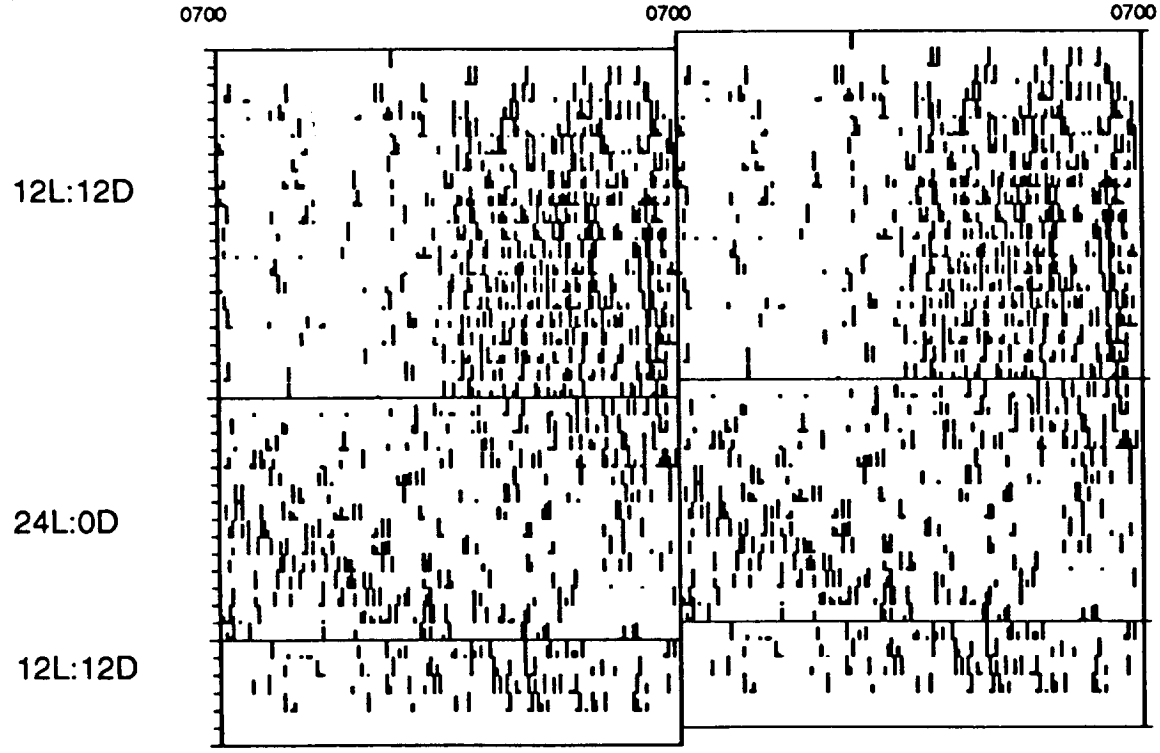
Light experiment 5 (9107Light10) Rat 6 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



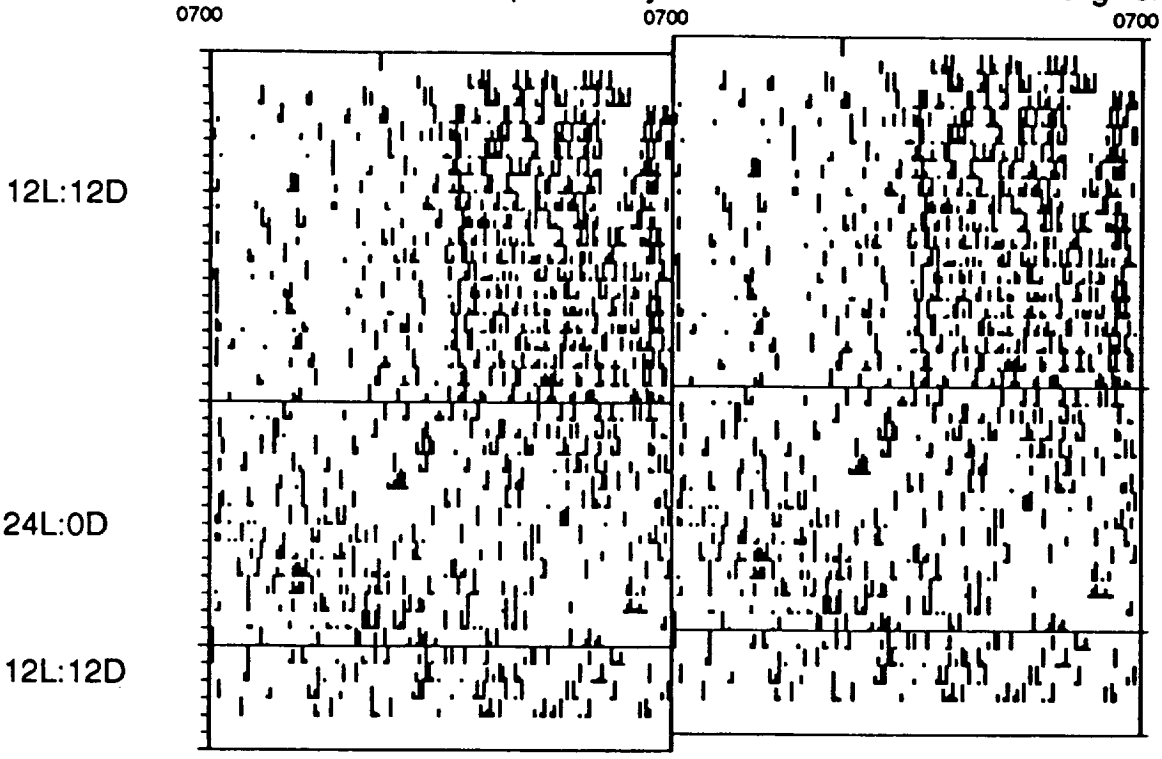
Light experiment 5 (9107Light10) Rat 1 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



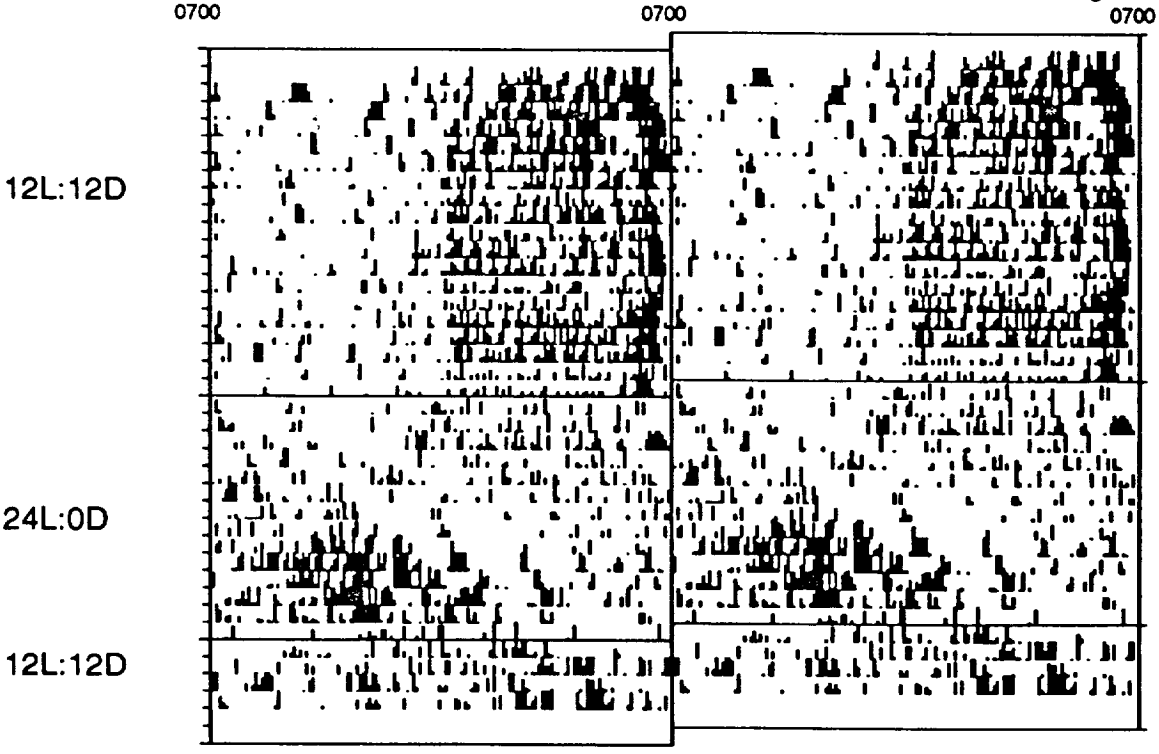
Light experiment 5 (9107Light10) Rat 2 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



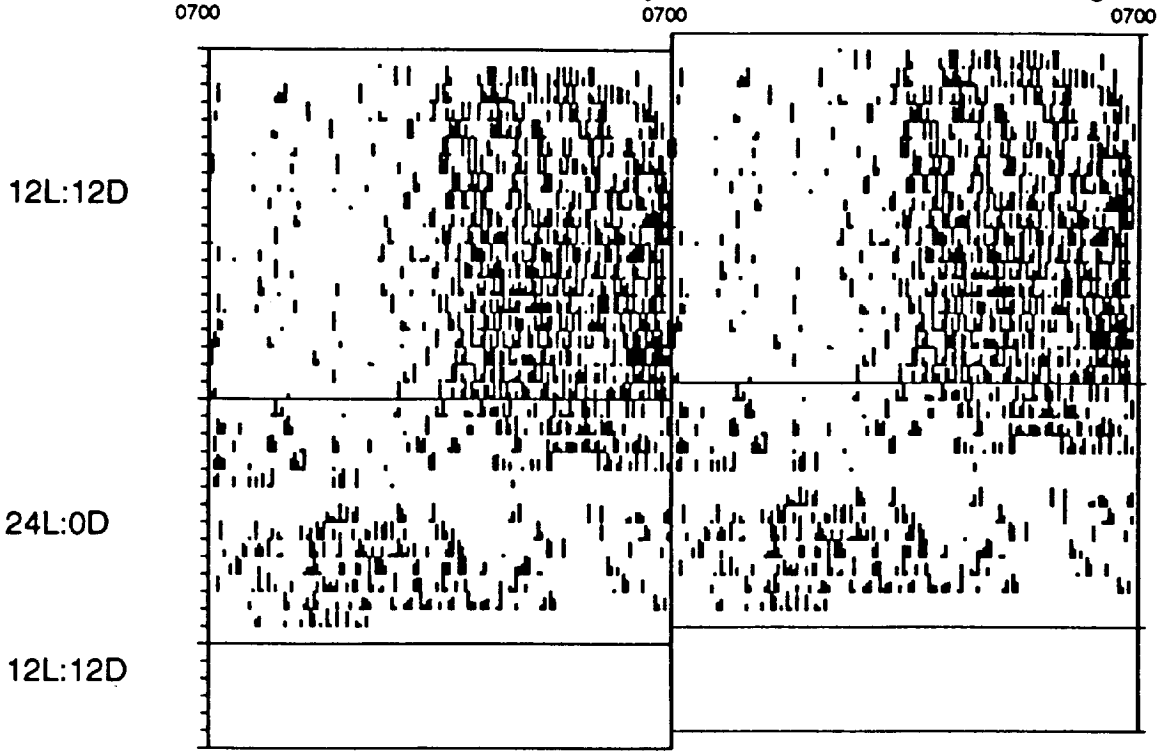
Light experiment 5 (9107Light10) Rat 3 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



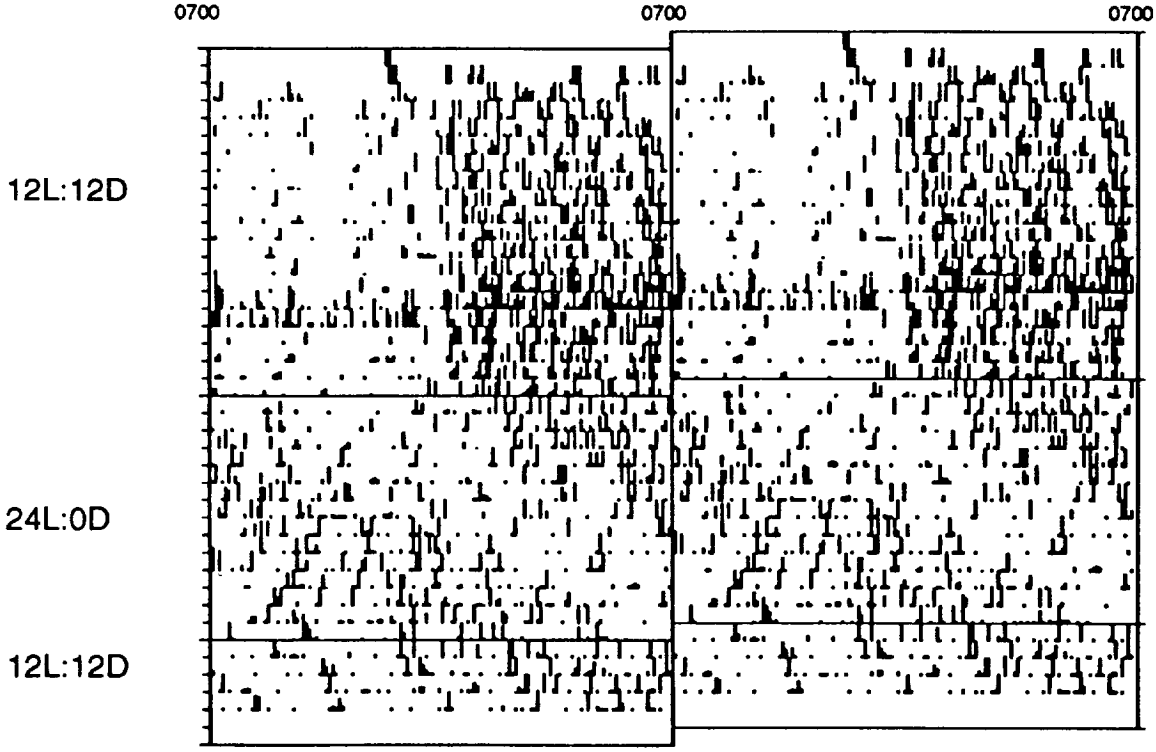
Light experiment 5 (9107Light10) Rat 4 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



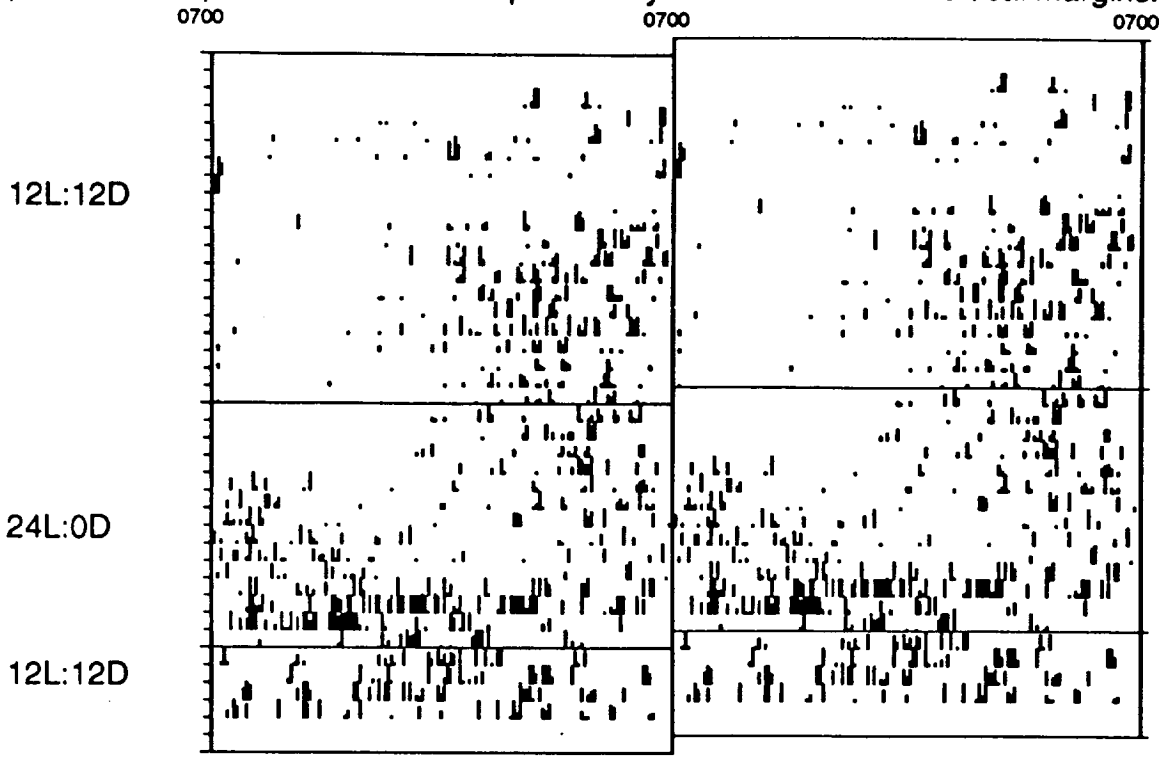
Light experiment 5 (9107Light10) Rat 5 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



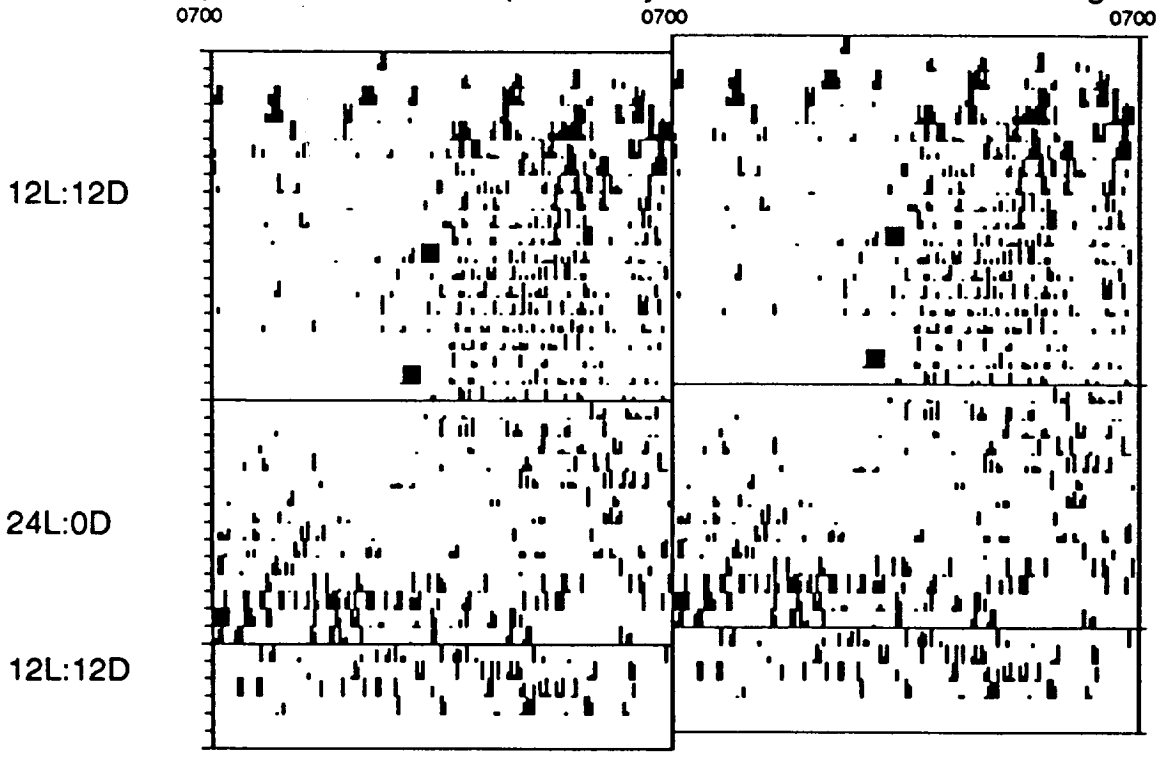
Light experiment 5 (9107Light10) Rat 6 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



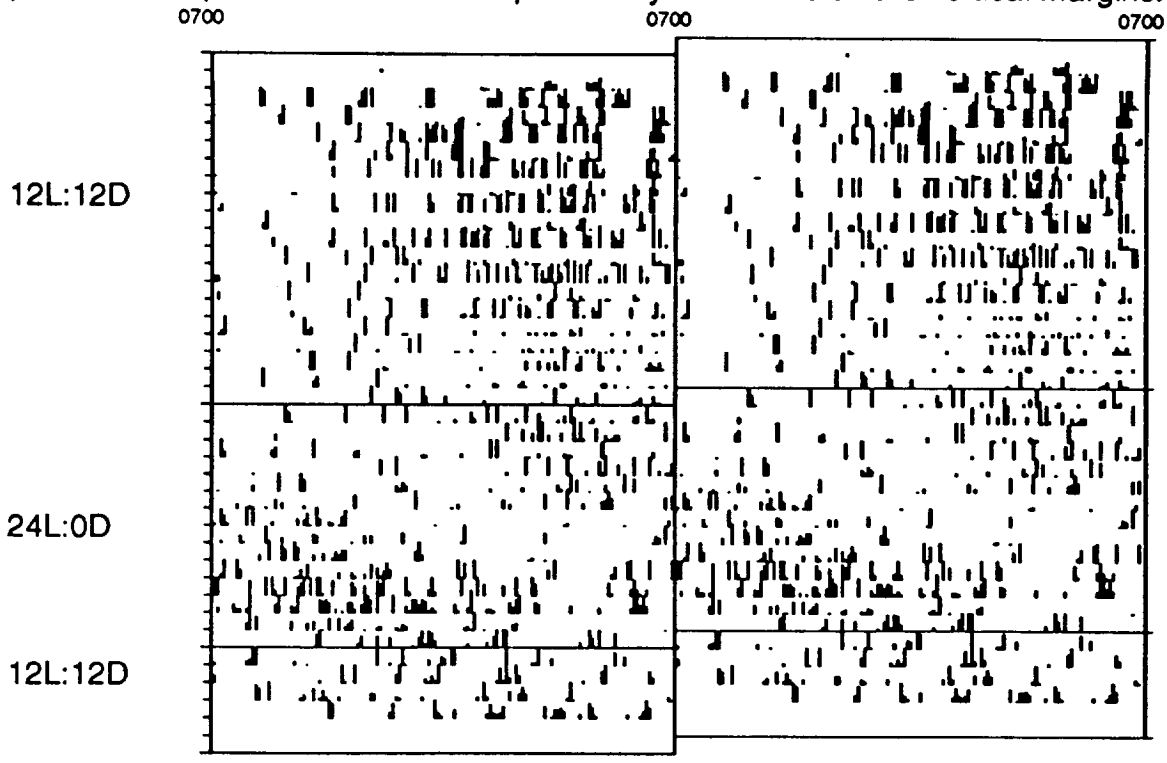
Light experiment 5 (9107Light10) Rat 1 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



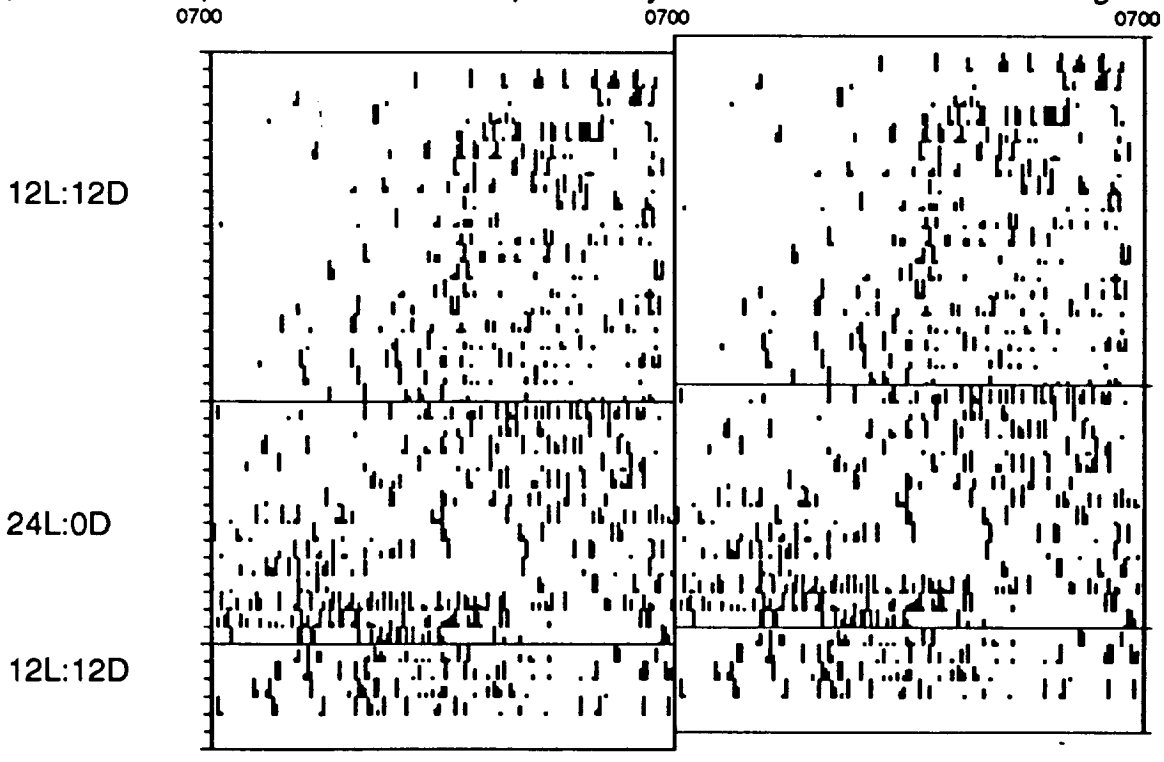
Light experiment 5 (9107Light10) Rat 2 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



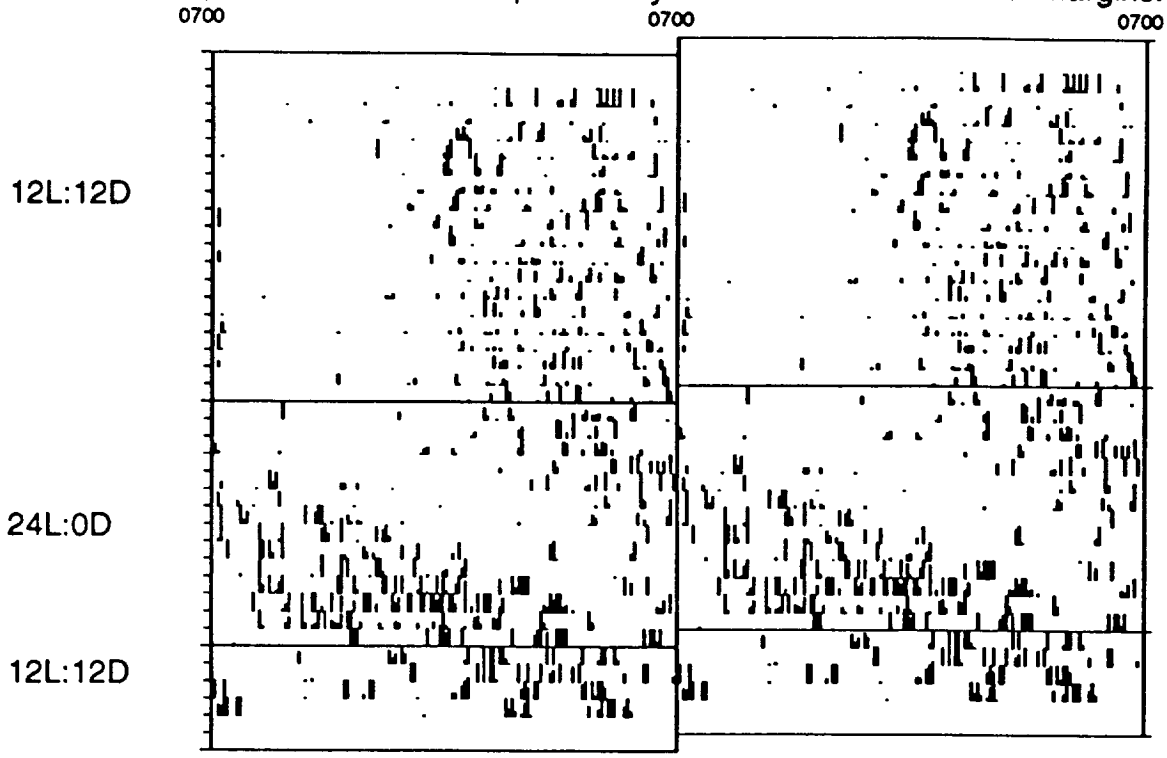
Light experiment 5 (9107Light10) Rat 3 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



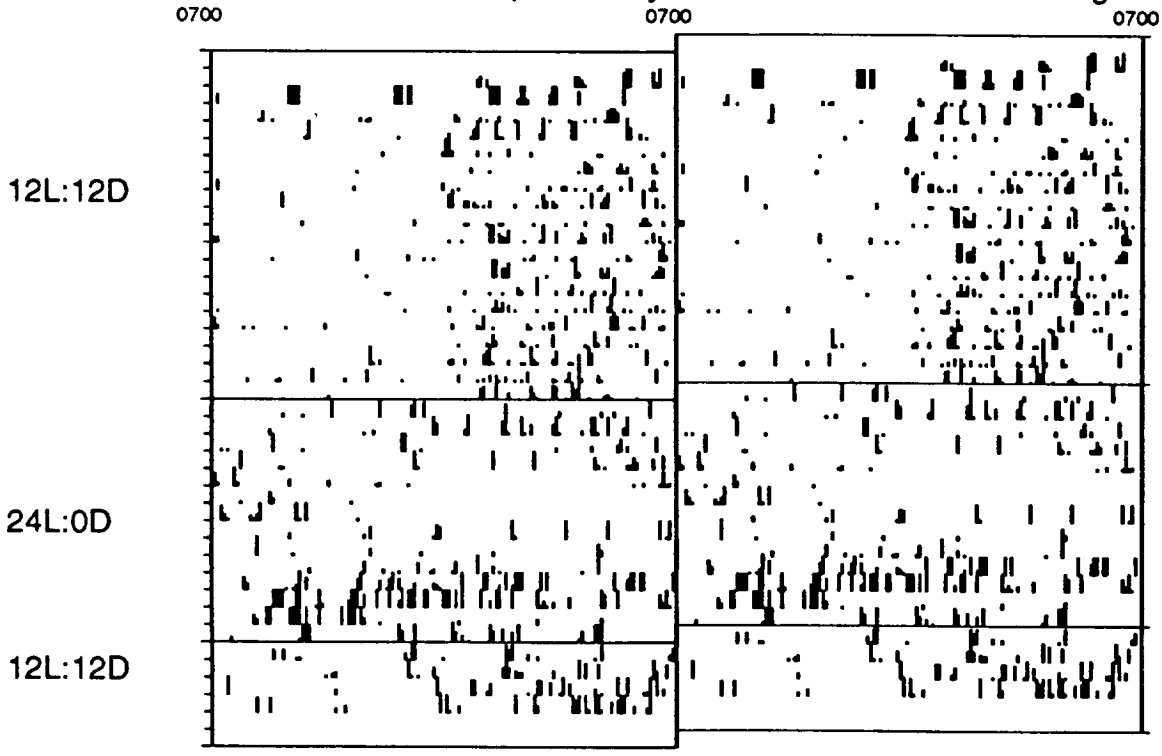
Light experiment 5 (9107Light10) Rat 4 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



Light experiment 5 (9107Light10) Rat 5 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



Light experiment 5 (9107Light10) Rat 6 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



Final Report for NASA COOPERATIVE AGREEMENT #NCC2-593
June 3, 1994
San Jose State University

C) Effects of Sound/Noise on the Circadian System of Rats

Final Subproject Report for Cooperative Agreement #NCC2-593

Final Report for NASA COOPERATIVE AGREEMENT #NCC2-593
June 3, 1994
San Jose State University

Part 1. Sound and its Effects on Circadian Free-Running Periods of Sprague-Dawley Rats

K.A. Moeller, D.C. Holley, C.W. DeRoshia, G. Mele, J. Gott, S. Okumura, C.M. Winget, and P.X. Callahan, 1992

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SOUND AND ITS EFFECTS ON CIRCADIAN FREE-RUNNING PERIODS OF SPRAGUE-DAWLEY RATS.

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This study was conducted to determine if 90 decibel white noise (N) can produce non-photic circadian rhythm entrainment in the rat. Rats (n=6) were housed individually in isolation chambers (22°C, food and water ad lib). They were entrained to 5 Lux (Lx) sunlight simulating light (Vitalite), 12L:12D photoperiod. Subsequently, animals were released to constant dark (DD, <0.01 Lx). Once a stable free-running period was established (8 days), N was delivered to each animal with a period of 12 hrs on/12 hrs off (12N:12Q) while they remained in constant DD conditions. The N regimen consisted of repeating cycles of 2 min. on/13 min. off. This DD-NQ routine lasted 60 d. Gross locomotor activity, feeding, and drinking were monitored continuously. Following about 10 days of rhythm relative coordination, all rats reached a new steady state between days 17 and 25 of N/Q. This steady state period persisted until the sound was switched off on day 60. The period (Tau) during the N/Q regimen was less than or equal to the control periods in all rats, for all parameters (periodogram analysis). The drinking periods during this time varied between 23.7 and 24.0 hrs. This study indicates that periodic 90 dB white noise results in lower than expected periods for rats placed in constant dark (0L:24D controls,Tau= 24.3 hr).(NASA Coop. Agree.#NCC2-593.)

Final Report for NASA COOPERATIVE AGREEMENT #NCC2-593
June 3, 1994
San Jose State University

Part 2. The Effects of Applied Periodic Sound on the Circadian Timing System of the Sprague-Dawley Rat

K.A. Moeller, 1993

Masters Thesis, San Jose State University, San Jose, CA

THE EFFECTS OF APPLIED PERIODIC SOUND ON THE CIRCADIAN TIMING SYSTEM OF THE SPRAGUE-DAWLEY RAT

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San Jose State University

In partial fulfillment

of the Requirements for the Degree

Master of Arts

By

Karen Audrey Moeller

May, 1993

ABSTRACT

We examined whether regularly scheduled white noise (N) (80-90 dB) could synchronize or influence the free-running drinking, feeding, and locomotor circadian rhythms of male Sprague-Dawley rats. Individually housed animals were entrained to a light-dark cycle (12L:12D), with no sound (Q), followed by constant darkness (DD1). Subsequently, a noise regimen (12N:12Q) was administered for 60 days (minimum). A second population of rats was similarly treated, but exposed to a 4N:20Q cycle. Both populations were finally exposed to a DD2 epoch. All rats from the first population and 1/3 of rats from the second population exhibited significantly shortened periods during DD2 relative to periods during DD1. The noise influence on rhythm period was not attributable to "masking effects," as verified in a third study. Our findings indicate that periodic noise influences, but does not entrain, the circadian rhythm of rats.

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INTRODUCTION

Most physiological and behavioral parameters in animals exhibit rhythms with a period of about 24 hours. These circadian rhythms normally synchronize to various periodic environmental stimuli. However, when an animal is subjected to controlled conditions where environmental variables are held constant (e.g. light, food availability, temperature, and sound) the animal will generate cellular and overt rhythms with periods that differ slightly from 24 hours. These rhythms are referred to as free-running and display non-stationary phase angles with regard to sidereal time (Aschoff, 1960).

Photic and non-photic environmental variables have been categorized according to their ability to synchronize physiological and behavioral rhythms; however, light/dark (LD) cycles, are thought to be the dominant environmental zeitgeber entraining biological rhythms in most species, including man (Aschoff et al., 1982; Bruce, 1960; Czeisler et al., 1981). Non-photic zeitgebers including food availability cycles (Aschoff et al., 1983; Edmonds & Adler, 1977; Mistlburger et al., 1990; Sulzman et at., 1977; Rusak, 1989), environmental temperature fluctuations (Sweeney, 1960; Lindberg & Hayden, 1974), barometric pressure changes (Hayden, 1969), electrostatic field cycles (Dowse, 1969) and scheduled volitional exercise (Edgar & Dement, 1991) are reported to synchronize circadian rhythms in various

species.

In addition to the non-photic zeitgebers mentioned above, social interactions are thought to systematically influence the circadian system. Social time cues (visual, acoustical, pheromonal, etc.) have been shown to function as zeitgebers in bats (Marimuthu & Chandreshakaran, 1978, 1979, 1981), mice (*Mus musculus*), antelope, wolf-coyote hybrid, beaver (*Castor canedensis*) colonies, macaque monkeys (Chandreshakaran, 1982), golden hamsters (*Mesocricetus auratus*)(Mrosovsky, 1988), and deer mice (*Peromyscus maniculatus*)(Crowley et al., 1980). However, similar studies performed on the common marmoset (*Callithrix jacchus*) (Erkert et al., 1986), Australian sugar glider (*Petaurus breviceps*) (Kleinknecht, 1985), and the laboratory rat (Borbély, 1982) all failed to show social entrainment. Research using humans has indicated that social factors may act as circadian rhythm zeitgebers in man (Wever, 1979; Winget et al., 1989; Aschoff, 1971).

Studies that have investigated sound as a zeitgeber suggest that acoustical disturbances can entrain birds (Gwinner, 1966; Menaker & Eskin, 1966; Lohman & Enright, 1967; Reebs, 1989), golden hamsters (*Mesocricetus auratus*) (Meyer, 1968), and cats (*Felis catus* L.) (Randall, 1990) but fail to entrain the squirrel monkey (*Saimiri sciureus*) (Sulzman et al., 1977).

In the controlled environment of the proposed U.S. Space Station Freedom, animals will be maintained and studied for extended periods. Simultaneously, crews may have various work-

rest schedules which produce periodic noises. The present study was undertaken to established whether sound or social noises influence the circadian system of the Sprague-Dawley rat. Our findings suggest that periodic noise does not entrain drinking, feeding, or locomotor activity (LMA) rhythms, but may influence the stability of the free-running circadian period.

MATERIALS & METHODS

Animals and apparatus

Male Sprague-Dawley rats, purchased from Simonsen Laboratories (Gilroy, CA) weighing approximately 150 g at the start of the experiment, were housed individually in Nalgene metabolic cages (Nalge Co., Rochester, NY). The cages were placed inside separate ventilated, radio frequency shielded, wooden hives (I.D. 66cm X 66cm X 76cm). These hives were contained in a sound attenuated, environmental chamber at constant temperature (20-22 C°). Light was provided to each hive via broad spectrum fluorescent lights (Vita-Lites, Duratest Corp., Fairfield, NJ) with an intensity of 5.0 ± 1.0(SD) lux measured at the height of the animal's head when standing. Light measurements were taken with a calibrated IL-1700 Research Radiometer (International Lighting Co., Newburyport, MA).

The amplified sound used during this study was produced by a Synthi-AKS (EMS Ltd., London, UK) sound generator with a maximum frequency of 10kHz and was distributed to each hive through individual speakers (Radio Shack #40-1248B, Tandy Corp., TX). Though the white noise function was used, the measured decibel intensity at each octave band frequency (31.5 Hz - 8000Hz) ranged from 70 dB to 92 dB. Therefore, the projected noise should be considered indiscriminate and not pure "white" noise. The sound

intensities were adjusted to 80-90 dB (See Protocols) with a Quest #215 sound level meter and octave band intensities were measured with a Quest octave band analyzer (Quest Electronics, Oconomowoc, WI).

The behavioral rhythms monitored were locomotor activity (LMA), drinking activity, and feeding activity. LMA was recorded using a three-axis accelerometer (Straindyne Eng. Co., Los Altos, CA) which was attached to the cage stand. The stand was positioned atop a plexiglas platform supported by semi-rigid springs which allowed omnidirectional cage movement in response to animal activity. The accelerometer transduces the physical motion of the cage to a voltage that is proportional to the cage displacement (displacement units). This voltage was then digitized and recorded. Drinking activity was recorded by the completion of an electrical circuit that ran from the metal grid cage bottom to the water lick. When the rat drank, the circuit was completed and duration of drinking activity was recorded. Water was available *ad libitum*. Feeding activity was recorded via an infrared light beam located above the food tray (Model #FM8, Omnitech Electronics, Inc., Columbus, OH). When the rat interrupted the beam, the monitoring system recorded duration of feeding activity. Throughout all phases of the investigation food (Wayne Rodent BLOX 8604) was available *ad libitum*.

All monitored activities (locomotor activity, feeding, and drinking) were recorded using a Keithley Data Acquisition System (DAS) located in a room separate from the experimental chamber.

Behavioral events were summed and recorded every ten minutes.

Animal maintenance was performed on alternate days throughout the experiment to minimize disturbances of the animal's circadian system. During the constant dark (DD) epochs of the study animal maintenance was performed under a red light filter (Roscolux RoscoeSun #27 Medium Red, Musson Theatrical, Santa Clara, CA) with 50% cutoff at 660 nanometers; 4% transmission.

Data Analysis

Data collected from all experiments were transferred from the Keithley (MS-DOS based) data acquisition system to a Hewlett Packard 9836 computer for editing, filtering, and time series analysis. Raw data for all parameters was initially processed using a box-and-whisker (Tukey, 1977) editing program which objectively removed outliers, editing an average of 1.04 ± 1.26(SD)% of the data. The feeding and drinking values were then converted from 10 minute sample point values to numbers of non-zero 10 minute sample point values per each one hour bin. The locomotor activity (LMA) data were reduced through decimation. This procedure converts six 10 minute recordings into hourly means.

The data then underwent robust locally weighted regression (RLWR), a non-parametric detrending process (Cleveland, 1979). This filter (72 terms, filters data > 3 cycles) removes low frequency rhythms or trends which may obscure periodicities of

interest. After editing and filtering, the data were subjected to periodicity analysis.

To obtain an accurate estimation of phase and amplitude of the circadian rhythm during the various environmental conditions the data were folded out 20% on each end and then underwent complex demodulation (Sing, 1980). The period of the rhythm (Tau) for each experimental condition was calculated by performing a linear regression on the first determined acrophase (peak time of best fit sine function) of each cycle. The estimated period of the rhythm during each experimental epoch was confirmed by processing the detrended data through FFT spectral analysis (Bloomfield, 1976) with 5 day windows and 2 day moving increments. Experiment 3 data initially underwent Z-score normalization (Zar, 1984) followed by a mean educed cycle procedure. The mean educed cycle averages the data for each rat across each day at each of the 144 ten minute sample points. This smooths the circadian rhythm and allows for visualization of phase-dependent masking effects which would produce peaks or depressions in response to the applied noise/quiet cycle.

Statistical significance of period, phase, and amplitude changes for the entrainment studies were determined through a repeated measures two-way analysis of variance (ANOVA). Significance of changes in activity levels during various conditions in experiment 3 were determined by a four-way repeated measures ANOVA. Null hypothesis was rejected for $p < 0.025$. Data are presented using mean ± SD unless otherwise indicated.

Entrainment Experiments

Experiment 1:

Six rats were acclimated for 14 days to a 12L:12D photo period. The average light intensity was 5.08 ± 0.42 lux. Following acclimation, the experiment began with a 6 day control period having 12L:12D lighting and no applied sound. Baseline ambient noise within the cages was 64.6 ± 3.3 dB. The baseline period was followed by an 8 day period of constant dark (DD-1; <0.01 Lux). After the DD-1 eight day period, the animals were subjected to a 12N:12Q cycle (noise/quiet) while they remained in DD conditions. The sound "on" period began at 19:00 and ended at 07:00. In order to avoid habituation to monotonous sound stimulation intermittent noise was chosen. The twelve hour sound "on" cycle, therefore consisted of 2 minutes ON (83.4 ± 1.34 dB) followed by 13 minutes OFF (64.6 ± 3.3 dB). This schedule lasted for 60 days. Following this regimen, a 32 day DD freerun period (DD-2) was re-established.

Experiment 1 Timeline:

Acclimation	14 days
Control (12L:12D)	06 days
DD-1 (freerun)	08 days
DD-N:Q	60 days
DD-2 (freerun)	32 days

Experiment 2:

The protocol for this experiment differs from the first in that the 2 min. ON:13 min. OFF sound (88 ± 0.82 dB), was applied in concentrated 4 hour periods (4 ON: 20 OFF) starting at circadian time (CT) 10. This schedule was chosen based on Mrosovsky's (1988) non-photic phase response curve (PRC)(See Discussion). Circadian time (CT) is determined by taking the animals circadian cycle and dividing it into 24 equal parts. Each part represents an "hour." By convention, CT 0 is defined as the onset of activity in diurnal animals and CT 12 is defined as the onset of activity in nocturnal animals. In order to expose the rats to sound during their CT 10 - CT 14 window, the rats were monitored during baseline in DD and their mean activity onset (CT 12) was determined . By convention, CT 10 was defined as 2 hours prior to activity onset.

Light intensity was measured in the same manner as in all other experiments with the average intensity measuring 4.95 ± .05 lux. Light intensity was <0.01 lux in DD conditions.

Experiment 2 Timeline:

Acclimation	10 days
Control (12L:12D)	14 days
DD-1 (freerun)	17 days
DD-NQ*	69 days
DD-2 (freerun)	11 days

Masking Experiment

Experiment 3 determined whether sound elicited masking effects in the monitored behavioral parameters. Rats were initially exposed to a 10 day acclimation period in LD. Following acclimation, the rats were monitored for 5 days in 12L:12D with no applied sound. This baseline period was followed by a 6 day 12L:12D light schedule accompanied with a 2 hour ON: 2 hour OFF (ambient) sound cycle. The applied sound cycle (N) was comprised of intermittent "white" noise with a 2 minute ON (89 ± 0.58 dB): 13 minute OFF (65.8 ± 2.7 dB) repeating schedule. By using a periodic sound regimen, any masking effects produced should appear clearly in the educed cycle.

Following the 12L:12D -N/Q epoch the rats were monitored for 7 days in 12L:12D schedule with no applied sound. Subsequently,

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* The sound epoch incorporated the average CT 12 in the middle of the 4 hour administration. Specifically, activity onset was extrapolated to be approximately 21:15 at the end of the first DD epoch. In accordance with this estimation, the sound timer was set to turn on at 19:15 and turn off at 23:15.

the rats were subjected to a DD-N/Q cycle. This routine was similar to the above mentioned noise:quiet cycling schedule and ran for 4 days. Transition of lights, ON/OFF, took place at 07:00 and 19:00, respectively. The sound cycling routine was turned ON at 19:00 on day 1, and turned OFF at 19:00 four days later.

Experiment 3 Timeline:

Acclimation	10 days
Control (12L:12D)	05 days
N/Q - 12L:12D	06 days
Control (12L:12D)	07 days
N/Q - DD	04 days

RESULTS

Entrainment studies

Figure 1 shows double raster plots of the three monitored parameters from a representative animal (animal 2, experiment 1). Entrainment of the rhythms is demonstrated during the LD cycle in all parameters. Upon release to constant conditions (DD1) the average free-running period significantly increased (24.39 ± 0.09 h, ANOVA, $p < 0.025$). In calculating the mean Tau, the period values estimated for rat #4 (LMA) during the LD and DD1 epochs were not used because they were outliers, deviating from the mean by more than 3 standard deviations. All other data were used. The period from the 60 day sound window is represented in 20 day increments (DDNQ-1 = days 1-20, etc.) (Tables 1 & 2 and Figures 3 & 4) for a more accurate description of the period dynamics during that epoch. Within the first twenty days of the applied sound condition (DD-NQ1) the rhythm shows an initial lengthening followed by a shortening of the period which resulted in a "scalloping" appearance of the rhythm (See Fig. 1 a,b,c). Following these transients, Tau gradually decreased 0.38h to an average period of 23.93 ± .13h in the last 20 days (DD-NQ3) of the sound epoch. The second freerun condition following the sound application continued to show a significant decrease in Tau (ANOVA, $p < 0.025$) in all three parameters relative to the initial

free-running period. The final freerun conditions resulted in an average Tau of 23.86 + 0.08h for all rats, all parameters, which represents a 0.45h decrease from the original freerun period (DD1)(Figure 2a,b). Table 1 shows the corresponding period lengths of each rat during all experimental conditions. The 60 day sound window was separated into 20 day increments (DDNQ-1 = days 1-20, etc.) for a higher resolution of the period changes during that epoch.

Figure 3 shows double raster plots from the drinking activity obtained from each animal in experiment 2. Figure 3 b,c,d, & f shows non-stationary circadian rhythms (with respect to phase and period) with similar patterns while Fig.3 a & e show smaller changes in Tau over time. Table 2 shows the periods of the drinking activity corresponding with the raster plots displayed in Fig. 3. Estimated Tau from the feeding activity rhythm corresponded with the estimated Tau of the drinking activity rhythm but is not shown. Due to a technical malfunction, the LMA data for all rats was not available for this portion of the study.

All six rats in experiment 2 exhibited entrainment to the LD cycle. In freerun conditions (DD1), four of the six rats (#2,#3,#4,#6) displayed an average Tau of 24.19 ± 0.08h for the drinking activity rhythm while the other two rats (#1,#5) maintained period lengths of 24.04h and 24.00h for drinking, respectively. During the final freerun condition (DD2), drinking and feeding periods ranged from 23.66 h to 24.38h and from 23.85h to 24.48, respectively.

Figure 4 (a,b,c) shows the mean period length, acrophase, and rhythm amplitude for the drinking parameter measured in experiments 1 and 2. Though not presented, feeding activity and LMA showed similar results.

Figure 4a offers a comparison of the average changes observed in Tau over the length of the two experiments. The graph indicates that experiment 1 rats demonstrated a free-running period that differed more from 24 hours than rats in experiment 2. Experiment 2 results also differed from experiment 1 in that upon presentation of the sound, the rhythm period length continued to increase through the first twenty days of the sound schedule, while experiment 1 shows that the period had already begun to decrease during that time. From the first 20 days of the sound epoch up through the end of the sound epoch, the period from experiment 2 displayed a linear decrease while experiment 1 showed the primary decrease in period occurred between days 21-40 of the sound epoch and then decreased slowly in the final 29 days. The period lengths during the final freerun of the two protocols diverge. Experiment 1 shows that Tau continues to decrease while the mean period in experiment 2 increases back toward the initial freerun period.

Figure 4b compares the mean acrophases of the drinking rhythms between the two experiments during all experimental conditions. A mean drinking acrophase of 264.6 ± 3.3 degrees was obtained during the LD entrainment period (Expt. 1). Corresponding to the increasing Tau during the DD1 free-running condition, the

acrophase delayed to 281.8 ± 5.9 degrees. During the first 20 days of the sound epoch (N/Q 1), the acrophase advanced significantly (ANOVA, $p < 0.025$). This trend continued, for the next 20 days until the mean peak acrophase was 342.7 ± 8.4 degrees. The acrophase then displayed a marked delay during the final 20 days of the sound regimen to a mean of 326.8 ± 22.4 degrees. This trend continued through the 30 day freerun condition to a final mean acrophase of 301.3 ± 24.2 degrees.

Experiment 2 displays trends similar to those represented by the first experiment except in the final phase during the last freerun condition (DD2). The mean acrophase for the LD entrainment condition was 255.9 ± 8.5 degrees. The initial free-running acrophases in this experiment ranged from 255.1 to 287.8 degrees. This range illustrates the variety of acrophases found in the individual rats (See Fig. 3). The mean acrophase showed a maximum delay between days 21-40 of the sound epoch. The delaying acrophase from DD1 to N/Q 2 displayed a slope that was greater than that seen during the free-running condition. The mean acrophase advanced during the last N/Q 3 window. Standard error for the mean during this time was large due to the fact that the rhythms of the individual rats varied markedly (Fig. 3). From the graph it is clear that phase control was not demonstrated by the zeitgeber in either protocol.

Figure 4c illustrates the trend of the rhythm amplitude over the course of the two experiments. The mean amplitudes from both experiments decrease at nearly the same rate from LD to DD1

condition. Experiment 1 showed a linear decrease in the amplitude throughout the sound epoch, followed by a slight increase in amplitude during the final DD2 condition. Experiment 2 displayed a decrease in the drinking rhythm amplitude up through the first 40 days of the sound window (Fig 4c). The decrease, however, was not linear; amplitude dropped 0.46 units (number of events/hour) from DD1 to N/Q 1, but only decreased by 0.09 units from N/Q 1 to N/Q 2. The last 29 days of the sound window (N/Q 3) showed a significant increase in the rhythm amplitude (ANOVA, $p < .025$) relative to the previous 20 days (N/Q 2). The total decrease of the mean rhythm amplitudes from the start to the end of both experiments are not statistically significant.

Masking Experiment

Figure 5 compares the mean LMA levels of the 12L:12D epoch with the 12L:12D; 24 N/Q regimen (See Methods). The mean amplitude for the group (n=6) was determined by Z-score normalization of the values.

The masking effects produced by the light cycle combined with the underlying circadian rhythm of the LMA are evident by the marked changes in the activity levels. In the dark, without the sound application, the mean activity level was 3223.83 ± 1.51 (S.E.M.) displacement units (d.u.) (See Methods). This value is significantly higher than the mean activity value, 3178.49 ± 1.60 (S.E.M.) d.u., found when the rats were exposed to light (ANOVA, p

< 0.025). The mean activity level of the animals during 12L:12D, 2 hour "on" sound schedule, was 3190.89 ± 1.64 (S.E.M.) d.u.. The mean activity value for the 12L:12D no applied sound was 3194.08 ± 1.65 (S.E.M.) d.u.; these values do not differ significantly. Had sound produced a masking effect, two hour peaks or depressions in the mean activity that correspond with the administered sound cycle would be evident in the educed cycle.

Table 1. Experiment 1. Periods (hours) for each rat, during each experimental condition.

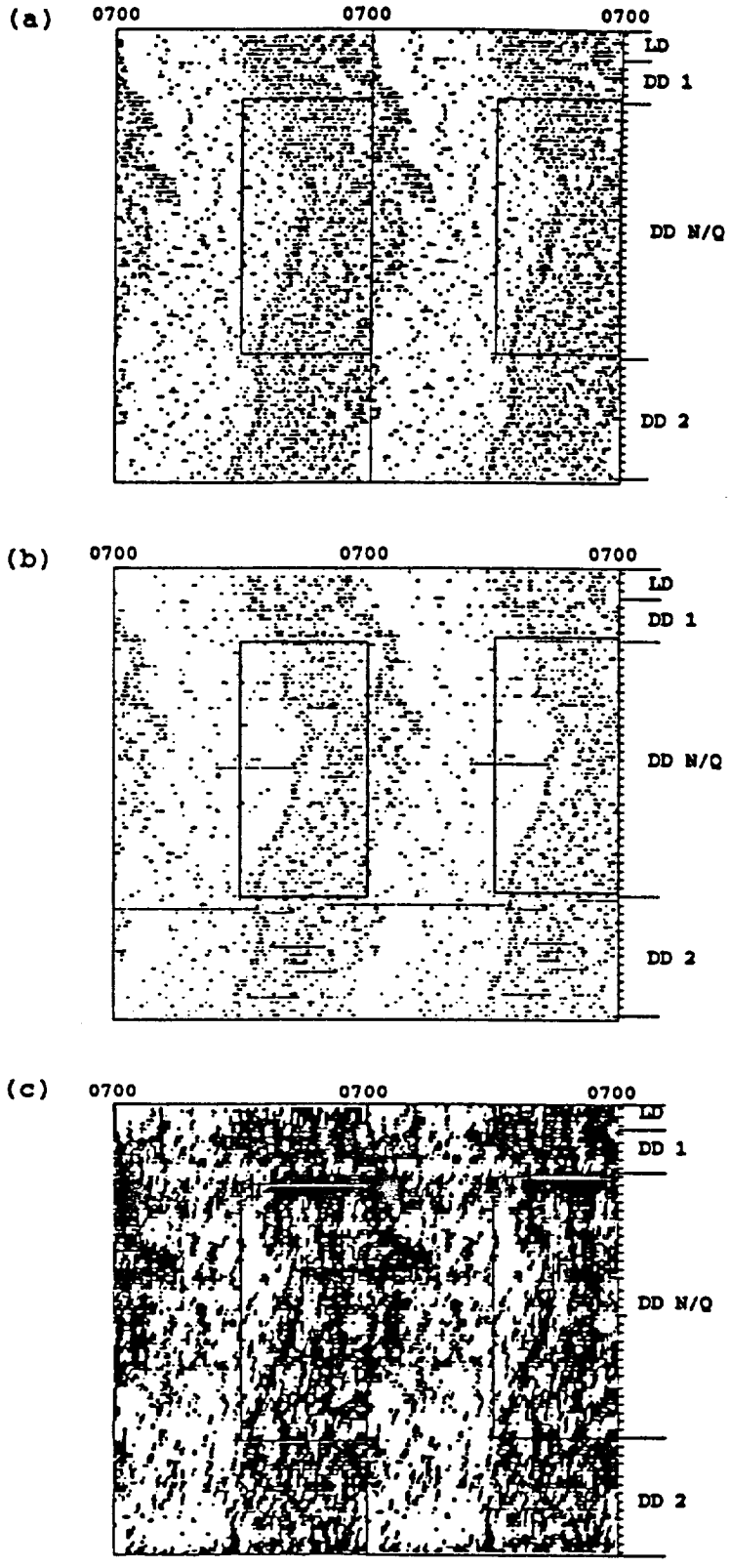
Rat #	1	2	3	4	5	6	mean ± S.D
LD	23.87	24.00	23.83	24.07	23.65	23.83	23.88 ± 0.14
DD1	24.43	24.39	24.32	24.25	24.39	24.40	24.36 ± 0.07
DD/NQ-1	24.06	24.19	24.20	24.20	24.11	24.14	24.15 ± 0.06
DD/NQ-2	23.96	24.01	24.00	23.94	24.00	23.93	23.97 ± 0.03
DD/NQ-3	24.00	23.87	23.97	23.97	24.00	23.94	23.96 ± 0.05
DD2	23.96	23.84	23.92	23.77	23.82	23.87	23.86 ± 0.07

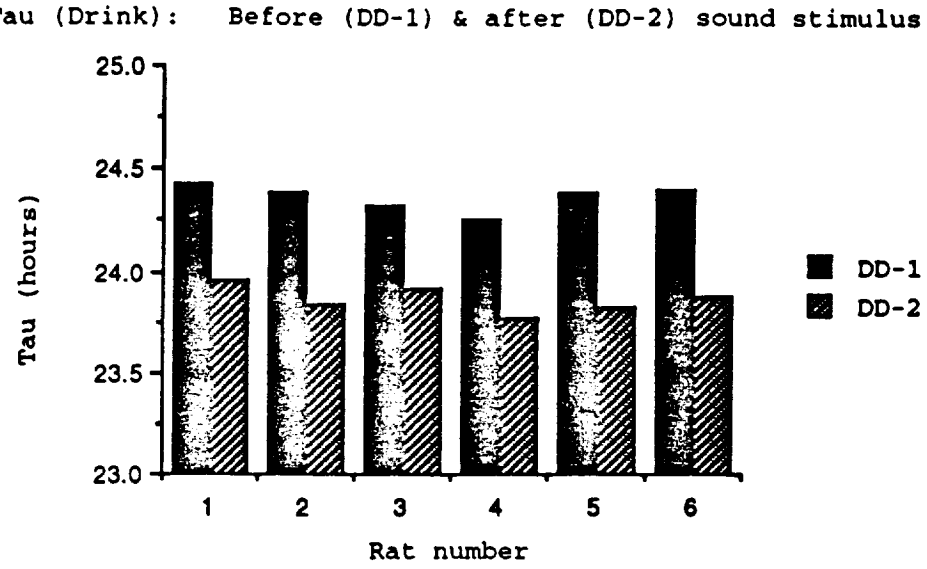
Table 2. Experiment 2. Periods (hours) for each rat, during each experimental condition.

Rat #	1	2	3	4	5	6	mean ± S.D
LD	24.00	23.93	23.93	23.88	23.97	24.00	23.95 ± 0.05
DD1	24.04	24.16	24.28	24.20	24.00	24.10	24.13 ± 0.10
DD/NQ-1	24.07	24.24	24.31	24.20	24.09	24.22	24.19 ± 0.09
DD/NQ-2	23.97	24.13	24.15	24.15	24.08	23.97	24.08 ± 0.08
DD/NQ-3	23.81	24.06	24.08	24.00	23.83	23.90	23.95 ± 0.11
DD2	24.38	23.72	24.15	23.66	24.38	24.24	24.09 ± 0.32

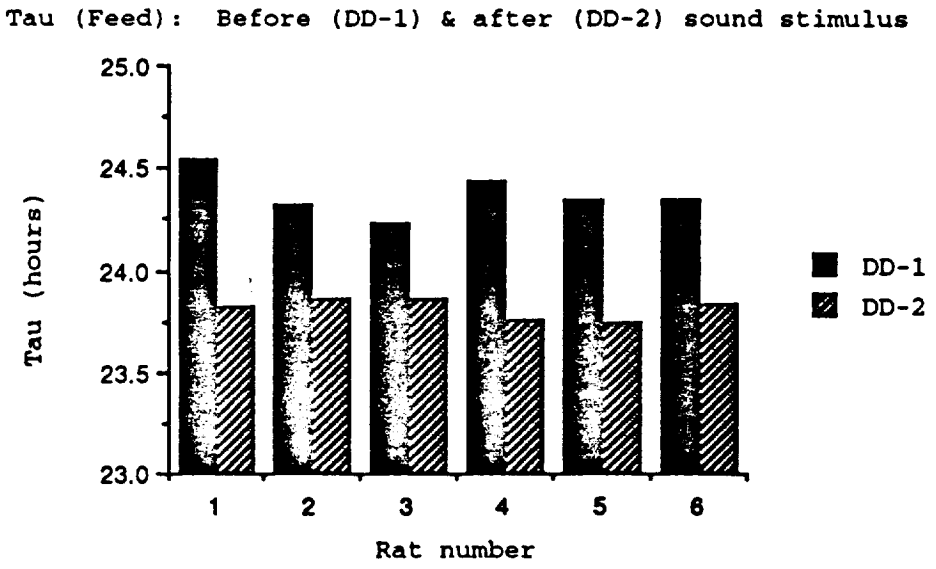
Table 1 & 2. Tau (h) for drinking activity rhythm across all experimental conditions. LD-12L:12D; DD1- 24D, first free-run condition; DD/NQ(-1,-2,-3) 12N:12Q cycle separated into three sections: first, second, third; DD2- 24D, final freerun condition.

Fig. 1(a,b,c). Standard raster plot from experiment 1 of drinking, feeding, and LMA, respectively (rat #2). Activity from each day is shown by vertical tick marks plotted horizontally with respect to time of day in hours. For optimum rhythm visualization the record has been double plotted. The light entrainment period is designated by LD; first freerun condition is designated by DD1; sound application (DD N/Q) epoch is represented by the boxed areas; and the final freerun is designated by DD2. Dotted horizontal lines (Fig. 1b) represent blockage of the feed tray sensors.





(a)



(b)

Figure 2(a,b). Histogram of estimated periods obtained from each rat in experiment 1 before and after the sound regimen was administered. (a) drinking data, (b) feeding data.

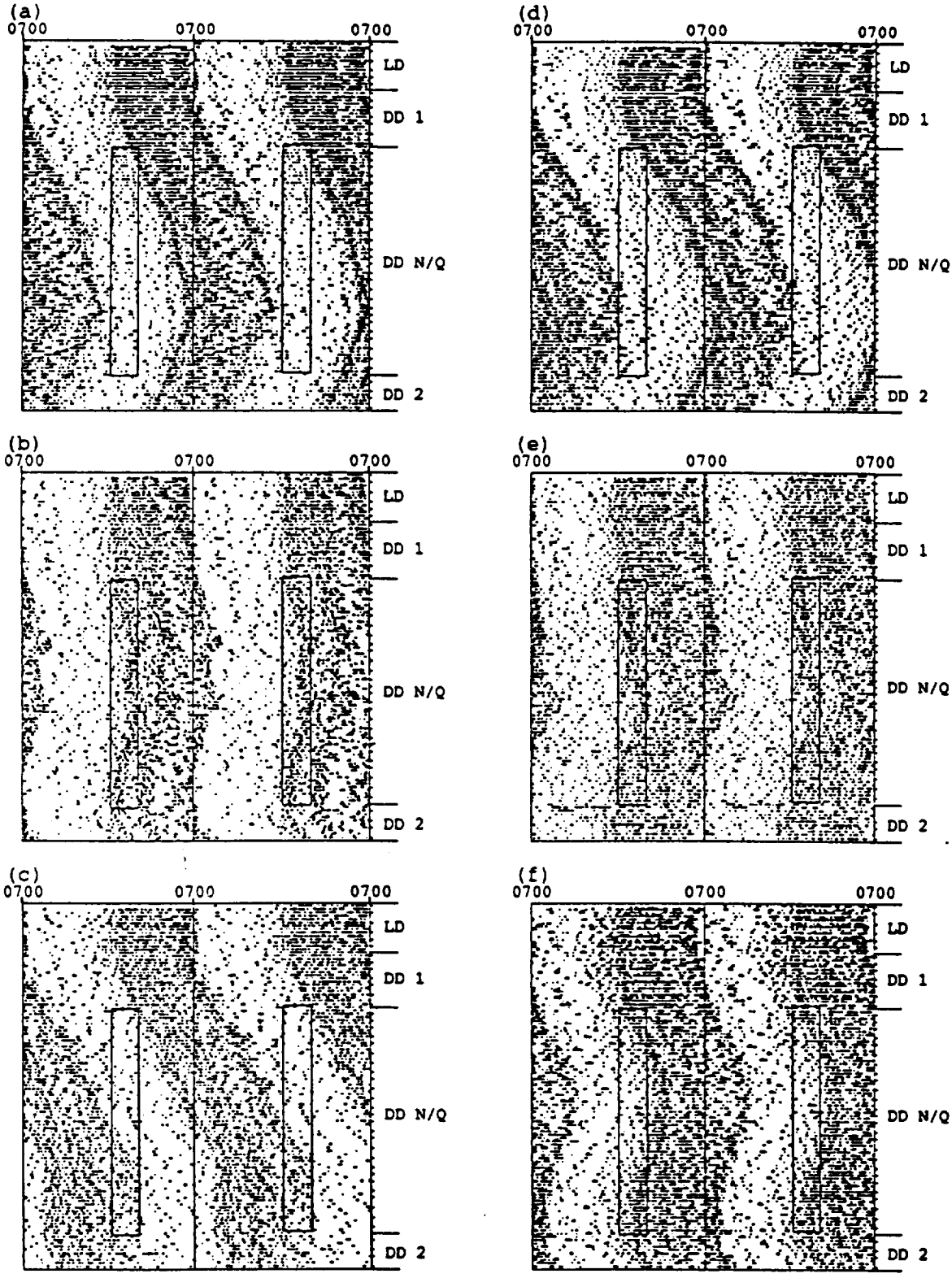


Figure 3a-f. Double plotted actograms of experiment 2 drinking activity for all six rats. Boxed areas indicate when sound was on.

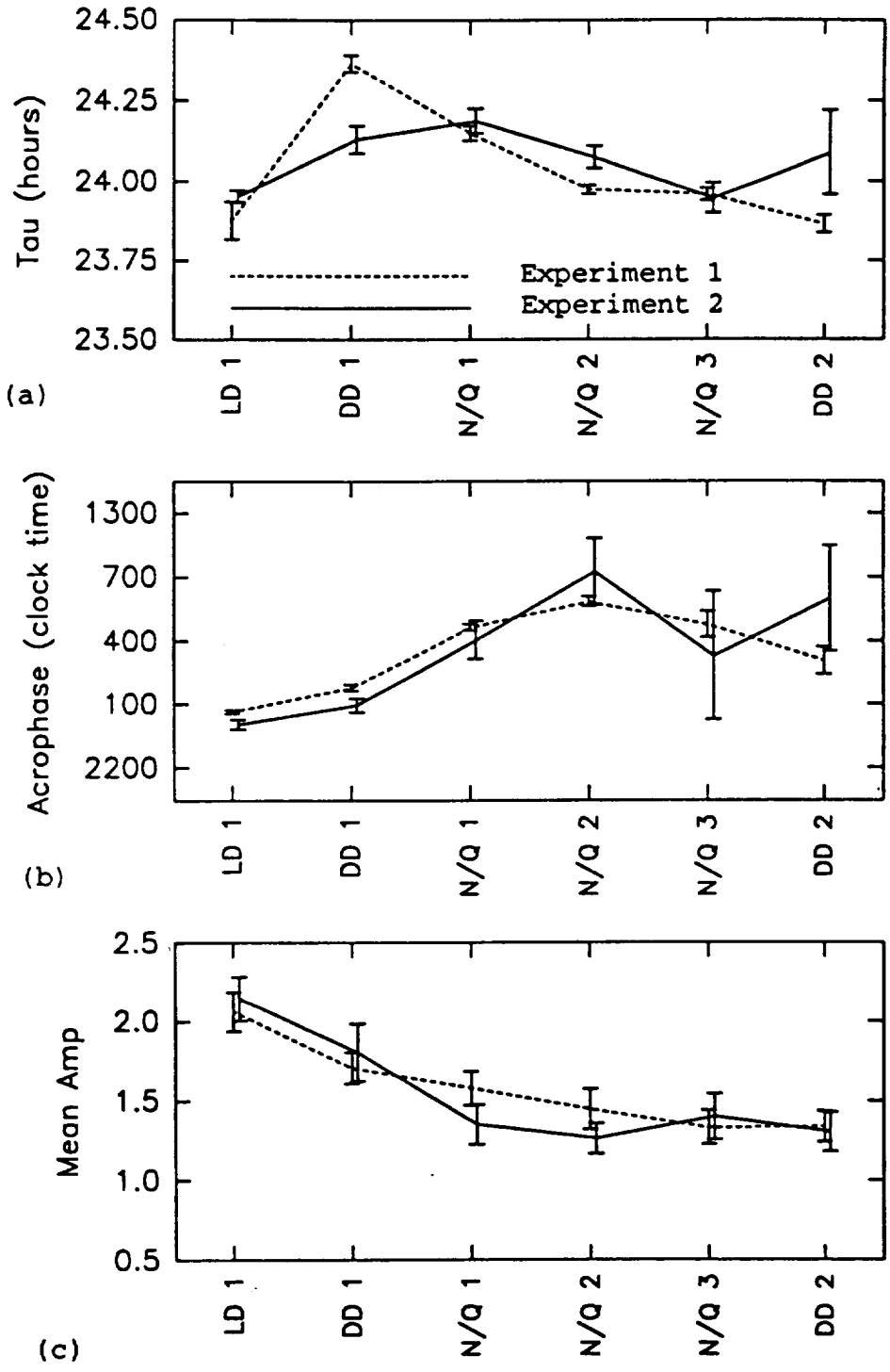


Figure 4. Comparisons between experiments 1 and 2 of mean and standard error of drinking activity values during each experimental condition. (a) Tau (hours), (b) acrophase (clock time), (c) amplitude (number of drinking events per 1 hour bin). Dashed line represents experiment 1. Solid line indicates Experiment 2.

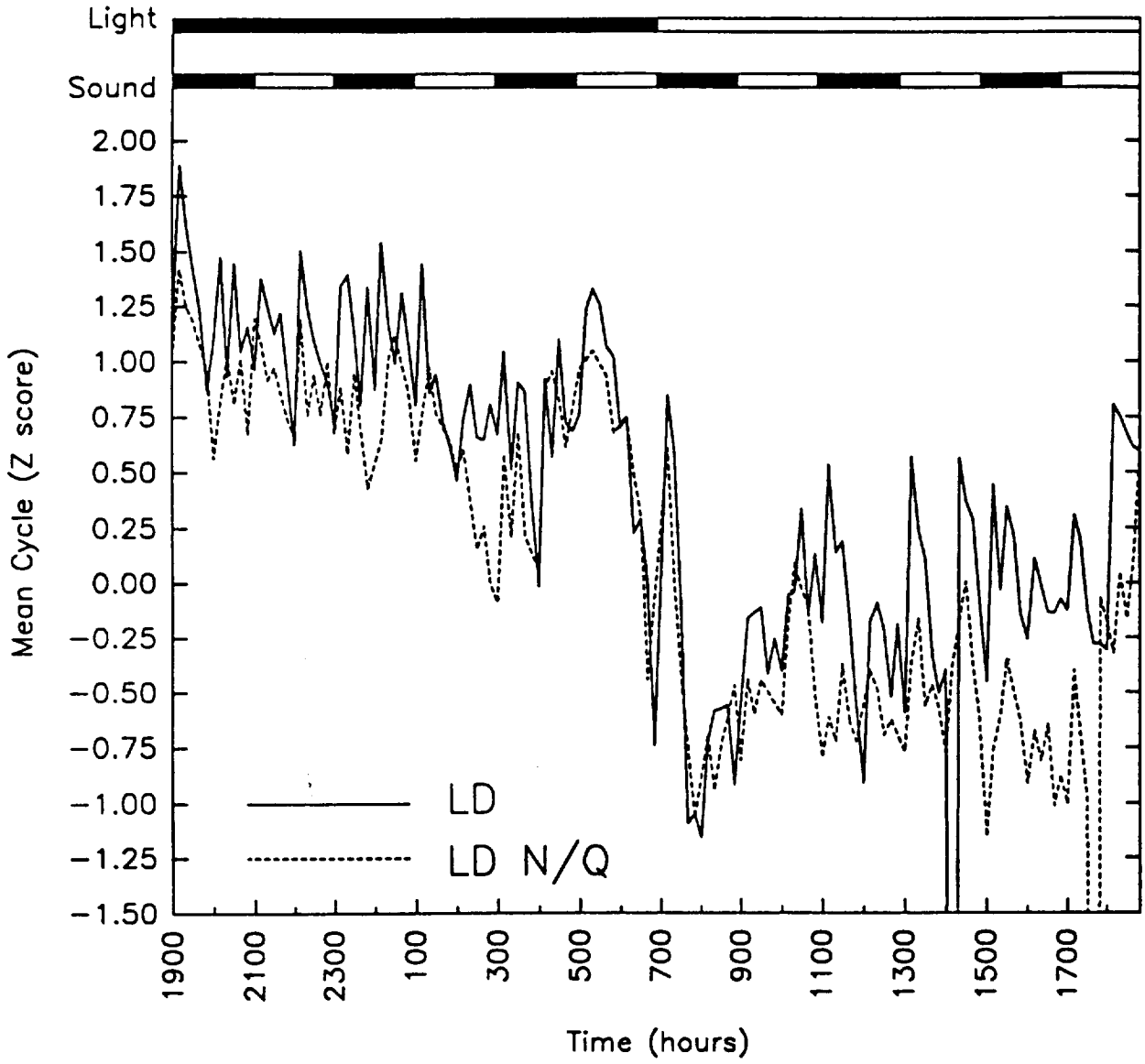


Figure 5. Experiment 3 mean cycle of LMA. Bars indicate light and sound schedules. Lights off denoted by dark bar. Sound on denoted by dark bar.

DISCUSSION

Our findings indicate that sound applied periodically can influence the circadian timing system of the Sprague-Dawley rat. In response to the imposed sound cycles, the circadian rhythm of activity/behavior expressed initial instability in period and phase (Experiment 1) followed by a shortened period; some periods measuring <24h.

Upon release from the sound regimen back to free-running conditions all six rats in experiment 1 and two rats from experiment 2 continued to display periods of <24h. This continuation of shortened periods following the removal of the stimulus supports the notion that the sound cycle disturbed the timing process of the underlying circadian pacemaker causing aftereffects in the circadian rhythm (Pittendrigh, 1960).

Experiment 3 was performed to determine if the observed shifts in phase and period were expressions of a masking effect in response to the applied sound. This experiment showed no significant indication of the presence of this type of effect. Moreover, it can be argued that if the endogenous pacemaker remained unaltered by the noise, upon removal of the sound the rhythm would resume its previously established free-running period length (>24h) from the phase predicted by the endogenous clock and not by the environmental cycle. It did not.

The amplitude of the rhythms in the entrainment experiments

declined at the same rates over the first freerun epoch (Fig. 4c). During the sound regimen and thereafter, however, the decrease of the amplitude did not remain constant. Speculatively, this could be due to some interactive property of sound on the strength of the rhythm. However, this cannot be conclusively explained until an experiment is performed to show the change in amplitude of the rhythm as a function of time.

An intriguing aspect of the results is that the period length shortened to < 24 h in 8 out of 12 rats exposed to the exogenous sound stimulus. Normally, Sprague-Dawley rats only show a Tau of <24 h as an aftereffect to short photoperiods (Stephan, 1983). Wistar rats, which also normally freerun with periods of >24h, have also been shown to express profound decreases in their free-running period (<24h) in response to intercerebral ventricular administrations of serotonergic agonists (Edgar et al., 1993). In addition to these findings, it has been shown that auditory stimulation leads to excitation, without habituation, of serotonergic cells in the Raphe Nucleus in cats (Rasmusson, 1984, 1986). If this information can be extrapolated to the rat, a possible neuromodulatory mechanism can be proposed: sound stimulation excites serotonergic cells in the Raphe Nuclei which sends ascending, efferent projections to the SCN (Steinbusch, 1981) thus providing information to the primary circadian pacemaker. This proposed hypothesis is not specific to sound (e.g. several investigators suspect serotonin mediation of exercise-dependent non-photic zeitgebers (Edgar et al., 1993)), but

provides a possible mechanism consistent with Mrosovsky's (1988) proposed hypothesis for a non-photic "non-specific, arousal oscillator" which may influence the primary photic oscillator. Regardless of the actual mechanism, it is apparent that the circadian rhythm periods of the overt behaviors change when subjected to a temporal acoustic disturbance.

In this study, noise cycles did not meet all four standard criteria for entrainment (Moore-Ede et al., 1982). First, the overt rhythms must show a free-running period, independent of the environment, before the putative entrainer is administered and again after the temporal cue is removed. Second, in conjunction with the stimulus, the period of the monitored rhythm must adjust to become equal to that of the stimulus. Third, a stable phase relationship must be demonstrated. That is, the phase angle of the rhythm must be reproducible and constant in relationship to the timing of the zeitgeber. And finally, the zeitgeber must show phase control until it is removed. Once removed, the rhythm should begin free-running from the time in which it was being held.

In the first experiment of the entrainment study the majority of animals showed a decrease followed by an increase in Tau during the first 20 days of the sound epoch. Though "entrainment" was not evident, the resulting "scalloping" in the rhythm may indicate relative coordination (Pittendrigh & Daan, 1976). The design of experiment 2 was based on: 1) the findings of Menaker and Eskin (1966) which indicated that noise entrainment

was best achieved when the zeitgeber was presented at or near the onset of the animal's activity, and 2) information provided by the non-photic phase response curve (PRC) proposed by Mrosovsky (1988). According to this PRC, a zeitgeber applied between CT 10 and CT 14 would not allow for entrainment until the free-running rhythm advanced, or delayed, sufficiently to coincide with a position on the PRC that would result in rhythm phase control. Experiment 2 showed transient shifts in Tau late in the sound epoch but did not produce a "scalloping" of the rhythm as observed in experiment 1. This could possibly be a function of zeitgeber duration. The 12h sound application interacts with more phases of the animal's rhythm, thus producing a mixed combination of phase advances and delays. The 4h sound application interacts with a smaller portion of the animal's rhythm, possibly accounting for the smaller net changes in phase and lack of scalloping in the data. Alternatively, the strength of the zeitgeber during the second experiment of the entrainment study may have been weaker due to the shorter stimulus duration.

The inability to show white noise-dependent entrainment in rats is inconsistent with the results of related studies performed using birds. For example, Siskins (*Carduelis spinus*), Serins (*Serinus serinus*), and House sparrows (*Passer domesticus*) were able to entrain to species-specific song cycles (Gwinner, 1966; Menaker and Eskin, 1966; Reebs, 1989). In a study using greenfinches (*Carduelis chloris*) and chaffinches (*Fringilla coelebs*), Lohman & Enright (1967) removed the "social" aspect of

the acoustical zeitgeber by replacing the species-specific song cycles with cycles of continuous and intermittent noise produced by electrical buzzers and frequency generators. These birds showed weak entrainment to both types of sound stimuli. Thus, it is not the content of the sound stimulus that is crucial for entrainment, rather it is the temporal stimulation of the auditory pathways which is the crucial factor. Since our study did not demonstrate entrainment of rat circadian rhythms to electrically generated white noise, it is plausible that the ability to synchronize to general sound stimulation is specific to birds.

Birds released from sound entrainment consistently demonstrate lengthening of Tau (Menaker & Eskin, 1966; Reebs, 1989). In contrast, Tau continued to shorten after the stimulus was removed in our rats. It is likely that these period effects reflect latent influences of the preceding periodic stimuli although additional studies are needed for a definitive conclusion.

Reebs (1989) has reported an "acoustical" PRC for birds (*Passer domesticus*) using single, 2h "pulses" of conspecific vocalization. That PRC suggests minimal changes in phase between CT 0 and CT 18 and suggests that advances occur between CT 18 and CT 24. Unfortunately, however, that PRC lacks sufficient data to be unequivocal.

Only a few studies have addressed acoustical entrainment using mammals (Meyer, 1968; Randall et al., 1990; Sulzman et al., 1977). Sulzman et al.(1977) examined the ability of 12h

intermittent (2 min. ON:13 min. OFF) white noise to entrain the circadian rhythm of the squirrel monkey (*Saimiri sciureus*). Our first experiment (Exp. 1) was designed similarly to that of Sulzman et al., except that they used diurnal squirrel monkeys (*Saimiri sciureus*) which were held in constant light and subjected to a 10 day sound epoch. Acoustical entrainment was not observed in the squirrel monkeys, although one of the three experiments showed a shortening of Tau amongst the animals (Sulzman et al., 1977). Thus it would appear that nocturnal and diurnal mammals may respond similarly to periodic acoustical stimuli.

Randall et al. (1990) studied sound entrainment in cats. They subjected 4 individually isolated cats to an 8 hour recording of cat colony sounds during maintenance. In response to this daily stimulus, 3 of the 4 cats showed "weak" entrainment of their locomotor activity rhythms. The fourth cat demonstrated relative coordination. All cats showed immediate phase control when the sound stimulus onset corresponded with the activity onset. Though our study did not show phase control when the stimulus was presented during the activity onset, the Randall et al. (1990) findings support those reported by Menaker & Eskin (1966), specifically that entrainment occurred more readily when the onset of the sound stimulus corresponded with the onset of activity.

The lack of period stability during the course of this study need not be attributed to the effects of sound alone. To the best of our knowledge, the longest freerun study performed on Sprague-Dawley rats in constant darkness was 44 days which resulted in an

average Tau greater than 24 h (Stephan, 1983). It is not clear whether Tau remains stable beyond six weeks. Since it has been established that aging tends to shorten circadian free-running periods (Pittendrigh & Daan, 1974) and our animals were maintained in constant dark (DD) for 110 days, additional work may be necessary to differentiate longitudinal study effects from the effects of periodic acoustical stimuli.

In summary, we have found that temporal noise disturbances can influence the circadian pacemaker in the rat. Changes in phase in response to the noise stimulation ultimately resulted in profound rhythm variability and decreases in Tau, the latter persisted after the stimulus was removed.

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Final Report for NASA COOPERATIVE AGREEMENT #NCC2-593
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San Jose State University

Part 3. Experiment protocols, Methods, and Data

Effects of Sound on the Circadian System of the Rat

FINAL REPORT of sub project for COOPERATIVE AGREEMENT #NCC2-593.

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Effects of Sound on the Circadian System of the Rat
Final Subproject Report 06/02/94
(Cooperative agreement NCC2-593)
San Jose State University

INTRODUCTION

This series of experiments was intended to determine whether periodic sound can affect the circadian timing system of white laboratory rats.

MATERIALS AND METHODS

All experiments were conducted in a temperature and humidity controlled environmental chamber. Within this chamber six ventilated radio frequency shielded 3/8" plywood cabinets (I.D. 66 cm x 66 cm x 76 cm) each held one Nalgene plastic metabolism cage. Each metabolism cage held one Sprague-Dawley rat for a total of six animals per experiment. Light levels from Vita-lite (Duro-Test Corp., Fairfield, NJ) broad spectrum fluorescent lights were adjusted to 5 ± 1.0 (SD) lux measured at the height of a test animals head when standing. We measured light intensity with a calibrated IL-1700 Research Radiometer (International Lighting Co., Newburyport, MA). A Keithley Series 500 Data Acquisition System monitored gross locomotor activity, drinking duration, and feeding duration. A Sperry PC (80286/MS-DOS) recorded one set of parameters onto floppy disks every 10 minutes. Applied noise in all experiments consisted of 90 dBA noise with the characteristics shown in Figure 1 applied in 15 minute cycles (2 minutes noise on, 13 minutes noise off) for the duration of any "sound on" period. Sound levels were measured with either a Quest #215 sound level meter or a calibrated Brüel and Kjær type 2219 sound level meter. A Quest octave band analyzer (Quest Electronics, Oconomowoc, WI) measured sound spectral characteristics.

SOUND PROTOCOL 1

Sound experiment protocol 1 was designed to demonstrate entrainment to periodic noise during continuous low light conditions (5 lux, constant light). We conducted two experiments, #1.1 (9002Sound05) and #1.2 (9007Sound05), using this protocol. These two experiments consisted of four time periods, each period with light and/or sound stimuli differing from the previous period. The total length of each experiment respectively was 56 and 76 days. The rats were first subjected to respectively 14 and 23 days of light with a 12L:12D cycle and no applied sound. The lights came on at 0700 and went off at 1900. Period 2 consisted of respectively 15 and

18 days of constant light (24L:0D) with no applied sound. Period 3 was respectively 13 and 16 days of constant light (24L:0D) with the previously defined sound cycle applied on a 12N:12Q noise:quiet cycle at an intensity of 90-92 dB. Sound came on at 0700 and went off at 1900. Period 4 consisted of respectively 14 and 10 days of light with a 12L:12D cycle with applied sound the same as period 3. Both light and sound started at 0700 and ended at 1900. Ambient noise (mostly fans) during times with no applied sound was 65 to 70 dB.

SOUND PROTOCOL 2

Sound experimental protocol 2 was designed to demonstrate entrainment to periodic noise during conditions of no light (<0.01 lux). We conducted four experiments, #2.1 (9011Sound05), #2.2 (9102Sound05), #2.3 (9112Sound05, rats #4, #5, and #6 only), and #2.4 (9205Sound05), using sound protocol 2. The total length of each experiment respectively was 66, 120, 123, and 110 days. Each experiment consisted of 4 or 5 time periods. The rats were first subjected to respectively 35, 18, 23 and 14 days of light with a 12L:12D cycle and no applied sound. The lights came on at 0700 and went off at 1900. Period 2 consisted of respectively 7, 10, 8, and 17 days of constant dark (0L:24D) with no applied sound. Period 3 was respectively 10, 60, 60, and 68 days of constant dark (0L:24D) with the previously defined sound cycle applied on a 12N:12Q noise:quiet cycle at an intensity of 90-92 dB. Sound came on at 1900 and went off at 0700. Period 4 consisted of respectively 8, 32, 32, and 11 days of constant dark (0L:24D) with no applied sound. The first experiment using protocol 2, #2.1 (9011Sound05), contained a fifth time period with a light cycle of 12L:12D.

SOUND PROTOCOL 3

Sound experiment protocol 3 was used to determine whether sound elicited any masking effects in the measured parameters. We conducted one experiment, #3.1 (9109Sound05), lasting a total of 33 days using this protocol. The protocol consisted of 4 time periods. The rats were first subjected to 17 days of light with a 12L:12D cycle and no applied sound. The lights came on at 0700 and went off at 1900. Period 2 consisted of 6 days of light with a 12L:12D cycle with the previously defined sound cycle applied on a 2N:2Q noise:quiet cycle at an intensity of 90-92 dB. Sound cycling started at 1900 and stopped at 0700. Period 3 was 7 days of light with a 12L:12D cycle

and no applied sound. Period 4 consisted of 3 days of constant dark (0L:24D) with the previously defined sound cycle applied on a 2N:2Q noise:quiet cycle at an intensity of 90-92 dB. Sound cycling started at 1900 and stopped at 0700.

SOUND PROTOCOL 4 (CONTROL)

Sound protocol 4 was used to determine the effect of long term darkness on rat circadian rhythms. We compared sound protocols 1 and 2 to this one in order to verify that the observed modifications to circadian rhythms were caused by the applied sound and not as a natural result of rhythm degradation over time. Two experiments used protocol 4, #4.1 (9306Sound05), and #4.2 (9112Sound05, rats #1, #2, and #3). The total length of each experiment respectively was 123 and 106 days. Each experiment contained 2 time periods. The rats were first subjected to respectively 23 and 17 days of light with a 12L:12D cycle and no applied sound. The lights came on at 0700 and went off at 1900. Period 2 consisted of respectively 100 and 89 days of constant dark (0L:24D) with no applied sound.

DATA ANALYSIS

Actograms (raster plots) were produced for all measured parameters for each rat. The abscissa represents a time span of 48 hours. We used the following criteria to prove entrainment (from Dale M. Edgar, Sleep Disorders Center, Stanford University, personnel communication, 1991):

1) Prior to presentation of the entraining stimulus the organism must demonstrate a free-running rhythm with a period independent from the entraining stimulus: this free-running period must be restored once the entraining stimulus is removed.
2) The entraining stimulus must result in period control such that the period of the rhythm must systematically adjust to the period of the stimulus.
3) A constant and stable phase relationship must be achieved between the entraining stimulus and the circadian rhythm being monitored.
4) The phase of the circadian rhythm must be determined by the stimulus and not by the rhythm prior to entrainment (proof of phase control), i.e. if the entraining stimulus is removed, the organism should free-run from the time of stimulus removal and should not phase-jump to a phase that would have existed had the stimulus not been applied.

5: The endogenous wave form of the observed (monitored) circadian rhythm must be distinguishable from any masking effects which may be imposed by the entraining stimulus. [This distinguishes a coincidental effect of the stimulus on the overt measured rhythm that is not due to actual phase control being exerted by the physiological biological clock mechanism of the animal. An example of this would be the animal moving or jumping in response to the noise coming on at the same time each day because it was startled, and not because of a biological clock control signal.]

Entrainment of the observed parameters was determined by examination of each actogram and by the period determination method described below. Rats should be entrained to a 24 hour cycle by the 12L:12D light cycle of period 1. During period 2 during conditions of constant light or dark the rats circadian cycle should be longer than 24 hours. The length of this free-run period is determined by the intensity of light present (Aschoffs Rule). If sound is an entraining element then the 12Q:12N quiet/noise cycle of period 3 should entrain the rat to a 24 hour daily cycle with a stable phase relationship to the applied sound. The 12L:12D light cycle of period 4 should re-entrain the rats daily rhythm to the same phase as during time period 1.

Entrainment was also determined by the following method which compared the period length, amplitude, and phase of circadian and entrained cycles and applied the preceding rules for entrainment. The procedure consisted of steps to select data, replace missing data points, smooth and detrend data, and calculate rhythm parameters.

For each experiment we examined all actograms to find a set of data sections to analyze. The analysis procedure needed sections of data of equal length from each time period. Data sets needed to be an integral number of days and cover the same time period for all rats. Data sections did not include the first two days of a time period unless this forced a data section to be shorter than 7 days. In addition, sections of the record with missing data points or sections of the feeding record where the sensor was blocked were excluded if possible. We also excluded data sections for a single measured parameter where the actogram for that pattern did not closely resemble actograms for the other two measured parameters.

RESULTS

Table 1 contains a summary of all experiment dates and times with a description of the applied light and noise. Sound experiments ran for a total of 710 days between February 1990 and June 1993. Table 2 contains rat initial and final weights, light levels, and sound levels associated with each rat for all experiments. Appendix 1 contains all the actograms ordered by experiment number, parameters and rat number.

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Table 1. Summary of all experiment times and applied light and noise

Experiment	Start	End	Type	Days
1.1 (9002Sound05)	08-Feb-90	26-Feb-90	LD 5	14
	26-Feb-90	13-Mar-90	LL 5	15
	13-Mar-90	26-Mar-90	LL 5 NQ	13
	26-Mar-90	09-Apr-90	LD 5 NQ	14
Total Days				56

Expt	Start	End	Type	Days
1.2 (9007Sound05)	16-Jul-90:1633	08-Aug-90	LD 5	23
	08-Aug-90	26-Aug-90	LL 5	18
	26-Aug-90	11-Sep-90	LL 5 12N:12Q	16
	11-Sep-90	21-Sep-90:0815	LD 5 12N:12Q	10
Total Days				67

Expt	Start	End	Type	Days
2.1 (9011Sound05)	31-Oct-91	05-Nov-90	LD 5	5
	05-Nov-90:1845	10-Dec-90	LD 5	35
	10-Dec-90	17-Dec-90	DD	7
	17-Dec-90	27-Dec-90	DD 12Q:12N	10
	27-Dec-90	04-Jan-91	DD	8
	04-Jan-91	10-Jan-91:1250	LD 5	6
Total Days				71

Expt	Start	End	Type	Days
2.2 (9102Sound05)	25-Feb-91:1347	17-Mar-91	LD 5	20
	17-Mar-91	25-Mar-91	DD	8
	25-Mar-91	24-May-91	DD 12Q:12N	60
	24-May-91	22-Jun-91:0650	DD	29
	22-Jun-91:0700	23-Jun-91:0655	DD	1
	23-Jun-91:0700	25-Jun-91:1427	DD 12N:12Q	3
	25-Jun-91:1427	?	DD	?
Total Days				121

Table 1 (cont.). Summary of all experiment times and applied light and noise

Expt	Start	End	Type	Days
2.3 (9112Sound05)	11-Dec-91:1615	03-Jan-92	LD 5	23
	03-Jan-92	11-Jan-92	DD	8
	11-Jan-92	11-Mar-92	DD 12Q:12N	60
	11-Mar-92	12-Apr-92	DD	32
Total Days				123

Expt	Start	End	Type	Days
2.4 (9205Sound05)	11-May-92:2315	13-May-92:0215	LD 5	2
	13-May-92	07-Jun-92	LD 5	25
	07-Jun-92	24-Jun-92	DD	17
	24-Jun-92	01-Sep-92	DD 10Q:4N:10Q	68
	01-Sep-92	12-Sep-92:1335	DD	11
	12-Sep-92:1340	23-Sep-92:1210	DD	11
Total Days				134

Expt	Start	End	Type	Days
3.1 (9109Sound05)	25-Sep-91:1012	11-Oct-91	LD 5	17
	11-Oct-91:1851	17-Oct-91:1850	LD 5 2Q:2N	6
	17-Oct-91:1900	24-Oct-91:1850	LD 5	7
	24-Oct-91:1900	27-Oct-91:1850	DD 2Q:2N	3
	27-Dec-91:1900	?	DD	?
Total Days				33

Expt	Start	End	Type	Days
4.1 (9306Sound05)	04-Jun-93	22-Jun-93	LD 5	17
	22-Jun-93	18-Sep-93	DD	88
Total Days				105

Total of all experiments 804

Table 2. Initial and final weights, light levels, and sound levels.

Rat Number	Initial Weight (grams)	Final Weight (grams)	Initial Light Level (lux)	Final Light Level (lux)	Initial Ambient Noise (dBA)	Final Ambient Noise (dBA)	Initial Applied Sound (dBA)	Final Applied Sound (dBA)	Adrenal Weight (mg)	
Experiment 1.1 (9002Sound05)										
1	187	383								
2	182	374								
3	175	365								
4	186	342								
5	181	380								
6	184	N/A								
Experiment 1.2 (9007Sound05)										
1	174	430	4.91				90			
2	172	423	5.01				90			
3	171	416	5.06				90			
4	169	374	5.17				91			
5	175	395	4.94				91			
6	170	362	5.11				90			
Experiment 2.1 (9011Sound05)***										
1	264	389	4.90	8.25						
2	265	407	4.98	4.23						
3	259	355	5.01	4.79						
4	271	380	5.00	4.26						
5	266	397	5.02	4.20						
6	260	395	5.05	5.65						
Experiment 2.2 (9102Sound05)***										
1	233	400	5.08		58	60	93	83	39	46
2	248	434	5.90		73	70	91	84	40	43
3	235	412	5.36		64	68	91	83	42	35
4	255	460	4.32		68	66	91	83	43	42
5	237	411	4.55		63	64	90	83	30	20*
6	226	387	5.15		64	64	89	81	54	41

*** Experiment number includes the protocol and experiment done in that sequence; *e.g.* Experiment #3.1 is experiment 1 of protocol 3.

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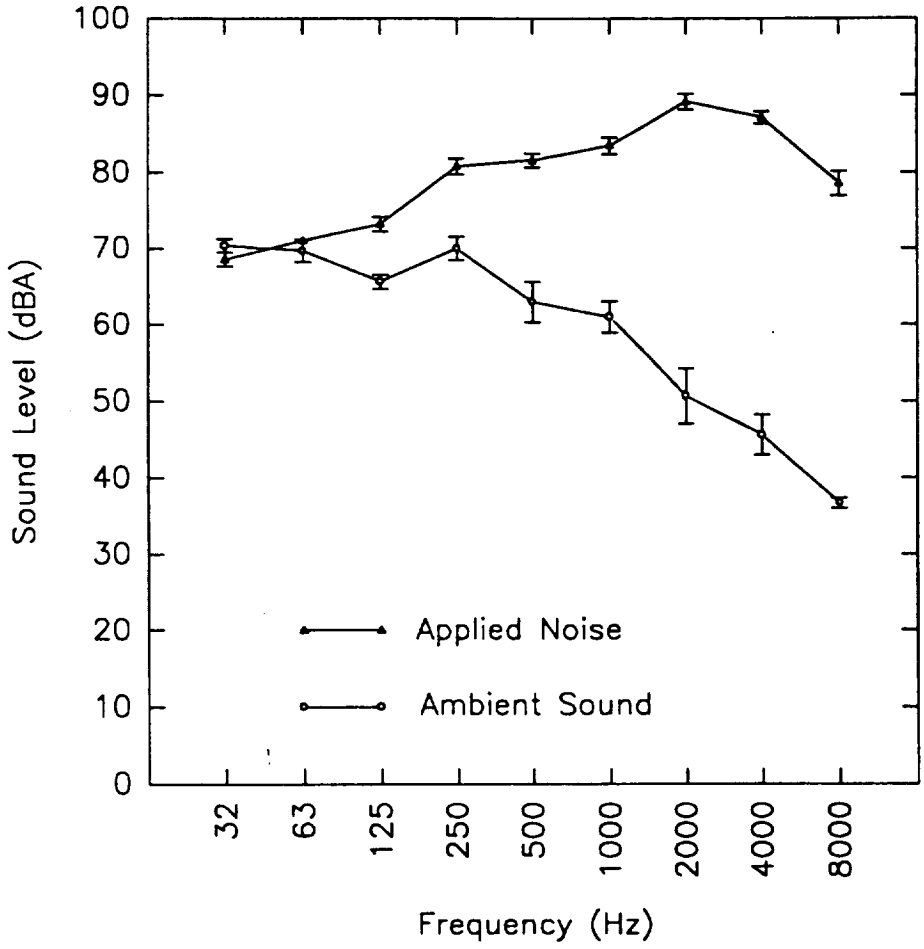
(Cooperative agreement NCC2-593)
San Jose State University

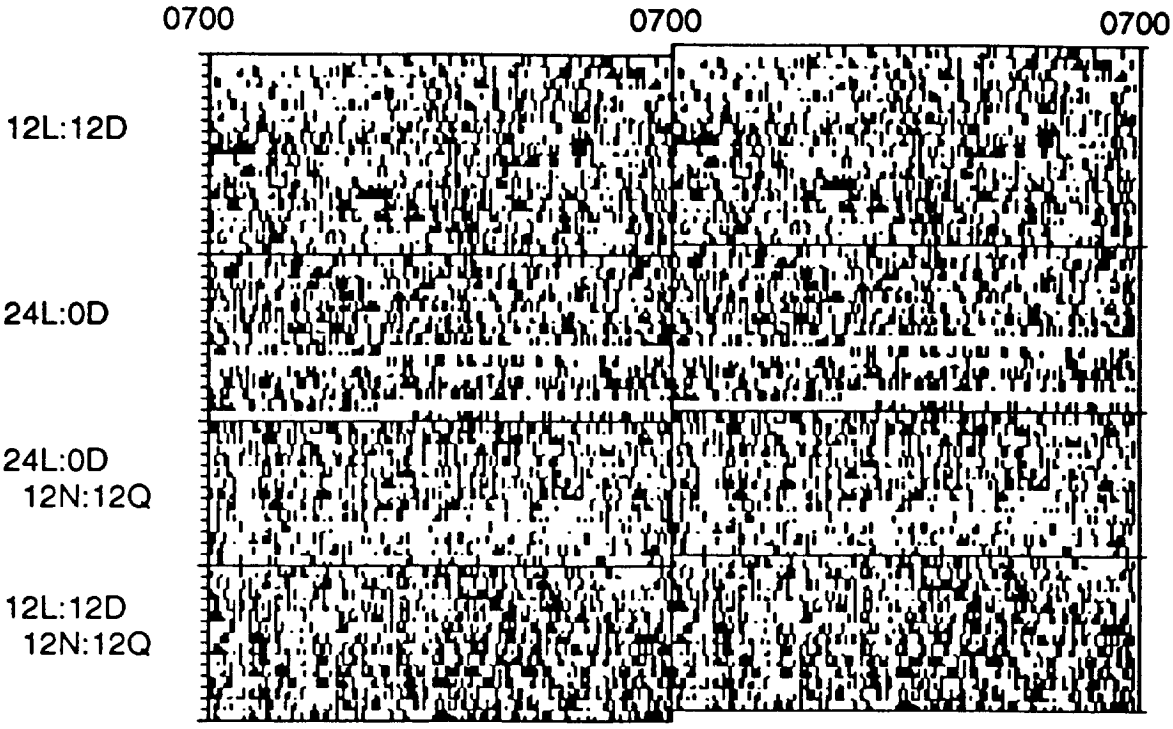
Rat Number	Initial Weight (grams)	Final Weight (grams)	Initial Light Level (lux)	Final Light Level (lux)	Initial Ambient Noise (dBA)	Final Ambient Noise (dBA)	Initial Applied Sound (dBA)	Final Applied Sound (dBA)	Adrenal Weight (mg)	
Experiment 2.3 (9112Sound05)***										
1	180	389	5		64	N/A	N/A	N/A	30	30
2	180	369	5		64	N/A	N/A	N/A	30	20**
3	192	388	5		62	N/A	N/A	N/A	30	30
4	174	359	5		66	62	92	86	30	30
5	190	385	5		65	63	90	85	30	10*
6	191	413	5		64	62	90	83	40	40
Experiment 2.4 (9205Sound05)***										
1	190	378	4.9		67	69	90	88	30	20
2	187	386	4.9		63	61	90	88	10	20
3	183	401	5.0		63	64	89	86	40	30
4	181	430	5.0		64	64	90	87	19	20
5	194	404	4.9		62	63	87	86	20	10
6	194	433	5.0		64	65	90	87	20	10
Experiment 3.1 (9109Sound05)***										
1	264	336	5.07		66		90			
2	280	346	N/A		71		89			
3	274	370	N/A		63		89			
4	258	358	N/A		67		89			
5	289	406	N/A		63		89			
6	276	334	N/A		65		88			
Experiment 4.1 (9306Sound05)***										
1	157	440	5.1						14.5*	
2	153	396	5.0						25.0	
3	166	435	5.0						26.2	
4	178	430	5.0						23.6	
5	172	478	5.1						27.9	
6	160	407	5.4						17.4	

* partial cleavage of the adrenal may have occurred during its removal.
** very little fat pad/no fat
*** Experiment number includes the protocol and experiment done in that sequence; *e.g.* Experiment #3.1 is experiment 1 of protocol 3.

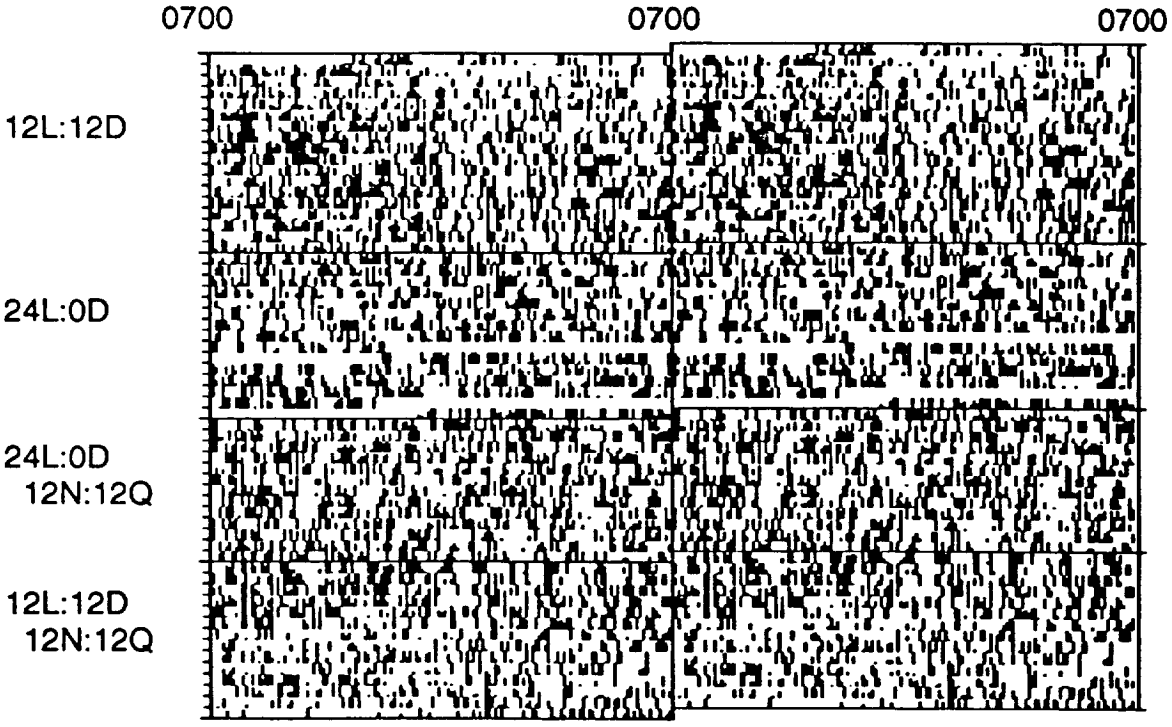
Figure 1. Ambient sound and applied noise spectra taken from Table 2.



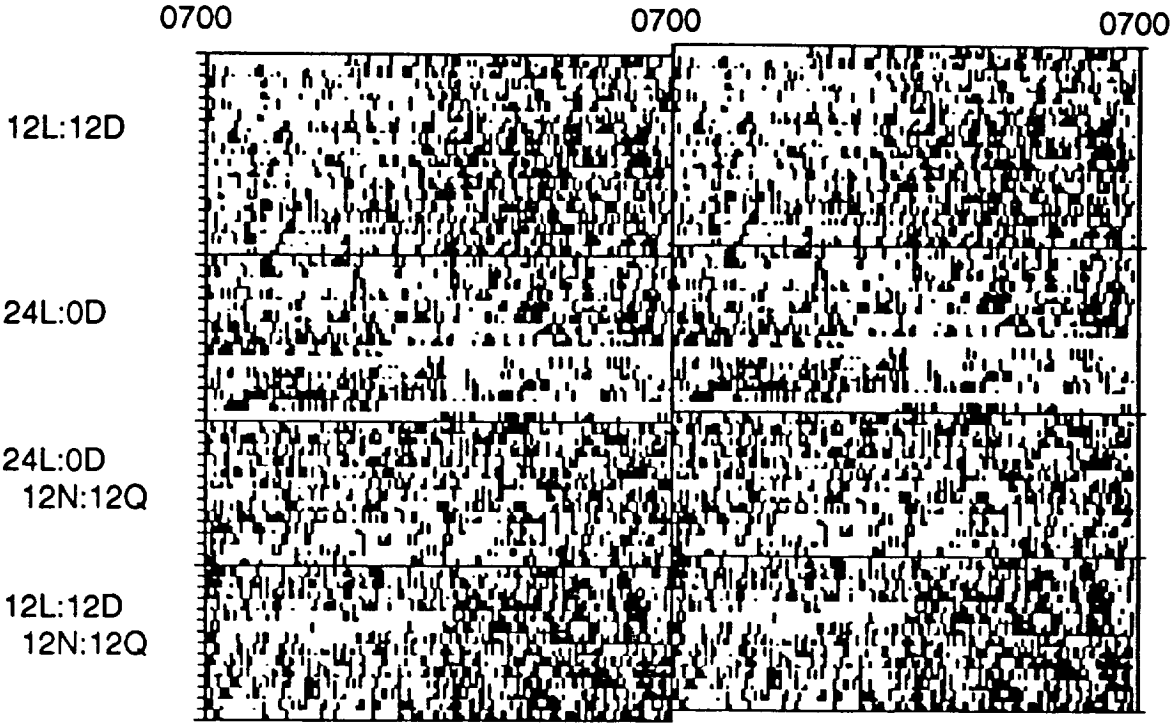
Experiment 1.1 (9002Sound05) Rat 3 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



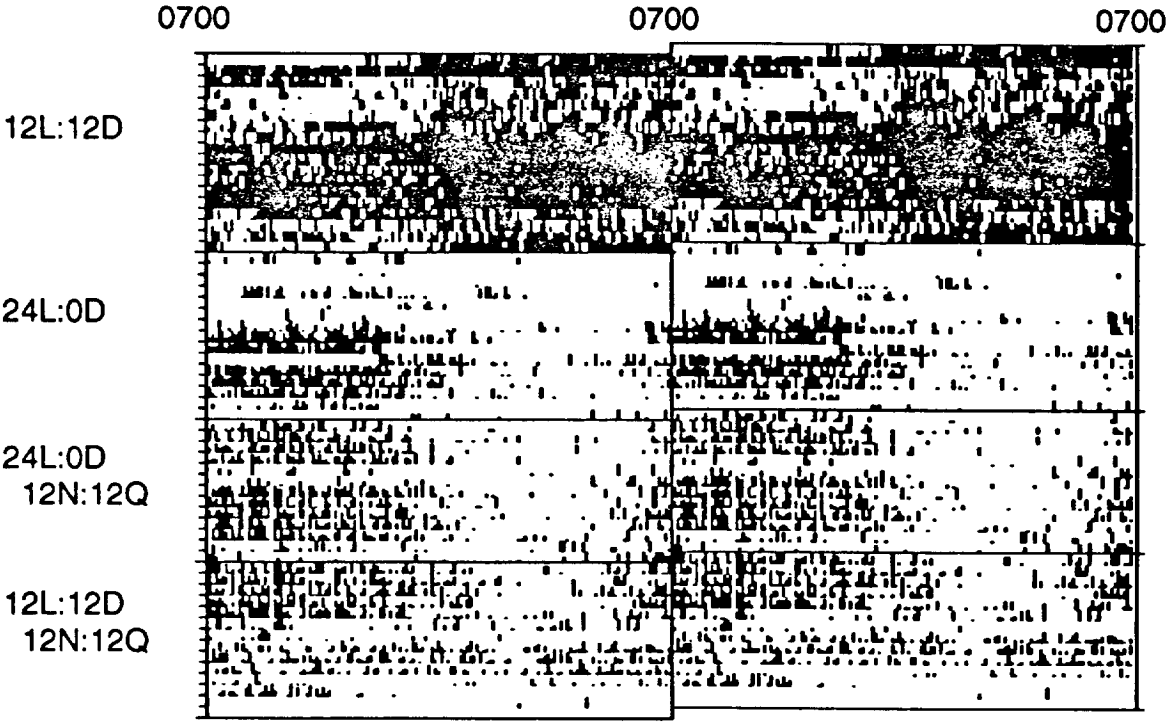
Experiment 1.1 (9002Sound05) Rat 4 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



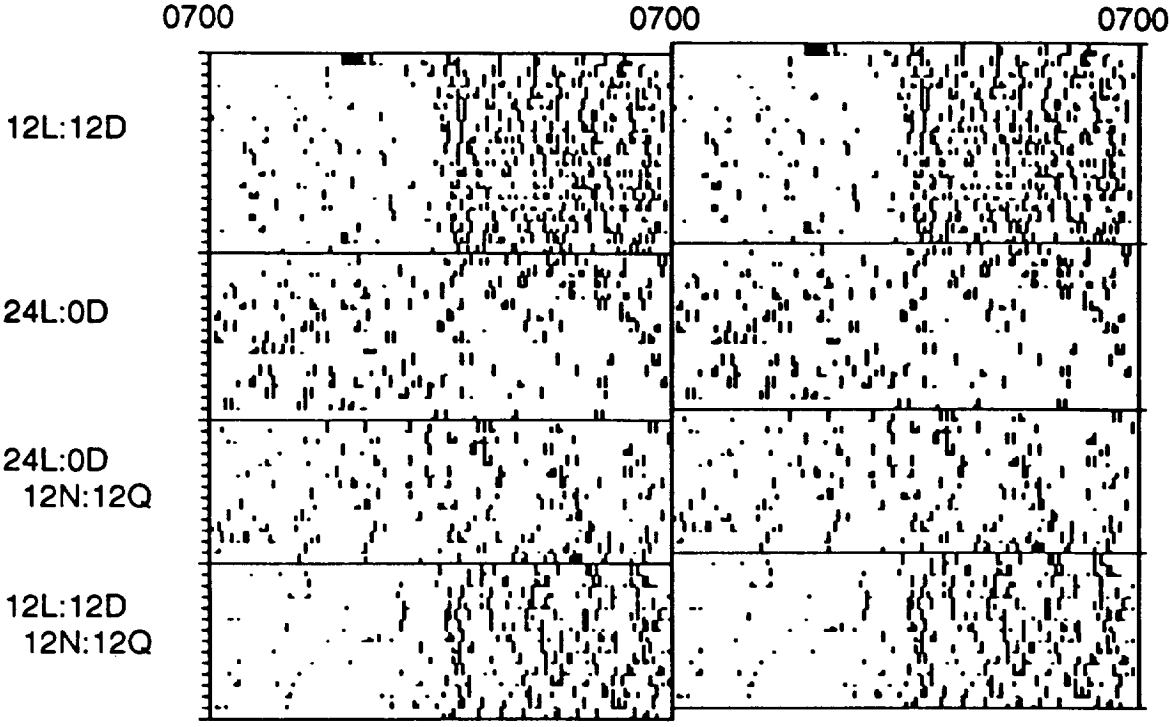
Experiment 1.1 (9002Sound05) Rat 5 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



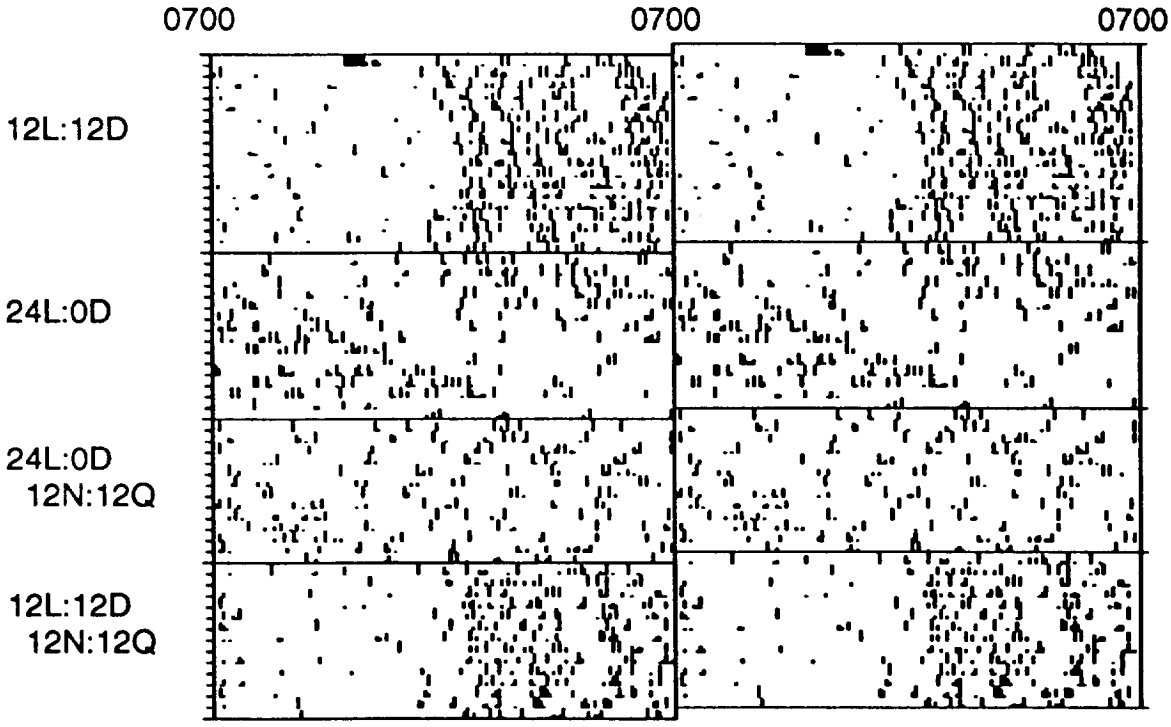
Experiment 1.1 (9002Sound05) Rat 6 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



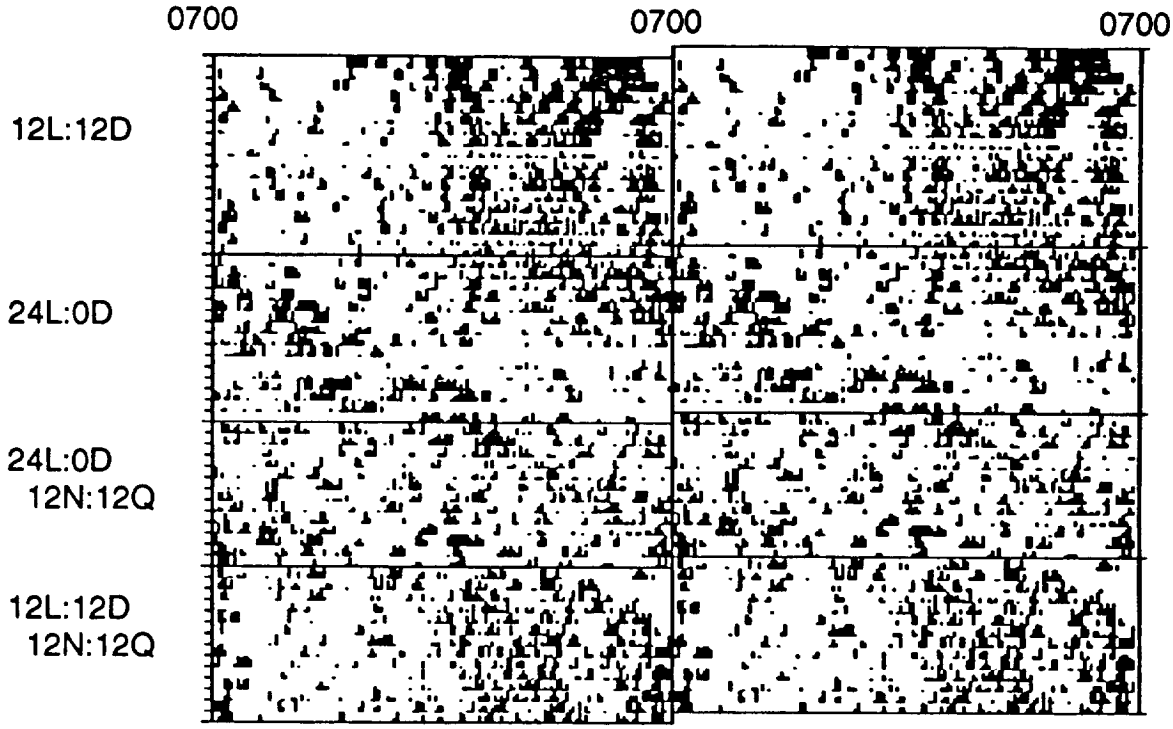
Experiment 1.1 (9002Sound05) Rat 1 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



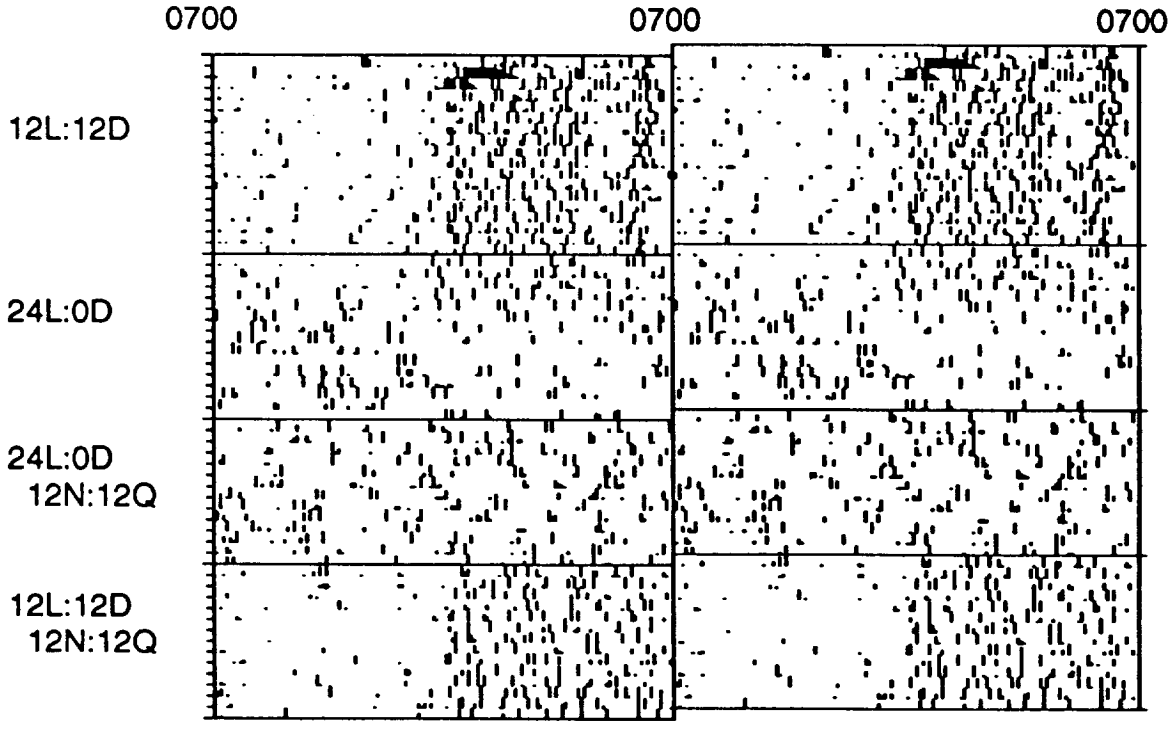
Experiment 1.1 (9002Sound05) Rat 2 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



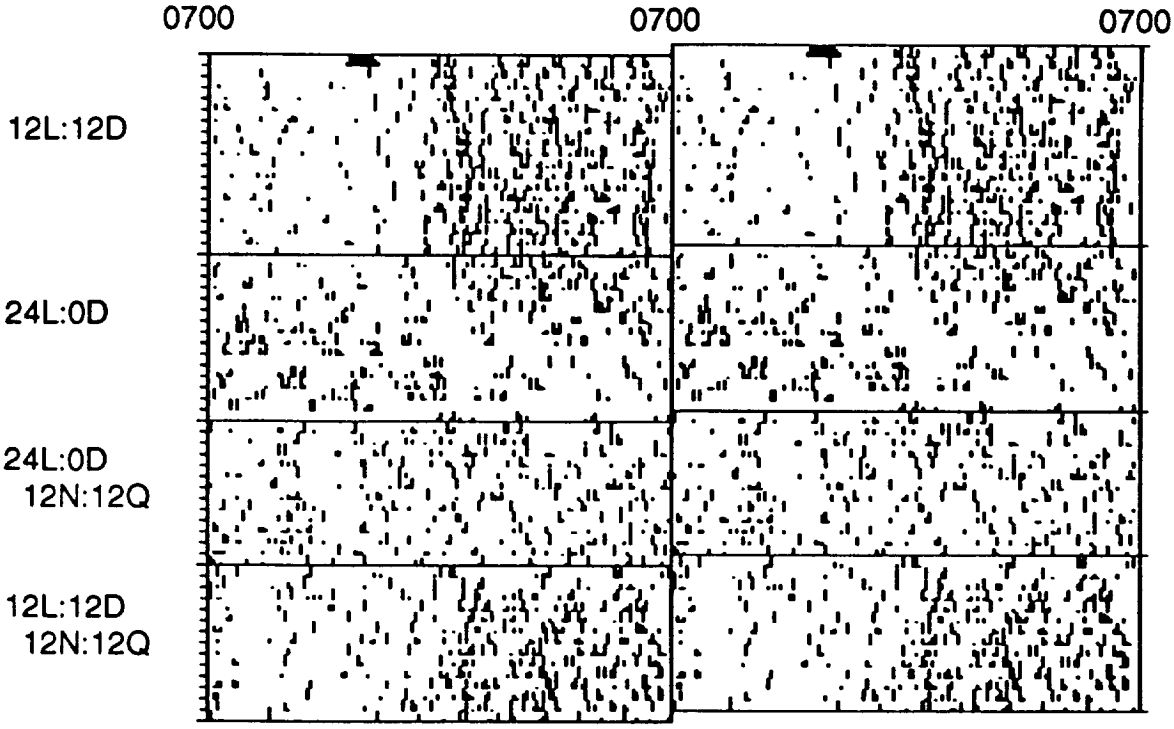
Experiment 1.1 (9002Sound05) Rat 3 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



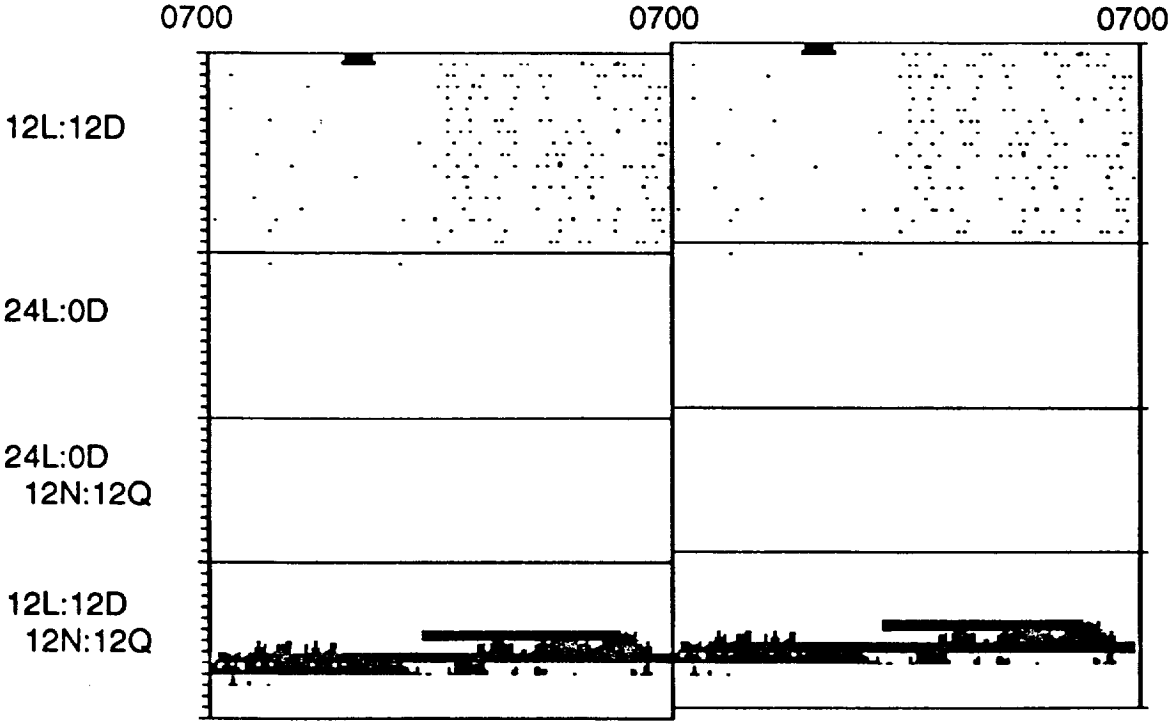
Experiment 1.1 (9002Sound05) Rat 4 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



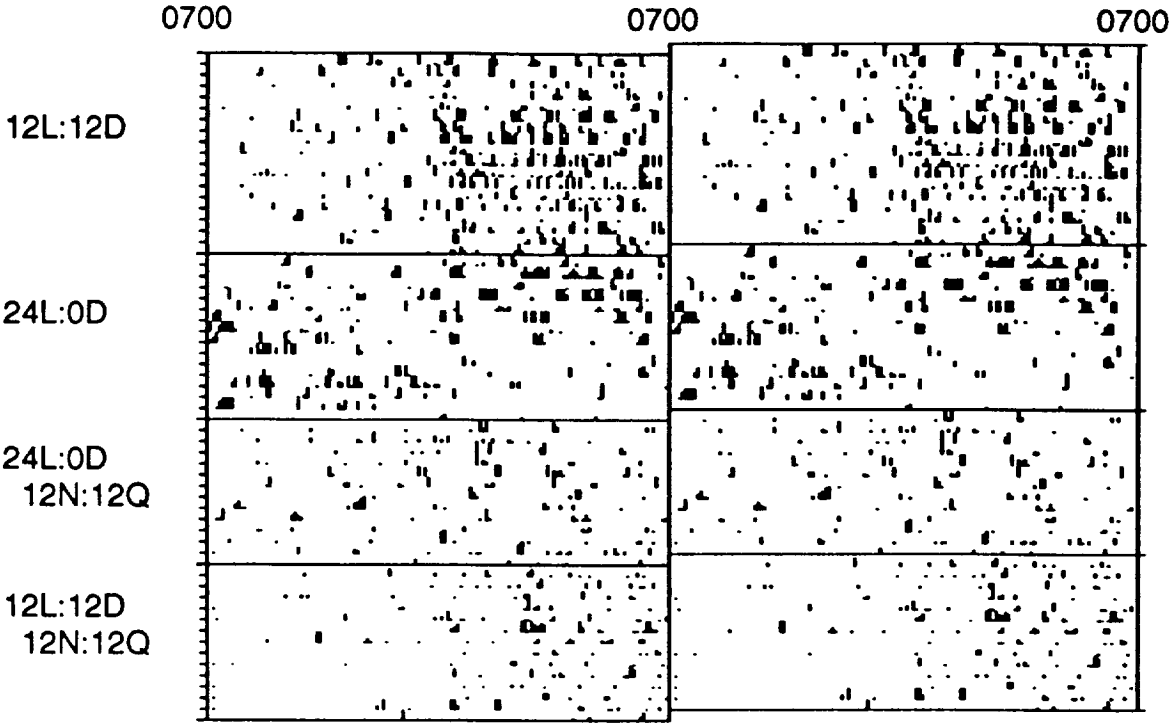
Experiment 1.1 (9002Sound05) Rat 5 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



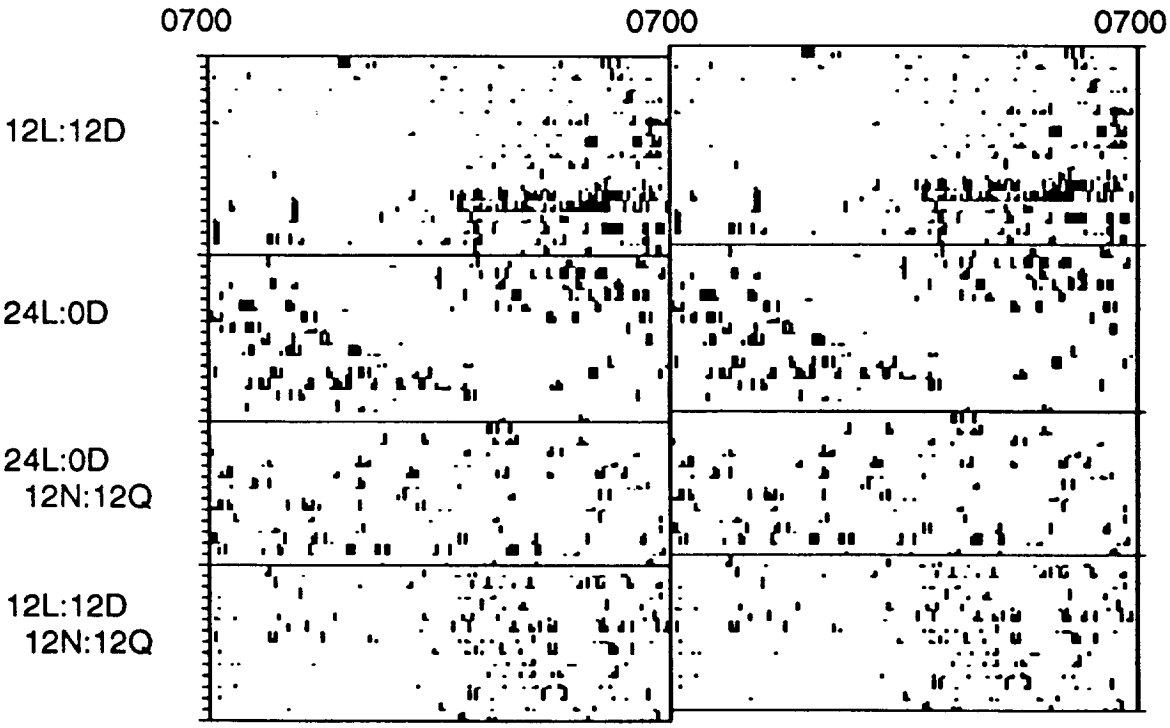
Experiment 1.1 (9002Sound05) Rat 6 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



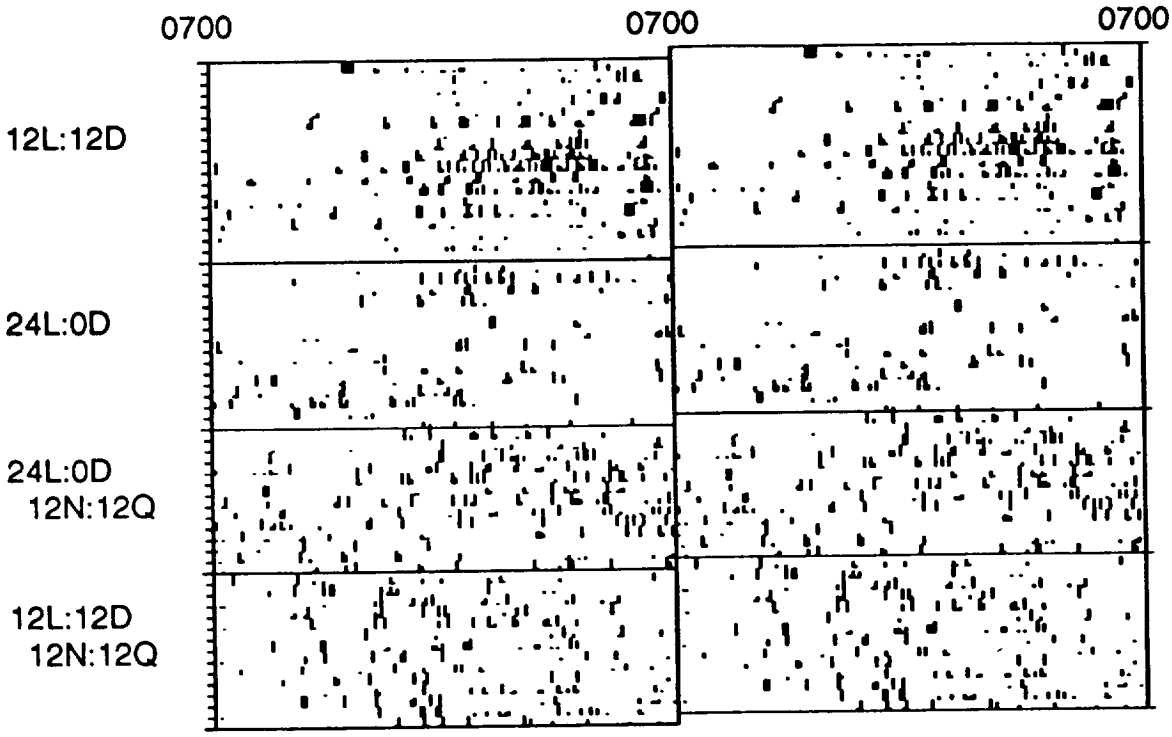
Experiment 1.1 (9002Sound05) Rat 1 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



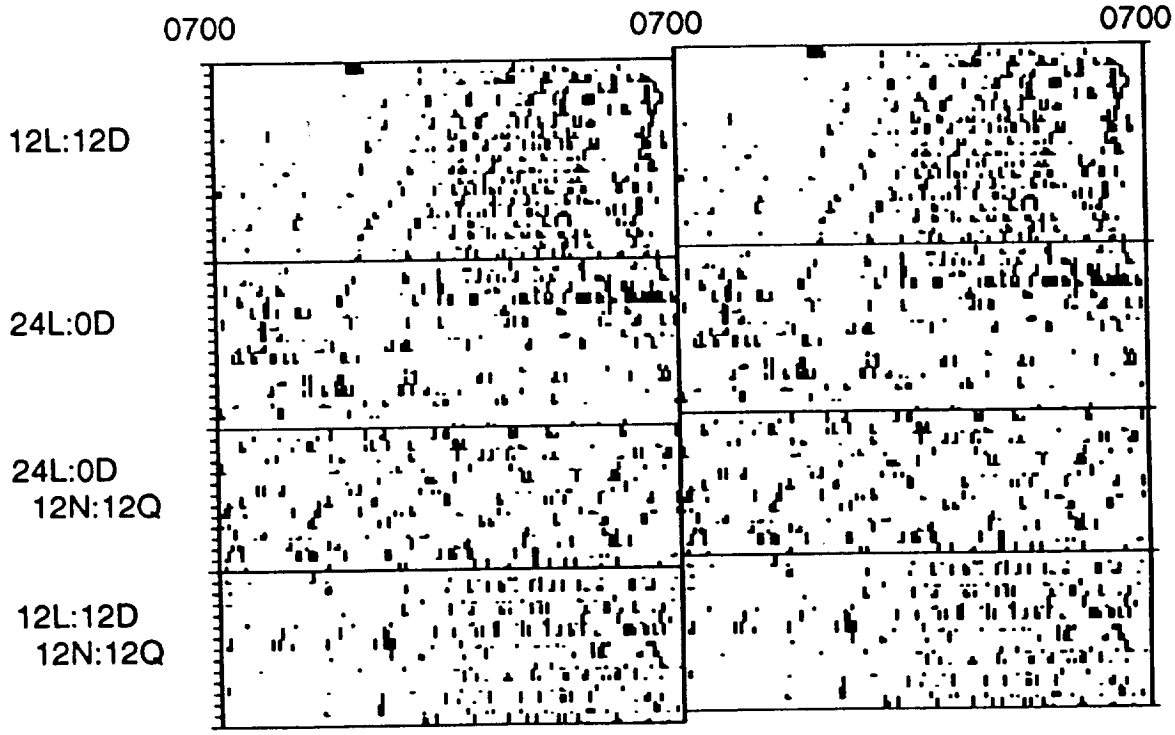
Experiment 1.1 (9002Sound05) Rat 2 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



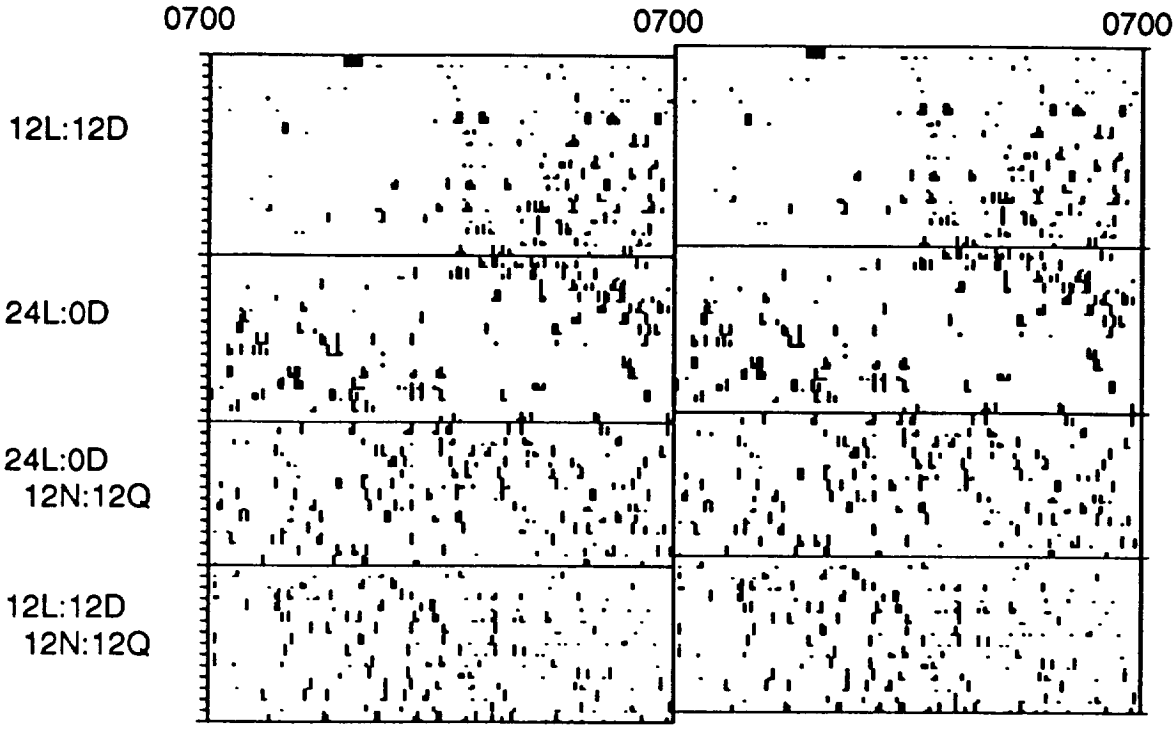
Experiment 1.1 (9002Sound05) Rat 3 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



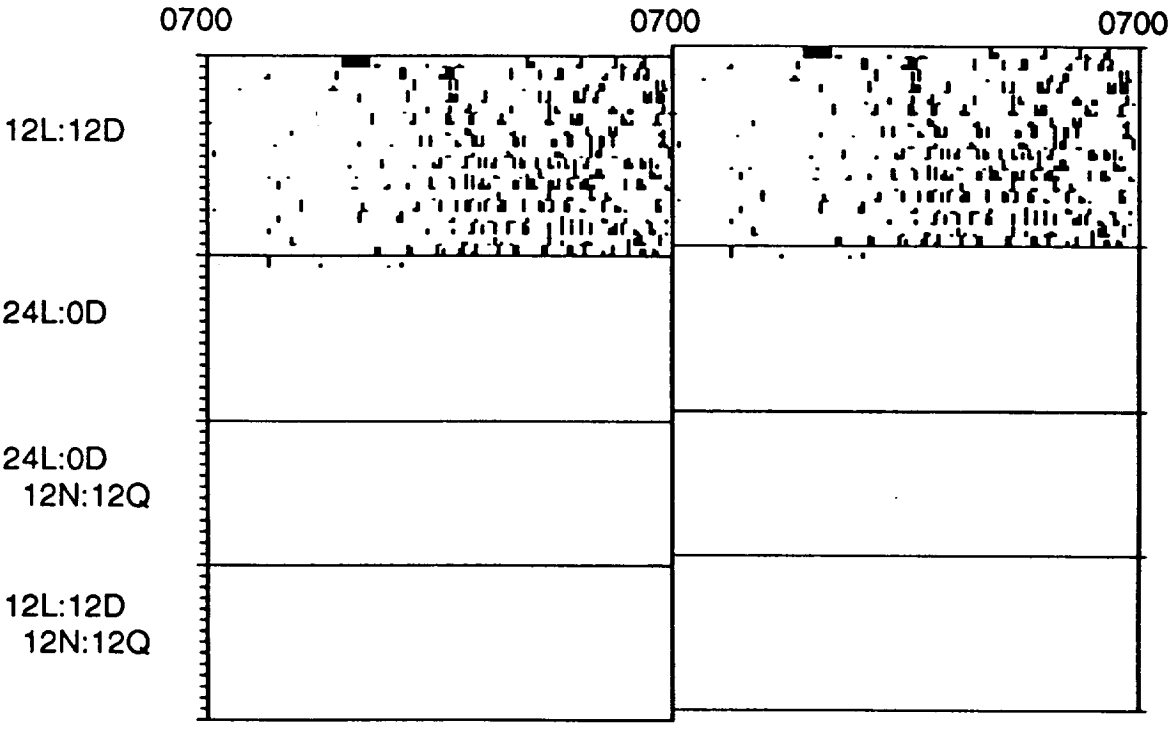
Experiment 1.1 (9002Sound05) Rat 4 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



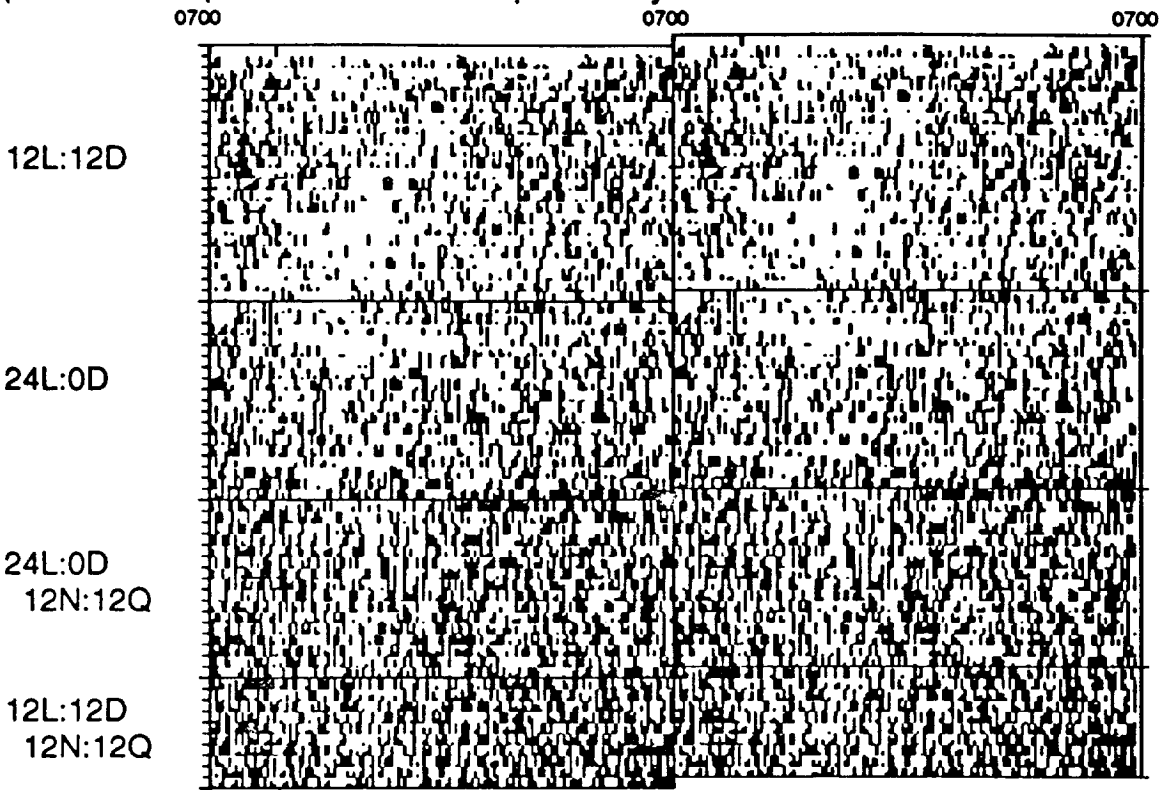
Experiment 1.1 (9002Sound05) Rat 5 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



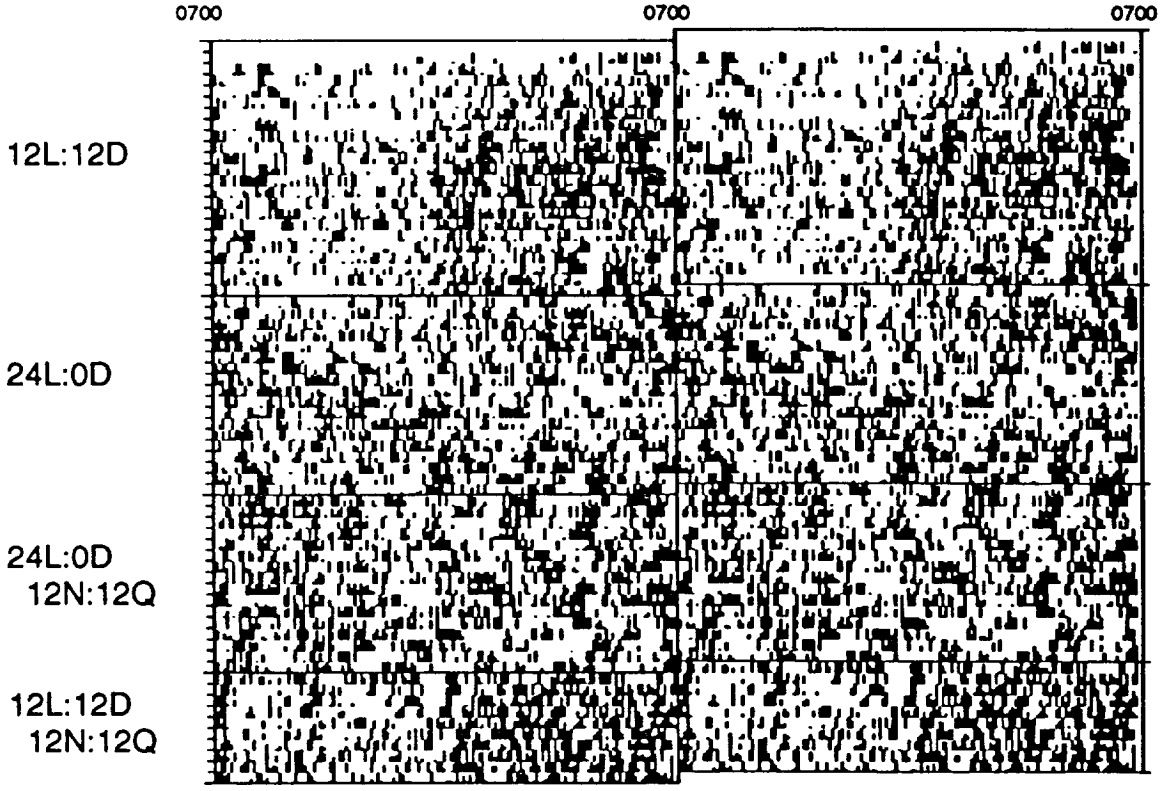
Experiment 1.1 (9002Sound05) Rat 6 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



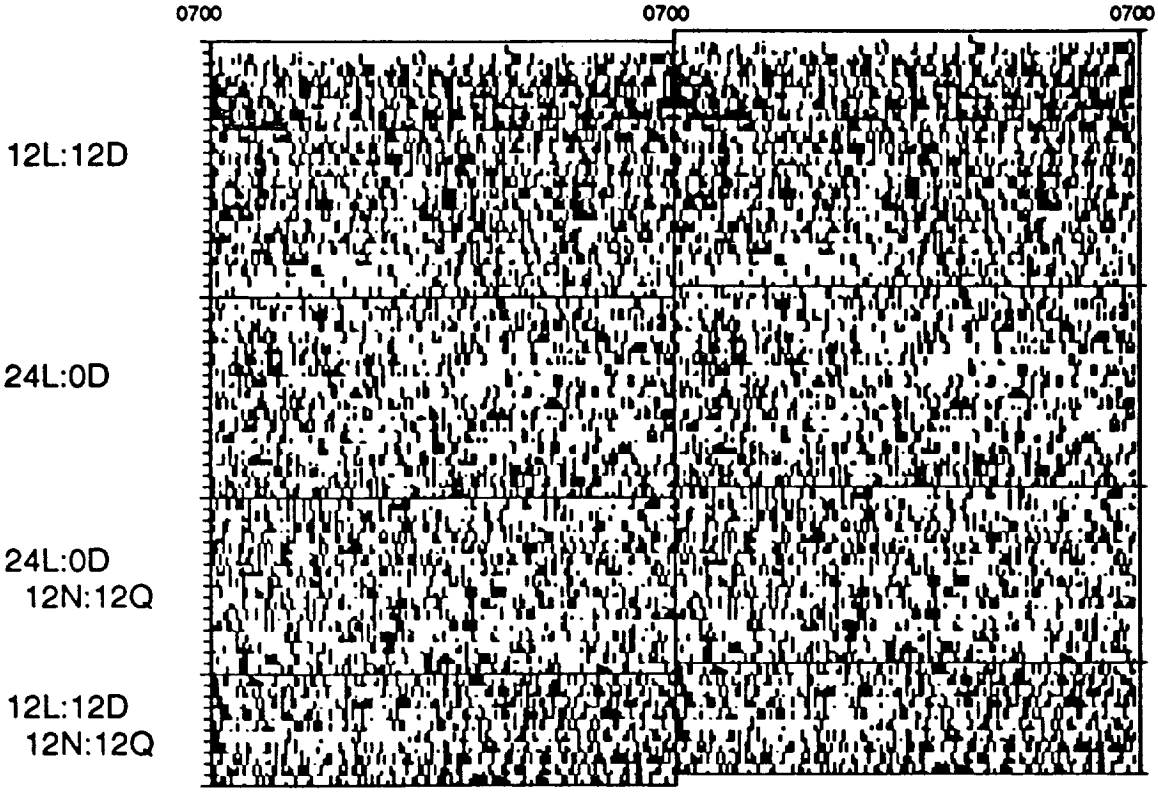
Experiment 1.2 (9007Sound05) Rat 1 LMA actogram. All values above the median are plotted. Four periods of time are separated by horizontal lines.



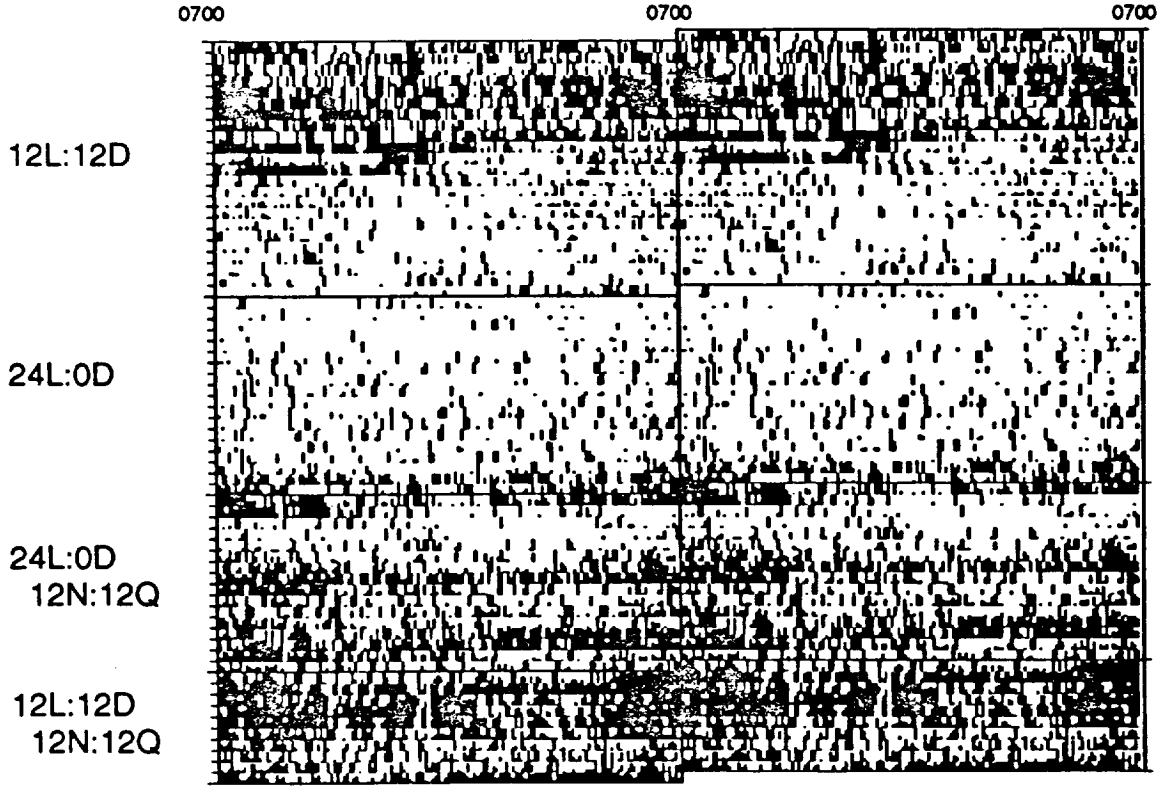
Experiment 1.2 (9007Sound05) Rat 2 LMA actogram. All values above the median are plotted. Four periods of time are separated by horizontal lines.



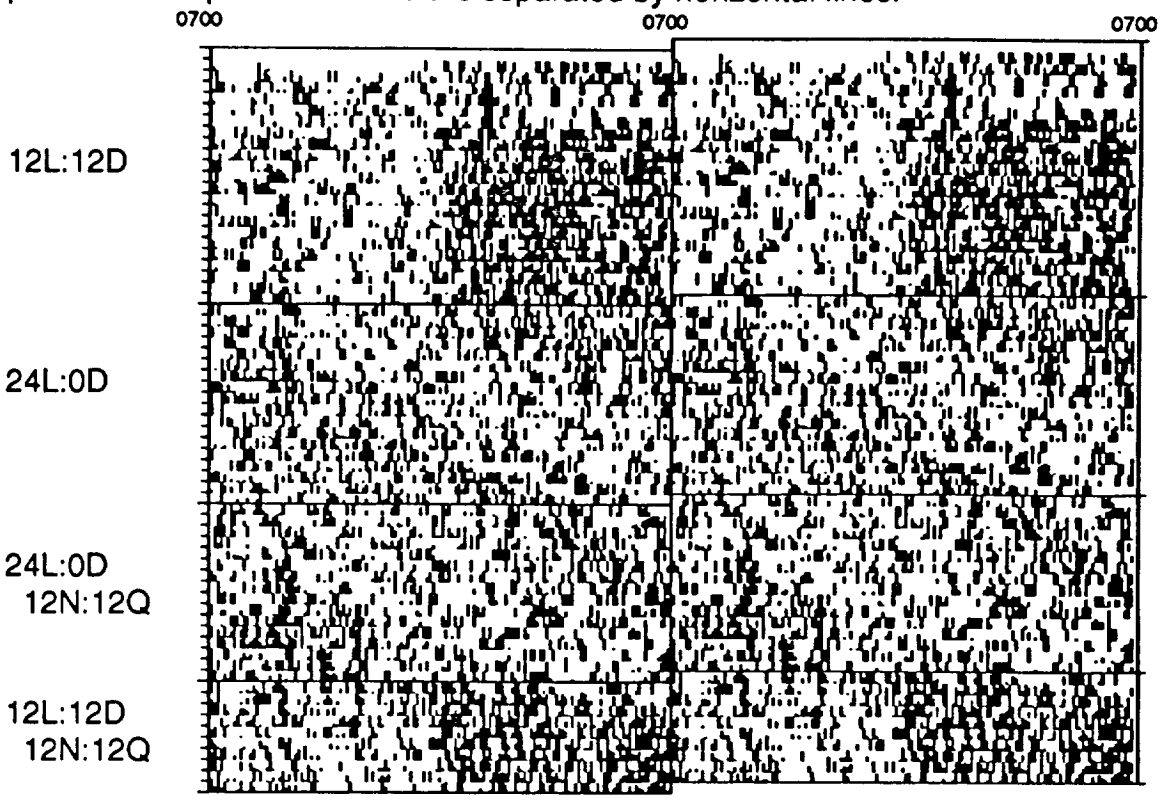
Experiment 1.2 (9007Sound05) Rat 3 LMA actogram. All values above the median are plotted. Four periods of time are separated by horizontal lines.



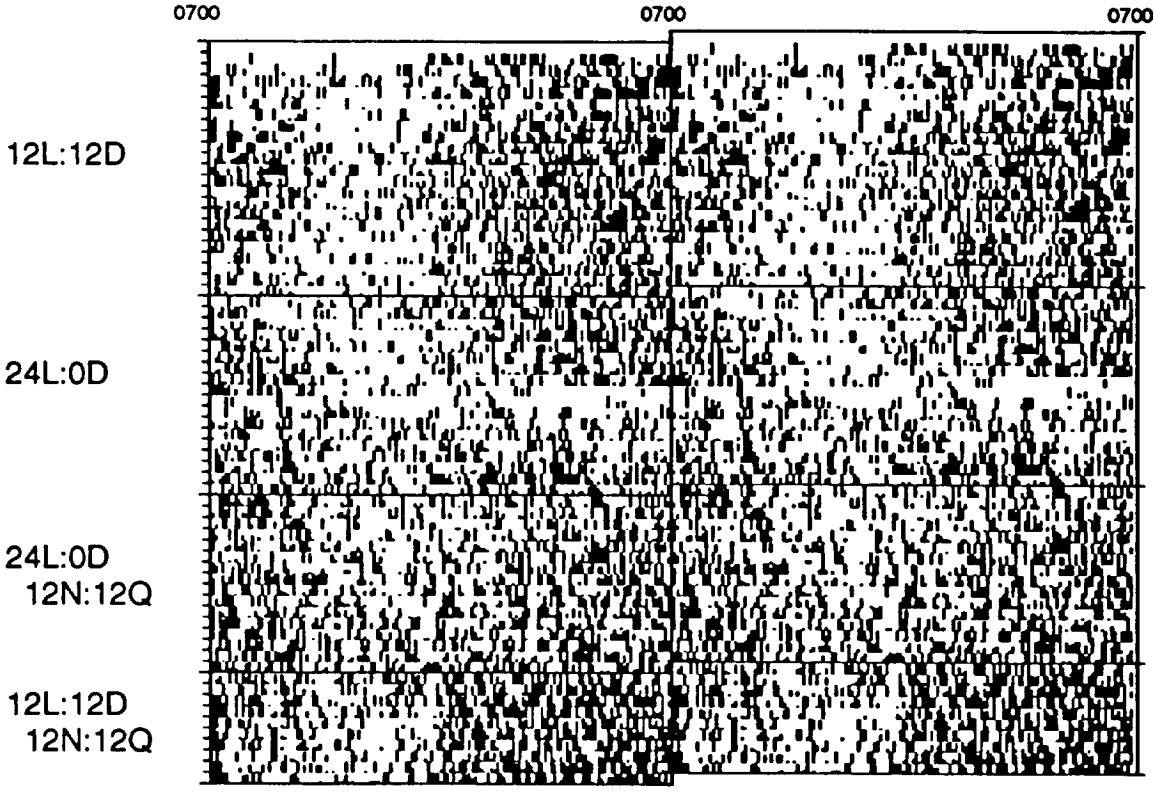
Experiment 1.2 (9007Sound05) Rat 4 LMA actogram. All values above the median are plotted. Four periods of time are separated by horizontal lines.



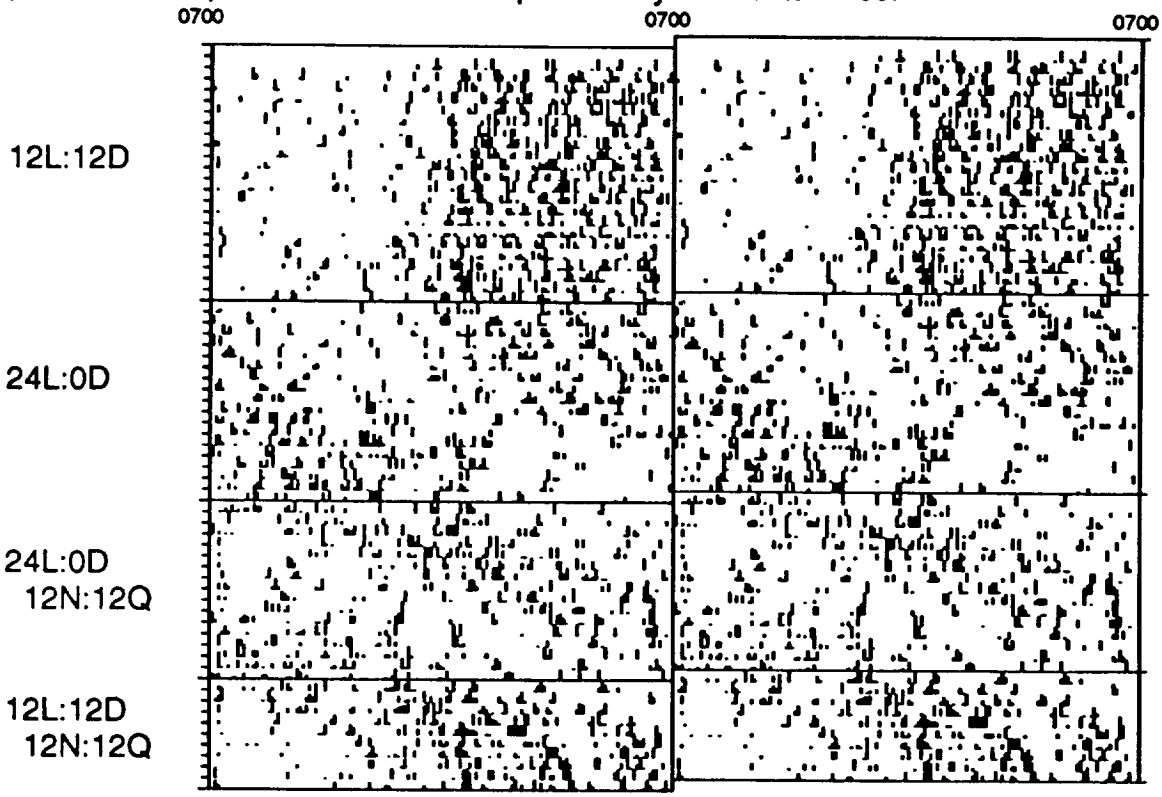
Experiment 1.2 (9007Sound05) Rat 5 LMA actogram. All values above the median are plotted. Four periods of time are separated by horizontal lines.



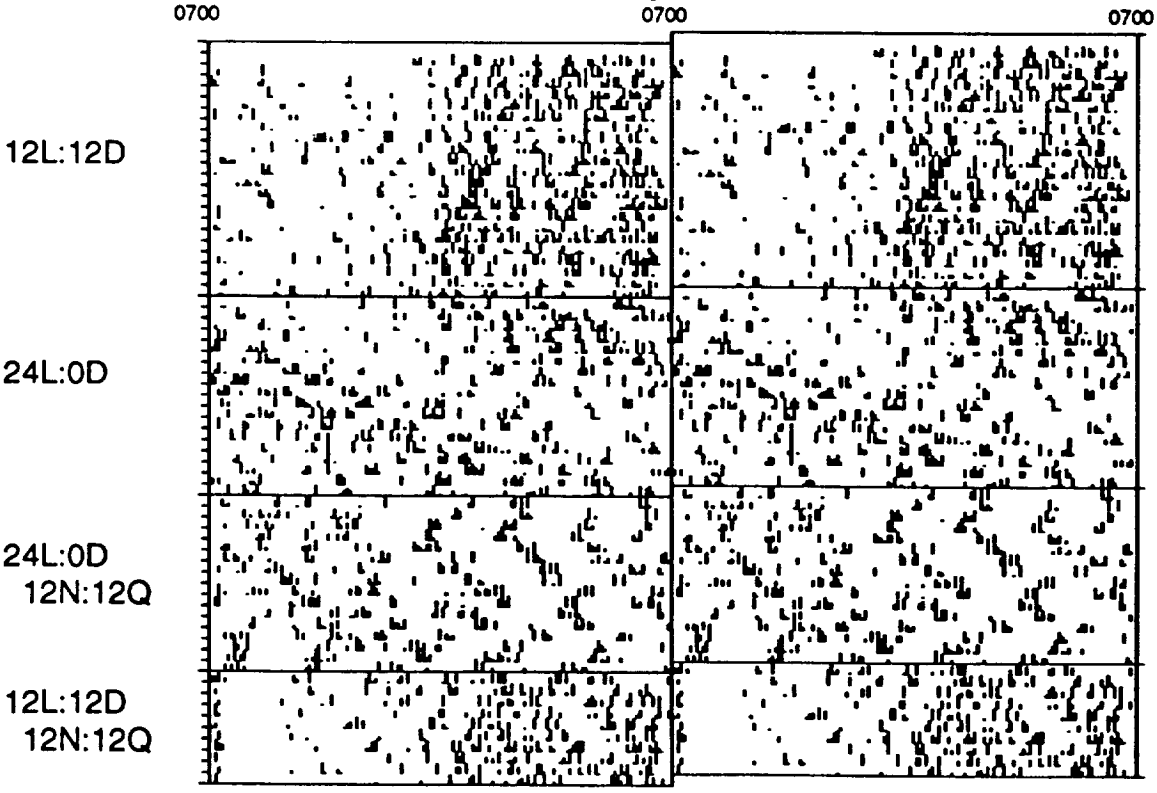
Experiment 1.2 (9007Sound05) Rat 6 LMA actogram. All values above the median are plotted. Four periods of time are separated by horizontal lines.



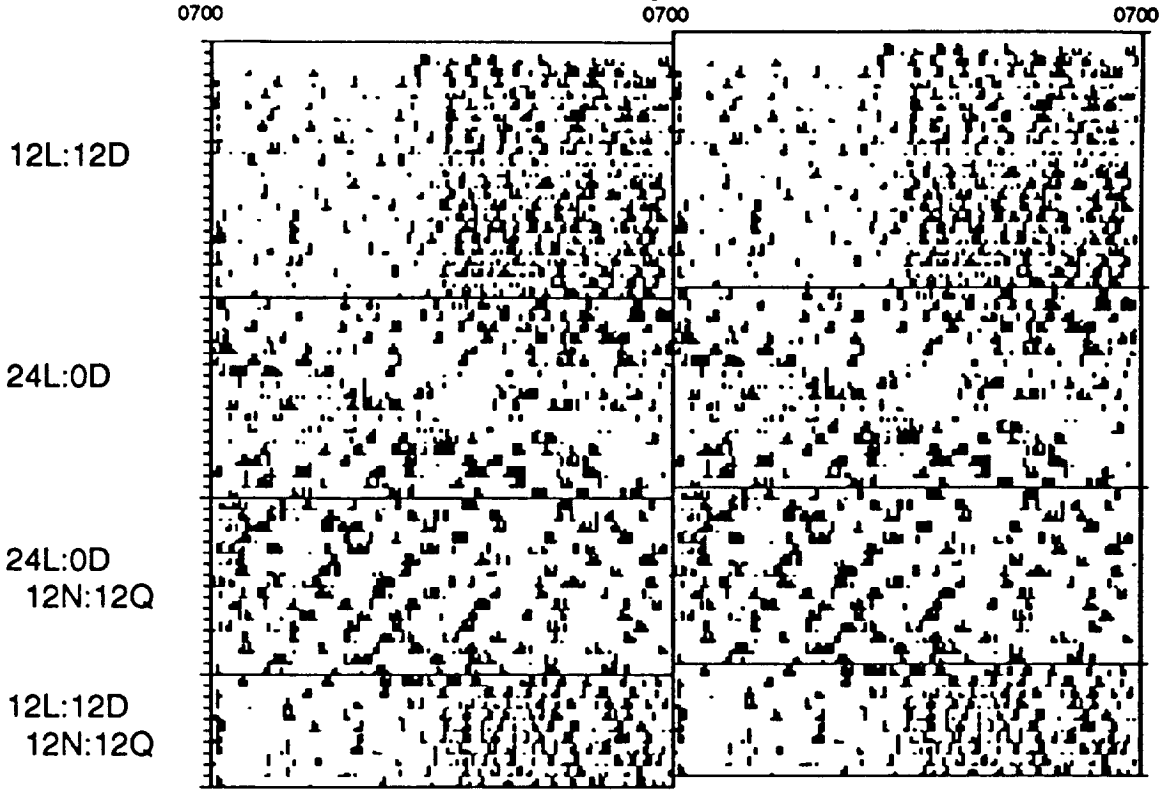
Experiment 1.2 (9007Sound05) Rat 1 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



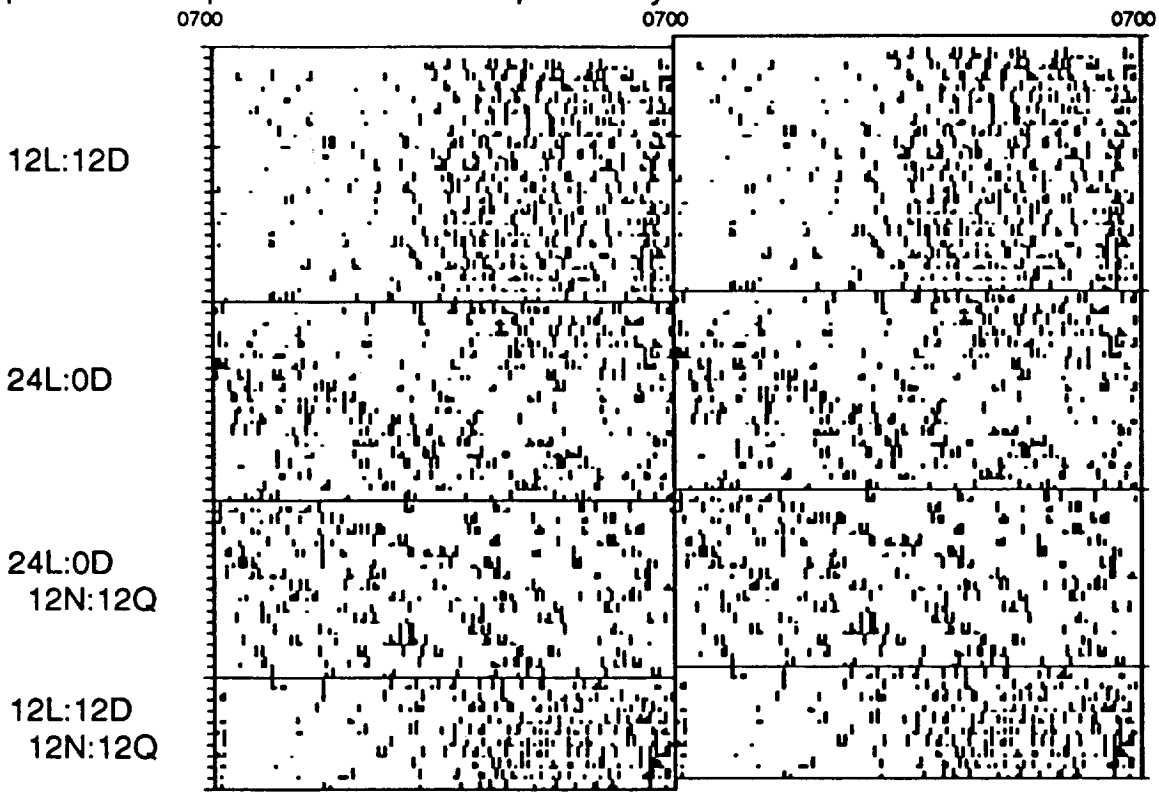
Experiment 1.2 (9007Sound05) Rat 2 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



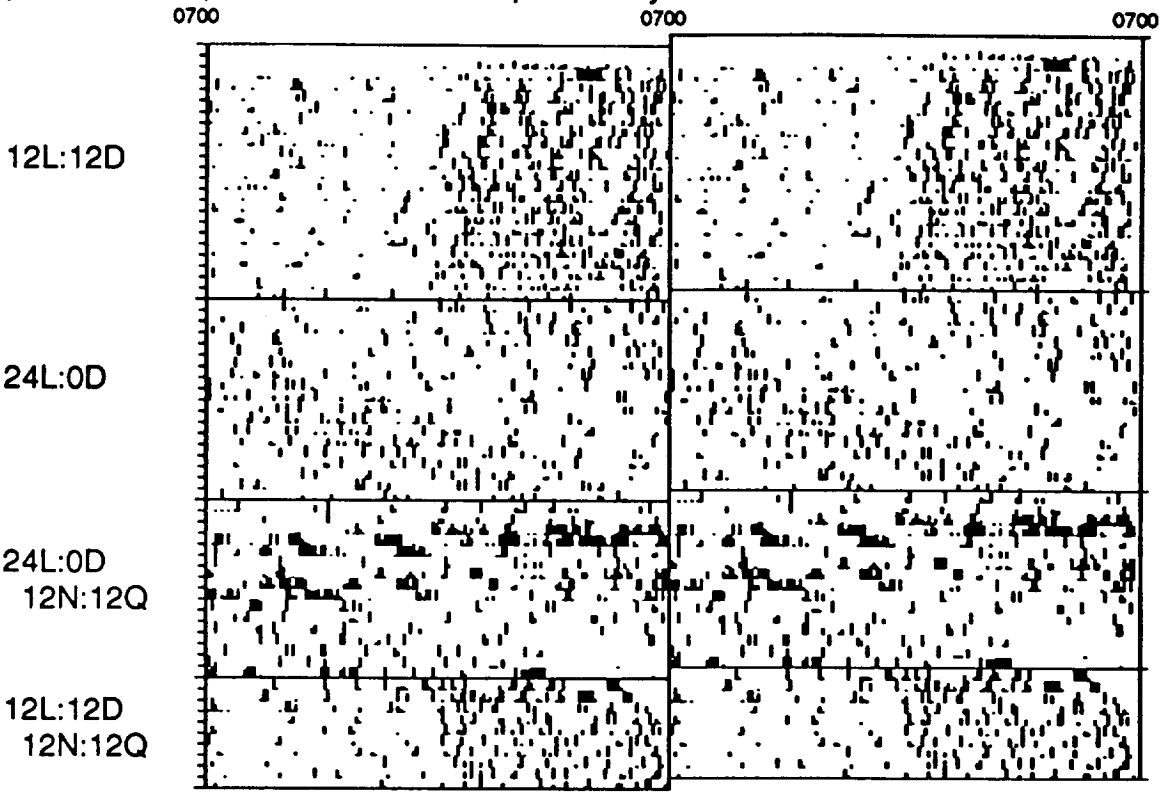
Experiment 1.2 (9007Sound05) Rat 3 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



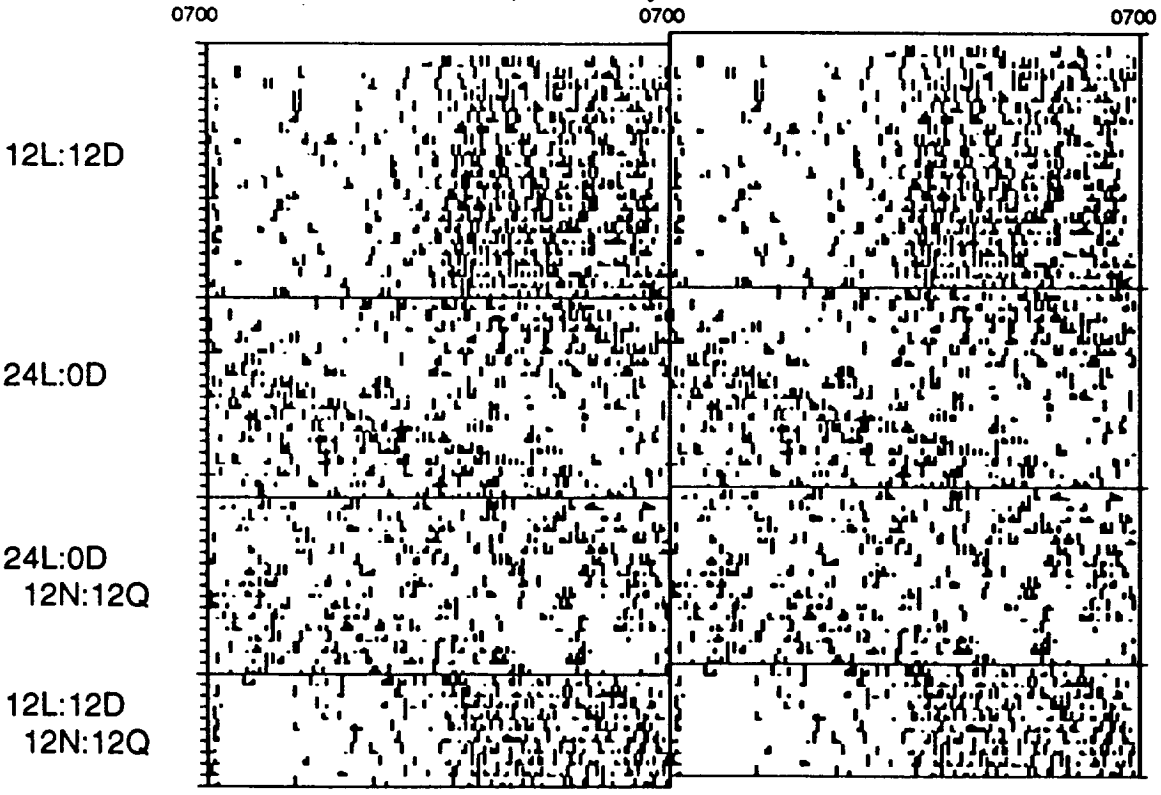
Experiment 1.2 (9007Sound05) Rat 4 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



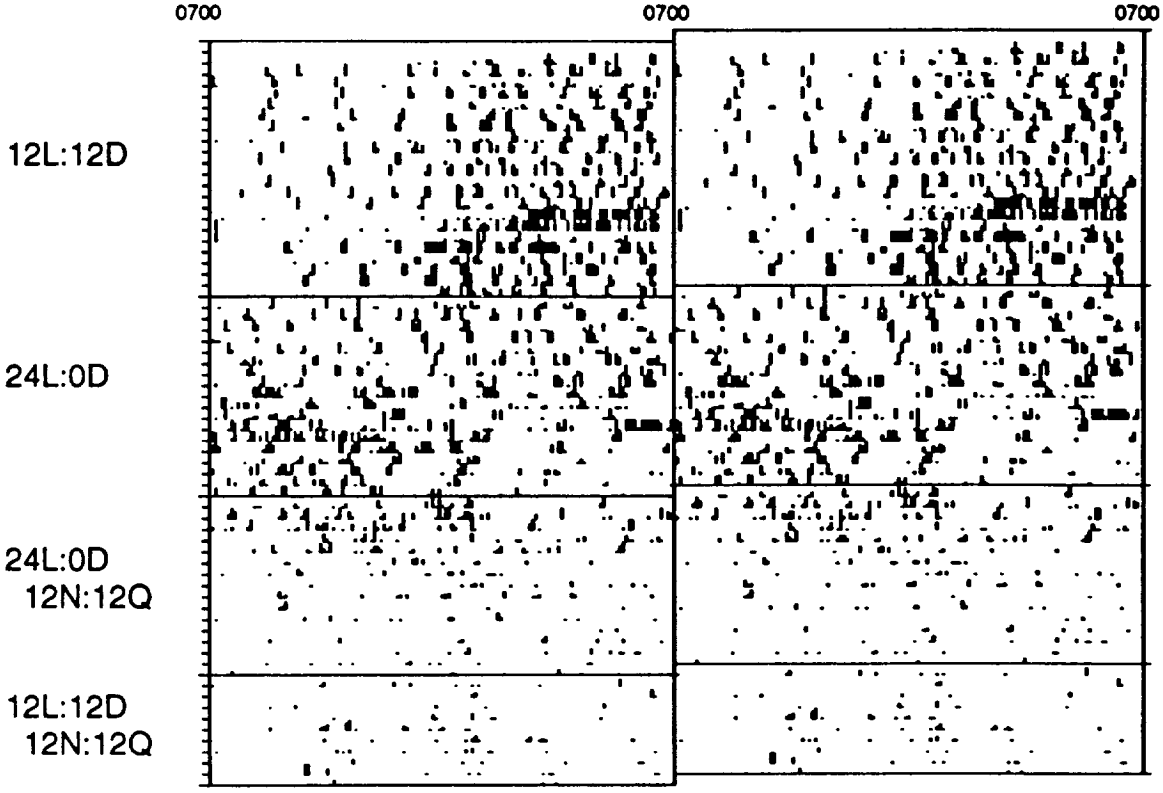
Experiment 1.2 (9007Sound05) Rat 5 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



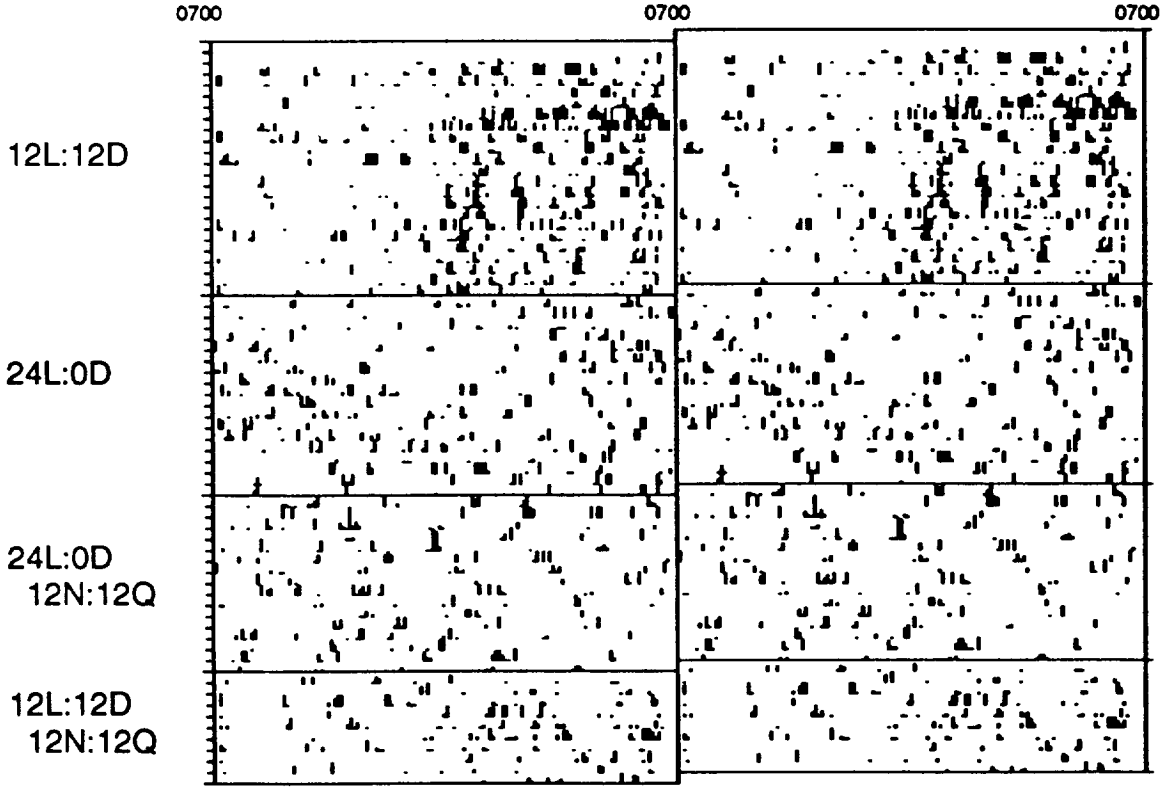
Experiment 1.2 (9007Sound05) Rat 6 drinking actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



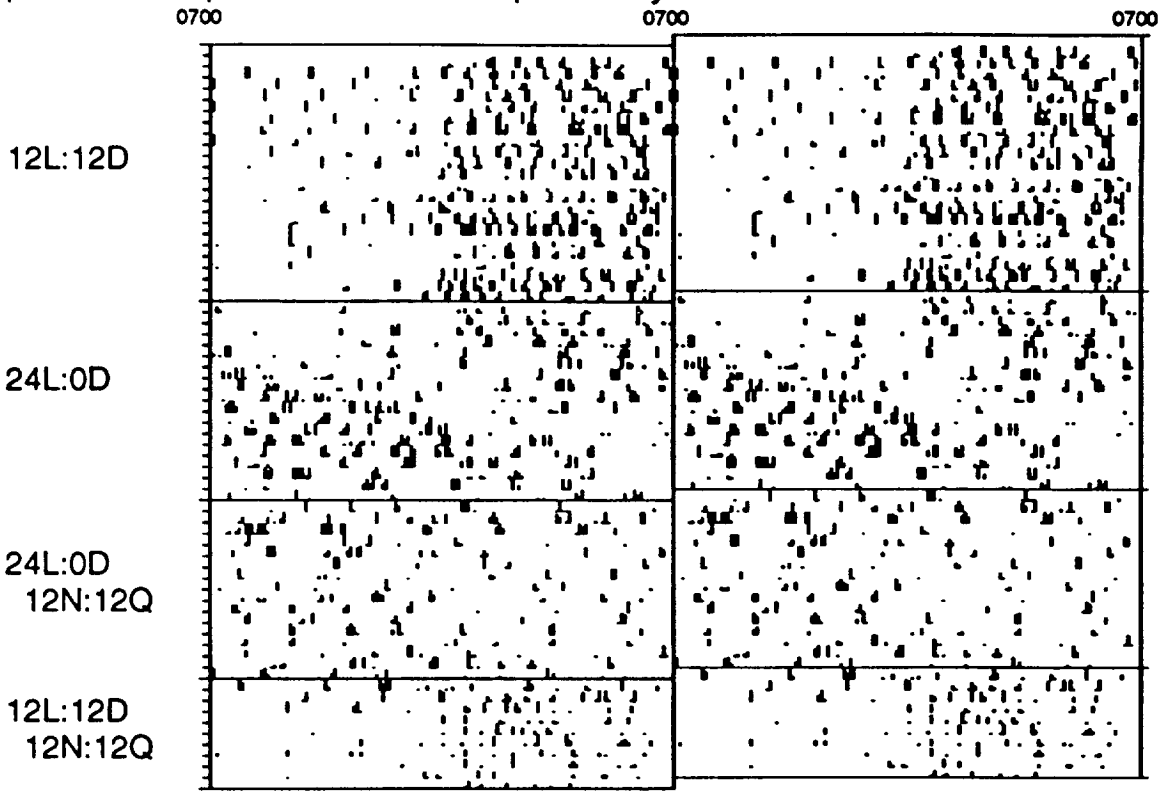
Experiment 1.2 (9007Sound05) Rat 1 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



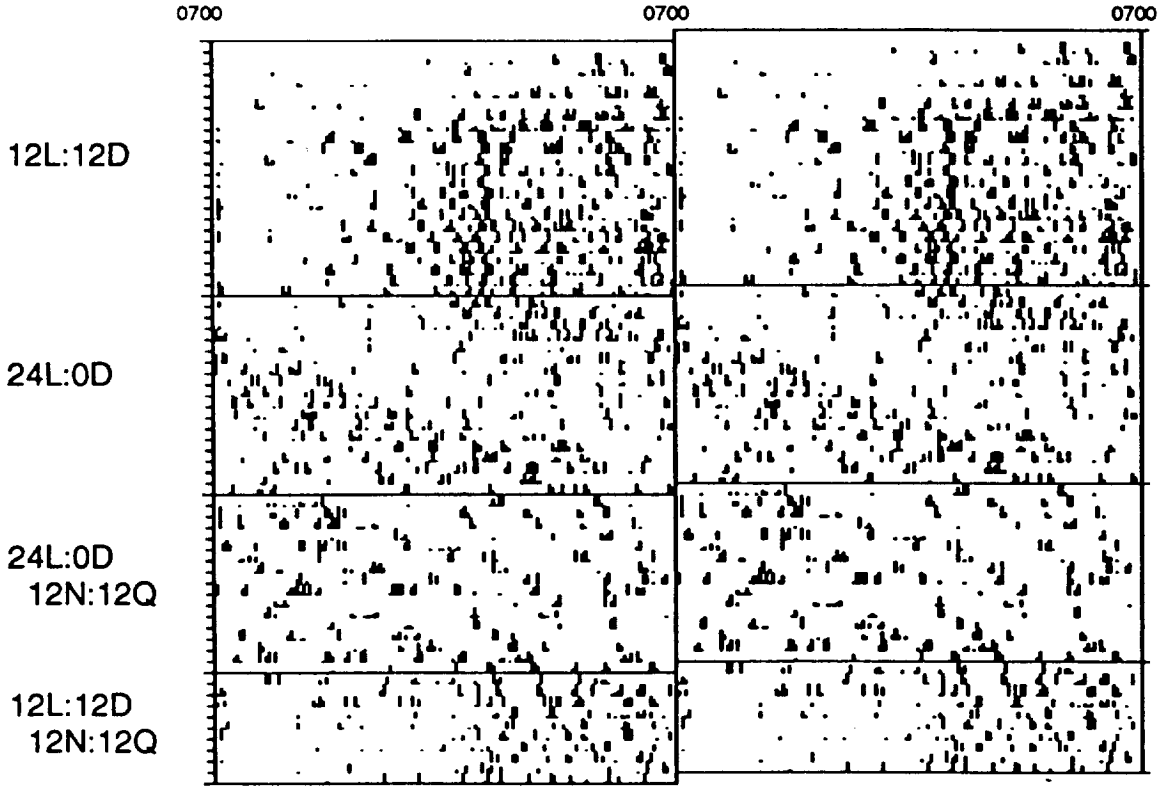
Experiment 1.2 (9007Sound05) Rat 2 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



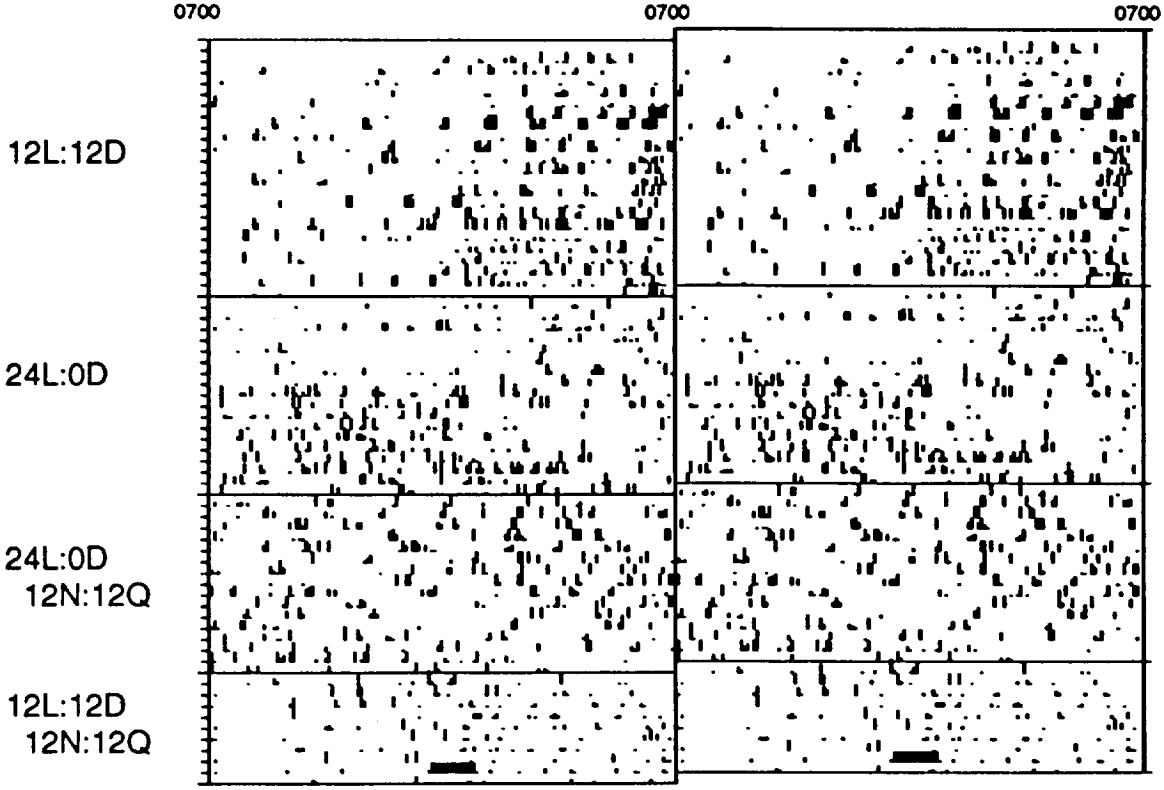
Experiment 1.2 (9007Sound05) Rat 3 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



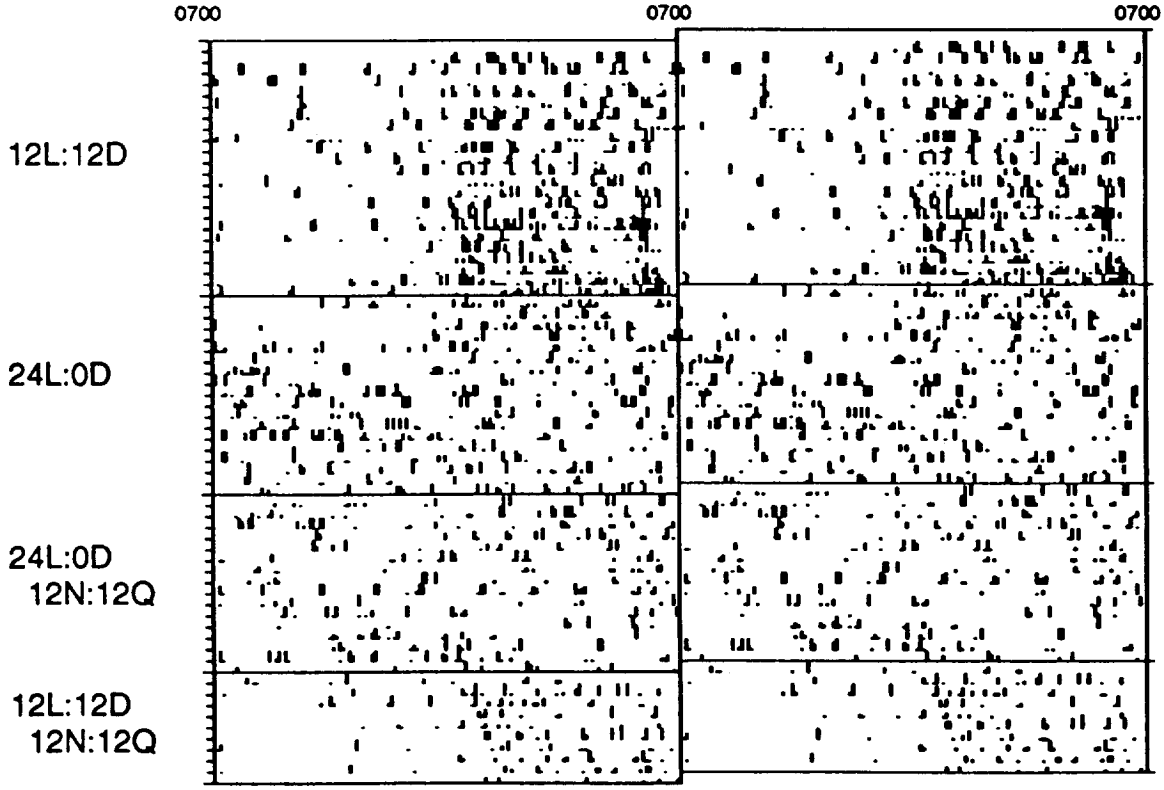
Experiment 1.2 (9007Sound05) Rat 4 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



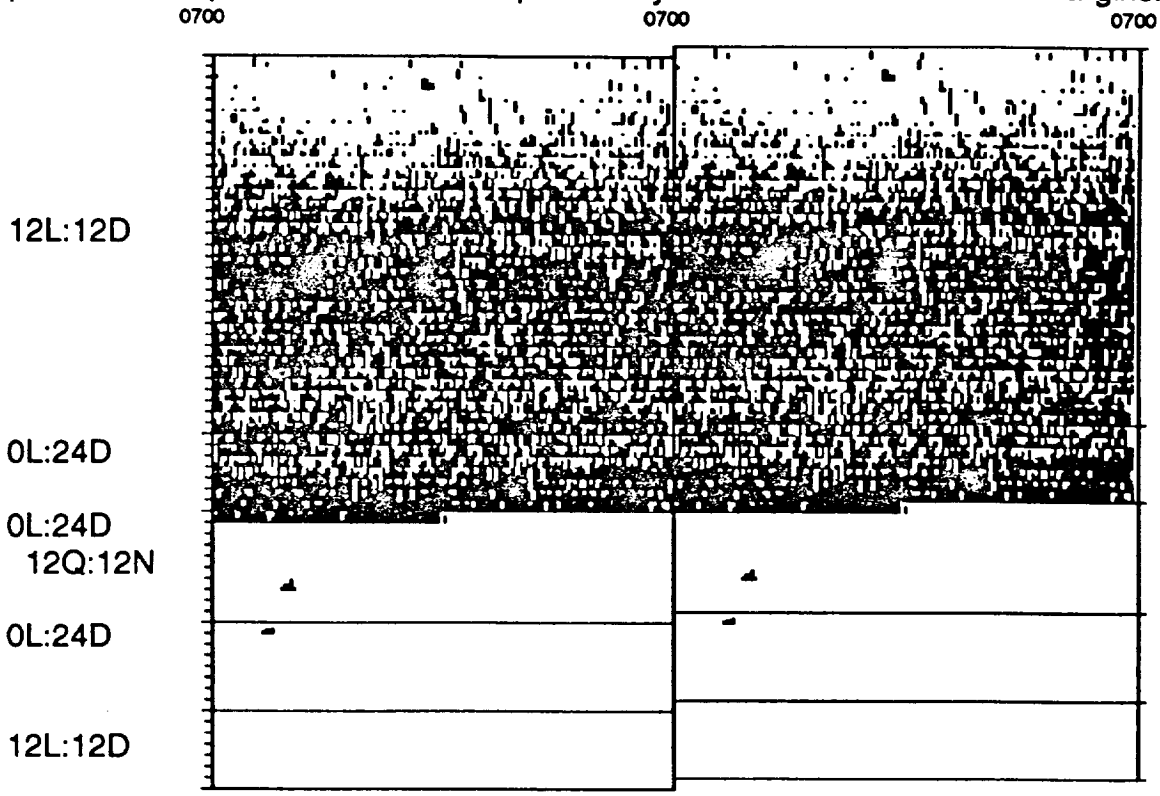
Experiment 1.2 (9007Sound05) Rat 5 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



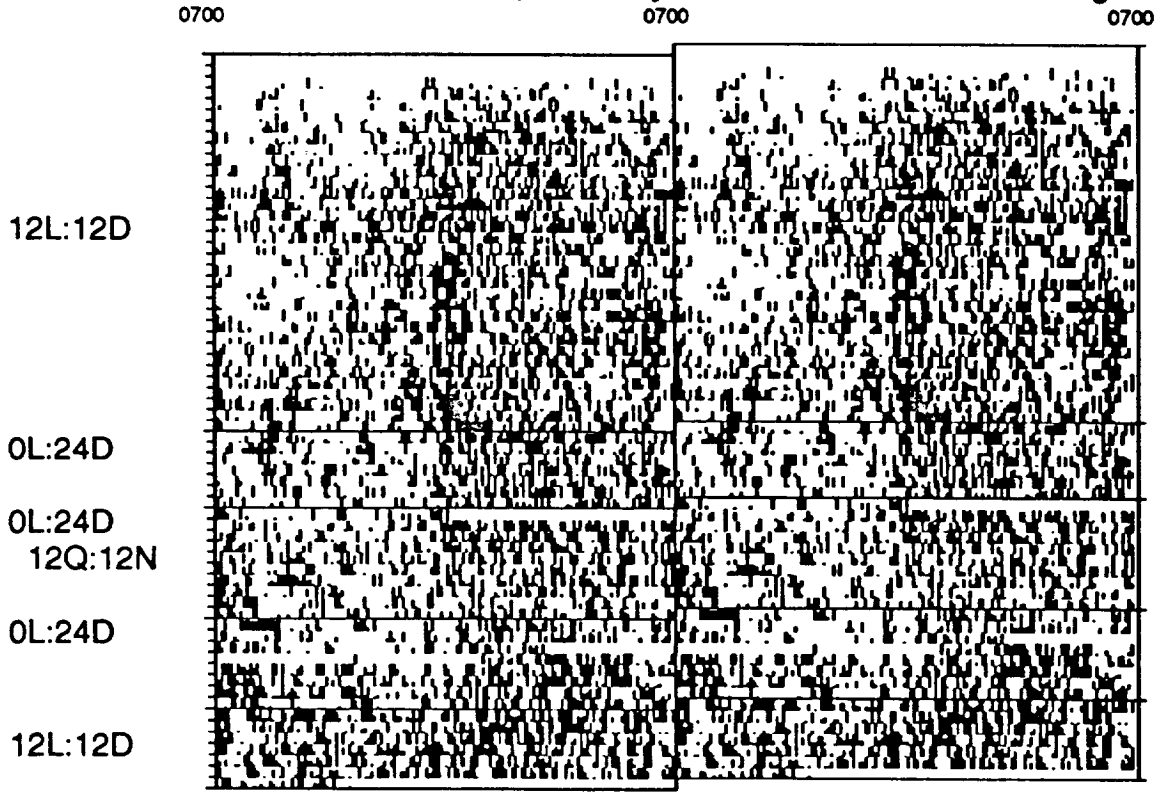
Experiment 1.2 (9007Sound05) Rat 6 feeding actogram. All non-zero values are plotted. Four periods of time are separated by horizontal lines.



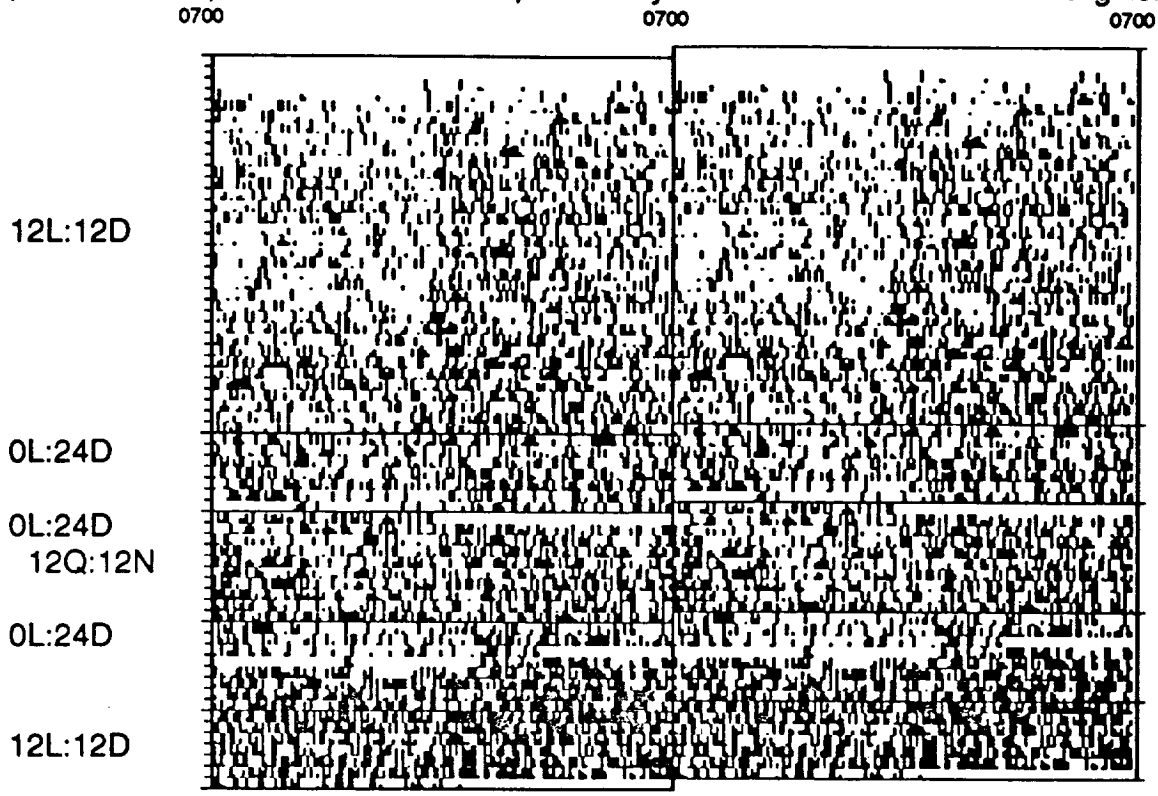
Experiment 2.1 (9011Sound05) Rat 1 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



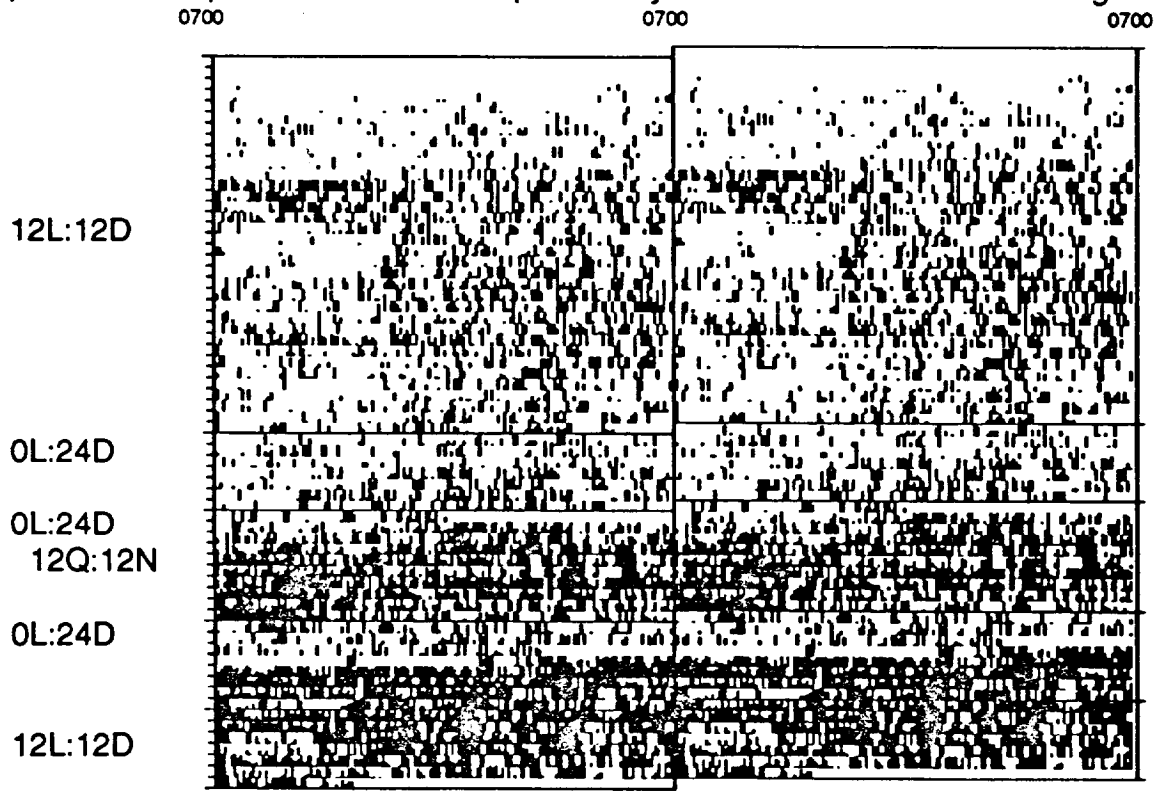
Experiment 2.1 (9011Sound05) Rat 2 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



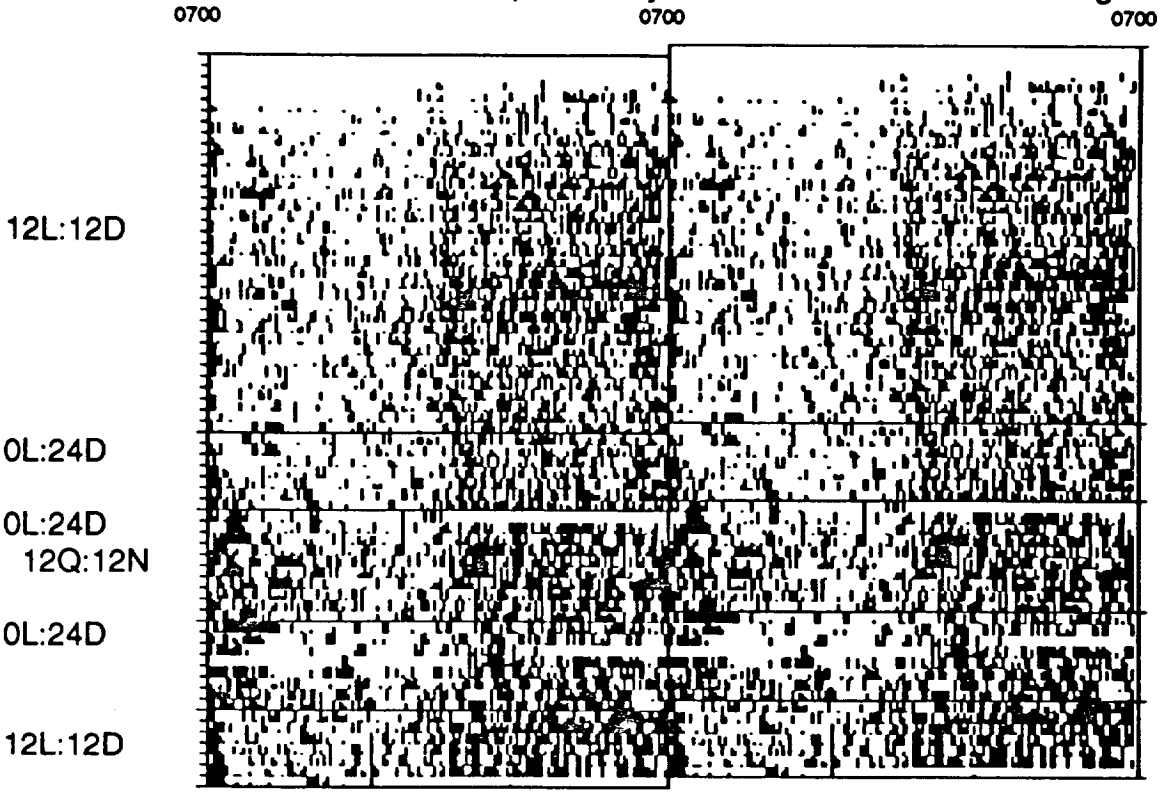
Experiment 2.1 (9011Sound05) Rat 3 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



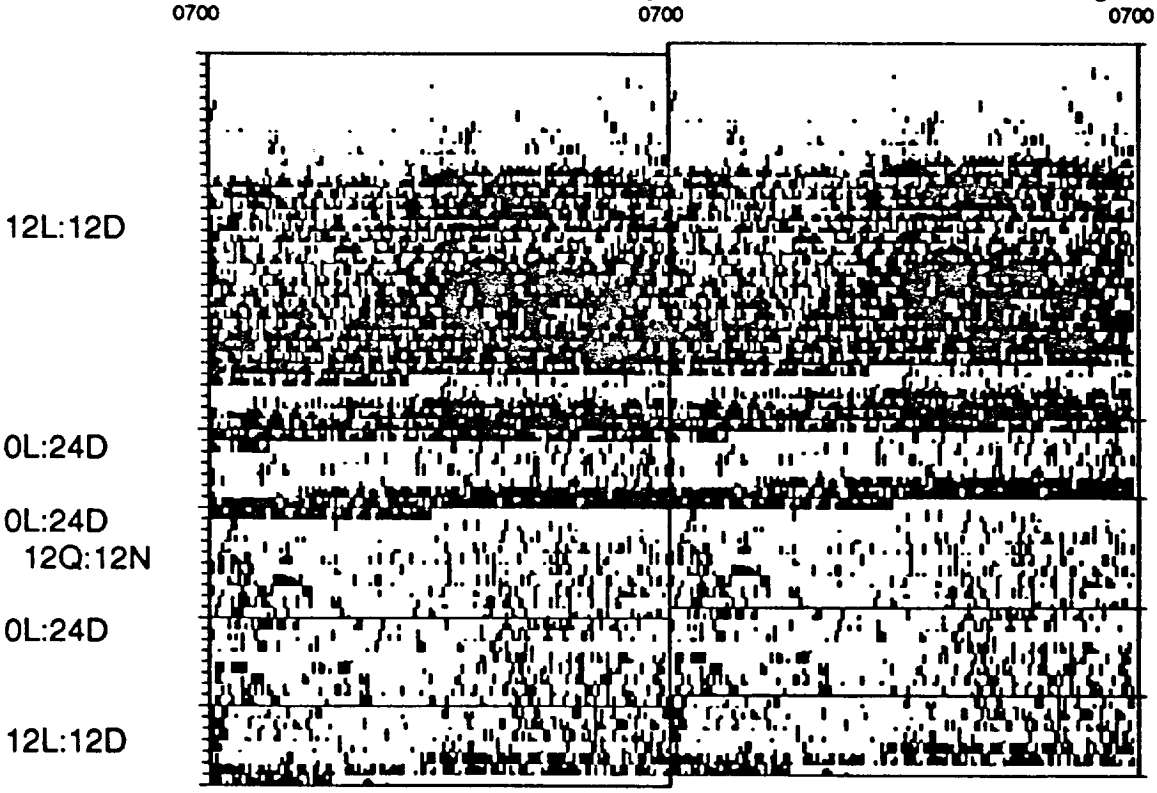
Experiment 2.1 (9011Sound05) Rat 4 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



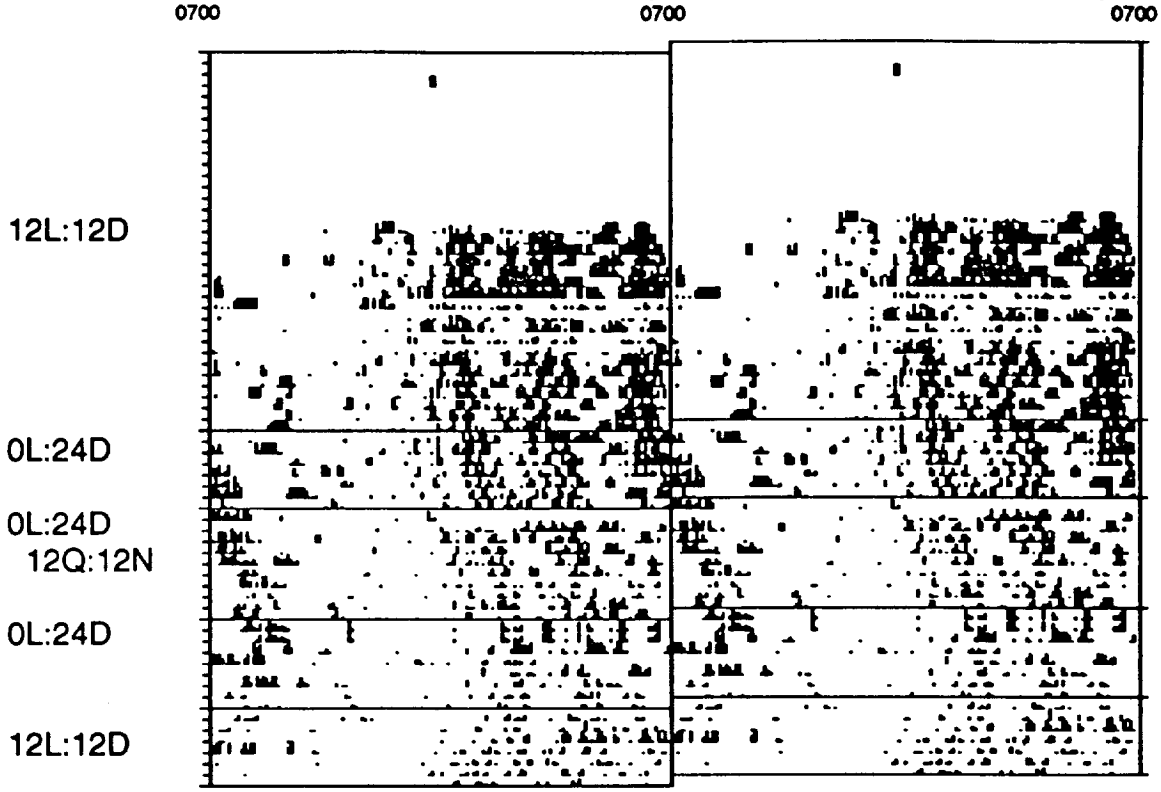
Experiment 2.1 (9011Sound05) Rat 5 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



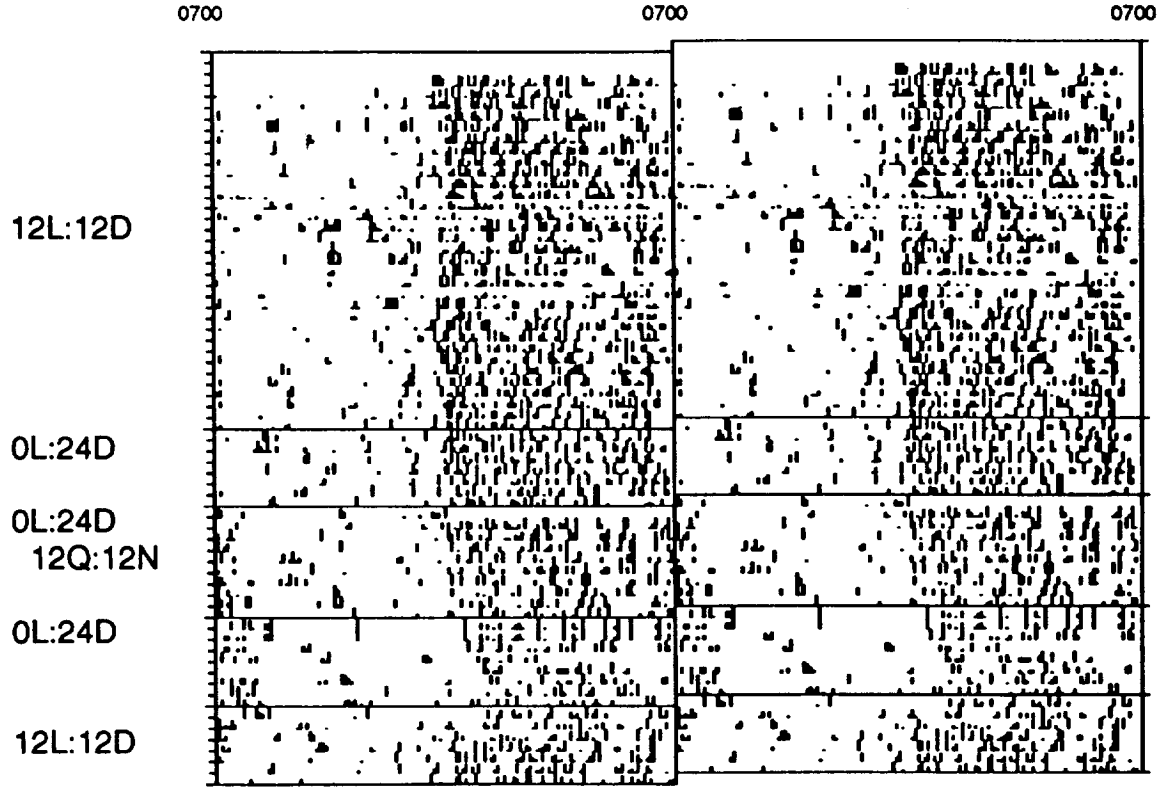
Experiment 2.1 (9011Sound05) Rat 6 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



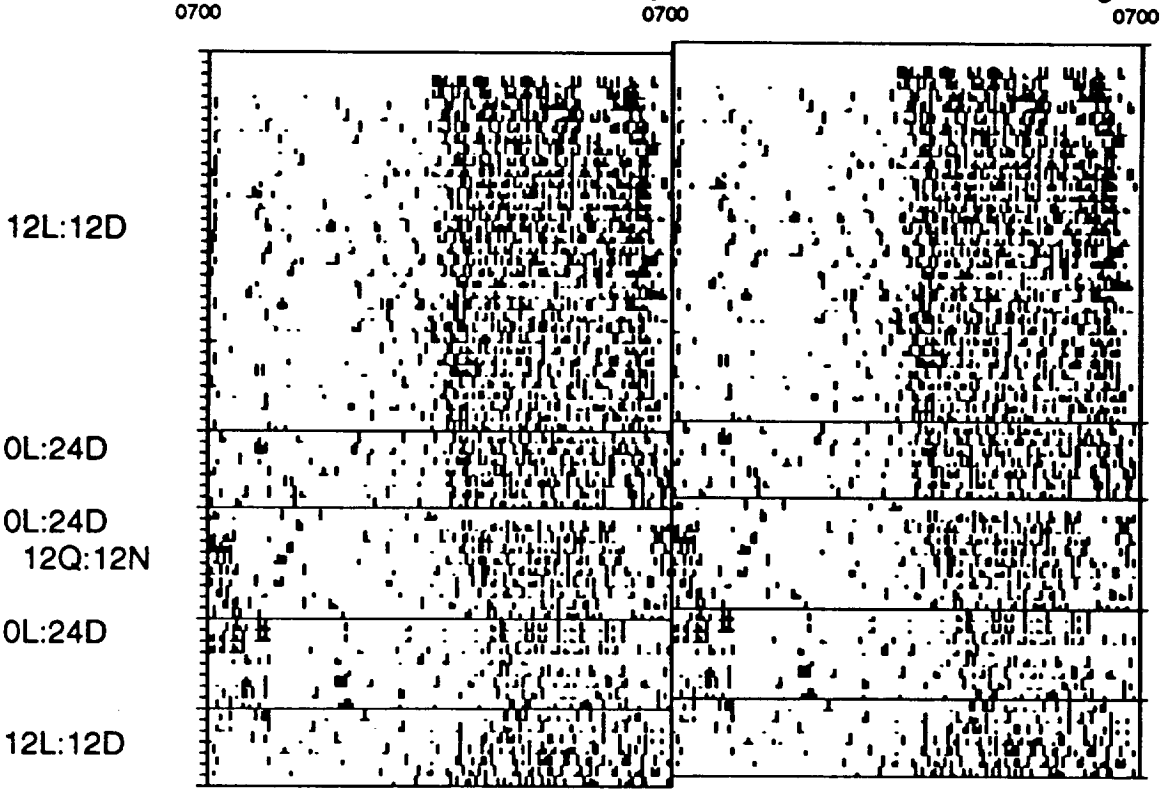
Experiment 2.1 (9011Sound05) Rat 1 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



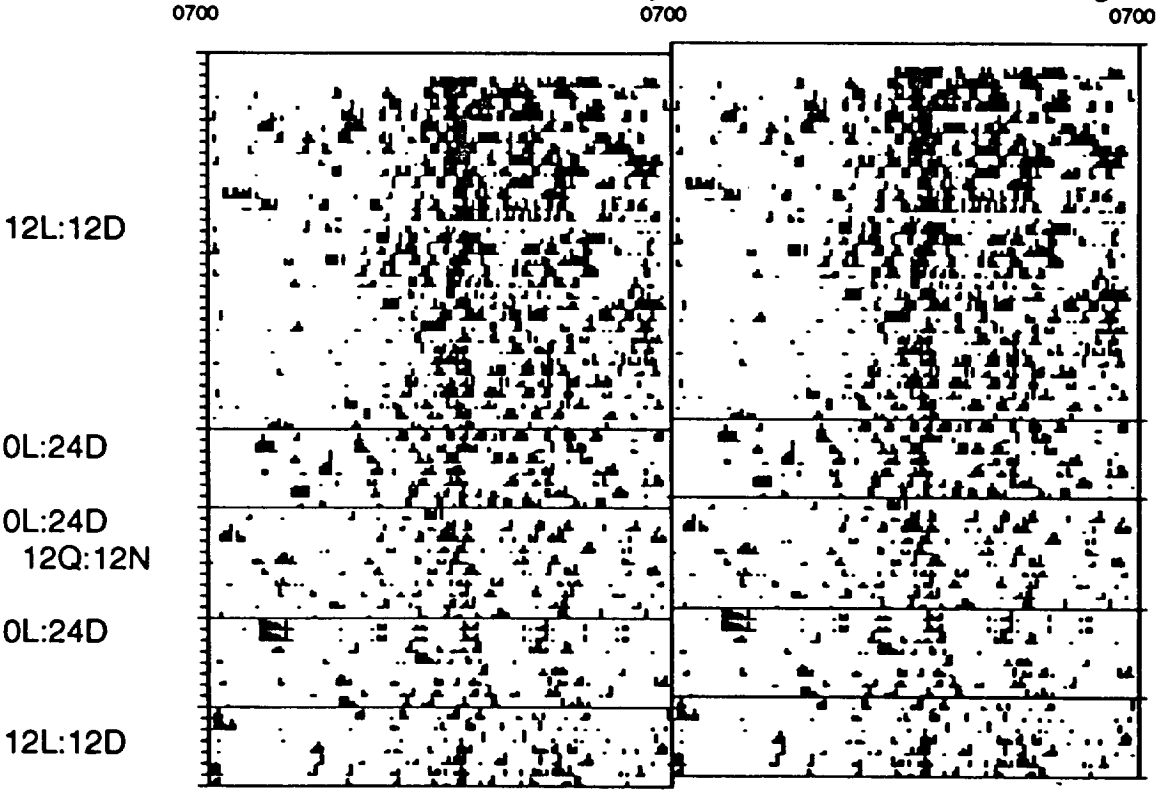
Experiment 2.1 (9011Sound05) Rat 2 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



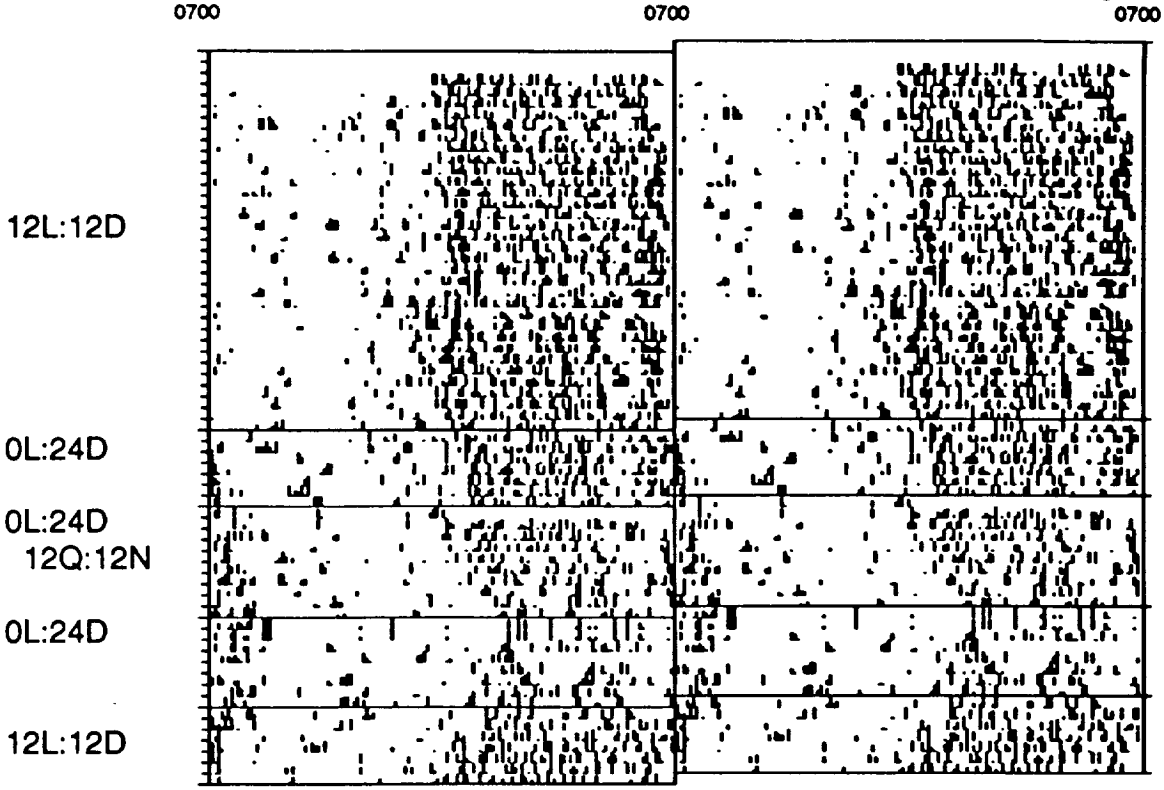
Experiment 2.1 (9011Sound05) Rat 3 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



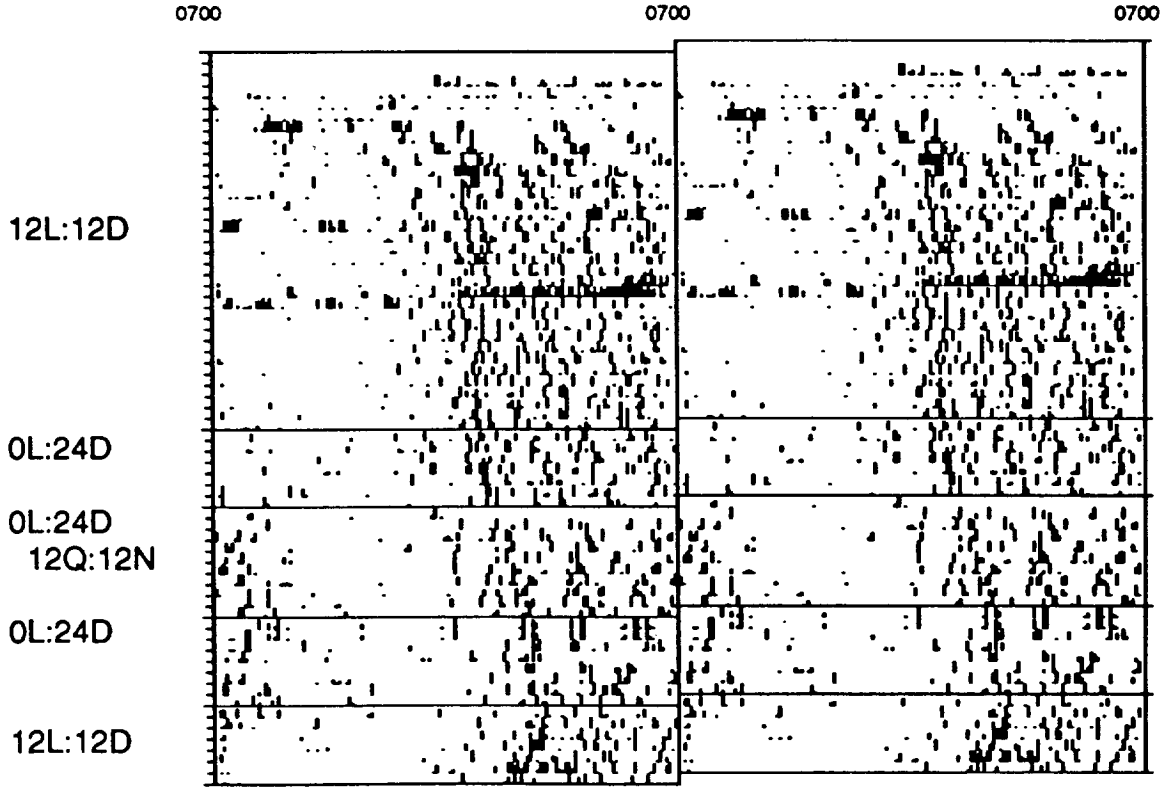
Experiment 2.1 (9011Sound05) Rat 4 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



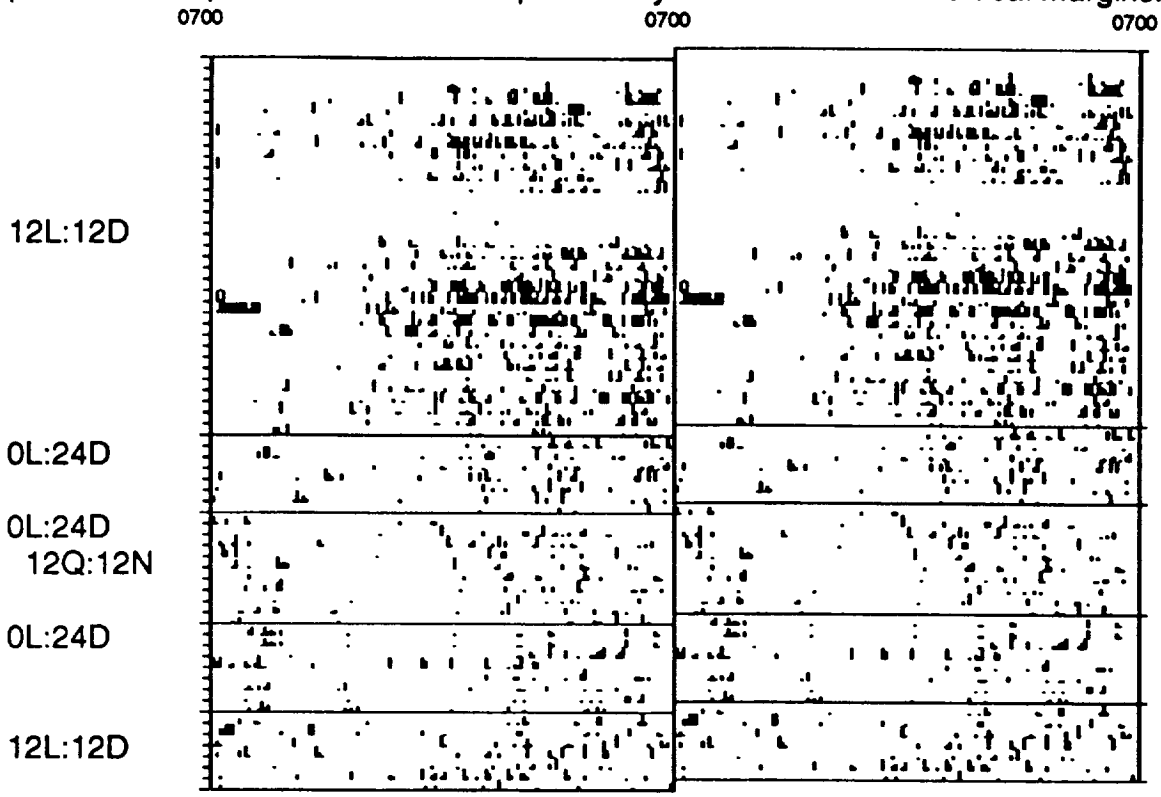
Experiment 2.1 (9011Sound05) Rat 5 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



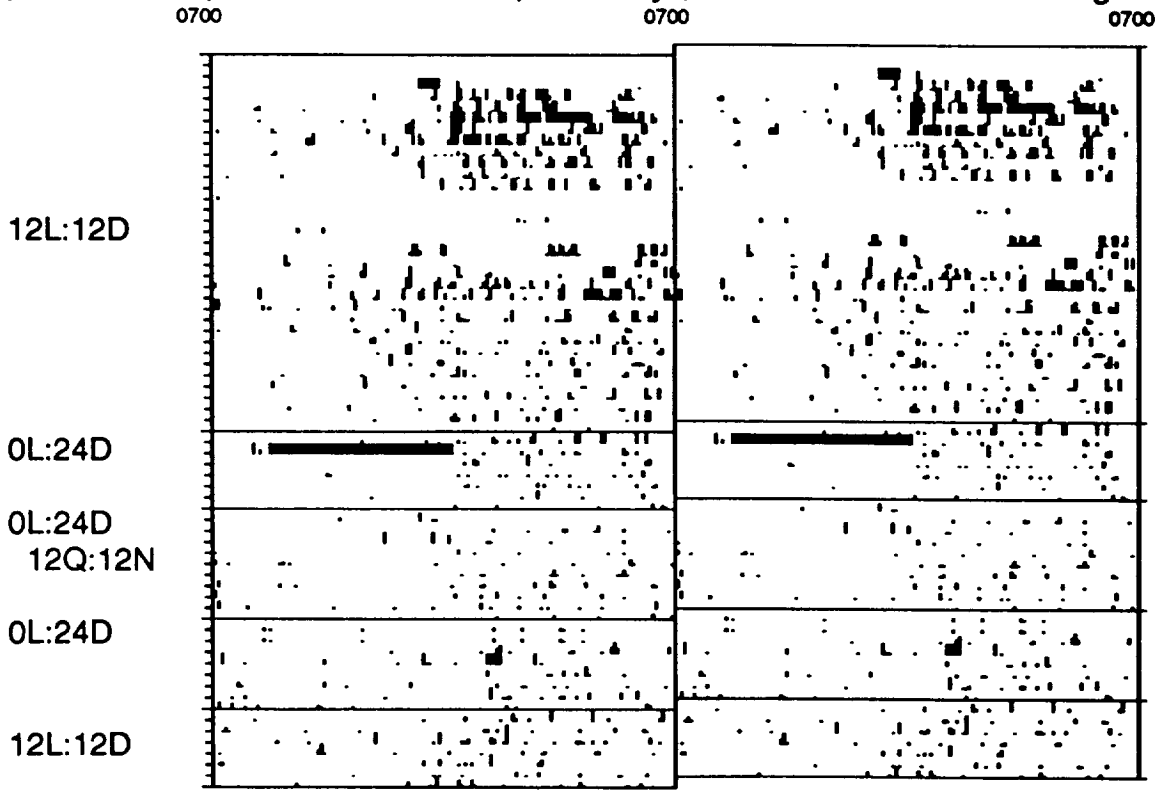
Experiment 2.1 (9011Sound05) Rat 6 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



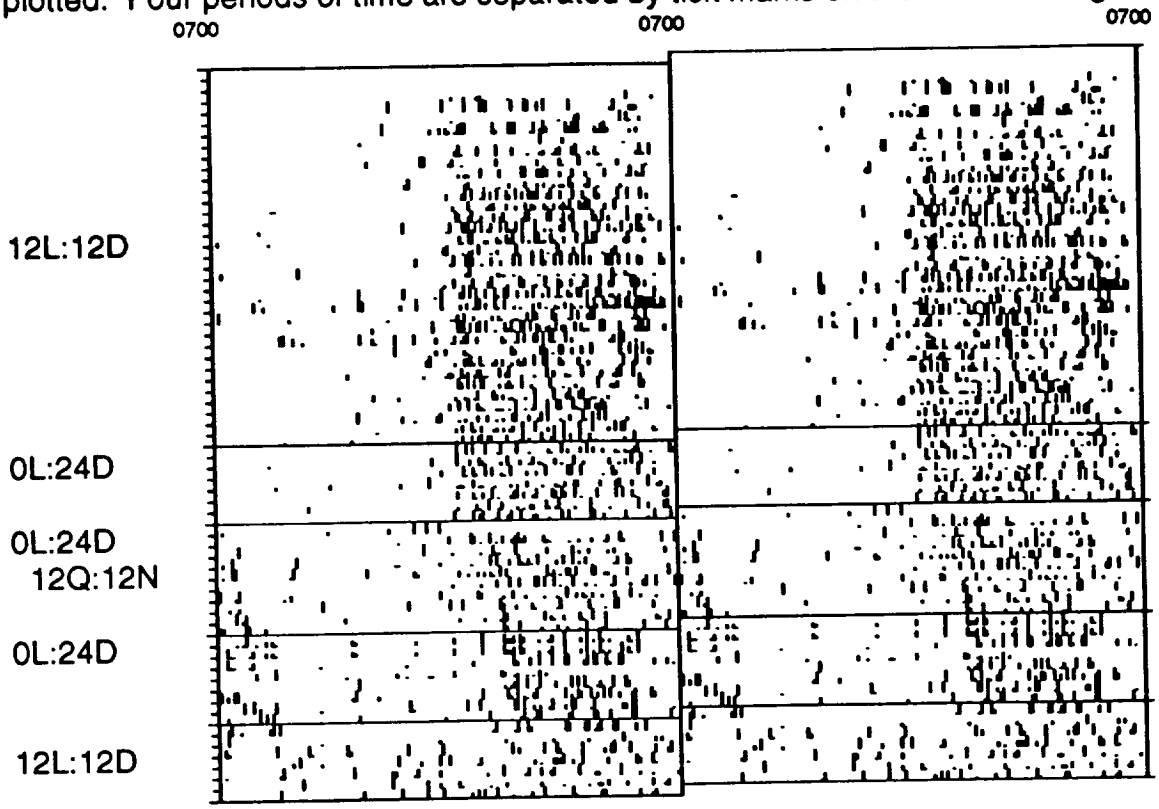
Experiment 2.1 (9011Sound05) Rat 1 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



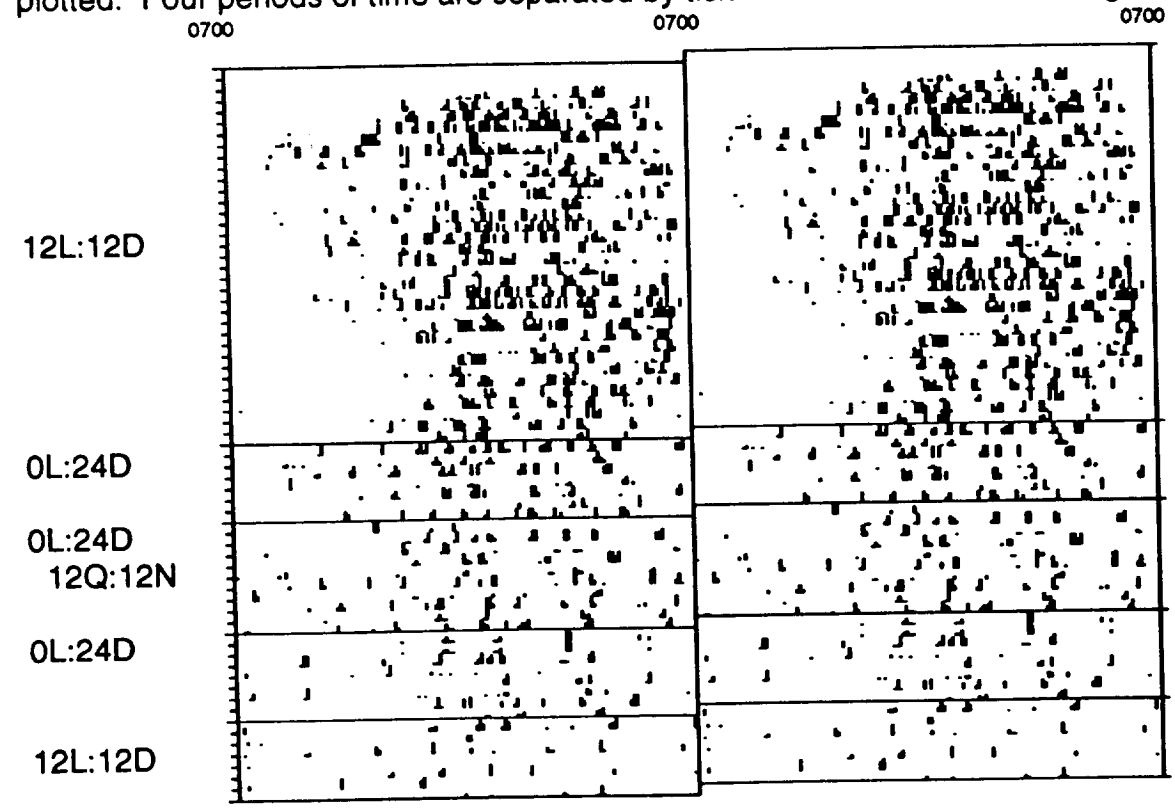
Experiment 2.1 (9011Sound05) Rat 2 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



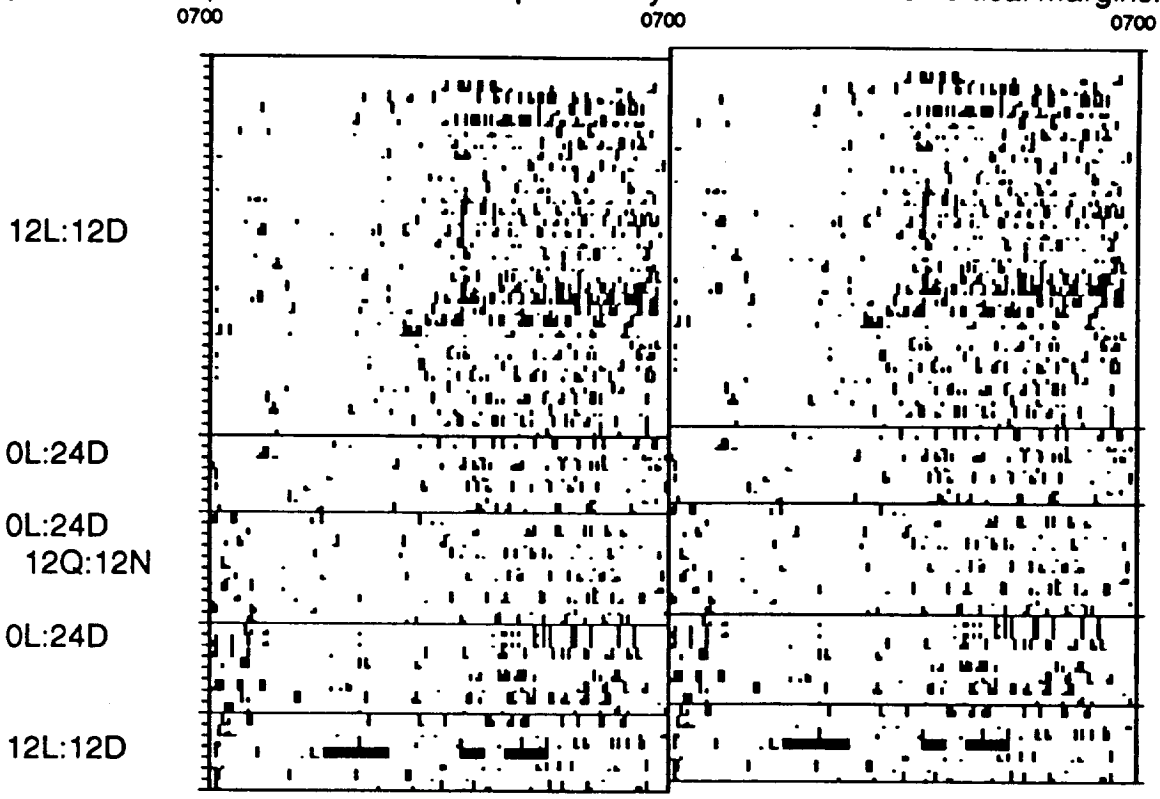
Experiment 2.1 (9011Sound05) Rat 3 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



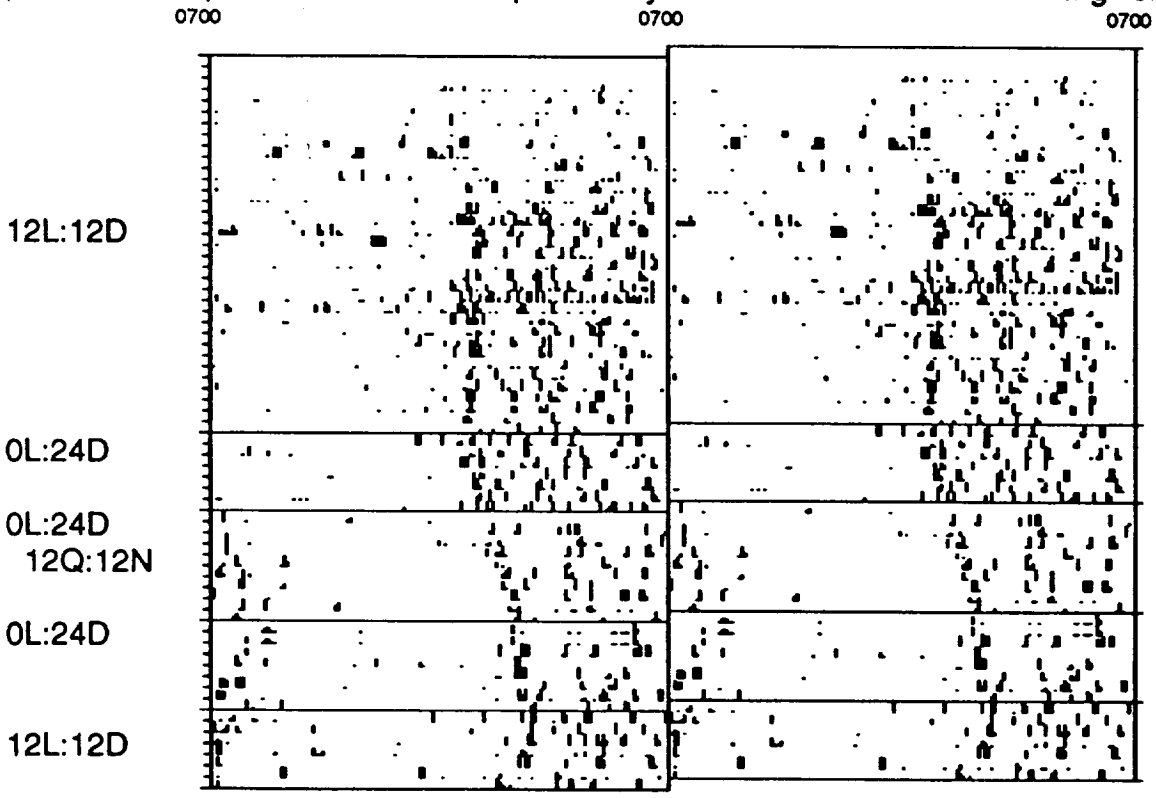
Experiment 2.1 (9011Sound05) Rat 4 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



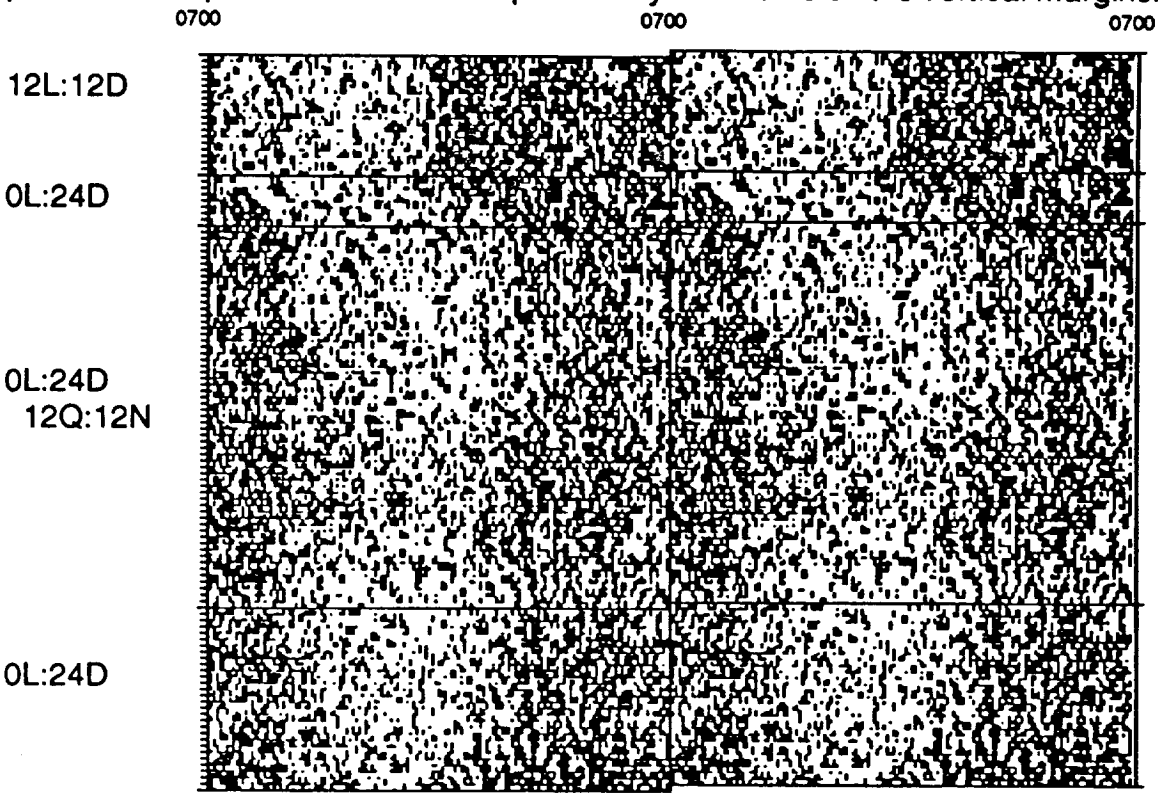
Experiment 2.1 (9011Sound05) Rat 5 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



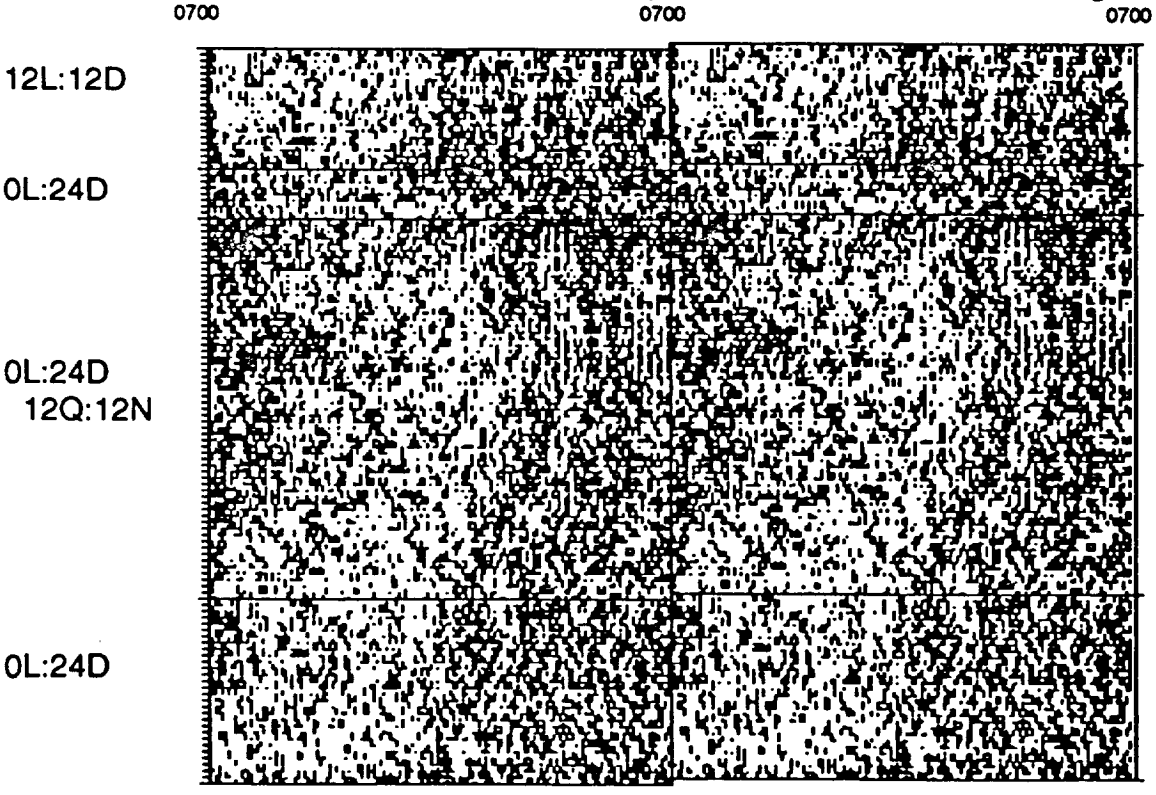
Experiment 2.1 (9011Sound05) Rat 6 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



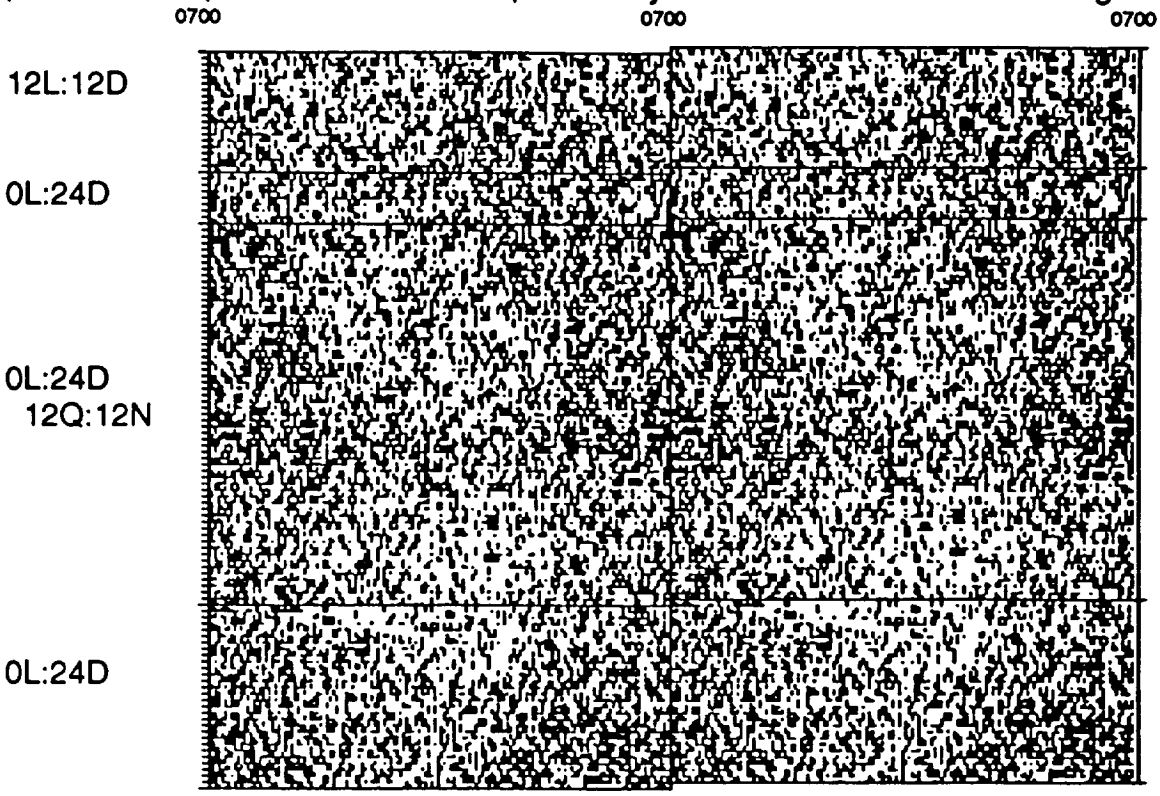
Experiment 2.2 (9102Sound05) Rat 1 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



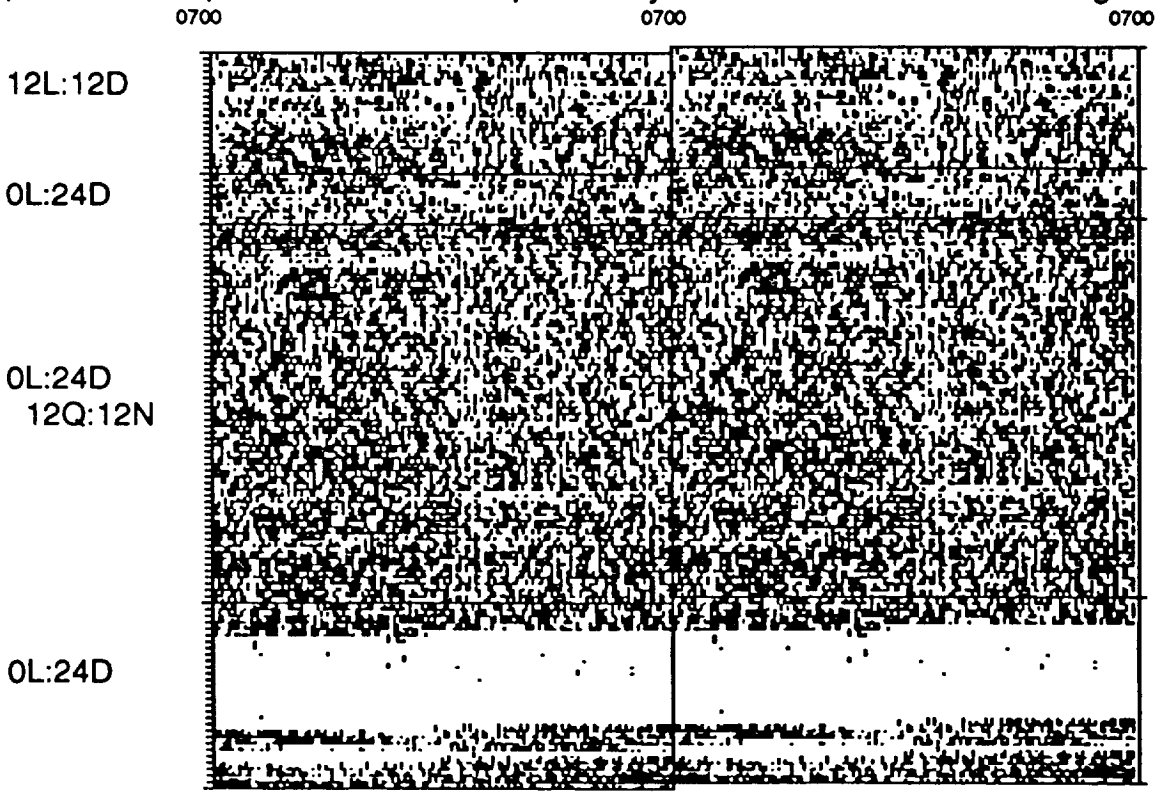
Experiment 2.2 (9102Sound05) Rat 2 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



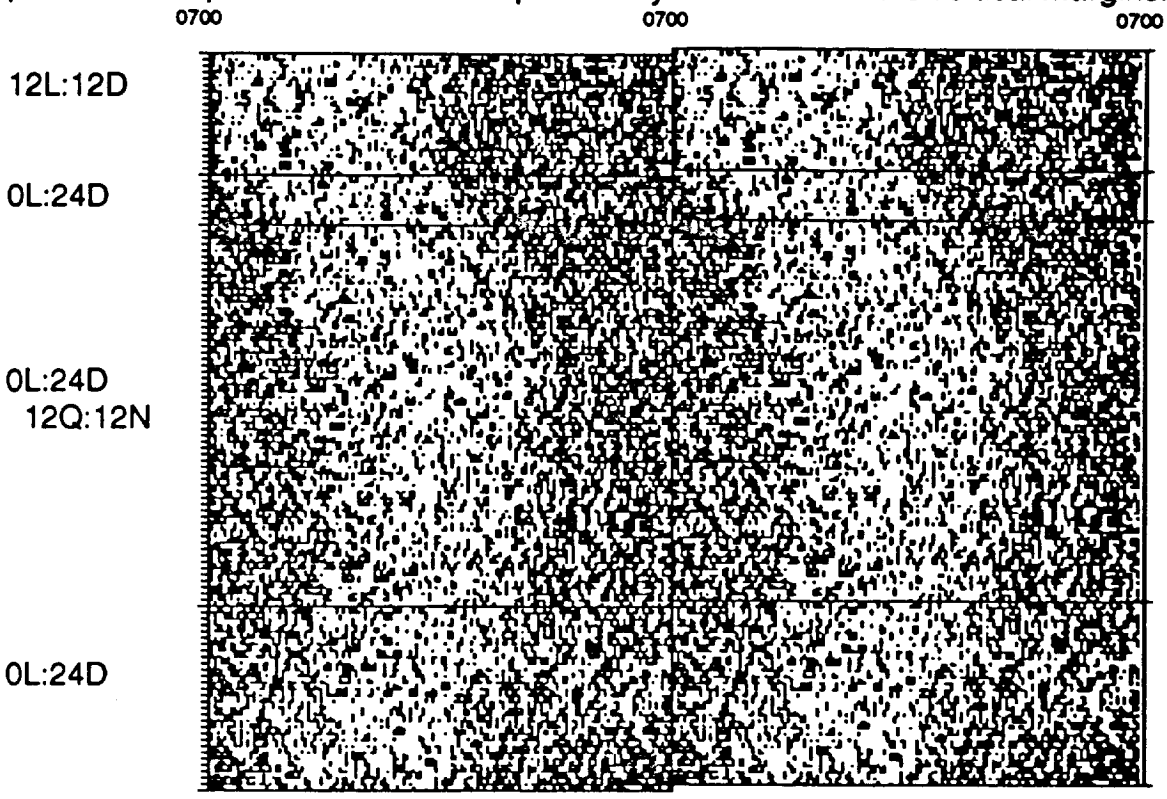
Experiment 2.2 (9102Sound05) Rat 3 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



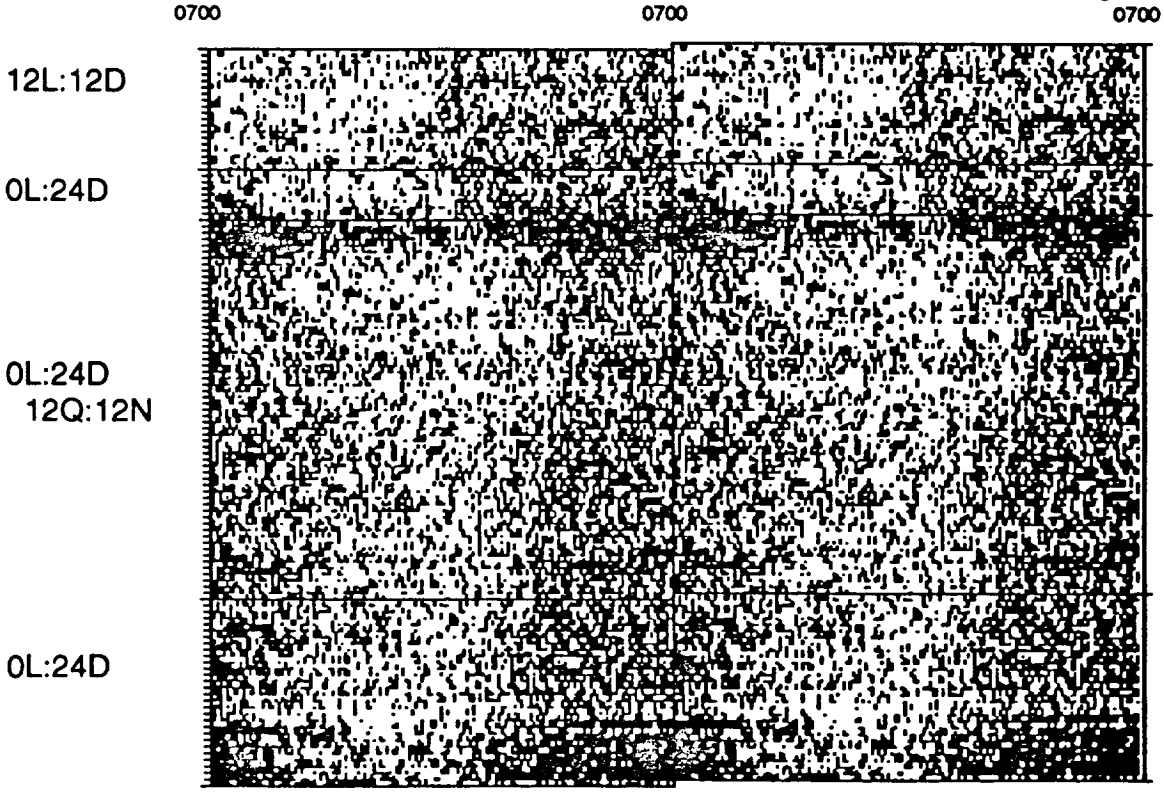
Experiment 2.2 (9102Sound05) Rat 4 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



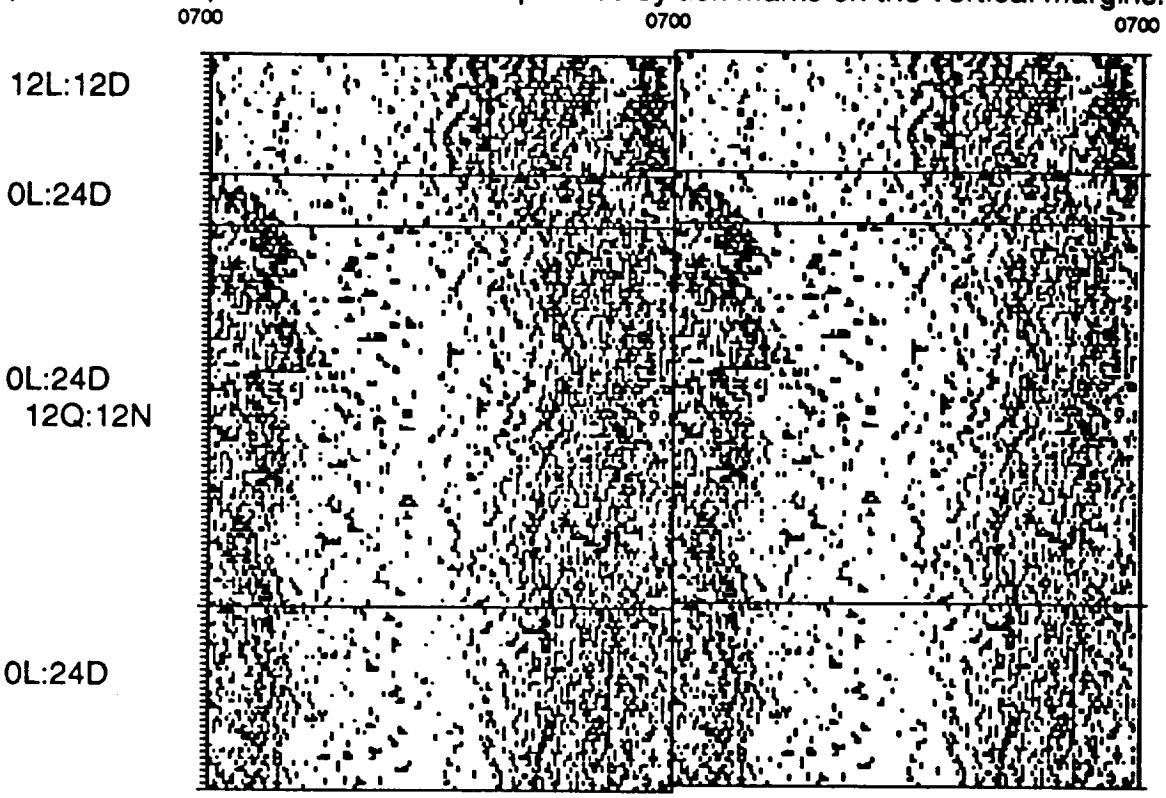
Experiment 2.2 (9102Sound05) Rat 5 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



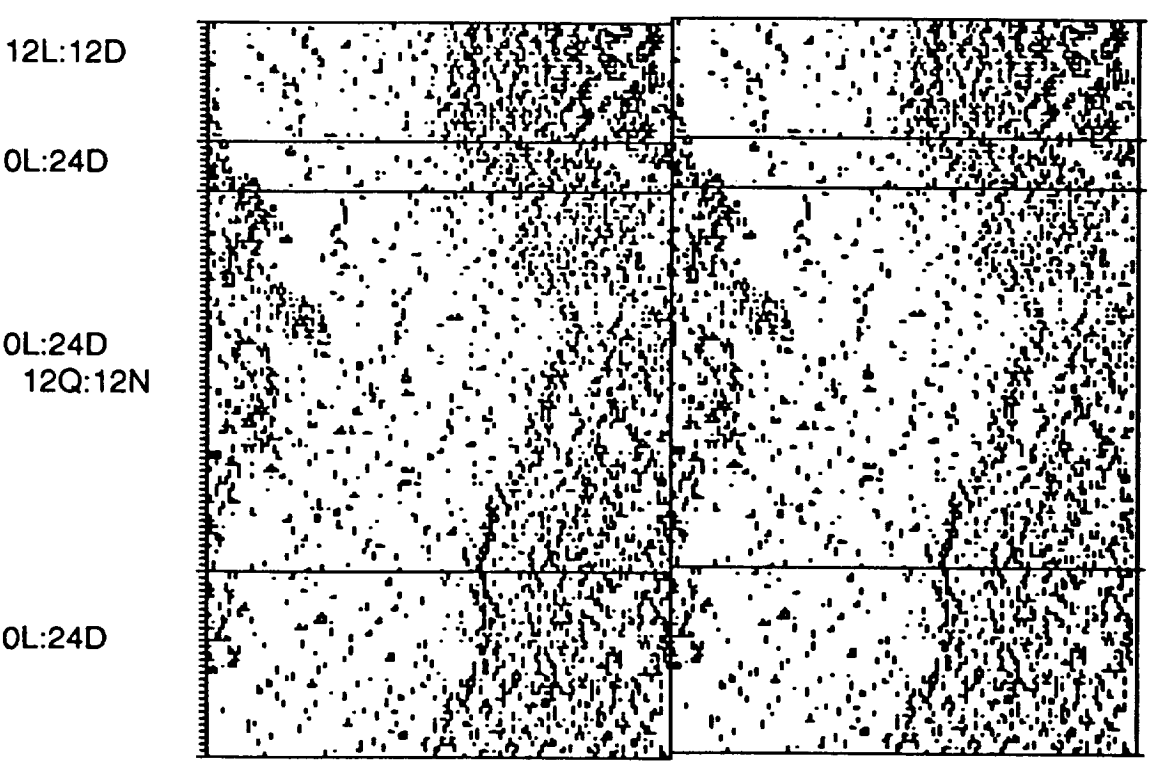
Experiment 2.2 (9102Sound05) Rat 6 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



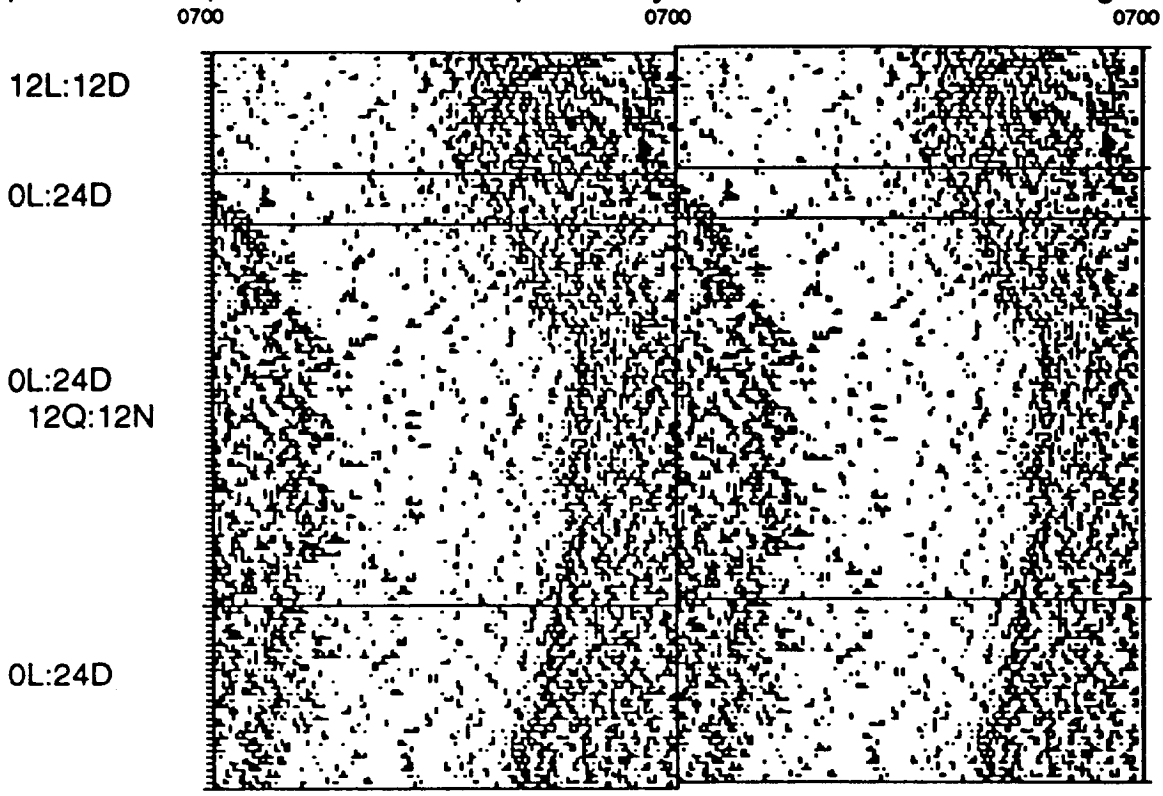
Experiment 2.2 (9102Sound05) Rat 1 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



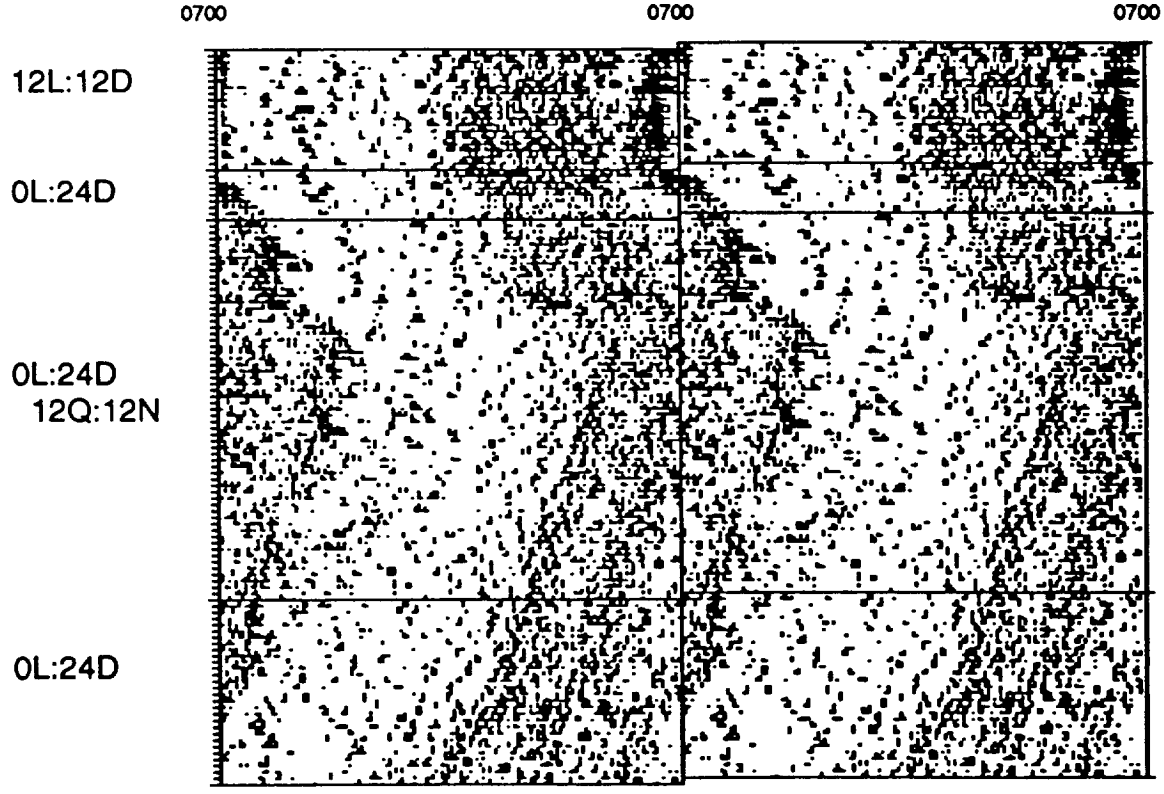
Experiment 2.2 (9102Sound05) Rat 2 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



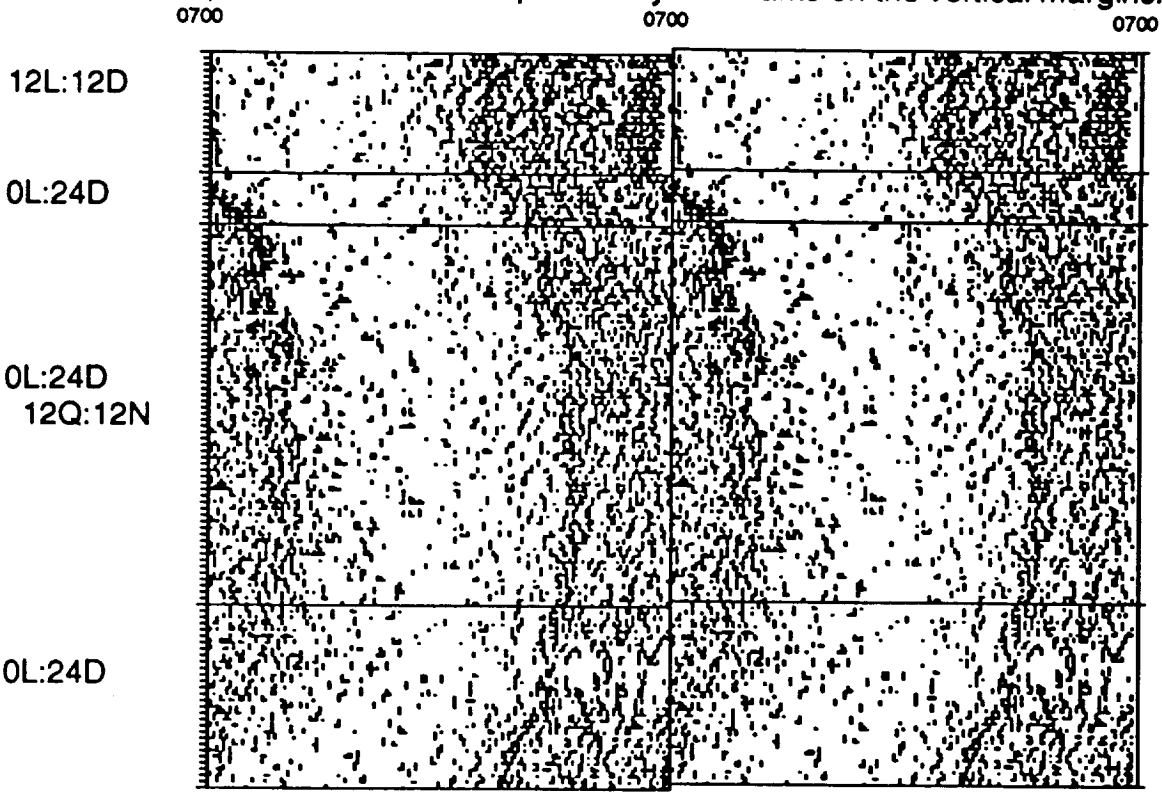
Experiment 2.2 (9102Sound05) Rat 3 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



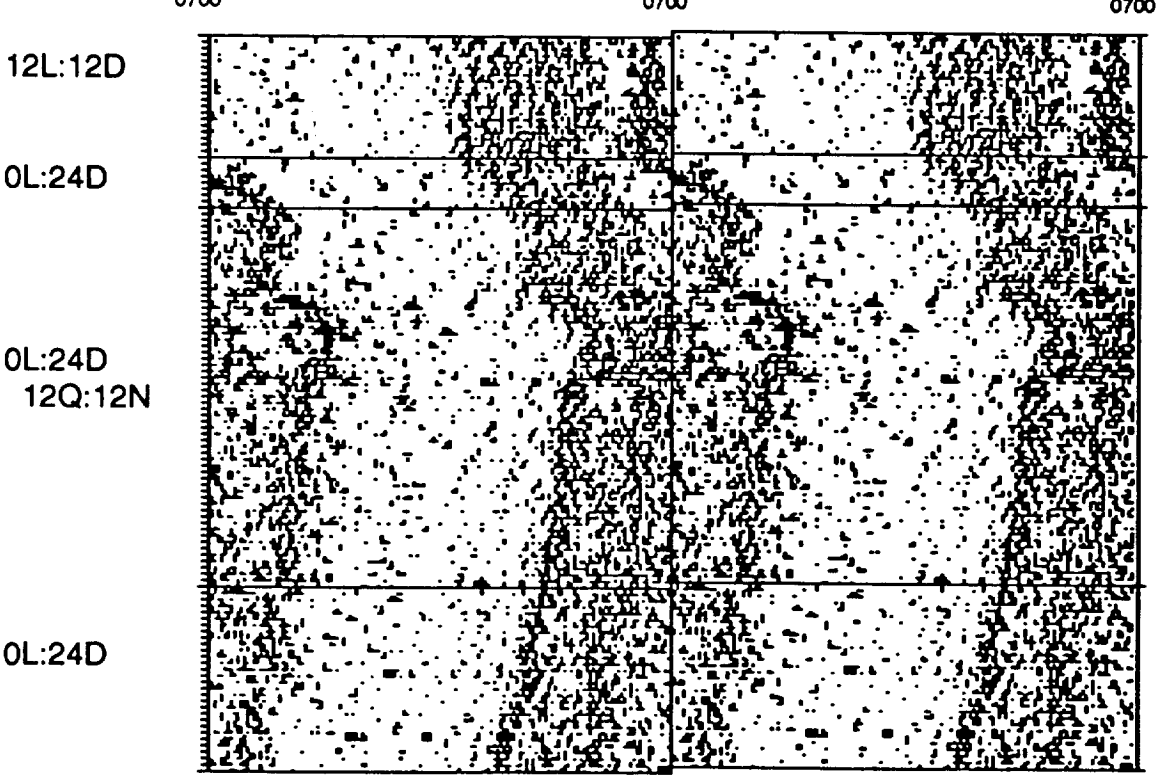
Experiment 2.2 (9102Sound05) Rat 4 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



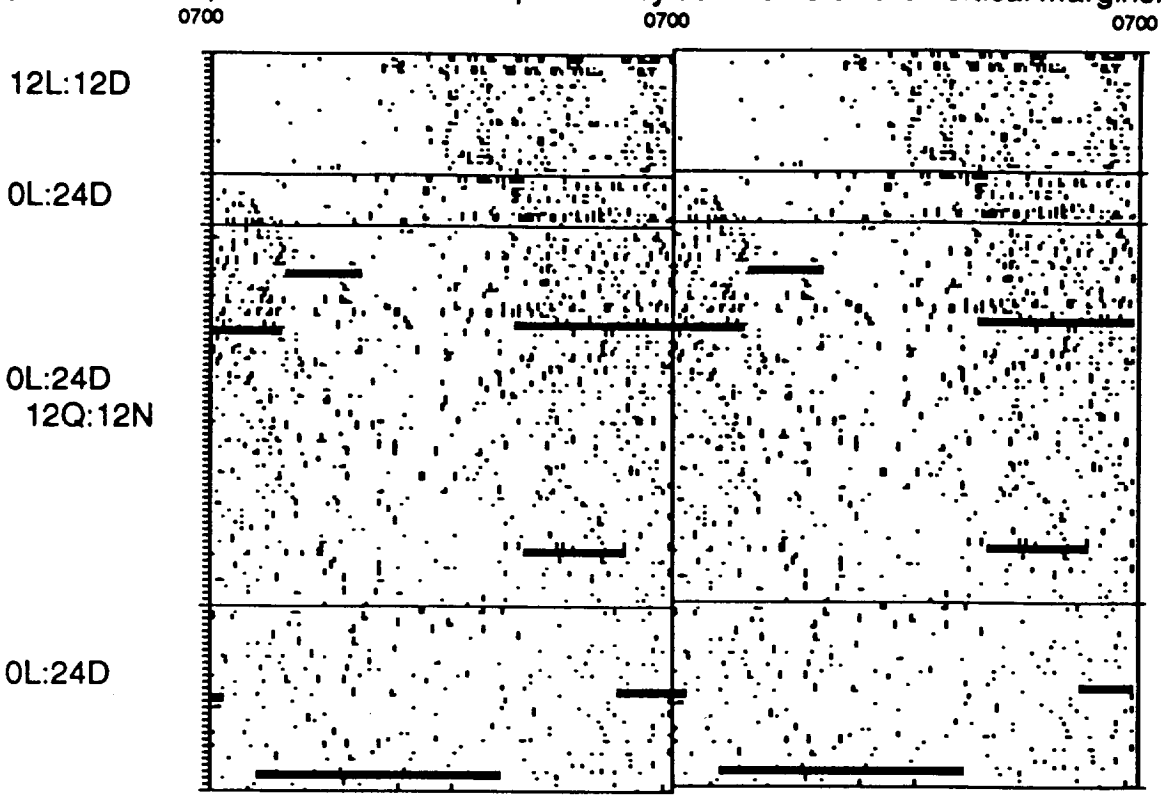
Experiment 2.2 (9102Sound05) Rat 5 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



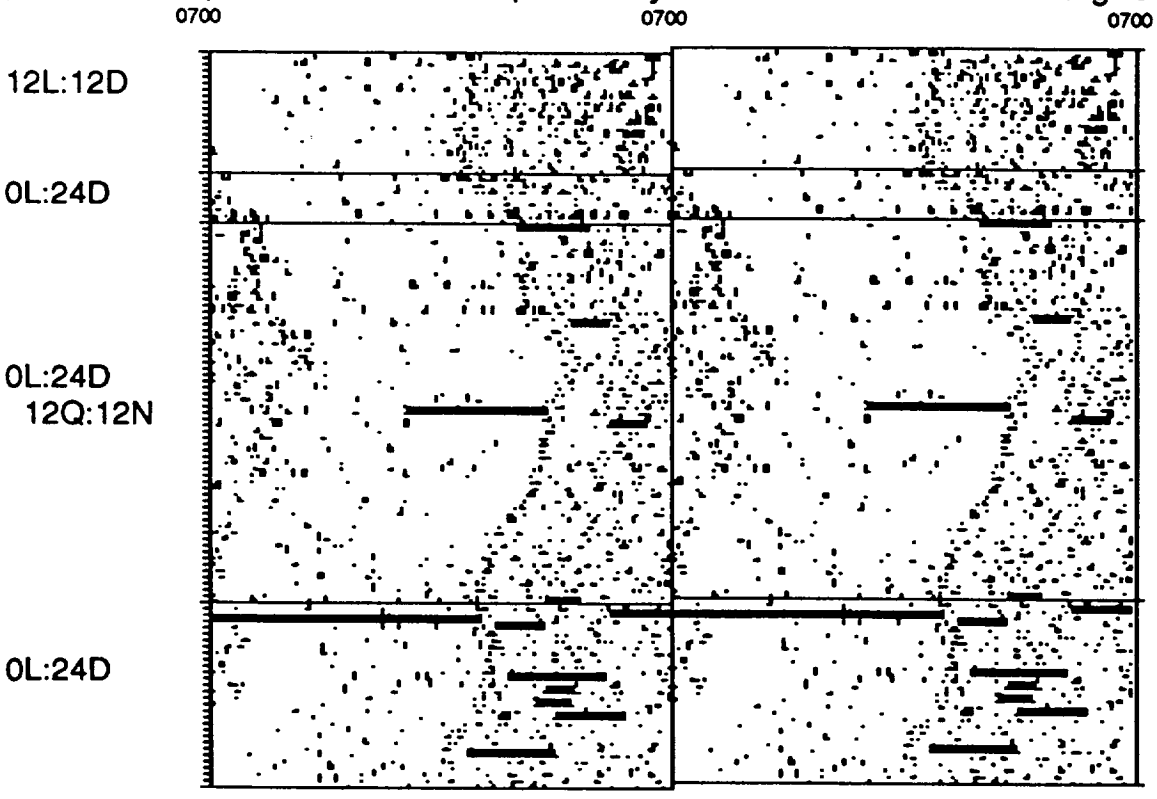
Experiment 2.2 (9102Sound05) Rat 6 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



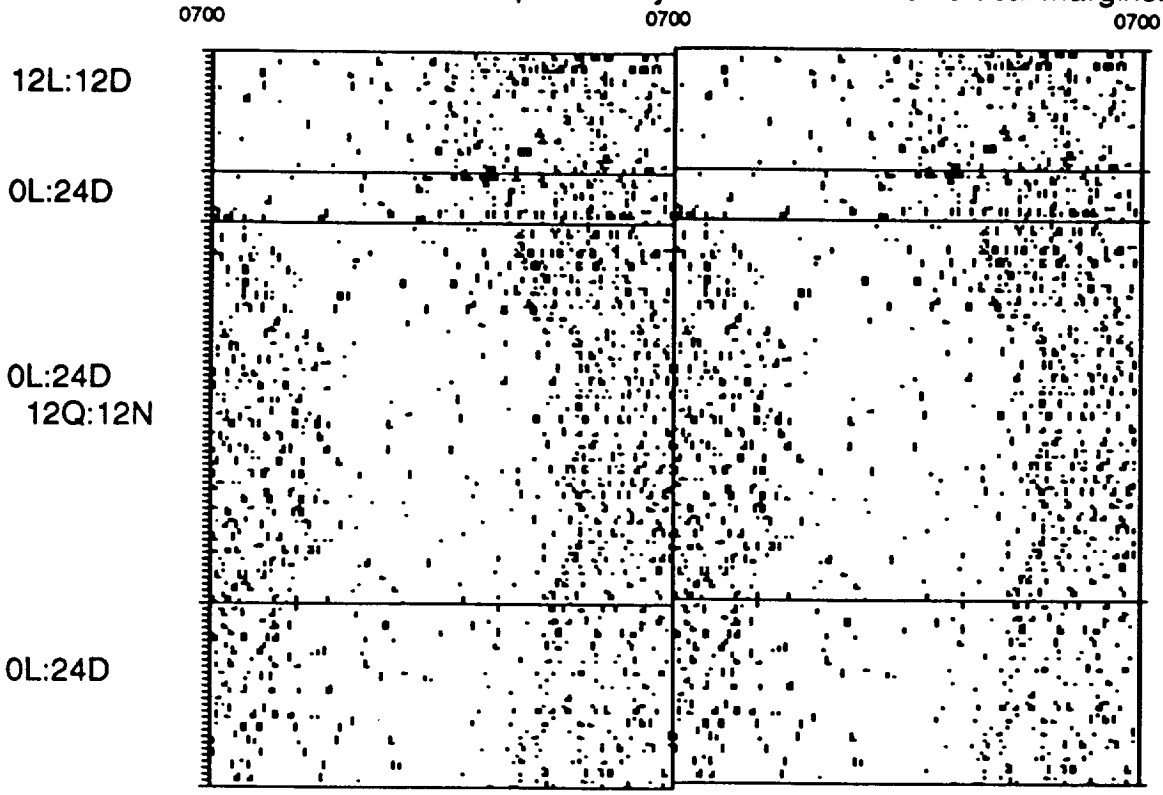
Experiment 2.2 (9102Sound05) Rat 1 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



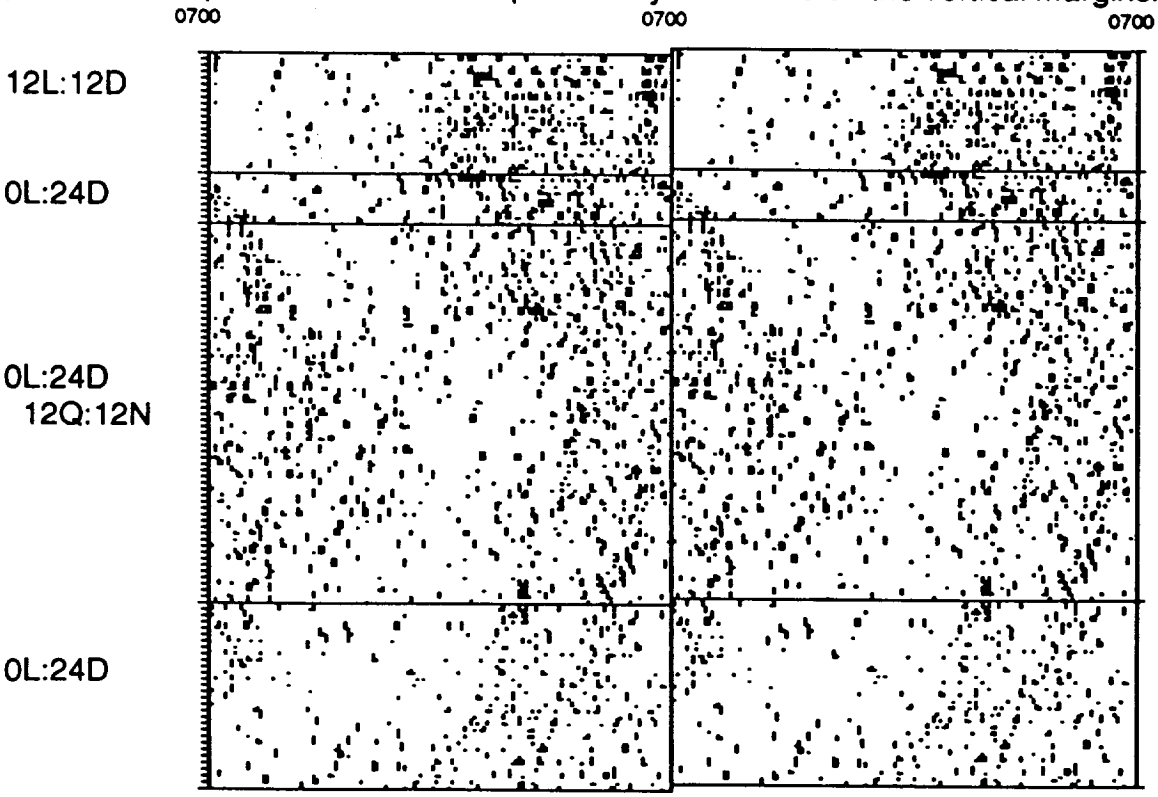
Experiment 2.2 (9102Sound05) Rat 2 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



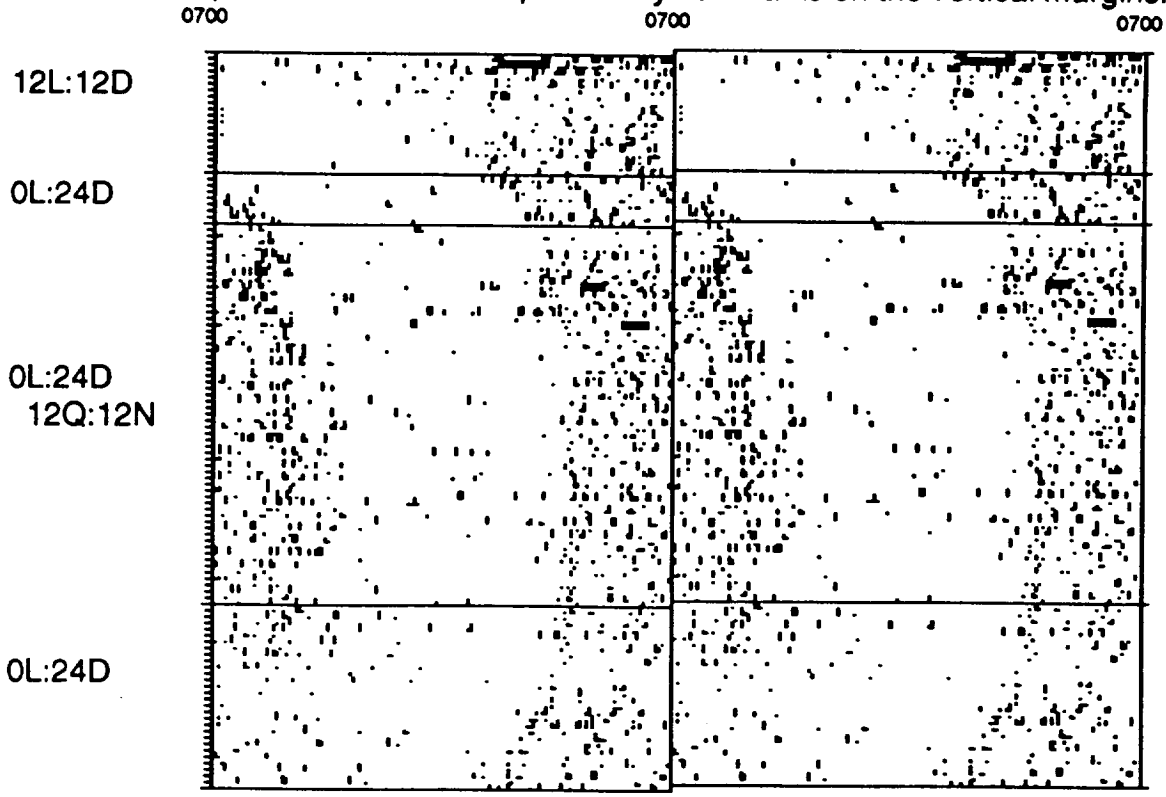
Experiment 2.2 (9102Sound05) Rat 3 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



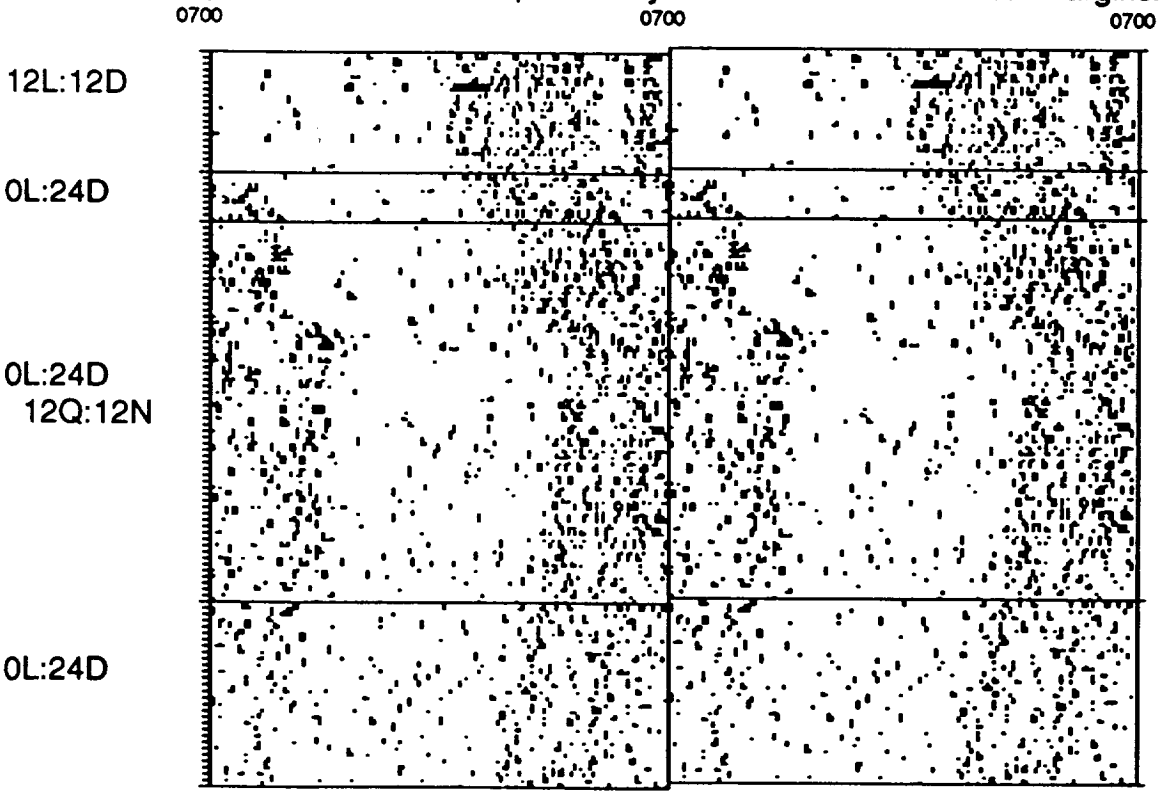
Experiment 2.2 (9102Sound05) Rat 4 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



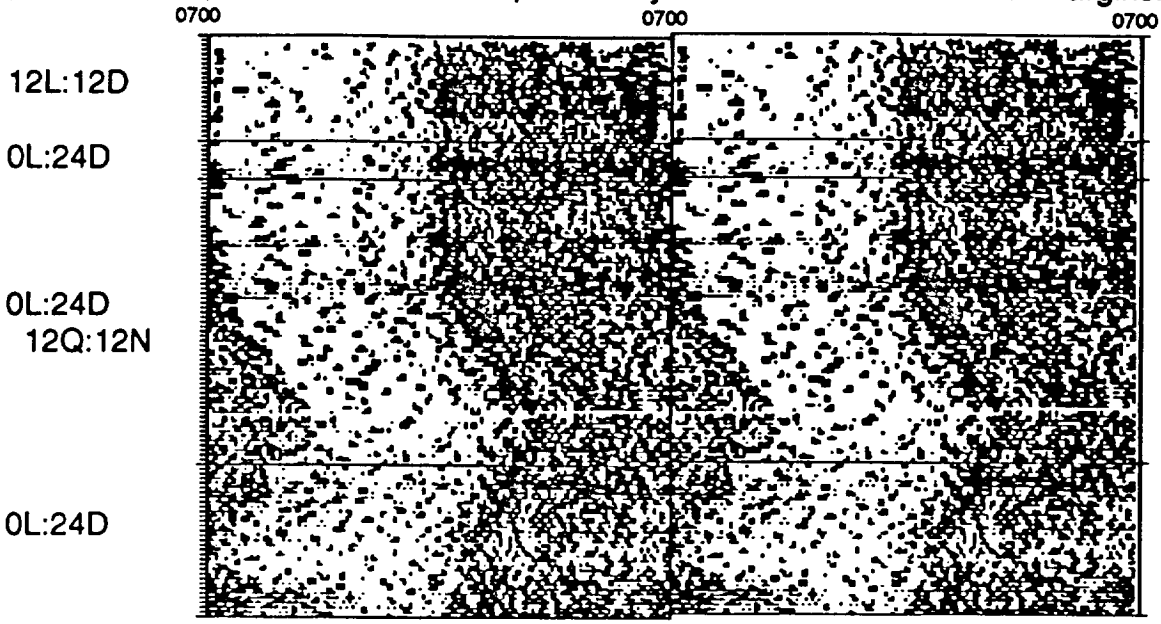
Experiment 2.2 (9102Sound05) Rat 5 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



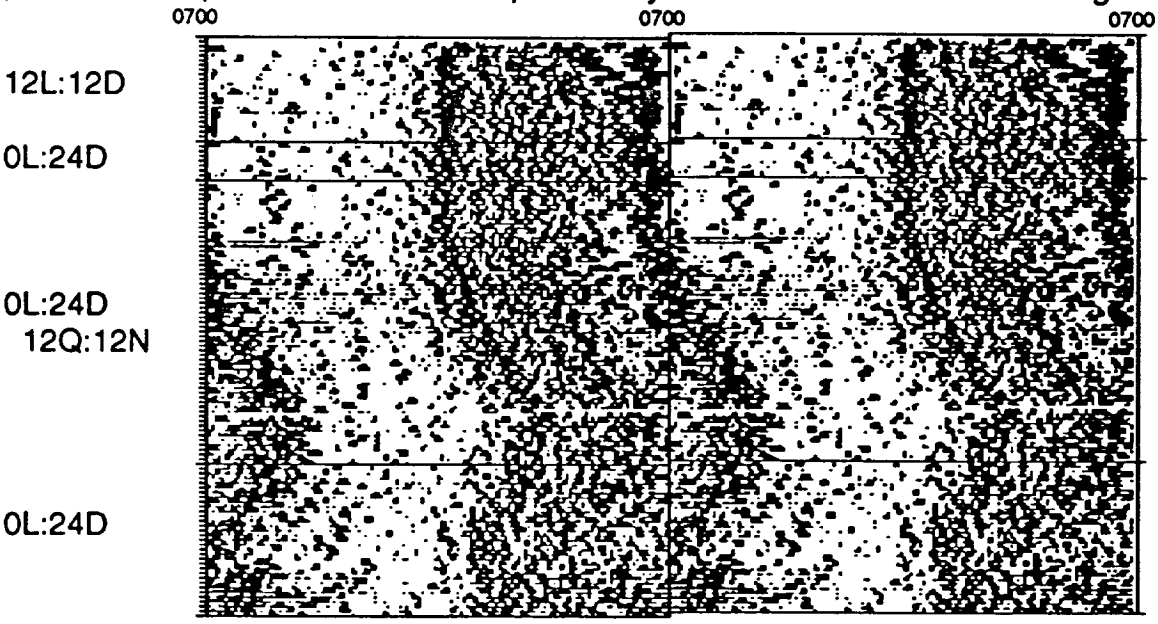
Experiment 2.2 (9102Sound05) Rat 6 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



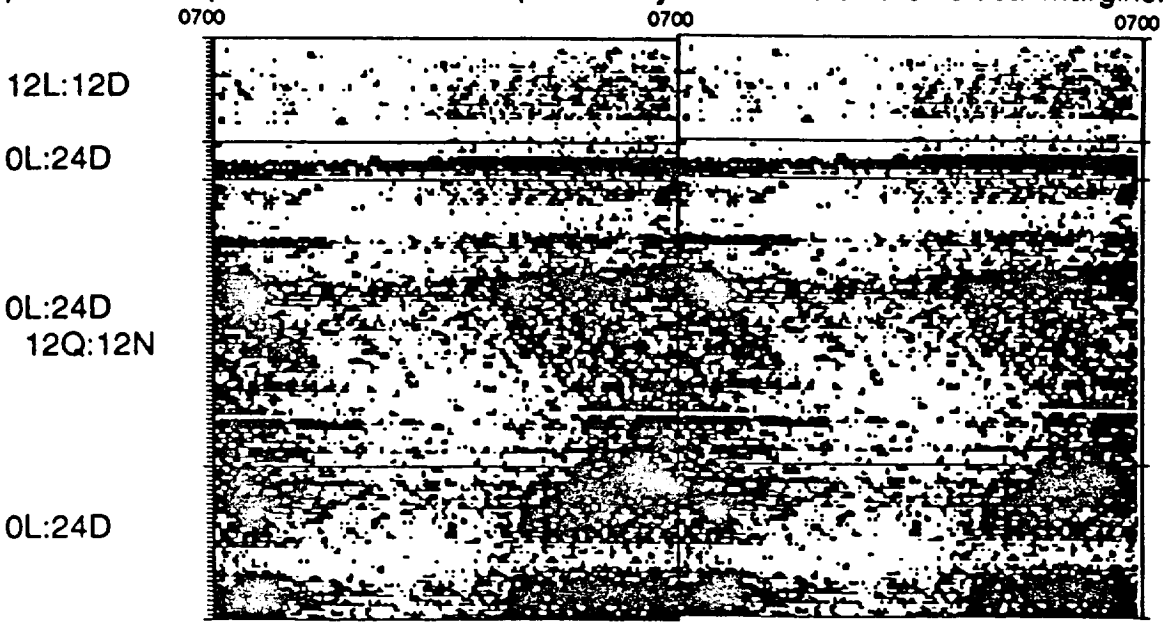
Experiment 2.3 (9112Sound05) Rat 4 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



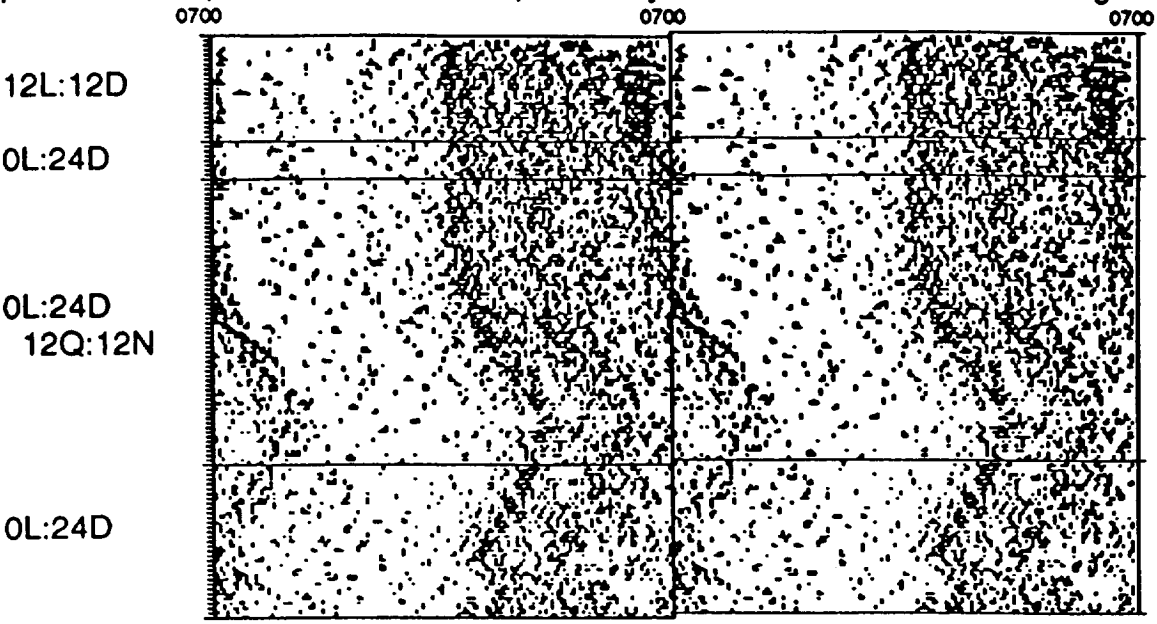
Experiment 2.3 (9112Sound05) Rat 5 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



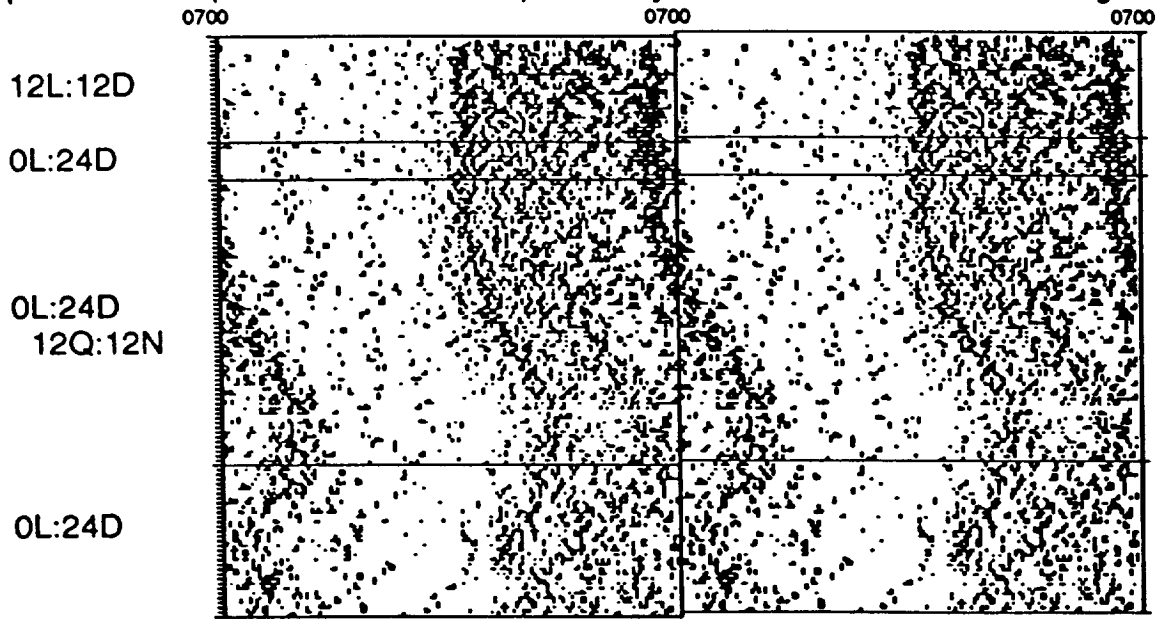
Experiment 2.3 (9112Sound05) Rat 6 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



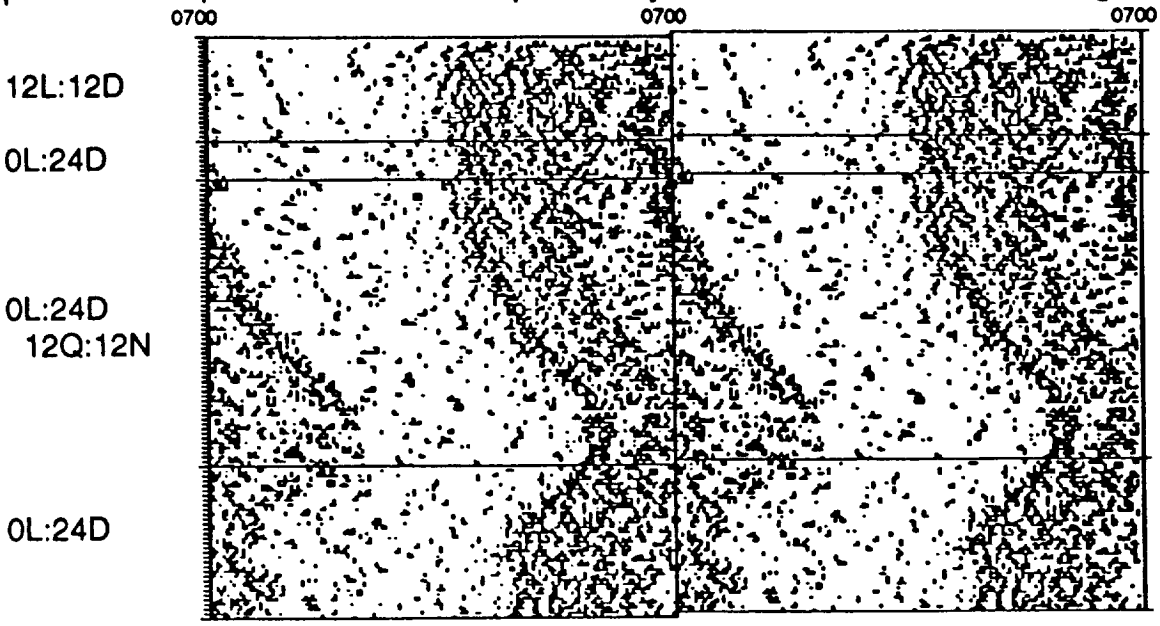
Experiment 2.3 (9112Sound05) Rat 4 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



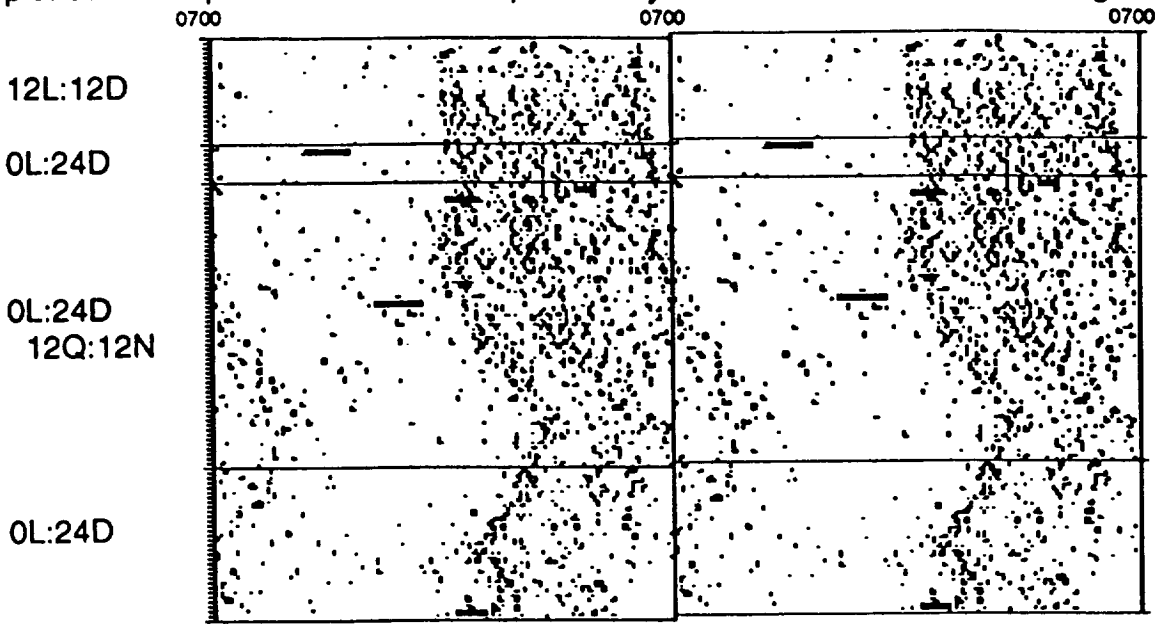
Experiment 2.3 (9112Sound05) Rat 5 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



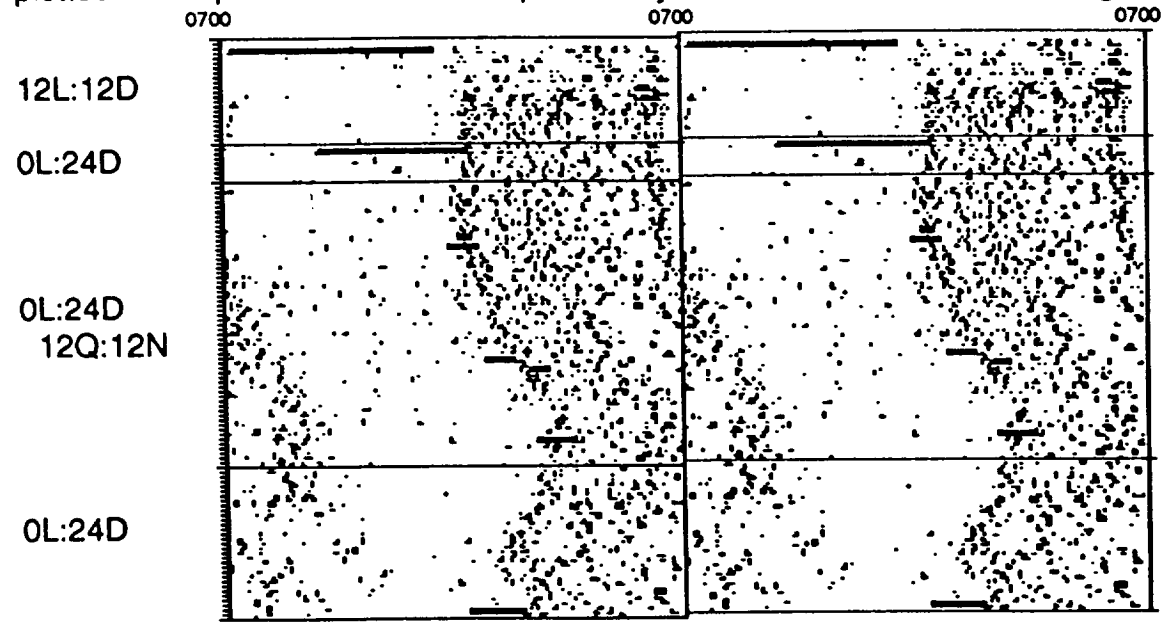
Experiment 2.3 (9112Sound05) Rat 6 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



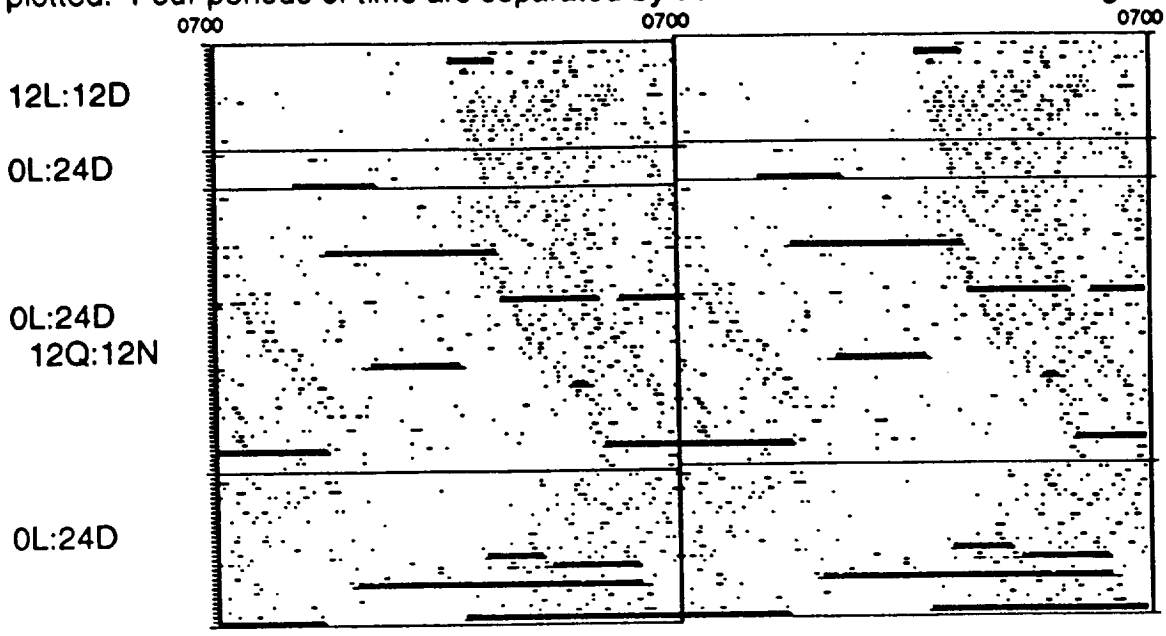
Experiment 2.3 (9112Sound05) Rat 4 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



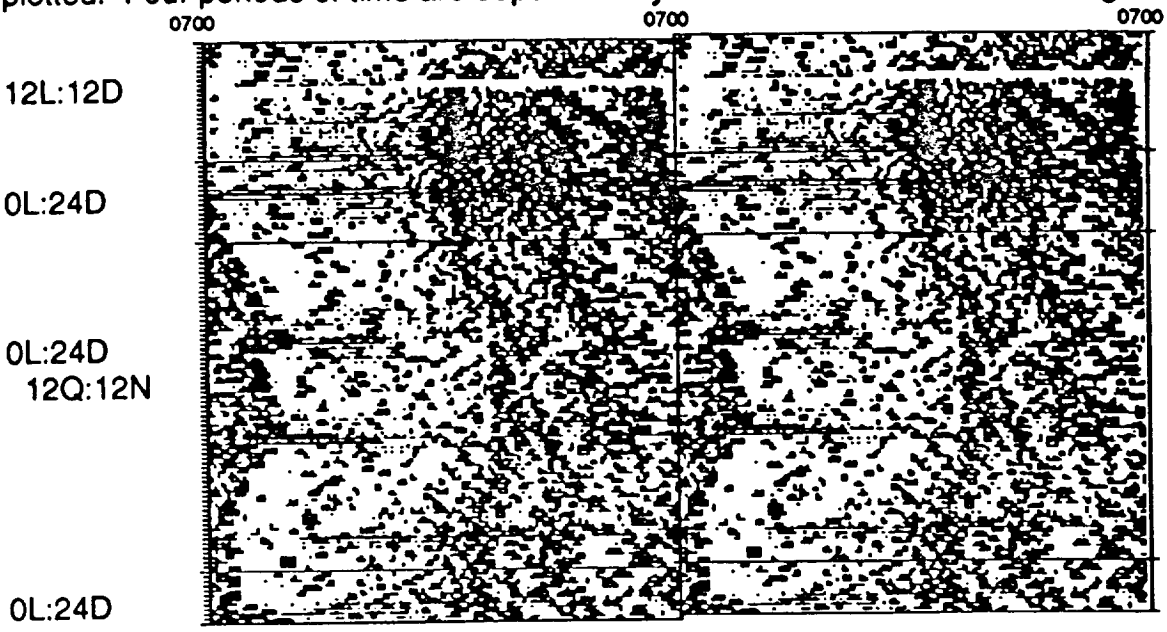
Experiment 2.3 (9112Sound05) Rat 5 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



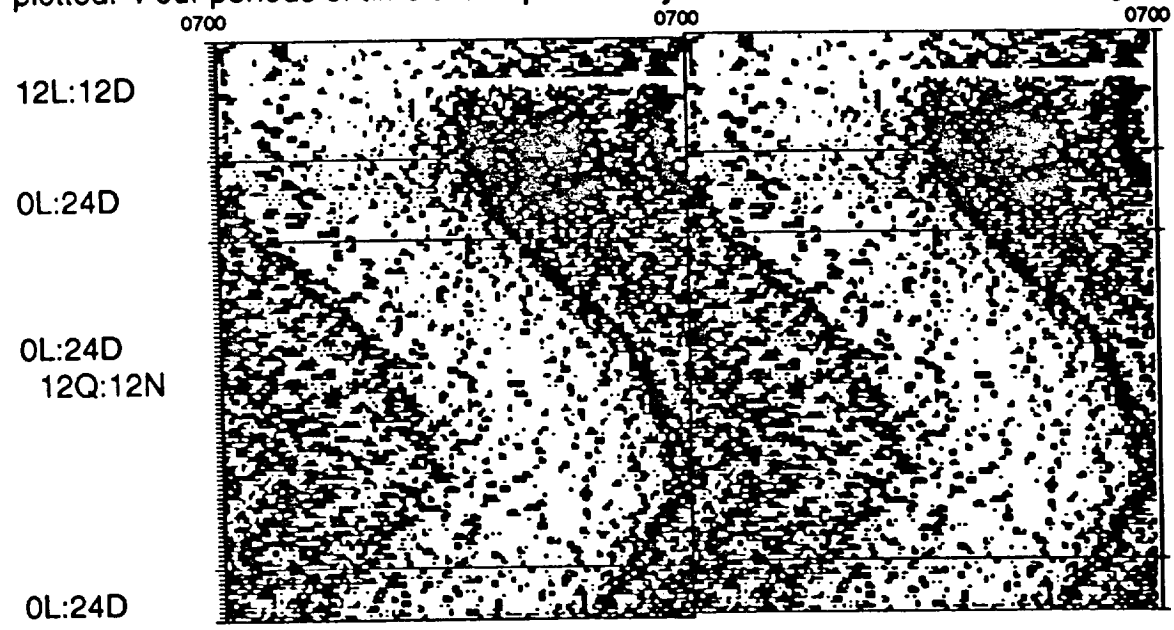
Experiment 2.3 (9112Sound05) Rat 6 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



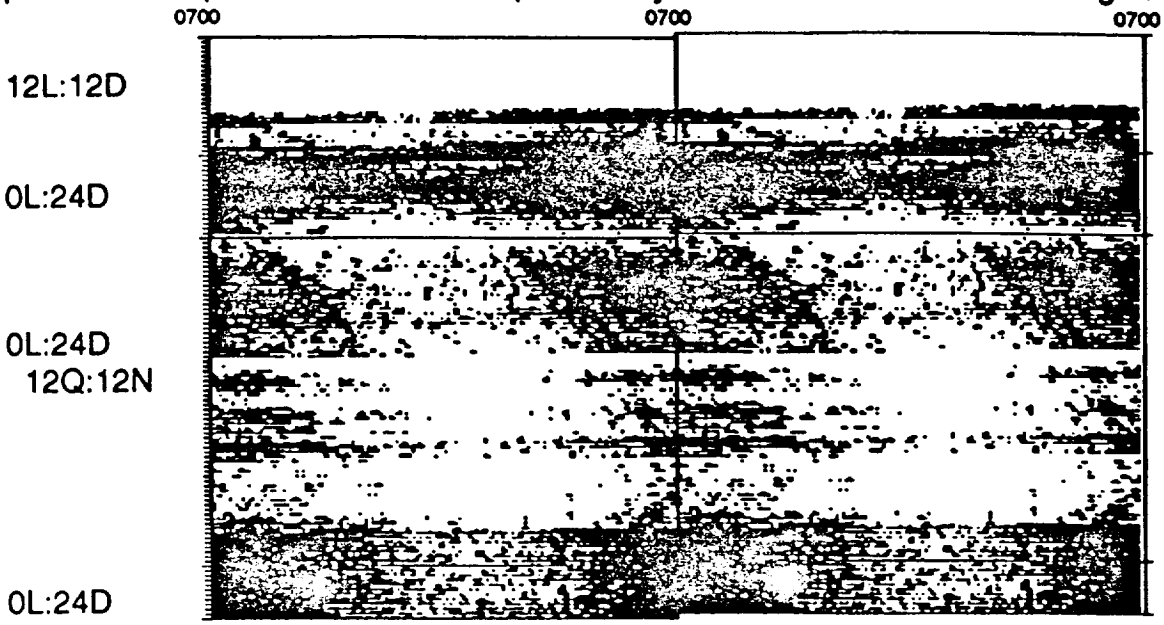
Experiment 2.4 (9205Sound05) Rat 1 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



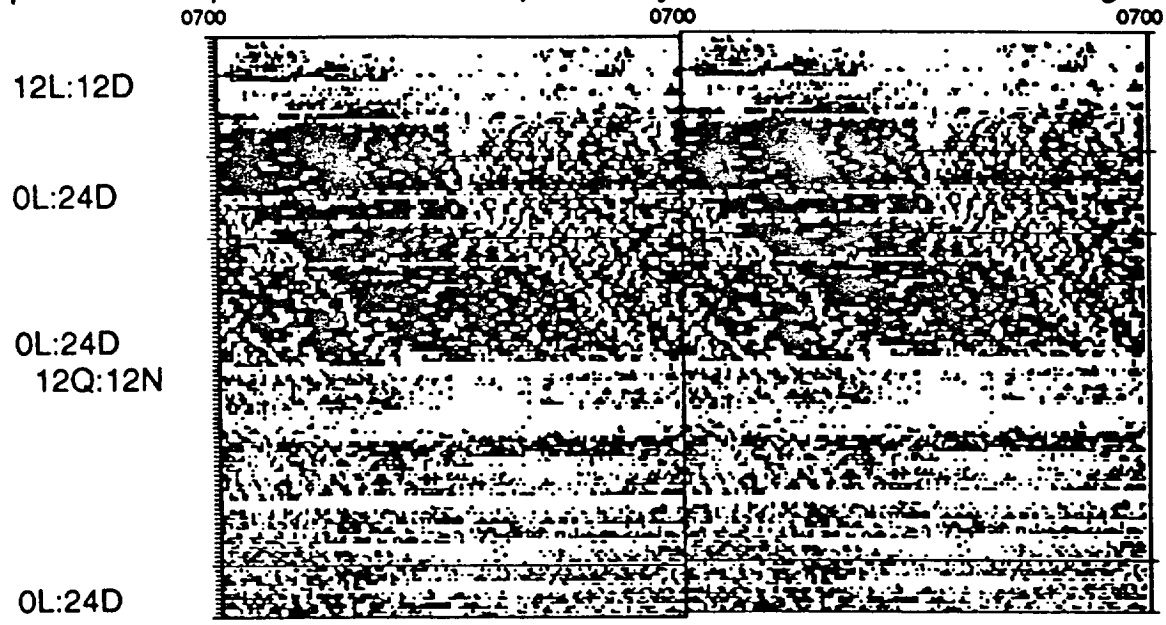
Experiment 2.4 (9205Sound05) Rat 2 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



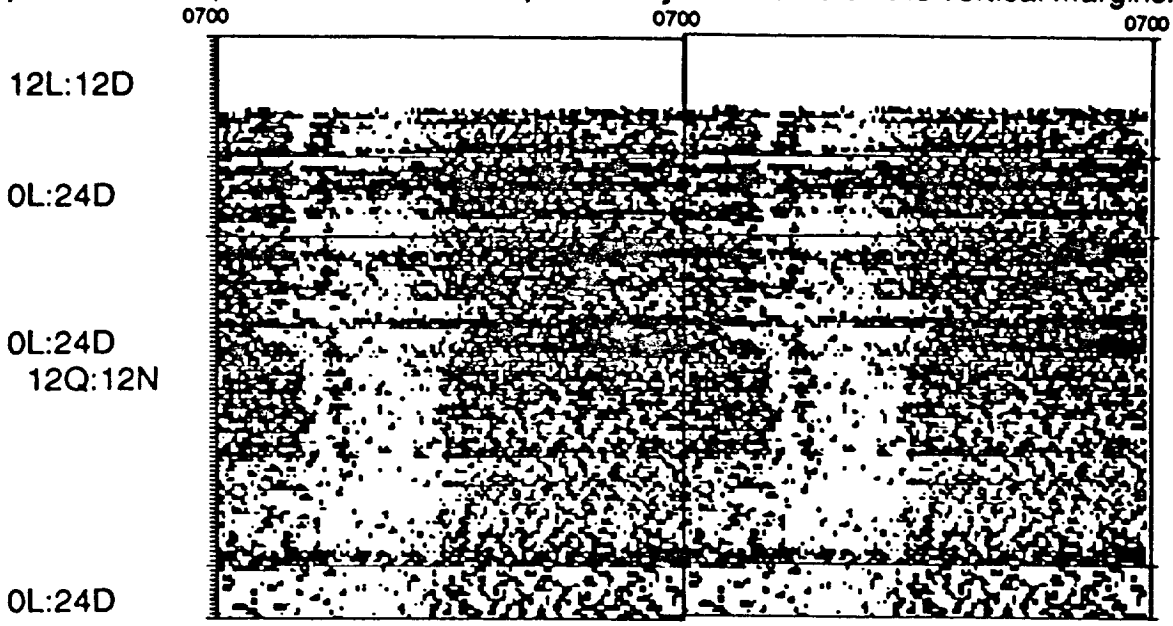
Experiment 2.4 (9205Sound05) Rat 3 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



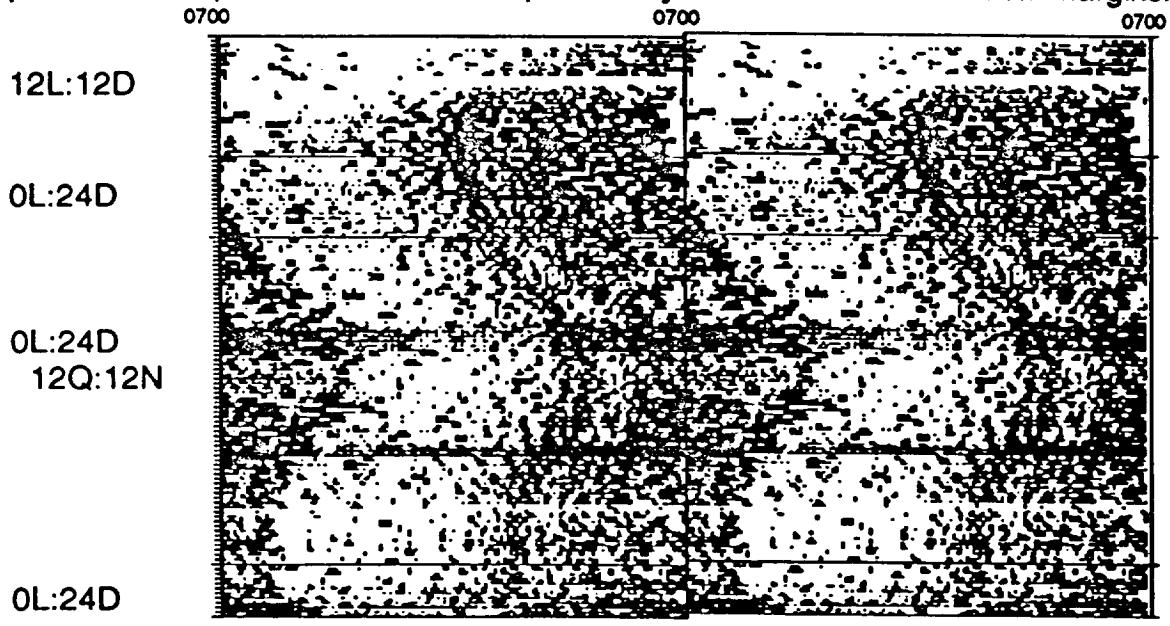
Experiment 2.4 (9205Sound05) Rat 4 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



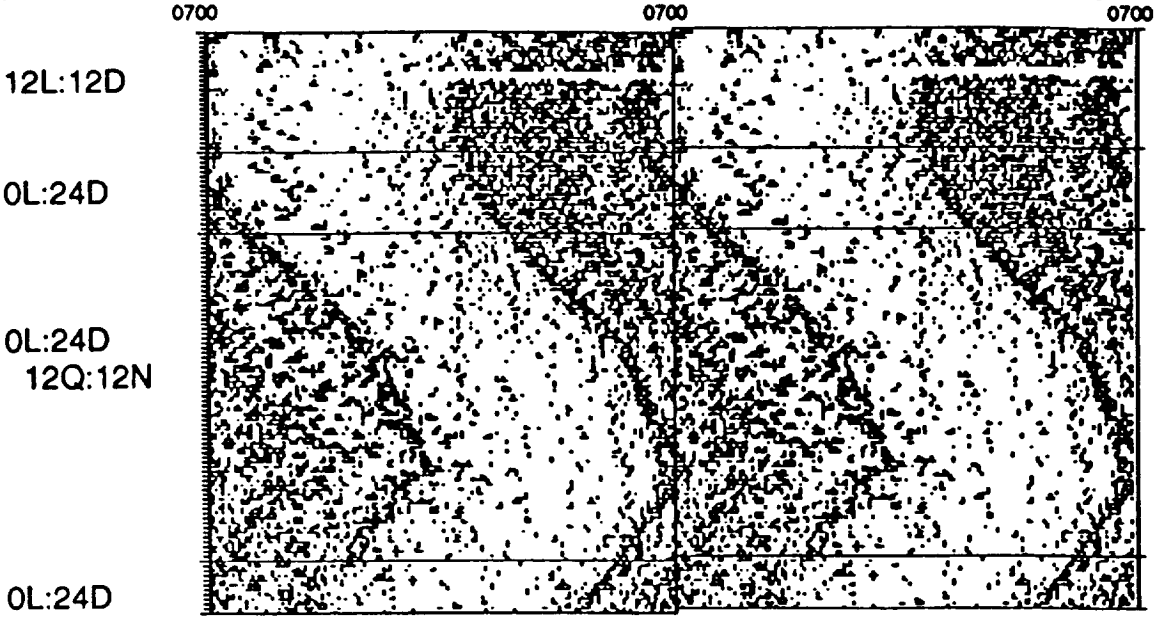
Experiment 2.4 (9205Sound05) Rat 5 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



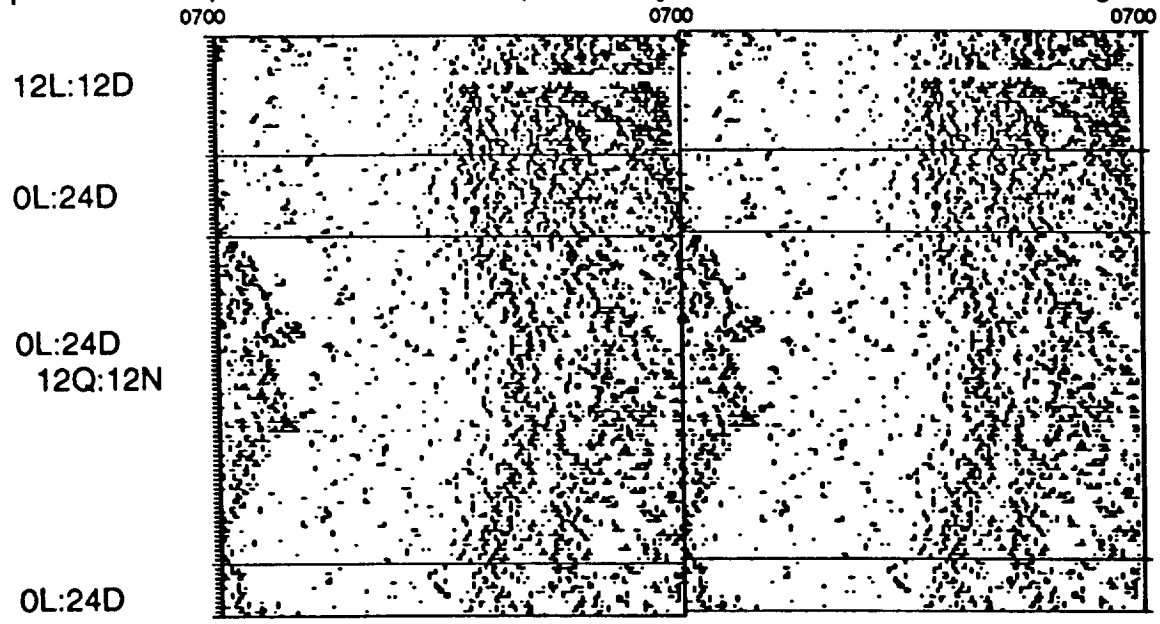
Experiment 2.4 (9205Sound05) Rat 6 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



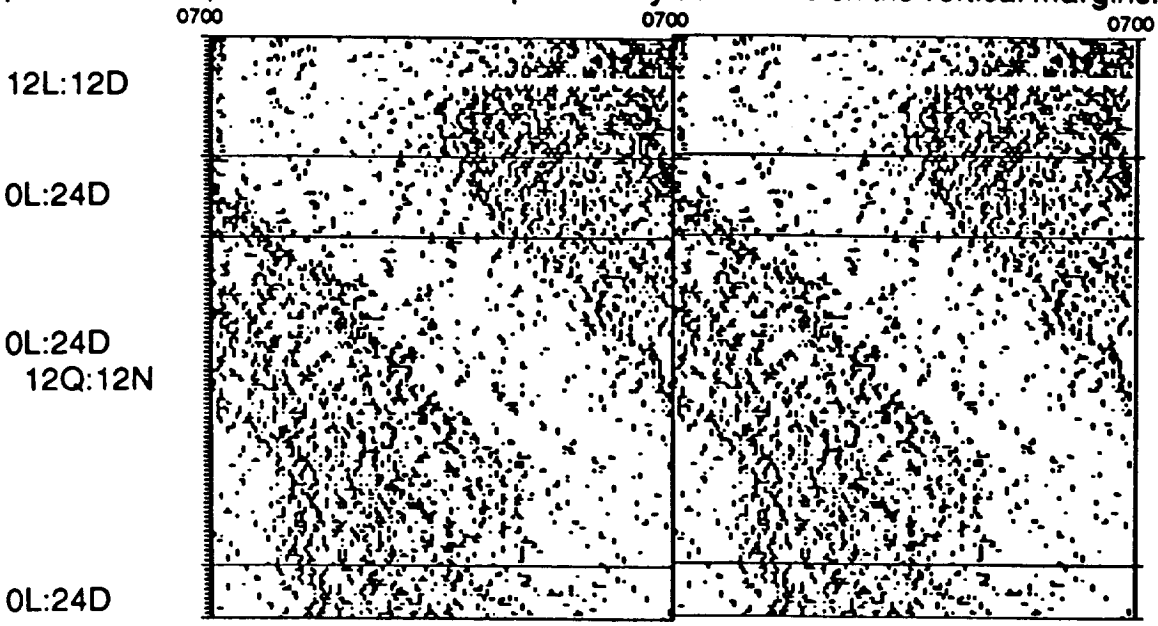
Experiment 2.4 (9205Sound05) Rat 1 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



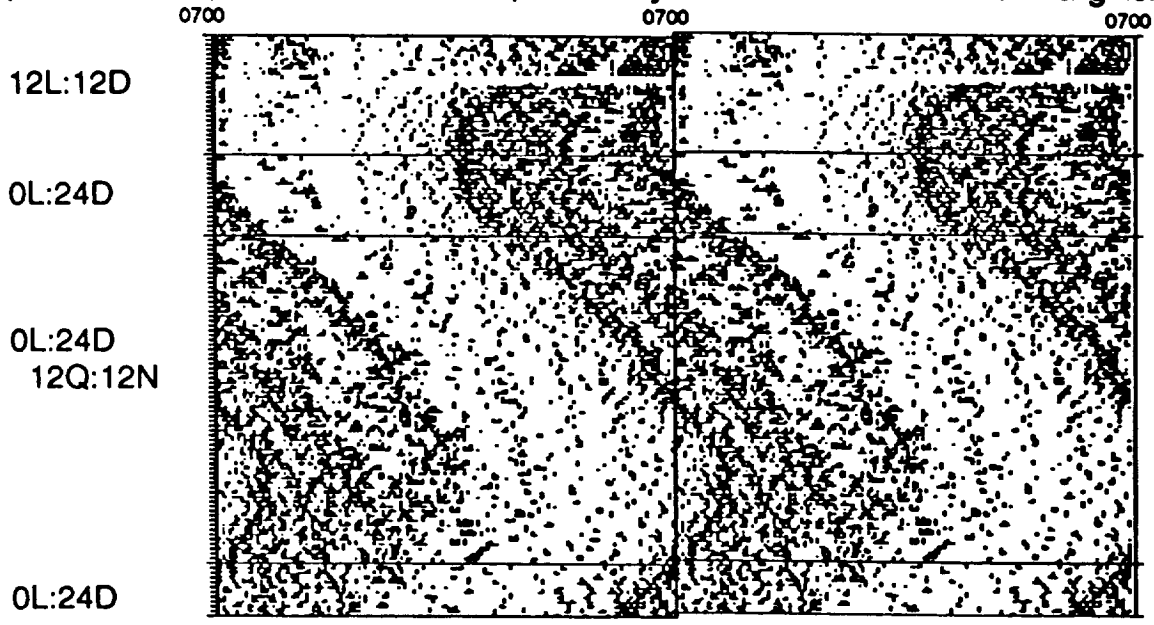
Experiment 2.4 (9205Sound05) Rat 2 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



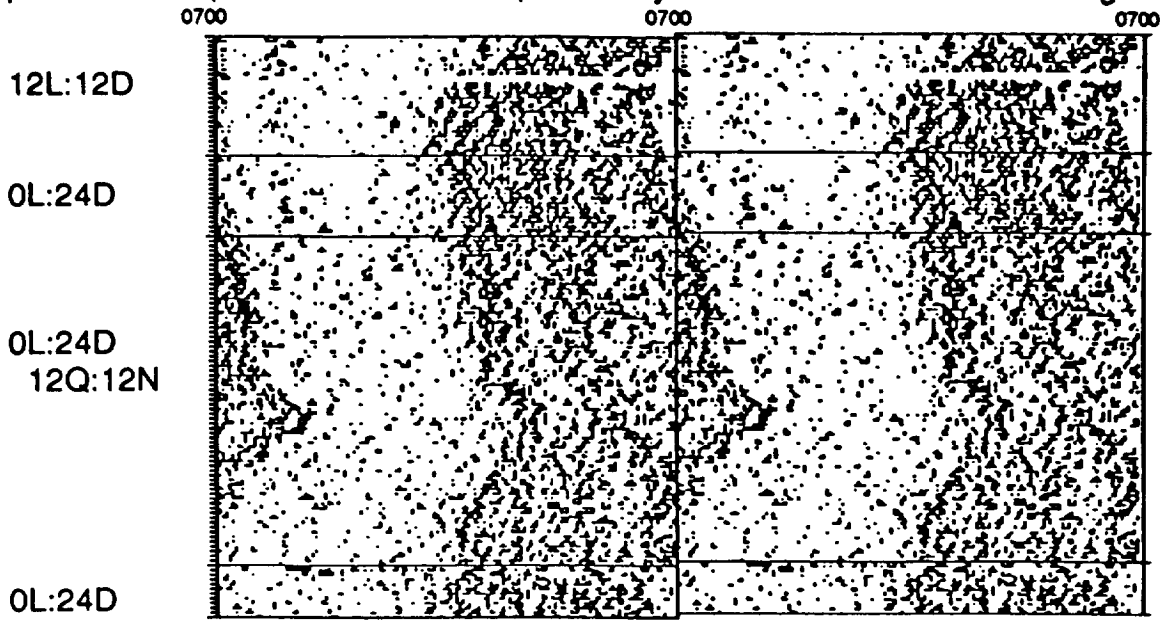
Experiment 2.4 (9205Sound05) Rat 3 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



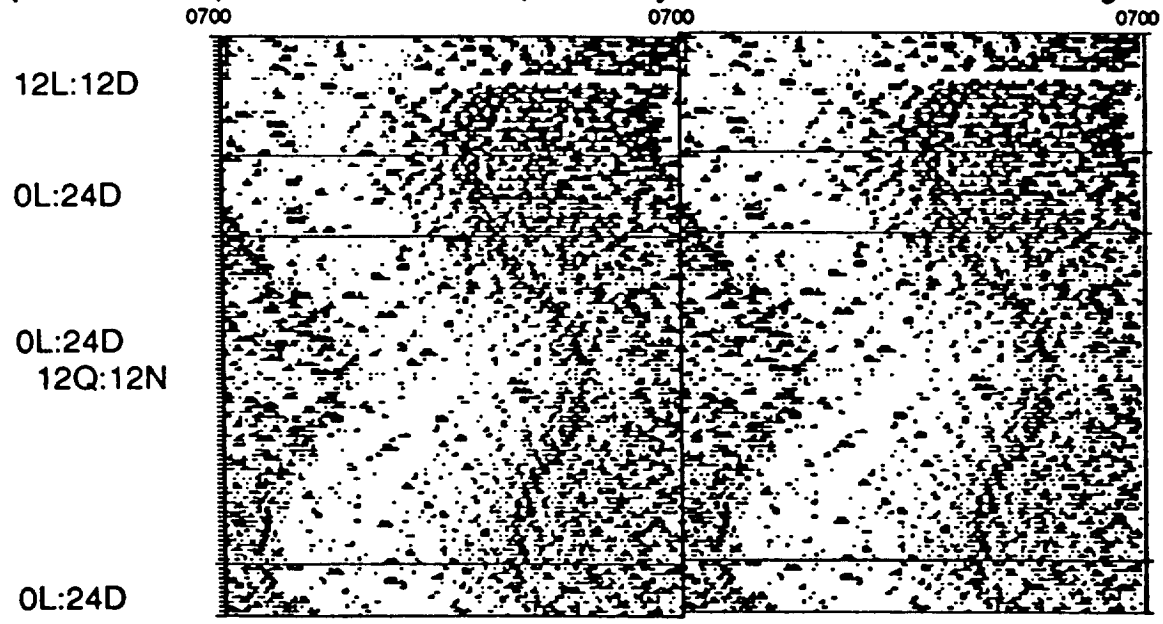
Experiment 2.4 (9205Sound05) Rat 4 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



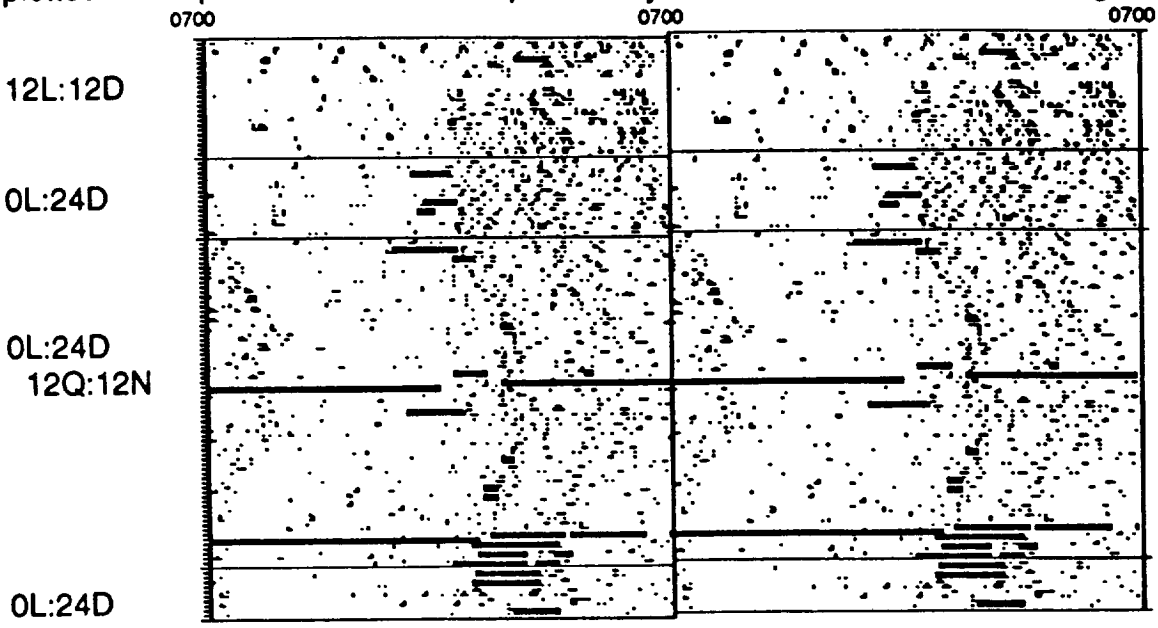
Experiment 2.4 (9205Sound05) Rat 5 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



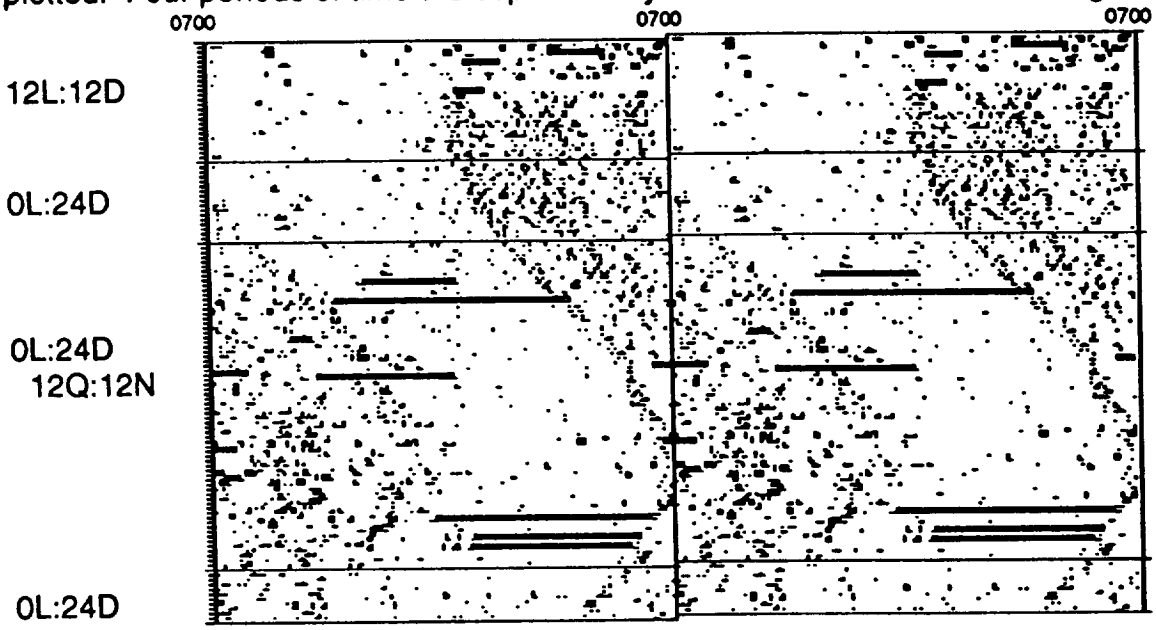
Experiment 2.4 (9205Sound05) Rat 6 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



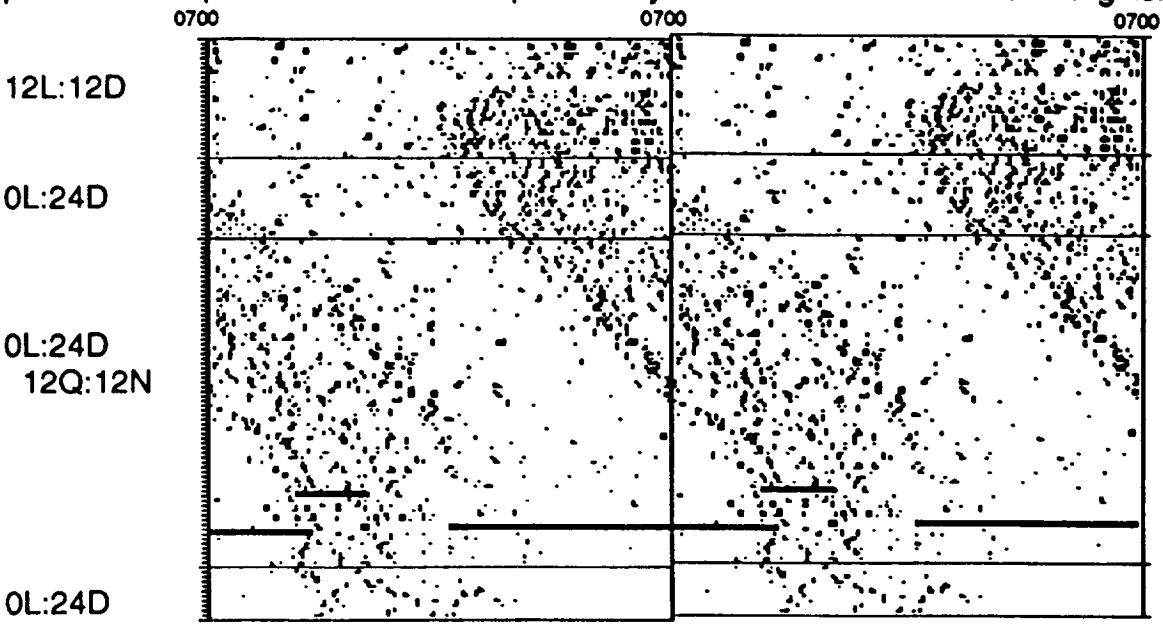
Experiment 2.4 (9205Sound05) Rat 1 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



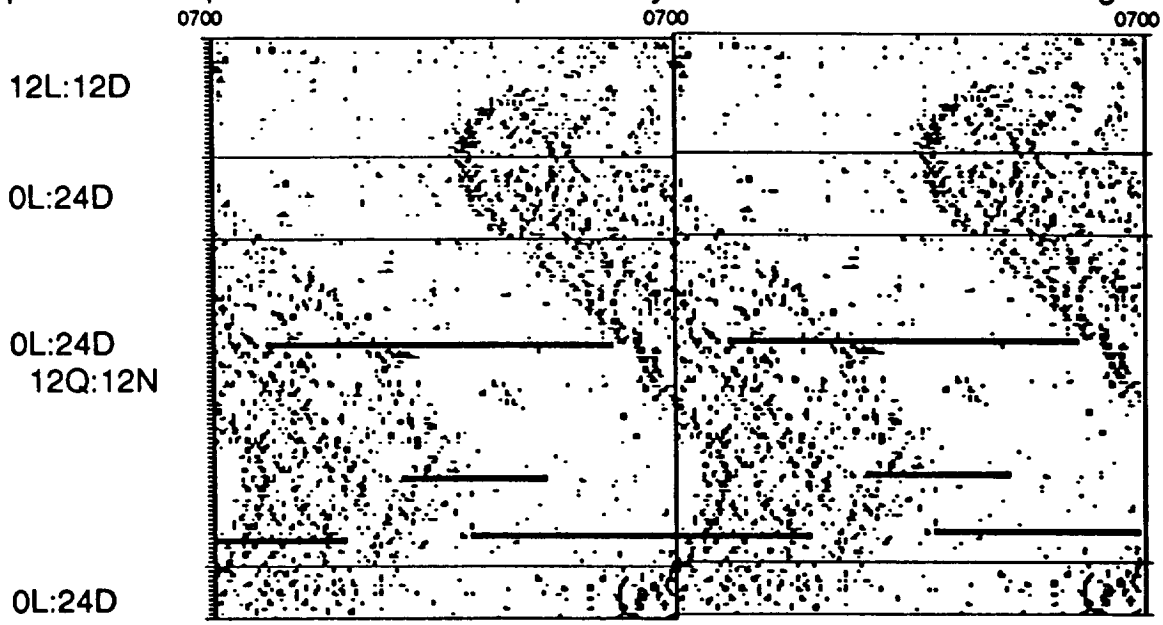
Experiment 2.4 (9205Sound05) Rat 2 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



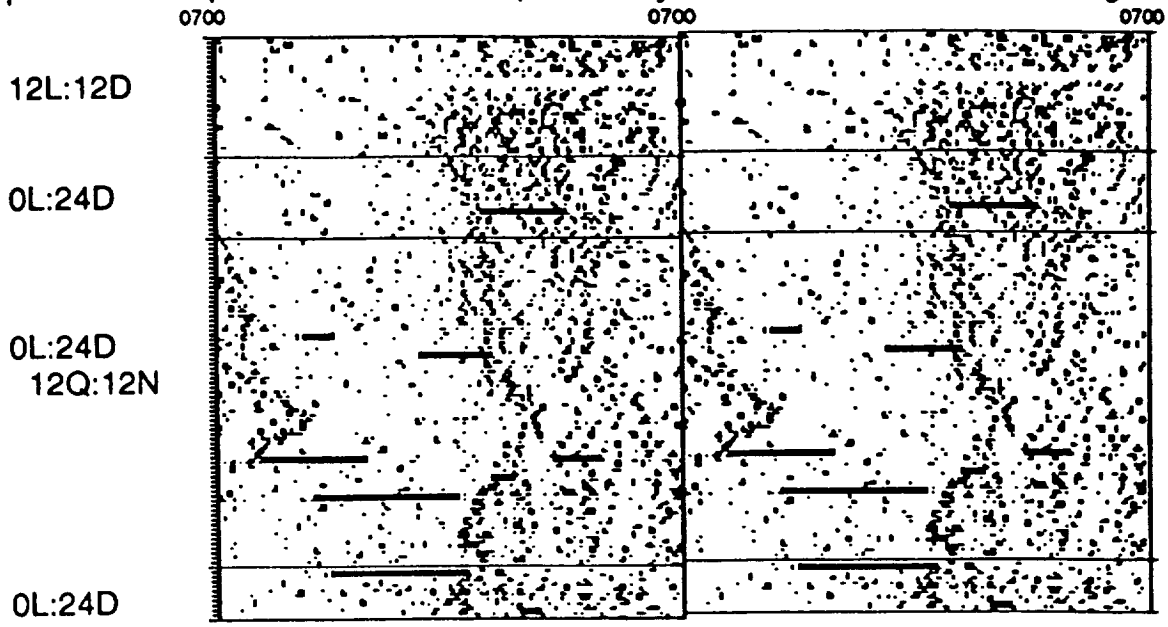
Experiment 2.4 (9205Sound05) Rat 3 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



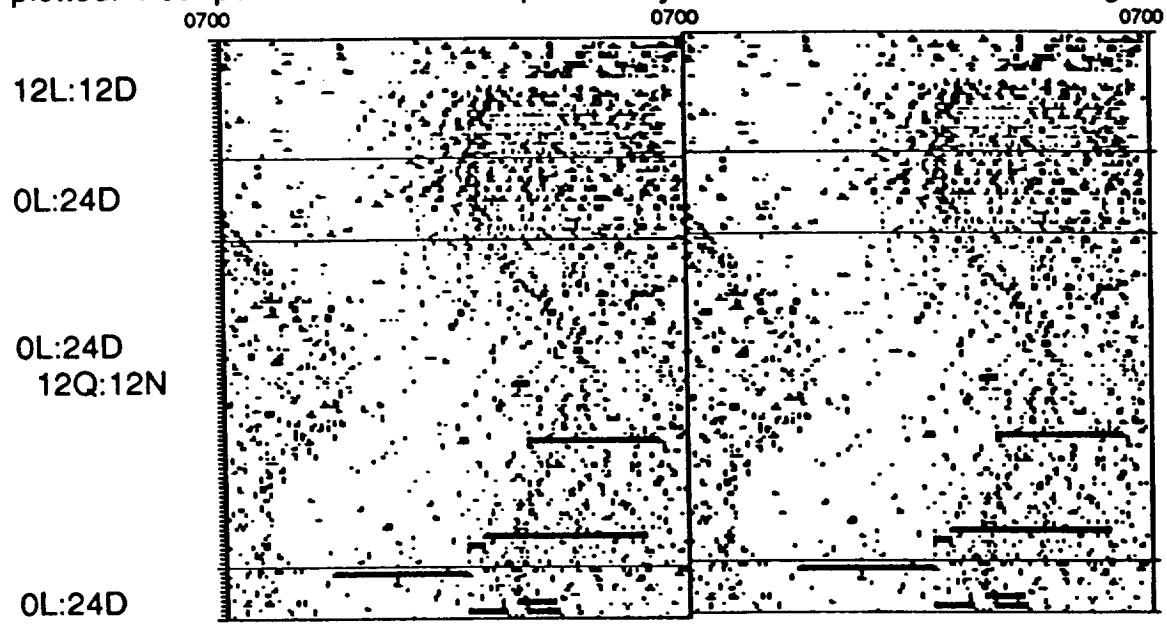
Experiment 2.4 (9205Sound05) Rat 4 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



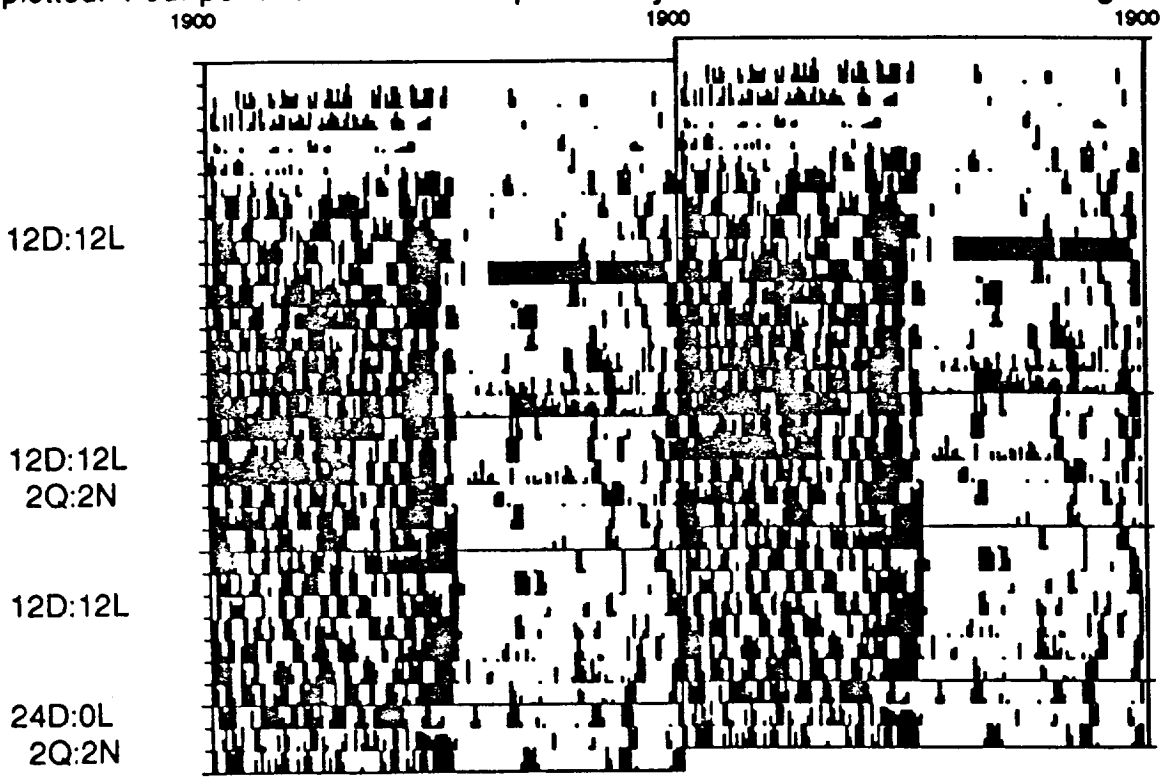
Experiment 2.4 (9205Sound05) Rat 5 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



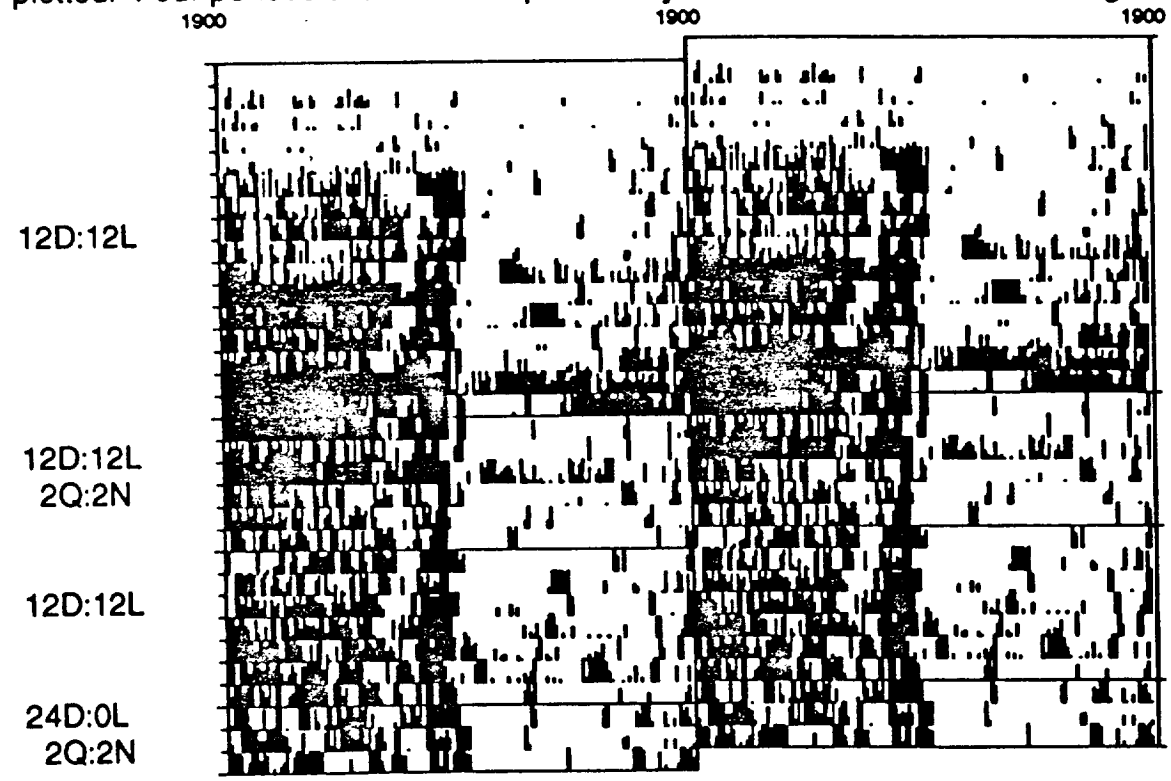
Experiment 2.4 (9205Sound05) Rat 6 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



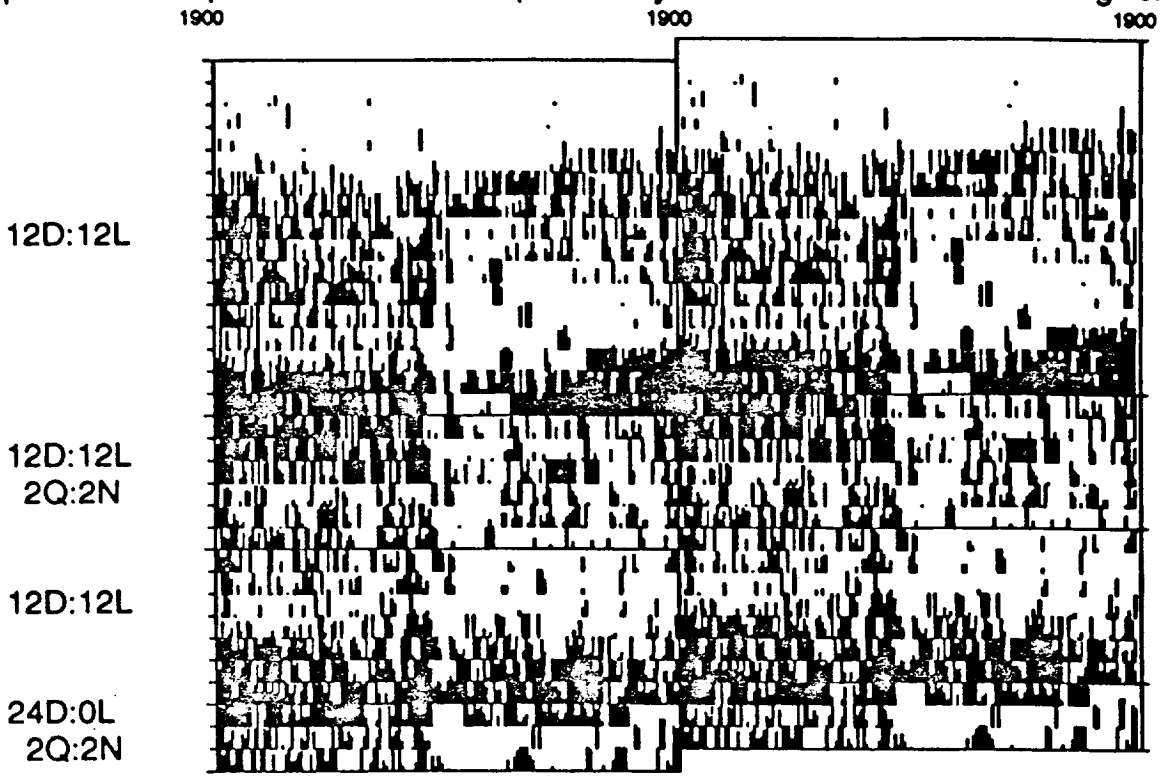
Experiment 3.1 (9109Sound05) Rat 1 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



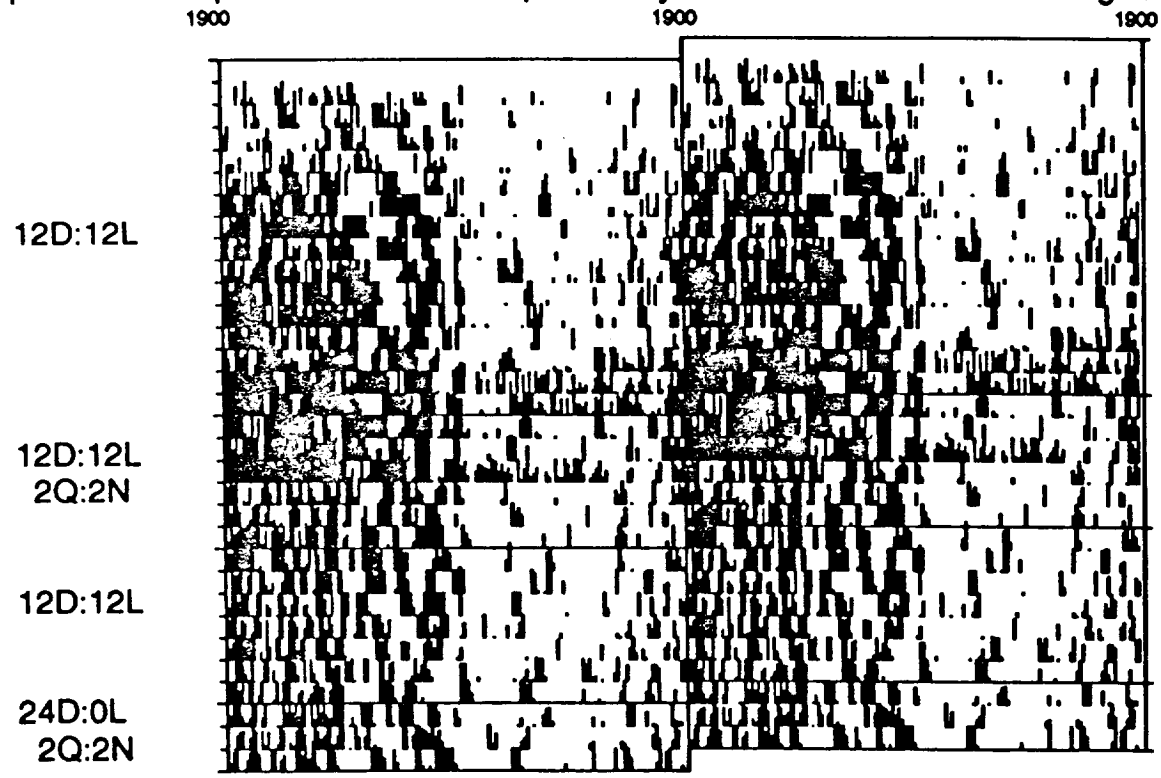
Experiment 3.1 (9109Sound05) Rat 2 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



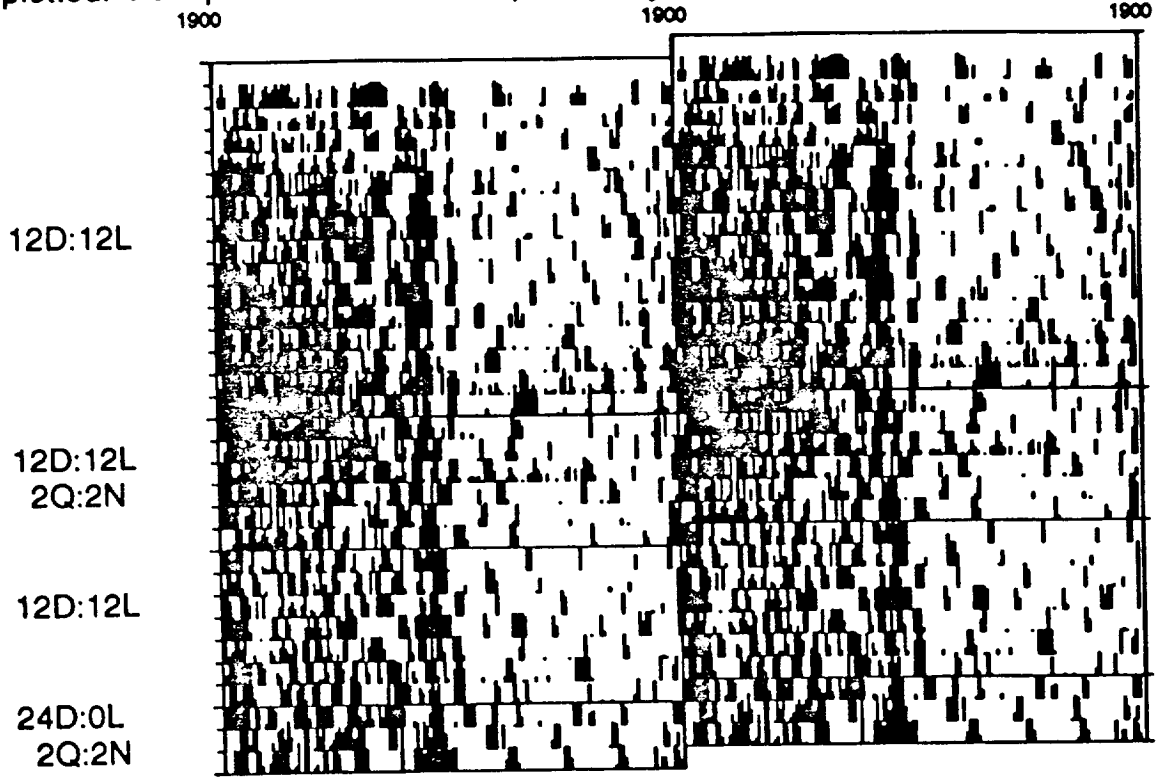
Experiment 3.1 (9109Sound05) Rat 3 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



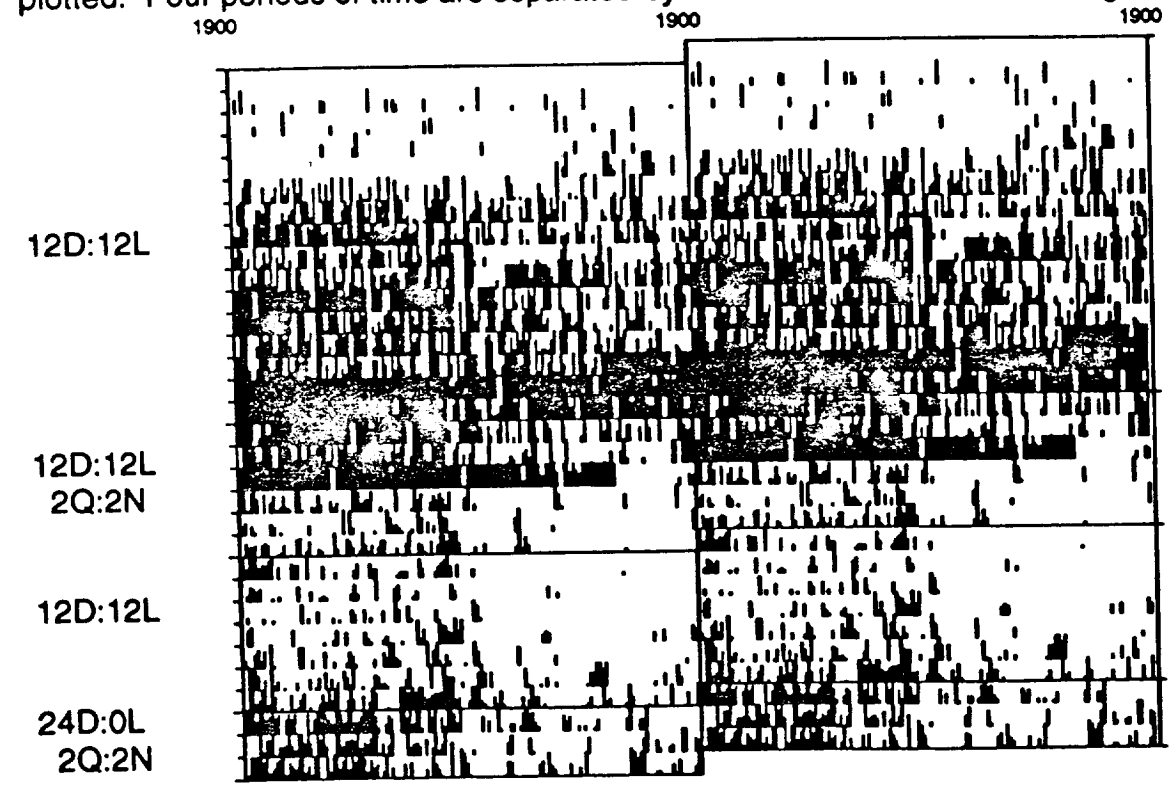
Experiment 3.1 (9109Sound05) Rat 4 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



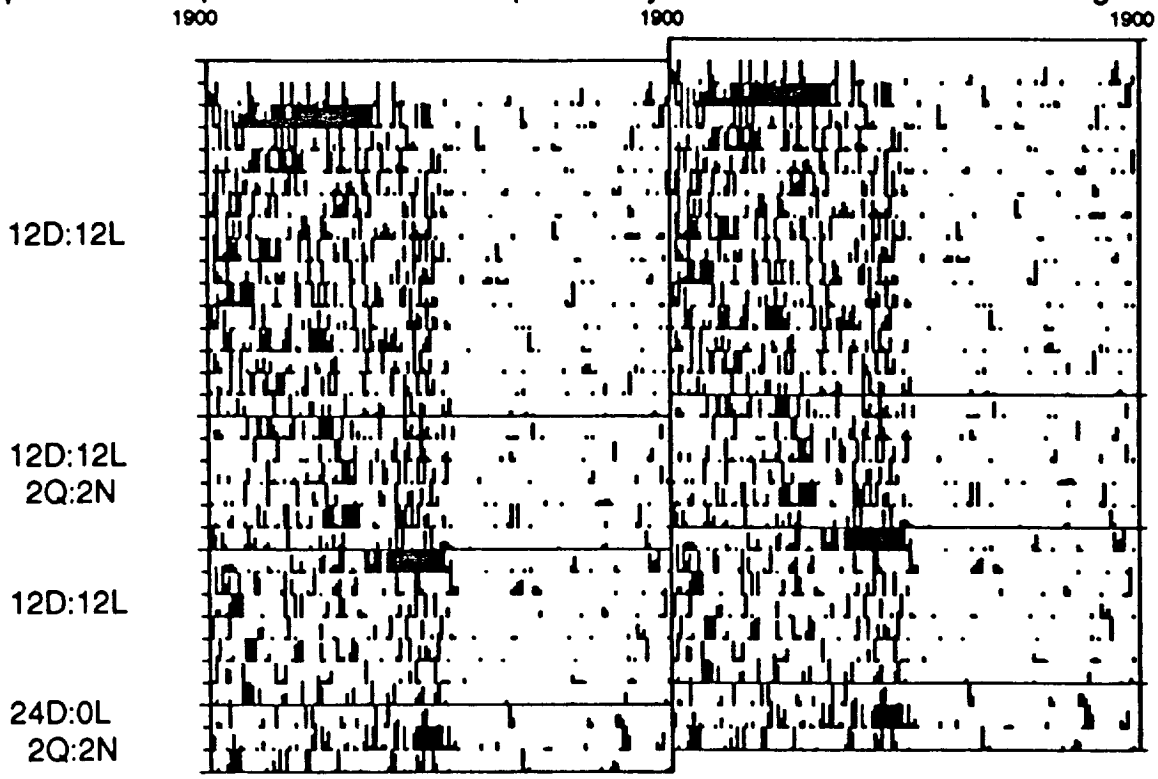
Experiment 3.1 (9109Sound05) Rat 5 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



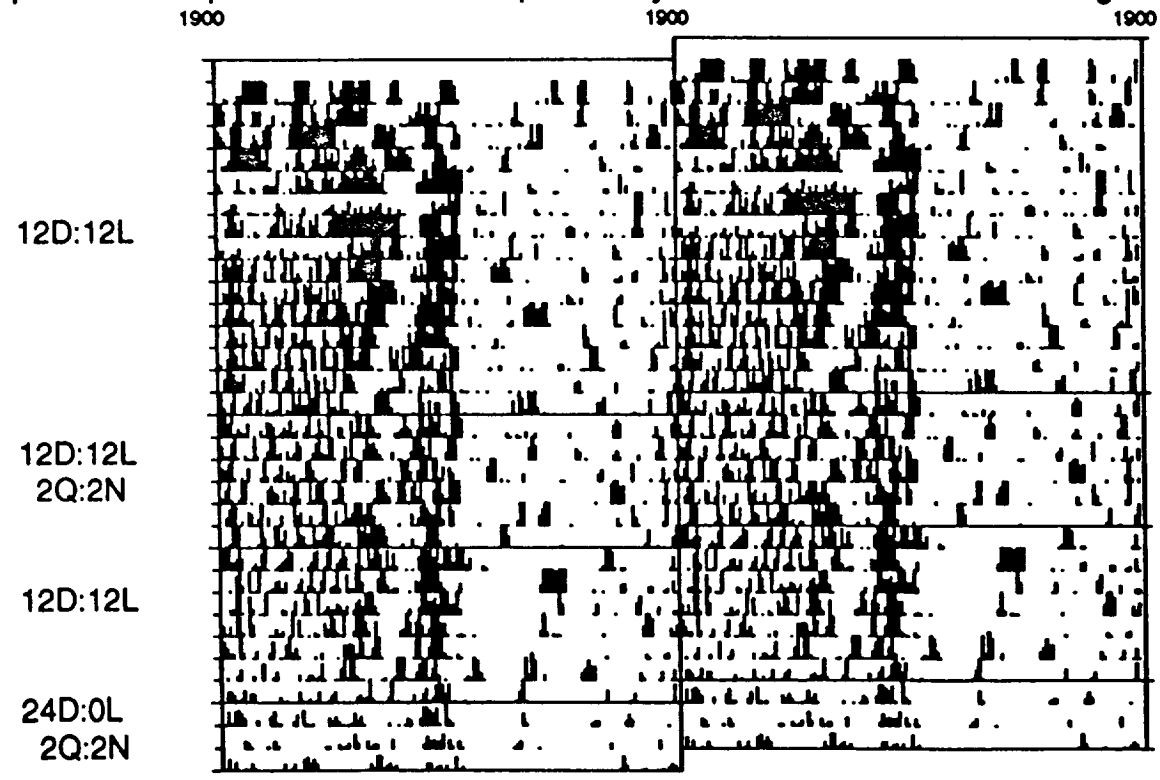
Experiment 3.1 (9109Sound05) Rat 6 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



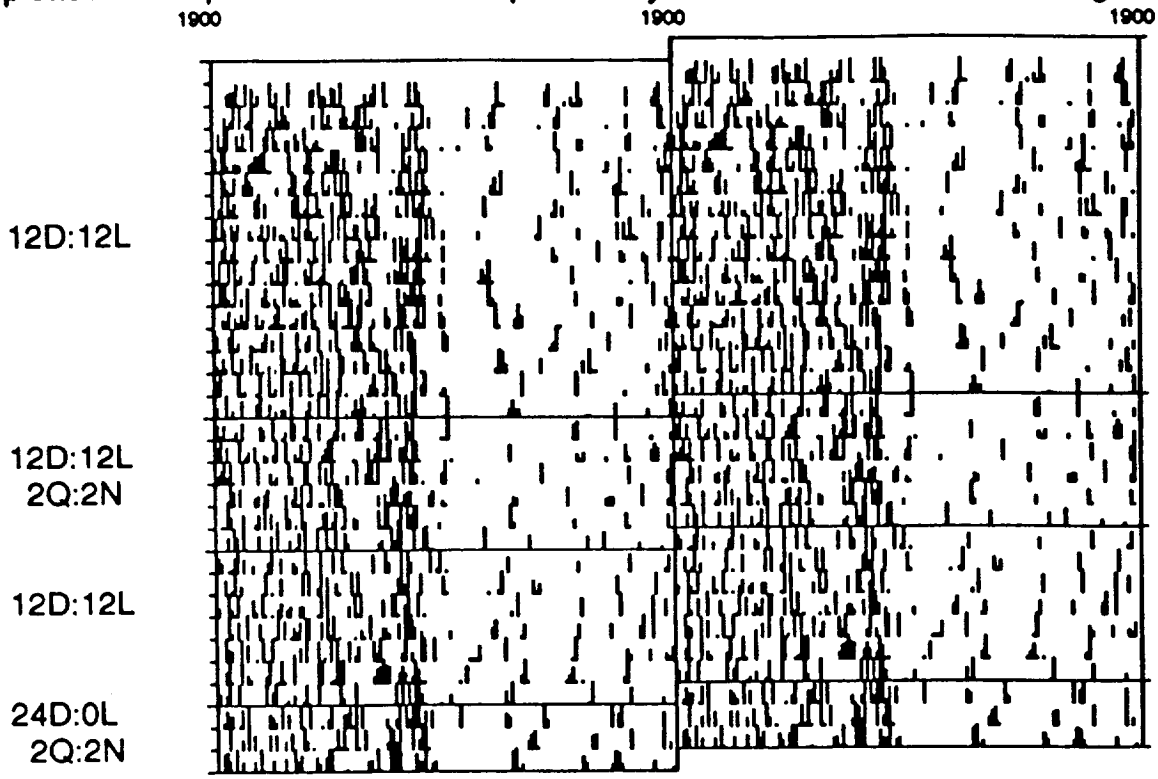
Experiment 3.1 (9109Sound05) Rat 1 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



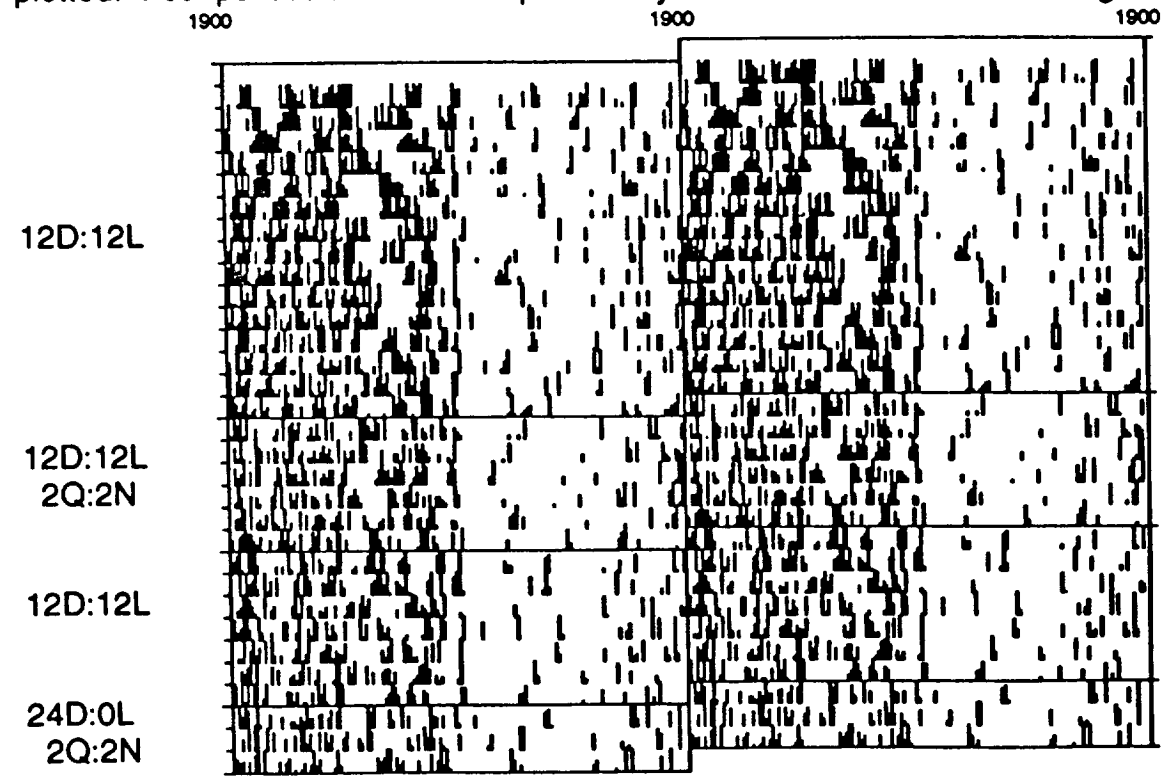
Experiment 3.1 (9109Sound05) Rat 2 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



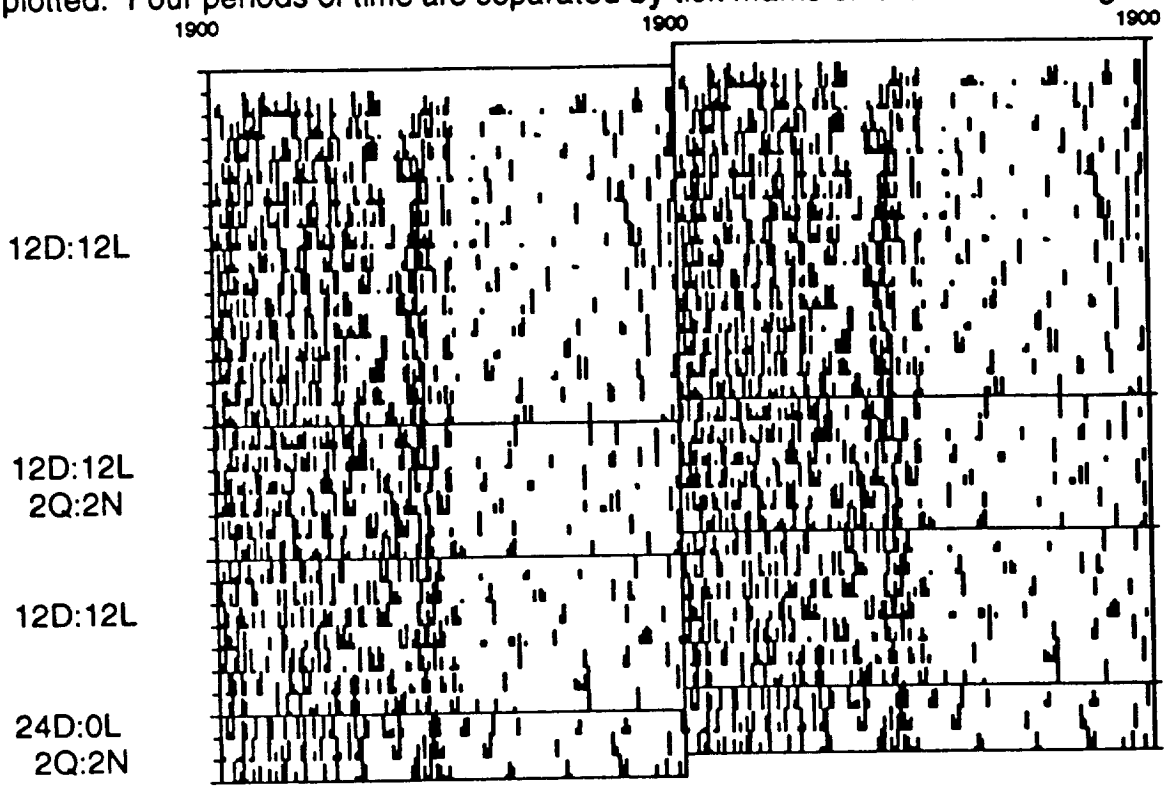
Experiment 3.1 (9109Sound05) Rat 3 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



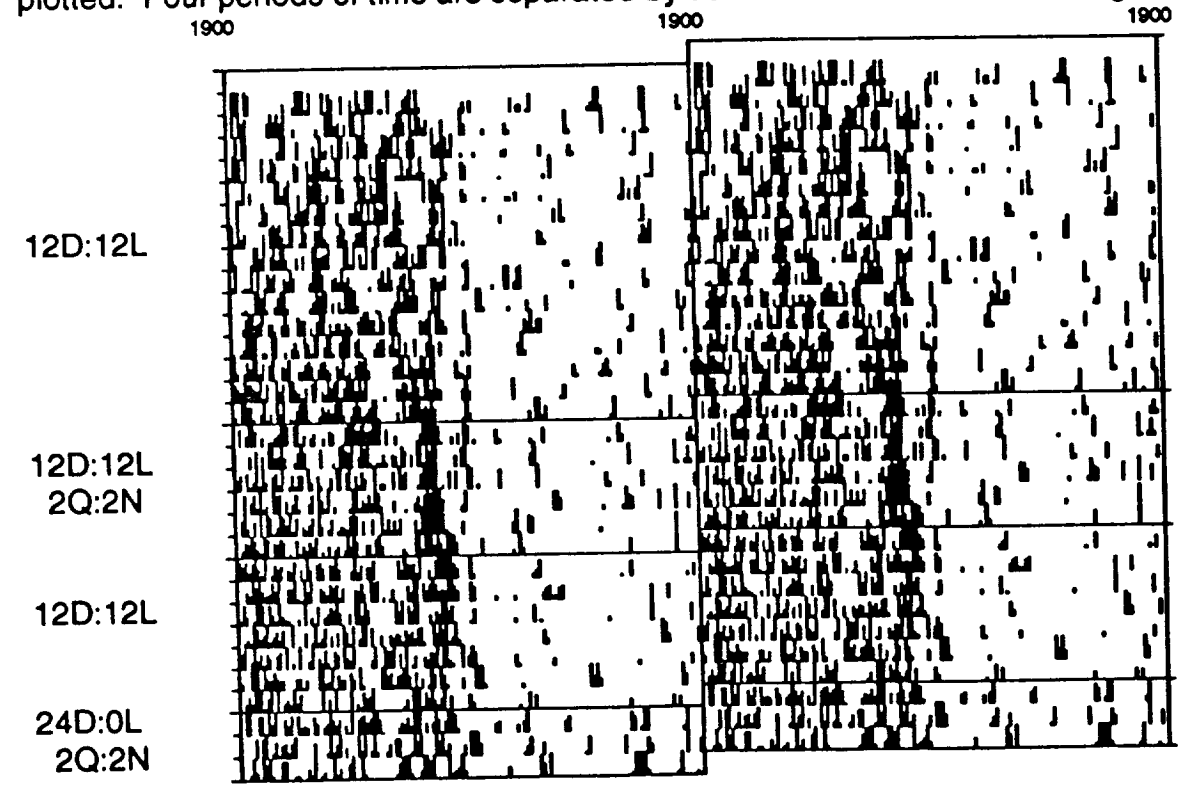
Experiment 3.1 (9109Sound05) Rat 4 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



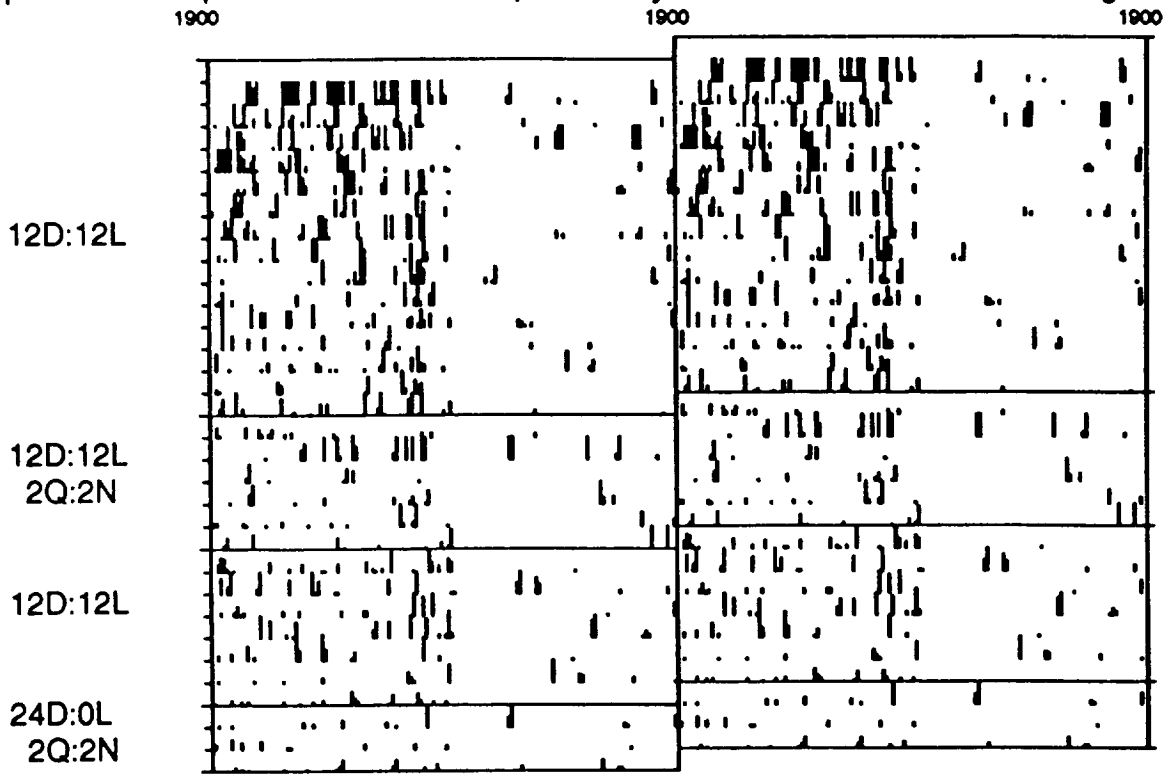
Experiment 3.1 (9109Sound05) Rat 5 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



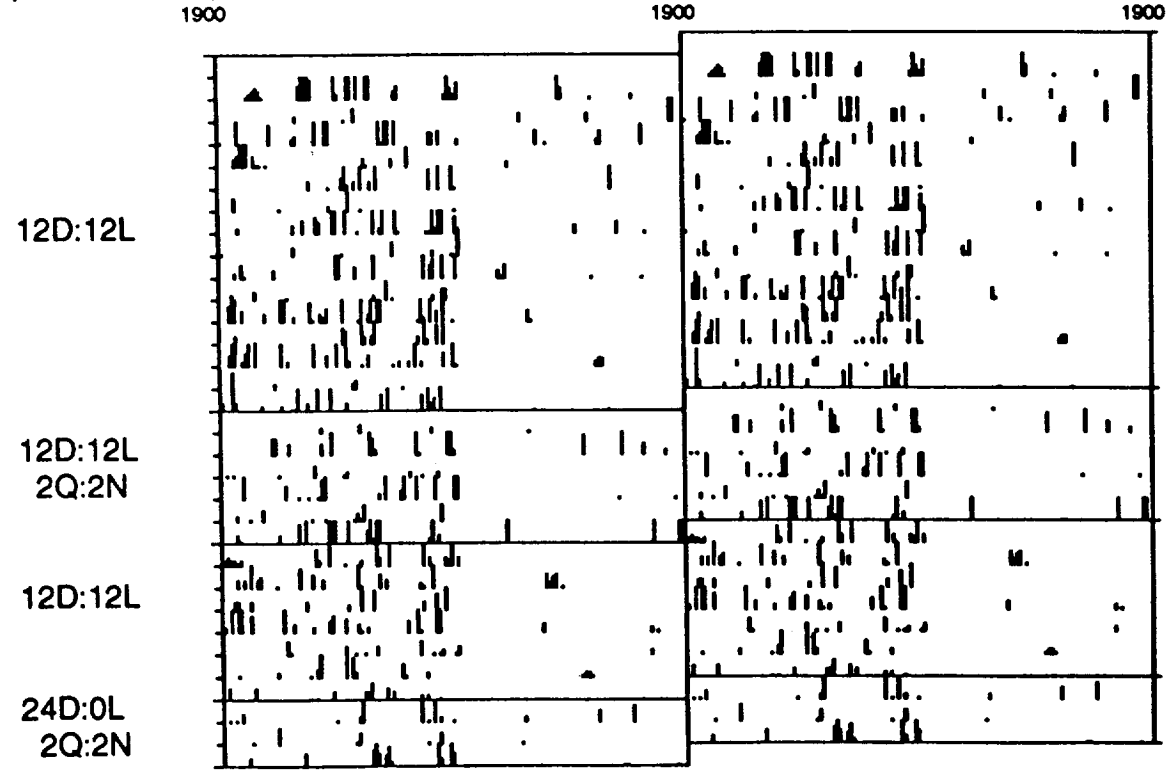
Experiment 3.1 (9109Sound05) Rat 6 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



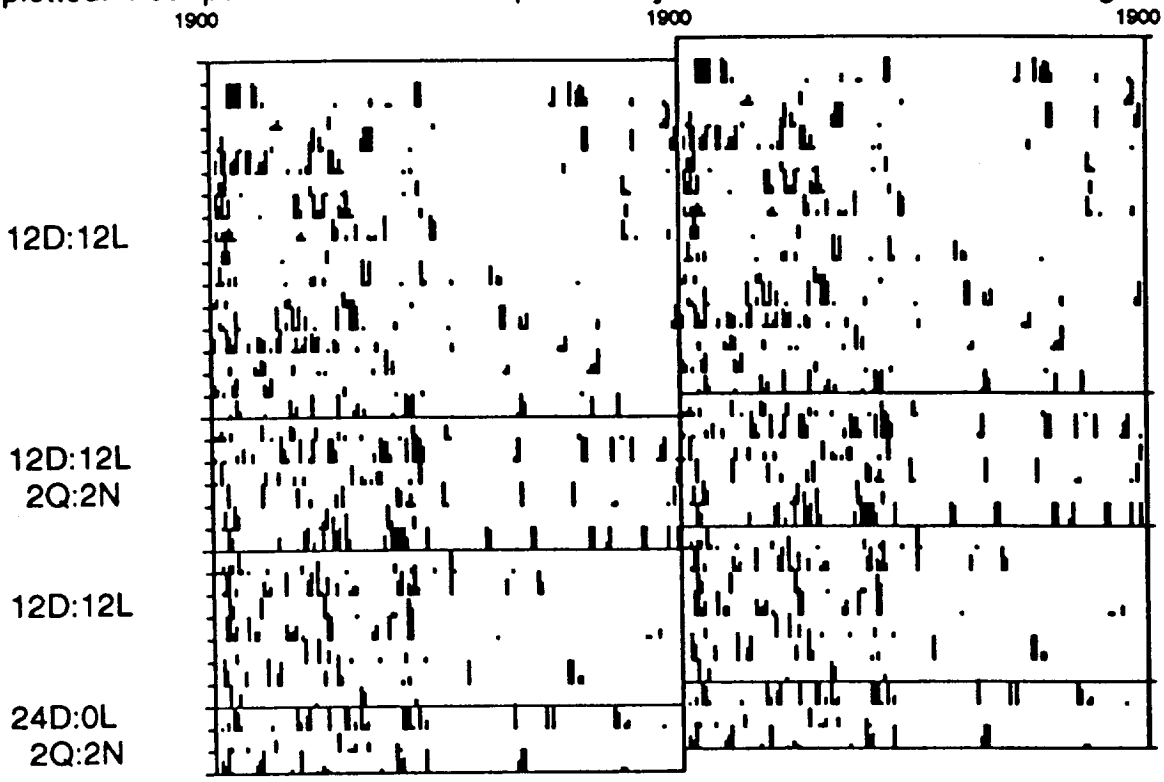
Experiment 3.1 (9109Sound05) Rat 1 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



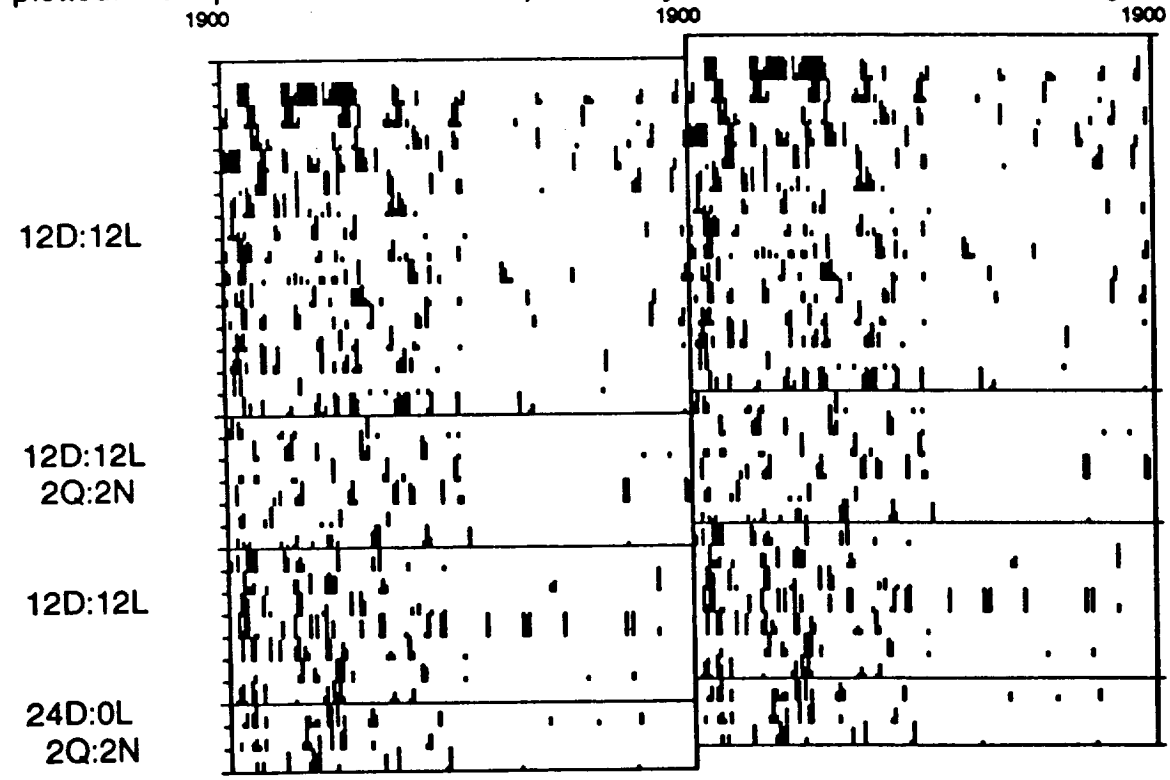
Experiment 3.1 (9109Sound05) Rat 2 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



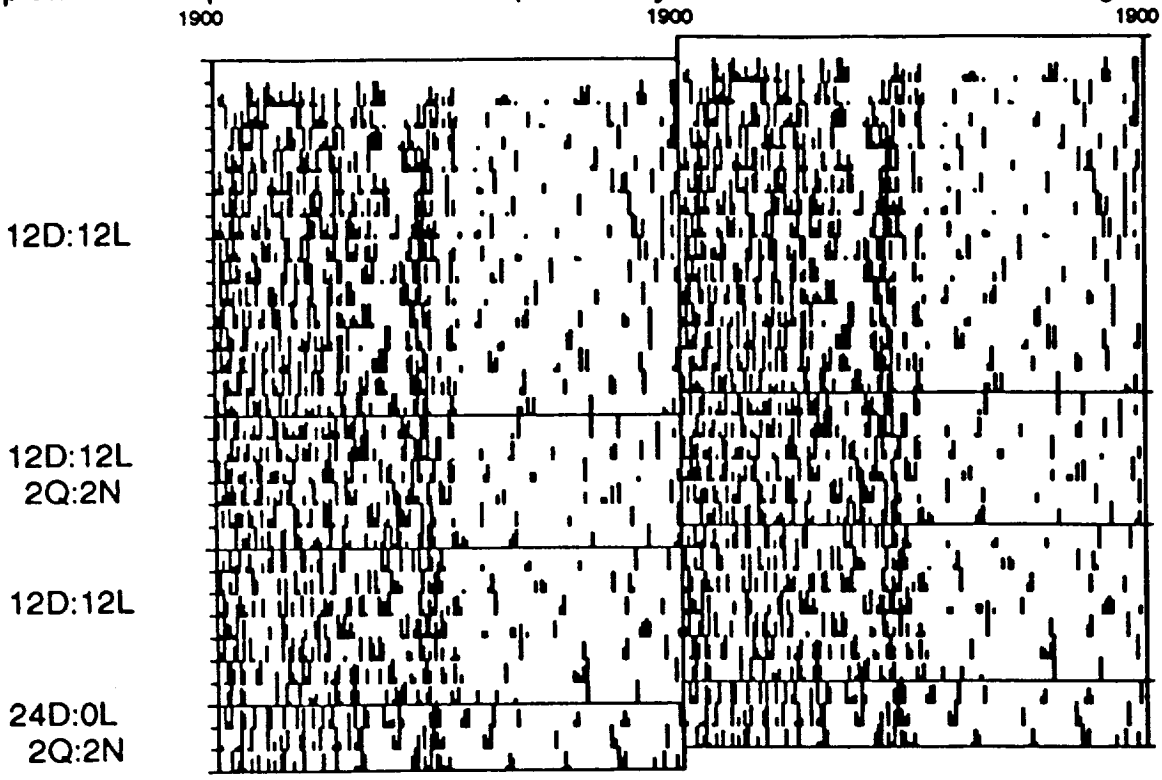
Experiment 3.1 (9109Sound05) Rat 3 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



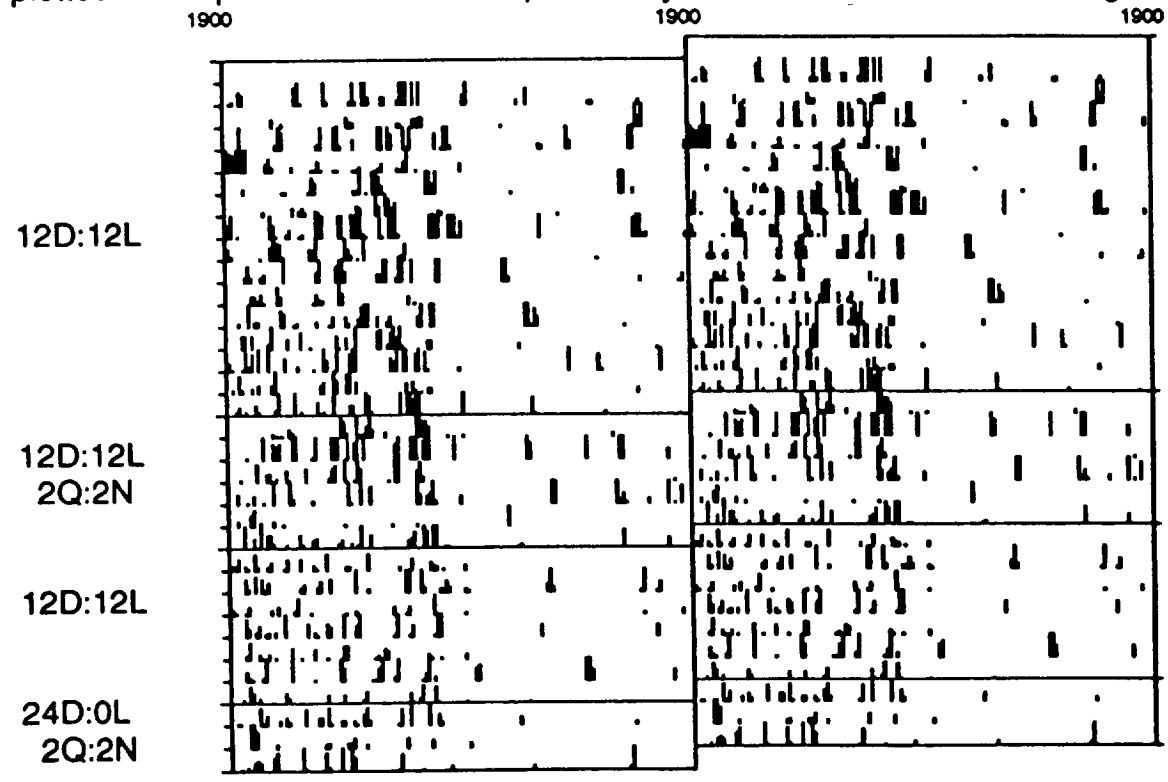
Experiment 3.1 (9109Sound05) Rat 4 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



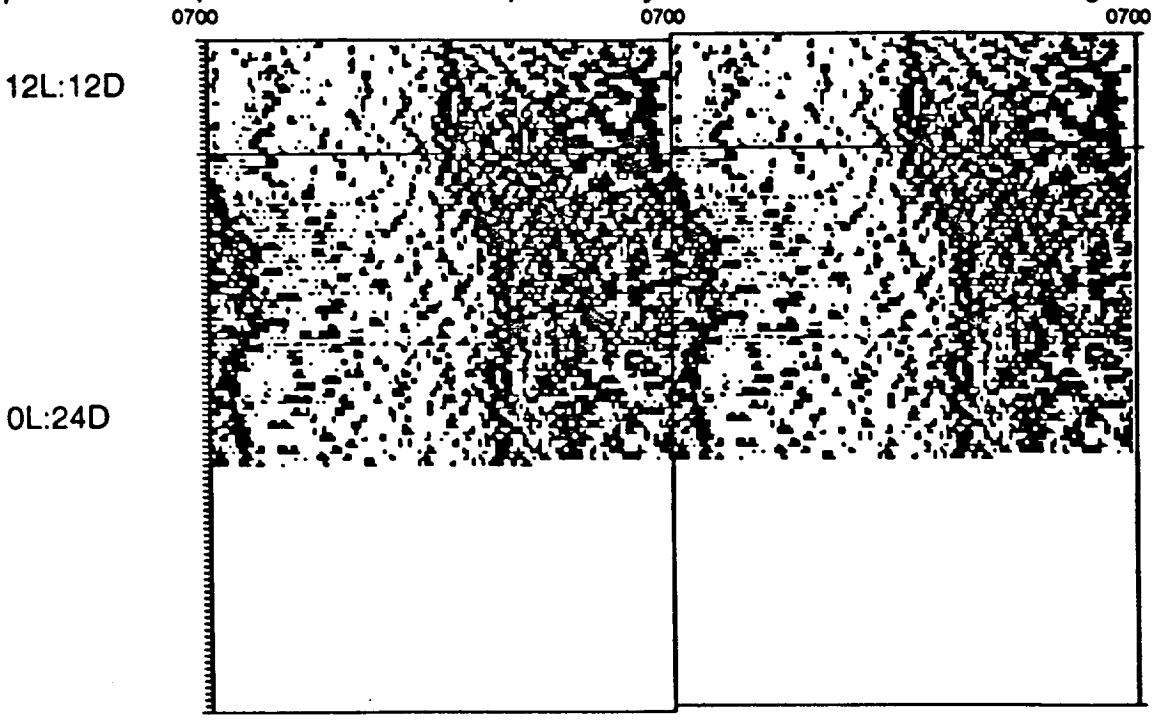
Experiment 3.1 (9109Sound05) Rat 5 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



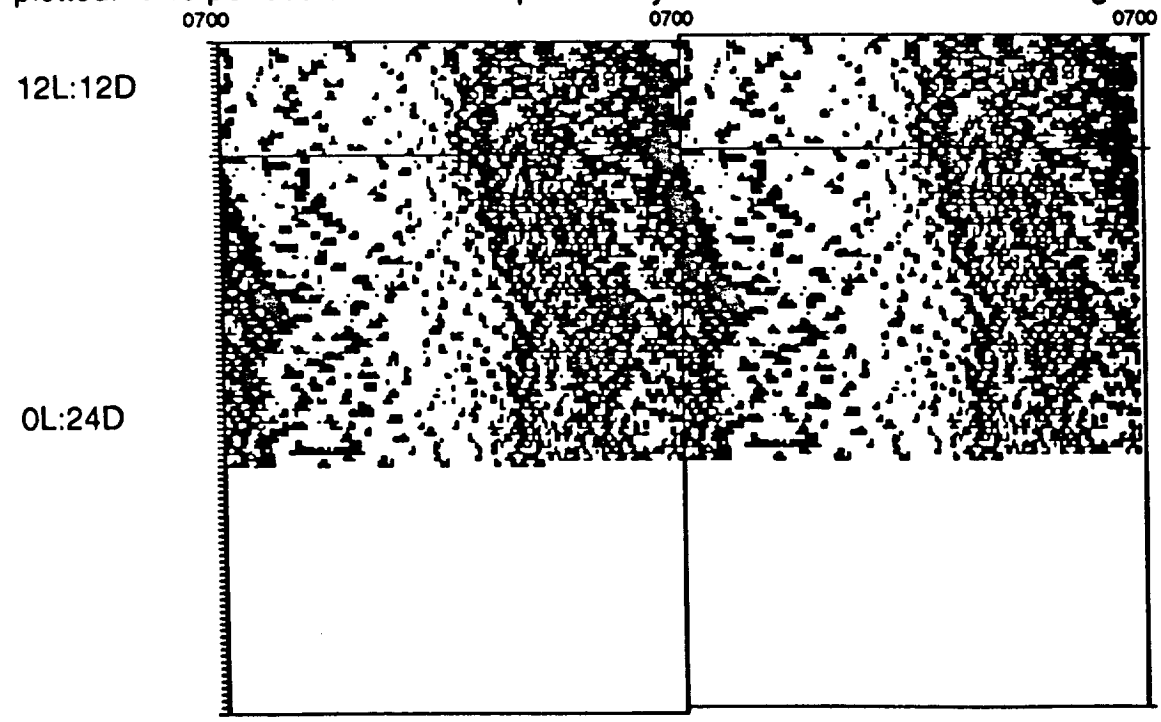
Experiment 3.1 (9109Sound05) Rat 6 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



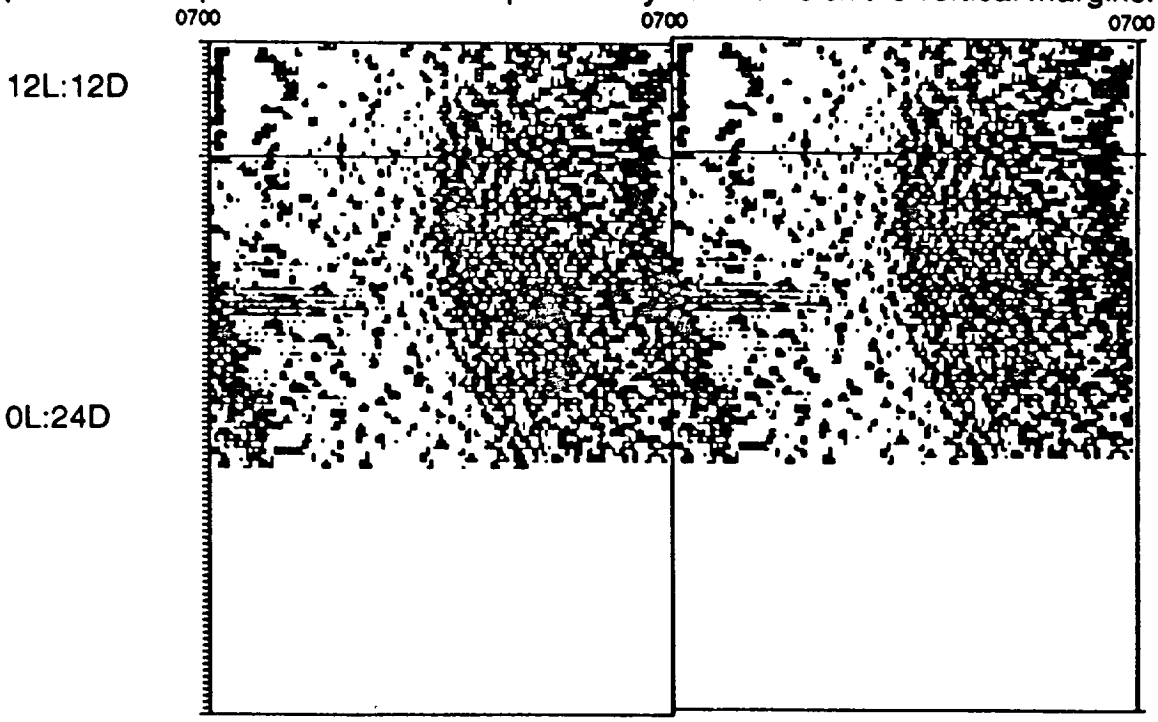
Experiment 4.1 (9306Sound05) Rat 1 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



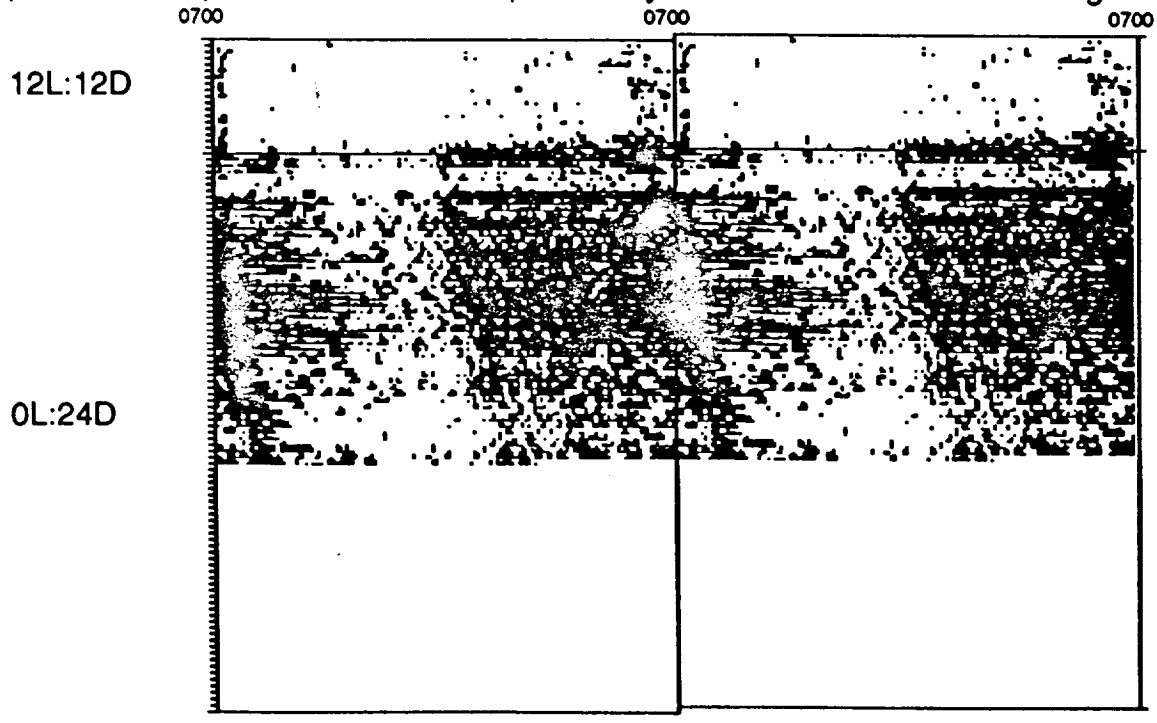
Experiment 4.1 (9306Sound05) Rat 2 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



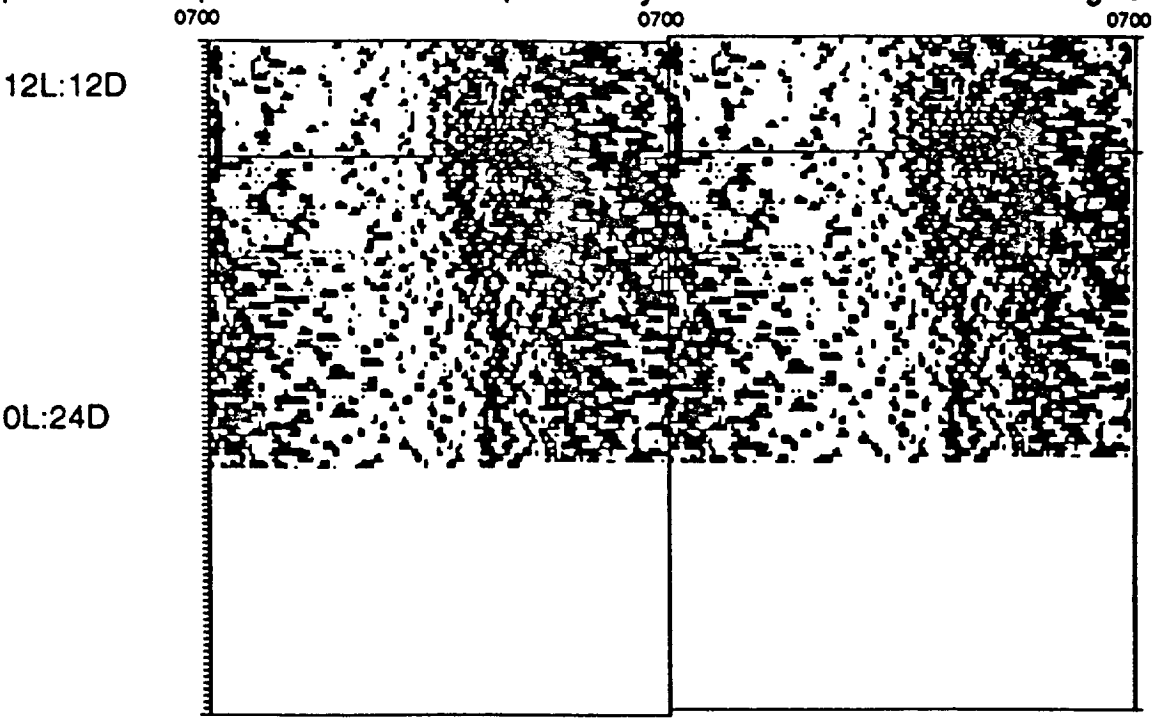
Experiment 4.1 (9306Sound05) Rat 3 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



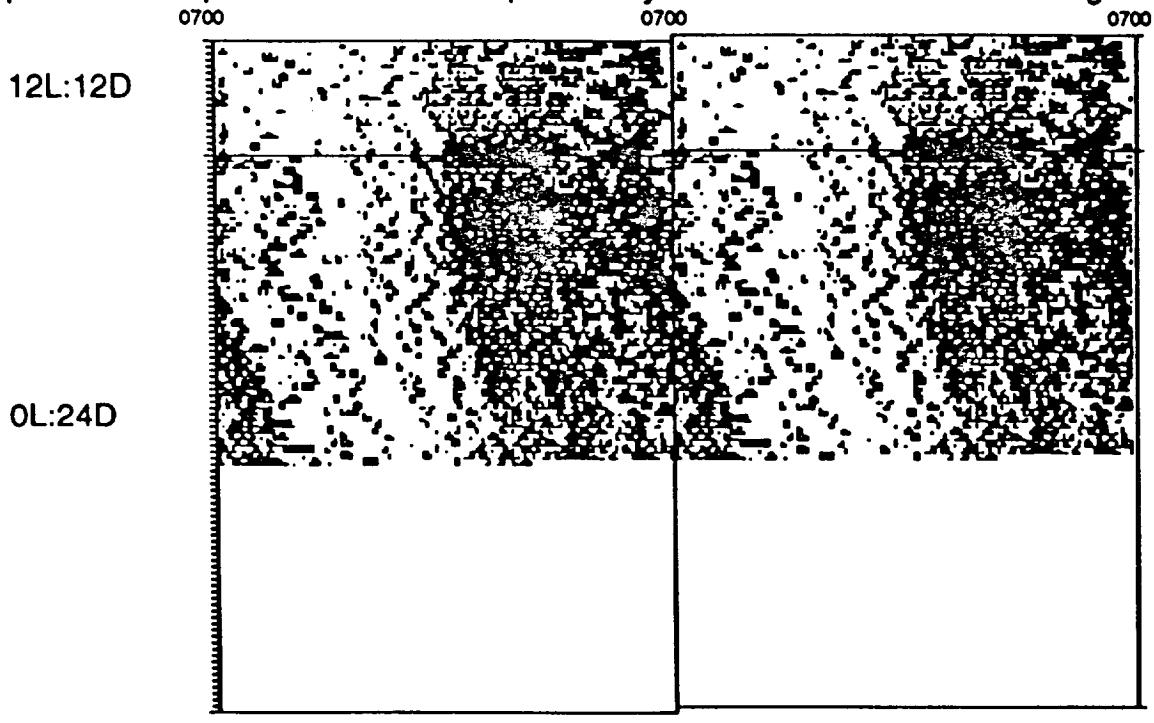
Experiment 4.1 (9306Sound05) Rat 4 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



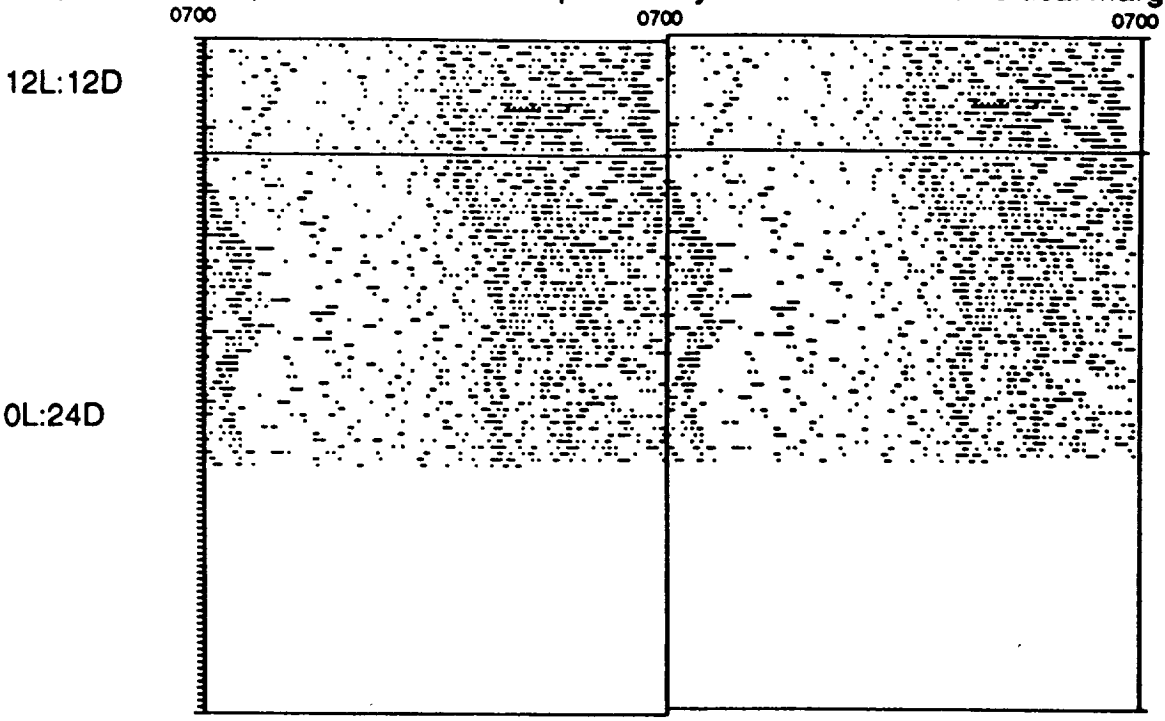
Experiment 4.1 (9306Sound05) Rat 5 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



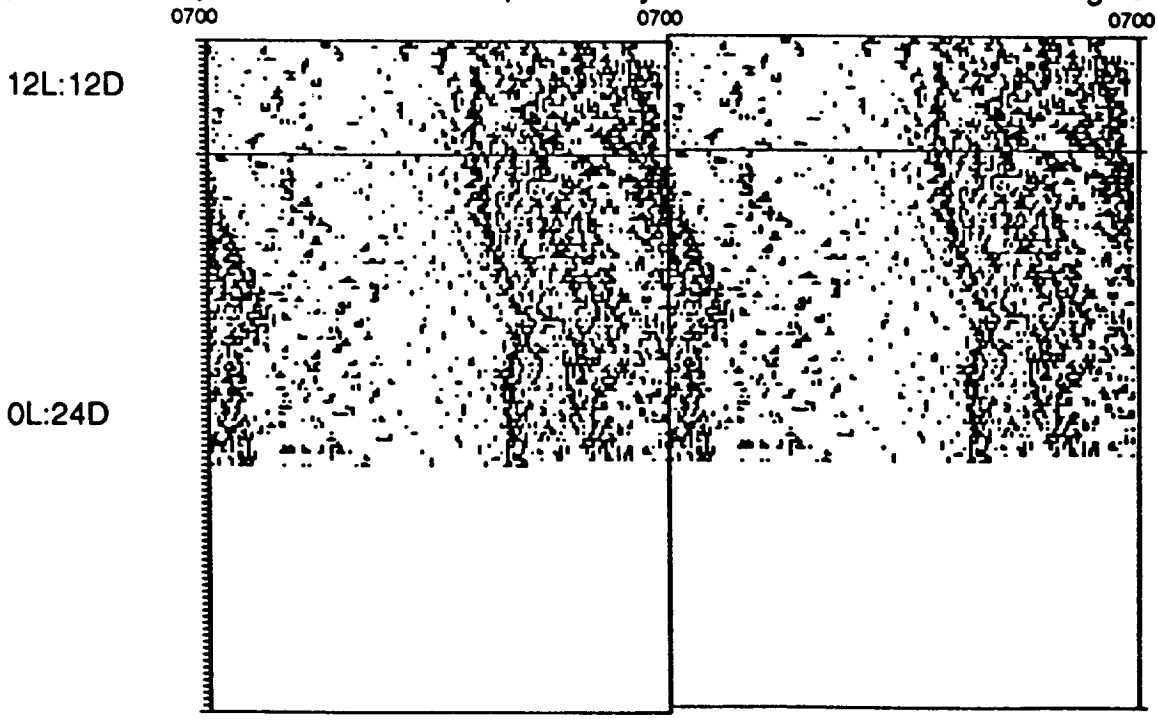
Experiment 4.1 (9306Sound05) Rat 6 LMA actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



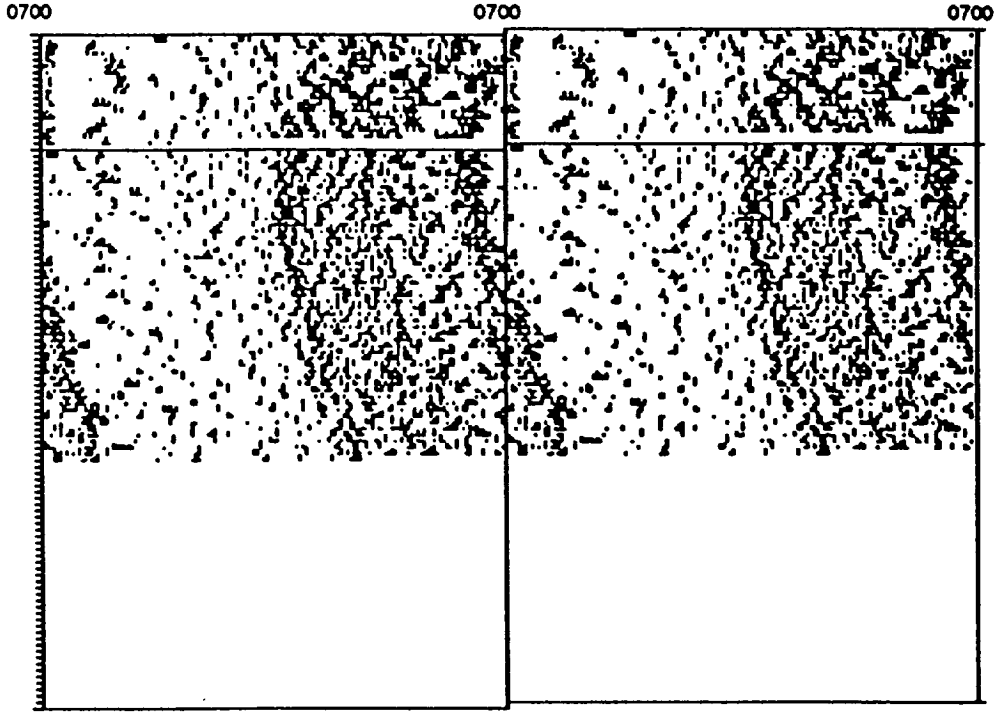
Experiment 4.1 (9306Sound05) Rat 1 drinking actogram. All values above the median are plotted. Two periods of time are separated by tick marks on the vertical margins.



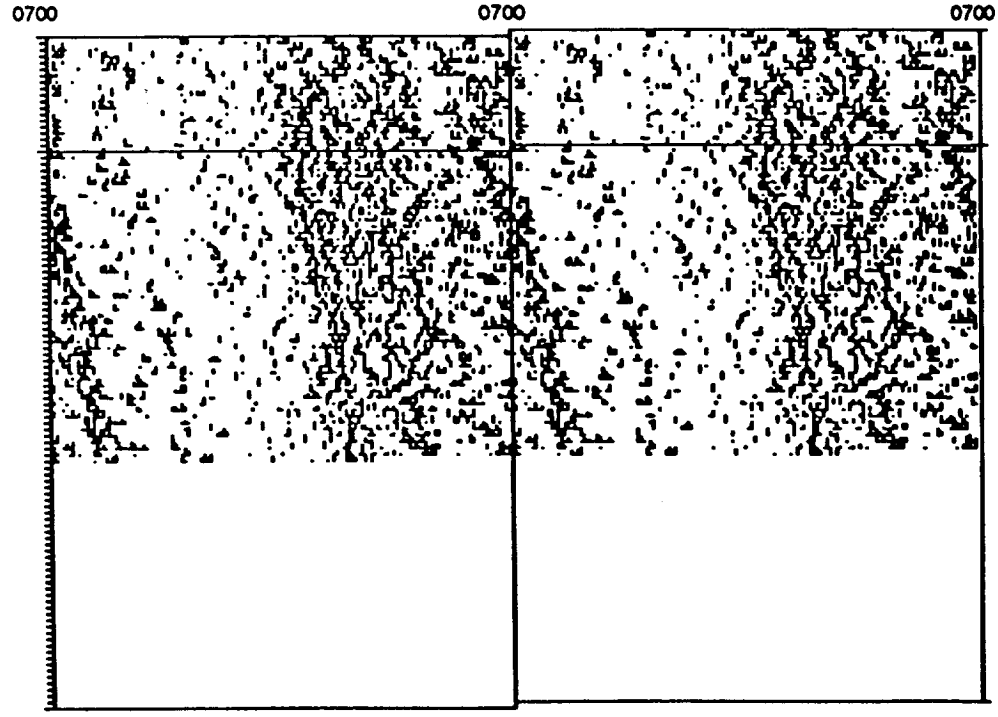
Experiment 4.1 (9306Sound05) Rat 2 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



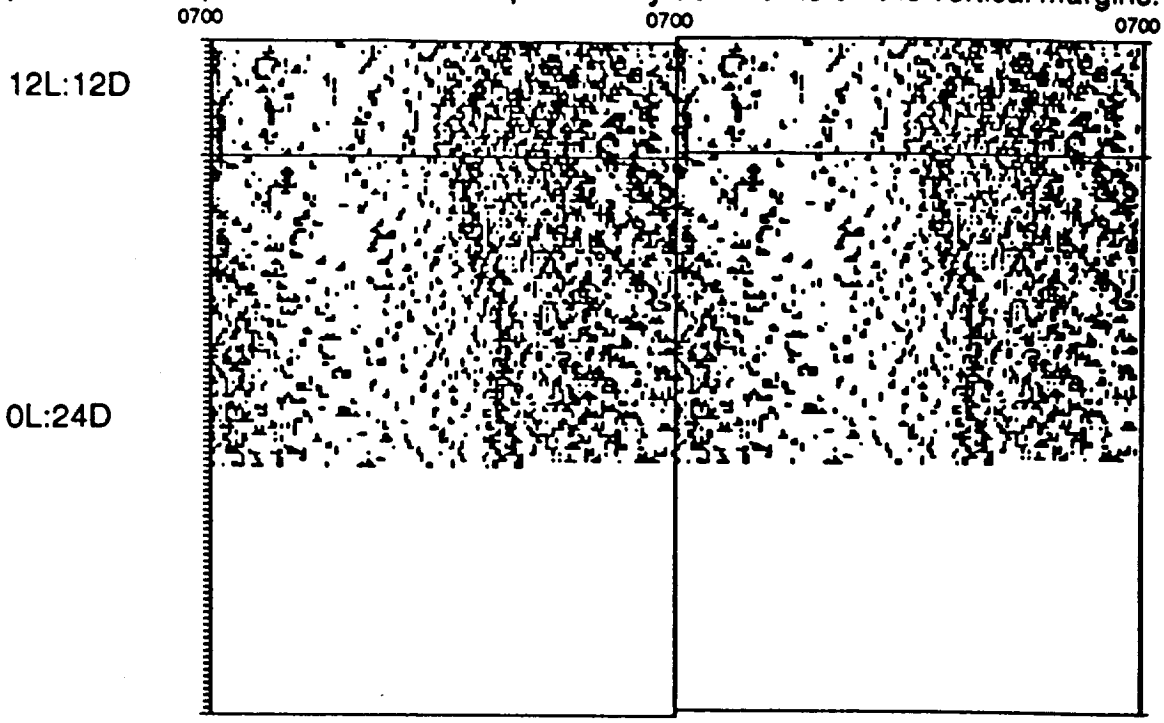
Experiment 4.1 (9306Sound05) Rat 3 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



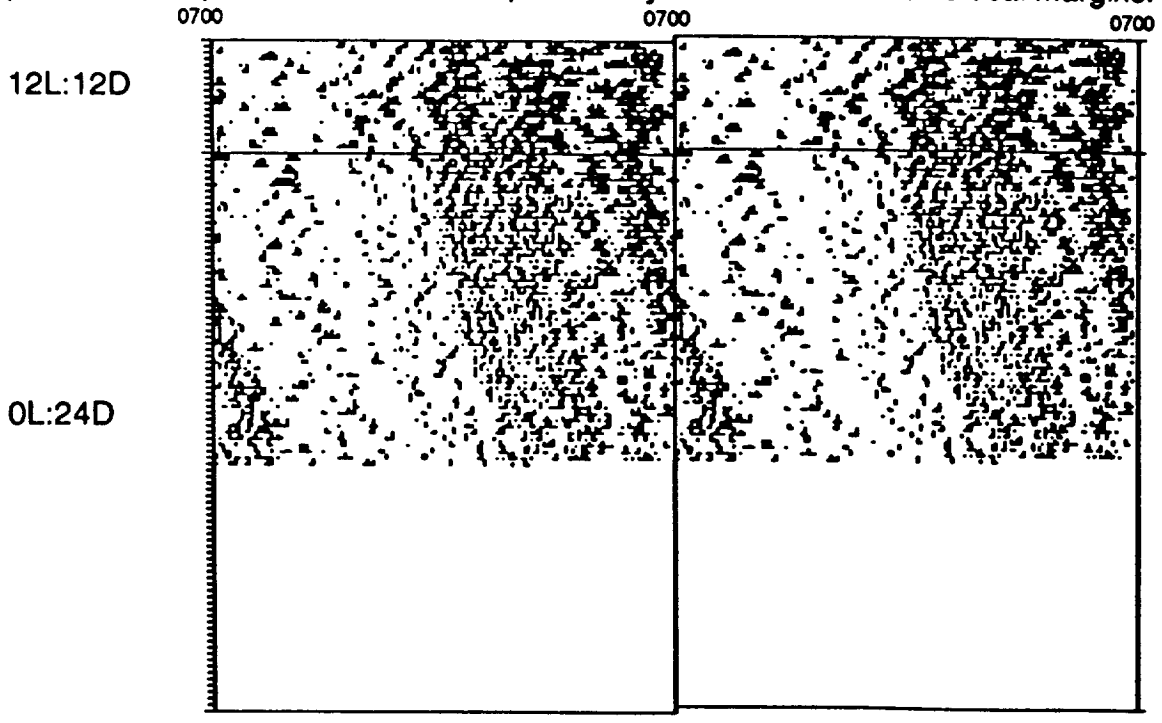
Experiment 4.1 (9306Sound05) Rat 4 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



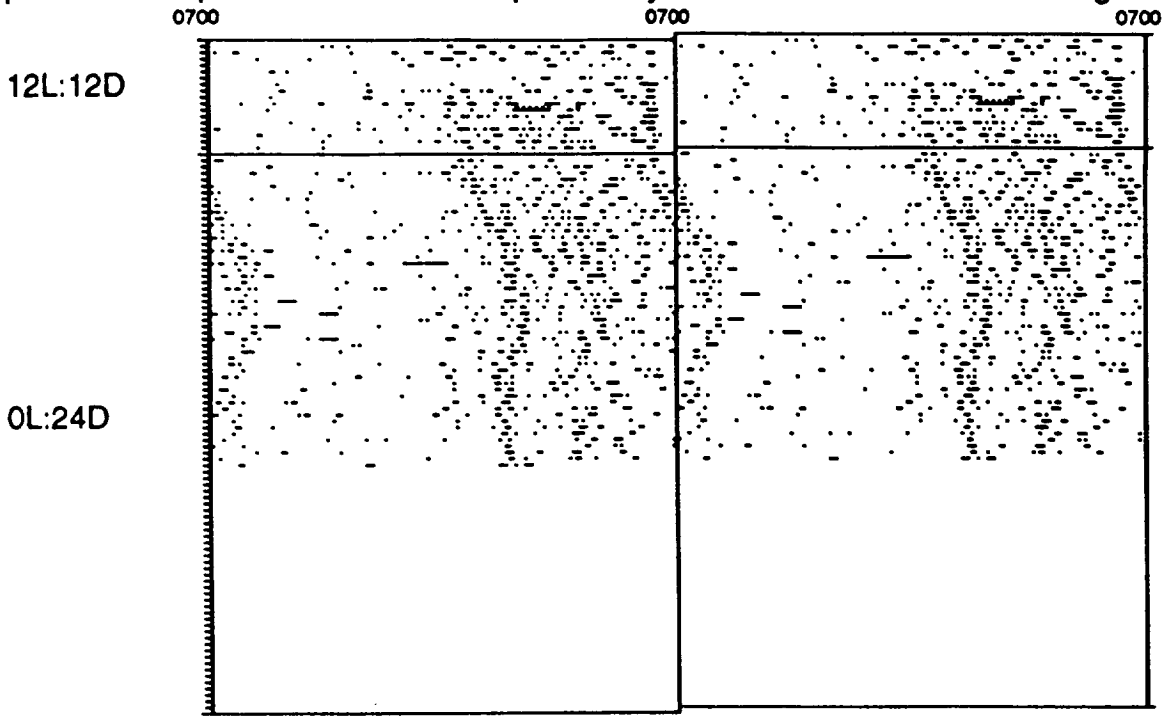
Experiment 4.1 (9306Sound05) Rat 5 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



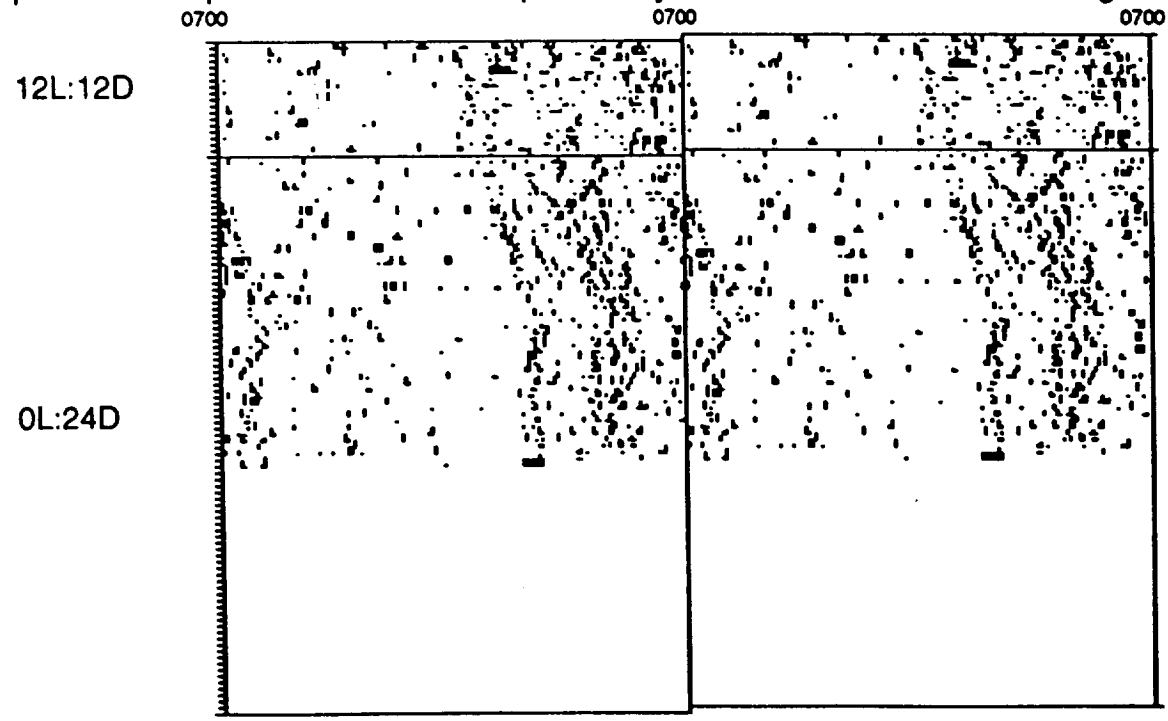
Experiment 4.1 (9306Sound05) Rat 6 drinking actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



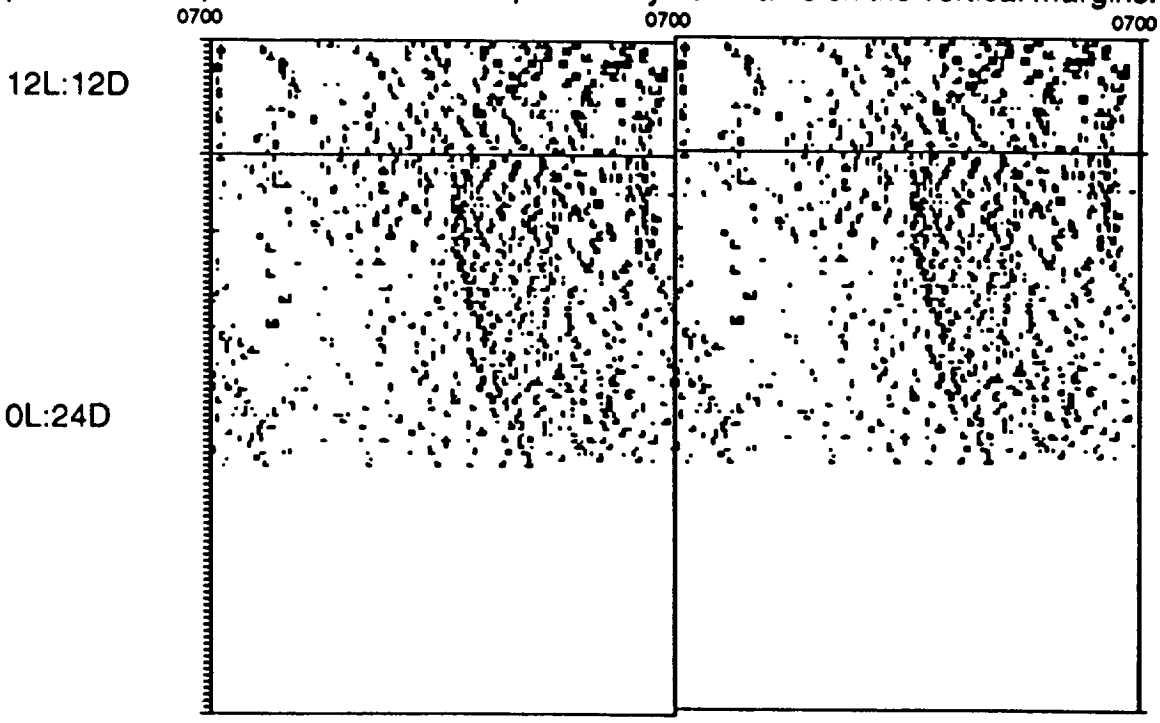
Experiment 4.1 (9306Sound05) Rat 1 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



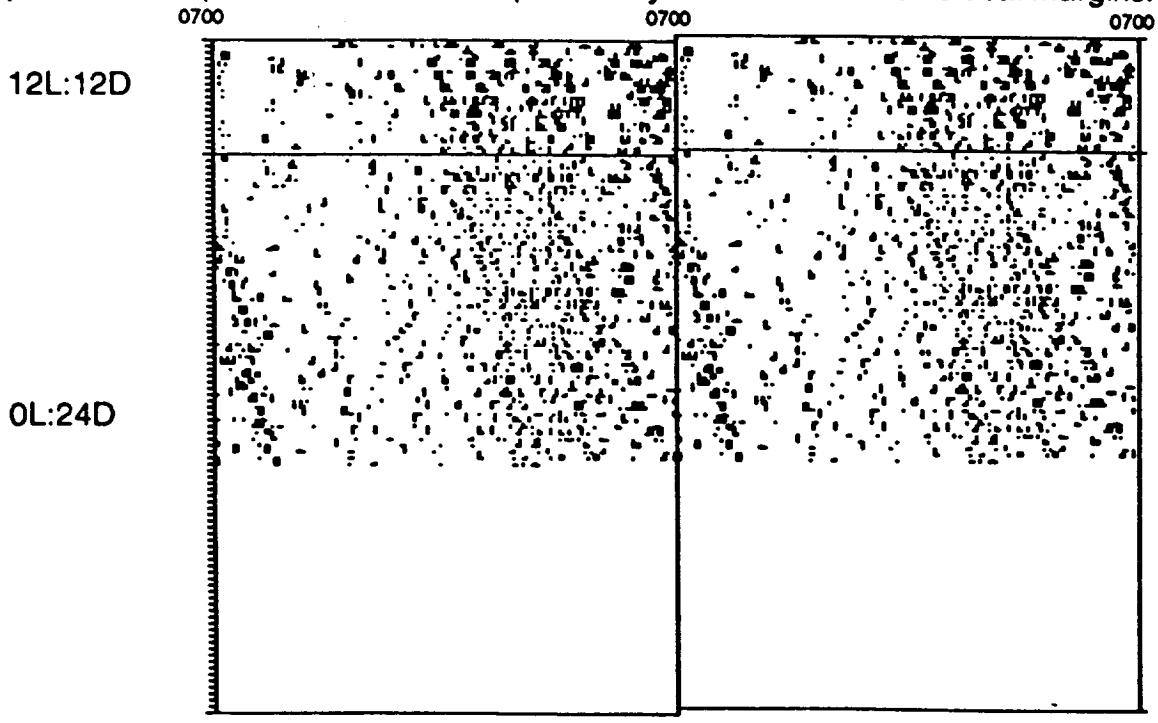
Experiment 4.1 (9306Sound05) Rat 2 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



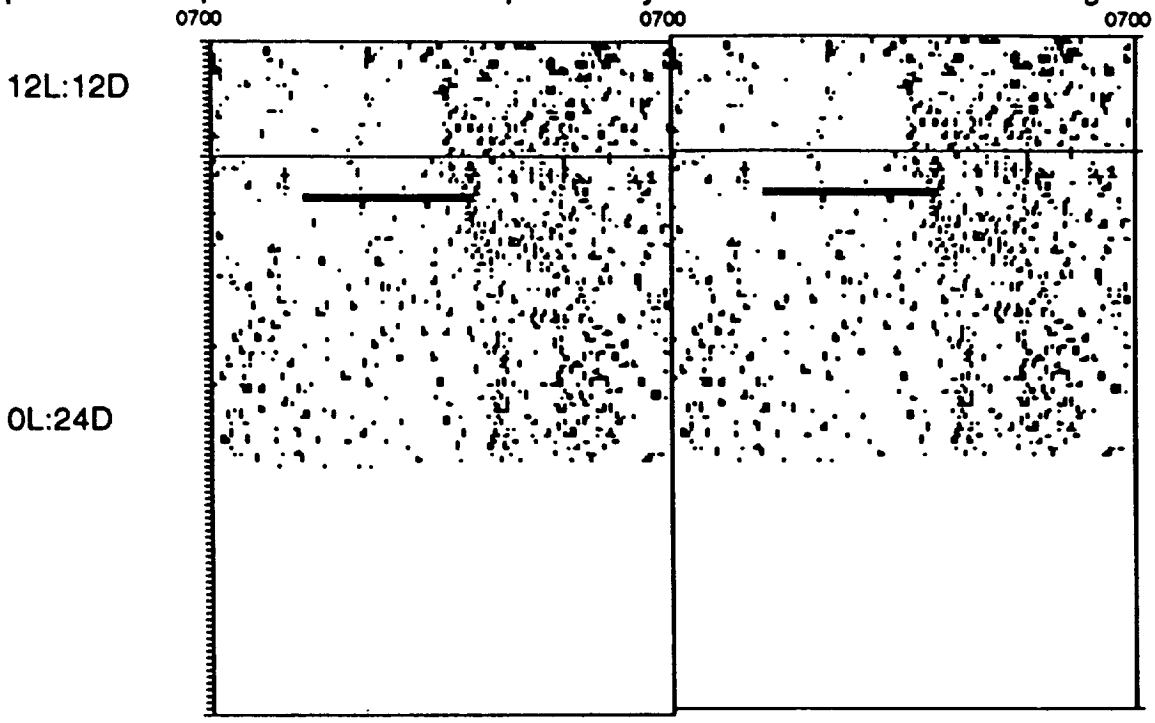
Experiment 4.1 (9306Sound05) Rat 3 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



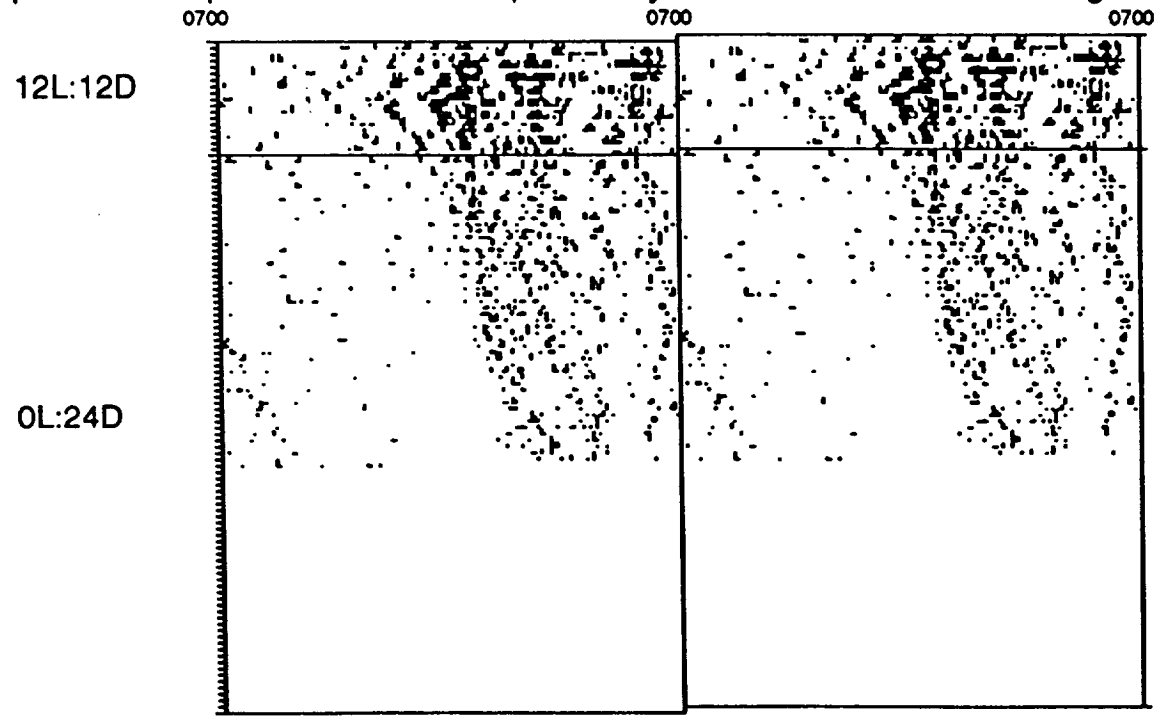
Experiment 4.1 (9306Sound05) Rat 4 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



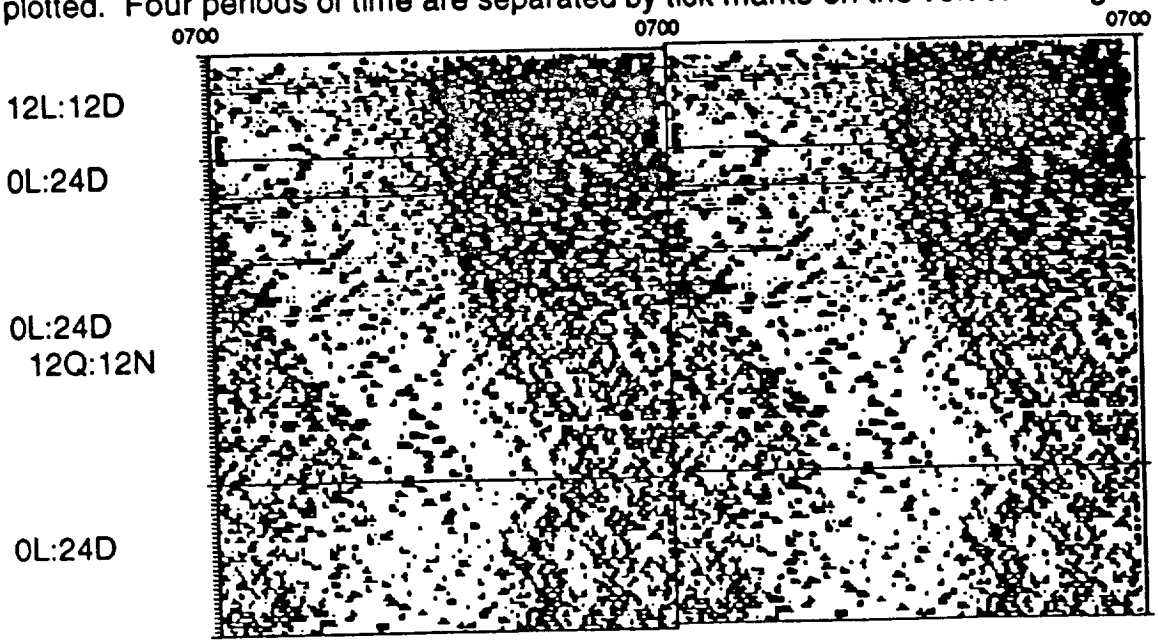
Experiment 4.1 (9306Sound05) Rat 5 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



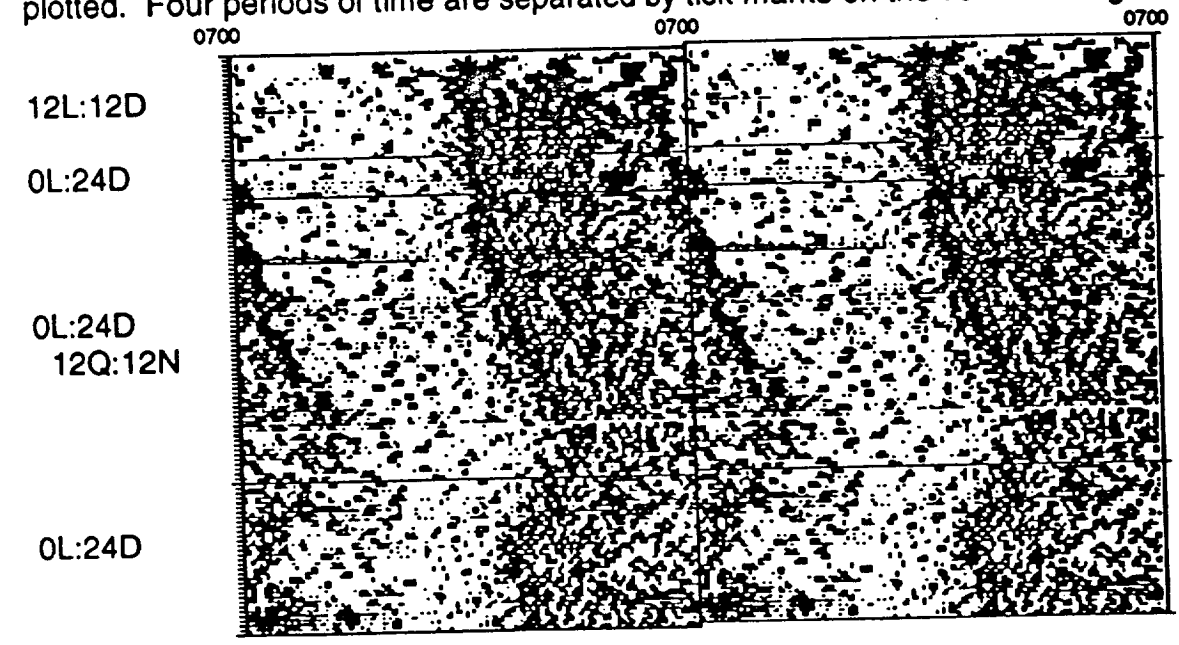
Experiment 4.1 (9306Sound05) Rat 6 feeding actogram. All non-zero values are plotted. Two periods of time are separated by tick marks on the vertical margins.



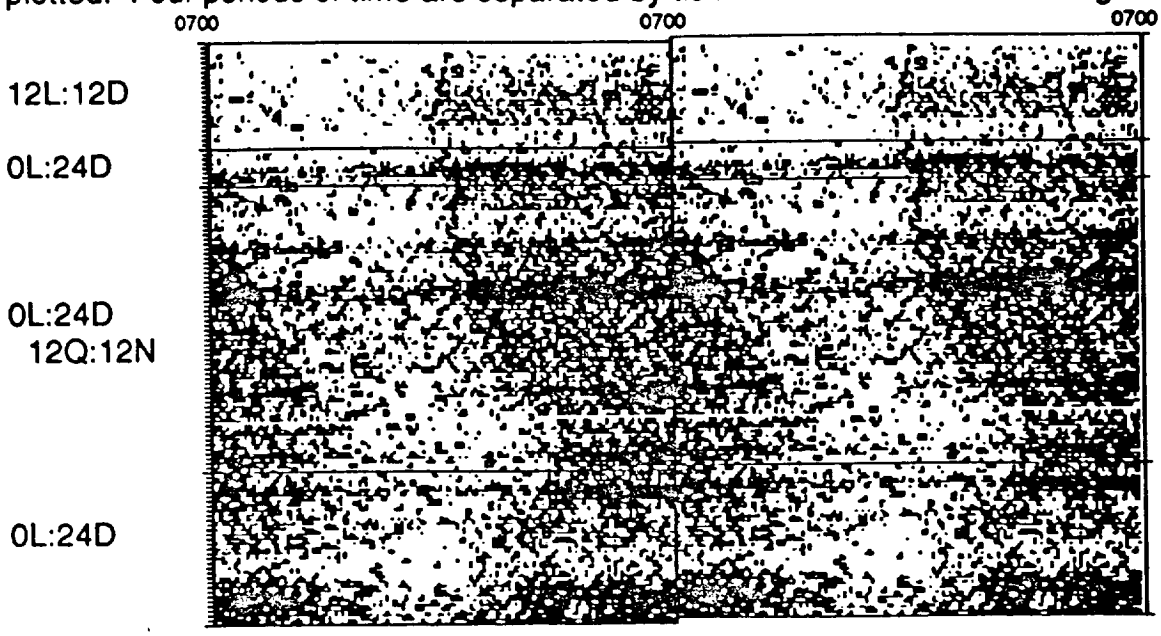
Experiment 4.2 (9112Sound05) Rat 1 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



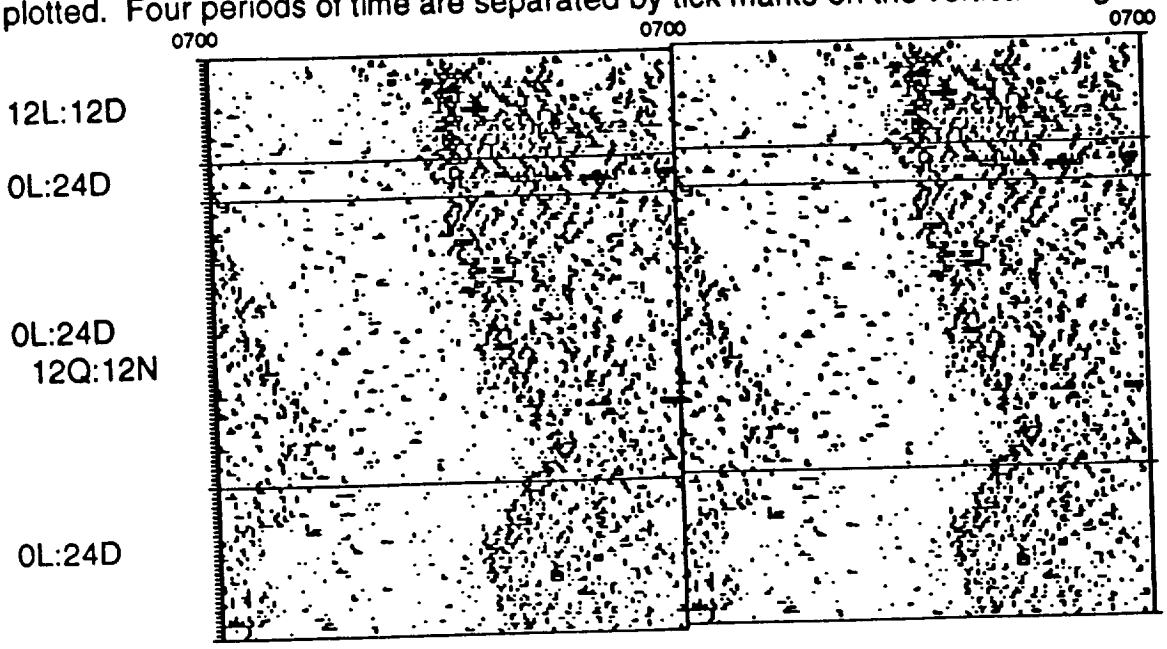
Experiment 4.2 (9112Sound05) Rat 2 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



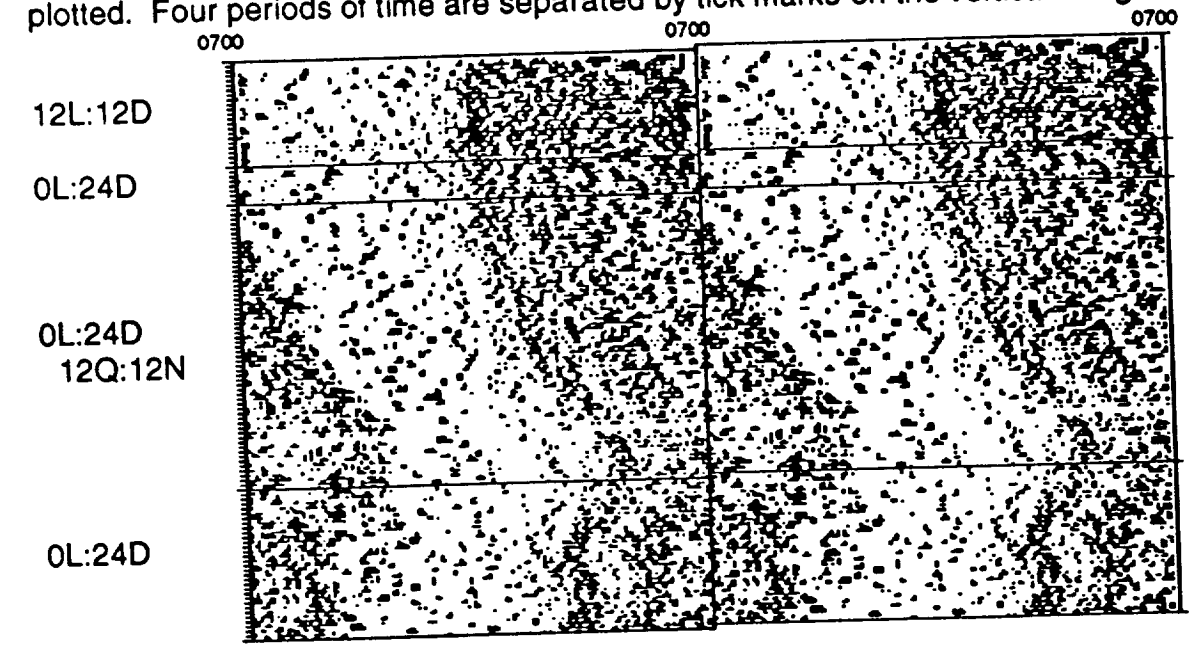
Experiment 4.2 (9112Sound05) Rat 3 LMA actogram. All values above the median are plotted. Four periods of time are separated by tick marks on the vertical margins.



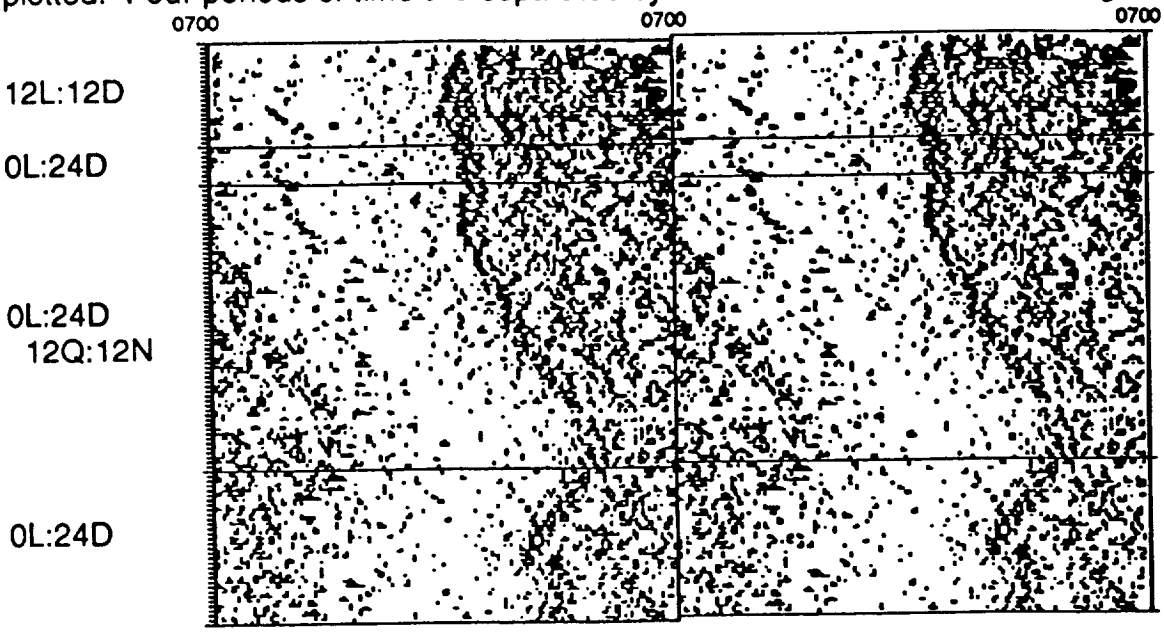
Experiment 4.2 (9112Sound05) Rat 1 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



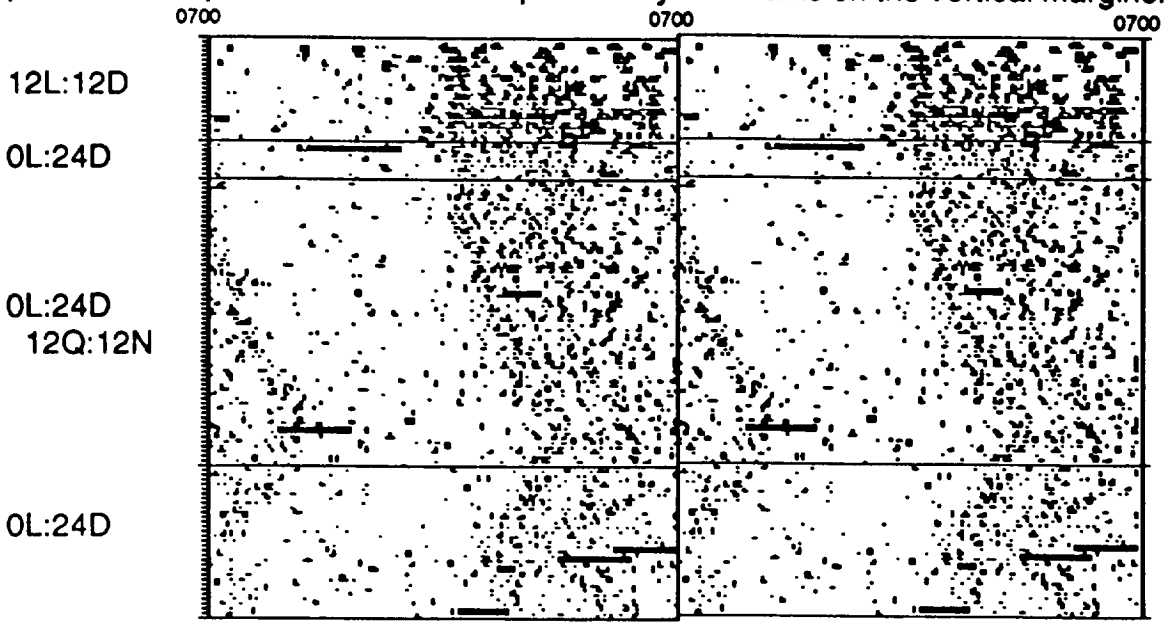
Experiment 4.2 (9112Sound05) Rat 2 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



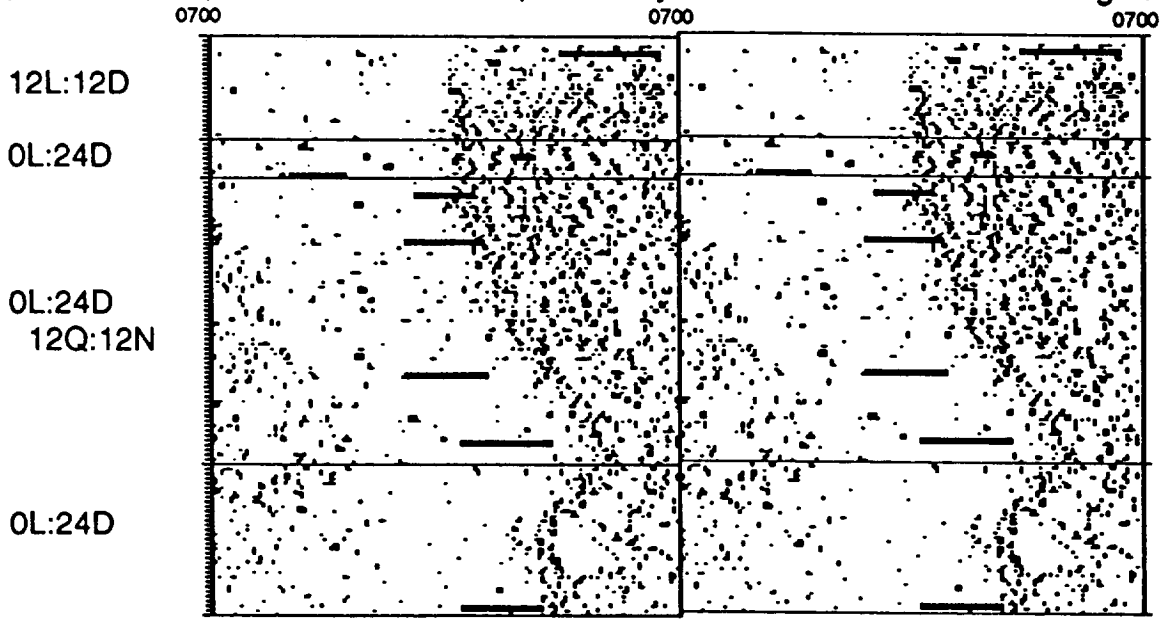
Experiment 4.2 (9112Sound05) Rat 3 drinking actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



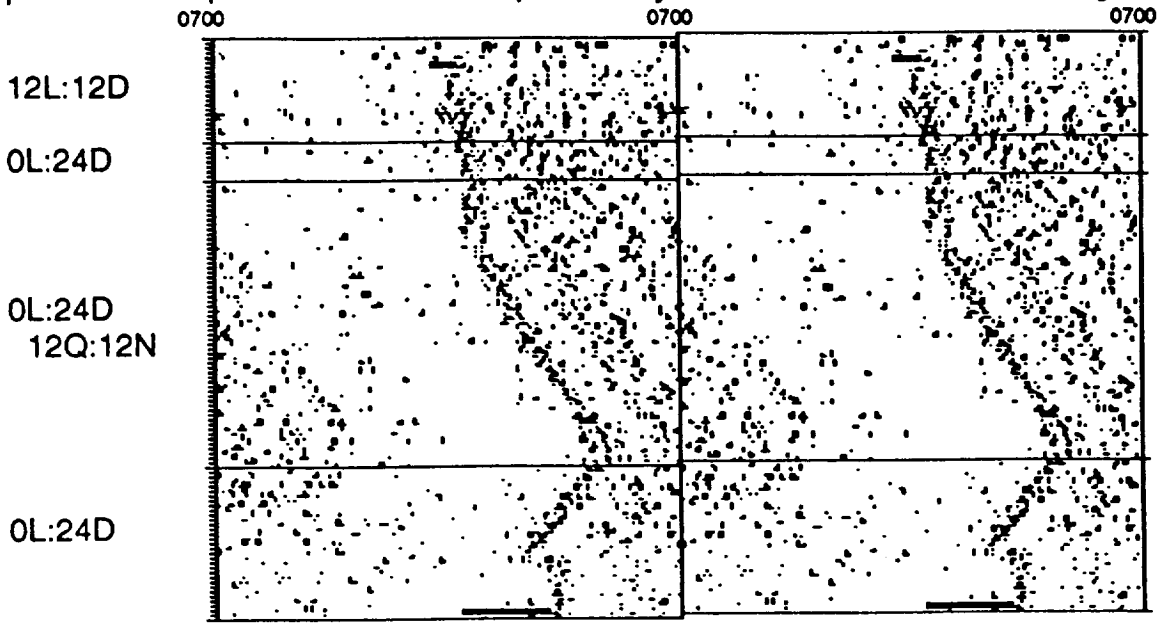
Experiment 4.2 (9112Sound05) Rat 1 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



Experiment 4.2 (9112Sound05) Rat 2 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



Experiment 4.2 (9112Sound05) Rat 3 feeding actogram. All non-zero values are plotted. Four periods of time are separated by tick marks on the vertical margins.



D) Temperature Related Problems Involving the AEM Lighting System

Final Subproject Report for Cooperative Agreement #NCC2-593

FINAL REPORT

AEM TEMPERATURE and LIGHTING PROBLEM

February 15, 1992

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Relative to Cooperative Agreement # NCC2-593

I. BACKGROUND

It has been ascertained that the internal temperatures of the animal enclosure modules (AEMs) used during certain space missions have been occasionally high (out of expected normal range) e.g., PAR E.01, PSE-01, and SLS-1. Since the AEMs are stowed in the shuttle mid-deck lockers, and since they draw ambient cabin air, their internal air temperature is a function of 1) temperature of ambient air being drawn from the crew cabin and partially recycled within the containment locker, 2) heat being produced by the living inhabitants within the AEMs (the rats), 3) heat generated by the electronic devices within the AEMs (e.g., fan motors, timer circuits, etc.), and 4) heat generated by the AEM internal lighting system.

Given that most shuttle crews keep cabin temperature fairly warm (approx. 24-28 ^{o}C), when animals are within the units and the AEM lights are on, the internal temperature can exceed desired levels per experiment requirements. If it is assumed that items 1-3 above are fairly constant and not easily subject to modification, then item number 4 (lighting) is the logical system to modify to alleviate the high temperature problem. Accordingly, it was recommended by Dr. Louis Ostrach, that the normal AEM lighting configuration of four incandescent G.E. #313 bulbs be replaced by two incandescent G.E #1818 bulbs. The reasoning was that the lower wattage G.E #1818 bulb would produce less heat, therefore, result in lower lights-on internal AEM temperatures. The disadvantage of this concept is that the bulbs would necessarily produce less light.

Subsequent to discussions in late May and June 1991 about this issue, our current Cooperative Agreement (NCC2-593) was modified and we were given the task of further defining this problem. We were to test various light bulb configurations and to provide hard data to substantiate subsequent hardware modification recommendations. In addition, we undertook some development work aimed at finding and testing alternate light sources (LEDs) that might alleviate the high temperature/low light intensity situation. This report summarizes our findings and includes our recommendations on these issues.

II. METHODS AND PROCEDURES

A. Simulated AEM: Wooden Box.

Due to the unavailability of a high fidelity flight-like AEM, and the urgency of this problem, preliminary studies were performed using a "simulated" AEM, constructed of wood. The wooden box was made of unfinished 3/4" pine (internal dimensions 29.2 x 29.2 x 29.2 cm) with a non air-tight lid, also unfinished 3/4" pine. This size was used as an approximation of the flight AEM internal volume. We believed that by using this test model preliminary data could be obtained that would indicate relative differences between bulb configurations. We also wanted to use the box to evaluate the LED arrays that we were developing as an alternative to the existing incandescent bulbs used in the current AEMs.

B. High Fidelity AEM Prototype.

In August 1991, we obtained from Mike Hines (code SPD), the high fidelity prototype AEM that was constructed prior to the first four flight qualified AEMs (AEM prototype #1). We used this prototype AEM to verify our preliminary findings on temperature and lighting intensity using the wooden box. We also tested our linear LED arrays in this prototype.

C. Light Intensity Measurements On The Lab Bench.

Light intensity measurements were made using a calibrated IL-1700 Research Radiometer (Industrial Light, Inc., Newburyport, MA).

When testing individual light sources, light intensity was measured in two ways. First, illuminance measurements were taken at several distances (90, 70, 50, 30, 15,and 8 cm) from the light bulb being evaluated. The bulb was mounted vertically, about 100 cm above a black lab bench in a darkened room. The sensor was moved incrementally from the bench upward towards the bulb. Second, illuminance readings were taken from various polar coordinates in the perpendicular plane (mid-bulb). The light bulb was also mounted vertically in this case about 60 cm from the black lab bench.

When measuring light intensity within the prototype AEM, readings were made at 27 different locations throughout the three dimensional area of the AEM: 9 readings on the floor of the AEM, 9 in the middle plane, and 9 at the top plane (i.e., the probe was touching the cage top). All measurements were made with the radiometer probe directed at the rear of the AEM (away from the fans, pointing towards the light sources). The mean of the 27 values was reported for each light source.

D. Temperature Measurements.

Temperature recordings were made using a Rustrak Ranger data logger with temperature sensor type 03 (Rustrak, East Greenwich, RI). This was calibrated using a standard laboratory mercury thermometer. This unit can be interfaced with a PC (MS-DOS) for data manipulation and graphics.

1. Wooden box.

Temperature measurements were made of the various light bulb configurations and LED arrays, initially using the wooden box test unit. During

these procedures, the wooden box was closed and the light source being evaluated was activated for one hour. At that time the temperature in the box was compared with the ambient room temperature to obtain a temperature difference. The position of the temperature probe for these measurements was the center of the box (no contact with the box).

2. Prototype AEM.

Temperature measurements within the AEM were recorded continuously for 24 hours using the Rustrak Ranger data logger. The probe for the data logger was placed in front of the water container, but not in direct contact with it or any other surface. The AEM filters were in place and the fans were operating per specifications. It should be noted that food bars were not in place for these studies, and no animals were in the AEM.

E. Light Emitting Diode (LED) Arrays.

We consulted David Bubenheim regarding his Director's Discretionary Fund project to develop LED light sources for plant growth chambers in space. Subsequently, we discovered that two manufacturers produced "high intensity" LEDs that might fit our application. These manufacturers were 1) Hewlett Packard, and 2) Siemens. The red, green, and yellow LEDs are reasonably priced (about $0.65 per LED), but the blue LEDs are approximately $65.00 each and were not purchased. It should be noted that the Siemens LEDs are of lower intensity and we did not test these.

1) LED arrays for comparison testing: intensity, power consumption.

We developed several LED test arrays, consisting of different numbers and types of LEDs, which we subjected to light intensity and temperature measurements. The LEDs used were a combination of green, red, and yellow high intensity LEDs (#HLMP-3750, -3850, -3950, Hewlett Packard, Sunnyvale, CA). Table 7 describes the composition and dimensions of the various LED arrays tested. Figure 1 shows the wiring diagram for these test arrays. It should be noted that we tried to develop a wiring design that would assure uniform power consumption by each LED. Also, we tried to develop an array that would not black out if one to several LEDs in the array burned out. We estimate that with this wiring design, 4 LEDs in a given row would have to fail to bring down the entire array.

2) LED arrays tested within the prototype AEM: intensity, temperature.

Two LED arrays were constructed using a combination of red, yellow, and green high intensity LEDs (#HLMP-3750, -3850, -3950, Hewlett Packard, Sunnyvale, CA). These arrays measured 2.5 x 2.0 x 18.0 cm, and were designated "set H". Each array was built using 42 LEDs (14 of each color, and consisting of three circuits in parallel, see Figure 2). These LEDs are rated by the manufacturer as producing 120 millicandela (mcd), 125 mcd, and 140 mcd, at 20 milliamps, for green, red, and yellow LEDs, respectively. Using this design, this circuit distributed 15.7 milliamps to each LED (78.5% of rated capacity).

It was not possible to place the test LED arrays into the normal position occupied by the incandescent bulbs inside the perforated metal containment housing within the AEM prototype. This was because the mini

base sockets for the incandescent bulbs were spot-welded into place. Therefore, the arrays to be tested were taped onto the metal containment housing with the LEDs facing towards the front of the cage.

F. Measurement Of Spectral Power Distribution Of Individual LEDs, LED Test Array (Set H), and G.E. Bulb #1819.

Spectral power distributions of the various individual LEDs and the 42 lamp LED array (set H) used in the AEM prototype test were made using a Bauch and Lomb monochronometer and photomultiplier obtained from Dr. J. Becker, Physics Dept., San Jose State University. Initial readings were corrected using a factor obtained by scanning a calibrated reference standard lamp (type 30A/T2417, G.E. # EPT 1334). The manufacturer's published spectral power distribution (intensity) for this lamp powered at 38.0 Amps (D.C.) is shown in Figure 3. Figure 4 shows the voltage output from the monochronometer/photomultiplier system used in our application when the standard lamp (38.0 Amps D.C.) was scanned. These data were used to correct the spectral power distribution curves presented in Figures 5a-c, 6, and 7.

III. RESULTS AND DISCUSSION

A. Relative Illuminance Of The Various Light Sources.

From illuminance data gathered at distances of 8 cm to 90 cm from the light bulb, it was determined that the G.E. 313, currently in use in the AEM, is approximately four to five times as bright as the G.E. 1819, a proposed replacement (Table 1). Light intensity measurements were also taken of the G.E. 1820 and G.E. 757 bulbs. The G.E. 1820 was shown to have a light intensity in the intermediate range between the G.E. 313 and the G.E. 1819, while the G.E. 757 had an illuminance below that of the G.E. 1819 bulb.

Data in Table 1 was collected with light bulbs operating from a 28 volt A.C. power supply, rather than a 28 volt D.C. power supply, the power source from which the AEM operates. To test the validity of data collected from the A.C. power supply, comparable data were collected for the G.E. 313 using a 28 volt D.C. power source (Table 3). These data indicate that the measurements made using the A.C. power supply were, indeed, comparable to those collected using a D.C. power source, with the D.C. configuration producing 2-10% greater absolute intensity.

Light intensity measurements made around the bulbs perpendicular to their long axis at 30 and 60 cm distance showed that, in this respect, the G.E. 1819 was, on the average, 12.7% and 12.6% the brightness of the G.E. 313 at 30 cm and 60 cm, respectively (Tables 4 and 5). This is a particularly important consideration since the light bulbs are situated in the AEM in a manner similar to the way in which they were situated for these measurements, i.e. mounted vertically, giving off light to the AEM from the perpendicular (side of the bulb). Surprisingly, data collected in this manner for the G.E. 757 light bulb shows that this light bulb provided greater brightness than the G.E. 1819 when illuminance was measured from the perpendicular or side of the bulb (Tables 4 and 5), but provided less brightness when illuminance was measured vertically or directly above the bulb (Table 1).

The LED arrays (sets D-G) were made of 60 to 86 LEDs and their composition is shown in Table 7. Light intensity measurements taken from 8 to 90 cm indicated that illuminance surpassing that of the G.E. 313 light bulb could be obtained with an LED array containing as few as 63 LEDs and measuring 18.8 square cm (sets E and G, Table 2). However, when illuminance measurements were taken of these LED arrays from the perpendicular at various polar coordinates (Table 6), light intensity was well below that of the G.E. 313 light bulb (Tables 4 and 5). This is due to the fact that the majority of light emitted by the LEDs is projected in a 24 degree beam out of the end (the long axis of the LED), i.e., little light is emitted perpendicularly from the sides of the LEDs.

B. Temperature In The Wooden Box Test Fixture Using The Various Light Source Configurations.

Temperature measurements taken within the simulated AEM (wooden box) revealed that two G.E. 313 bulbs (6.4 ^{o}C change) produce 4.57 times as much temperature change as two G.E. 1819 bulbs (1.4 ^{o}C change) (Table 8). Table 8 also shows that four G.E. 313 light bulbs raised the box temperature by 10.4 ^{o}C, while four G.E. 1819 light bulbs increased the temperature by 4.3 ^{o}C (41.3% difference).

The LED arrays D, F, and G raised the box temperature between 0.2 and 0.6 ^{o}C, and array E raised the temperature 0.8^{o}C (Table 8).

C) Light Intensity, Temperature, And Power Consumption When Using Various Light Sources In The Prototype AEM.

1. Power consumption.

When considering the most desirable light source configuration to be used in the AEMs, it is important to weigh three parameters: power consumption, light intensity produced, and heat production. The light source that produces the greatest light intensity, together with the least heat production and power consumption, is the most preferable.

The current flight AEMs are configured with four G.E. 313 incandescent light bulbs. Each of these light bulbs consumes 4.76 Watts and produces 5 candela (as rated by manufacturer). This results in a total output of 1.05 candela/Watt (Table 9). Because the majority of the power that incandescent light bulbs draw is converted to heat, they are inherently inefficient. This fact is more pronounced for the alternate light bulb, the G.E. 1819. While consuming 23.5% (1.12 Watts) of the amount of power used by the G.E. 313, this light bulb produces 0.5 candela (as rated by manufacturer). In this regard, the resulting efficiency is a mere 0.45 candela/Watt (Table 9). These data indicate that the G.E. 313 light bulb is more than two times as efficient as the G.E. 1819, producing more than twice as much light per Watt. Note, it is due to the fact that the G.E. 1819 consumes less power than the G.E. 313 that it produces less heat.

Both of the G.E. light bulbs (313 and 1819) pale in comparison with the constructed LED array in terms of efficiency. Each LED array was designed to be placed in a corner of the AEM. Currently each corner is occupied by two incandescent light bulbs. For this reason we compare

one LED array to two incandescent light bulbs. Each of the LED arrays consumes 1.36 Watts. This represents only 14.3% of the power usage by the two G.E. 313 light bulbs (9.52 Watts), and 60.7% of the power consumption of the two G.E. 1819 (2.24 Watts). Dividing 4.04 candela (78.5% of manufacturers rating, see Methods and Procedures) by 1.36 Watts results in an efficient 2.97 candela/Watt (Table 9). This represents 2.83 times the efficiency of the G.E. 313, and 6.60 times the efficiency of the G.E. 1819.

We conclude that the test LED array (set H) is by far the most power efficient, followed by the G.E. 313 light bulb and the G.E. 1819.

2. Light intensity.

A great deal of variation was noted in measuring light intensity at different locations within the AEM (Table 10). This is due to the fact that while locations close to the light source were well lit, others, away from the light source, often behind the water container, were very dim (as low as 0.2 lux for G.E. 1819). This variation is expected when taking into consideration the AEM structure.

In considering the data presented in Table 10, it is important to recall that the recommended minimum light intensity for rodents is 5 lux (NASA Technical Memorandum #101077, Lighting Requirements in Microgravity - Rodents and Nonhuman Primates, December 1988). **Based on this information, the use of two G.E. 1819 light bulbs should be reconsidered since mean intensity was only 3.3 lux (S.D. ± 2.3 lux).**

Though the placement of the 2 LED arrays on the outside of the lighting containment housing probably produced illuminance readings higher than would be obtained if they were within the housings (see Methods and Procedures section), the values were encouraging (mean=22.6 lux, SD ± 22.0 lux).

It should be noted that the standard deviation in measuring the illuminance of the LED arrays was greater than for any of the other configurations (Table 10). This is due to the fact that the majority of the light emitted by an individual LED is within an angle of 24 degrees of its long axis. As a result, some locations within the AEM received the full brightness of the LED, while others received little. Corrective lenses may be used to rectify this observation.

3. Temperature within the prototype AEM using various lighting configurations.

Temperature inside the AEM is a function of the temperature outside of the AEM since the fans continually draw outside air. When heat is produced in the AEM from light bulbs or the presence of animals, the temperature inside the AEM rises and may exceed the outside temperature. However, if there are no light bulbs on and no animals present inside the AEM, the temperature inside the AEM will be approximately the same as the temperature outside. There will be a slight delay (due to mixing and thermal inertia of the inner mass) in time between temperature changes that occur outside and those recorded inside.

Twenty-four hour temperature recordings were made of the following configurations: four G.E. 313 light bulbs, two G.E. 313 light bulbs, four G.E. 1819 light bulbs, two G.E. 1819 light bulbs, and two LED arrays, set H (Table 11). As expected, the greatest temperature change between mean lights-on and mean lights-off periods was for the configuration that consumed the most power, the four G.E. 313 bulbs (2 x 9.52 = 19.04 Watts). However, the configuration that used the least power, the two G.E. 1819 bulbs (2.24 Watts), produced more of a temperature change (0.22 ^{o}C) than the two LED arrays (0.18 ^{o}C), which consume 2.72 Watts. This is attributed to the greater efficiency of the LED arrays than either of the other two bulbs.

The standard deviation about each mean was approximately 0.2 ^{o}C, and is considered to be a function of the fluctuations in the room temperature of the environmental chamber housing the AEM as the chamber thermostat cycled off and on. The greater standard deviation noted for the four G.E. 313 mean on-temperature (0.442 ^{o}C) was probably due to the greater fluctuations in temperature as a result of the greater heat output. Mean off-temperature for all configurations was 23.52 ^{o}C, which was the ambient temperature of the environmental chamber where the tests were performed.

The maximum and minimum operating temperatures during the recorded 24 hour periods (Table 12) follow patterns similar to those evident in the mean lights on/off temperatures (Table 11). However, maximum and minimum values show more pronounced differences between the various configurations.

The difference between the maximum and minimum temperatures recorded for the four G.E. 313 bulbs was 3.6 ^{o}C. On the other hand, the two LED arrays produced a difference of 0.3 ^{o}C. It is noteworthy that the temperature change for the two G.E. 313 bulbs was 2.1 ^{o}C, while the temperature change for the four G.E. 1819 bulbs was 1.7 ^{o}C. In other **words, the two G.E. 313 bulbs produced a heat load 23.5% higher (Table 12), but light intensity 3 times greater than four G.E. 1819 bulbs (Table 10).**

D. Spectral Power Characteristics Of The Various Light Sources Tested.

1. Spectral power distribution of individual LEDs, LED array (set G) and G.E. Bulb 1819.

The corrected spectral power distribution curves obtained for the LEDs were similar to those published by the manufacturer (see Figures 5a-c). The green LED peak intensity was approximately 565 nm with a range of 515-655 nm (Figure 5a). The yellow LED peaked at approximately 580 nm with a range of 525-675 nm (Figure 5b). The red LED peaked at approximately 650 nm, and ranged from 575 to an estimated value of 725 nm on the high end (we note that our equipment was limited in the upper range thus requiring this high end estimation) (Figure 5c).

The LED array (set G, Figure 6) produced a spectral distribution with low power in the blue/ultra-violet range. However, this may be compensated for by using blue LEDs and/or filters to obtain a spectral power distribution more closely approximating sunlight than the spectral power

distribution of the incandescent bulbs currently being used (Figure 7).

2. "Full spectrum" red/green/blue LEDs now commercially available.

We have recently learned that a company in southern California has begun manufacturing a blue, and a full spectrum (red/green/blue) LED. These LEDs, particularly the full spectrum LED, may be excellent candidates for future AEM applications if their intensity characteristics are adequate. We recommend that they be investigated further. The specification sheets for these items are included as Appendix A. Order information is available through Ledtronics Co., 4009 Pacific Coast Highway, Torrance, CA 90505, (213) 549-9995.

IV. SUMMARY AND CONCLUSIONS

In comparing the G.E. 313 light bulb, currently in the AEM, to the G.E. 1819 light bulb, a proposed replacement, several facts are evident.

First, although the G.E. 313 light bulb consumes more power, it is more efficient than the G.E. 1819 light bulb, producing 2.33 times as much light per Watt consumed (Table 9). Second, configurations using the G.E. 1819 either did not meet the recommended minimum light intensity of 5 lux, or came marginally close (Table 10). Third, though the use of two G.E. 313 bulbs produced a heat load 23.5% higher than four G.E. 1819 bulbs, light intensity was 3 times greater.

For these reasons, we feel that the best lighting configuration using incandescent light bulbs, which maximizes light intensity while minimizing heat load, is the two G.E. 313 light bulb configuration.

However, in comparing this "optimum incandescent bulb configuration" to the two LED array that we developed, the LED array configuration is more desirable for the following reasons.

First, the two LED array configuration consumed only 28.6% of the power that two G.E. 313 bulbs used (2.72 Watts vs. 9.52 Watts). Second, the array was 182.9% as efficient (2.97 candela/Watt) as two G.E. 313 bulbs (1.05 candela/Watts). Third, it produced 37.0% more light (mean = 22.6 lux, S.D.$\pm$0.5 lux) than the two G.E. 313 bulbs (mean = 16.5 lux, S.D.$\pm$.0 lux). Fourth, the two LED array configuration produced only 14.3% of the heat (0.3^{o}C change) produced by two 313 G.E. bulbs (2.1^{o}C change). Finally, though the spectral power distribution of the LED array tested is not ideal due to low energy in the blue/ultra-violet range, this may be corrected by adding blue LEDs, using filters, or using the newly available full spectrum LEDs (see Results and Discussion section D.2. and Appendix A).

Given these encouraging results, we recommend that studies to explore the feasibility of LED use in animal habitats be performed and that their effects on physiological systems be investigated.

The LED arrays had many positive characteristics that would warrant their use in the current AEMs (as a retrofit) or in the phase two AEM under development, including: 1) ample illumination, 2) low heat production, 3) minimal power consumption, and 4) with modifications, the ability to meet spectral power distribution requirements.

Despite the use of the most efficient light sources, AEM temperature is dependent on cabin temperature. If cabin temperature can be lowered, so too will the AEM temperature.

Table 1. Average illuminance of various light bulbs as measured from several distances.

Distance* (cm)	Illuminance (lux)			
	GE 313 (AC)	GE 1820 (AC)	GE 1819 (AC)	GE 757 (AC)
90	3.50	1.97	0.78	0.54
70	4.55	2.68	1.05	0.75
50	6.60	4.56	1.91	1.42
30	23.25	9.83	4.86	3.34
15	91.40	43.5	19.61	11.29
8	337.1	152.8	66.7	57.70

*Measurements were directly above the light bulb end (bulb mounted vertically).

TABLE 2. Average illuminance of various LED arrays as measured from several distances*.

Distance (cm)	Illuminance (lux)			
	Set D	Set E	Set F	Set G
90	2.03	6.23	4.39	8.2
70	2.95	9.37	6.77	12.6
50	5.20	19.47	13.48	23.3
30	13.58	49.4	38.47	58.6
15	39.8	149.1	116.3	191.8
8	94.7	366	198.1	356

* LEDs were arranged in a contiguous, square honeycomb pattern. Illuminance measurements were made directly above the mid-point of the array at various distances.

TABLE 3. Illuminance comparison of GE 313 using 28V AC and 28V DC.

Distance* (cm)	Illuminance (lux)		
	GE 313 28V AC	GE 313 28V DC	Percent Difference
90	3.50	3.44	1.71
70	4.55	4.73	3.95
50	6.60	6.17	6.52
30	23.25	22.43	3.53
15	91.40	100.1	9.52
8	337.1	349.3	3.62

*Measurements were made dircetly above the light bulb end (bulb mounted vertically).

TABLE 4. Average illuminance of various light bulbs at 30 cm in the perpendicular plane at several polar coordinates.

Angle (deg)	Illuminance* (lux)			
	GE 313	GE 1820	GE 1819	GE 757
0	39.95	39.75	5.29	8.25
45	38.25	39.95	5.17	8.96
90	50.30	31.25	6.55	8.09
135	39.85	26.5	5.51	7.85
180	47.65	25.7	5.61	7.65
225	39.20	27.7	4.70	6.38
270	42.25	24.7	4.51	6.55
315	39.80	32.95	5.39	7.36
Mean	42.16	31.06	5.34	7.64
Std.Dev.	4.41	6.09	0.62	0.86

*Illuminance measurements ere obtained along the perpendicular plane (mid bulb) at various polar coordinates (angles).

TABLE 5. Average illuminance of various light bulbs at 60 cm in the perpendicular plane at several polar coordinates.

Angle (deg)	Illuminance* (lux)			
	GE 313	GE 1820	GE 1819	GE 757
0	11.93	9.04	1.550	2.41
45	11.71	10.37	1.612	2.89
90	15.03	10.65	1.898	2.82
135	12.66	8.21	1.742	2.60
180	13.13	8.86	1.499	2.18
225	12.31	10.20	1.458	2.63
270	13.09	7.25	1.533	2.41
315	13.05	10.46	1.705	2.44
Mean	12.86	9.38	1.624	2.55
Std.Dev.	1.03	1.24	0.148	0.23

*Illuminance measurements were obtained along the perpendicular plane (mid-bulb) at various polar coordinates (angles).

TABLE 6. Average Illuminance of various LED arrays* as measured from several polar coordinates in the perpendicular plane at distances of 8, 15, and 30 cm.

LED Array	Illuminance (lux)					
	8 cm		15 cm		30 cm	
	Mean	SD	Mean	SD	Mean	SD
Set D	2.28	0.43	1.067	0.337	0.307	0.028
Set E	5.51	0.42	1.738	0.159	0.648	0.065
Set F	3.83	0.83	0.845	0.063	0.260	0.024
Set G	4.94	0.28	1.83	0.10	0.81	0.08

*LEDs were arranged in a contiguous, square honeycomb pattern. Illuminance measurements obtained along the perpendicular plane (mid-bulb); similar to tables 4 and 5.

TABLE 7. Descriptions of various LED arrays.

LED Array	Total No. of LEDs	Composition			Dimensions (cm)	Size (sq.cm)
		Red	Green	Yellow		
Set D	86	43	43	0	5.34 X 5.34	28.5*
Set E	63	31	32	0	4.34 X 4.34	18.8*
Set F	60	20	20	20	4.25 X 4.25	18.1*
Set G	63	21	21	21	4.34 X 4.34	18.8*
Set H	42	14	14	14	2.50 X 18.0	45.0

* Arrays were square shaped.

TABLE 8. Temperature differences* of several Light bulbs and LED array designs measured after one hour from point of activation.

Bulb Type	No. of Bulbs	Ambient Temp (C)	Box Temp. After 1 Hr. (C)	Temp. Difference (C)
GE 313	1	21.5	23.8	2.3
GE 1820	1	28.4	30.5	2.1
GE 757	1	22.9	21.8	1.1
GE 1819	1	22.4	21.7	0.7
GE 313	2	28.4	34.8	6.4
GE1820	2	28.4	33.0	4.6
GE 757	2	28.3	31.8	3.5
GE 1819	2	28.0	29.4	1.4
GE 313	4	27.7	38.1	10.4
GE 1819	4	27.4	31.7	4.3
GE 313	6	27.8	43.6	15.8
GE 1819	6	27.5	33.6	6.2
Set D (AC)	86 LEDs	28.1	28.3	0.2
Set E (AC)	63 LEDs	28.1	28.9	0.8
Set F (AC)	60 LEDs	28.2	28.6	0.4
Set G (AC)	63 LEDs	28.0	28.6	0.6
Set G (DC)	63 LEDs	27.7	28.1	0.4

*Bulbs were placed in an unfinished wooden box (3/4", pine: internal dimensions 29.2 x 29.2 x 29.2 cm) and temperature difference recorded one hour after bulbs were activated.

TABLE 9. Power consumption and efficiency of GE 313 and GE 1819 light bulbs compared with LED array Set H used in the prototype AEM (Note that one LED array replaces 2 light bulbs).

CONFIGURATION	POWER CONSUMPTION (WATTS)	EFFICIENCY* (CANDELA/WATTS)
2-GE-313	9.52	1.05
2-GE-1819	2.24	0.45
LED ARRAY SET H	1.36	2.97

*Illuminance data (candela) provided by manufacturers. Power (Watts) measured in our application.

TABLE 10. Mean illuminance measured at 27 positions within AEM using various lighting configurations.

CONFIGURATION	MEAN ILLUMINANCE (LUX)	STD. DEV (N=27)
4 GE-313	26.1	14.8
2 GE-313	16.5	12.5
4 GE-1819	5.5	3.1
2 GE-1819	3.3	2.3
2 LED ARRAYS (2 SET H)	22.6	22.0

Table 10a. Illuminance readings made at 27 different positions throughout AEM. Positions 1-9 are on the floor (2.5 cm above floor), 10-18 are at the mid-level (9.0 cm above floor), and 19-27 are at the highest levels in the AEM (18.0 cm above floor). Refer to figure 8 for spatial arrangement of positions in AEM.

Position	2-LED Array	4-GE 313	4-GE 1819	2-GE 313*	2-GE 1819*
1	61.2	20.2	3.4	2.0	0.7
2	18.2	18.7	3.0	3.4	1.0
3	7.4	13.6	2.5	3.6	1.1
4	8.0	10.2	4.4	4.1	1.1
5	7.6	4.8	1.2	1.7	0.4
6	16.4	11.3	1.8	4.3	1.6
7	11.0	12.1	2.2	22.9	1.3
8	48.1	15.7	2.9	12.8	2.7
9	28.6	20.5	4.1	28.4	3.5
10	55.6	53.6	8.6	12.0	3.4
11	19.8	35.9	11.7	14.3	3.0
12	8.7	26.2	10.7	9.4	2.4
13	8.0	9.4	6.3	8.4	1.7
14	2.6	5.4	1.2	1.4	0.5
15	16.6	12.3	3.6	7.0	2.1
16	24.5	25.3	7.3	22.0	10.1
17	61.5	38.1	6.6	25.0	5.4
18	16.1	50.5	5.3	26.6	4.1
19	42.4	58.7	13.8	45.9	7.5
20	6.2	41.3	7.7	44.6	4.3
21	5.4	26.8	4.9	31.1	2.2
22	4.0	10.5	4.3	25.0	1.5
23	0.8	5.9	1.0	1.1	0.5
24	13.4	12.7	3.5	7.9	6.3
25	11.4	32.5	3.8	11.2	3.4
26	52.0	37.4	4.3	11.4	3.2
27	54.5	32.5	5.5	11.9	2.3

* Two light bulb configuration measurements were made with light bulbs "A" (upper left) and "D" (lower right) active.

TABLE 11. Mean AEM lights On/Off operating temperatures during 24 hr period with various lighting configurations.

CONFIGURATION	MEAN-ON TEMP (C)	STD. DEV (N=288)	MEAN-OFF TEMP (C)	STD.DEV. (N=288)	CHANGE (C)
4 GE-313	25.49	0.442	23.70	0.242	1.79
2 GE-313	24.31	0.233	23.40	0.200	0.91
4 GE-1819	23.99	0.253	23.39	0.216	0.60
2 GE-1819	23.79	0.186	23.57	0.135	0.22
2 LED ARRAYS (2 SET H)	23.70	0.263	23.52	0.173	0.18

TABLE 12. Maximum and minimum AEM operating temperatures during a 24 hr period with various lighting configurations.

CONFIGURATION	MAX. TEMP. (C)	MIN. TEMP. (C)	CHANGE (C)
4 GE-313	26.2	22.6	3.6
2 GE-313	25.0	22.9	2.1
4 GE-1819	24.6	22.9	1.7
2 GE-1819	24.3	23.2	1.1
2 LED ARRAYS(2 SET H)	23.6	23.3	0.3

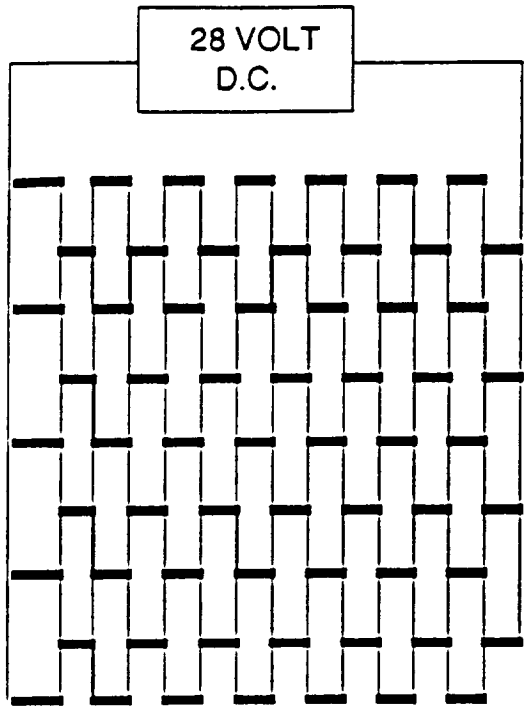


Figure 1. Schematic wiring diagram for LED array Set G. Dark lines represent LEDs, which were arranged in an alternating color pattern (red, green, yellow).

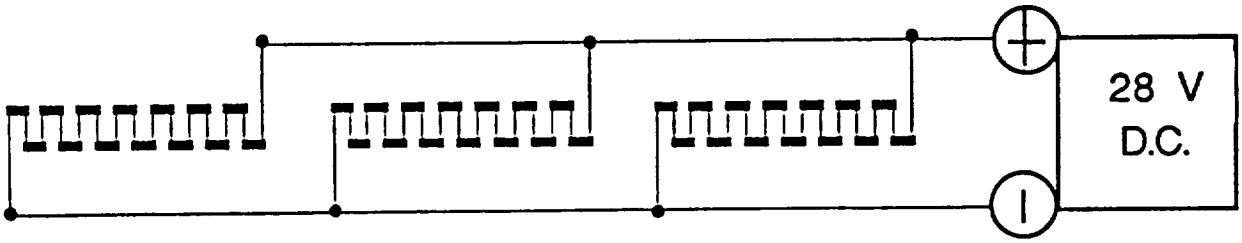


Figure 2. Schematic wiring diagram for LED array Set H (used in prototype AEM tests). Dark lines represent LEDs, which were arranged in an alternating color pattern (red, green, yellow).

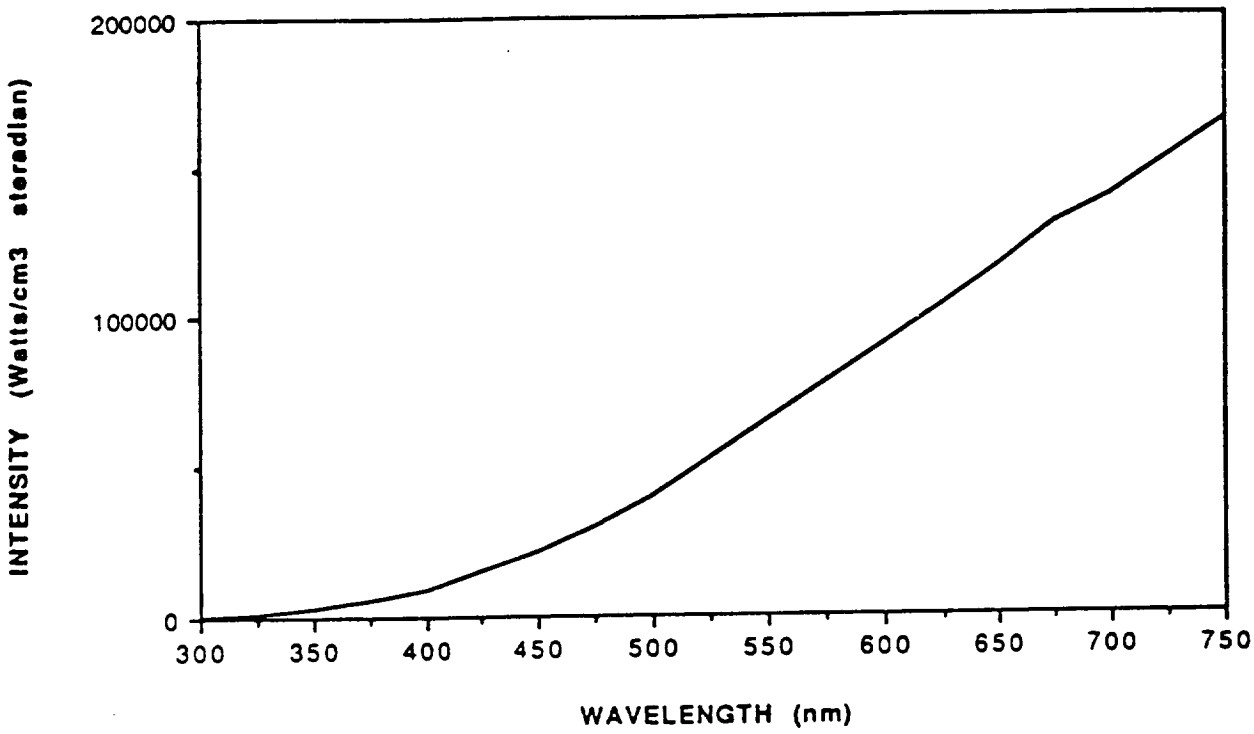


Figure 3. Spectral power distribution of standard lamp No. EPT-1334 operated at 38.0 amperes D.C., as supplied by manufacturer.

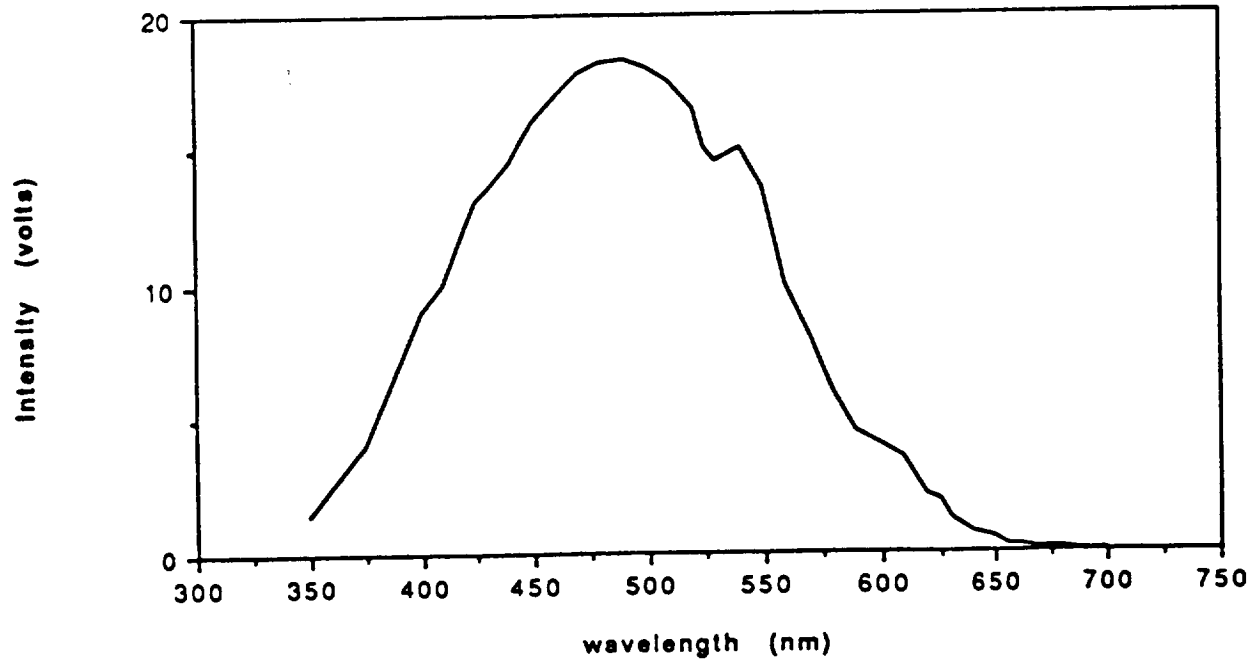


Figure 4. Recorded spectral power distribution of standard lamp No. EPT-1334 operated at 38.0 amperes D.C.

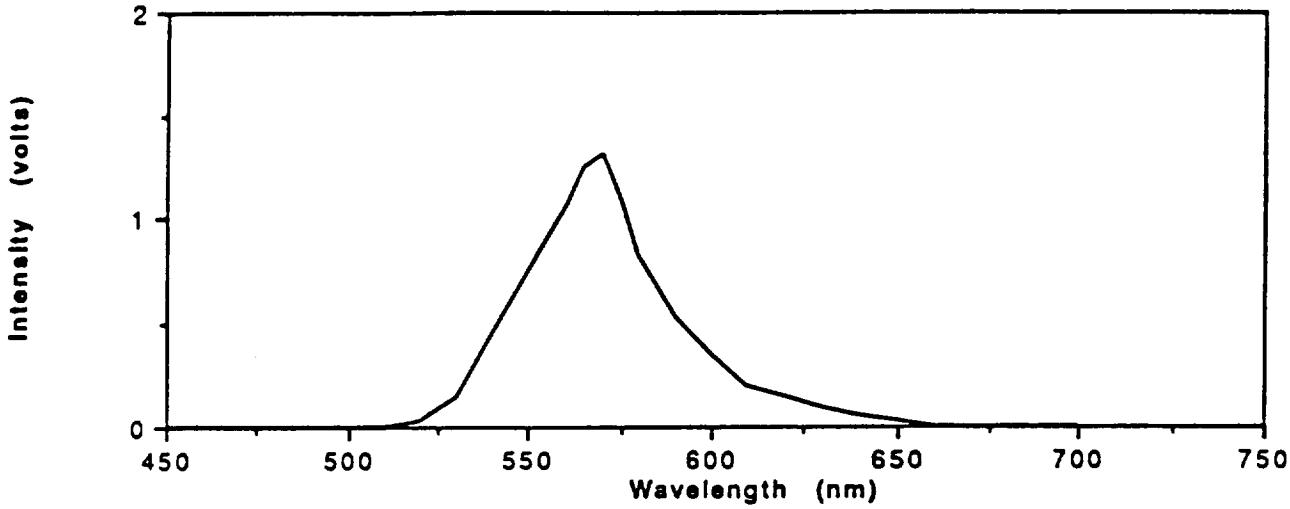


Figure 5a. Recorded spectral power distribution of green LED.

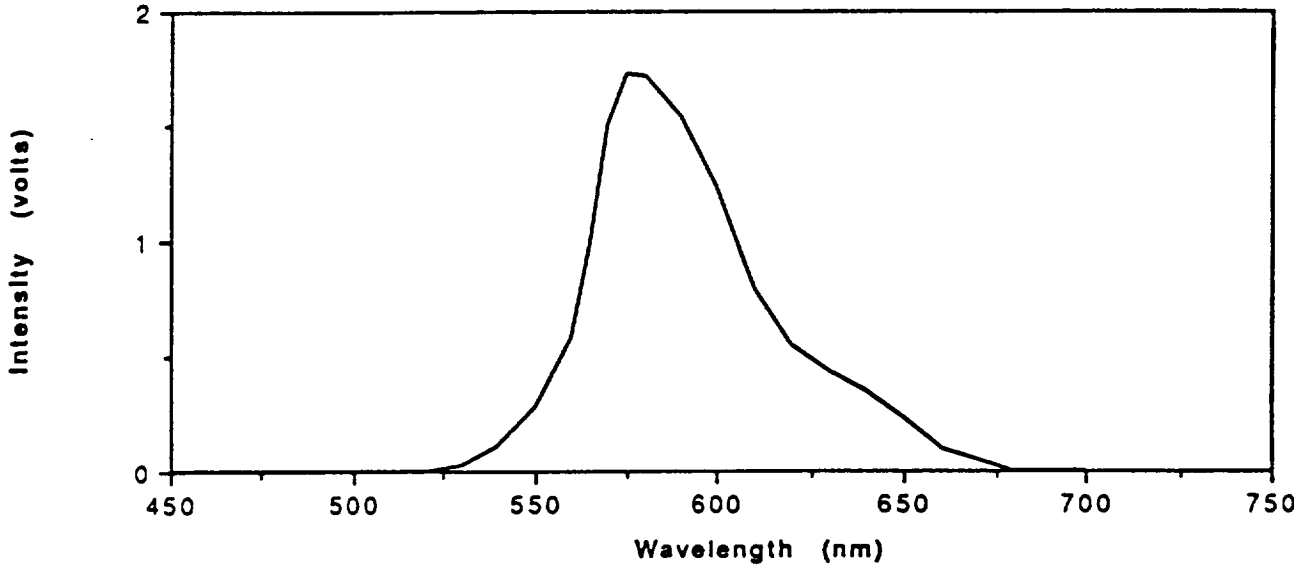


Figure 5b. Recorded spectral power distribution of yellow LED.

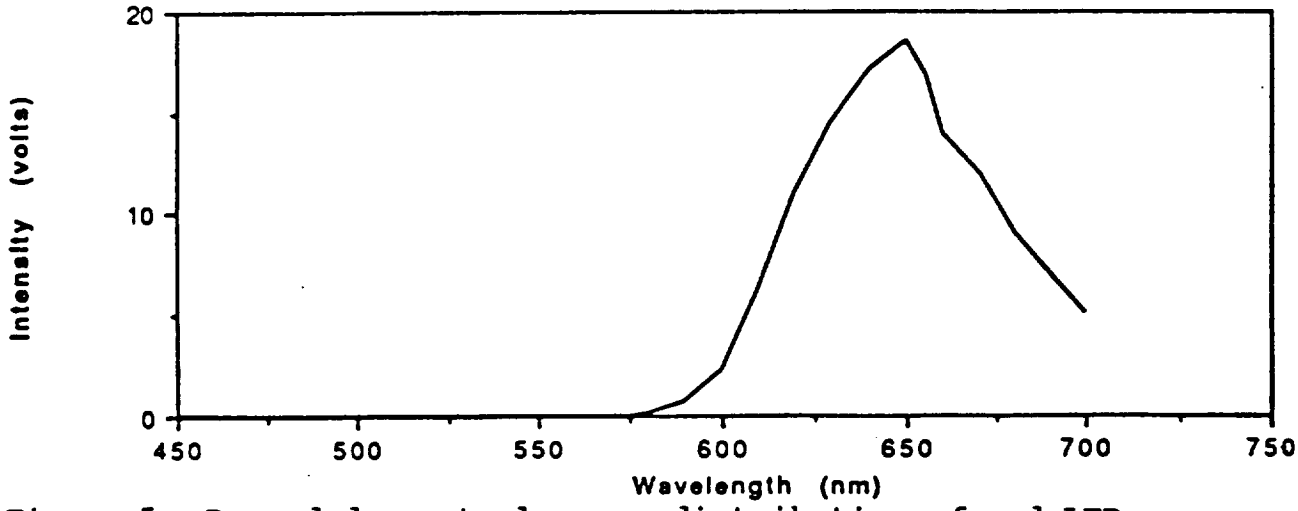


Figure 5c. Recorded spectral power distribution of red LED.

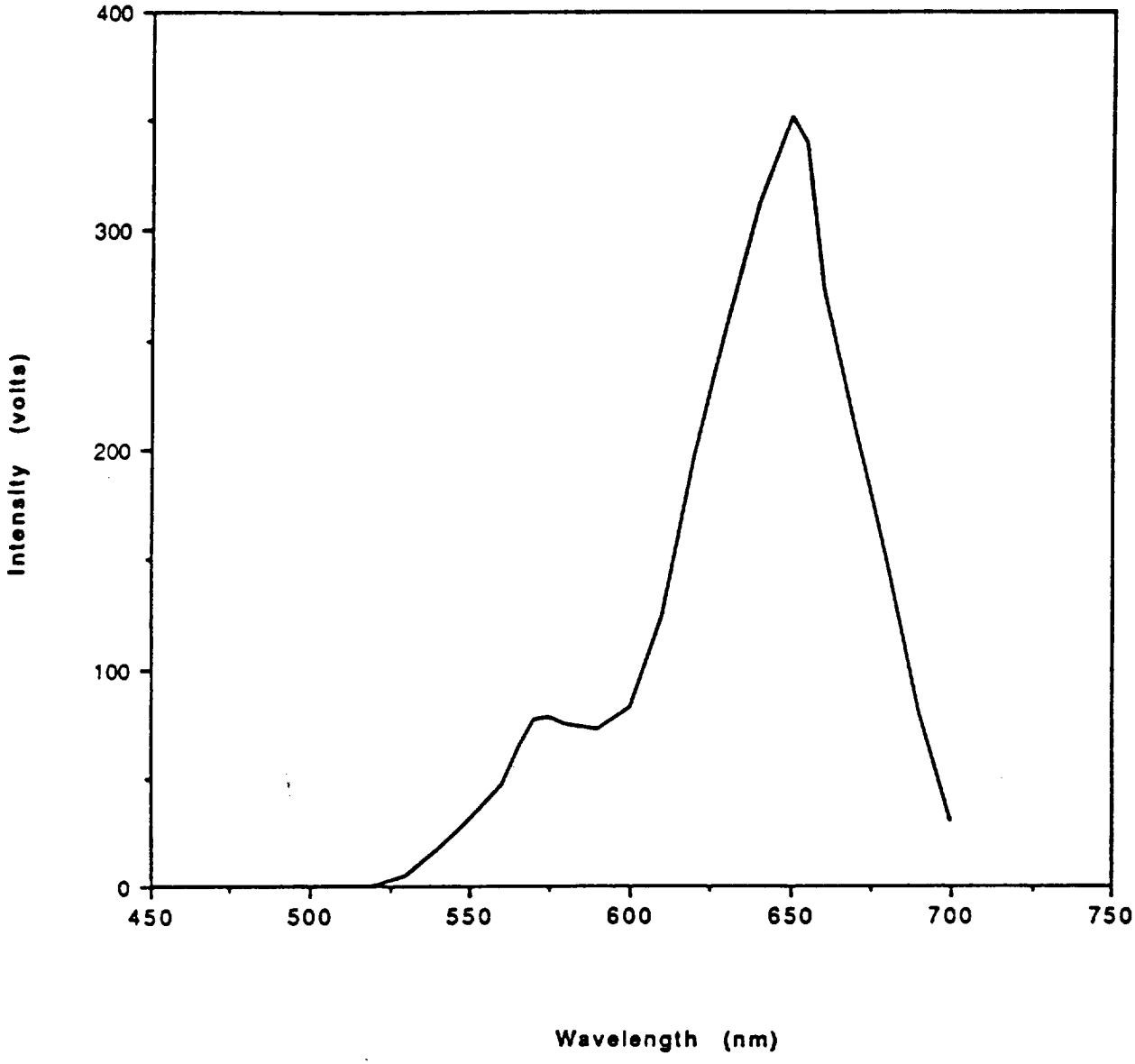


Figure 6. Recorded spectral power distribution of an LED array composed of an equal number of green, yellow, and red LEDs (model numbers HLMP-3950, HLMP-3850, HLMP-3750, respectively).

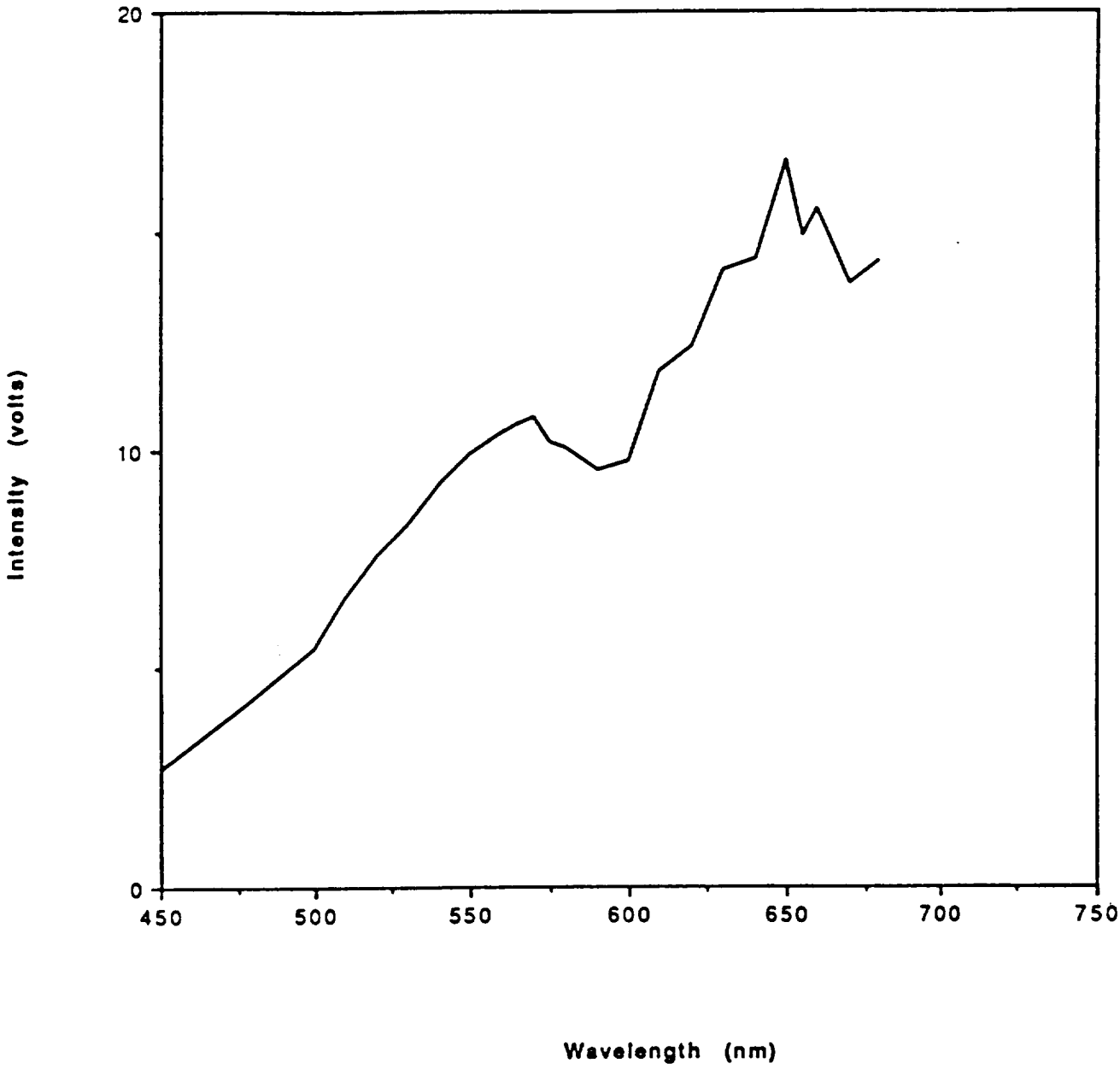


Figure 7. Recorded spectral power distribution of an incandescent light bulb, model number G.E. 1819.

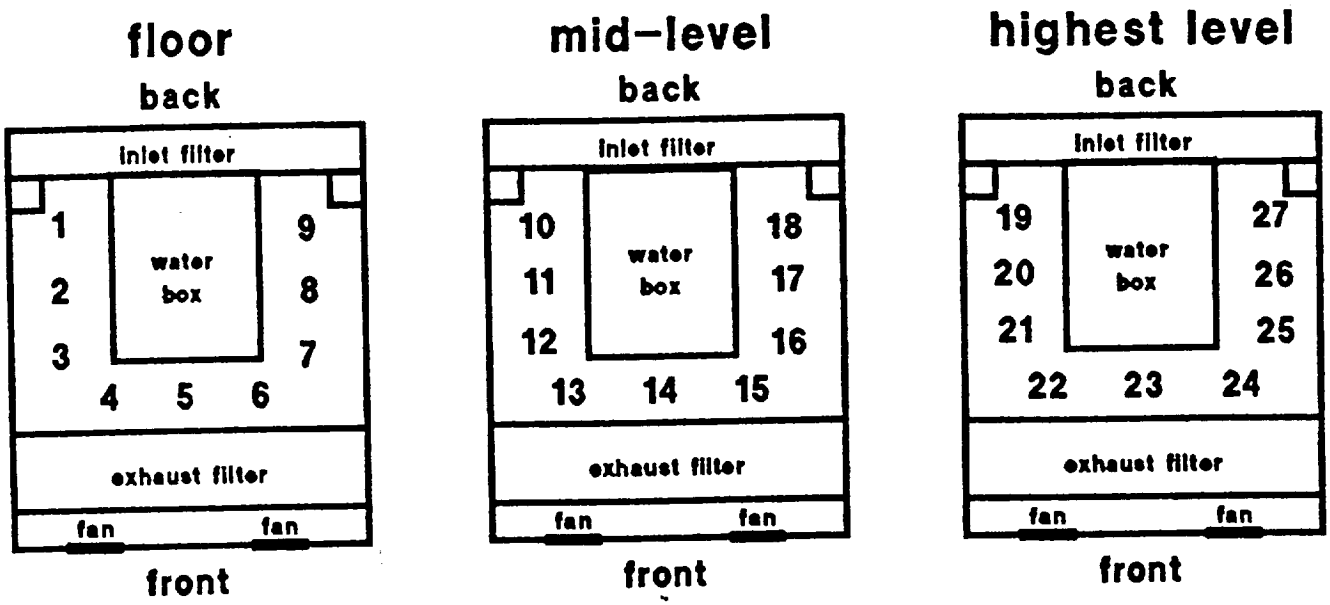


Figure 8. Locations of positions in AEM where illuminance measurements were taken. Radiometer probe was facing the back of the AEM for all readings. Floor measurements were made with probe center 2.5 cm above floor. Mid-level measurements were made with probe center 9.0 cm above floor. Highest level measurements were made 18.0 cm above floor of AEM. Refer to Table 10a for results of these measurements.

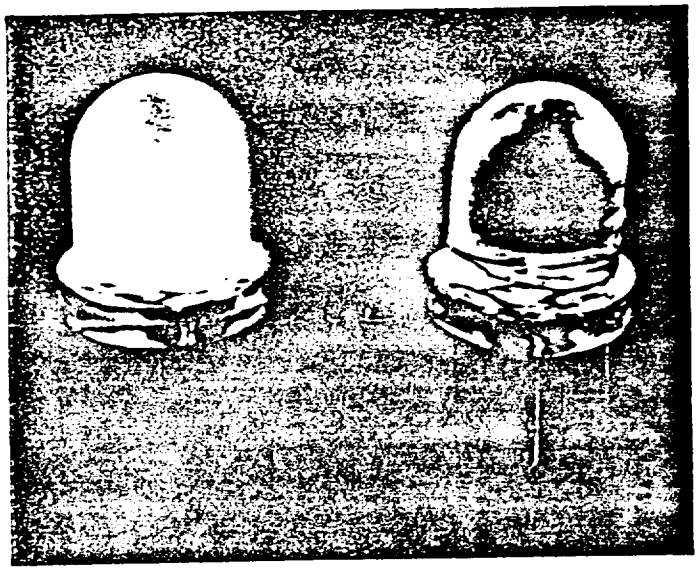
APPENDIX A

Blue and RGB LED Specification Sheets

per

Ledtronics
4009 Pacific Coast Highway
Torrance, CA 90505

LLED™



FULL SPECTRUM RGB LEDs

A RAINBOW OF COLOR

GENERAL INFORMATION

The industry's first **INTEGRATED RED, GREEN, BLUE (RGB) LED** is available for the wide range of applications anticipated since last years introduction of the blue LED. Designated the L300RGB series, this **DISCRETE** is packaged in a clear or diffused 0.300" (8mm) case. Incorporating state of the art chip mounting technology that dissipates heat in closely spaced chip arrays. Within the next six (6) months, this **RGB DISCRETE** will be available in a T1-3/4 (5mm) package. Design analyses for the T1 (3mm) package are progressing. Additionally, total flexibility in chip selection has been retained. Thus, this device can be manufactured with deep red (660nm), pure green (555nm), and 470nm blue chips.

APPLICATIONS

The potential applications for this device are as varied as the colors available from it. First, of course, is the best rendition of a white color available in the LED market. The L300RGB also serves as the fundamental pixel for large area full color screens/monitors/displays. Additionally, the L300RGB can be used in full color moving signs/displays, as a light source for a variety of scanners such as used in color copiers and equipment which senses a paper's color differences to detect counterfeit currency, as a spectral analysis reference or source in color scanning and high speed document reading, and color synthesis for photic stimulation/simulation.

SPECIFICATIONS

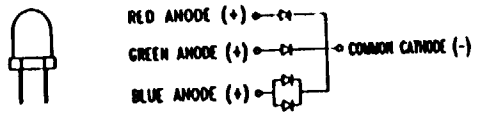
This **DISCRETE** utilizes high efficiency red chips (635nm), high efficiency green chips (565nm), and 470nm blue chips. Direct access to the RGB chips and a common cathode provide a virtual cornucopia of color to the applications engineer. Data sheets for the L300RGB are available describing the brightness of each chip and related electro-optical characteristics. Necessary bias requirements to operate the L300RGB as a 5 VDC discrete to obtain a white color are also provided. Application notes regarding other colors/characteristics are currently in preparation. Gather up the power supply and decade resistance box-develop your rainbow of color.

ORDERING INFORMATION

Prototype quantities are immediately available. The L300TRGB3 is configured in a diffused epoxy case, the L300CRGB3 is configured in a water clear epoxy package.

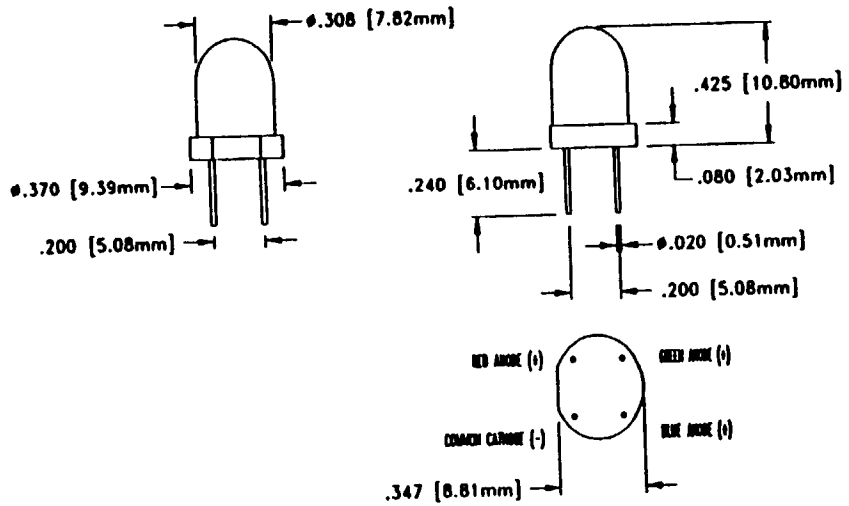
4009 PACIFIC COAST HWY., TORRANCE, CA 90505 PH: (213) 549-9995 • FX: (213) 549-4820

LTR	REVISION	DATE	APPD
–	ORIGINAL ISSUE	8.15.91	R. KUCHTA



LEDTRONICS, INC.
COMPANY PROPRIETARY

Actual Size



NOTES:
1. Ifp .1 msec PULSE 0.1% DUTY CYCLE.
2. TO PRODUCE A WHITE LIGHT, THE FOLLOWING RESISTOR VALUES AND CURRENTS ARE RECOMMENDED FOR 5V APPLICATIONS: RED ANODE: 1200Ω/3mA
   GREEN ANODE: 560Ω/10mA
   BLUE ANODE: 33Ω/60mA
3. SPECIAL CONFIGURATIONS OF THIS PRODUCT CAN BE PRODUCED WITH 660nm RED AND 555nm PURG GREEN LED CHIPS.

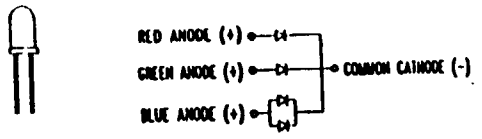
L.E.D. RADIATION COLOR	ABSOLUTE MAXIMUM RATINGS (Ta=25°C) Pd mW	Ifp mA	If mA	Vr V	Topr	DIFFUSED -101 ELECTRO-OPTICAL CHARACTERISTICS (Ta=25°C) If mA	Iv mcd min/typ	Vf/Vf typ/max	2θ½ DEGREES	Ir max µA	λP nm	CLEAR -001 ELECTRO-OPTICAL CHARACTERISTICS (Ta=25°C) If mA	Iv mcd min/typ	Vf/Vf typ/max	2θ½ DEGREES	Ir max µA	λP nm
RED	100	100	30	5	-20°C TO 60°C	20	2/4	2.0/2.8	50	100	635	20	20	2.0/2.8	25	100	635
GREEN	100	100	30	5	-20°C TO 60°C	20	2/4	2.1/2.8	50	100	565	20	20	2.1/2.8	25	100	565
BLUE	200	300	60	5	-20°C TO 60°C	50	1/2	3.0/3.7	50	100	470	50	5	3.0/3.7	25	100	470
WHITE						MIN	1.5/3	5.0/8.0	50			MIN	8	5.0/8.0	25		

Tstg: -35°C TO +100°C

TITLE	DRAWING NO	DR	DATE
DIS-1018-001 (CLEAR) DIS-1018-101 (DIFFUSED)	DIS1018C	ROBERT KUCHTA	8.15.91
	MATERIAL: AS SHOWN	CHKD	REV. –
	FINISH: –	USED ON: –	
LEDTRONICS 4009 Pacific Coast Hwy. Torrance, CA 90505	CODE IDENT NO: 8Z410	SCALE: 2:1	SHEET 1 OF 1

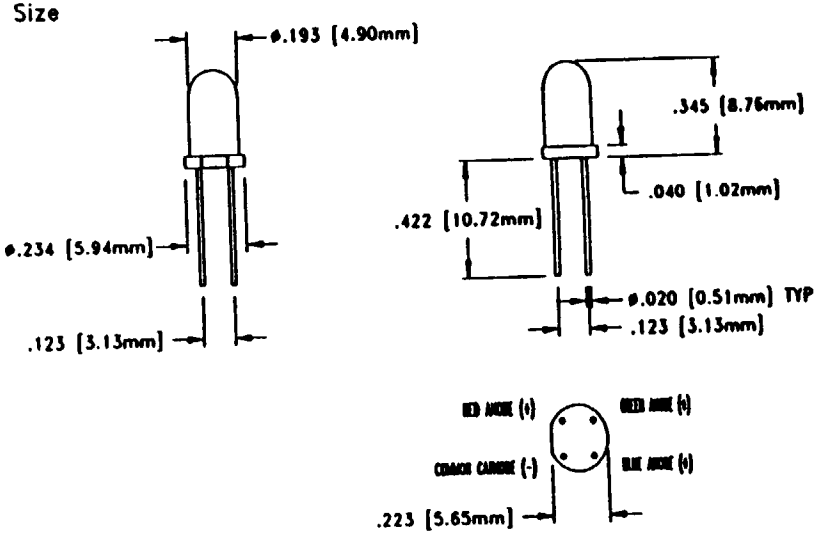
TOLERANCES UNLESS OTHERWISE SPECIFIED
DIMENSIONS IN INCHES PER ANSI-Y14.5
.XXX ±.010
.XX ±.025
ANGLES ± 0°.30'
FRACT. ± 1/32

LTR	REVISION	DATE	APPD
–	ORIGINAL ISSUE	8.14.91	R. KUCHTA



LEDTRONICS, INC.
COMPANY PROPRIETARY

Actual Size



NOTES:
1. Ifp .1 msec PULSE 0.1% DUTY CYCLE.
2. TO PRODUCE A WHITE LIGHT, THE FOLLOWING RESISTOR VALUES AND CURRENTS ARE RECOMMENDED FOR 5V APPLICATIONS: RED ANODE: 1200Ω/3mA
   GREEN ANODE: 560Ω/10mA
   BLUE ANODE: 33Ω/60mA
3. SPECIAL CONFIGURATIONS OF THIS PRODUCT CAN BE PRODUCED WITH 660nm RED AND 555nm PURG GREEN LED CHIPS.

L.E.D. RADIATION COLOR	ABSOLUTE MAXIMUM RATINGS (Ta=25°C) Pd mW	Ifp mA	If mA	Vr V	Topr	DIFFUSED -101 ELECTRO-OPTICAL CHARACTERISTICS (Ta=25°C) If mA	Iv mcd typ	Vf/Vf typ/max	2θ½ DEGREES	Ir max µA	λP nm	CLEAR -001 ELECTRO-OPTICAL CHARACTERISTICS (Ta=25°C) If mA	Iv mcd min/typ	Vf/Vf typ/max	2θ½ DEGREES	Ir max µA	λP nm
RED	100	100	30	5	-20°C TO 60°C	20	8	2.0/2.8	12	100	635	20	40	2.0/2.8	25	100	635
GREEN	100	100	30	5	-20°C TO 60°C	20	8	2.1/2.8	12	100	565	20	40	2.1/2.8	25	100	565
BLUE	200	300	60	5	-20°C TO 60°C	20	4	3.0/3.7	12	100	470	20	10	3.0/3.7	25	100	470
WHITE						MIN	6	5.0/8.0	12			MIN	16	5.0/8.0	25		

Tstg: -35°C TO +100°C

TITLE	DRAWING NO	DR	DATE
DIS-1024-001 (CLEAR) DIS-1024-101 (DIFFUSED)	DIS1024C	ROBERT KUCHTA	8.14.91
	MATERIAL: AS SHOWN	CHKD	REV. –
	FINISH: –	USED ON: –	
LEDTRONICS 4009 Pacific Coast Hwy. Torrance, CA 90505	CODE IDENT NO: 8Z410	SCALE: 2:1	SHEET 1 OF 1

TOLERANCES UNLESS OTHERWISE SPECIFIED
DIMENSIONS IN INCHES PER ANSI-Y14.5
.XXX ±.010
.XX ±.025
ANGLES ± 0°.30'
FRACT. ± 1/32

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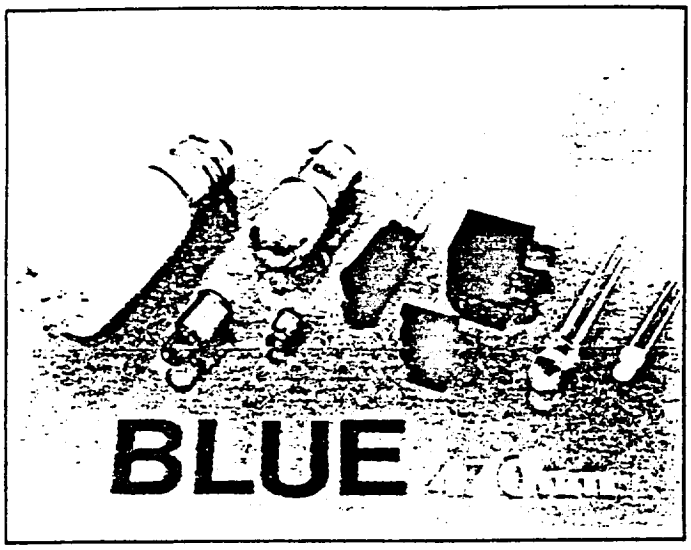
LLED™



INDUSTRY'S BRIGHTEST

Immediately Available

These ***BLUE*** (470nm) ***LEDs*** are available in standard T1 (3mm) and T1-¾ (5mm) Discrete packages. The T1-¾ Discretes are available in single and 6-chip configurations to accommodate most applications. The T1 package is configured as a single or dual chip device. Both Discretes can be obtained in a clear, focused beam, or diffused, wide angle, epoxy case. The 6-chip Discrete provides an extra wide angle output.

The ***BLUE LEDs*** are being used in the medical field (e.g., blood gas analysis) military avionics (MIL-L-85762A), underwater detection, digital color printing and reproduction, spectrometry, displays, and as medium priced indicators.

Chip material:	Silicon Carbide
Peak Wavelength:	470nm.
Forward voltage:	3.0 vdc, typical; 3.7 vdc max
Forward current:	30 to 50 mA for max brightness
Brightness Levels:	4 to 20 mcd. Pulsed modes can be used to achieve higher light output levels

T1 DISCRETES:

L120CWB2/L120TWB2-3V/30	(4mcd, water clear case/ diffused case, single chip– L122 for dual chip)
L120CWB4/L120TWB4-3V/30	(8mcd, water clear case/diffused case) single chip–L122 for dual chip

T1-¾ DISCRETES:

L200CWB3/L200TWB3-3V/50	(7mcd, water clear/diffused case, single chip)
L200CWB4/L200TWB4-3V/50	(11mcd, water clear/diffused case, single chip)
L200CWB5/L200TWB5-3V/50	(20mcd, water clear/diffused case, single chip)
L206CWB3-18V/15	(7mcd, water clear case, 6-chip)

The ***BLUE LED*** Discretes are also available in narrow beam (6 degree viewing angle), Rectangular (1.7x4mm, 2x5mm, 1x5mm, 2.5x7mm) and Square (5x5mm) configurations. All Discretes can be configured as standard socket mount Based LEDs for direct incandescent lamp replacement, PC Board vertical and right angle indicators, 7 segment displays, Lightbars, etc. . . .

4009 PACIFIC COAST HWY., TORRANCE, CA 90505 PH: (213) 549-9995 • FX: (213) 549-4820

DISCRETE BLUE LEDs

T1 (3mm), T1 3/4 (5mm), RECTANGULARS, SQUARES

LEDTRONICS PART NO.	LENS COLOR	LED COLOR	ABSOLUTE MAX. RATINGS Ta=25 °C				ELECTRO - OPTICAL CHARACT. If=50mA Ta=25°C			FIG
			Pd mW	Ifp mA	If mA	Vr V	IvTyp mcd	Vf Typ/Max	View Angle	
T1 (3mm) DISCRETE BLUE LEDs, DIFF. & CLEAR										
L120CWB2-3V/30	CLEAR	BLUE	100	180	30	5	4	2.8 / 3.7	12	1
L120CWB4-3V/30	CLEAR	BLUE	100	180	30	5	8	2.8 / 3.7	12	1
L120TWB2-3V/30	WHITE	BLUE	100	180	30	5	4	2.8 / 3.7	30	1
L120TWB4-3V-30	WHITE	BLUE	100	180	30	5	8	2.8 / 3.7	30	1
T1-3/4 (5mm) DISCRETE BLUE LEDs, DIFF. & CLEAR										
L200CWB3-3V/50	CLEAR	BLUE	200	300	60	5	7	2.9 / 3.7	12	2
L200CWB4-3V/50	CLEAR	BLUE	200	300	60	5	11	2.9 / 3.7	12	2
L200CWB5-3V/50	CLEAR	BLUE	200	300	60	5	20	2.9 / 3.7	12	2
L200CWB4-3V/50-NB	CLEAR	BLUE	200	300	60	5	11	2.9 / 3.7	6	3
L200CWB5-3V/50-NB	CLEAR	BLUE	200	300	60	5	20	2.9 / 3.7	6	3
L200TWB4-3V/50	WHITE	BLUE	200	300	60	5	11	2.9 / 3.7	30	2
L200TWB5-3V/50	WHITE	BLUE	200	300	60	5	20	2.9 / 3.7	30	2
1.7 x 4mm RECTANGULAR BLUE LEDs, DIFF.										
RL070TB4-3V/50	DIFF.	BLUE	100	180	30	5	2	2.8 / 3.7	130	4
2 x 5mm RECTANGULAR BLUE LEDs, DIFF.										
RL280TB4-3V/50	DIFF.	BLUE	200	300	60	5	2	2.9 /3.7	140	5
1 x 5mm RECTANGULAR BLUE LEDs, DIFF.										
RL240TB4-3V/50	DIFF.	BLUE	200	300	69	5	2	2.9 / 3.7	120	6
2.5 x 7mm RECTANGULAR LEDs, DIFF.										
RL310TB4-3V/50	DIFF.	BLUE	200	300	60	5	2	2.9 / 3.7	130	7
5 x 5mm RECTANGULARLEDs, DIFF.										
SL190TB4-3V/50	DIFF.	BLUE	200	300	60	5	2	2.9 / 3.7	150	8
T 3/4 SUBMINIATURE AXIAL LEAD LEDs										
L080CWB4-3V/50	CLEAR	BLUE	100	180	40	5	8	2.8 / 3.7	30	

FOR MORE INFORMATION - PLEASE CALL FACTORY

DESCRIPTION

FIG. 1

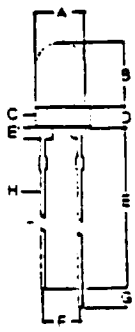


FIG. 2

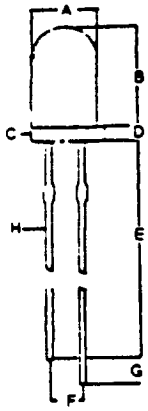


FIG. 3

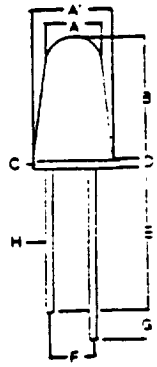


FIG. 4

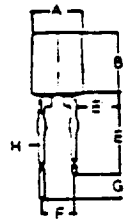


FIG. 5

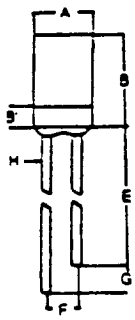


FIG. 6

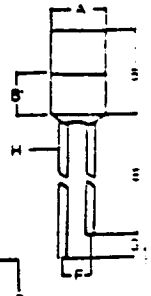


FIG. 7

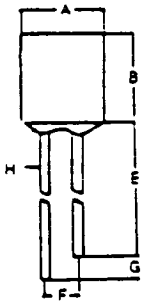
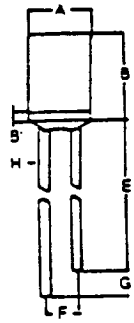


FIG. 8



MECHANICAL SPECIFICATIONS

FIG	A	A'	B	B'	C DIA	D	E	E'	F CTR	G TYP	H	I MAX
1	.115 (2.92)	---	.220 (5.59)	---	.150 (3.81)	.015 (.37)	.690 (17.53)	.05 (1.27)	.100 (2.56)	.075 (1.91)	.020 (.5)	.030 (.76)
2	.190 (5.00)	---	.350 (8.91)	---	.231 (5.85)	.04 (.94)	1.000 (25.4)	---	.100 (2.56)	.050 (1.27)	.020 (.5)	.030 (.76)
3	.160 (4.10)	.195 (4.95)	.358 (9.10)	---	.231 (5.85)	.04 (.94)	1.000 (25.4)	---	.100 (2.56)	.100 (2.56)	.020 (.5)	---
4	.154 x .070 (3.93 x 1.89)	.154 x .070 (3.92 x 1.79)	.205 (5.22)	---	---	---	.945 (24.)	.031 (.76)	.100 (2.56)	.079 (2.01)	.018 (.05)	.028 (.72)
5	.196 x .080 (5.00 x 2.03)	.196 x .080 (5.0 x 2.80)	.295 (7.50)	---	---	---	1.040 (26.43)	---	.100 (2.56)	.040 (1.03)	.002 (.05)	---
6	.109 x .040 (2.77 x 1.02)	.200 x .110 (5.08 x 2.80)	.315 (8.00)	.138 (3.51)	---	---	1.040 (26.43)	---	.100 (2.56)	.040 (1.03)	.020 (.5)	---
7	.300 x .094 (7.62 x 2.39)	.300 x .096 (7.62 x 2.44)	.300 (7.62)	---	---	---	1.040 (26.43)	---	.100 (2.56)	.040 (1.03)	.002 (.05)	---
8	.197 x .197 (5.0 x 5.0)	.197 x .240 (5.0 x 6.10)	.276 (7.0)	.03 (.7)	---	---	1.040 (26.43)	---	.100 (2.56)	.040 (1.03)	.020 (.51)	---

NOTES:

1. "A" DIMENSION IS O.D. IN FIGS. 1,2,3
2. "A" DIMENSION IS LENGTH & WIDTH OF THE LED ILLUMINATION AREA IN FIGS. 4,5,6,7,8
3. A' IS THE MAX LENGTH & WIDTH OF THE LED PACKAGE IN FIGS. 4,5,6,7,8
4. PROTRUDED RESIN UNDER FLANGE IS 0.059" (1.5) MAX.
5. ALL DIMENSIONS ARE IN INCHES / (mm).
6. Ir MAX = 100 µA FOR ALL DEVICES.
7. LONG LEAD ANODE, SHORT LEAD CATHODE.

Final Report for NASA COOPERATIVE AGREEMENT #NCC2-593
June 3, 1994
San Jose State University

E) NASA AEM Filter Test 92/93 (Rats)

Final Subproject Report for Cooperative Agreement #NCC2-593

NASA AEM FILTER TEST 92/93 (Rats)

FINAL REPORT of subproject for COOPERATIVE AGREEMENT #NCC2-593.

July 1, 1993

Daniel C. Holley, Ph.D. and Gary D. Mele, M.A. (cand.)

Department of Biological Sciences
San Jose State University
1 Washington Square
San Jose, CA 95192-0100

NASA AEM Filter Test 92/93
Final Subproject Report 07/01/93
(Cooperative Agreement NCC2-593)
San Jose State University

MATERIALS AND METHODS

Odor Panel Selection

Sensory evaluation methods used throughout this project were done in accordance with the Manual on Sensory Testing (American Society for Testing and Materials, 1968). The initial odor screening test was held on Friday, August 11, 1992 from approximately 1515h to 1630h. In this period six testers presented 10 odor samples to 29 subjects. Testers presented samples to the subjects in a random order and paused a minimum of 30 seconds between samples. Odor samples, 10±4 ml, were presented in ten 20 ml screw capped liquid scintillation vials. Seven of the vials held the primary standards defined in Table 1 using de-ionized water for dilution (NASA, NHB 8060.1C). The remaining three vials held de-ionized water only. After the initial odor screening date, 67 additional subjects were tested using the same procedure. Overall, 24 of 96 subjects correctly distinguished 3 odorless samples from the 7 primary standards. This group became the primary tester pool (P prefix). The secondary subject pool (S prefix) contained 12 subjects who either rated the mint standard as having no odor or rated one water sample as having a slight odor (score of 1). Thirty other subjects not meeting the criteria for either primary or secondary pools were designated the tertiary subject pool (T prefix).

Odor panel membership varied over the course of each filter test (see Tables 2 and 3). Each participating panel member performed one set of odor evaluations per test day. Overall, primary pool testers performed 190 sets of odor evaluations. Secondary pool subjects performed 77 sets of odor evaluations. Tertiary pool members performed 5 sets of odor evaluations. Subject age and sex are listed in Table 4.

Panel Odor Evaluation Protocol

Odor panel evaluations of outlet air samples and standard odors occurred at 0700h - 0745h on days 1, 3, 6, 9, 12, and 14 for odor test #1, and on days 1, 3, 7, 14, 21, and 24 for odor tests #3 and #4. SJSU personnel performed odor evaluations during filter test #2, using the standard odor evaluation forms. Panel members evaluated three or four standard solutions in 20 ml scintillation vials (one blank, two primary standards, and occasionally a positive control containing either rat urine and feces or food bar pieces) and six cage air samples on each test day. Panel members also evaluated odors near the top, middle, and bottom of cages 1, 3, and 4 on day 21 of filter test #3 and of cage 4 on day 21 of filter test #4. Standard solutions were diluted with Millipore brand ultra pure water (resistivity > 10 megohm-cm). A physician examined all panel members both before and after each set of odor evaluations. Black plastic covered the test enclosures and hid their contents during each panel evaluation. Water reservoirs were filled to capacity prior to each panel evaluation. Panel members wore a plastic glove on the hand that handled the cork that sealed the air sample hole (see fig. 1).

The odor panel rated all odors by assigning a score from 0 to 4 to all air and liquid samples. The scores are defined in NASA NHB 8060.1C as; 0 = no odor detectable, 1 = odor barely detectable, 2 = odor easily detectable, 3 = odor objectionable (disagreeable), and 4 = odor revolting (extremely offensive).

Hardware Setup, Filters Tested, and Daily Measures

Rats were housed in Animal Enclosure Module odor test fixtures which were supplied by NASA. From air inlet to air outlet the test fixture was composed of: inlet filter, top frame, lexan enclosure, bottom frame, outlet filter, plenum spacer, fan mounting plate, outlet plenum, and adjustable support (Fig. 1). A wire mesh AEM cage (24x36x22 cm), minus the water box and supported in the lexan enclosure, contained the rats and their food. Two 250ml graduated reservoirs supplied water to two lixit valves mounted through the side of the lexan enclosure. Stainless steel hose clamps secured the lexan enclosure between top and bottom frames. Outlet filter, plenum spacer, fan mounting plate, and outlet plenum were compressed between the bottom frame and two adjustable supports. A short length of flexible plastic air duct connected the outlet plenum to a 3 inch i.d. ABS (plastic) coupler which fit inside a 4 inch i.d. by 48 inch long PVC (plastic) pipe. A brass fitting in the PVC pipe located 24 inches from the ABS coupler allowed air speed measurements to be made, using a hot wire air flow meter (see below). A 4 inch o.d. round to flat adapter connected the PVC pipe to the blower assembly air inlet. Another 4 inch round to flat adapter connected the blower assembly air outlet to a 4 inch o.d. ABS pipe followed by a 4 inch to 3 inch ABS adapter. A 15 foot length of flexible plastic air duct connected to the ABS reducer carried the waste air to a ceiling exhaust hood and out of the room. The 4 inch to 3 inch diameter reduction plus flow restriction due to the flexible plastic air duct produced enough back pressure to allow an air sample to be diverted from the main flow through a 1/2 inch hole in the 4 inch ABS pipe. *This provided the air stream used for all filtered cage air odor evaluations.* Between odor evaluations the hole was covered with black electrical tape in filters tests #1 and #2 and by a cork stopper in filter tests #3 and #4. The blower assembly consisted of a single inlet blower (EBM Industries, Inc, part number G2E108-AA05-44), 4 mfd motor capacitor (EBM Industries, Inc, part number 2161-4-7320), fan speed control (Power Controls Corp. #FS-301), and mounting flange (provided by NASA). After filter test #3, brass fittings were installed through one wall of the lexan enclosure and the outlet plenum of cage #5. Plastic tubing connected these to a low pressure differential air gauge (0-2 in. H_2O, Dwyer, Magnehelic® #2002). On day 12 of filter test #4, an AC current meter was installed in the wiring of cage #2 in order to measure the motor current.

Outlet filters were supplied by NASA. Filter test #1 used ALFCO #1 filters. Test #2 used ALFCO #2 filters: 51.1 gram D-Mark 300 for cage 3 and 54.7 gram Zeolite for cage 4. Test #3 used ALFCO #3 filters. Test #4 used APM/Pall #1 filters. Test #1, #2 and #3 outlet filters were weighed before being mounted in the odor test fixture. NASA

personnel weighed the test #4 outlet filters prior to installation. Test #1 and #2 outlet filters were weighed after their removal at the end of the experiment. NASA personnel removed the outlet filters at the conclusion of tests #3 and #4. During test #1 NASA personnel installed a supplementary filter, 4 layers of D-Mark Carbon 110, into the plenum spacer of test fixture #4 on day 9 of the test.

Daily measurements were made during all experiments of water consumption, air speed in the 4 foot PVC pipe, minimum, maximum, and current room temperature, and room humidity. Each day the number of food bars remaining in each cage was estimated. Motor #2 AC current and air pressure across outlet filter #5 were measured during filter test #4. Air speed in the 4 foot PVC pipe was measured with a TSI Inc. VelociCalc model 8350 hot wire air flow meter. Since the measured air speed fluctuated continuously, minimum and maximum values over a 2 minute sample period were recorded during test #1 The minimum and maximum values were then used to calculate the mean air speed. In subsequent tests, 20 values read from the air flow meter at two second intervals were used to calculate the mean air speed. Mean air speed multiplied by cross section area of the 4 inch i.d. pipe was used to estimate air flow volume. Air flow volume in all cages was set to 15±0.5 cfm at the start of each experiment.

Fluorescent light levels measured outside the cages at the brightest position adjacent to each cage face were 330-470 lux. Photoperiod was 12 hours lights on (0700), 12 hours lights off.

Food, Animals, Blank (control) Cage, and Dead Animal Test

Rats were placed into their cages 1 hour prior to the odor panel evaluation (day 1) for filter test #1; 9 hours and 15 minutes prior for test #2; 8 hours prior for test #3; and 9 hours prior for test #4.

Five NASA food bars were glued to each food bar plate using a thin layer of Hysol® EPK® Epoxi-Patch (Dexter Corporation, Hysol Division). In setting up for test #1, many bars became detached from the plates when they were inserted into the rat cages three hours later. Food bars were then re-glued using a thick layer of Epoxi-Patch and allowed to cure. After seven hours, two food bar plates were successfully inserted into each of 5 AEM cages. During subsequent tests, food bar plates were inserted into the AEM cages at least 7 hours after they were glued.

For filter tests #1, #3, and #4, a total of thirty male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) were placed in five of the six AEM cages (6 per cage). Filter test #2 used 8 rats from filter test #1 divided between two cages. In filter tests #1, #3, and #4, one control cage (cage 1) contained neither food bars nor rats. Rats were weighed before each test, when new food bar plates were inserted, and at the conclusion of each test. Test #1 rats were removed from cage 6 after 18 days, and from cages 2, 3, and 5 after 19 days when the food supply was exhausted. Extra food bars were dropped into cage 4 on days 20 and 23. Rats were removed from cage 4 on

day 25. All test #2 rats were removed on day 10. Test #3 rats were removed from cage 3, killed and returned to cage 3 on day 15. The fan motor in cage 3 was turned off on day 15 then on on day 20. We removed the rats from cage 3 on day 22 when their smell within the room became too strong to continue odor testing. Test #3 rats were removed from cages 2, 4, 5, and 6 on day 30. Test #4 rats were removed from cage 4 killed and returned to cage 4 on day 15. The fan motor in cage 4 was turned off on day 15 then on on day 20. We removed the rats from cage 4 on day 21 when their smell within the room became too strong to continue odor testing. Test #4 rats were removed from cages 2, 3, 5, and 6 on day 30.

Statistical Analysis

All cage odor evaluation scores were divided into two score classes, 0-1 and 2-4. Classed odor score frequencies were determined by counting the number of scores in each score class for each test day. A 2 by 2 test of independence using the G test (Sokal and Rohlf, 1987) compared the frequencies of classed odor scores of cage 1, the blank, to the frequencies of classed odor scores of each other cage for each test day. Results for 5 independence tests from one test day comparing 5 test cages to 1 control cage were considered significant for $P < .01$ for each test. This represents a test day error rate of .05. A significant result meant that the number of low and high odor scores differed between the test cage and the control (empty) cage.

RESULTS

Panel Odor Evaluations

Table 2 lists the number of odor evaluation sets for each tester and filter test. Table 3 lists the number of testers completing 1 to 6 sets of odor evaluations for each filter test. Table 4 shows tester sex and age. Tables 5 through 14 and Figures 2 through 7 show the panel odor evaluation results. Tables 5, 6, and 7 contain odor scores grouped by cage number for all panel evaluations for filter tests #1, #3, and #4 respectively. Tables 8, 9, and 10 contain odor scores grouped by test day number for all panel evaluations for filter tests #1, #3, and #4 respectively. Tables 11, 13, and 14 show odor score frequencies for each day of panel evaluation for each cage. Table 12 shows odor evaluations for test #2. Tables 11, 13, and 14 also give the test odor identities for each panel odor evaluation day. Figures 2, 3, and 4 contain a graphical representation of the score frequency data. The graph bars show the frequency of each odor score (0 - 4) for each day of odor evaluation. Different patterns represent different scores. Graph bars for all scores for each day are stacked to give cumulative odor score frequencies. Tables 31 through 33 and Figures 5, 6, and 7 show the mean (±S.D.) score of all cages containing live rats for each panel evaluation day.

Tables 15 through 17 show classed odor score frequencies. Tables 18 through 20 contain the results of the 2 by 2 independence tests. Filter test #1 cage 1 (empty) scores differ from test cage scores for 23 of 28 cage tests. Filter test #3 cage 1 (empty) scores do not differ from test cage scores for any cage on any test day. Filter test #4 cage 1 (empty) scores differ from test cage scores for 4 of 15 tests for the first three test days. Over the last 3 test days, cage 1 (empty) scores differ from test cage scores for 10 of 15 cage days.

Rat and Filter Weight Data, and Miscellaneous Observations

Table 21 shows mean rat weights and weight changes for each cage for all tests. The mean initial weight of all rats was 232±6 grams in test #1, 396±11 grams in test #2, 266±8 grams in test #3, and 202±10 grams in test #4. The mean final rat weights were 328±19 grams in test #1, 411±12 grams in test #2, 320±18 grams in test #3, and 287±10 grams in test #4.

Table 22 contains outlet filter weight data.

After filter test #1, a small quantity of a yellowish viscous liquid was found on the fan mounting plates of cages 2 and 3. It appeared to have leaked from the outlet filters of those cages.

Rats in all filter tests appeared to be healthy except for two instances. A dead rat was removed from cage 2 on day 26 of filter test #3. Inspection of the water consumption data suggests that the rat had been dead for 3 to 4 days before it was noticed and removed. The rat appeared to have died while sleeping but the cause of death is unknown. In a separate incident, one rat in cage 4 lost a small quantity of blood from an ear wound inflicted on day 16 of filter test #4. No action was taken as these rats were euthenized on day 16 of the test.

Daily data (water consumption, air flow, room temp, humidity) for each test fixture are shown in Tables 23 through 26 and Figures 8 through 11. Tables 27 through 30 contain the number of food bars remaining in each occupied cage for all filter tests. Filter test #4 motor current data for cage 2 is in Table 26

CONCLUSIONS

Rats appeared normal in behavior and in eating and drinking habits over the course of these filter experiments. Weight gained by rats was similar in all tests. Filter efficiency varied amoung tests. In test #1 using ALFCO #1 filters, animal odors were detected in odor panel test on days 1, 6, 9, 12, and 14 (see Table 18). The supplemental carbon filters added to cage #4 eliminated the odors. Test #2 was a short test to check ALFCO #2 filter performance. Test #3 using ALFCO #3 filters found no difference between the empty and occupied cages in any odor panel evaluation over 24 days. Test #4 using APM/Pall #1 filters showed no difference between empty and

occupied cages for the first 7 test days. Panel evaluations on days 14, 21, and 24 showed differences in 4, 1, and 3 cages, respectively. These results indicate that the ALFCO #3 filter is effective in containing odor, urine, feces, animal hair, and dander from six (approximately 266 g initial weight) male rats for 24 days.

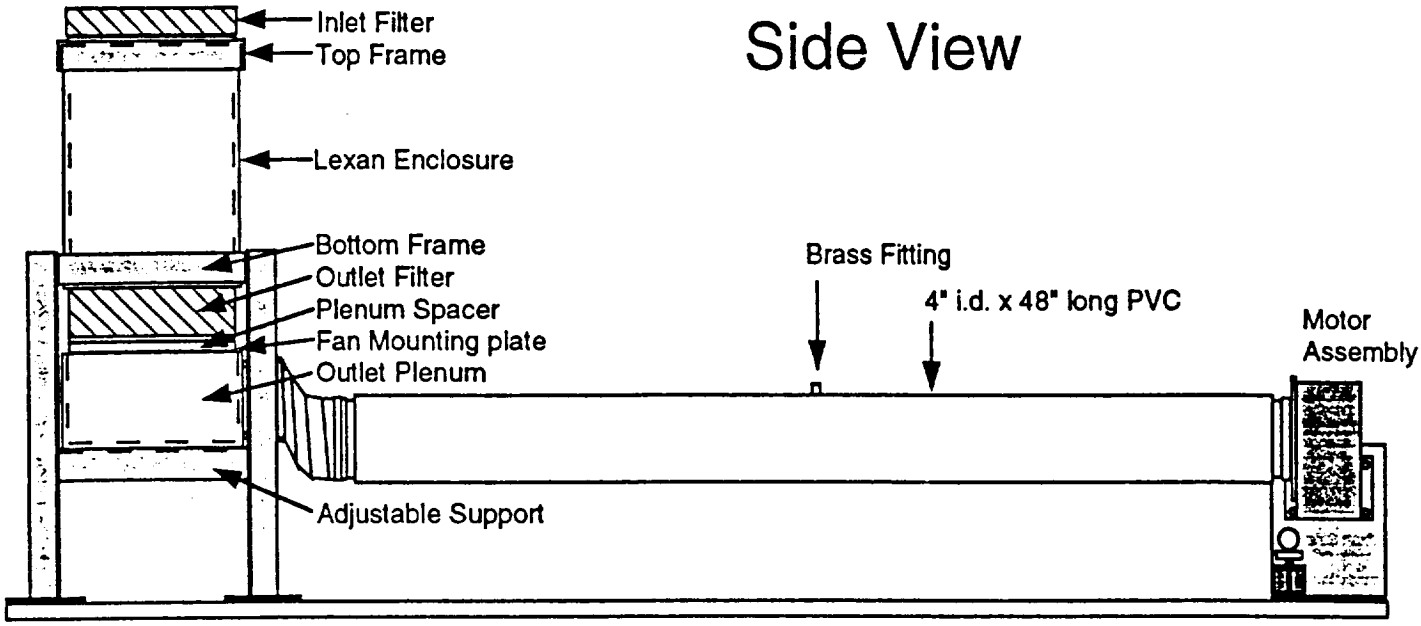
REFERENCES

American Society for Testing and Materials, ASTM Committee E-18. 1968. Manual on Sensory Testing Methods, ASTM Special Publication 434. American Society for Testing and Materials, Philadelphia, PA.

National Aeronautics and Space Administration. Handbook NHB 8060.1C: Office of Safety and Mission Quality. Flammability, Odor, Offgassing, and Compatability Requirements and Test Procedures for Materials in Environments that Support Combustion. April, 1991.

Sokal, R. R and F. J. Rohlf. 1987. Introduction to Biostatistics (second edition). W. H. Freeman and Compay, New York.

Figure 1. AEM Filter Odor Test Fixture (1" = 10")



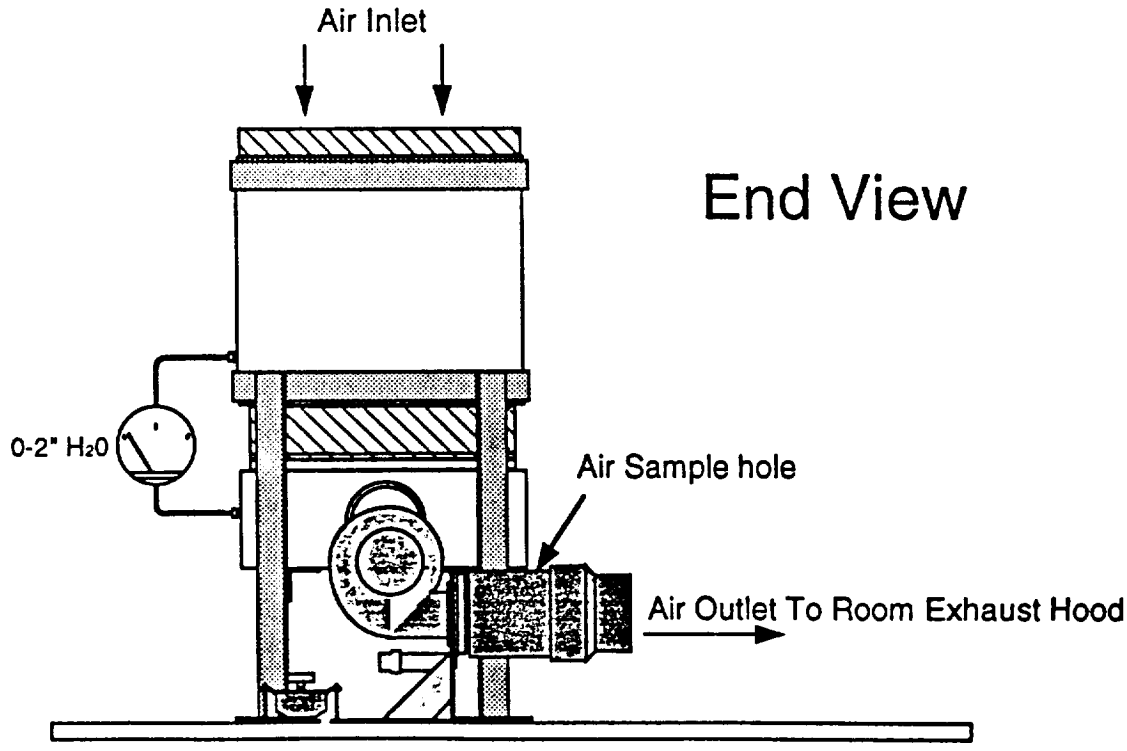


Table 1. Primary Odor Standards.

Odor	Standard compound*	Dilution in Water**
Ether	Diethyl ether	90 µl/333 ml
Camphor	1, 8-Cineole	5 µl/500 ml
Musk	15-Hydroxypentadecanoic acid lactone	1 mg/1000 ml
Floral	1-Methyl-1-ethyl-2-phenyl propanol-1	75 µl/500 ml
Mint	Menthone (dl)	2 µl/333 ml
Pungent	Acetic Acid	2 ml/333 ml
Putrid	Methyl disulfide	1 µl/10 L

* - From NASA NHB 8060.1C.
** - Highly purified water (resistivity > 10 megohm-cm)

Dilution procedure for 100ml substock

Odor	Chemical Name (from packaging)	1st dilution	2nd Dilution
Ether	Ether, anhydrous	27.0 µl/100 ml	
Camphor	Cineole (Eucalyptol)	0.1 ml/10 ml	0.1 ml/100 ml
Musk	15-Hydroxypentadecanoic acid lactone	10 mg/10 ml	0.1 ml/100 ml
Floral	ß-Phenylethylmethylethylcarbinol	15 µl/100 ml	
Mint	Menthone	60 µl/10 ml	0.1 ml/100 ml
Pungent	Acetic acid, glacial	0.6 ml/100 ml	
Putrid	Dimethyl disulfide (2,3-Dithiabutane)	10 µl/10 ml	10 µl/100 ml

Chemical suppliers

Odor	Order Name (from catalog)	Amount	Purity	Order Number	Supplier
Ether	Ether, anhydrous	250 ml	>99.0%	9244-01	Baker
Camphor	Cineole	100 ml		C-8144	Sigma
Musk	15-Pentadecanolide	1 g		P-5909	Sigma
Floral	ß-Phenylethylethylmethyl carbinol	100 g		217384	ICN
Mint	Menthone	100 ml	>97%	63680	Fluka
Pungent	Acetic acid, glacial	500 ml	>99.7%	9508-01	Baker
Putrid	Dimethyl disulfide	100 ml	>99%	D-8501	Sigma

Fluka Chemical
980 South Second St.
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Table 2. Number of odor evaluation days per tester for each filter test.

	Tester #	Filter Test #1	Filter Test #3	Filter Test #4	Total
	P1	6	6	6	18
	P2	6	1		7
	P3	5		2	7
	P4	5	5	6	16
	P5	6	5	6	17
	P6	4	3	3	10
	P7	5	4	6	15
	P8	6	5	3	14
	P9	3	3	1	7
	P10	6	2	5	13
	P11	3			3
	P12		4	6	10
	P14		3	5	8
	P15		5	6	11
	P16		2		2
	P17		6	6	12
	P18		2		2
	P19		2	6	8
	P20			1	1
	P21		1		1
	P22		3		3
	P23			5	5
	S2	5	3	6	14
	S4	2	1		3
	S5	5	3	3	11
	S6	6			6
	S7	6	5	6	17
	S8	2			2
	S9	5			5
	S11		5	5	10
	S12		3	6	9
	T21		3		3
	T30		2		2
Total	30	86	87	99	272

Table 3. Number of testers completing a specified number of odor evaluations

Number of odor evaluations performed	Filter Test #1 Number of Testers	Filter Test #3 Number of Testers	Filter Test #4 Number of Testers
6	7	2	11
5	6	6	4
4	1	2	0
3	2	8	3
2	2	5	1
1	0	3	2
Total	18	26	21

Table 4. Subject information.

	Tester #	Sex	Age
	P1	F	28
	P2	M	31
	P3	M	23
	P4	M	23
	P5	M	33
	P6	F	19
	P7	F	22
	P8	M	32
	P9	F	33
	P10	F	26
	P11	F	21
	P12	M	22
	P14	F	23
	P15	F	36
	P16	M	24
	P17	M	---
	P18	M	---
	P19	F	32
	P20	F	19
	P21	M	21
	P22	F	43
	P23	M	20
	S2	F	26
	S4	M	24
	S5	F	29
	S6	M	27
	S7	F	39
	S8	M	39
	S9	F	45
	S11	M	20
	S12	F	44
	T21	F	36
	T30	M	31
Total	33	M = 16 F = 17	Mean=28.7 ± 7.7 S.D.

Table 5. Scores of filter test #1 (ALFCO #1 filters) odor evaluations are listed by odor evaluation number, tester ID number and filter test number. Each 6 digit number represents all 6 odor evaluations for a single filter test. Underscores mean that a tester did not participate in that test. For example, 01_340 states that odor evaluations number 1 and 6 were scored 0, number 2 was scored 1, number 3 was not scored, etc. Cage 1 in each filter test contained neither rats nor food bars.

	Cage 1	Cage2	Cage 3	Cage 4	Cage 5	Cage 6
Tester	Evaluation	Evaluation	Evaluation	Evaluation	Evaluation	Evaluation
ID #	123456	123456	123456	123456	123456	123456
P1	000000	120112	111102	100120	111202	110212
P3	0____1	1____2	2____1	1____3	1____2	1____0
P4	_00000	111222	011222	011232	000212	001221
P5	110000	131334	311434	113442	323434	103133
P6	100___	021___	001___	000___	111___	121___
P7	101021	022223	010223	221243	212213	221233
P8	001___	000___	000___	000___	001___	001___
P9	0_____	1_____	1_____	1_____	1_____	1_____
P10	0_0000	2_2122	2_2222	1_2232	0_2222	1_1222
P12	102021	222222	222222	322243	222222	222222
P14	1010_0	0101_2	0101_1	0001_3	0011_0	0012_1
P15	100000	010022	110111	111121	111100	111210
P17	011102	022122	122222	211133	002222	322222
P19	000001	100002	110112	100121	111011	21121_
P20	2_____	3_____	3_____	3_____	2_____	4_____
P23	0000_1	0111_0	1000_1	0010_3	0020_1	0110_0
S2	122100	223222	412321	022233	122212	122321
S5	011___	111___	221___	102___	211___	221___
S7	000001	121112	222222	121241	222222	222222
S11	00000_	00001_	10001_	00004_	00011_	00001_
S12	000100	011200	122200	132302	132302	232201

Evaluation 1 = day 1, 2 = day 3, 3 = day 6, 4 = day 9, 5 = day 12, and 6 = day 14.

Table 6. Scores of filter test #3 (ALFCO #3 filters) odor evaluations are listed by odor evaluation number, tester ID number and filter test number. Each 6 digit number represents all 6 odor evaluations for a single filter test. Underscores mean that a tester did not participate in that test. For example, 01_340 states that odor evaluations number 1 and 6 were scored 0, number 2 was scored 1, number 3 was not scored, etc. Cage 1 in each filter test contained neither rats nor food bars.

	Cage 1	Cage2	Cage 3	Cage 4	Cage 5	Cage 6
Tester	Evaluation	Evaluation	Evaluation	Evaluation	Evaluation	Evaluation
ID #	123456	123456	123456	123456	123456	123456
P1	010010	000000	101001	001000	000101	000000
P2	___1__	___1__	___1__	___2__	___1__	___1__
P4	0_0100	0_0000	0_1000	0_0001	0_0010	1_1100
P5	000_00	000_00	100_00	101_00	101_11	111_01
P6	0_00__	1_00__	0_01__	1_01__	0_00__	1_10__
P7	0001__	0122__	2220__	1112__	0121__	1222__
P8	00_000	00_000	00_000	00_000	00_000	10_000
P9	01___0	00___0	00___0	11___1	00___0	11___1
P10	__1_0_	__0_0_	__0_1_	__0_0_	__0_1_	__1_0_
P12	1212__	0101__	1110__	1111__	0111__	1022__
P14	_0__01	_1__01	_0__00	_0__00	_0__00	_0__00
P15	0000_0	1000_1	1000_0	2101_0	3111_0	3010_0
P16	__01__	__00__	__01__	__11__	__00__	__21__
P17	011002	202020	000120	112211	000110	111200
P18	____10	____01	____01	____00	____00	____10
P19	___10_	___01_	___11_	___00_	___00_	___11_
P21	___1__	___0__	___0__	___0__	___0__	___0__
P22	___001	___000	___000	___000	___100	___010
S2	033___	122___	101___	313___	000___	012___
S4	1_____	1_____	2_____	0_____	0_____	0_____
S5	112___	011___	110___	111___	110___	010___
S7	01000_	01010_	00001_	00010_	01101_	11110_
S11	_00000	_01000	_00000	_00000	_00000	_00000
S12	___000	___001	___000	___000	___020	___001
T21	_0_11_	_0_10_	_1_00_	_0_00_	_0_01_	_1_21_
T30	____10	____10	____10	____00	____00	____00

Evaluation 1 = day 1, 2 = day 3, 3 = day 7, 4 = day 14, 5 = day 21, and 6 = day 24.

Table 7. Scores of filter test #4 (APM/Pall #1 filters) odor evaluations are listed by odor evaluation number, tester ID number and filter test number. Each 6 digit number represents all 6 odor evaluations for a single filter test. Underscores mean that a tester did not participate in that test. For example, 01_340 states that odor evaluations number 1 and 6 were scored 0, number 2 was scored 1, number 3 was not scored, etc. Cage 1 in each filter test contained neither rats nor food bars.

	Cage 1	Cage2	Cage 3	Cage 4	Cage 5	Cage 6
Tester	Evaluation	Evaluation	Evaluation	Evaluation	Evaluation	Evaluation
ID #	123456	123456	123456	123456	123456	123456
P1	100000	212122	212122	221110	221211	211121
P2	021211	211121	111212	111201	111112	102212
P3	12112_	22222_	22222_	22221_	22222_	22222_
P4	00_010	22_221	22_222	22_200	22_222	22_222
P5	000111	432333	332323	342311	234433	234333
P6	000__0	333__1	232__1	124__1	134__2	134__2
P7	0000_0	1222_2	1121_1	0321_0	2122_2	1231_2
P8	000000	013132	012213	013100	022121	021122
P9	000___	211___	211___	111___	111___	111___
P10	000100	122222	212222	222200	222222	212222
P11	___010	___222	___222	___200	___222	___222
S2	3_1111	2_3331	1_2222	2_2212	2_2232	2_3223
S4	1___0_	2___2_	2___1_	1___1_	1___1_	1___2_
S5	01100_	22232_	23222_	22220_	23231_	23322_
S6	020200	222321	222222	211200	212122	222222
S7	000000	222122	122222	232200	222222	222222
S8	10____	21____	22____	21____	11____	21____
S9	0_0000	1_1132	2_1212	1_1200	2_3223	4_3223

Evaluation 1 = day 1, 2 = day 3, 3 = day 7, 4 = day 14, 5 = day 21, and 6 = day 24.

Table 8. Scores of filter test #1 (ALFCO #1 filters) odor evaluations are listed by test day number, cage number, and tester ID number. Each 6 digit number represents odor evaluations all 6 cages for a single filter test day. Underscores mean that a tester did not participate in that test. For example, 021111 states that cage number 1 was scored 0, cage 2 was scored 2, and cages 3, 4, 5, and 6 were scored 1. Cage 1 in each filter test contained neither rats nor food bars.

	Day 1	Day 3	Day 6	Day 9	Day 12	Day 14
Tester	Cage #	Cage #	Cage #	Cage #	Cage #	Cage #
ID #	123456	123456	123456	123456	123456	123456
P1	122222	011221	022111	011121	022112	022011
P2	021111	211110	111112	212212	121011	112122
P3	122222	222222	122222	122222	222122	_____
P4	022222	022222	_____	022222	122022	012022
P5	043322	033433	022244	133343	132133	133133
P6	032111	033233	032444	_____	_____	011122
P7	011021	021312	022223	021121	_____	021022
P8	000000	011122	032321	012111	031022	023012
P9	022111	011111	011111	_____	_____	_____
P10	012222	021221	022222	122222	022022	022022
P11	_____	_____	_____	022222	122022	022022
S2	321222	_____	132223	132222	132132	112223
S4	122111	_____	_____	_____	021112	_____
S5	022222	123233	122223	032232	022012	_____
S6	022222	222112	022122	232212	022022	012022
S7	021222	022322	022222	012222	022022	022022
S8	122212	012111	_____	_____	_____	_____
S9	012124	_____	011133	012222	031022	022033

Table 9. Scores of filter test #3 (ALFCO #3 filters) odor evaluations are listed by test day number, cage number, and tester ID number. Each 6 digit number represents odor evaluations all 6 cages for a single filter test day. Underscores mean that a tester did not participate in that test. For example, 021111 states that cage number 1 was scored 0, cage 2 was scored 2, and cages 3, 4, 5, and 6 were scored 1. Cage 1 in each filter test contained neither rats nor food bars.

	Day 1	Day 3	Day 7	Day 14	Day 21	Day 24
Tester	Cage #	Cage #	Cage #	Cage #	Cage #	Cage #
ID #	123456	123456	123456	123456	123456	123456
P1	001000	100000	001100	000010	100000	001010
P2	_____	_____	_____	111211	_____	_____
P4	000001	_____	001001	100001	000010	000100
P5	001111	000001	000111	_____	000010	000011
P6	010101	_____	000001	001100	_____	_____
P7	002101	012112	022122	120212	_____	_____
P8	000001	000000	_____	000000	000000	000000
P9	000101	100101	_____	_____	_____	000101
P10	_____	_____	100001	_____	001010	_____
P12	101101	211110	101112	210112	_____	_____
P14	_____	010000	_____	_____	000000	110000
P15	011233	000110	000011	000110	_____	010000
P16	_____	_____	000102	101101	_____	_____
P17	020101	100101	120201	001212	022110	200100
P18	_____	_____	_____	_____	100001	011000
P19	_____	_____	_____	101001	011001	_____
P21	_____	_____	_____	100000	_____	_____
P22	_____	_____	_____	000010	000001	100000
S2	011300	320101	321302	_____	_____	_____
S4	112000	_____	_____	_____	_____	_____
S5	101110	111111	210100	_____	_____	_____
S7	000001	110011	000011	010101	001010	_____
S11	_____	000000	010000	000000	000000	000000
S12	_____	_____	_____	000000	000020	010001
T21	_____	001001	_____	110002	100011	_____
T30	_____	_____	_____	_____	111000	000000

Table 10. Scores of filter test #4 (APM/Pall #1 filters) odor evaluations are listed by test day number, cage number, and tester ID number. Each 6 digit number represents odor evaluations all 6 cages for a single filter test day. Underscores mean that a tester did not participate in that test. For example, 021111 states that cage number 1 was scored 0, cage 2 was scored 2, and cages 3, 4, 5, and 6 were scored 1. Cage 1 in each filter test contained neither rats nor food bars.

Filter test #4

	Day 1	Day 3	Day 7	Day 14	Day 21	Day 24
Tester ID #	Cage # 123456	Cage # 123456	Cage # 123456	Cage # 123456	Cage # 123456	Cage # 123456
P1	011111	021011	001010	011122	010201	022022
P3	012111	_____	_____	_____	_____	121320
P4	_10000	011100	011101	022222	022312	022221
P5	113131	131120	011333	034441	033433	044243
P6	100011	020012	011011	_____	_____	_____
P7	100222	021212	120121	022222	222413	133333
P8	000000	000000	100011	_____	_____	_____
P9	011111	_____	_____	_____	_____	_____
P10	022101	_____	022221	012222	022322	022222
P12	122322	022222	222222	022222	222422	122322
P14	100000	011000	100011	011112	_____	021301
P15	101111	011111	000111	001112	021201	021100
P17	001203	122102	122122	112122	022322	222322
P19	011112	001011	000011	001102	001211	12211_
P20	233324	_____	_____	_____	_____	_____
P23	001000	010001	010121	010000	_____	101310
S2	124011	221222	232222	123223	022312	021321
S5	012122	112012	111211	_____	_____	_____
S7	012122	022222	012122	012222	012422	122122
S11	001000	000000	000000	000010	011411	_____
S12	001112	012333	012222	122332	000000	000221

Table 11. Odor score frequencies from filter test #1 (ALFCO #1 filters).

	Odor Score				
Day 1 odor evaluation	0	1	2	3	4
Standard 1 (floral)	2	7	6	1	0
Standard 2 (acetic acid)	0	2	12	2	0
Standard 3 (DI water)	14	2	0	0	0
Standard 4 N. A					
Cage 1 (empty)	12	4	0	1	0
Cage 2	1	3	11	1	1
Cage 3	1	4	11	1	0
Cage 4	2	5	9	1	0
Cage 5	1	5	11	0	0
Cage 6	1	5	10	0	1

	Odor Score				
Day 3 odor evaluation	0	1	2	3	4
Standard 1 (floral)	2	7	5	0	0
Standard 2 (acetic acid)	0	0	13	1	0
Standard 3 (DI water)	14	0	0	0	0
Standard 4 (food bars)	0	0	8	5	1
Cage 1 (empty)	10	1	3	0	0
Cage 2	0	5	7	2	0
Cage 3	0	6	5	3	0
Cage 4	0	5	6	2	1
Cage 5	0	5	6	3	0
Cage 6	1	4	6	3	0

	Odor Score				
Day 6 odor evaluation	0	1	2	3	4
Standard 1 (Camphor)	0	1	13	0	0
Standard 2 (DI Water)	14	0	0	0	0
Standard 3 (Mint)	0	1	13	0	0
Standard 4 (rat urine & feces)	0	0	2	4	8
Cage 1 (empty)	10	4	0	0	0
Cage 2	0	3	8	3	0
Cage 3	0	3	11	0	0
Cage 4	0	5	7	1	1
Cage 5	0	3	8	1	2
Cage 6	0	3	5	4	2

Table 11. Filter test #1. (cont.)

	Odor Score				
Day 9 odor evaluation	0	1	2	3	4
Standard 1 (Camphor)	0	4	10	0	0
Standard 2 (DI Water)	13	0	1	0	0
Standard 3 (Mint)	0	0	13	1	0
Standard 4 N.A.					
Cage 1 (empty)	8	4	2	0	0
Cage 2	0	5	5	4	0
Cage 3	0	2	11	1	0
Cage 4	0	3	10	1	0
Cage 5	0	3	9	1	1
Cage 6	0	3	10	1	0

	Odor Score				
Day 12 odor evaluation	0	1	2	3	4
Standard 1 (Camphor)	0	3	11	0	0
Standard 2 (DI Water)	14	0	0	0	0
Standard 3 (Musk)	2	5	7	0	0
Standard 4 N.A.					
Cage 1 (empty)	8	5	1	0	0
Cage 2	0	0	10	4	0
Cage 3	0	4	10	0	0
Cage 4	9	5	0	0	0
Cage 5	0	4	8	2	0
Cage 6	0	1	12	1	0

	Odor Score				
Day 14 odor evaluation	0	1	2	3	4
Standard 1 (DI Water)	12	1	0	0	0
Standard 2 (Floral)	0	2	11	0	0
Standard 3 (Musk)	4	4	4	1	0
Standard 4 N.A.					
Cage 1 (empty)	10	3	0	0	0
Cage 2	0	5	7	1	0
Cage 3	0	2	9	2	0
Cage 4	9	3	1	0	0
Cage 5	0	2	9	2	0
Cage 6	0	1	9	3	0

Table 12. Odor score frequencies from Filter Test #2 (ALFCO #2 filters).

	Odor Score				
Day 1 odor evaluation	0	1	2	3	4
Cage 3	3	1	0	0	0
Cage 4	1	2	1	0	0

	Odor Score				
Day 4 odor evaluation	0	1	2	3	4
Cage 3	1	0	0	0	0
Cage 4	0	1	0	0	0

	Odor Score				
Day 7 odor evaluation	0	1	2	3	4
Cage 3	3	1	0	0	0
Cage 4	2	2	0	0	0

Table 13. Odor score frequencies from filter test #3 (ALFCO #3 filters).

	Odor Score				
Day 1 odor evaluation	0	1	2	3	4
Standard 1 (Musk)	4	6	4	0	0
Standard 2 (Millipore water)	13	0	1	0	0
Standard 3 (Ether)	0	4	5	5	0
Standard 4 (Acetic Acid)	0	1	12	1	0
Cage 1 (empty)	11	3	0	0	0
Cage 2	9	4	1	0	0
Cage 3	6	6	2	0	0
Cage 4	5	7	1	1	0
Cage 5	11	2	0	1	0
Cage 6	4	9	0	1	0

	Odor Score				
Day 3 odor evaluation	0	1	2	3	4
Standard 1 (Mint)	1	3	9	1	0
Standard 2 (Millipore Water)	14	0	0	0	0
Standard 3 (Food Bars)	0	0	6	6	2
Standard 4 (Camphor)	3	4	7	0	0
Cage 1 (empty)	7	5	1	1	0
Cage 2	8	5	1	0	0
Cage 3	10	3	1	0	0
Cage 4	7	7	0	0	0
Cage 5	9	5	0	0	0
Cage 6	6	7	1	0	0

	Odor Score				
Day 7 odor evaluation	0	1	2	3	4
Standard 1 (Mint)	1	6	6	1	0
Standard 2 (Millipore Water)	13	1	0	0	0
Standard 3 (Rat urine & feces)	1	3	1	7	2
Standard 4 (Camphor)	2	9	3	0	0
Cage 1 (empty)	9	3	1	1	0
Cage 2	9	2	3	0	0
Cage 3	9	4	1	0	0
Cage 4	6	6	1	1	0
Cage 5	9	4	1	0	0
Cage 6	3	7	4	0	0

Table 13. Filter test #3. (cont.)

	Odor Score				
Day 14 odor evaluation	0	1	2	3	4
Standard 1 (camphor)	8	5	4	0	0
Standard 2 (mint)	3	12	2	0	0
Standard 3 (Millipore water)	14	3	0	0	0
Standard 4 N.A.					
Cage 1 (empty)	9	7	1	0	0
Cage 2	12	4	1	0	0
Cage 3	12	5	0	0	0
Cage 4	9	5	3	0	0
Cage 5	10	7	0	0	0
Cage 6	8	5	4	0	0

	Odor Score				
Day 21 odor evaluation	0	1	2	3	4
Standard 1 (Food Bars)	0	1	1	6	6
Standard 2 (Millipore water)	12	0	2	0	0
Standard 3 (Acetic Acid)	1	0	9	4	0
Standard 4 N.A.					
Cage 1 (empty)	11	4	0	0	0
Cage 2	12	2	1	0	0
Cage 3 (dead animals)	10	4	1	0	0
Cage 4	14	1	0	0	0
Cage 5	8	6	1	0	0
Cage 6	11	4	0	0	0
Cage 3 (dead animals), A Top	7	4	3	0	1
Cage 3 (dead animals), A Middle	1	6	4	3	1
Cage 3 (dead animals), A Bottom	10	4	1	0	0
Cage 1 (empty), B Top	11	4	0	0	0
Cage 1 (empty), B Middle	8	6	1	0	0
Cage 1 (empty), B Bottom	11	4	0	0	0
Cage 4, C Top	13	2	0	0	0
Cage 4, C Middle	8	6	1	0	0
Cage 4, C Bottom	11	3	1	0	0

	Odor Score				
Day 24 odor evaluation	0	1	2	3	4
Standard 1 (musk)	0	4	8	1	0
Standard 2 (mint)	6	5	2	0	0
Standard 3 (Millipore water)	11	2	0	0	0
Standard 4 N.A.					
Cage 1 (empty)	10	2	1	0	0
Cage 2	9	4	0	0	0
Cage 3 (empty)	11	2	0	0	0
Cage 4	10	3	0	0	0
Cage 5	11	2	0	0	0
Cage 6	10	3	0	0	0

Table 14. Odor score frequencies from filter test #4 (APM/Pall #1).

	Odor Score				
Day 1 odor evaluation	0	1	2	3	4
Standard 1 (Ether)	0	1	14	6	0
Standard 2 (Camphor)	1	6	14	0	0
Standard 3 (Acetic Acid)	0	7	10	3	1
Standard 4 N.A.					
Cage 1 (empty)	12	7	1	0	0
Cage 2	9	8	3	1	0
Cage 3	5	8	5	2	1
Cage 4	7	10	2	2	0
Cage 5	7	8	5	1	0
Cage 6	5	8	6	1	1

	Odor Score				
Day 3 odor evaluation	0	1	2	3	4
Standard 1 (Camphor)	1	7	9	0	0
Standard 2 (Millipore water)	17	0	0	0	0
Standard 3 (Floral)	2	11	3	1	0
Standard 4 N.A.					
Cage 1 (empty)	13	3	1	0	0
Cage 2	3	6	7	1	0
Cage 3	4	8	5	0	0
Cage 4	8	4	4	1	0
Cage 5	6	6	4	1	0
Cage 6	5	4	7	1	0

	Odor Score				
Day 7 odor evaluation	0	1	2	3	4
Standard 1 (Mint)	0	0	17	1	0
Standard 2 (Dimethyl Disulfide)	8	10	0	0	0
Standard 3 (Millipore water)	12	6	0	0	0
Standard 4 (Rat urine)	0	0	1	7	10
Cage 1 (empty)	11	5	2	0	0
Cage 2	6	7	4	1	0
Cage 3	7	5	6	0	0
Cage 4	6	6	5	1	0
Cage 5	2	7	8	1	0
Cage 6	2	10	5	1	0

Table 14. Filter test #4. (cont.)

	Odor Score				
Day 14 odor evaluation	0	1	2	3	4
Standard 1 (Millipore water)	15	0	0	0	0
Standard 2 (Camphor)	0	4	11	0	0
Standard 3 (dimethyl disulfide)	14	1	0	0	0
Standard 4 N.A.					
Cage 1 (empty)	12	3	0	0	0
Cage 2	3	6	5	1	0
Cage 3	2	4	7	1	1
Cage 4	2	5	6	1	1
Cage 5	2	3	8	1	1
Cage 6	2	1	11	1	0

	Odor Score				
Day 21 odor evaluation	0	1	2	3	4
Standard 1 (Millipore water)	13	0	0	0	0
Standard 2 (Floral)	1	4	7	1	0
Standard 3 (Camphor)	1	4	8	0	0
Standard 4 N.A.					
Cage 1 (empty)	11	0	2	0	0
Cage 2	2	3	7	1	0
Cage 3	2	3	7	1	0
Cage 4 (dead animals)	1	0	3	4	5
Cage 5	3	5	4	1	0
Cage 6	1	4	6	2	0
Cage 4 Top	7	3	1	2	0
Cage 4 Middle	3	5	3	2	0
Cage 4 Bottom	5	4	3	1	0

	Odor Score				
Day 24 odor evaluation	0	1	2	3	4
Standard 1 (Millipore water)	15	0	0	0	0
Standard 2 (Floral)	1	7	7	0	0
Standard 3 (water)	2	6	7	0	0
Standard 4 N.A.					
Cage 1 (empty)	8	6	1	0	0
Cage 2	2	0	11	1	1
Cage 3	1	5	7	1	1
Cage 4 (empty)	1	3	4	7	0
Cage 5	2	2	9	1	1
Cage 6	3	4	5	2	0

Figure 2. Odor score histogram for filter test #1 (ALFCO #1 filters)

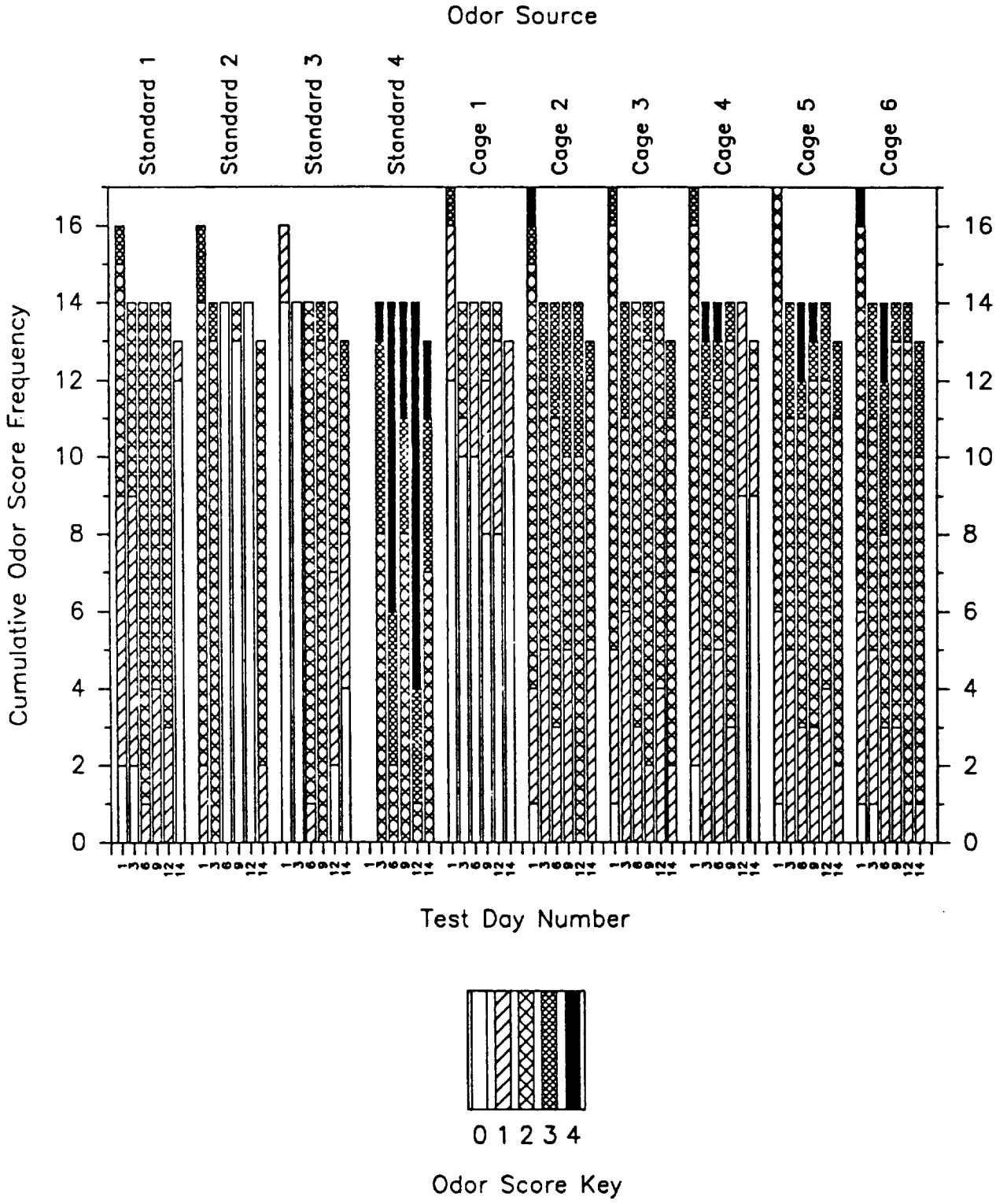

Figure 3. Odor score histogram for filter test #3 (ALFCO #3 filters).

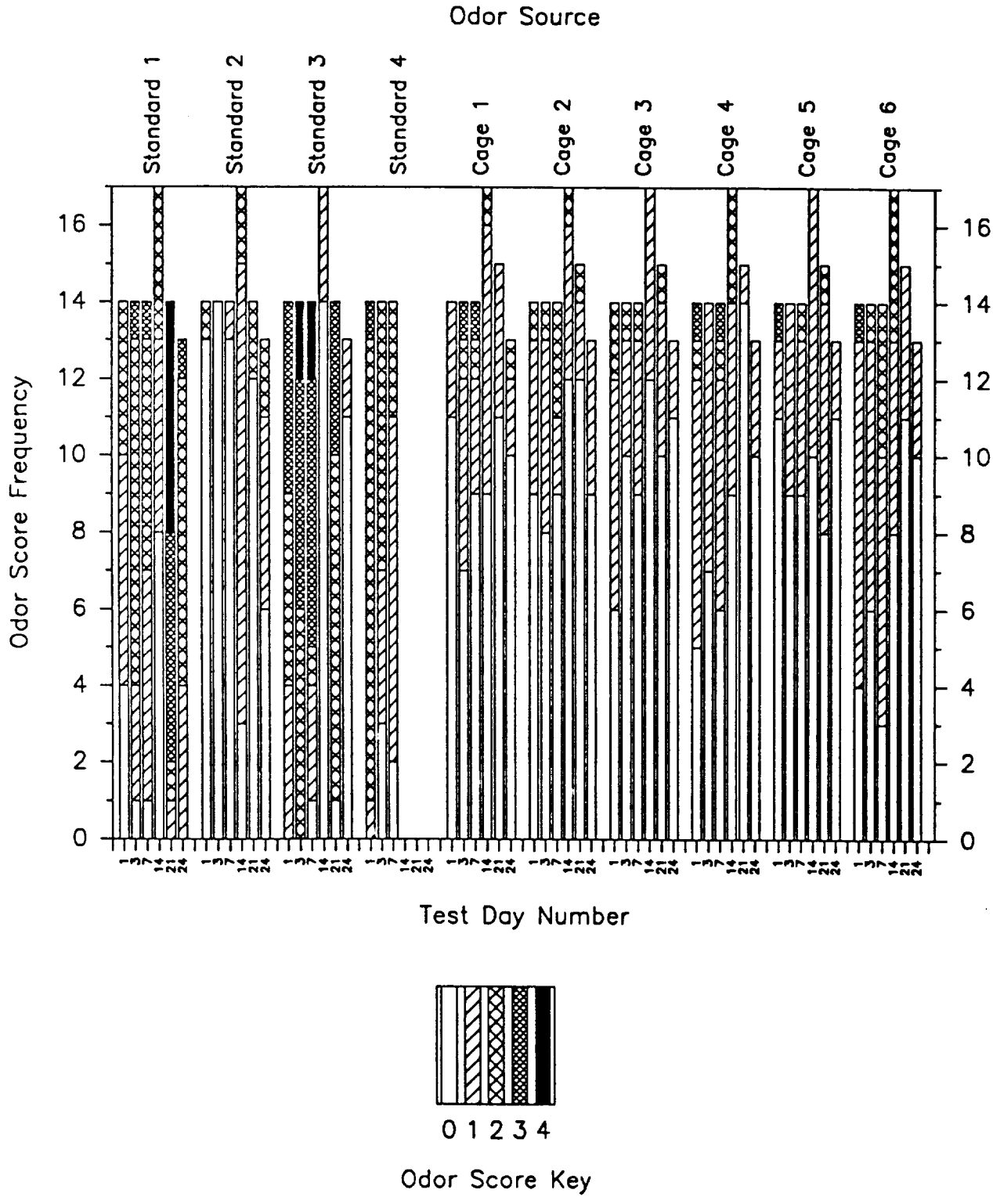

Figure 4. Odor score histogram for filter test #4 (APM/Pall #1 filters).

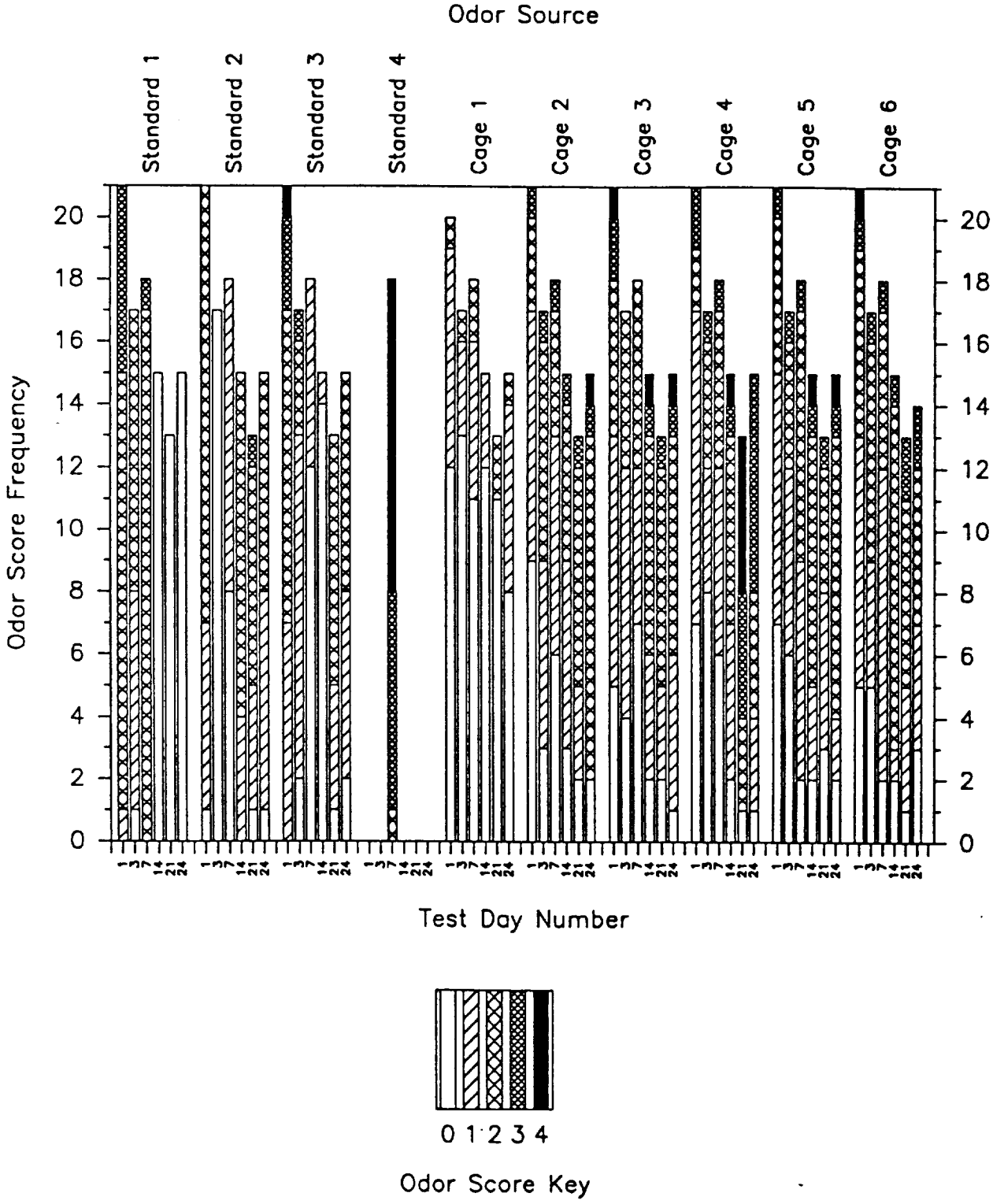

Figure 5. Mean odor score (± SD) for filter test #1 (ALFCO #1 filters). Open circle data is the mean odor score of all panel members for non-control cages (2-6). Closed circle data is for unoccupied (control) cage 1. These data are also included in Table 31.

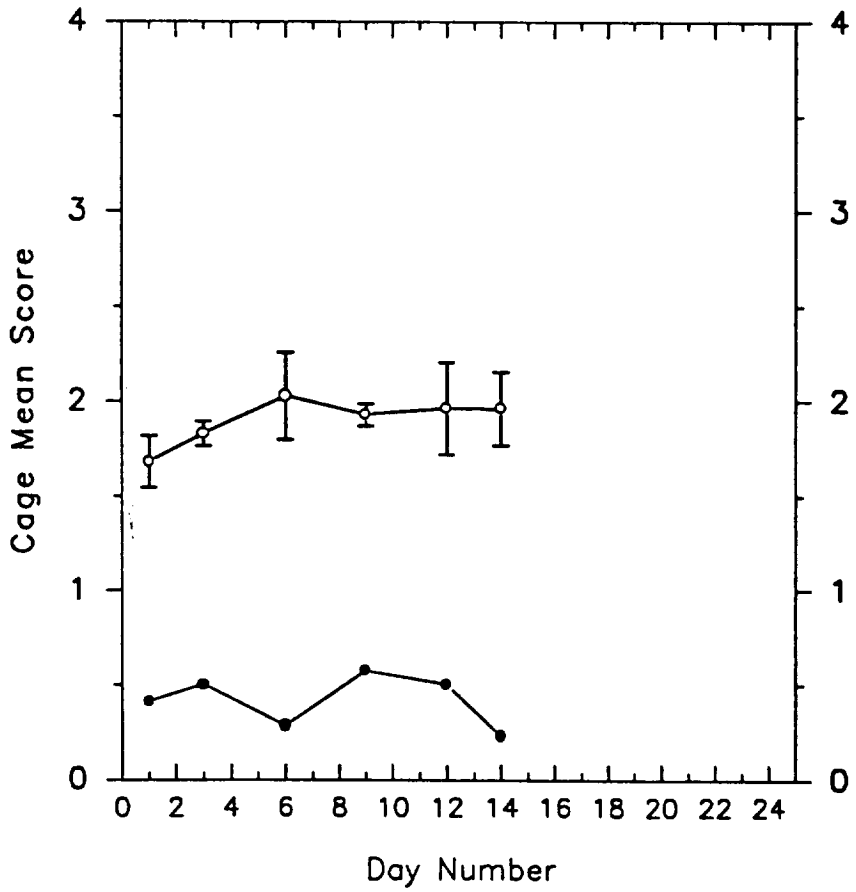

Figure 6. Mean odor score (± SD) for filter test #3 (ALFCO #3 filters). Open circle data is the mean odor score of all panel members for non-control cages (2-6). Closed circle data is for unoccupied (control) cage 1. These data are also included in Table 32.

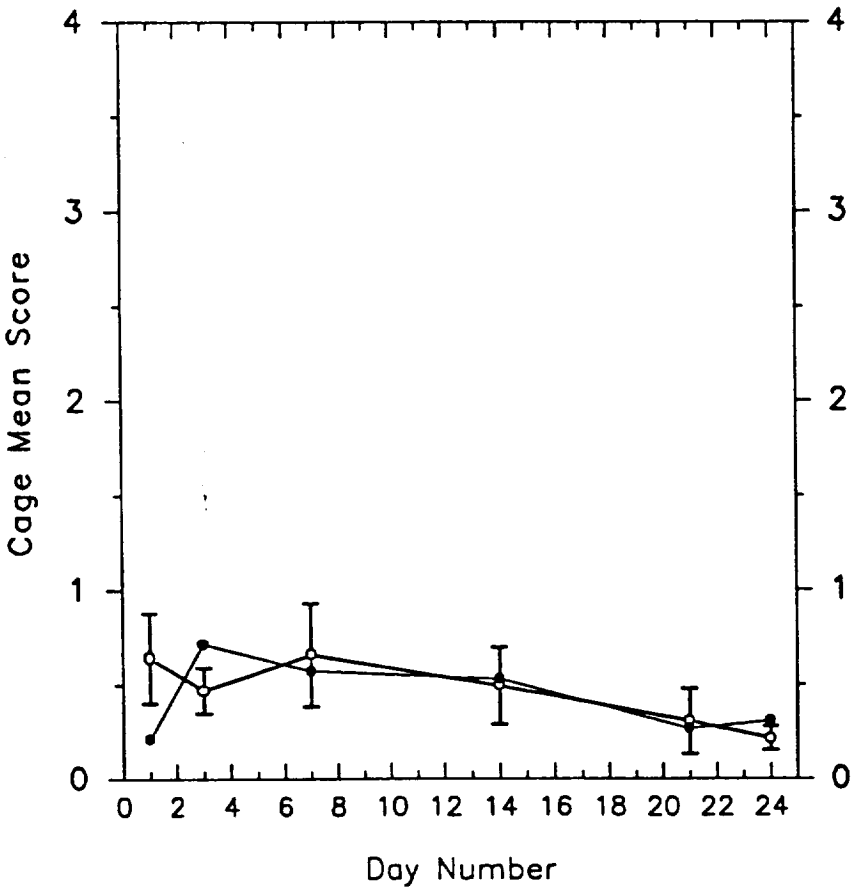

Figure 7. Mean odor score (± SD) for filter test #4 (APM/Pall #1 filters). Open circle data is the mean odor score of all panel members for non-control cages (2-6). Closed circle data is for unoccupied (control) cage 1. These data are also included in Table 33.

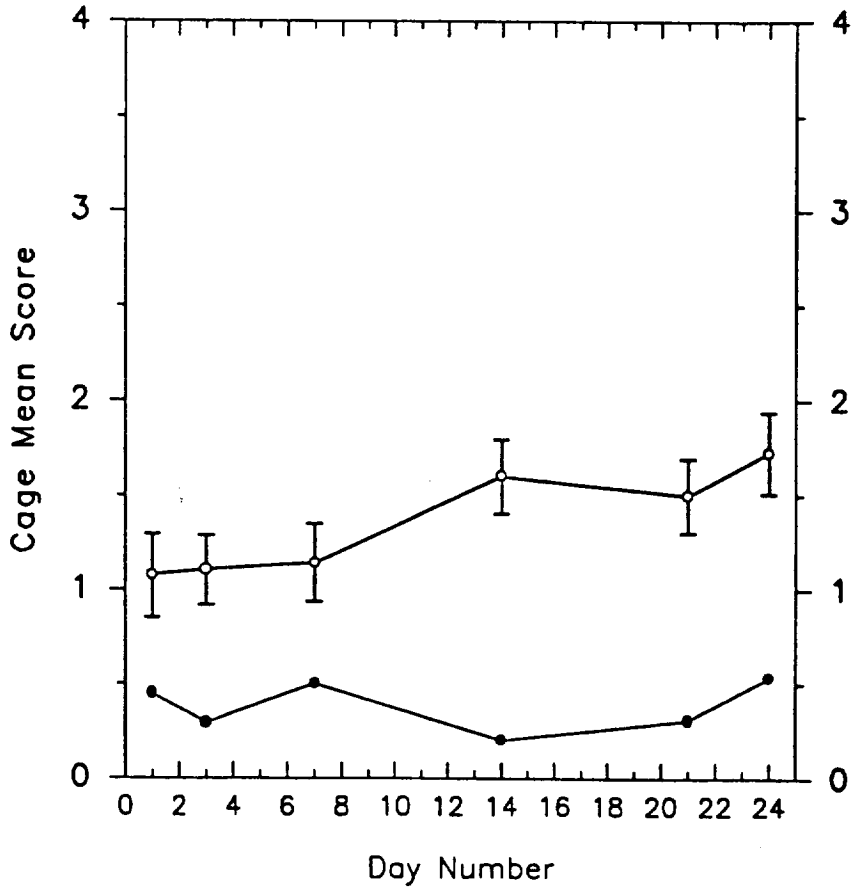

Table 15. Frequency of scores in the ranges 0-1 and 2-4 for filter test #1 (ALFCO #1 filters) .

	Day 1		Day 3		Day 6		Day 9		Day 12		Day 14	
Odor	0-1	2-4	0-1	2-4	0-1	2-4	0-1	2-4	0-1	2-4	0-1	2-4
Sample 1	9	7	9	5	1	13	4	10	3	11	13	0
Sample 2	2	14	0	14	14	0	13	1	14	0	2	11
Sample 3	16	0	14	0	1	13	0	14	7	7	8	5
Sample 4	0	0	0	14	0	14	0	14	0	14	0	13
Cage 1	16	1	11	3	14	0	12	2	13	1	13	0
Cage 2	4	13	5	9	3	11	5	9	0	14	5	8
Cage 3	5	12	6	8	3	11	2	12	4	10	2	11
Cage 4	7	10	5	9	5	9	3	11	14	0	12	1
Cage 5	6	11	5	9	3	11	3	11	4	10	2	11
Cage 6	6	11	5	9	3	11	3	11	1	13	1	12

Table 16. Frequency of scores in the ranges 0-1 and 2-4 for filter test #3 (ALFCO #3 filters).

	Day 1		Day 3		Day 7		Day 14		Day 22		Day 24	
Odor	0-1	2-4	0-1	2-4	0-1	2-4	0-1	2-4	0-1	2-4	0-1	2-4
Sample 1	10	4	4	10	7	7	13	4	1	13	4	9
Sample 2	13	1	14	0	14	0	15	2	12	2	11	2
Sample 3	4	10	0	14	4	10	17	0	1	13	13	0
Sample 4	1	13	7	7	11	3	0	0	0	0	0	0
Cage 1	14	0	12	2	12	2	16	1	15	0	12	1
Cage 2	13	1	13	1	11	3	16	1	14	1	13	0
Cage 3	12	2	13	1	13	1	17	0	14	1	13	0
Cage 4	12	2	14	0	12	2	14	3	15	0	13	0
Cage 5	13	1	14	0	13	1	17	0	14	1	13	0
Cage 6	13	1	13	1	10	4	13	4	15	0	13	0

Table 17. Frequency of scores in the ranges 0-1 and 2-4 for filter test #4 (APM/Pall #1 filters).

	Day 1		Day 3		Day 7		Day 14		Day 22		Day 24	
Odor	0-1	2-4	0-1	2-4	0-1	2-4	0-1	2-4	0-1	2-4	0-1	2-4
Sample 1	1	20	8	9	0	18	15	0	13	0	15	0
Sample 2	7	14	17	0	18	0	4	11	5	8	8	7
Sample 3	7	14	13	4	18	0	15	0	5	8	8	7
Sample 4	0	0	0	0	0	18	0	0	0	0	0	0
Cage 1	19	1	16	1	16	2	15	0	11	2	14	1
Cage 2	17	4	9	8	13	5	9	6	5	8	2	13
Cage 3	13	8	12	5	12	6	6	9	5	8	6	9
Cage 4	17	4	12	5	12	6	7	8	1	12	4	11
Cage 5	15	6	12	5	9	9	5	10	8	5	4	11
Cage 6	13	8	9	8	12	6	3	12	5	8	7	7

Table 18. Adjusted G from 2 by 2 independence test calculations for filter test #1 (ALFCO #1 filters). Odor evaluation scores for a test cage differ from the control, cage #1, if G_{adj} > Chi Square [df=1, P=.01] (6.635). * indicates a significant difference. From Sokal and Rohlf 1987.

Test cage pair	Day 1	Day 3	Day 6	Day 9	Day 12	Day 14
Cage 1 vs Cage 2	*19.037	5.161	*21.733	*7.368	*29.858	*13.793
Cage 1 vs Cage 3	*16.257	3.643	*21.733	*15.044	*12.832	*22.900
Cage 1 vs Cage 4	*11.558	5.161	*15.898	*11.995	‡0.948	‡0.950
Cage 1 vs Cage 5	*13.783	5.161	*21.733	*11.995	*12.832	*22.900
Cage 1 vs Cage 6	*13.783	5.161	*21.733	*11.995	*23.165	*27.254

‡ Suplemental filter, 4 layers of D-Mark Carbon 110, present in cage 4 plenum spacer.

Table 19. Adjusted G from 2 by 2 independence test calculations for filter test #3 (ALFCO #3 filters). Odor evaluation scores for a test cage differ from the control, cage #1, if G_{adj} > Chi Square [df=1, P=.01] (6.635). * indicates a significant difference. From Sokal and Rohlf 1987.

Test cage pair	Day 1	Day 3	Day 7	Day 14	Day 21	Day 24
Cage 1 vs Cage 2	0.948	0.325	0.222	0.000	0.947	0.950
Cage 1 vs Cage 3	2.339	0.325	0.325	0.944	★ 0.947	♣ 0.950
Cage 1 vs Cage 4	2.339	2.339	0.000	1.047	0.000	0.950
Cage 1 vs Cage 5	0.948	2.339	0.325	0.944	0.947	0.950
Cage 1 vs Cage 6	0.948	0.325	0.792	2.030	0.000	0.950

★ Dead animals present and the fan turned on in cage 3.
♣ Dead animals removed from cage 3 before this test.

Table 20. Adjusted G from 2 by 2 independence test calculations for filter test #4 (APM/Pall #1 filters). Odor evaluation scores for a test cage differ from the control, cage #1, if G_{adj} > Chi Square [df=1, P=.01] (6.635). * indicates a significant difference. From Sokal and Rohlf 1987.

Test cage pair	Day 1	Day 3	Day 7	Day 14	Day 21	Day 24
Cage 1 vs Cage 2	1.828	*7.715	1.525	9.043	5.801	*21.258
Cage 1 vs Cage 3	*6.898	3.207	2.500	*15.490	5.801	*10.065
Cage 1 vs Cage 4	1.828	3.207	2.500	*13.165	★*16.705	♣*14.852
Cage 1 vs Cage 5	4.106	3.207	6.471	*18.043	1.673	*14.852
Cage 1 vs Cage 6	*6.898	*7.715	2.500	*24.097	5.801	*6.927

★ Dead animals present and the fan turned on in cage 4.
♣ Dead animals removed from cage 4 before this test.

Table 21. Mean rat weights ± standard deviation (g) for each cage (n=6). Total rat weight per cage and weight gain.

	Filter Test #1		Filter Test #2		Filter Test #3		Filter Test #4	
Cage	Initial	Final	Initial	Final	Initial	Final	Initial	Final
#2	230 ± 4	317 ± 23	----------	----------	263 ± 10	320 ± 14	208 ± 5	284 ± 12
#3	229 ± 8	319 ± 25	394 ± 16	412 ± 18	267 ± 8	303 ± 16	201 ± 8	289 ± 13
#4	232 ± 6	342 ± 5	398 ± 4	409 ± 6	266 ± 12	321 ± 20	197 ± 4	286 ± 8
#5	235 ± 6	333 ± 14	----------	----------	268 ± 5	338 ± 14	202 ± 6	291 ± 9
#6	233 ± 6	327 ± 18	----------	----------	265 ± 7	317 ± 10	204 ± 4	285 ± 5
	232 ± 6	328 ± 19	396 ± 11	411 ± 12	266 ± 8	320 ± 18	202 ± 6	287 ± 10

Cage totals for Filter Test #1

		Total Rat Weight (grams)				
Cage	Days	Initial	Final	Gain	Gain\Day	Gain/Rat/Day
#1	24	____	____	___	__	_
#2	19	1381	1904	523	27.5	4.6
#3	19	1375	1913	538	28.3	4.7
#4	24	1392	2053	661	27.5	4.6
#5	19	1408	1999	591	31.1	5.1
#6	18	1399	1964	565	31.4	5.2

Cage totals for Filter Test #2

		Total Rat Weight (grams)				
Cage	Days	Initial	Final	Gain	Gain\Day	Gain/Rat/Day
#3	9	1576	1650	74	8.2	2.1
#4	9	1591	1637	46	5.1	1.3

Cage totals for Filter Test #3

		Total Rat Weight (grams)				
Cage	Days	Initial	Final	Gain	Gain\Day	Gain/Rat/Day
#1	16	____	____	___	__	_
#2	16	1577	1920	343	21.4	3.6
#3	15	1604	1816	212	14.1	2.4
#4	16	1596	1926	330	20.6	3.4
#5	16	1606	2027	421	26.3	4.4
#6	16	1588	1904	316	19.8	3.3

Cage totals for Filter Test #4

		Total Rat Weight (grams)				
Cage	Days	Initial	Final	Gain	Gain\Day	Gain/Rat/Day
#1	17	____	____	___	__	_
#2	17	1249	1707	458	26.9	4.5
#3	17	1208	1736	528	31.1	5.2
#4	15	1180	1716	536	35.7	6.0
#5	17	1214	1747	533	31.4	5.2
#6	17	1223	1710	487	28.6	4.8

Table 22. Filter weights.

Filter test #1 filter weight (grams)

Cage	Days	Filter	Initial	Final	Gain	Gain/day
#1	24	#3	734.0	731.9	-2.1	-0.1
#2	19	#1	728.1	1158.4	430.3	22.6
#3	19	#7	706.2	1102.7	396.5	20.9
#4	24	#6	694.8	1211.8	517.0	21.6
#5	19	#4	756.9	1180.2	423.3	22.3
#6	18	#5	715.2	1142.1	426.9	23.7

Filter test #2 filter weight (grams)

Cage	Days	Filter	Initial	Final	Gain	Gain/day
#3	9	#3	1234.3	1620.7	386.4	42.9
#4	9	#1	1289.3	1585.7	296.4	32.9

Filter test #3 filter weight (grams)

Cage	Days	Filter	Initial	Final	Gain	Gain/day
#1	16	#03	1035	*	*	*
#2	16	#04	1047	*	*	*
#3	15	#05	1009	*	*	*
#4	16	#06	1027	*	*	*
#5	16	#07	1050	*	*	*
#6	16	#08	1041	*	*	*

Filter test #4 filter weight (grams)

Cage	Days	Filter	Initial	Final	Gain	Gain/day
#1	17	#03	*	*	*	*
#2	17	#04	*	*	*	*
#3	17	#05	*	*	*	*
#4	15	#06	*	*	*	*
#5	17	#07	*	*	*	*
#6	17	#08	*	*	*	*

* - These data were recorded by NASA Quality Assurance Personnel.

Table 23. Daily data, AEM Filter Test #1: Water consumption, air flow, room temperature, and humidity.

Day		Water Consumption (ml)					Air FLow (cfm)						Room Temp. °F		Room Humidity
#	Cage	#2	#3	#4	#5	#6	#1	#2	#3	#4	#5	#6	Min	Max	%
1	14-Sep-92	-	-	-	-	--	14.9	14.8	14.8	14.8	14.9	14.8			50
2	15-Sep-92	178	186	160	320	332	0.0	14.7	15.1	14.4	13.8	14.0	70	84	48
3	16-Sep-92	146	172	148	68	150	14.6	14.9	14.8	14.1	13.4	13.8	70	75	54
4	17-Sep-92	149	164	144	170	167	7.3	14.3	14.7	14.0	13.5	13.8	70	74	55
5	18-Sep-92	146	149	140	174	154	7.8	14.2	14.5	14.2	13.2	13.7	70	72	56
6	19-Sep-92	150	236	141	171	144	14.4	14.7	15.1	14.2	14.0	14.3	68	73	58
7	20-Sep-92	167	168	158	194	174	10.0	14.7	14.7	14.0	13.8	14.0	68	80	53
8	21-Sep-92	159	152	158	177	176	0.0	14.1	14.3	13.4	13.3	13.2	73	80	
9	22-Sep-92	160	139	144	186	157	8.3	14.4	14.1	13.7	13.4	13.3	72	75	53
10	23-Sep-92	165	144	150	171	179	11.2	14.2	14.0	12.5	13.0	13.0	71	75	
11	24-Sep-92	166	146	153	162	160	7.9	13.6	13.7	12.2	12.4	12.9	72	75	59
12	25-Sep-92	150	142	149	170	159	18.1	14.1	13.8	12.6	13.0	13.0	70	75	52
13	26-Sep-92	192	168	186	204	188	17.4	14.0	13.9	12.5	13.1	13.0	70	79	52
14	27-Sep-92	190	177	212	218	205	19.0	14.0	14.0	12.4	13.2	13.0	74	86	45
15	28-Sep-92	223	178	204	226	222	16.9	13.6	12.4	12.0	13.6	12.6	74	86	42
16	29-Sep-92	233	192	209	244	212	13.2	13.2	13.3	11.5	11.9	12.1	77	84	50
17	30-Sep-92	176	144	178	183	190	0.0	13.3	13.0	11.2	11.9	11.2	72	78	54
18	1-Oct-92	169	155	164	194	184	20.3	12.7	12.4	10.7	11.1	10.3	71	79	56
19	2-Oct-92	174	150	159	192	164	20.9	13.0	13.0	11.1	11.8	10.9	70	80	51
20	3-Oct-92	162	138	141	157		21.3	13.0	13.3	11.3	12.0		67	72	53
21	4-Oct-92			140						11.2			68	78	50
22	5-Oct-92			158						10.8			73	80	46
23	6-Oct-92			154						10.4			70	80	48
24	7-Oct-92			150						10.6			70	83	42
25	8-Oct-92			186						10.0			70	84	38

Table 24. Daily data, AEM Filter Test #2: water consumption, air flow, room temperature, and humidity.

Day #		Water Consumption (ml)		Air FLow (cfm)		Room Temp. °F		Room Humidity
		Cage #3	#4	#3	#4	Min	Max	%
1	30-Oct-92	107	90	15.0	15.0	69	71	58
2	31-Oct-92	108	101	15.6	15.4	67	71	55
3	1-Nov-92	108	97	15.4	15.4	69	74	58
4	2-Nov-92	99	93	15.2	15.3	71	75	60
5	3-Nov-92	116	107	15.0	15.4	70	77	54
6	4-Nov-92	104	100	15.0	15.o	70	78	38
7	5-Nov-92	103	110	15.1	15.1	70	79	48
8	6-Nov-92			14.7	15.4	70	79	46

Table 25. Daily data, AEM Filter Test #3: water consumption, air flow, room temperature, and humidity.

Day		Water Consumption (ml)					Air FLow (cfm)						Room Temp. °F		Room Humidity
#	Cage	#2	#3	#4	#5	#6	#1	#2	#3	#4	#5	#6	Min	Max	%
1	1-Dec-92	171	162	168	168	162	15.2	15.4	14.7	15.2	15.4	15.1			44
2	2-Dec-92	122	129	142	124	128	14.7	14.9	15.1	14.1	15.0	14.3	61	70	50
3	3-Dec-92	156	147	155	152	148	15.0	15.2	16.2	14.9	15.3	15.0	62	65	57
4	4-Dec-92	183	178	197	168	189	15.0	15.4	15.6	15.0	15.1	14.7	60	68	48
5	5-Dec-92	198	194	207	182	179	14.0	14.7	14.4	13.7	14.8	14.3	63	80	38
6	6-Dec-92	174	141	155	170	161	14.0	14.7	14.7	13.6	14.7	14.5	72	82	43
7	7-Dec-92	177	160	164	164	165	14.0	14.8	16.0	14.5	15.1	14.8	68	82	49
8	8-Dec-92	180	174	190	183	195	13.4	14.4	14.2	13.3	14.7	14.4	68	78	39
9	9-Dec-92	176	160	170	170	162	13.1	14.3	14.7	13.4	14.3	14.3	74	78	47
10	10-Dec-92	166	154	185	175	162	13.3	14.3	14.9	13.3	14.6	14.2	72	78	39
11	11-Dec-92	175	159	194	187	169	13.4	14.3	14.6	13.3	14.3	14.5	68	78	44
12	12-Dec-92	190	172	213	178	182	14.0	14.6	15.0	14.0	14.6	14.5	68	78	42
13	13-Dec-92	191	164	207	204	184	14.4	14.8	14.4	14.2	14.7	15.0	68	78	34
14	14-Dec-92	186	154	168	180	165	13.4	14.3	14.3	13.4	14.4	14.5	73	76	36
15	15-Dec-92	165		182	196	165	13.5	14.7	14.7	14.0	14.7	14.7	69	76	40
16	16-Dec-92	196		205	220	218	13.4	14.4		14.0	14.5	14.7	69	78	33
17	17-Dec-92	168		214	192	170	13.8	14.2		14.7	13.9	15.7	70	78	42
18	18-Dec-92	176		194	181	168	14.1	14.7		14.7	14.3	16.1	70	78	36
19	19-Dec-92	176		194	165	182	14.2	14.7		14.8	14.2	16.9	68	78	36
20	20-Dec-92	195		219	215	190	14.5	14.7		13.7	14.5	16.9	70	77	36
21	21-Dec-92	152		180	172	169	14.2	14.3		14.7	14.4	16.5	72	78	38
22	22-Dec-92	144		189	174	172							72	78	38
23	23-Dec-92	144		182	191	184	13.7	14.5		14.5	14.0	16.4	69	77	37
24	24-Dec-92	122		177	170	200	14.9	14.7		14.8	14.2	16.3	70	77	38
25	25-Dec-92	129		184	157	167	14.7	14.9		14.8	14.4	16.7	68	77	37
26	26-Dec-92	154		180	164	171	14.1	14.4		14.5	14.1	16.1	68	76	37
27	27-Dec-92	121		126	151	148	14.1	14.7		14.7	14.2	16.0	69	75	40
28	28-Dec-92	148		149	130	166	14.7	14.8		14.7	14.6	16.4	60	70	56
29	29-Dec-92	149		151	171	173	13.7	14.3		13.8	13.6	16.0	59	74	42
30	30-Dec-92						14.7	14.8		14.6	14.4	16.3	68	74	45

Table 26. Daily data, AEM Filter Test #4: water consumption, air flow, room temperature, humidity, pressure drop across filter #5, and current through motor #2.

Day #		Water Consumption (ml) Cage #2	#3	#4	#5	#6	Air FLow (cfm) #1	#2	#3	#4	#5	#6	Room Temp °F Min	Max	Room Humidity %	Pres drop (in H_2O)	Current (ma)
1	27-Jan-93	142	140	140	160	142	15.6	15.9	15.4	15.8	15.9	15.4	65	71	43	0.3	
2	28-Jan-93	134	154	144	154	158	15.8	16.3	15.6	15.3	15.8	15.0	68	82	44	0.3	
3	29-Jan-93	164	156	159	159	153	15.9	15.9	15.1	14.8	15.7	14.3	66	82	43	0.3	
4	30-Jan-93	140	137	130	149	140	16.5	16.4	15.6	15.4	16.1	14.7	67	82	36	0.3	
5	31-Jan-93	128	147	149	146	143	15.8	15.5	15.0	14.5	15.6	14.2	64	81	34		
6	1-Feb-93	156	153	147	164	162	14.7	15.0	14.3	13.9	15.2	13.7	67	80	38	0.3	
7	2-Feb-93	132	138	121	139	146		15.4	14.5	15.0	15.1	13.4	67	82	40	0.3	
8	3-Feb-93	149	152	143	154	162		14.9	13.8	13.7	14.7	12.9	68	82	38	0.3	
9	4-Feb-93	135	133	121	136	144	16.0	14.7	13.6	13.3	14.0	13.0	68	82	40	0.3	
10	5-Feb-93	145	154	142	148	147	15.2	14.7	13.5	14.0	14.5	12.8	68	82	40	0.3	
11	6-Feb-93	132	136	129	132	135	18.1	16.4	14.7	14.7	15.0	13.6	68	82	48		
12	7-Feb-93	147	146	134	140	140	10.0	15.5	13.8	13.2	14.5	13.0	68	76	46	0.4	0.15
13	8-Feb-93	153	156	153	151	153	15.0	14.5	13.3	12.8	13.6	12.6	68	81	46	0.4	0.14
14	9-Feb-93	138	160	136	146	153	15.2	15.4	13.8	14.1	14.4	12.7	68	82	48	0.4	0.14
15	10-Feb-93	146	159	146	165	171	15.3	14.7	13.4	13.4	14.0	12.9	66	82	40	0.4	0.14
16	11-feb-93	97	100		90	104							66	82	42	0.4	0.14
17	12-Feb-93	162	170		158	157	16.1	15.4	13.7	0.0	14.6	13.0	65	82	43	0.4	0.16
18	13-Feb-93	115	124		125	126	17.0	16.5	14.5	0.0	14.7	13.4	62	82		0.4	0.14
19	14-Feb-93	137	139		140	155	16.7	15.4	14.0	0.0	14.2	13.3	60	82		0.4	0.14
20	15-feb-93	175	163		164	166	15.3	14.4	12.9		13.5	12.6	60	82		0.4	0.14
21	16-Feb-93	156	148		164	180	15.4	14.7	13.4	16.6	13.7	12.2	60	82		0.4	0.14
22	17-Feb-93	147	152		152	164							60	82		0.4	0.14
23	18-Feb-93	126	129		132	161	14.7	14.0	12.2	14.8	12.7	11.6	60	82		0.4	
24	19-Feb-93						15.2	15.2	12.7	15.9	13.1	11.9	60	82		0.4	0.14
25	20-Feb-93												61	80			0.14

Table 27. Filter test #1 (ALFCO #1 filters). Food bars remaining in occupied cages.

Day #		Cage Number 2	3	4	5	6
11	24-Sep-92	5.50	5.25	5.25	5.50	5.00
12	25-Sep-92	5.13	4.75	4.88	4.75	4.75
13	26-Sep-92	4.50	4.63	4.50	4.38	3.75
14	27-Sep-92	4.38	4.25	4.00	4.00	4.00
15	28-Sep-92	3.75	3.75	3.50	3.33	3.25
16	29-Sep-92	3.50	3.25	3.25	3.00	3.25
17	30-Sep-92	3.00	2.75	2.75	2.63	2.50
18	1-Oct-92	2.13	1.75	2.00	2.25	2.00
19	2-Oct-92	1.25	1.25	1.13	1.25	1.00
20	3-Oct-92	0.75	0.75	0.63	0.75	
21	4-Oct-92			1.00		
22	5-Oct-92			0.50		

Table 28. Filter test #2 (ALFCO #2 filters). Food bars remaining in occupied cages.

Day #		Cage Number 3	4
1	30-Oct-92	9.75	9.75
2	31-Oct-92	9.25	9.00
3	1-Nov-92	8.75	8.75
4	2-Nov-92	8.50	8.00
5	3-Nov-92	7.75	8.00
6	4-Nov-92	7.25	7.50
7	5-Nov-92	6.75	6.75
8	6-Nov-92	6.00	6.50

Table 29. Filter test #3 (ALFCO #3 filters). Food bars remaining in occupied cages.

Day #		Cage Number 2	3	4	5	6
1	1-Dec-92	10.00	10.00	10.00	10.00	10.00
2	2-Dec-92	8.75	9.00	9.00	8.75	9.00
3	3-Dec-92	8.25	8.25	8.25	8.25	8.00
4	4-Dec-92	7.75	7.75	7.50	7.50	7.50
5	5-Dec-92	6.50	7.00	7.00	6.75	7.00
6	6-Dec-92	6.50	7.00	6.75	6.25	6.50
7	7-Dec-92	6.00	6.25	6.00	5.75	6.00
8	8-Dec-92	5.25	5.50	5.25	5.00	5.50
9	9-Dec-92	4.50	5.00	4.50	4.50	4.75
10	10-Dec-92	4.00	4.50	4.25	4.25	4.50
11	11-Dec-92	4.50	4.25	3.50	3.50	4.00
12	12-Dec-92	3.25	3.50	2.34	3.00	3.14
13	13-Dec-92	2.50	3.25	2.25	2.50	3.00
14	14-Dec-92	2.00	3.00	2.00	2.00	2.75
15	15-Dec-92	1.75	2.50	1.50	1.00	1.50
16	16-Dec-92	1.50		0.75	0.50	1.50
17	17-Dec-92	9.50		9.50	9.50	9.25
18	18-Dec-92	9.25		8.75	9.00	9.00
19	19-Dec-92	8.00		8.25	8.50	8.25
20	20-Dec-92	7.50		8.25	7.75	7.50
21	21-Dec-92	6.50		7.50	7.25	7.00
22	22-Dec-92	6.00		6.75	6.50	6.50
23	23-Dec-92	5.75		6.00	6.25	6.00
24	24-Dec-92	5.25		6.00	5.50	5.25
25	25-Dec-92	4.25		5.00	4.75	5.00
26	26-Dec-92	4.25		4.00	4.25	4.25
27	27-Dec-92	3.50		3.50	3.50	3.50
28	28-Dec-92	3.00		3.00	2.50	2.75
29	29-Dec-92	2.75		2.00	1.75	2.00

Table 30. Filter test #4 (APM/Pall #1). Food bars remaining in occupied cages.

Day #		Cage Number 2	3	4	5	6
1	28-Jan-93	9.25	9.00	9.25	9.00	9.25
2	29-Jan-93	8.75	8.25	8.50	8.50	8.50
3	30-Jan-93	7.75	8.00	7.75	8.00	7.75
4	31-Jan-93	7.25	7.25	7.75	7.50	7.50
5	1-Feb-93	7.00	6.75	6.25	6.75	6.75
6	2-Feb-93	6.25	6.00	6.00	6.00	6.25
7	3-Feb-93	5.75	5.25	5.50	6.00	6.00
8	4-Feb-93	5.25	4.75	5.00	5.00	5.25
9	5-Feb-93	4.75	4.25	4.75	4.50	4.75
10	6-Feb-93	4.00	3.75	4.00	3.75	4.00
11	7-Feb-93	3.50	3.00	3.25	3.25	3.25
12	8-Feb-93	2.75	2.75	2.50	2.75	2.75
13	9-Feb-93	2.00	2.00	1.75	2.00	2.25
14	10-Feb-93	1.25	1.25	1.00	1.50	1.00
15	11-feb-93	0.75	0.50		0.50	0.50
16	12-Feb-93					
17	13-Feb-93	9.25	9.25		9.25	9.50
18	14-Feb-93	9.00	9.00		8.75	9.00
19	15-feb-93	8.00	8.50		8.00	8.50
20	16-Feb-93	7.75	8.00		7.50	8.00
21	17-Feb-93					
22	18-Feb-93	7.00	6.50		6.25	6.50
23	19-Feb-93	5.75	6.50		6.00	6.00

Figure 8. Mean air flow and daily mean water consumption (± SEM) for filter test #1 (ALFCO #1 filters).

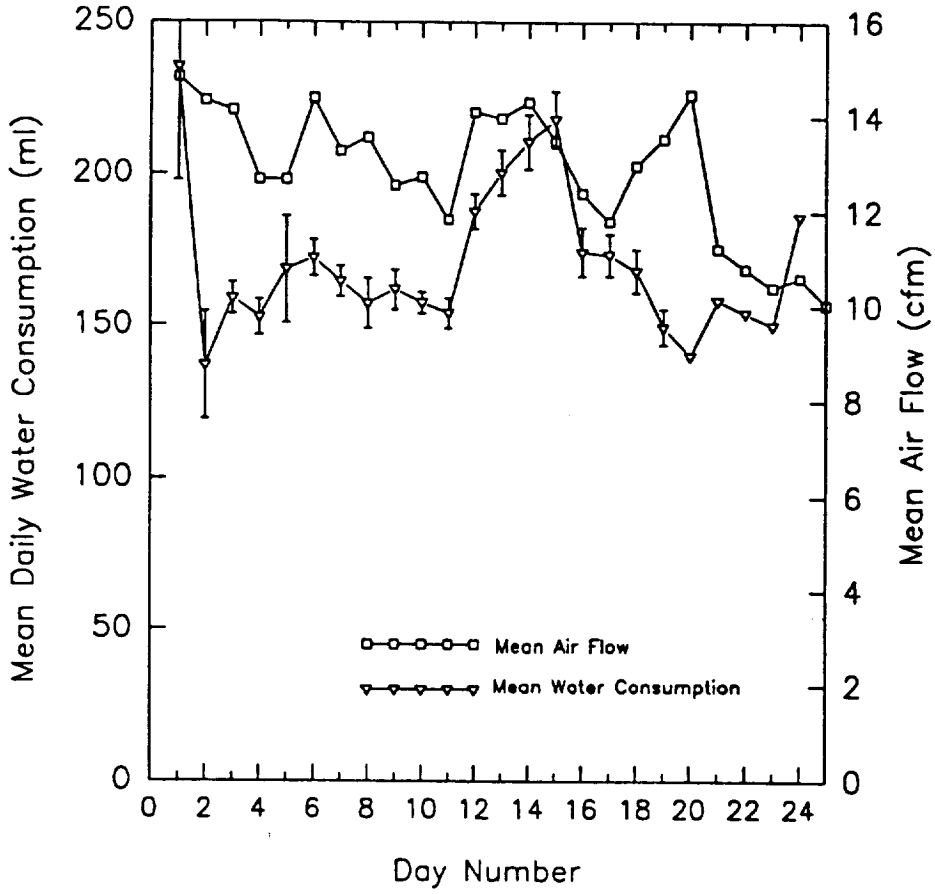

Figure 9. Mean air flow and daily mean water consumption (± SEM) for filter test #2 (ALFCO #2 filters).

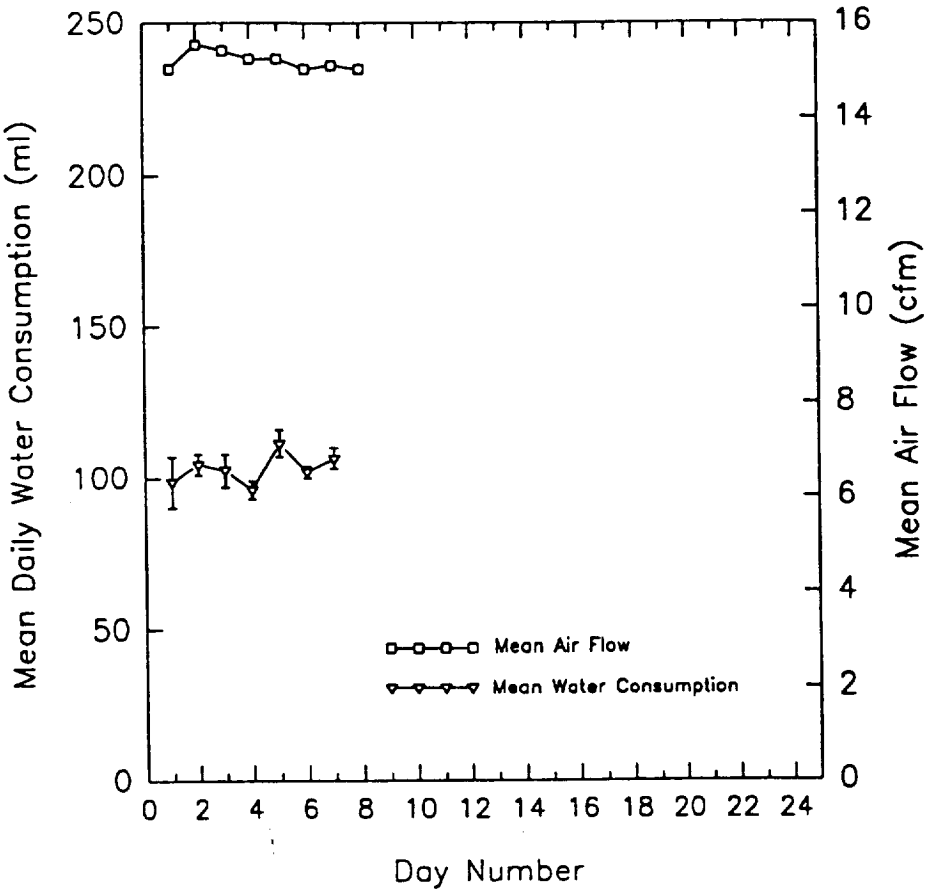

Figure 10. Mean air flow and daily mean water consumption (± SEM) for filter test #3 (ALFCO #3 filters).

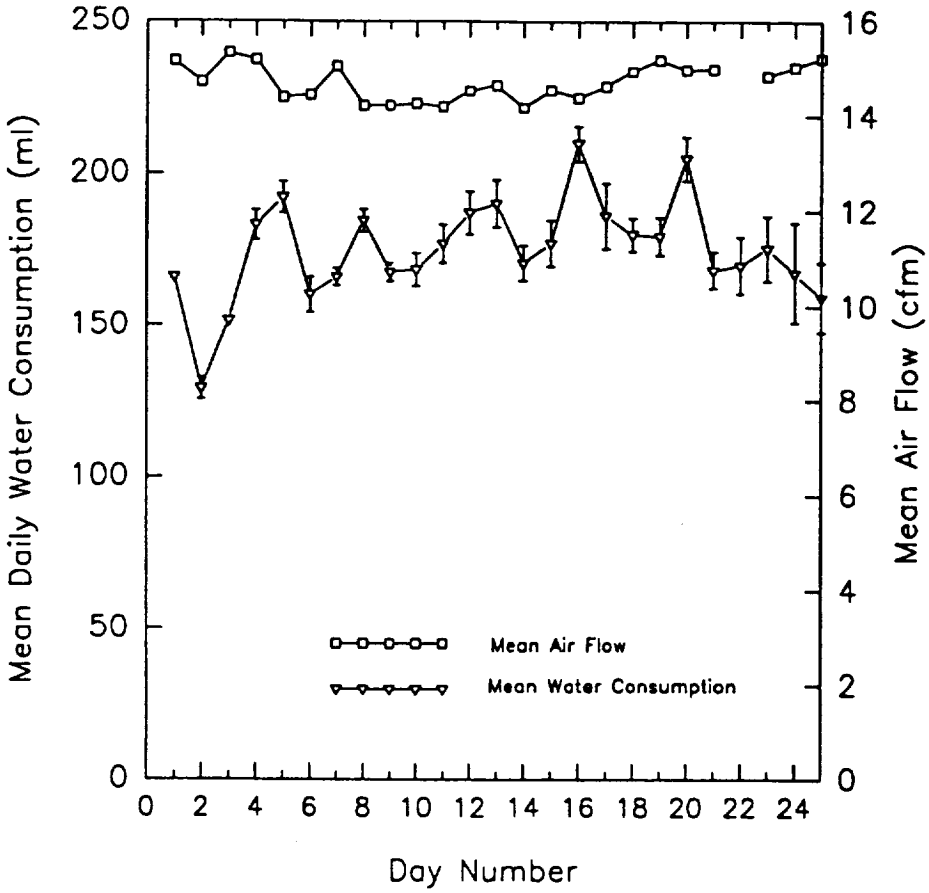

Figure 11. Mean air flow and daily mean water consumption (± SEM) for filter test #4 (APM/Pall #1 filters).

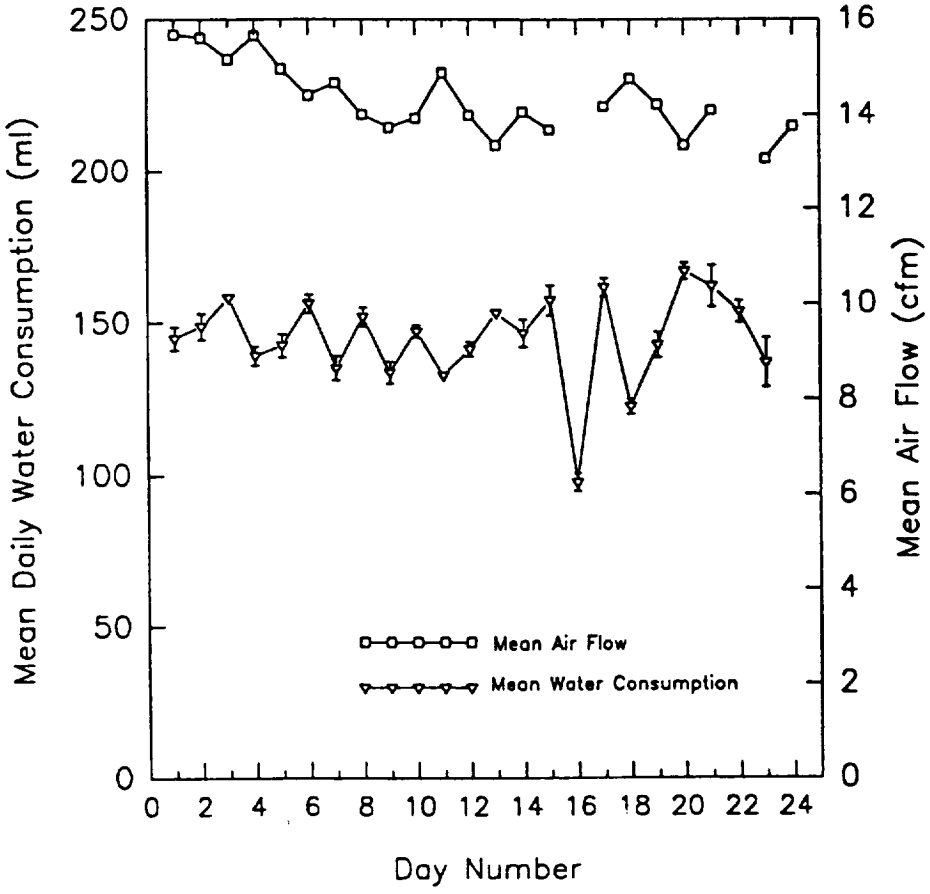

Table 31. Filter test #1 (ALFCO #1 filters). Mean scores and numbers of participants for odor panel members who passed a 3 sample test, members who failed a 3 sample test, and all odor panel members. Numbers are showN for each cage with totals for all occupied cages.

Day 1 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	12	12	12	12	12	12	0.151	StdDev pass
Mean pass	0.333	2.000	1.750	1.583	1.750	1.833	1.783	Mean Pass
N fail	5	5	5	5	5	5	0.167	StdDev Fail
Mean fail	0.600	1.600	1.600	1.400	1.200	1.400	1.440	Mean Fail
N all	17	17	17	17	17	17	0.135	StdDev All
Mean all	0.412	1.882	1.706	1.529	1.588	1.706	1.682	Mean All

Day 3 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	12	12	12	12	12	12	0.109	StdDev pass
Mean pass	0.583	1.667	1.667	1.917	1.750	1.667	1.733	Mean Pass
N fail	2	2	2	2	2	2	0.224	StdDev Fail
Mean fail	0	2.500	2.500	2.000	2.500	2.500	2.400	Mean Fail
N all	14	14	14	14	14	14	0.064	StdDev All
Mean all	0.500	1.786	1.786	1.929	1.857	1.786	1.829	Mean All

Day 6 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	14	14	14	14	14	14	0.229	StdDev pass
Mean pass	0.286	2	1.786	1.857	2.143	2.357	2.029	Mean Pass
N fail	0	0	0	0	0	0		StdDev Fail
Mean fail								Mean Fail
N all	14	14	14	14	14	14	0.229	StdDev All
Mean all	0.286	2.000	1.786	1.857	2.143	2.357	2.029	Mean All

Day 9 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	13	13	13	13	13	13	0.077	StdDev pass
Mean pass	0.615	2.000	1.923	1.846	2.000	1.846	1.923	Mean Pass
N fail	1	1	1	1	1	1	0.447	StdDev Fail
Mean fail	0.000	1.000	2.000	2.000	2.000	2.000	1.800	Mean Fail
N all	14	14	14	14	14	14	0.060	StdDev All
Mean all	0.571	1.929	1.929	1.857	2	1.857	1.914	Mean All

Day 12 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	12	12	12	12	12	12	0.767	StdDev pass
Mean pass	0.417	2.333	1.667	0.333	1.833	2	1.633	Mean Pass
N fail	2	2	2	2	2	2	0.671	StdDev Fail
Mean fail	1.000	2.000	2.000	0.500	2.000	2.000	1.700	Mean Fail
N all	14	14	14	14	14	14	0.749	StdDev All
Mean all	0.500	2.286	1.714	0.357	1.857	2	1.643	Mean All

Day 14 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	9	9	9	9	9	9	0.727	StdDev pass
Mean pass	0.222	1.667	2.000	0.333	1.889	2.111	1.600	Mean Pass
N fail	4	4	4	4	4	4	0.729	StdDev Fail
Mean fail	0.250	1.750	2.000	0.500	2.250	2.250	1.750	Mean Fail
N all	13	13	13	13	13	13	0.725	StdDev All
Mean all	0.231	1.692	2.000	0.385	2.000	2.154	1.646	Mean All

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Table 32. Filter test #3 (ALFCO #3 filters). Mean scores and numbers of participants for odor panel members who passed a 3 sample test, members who failed a 3 sample test, and all odor panel members. Numbers are showN for each cage with totals for all occupied cages.

Day 1 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	9	9	9	9	9	9	0.310	StdDev pass
Mean pass	0.333	0.333	1.000	1.000	0.444	0.778	0.711	Mean Pass
N fail	5	5	5	5	5	5	0.335	StdDev Fail
Mean fail	0.000	0.600	0.200	0.600	0.200	1.000	0.520	Mean Fail
N all	14	14	14	14	14	14	0.237	StdDev All
Mean all	0.214	0.429	0.714	0.857	0.357	0.857	0.643	Mean All

Day 3 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	13	13	13	13	13	13	0.122	StdDev pass
Mean pass	0.692	0.462	0.385	0.538	0.308	0.615	0.462	Mean Pass
N fail	1	1	1	1	1	1	0.548	StdDev Fail
Mean fail	1.000	1.000	0.000	0.000	1.000	1.000	0.600	Mean Fail
N all	14	14	14	14	14	14	0.120	StdDev All
Mean all	0.714	0.500	0.357	0.500	0.357	0.643	0.471	Mean All

Day 7 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	12	12	12	12	12	12	0.246	StdDev pass
Mean pass	0.417	0.500	0.417	0.667	0.417	1.000	0.600	Mean Pass
N fail	2	2	2	2	2	2	0.500	StdDev Fail
Mean fail	1.500	1.000	0.500	1.500	0.500	1.500	1.000	Mean Fail
N all	14	14	14	14	14	14	0.274	StdDev All
Mean all	0.571	0.571	0.429	0.786	0.429	1.071	0.657	Mean All

Day 14 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	7	7	7	7	7	7	0.424	StdDev pass
Mean pass	0.857	0.714	0.429	1.286	0.714	1.429	0.914	Mean Pass
N fail	10	10	10	10	10	10	0.071	StdDev Fail
Mean fail	0.300	0.100	0.200	0.200	0.200	0.300	0.200	Mean Fail
N all	17	17	17	17	17	17	0.202	StdDev All
Mean all	0.529	0.353	0.294	0.647	0.412	0.765	0.494	Mean All

Day 21 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	11	11	11	11	11	11	0.157	StdDev pass
Mean pass	0.364	0.364	0.455	0.091	0.455	0.364	0.318	Mean Pass
N fail	4	4	4	4	4	4	0.375	StdDev Fail
Mean fail	0.000	0.000	0.250	0.000	0.750	0.000	0.188	Mean Fail
N all	15	15	15	15	15	15	0.191	StdDev All
Mean all	0.267	0.267	0.400	0.067	0.533	0.267	0.283	Mean All

Day 24 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	2	2	2	2	2	2	0.289	StdDev pass
Mean pass	0.500	0.500	0	0.500	0.000	0.000	0.250	Mean Pass
N fail	11	11	11	11	11	11	0.052	StdDev Fail
Mean fail	0.273	0.273	0.182	0.182	0.182	0.273	0.227	Mean Fail
N all	13	13	13	13	13	13	0.063	StdDev All
Mean all	0.308	0.308	0.154	0.231	0.154	0.231	0.231	Mean All

Table 32. Filter test #4 (APM/Pall #1 filters). Mean scores and numbers of participants for odor panel members who passed a 3 sample test, members who failed a 3 sample test, and all odor panel members. Numbers are showN for each cage with totals for all occupied cages.

Day 1 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	19	20	20	20	20	20	0.225	StdDev pass
Mean pass	0.474	0.800	1.350	0.950	1.000	1.250	1.070	Mean Pass
N fail	1	1	1	1	1	1	0.447	StdDev Fail
Mean fail	0.000	1.000	1.000	1.000	1.000	2.000	1.200	Mean Fail
N all	20	21	21	21	21	21	0.225	StdDev All
Mean all	0.450	0.810	1.333	0.952	1	1.286	1.076	Mean All

Day 3 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	15	15	15	15	15	15	0.256	StdDev pass
Mean pass	0.333	1.333	0.933	0.667	0.800	1.067	0.960	Mean Pass
N fail	2	2	2	2	2	2	0.447	StdDev Fail
Mean fail	0	1.500	2.000	2.500	2.500	2.500	2.200	Mean Fail
N all	17	17	17	17	17	17	0.188	StdDev All
Mean all	0.294	1.353	1.059	0.882	1.000	1.235	1.106	Mean All

Day 7 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	7	7	7	7	7	7	0.359	StdDev pass
Mean pass	0.429	1.143	0.714	0.714	1.571	1.143	1.057	Mean Pass
N fail	11	11	11	11	11	11	0.197	StdDev Fail
Mean fail	0.545	0.909	1.091	1.273	1.364	1.364	1.200	Mean Fail
N all	18	18	18	18	18	18	0.210	StdDev All
Mean all	0.500	1.000	0.944	1.056	1.444	1.278	1.144	Mean All

Day 14 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	14	14	14	14	14	14	0.178	StdDev pass
Mean pass	0.214	1.286	1.643	1.571	1.714	1.714	1.586	Mean Pass
N fail	1	1	1	1	1	1	0.447	StdDev Fail
Mean fail	0.000	1.000	2.000	2.000	2.000	2.000	1.800	Mean Fail
N all	15	15	15	15	15	15	0.194	StdDev All
Mean all	0.200	1.267	1.667	1.600	1.733	1.733	1.600	Mean All

Day 21 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	12	12	12	12	12	12	0.210	StdDev pass
Mean pass	0.333	1.667	1.667	3.167	1.333	1.833	1.625	Mean Pass
N fail	1	1	1	1	1	1	0	StdDev Fail
Mean fail	0.000	0.000	0.000	0.000	0.000	0.000	0.000	Mean Fail
N all	13	13	13	13	13	13	0.194	StdDev All
Mean all	0.308	1.538	1.538	2.923	1.231	1.692	1.500	Mean All

Day 24 evaluation	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Occupied Cages only	
N pass	13	13	13	13	13	12	0.246	StdDev pass
Mean pass	0.615	2.077	1.923	2.077	1.769	1.500	1.817	Mean Pass
N fail	2	2	2	2	2	2	0.629	StdDev Fail
Mean fail	0.000	1.000	0.500	2.500	2.000	1.000	1.125	Mean Fail
N all	15	15	15	15	15	14	0.214	StdDev All
Mean all	0.533	1.933	1.733	2.133	1.800	1.429	1.724	Mean All